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M-253

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UNIT I: PHARMA-INFORMATICS

NOTES

★ STRUCTURE ★

1.1 LEARNING OBJECTIVES

After studying this unit, you will be able to:

- define bioinformatics
- discuss the aims of bioinformatics
- discuss about history of the pharmaceutical industry
- understand and organize the information

1.2 INTRODUCTION

After going through this unit, you will be able to discuss about aims of bioinformatics and the terms such as 'biological', 'biopharmaceutical' and 'products of biotechnology'. The term 'biologic' generally refers to medicinal product derived from blood, as well as vaccines, toxins and allergen products. Biotechnology refers to the use of biological systems or biological molecules.

1.3 INTRODUCTION TO BIOINFORMATICS

Biological data are being produced at a phenomenal rate. For example as of April 2001, the GenBank repository of nucleic acid sequences contained 11,546,000 entries and the SWISSPROT database of protein sequences contained 95,320. On average, these databases are doubling in size every 15 months.

In addition, since the publication of the *H. influenzae* genome, complete sequences for nearly 300 organisms have been released, ranging from 450 genes to over 100,000. Add to this the data from the myriad of related projects that study gene expression, determine the protein structures encoded by the genes, and detail how these products interact with one another, and we can begin to imagine the enormous quantity and variety of information that is being produced.

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As a result of this surge in data, computers have become indispensable to biological research. Such an approach is ideal because of the ease with which computers can handle large quantities of data and probe the complex dynamics observed in nature. Bioinformatics, the subject of the current review, is often defined as the application of computational techniques to understand and organise the information associated with biological macromolecules.

This unexpected union between the two subjects is attributed to the fact that life itself is an information technology; an organism's physiology is largely determined by its genes, which at its most basic can be viewed as digital information. At the same time, there have been major advances in the technologies that supply the initial data; Anthony Kervalage of Celera recently cited that an experimental laboratory can produce over 100 gigabytes of data a day with ease. This incredible processing power has been matched by developments in computer technology; the most important areas of improvements have been in the CPU, disk storage and Internet, allowing faster computations, better data storage and revolutionalised the methods for accessing and exchanging data.

1.4 AIMS OF BIOINFORMATICS

In general, the aims of bioinformatics are three-fold. First, at its simplest bioinformatics organises data in a way that allows researchers to access existing information and to submit new entries as they are produced *e.g.*, the Protein Data Bank for 3D macromolecular structures. While data-curation is an essential task, the information stored in these databases is essentially useless until analysed. Thus, the purpose of bioinformatics extends much further. The second aim is to develop tools and resources that aid in the analysis of data. For example, having sequenced a particular protein, it is of interest to compare it with previously characterised sequences.

This needs more than just a simple textbased search, and programs such as FASTA and PSI-BLAST must consider what constitutes a biologically significant match.

Development of such resources dictates expertise in computational theory, as well as a thorough understanding of biology.

The third aim is to use these tools to analyse the data and interpret the results in a biologically meaningful manner. Traditionally, biological studies examined individual systems in detail, and frequently compared them with a few that are related. In bioinformatics, we can now conduct global analyses of all the available data with the aim of uncovering common principles that apply across many systems and highlight novel features.

"...the INFORMATION associated with these Molecules..."

Table 1.1 lists the types of data that are analysed in bioinformatics and the range of topics that we consider to fall within the field. Here we take a broad view and include subjects that may not normally be listed. We also give approximate values describing the sizes of data being discussed.

We start with an overview of the sources of information. Most bioinformatics analyses focus on three primary sources of data: DNA or protein sequences,

macromolecular structures and the results of functional genomics experiments. Raw DNA sequences are strings of the four base-letters comprising genes, each typically 1,000 bases long. The GenBank repository of nucleic acid sequences currently holds a total of 12.5 billion bases in 11.5 million entries. At the next level are protein sequences comprising strings of 20 amino acidletters. At present there are about 400,000 known protein sequences, with a typical bacterial protein containing approximately 300 amino acids. Macromolecular structural data represents a more complex form of information. There are currently 15,000 entries in the Protein Data Bank, PDB, containing atomic structures of proteins, DNA and RNA solved by x-ray crystallography and NMR. A typical PDB file for a medium-sized protein contains the xyz-coordinates of approximately 2,000 atoms.

Table 1.1 Sources of data used in bioinformatics, the quantity of each type of data that is currently available, and bioinformatics subject areas that utilize this data.

Data source	*Data size*	*Bioinformatics topics*
Raw DNA sequence	11.5 million sequences (12.5 billion bases)	Separating coding and non-coding regions Identification of introns and exons Gene product prediction Forensic analysis
Protein sequence	400,000 sequences (~ 300 amino acids each)	Sequence comparison algorithms Multiple sequence alignments algorithms Identification of conserved sequence motifs
Macromolecular structure	15,000 structures (~ 1, 000 atomic coordinates each)	Secondary, tertiary structure prediction 3D structural alignment algorithms Protein geometry measurements Surface and volume shape calculations Intermolecular interactions Molecular simulations (force-field calculations, molecular movements, docking predictions)
Genomes	300 complete genomes (1.6 million – 3 billion bases each)	Characterization of repeats Structural assignments to genes Phylogenetic analysis Genomic-scale censuses (characterization of protein content, metabolic pathways) Linkage analysis relating specific genes to diseases
Gene expression	Largest: ~ 20 time point measurements for ~ 6,000 genes in yeast	Correlating expression patterns Mapping expression data to sequence, structural and biochemical data
Other data		
Literature	11 million citations	Digital libraries for automated bibliographical searches Knowledge databases of data from literature
Metabolic pathways		Pathway simulations

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Scientific euphoria has recently centred on whole genome sequencing. As with the raw DNA sequences, genomes consist of strings of base-letters, ranging from 1.6 million bases in *Haemophilus influenzae* 10 to 3 billion in humans. The Entrez database currently has complete sequences for nearly 300 archaeal, bacterial and eukaryotic organisms. In addition to producing the raw nucleotide sequence, a lot of work is involved in processing this data. An important aspect of complete genomes is the distinction between coding regions and non-coding regions -'junk' repetitive sequences making up the bulk of base sequences especially in eukaryotes. Within the coding regions, genes are annotated with their translated protein sequence, and often with their cellular function.

More recent sources of data have been from functional genomics experiments, of which the most common are gene expression studies.We can now determine expression levels of almost every gene in a given cell on a whole-genome level, however there is currently no central repository for this data and public availability is limited. These experiments measure the amount of mRNA that is produced by the cell under different environmental conditions, different stages of the cell cycle and different cell types in multicellular organisms. Much of the effort has so far focused on the yeast and human genomes. One of the largest dataset for yeast has made approximately 20 time-point measurements for 6,000 genes. However, there is potential for much greater quantities of data when experiments are conducted for larger organisms and at more time-points.

Further genomic-scale data include biochemical information on metabolic pathways, regulatory networks, proteinprotein interaction data from two-hybrid experiments, and systematic knockouts of individual genes to test the viability of an organism. What is apparent from this list is the diversity in the size and complexity of different datasets. There are invariably more sequence-based data than others because of the relative ease with which they can be produced. This is partly related to the greater complexity and information-content of individual structures or gene expression experiments compared to individual sequences. While more biological information can be derived from a single structure than a protein sequence, the lack of depth in the latter is compensated by analyzing larger quantities of data.

"...ORGANISE the Information on a LARGE SCALE..." : Redundancy and Multiplicity of Data

A concept that underpins most research methods in bioinformatics is that much of the data can be grouped together based on biologically meaningful similarities. For example, sequence segments are often repeated at different positions of genomic DNA Genes can be clustered into those with particular functions (*e.g.*, enzymatic actions) or according to the metabolic pathway to which they belong, although here, single genes may actually possess several functions. Going further, distinct proteins frequently have comparable sequences – organisms often have multiple copies of a particular gene through duplication and different species have equivalent or similar proteins that were inherited when they diverged from each other in evolution. At a structural level, we predict there to be a finite number of different tertiary structures–estimates range between 1,000 and 10,000 folds – and proteins adopt equivalent structures even when they differ greatly in sequence. As a result, although the number of structures in the PDB has increased

exponentially, the rate of discovery of novel folds has actually decreased. There are common terms to describe the relationship between pairs of proteins or the genes from which they are derived: analogous proteins have related folds, but unrelated sequences, while homologous proteins are both sequentially and structurally similar. The two categories can sometimes be difficult to distinguish especially if the relationship between the two proteins is remote. Among homologues, it is useful to distinguish between orthologues, proteins in different species that have evolved from a common ancestral gene, and paralogues, proteins that are related by gene duplication within a genome.

Normally, orthologues retain the same function while paralogues evolve distinct, but related functions. An important concept that arises from these observations is that of a finite "parts list" for different organisms: an inventory of proteins contained within an organism, arranged according to different properties such as gene sequence, protein fold or function. Taking protein folds as an example, we mentioned that with a few exceptions, the tertiary structures of proteins adopt one of a limited repertoire of folds. As the number of different fold families is considerably smaller than the number of genes, categorising the proteins by fold provides a substantial simplification of the contents of a genome. Similar simplifications can be provided by other attributes such as protein function. As such, we expect this notion of a finite parts list to become increasingly common in future genomic analyses.

Clearly, an essential aspect of managing this large volume of data lies in developing methods for assessing similarities between different biomolecules and identifying those that are related. There are well-documented classifications for all of the main types of data we described earlier. Although detailed descriptions of these classification systems are beyond the scope of the current review, they are of great importance as they ease comparisons between genomes and their products. Links to the major databases are available from our supplementary website.

1.5 DATA INTEGRATION

The most profitable research in bioinformatics often results from integrating multiple sources of data. For instance, the 3D coordinates of a protein are more useful if combined with data about the protein's function, occurrence in different genomes, and interactions with other molecules. In this way, individual pieces of information are put in context with respect to other data. Unfortunately, it is not always straightforward to access and crossreference these sources of information because of differences in nomenclature and file formats. At a basic level, this problem is frequently addressed by providing external links to other databases. For example in PDBsum, web-pages for individual structures direct the user towards corresponding entries in the PDB, NDB, CATH, SCOP and SWISS-PROT databases. At a more advanced level, there have been efforts to integrate access across several data sources. One is the Sequence Retrieval System, SRS, which allows flat-file databases to be indexed to each other; this allows the user to retrieve, link and access entries from nucleic acid, protein sequence, protein motif, protein structure and bibliographic databases. Another is the Entrez facility, which provides similar gateways to DNA and protein sequences, genome mapping data, 3D macromolecular structures and the PubMed bibliographic database.

A search for a particular gene in either database will allow smooth transitions to the genome it comes from, the protein sequence it encodes, its structure, bibliographic reference and equivalent entries for all related genes. In our own group, we have developed the SPINE and PartsList web resources; these databases integrate many types of experimental data and organise them using the concept of the finite "parts list" we described above.

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"...UNDERSTAND and Organise the Information..."

Having examined the data, we can discuss the types of analyses that are conducted. As shown in Table 1.1, the broad subject areas in bioinformatics can be separated according to the type of information that is used. For raw DNA sequences, investigations involve separating coding and non-coding regions, and identification of introns, exons and promoter regions for annotating genomic DNA. For protein sequences, analyses include developing algorithms for sequence comparisons, methods for producing multiple sequence alignments, and searching for functional domains from conserved sequence motifs in such alignments. Investigations of structural data include prediction of secondary and tertiary protein structures, producing methods for 3D structural alignments [49, 50], examining protein geometries using distance and angular measurements, calculations of surface and volume shapes and analysis of protein interactions with other subunits, DNA, RNA and smaller molecules.

These studies have lead to molecular simulation topics in which structural data are used to calculate the energetics involved in stabilising macromolecular structures, simulating movements within macromolecules, and computing the energies involved in molecular docking. The increasing availability of annotated genomic sequences has resulted in the introduction of computational genomics and proteomics – large-scale analyses of complete genomes and the proteins that they encode. Research includes characterisation of protein content and metabolic pathways between different genomes, identification of interacting proteins, assignment and prediction of gene products, and large-scale analyses of gene expression levels. Some of these research topics will be demonstrated in our example analysis of transcription regulatory systems.

Other subject areas we have included in Table 1.1 are: development of digital libraries for automated bibliographical searches, knowledge bases of biological information from the literature, DNA analysis methods in forensics, prediction of nucleic acid structures, metabolic pathway simulations, and linkage analysis – linking specific genes to different disease traits.

In addition to finding relationships between different proteins, much of bioinformatics involves the analysis of one type of data to infer and understand the observations for another type of data. An example is the use of sequence and structural data to predict the secondary and tertiary structures of new protein sequences.

These methods, especially the former, are often based on statistical rules derived from structures, such as the propensity for certain amino acid sequences to produce different secondary structural elements. Another example is the use of structural data to understand a protein's function; here studies have investigated the relationship different protein folds and their functions and analysed similarities between different binding sites in the absence of homology. Combined with similarity

measurements, these studies provide us with an understanding of how much biological information can be accurately transferred between homologous proteins.

1.6 BIOPHARMACEUTICALS AND PHARMACEUTICAL BIOTECHNOLOGY

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Terms such as 'biologic', 'biopharmaceutical' and 'products of pharmaceutical biotechnology' or 'biotechnology medicines' have now become an accepted part of the pharmaceutical literature. However, these terms are sometimes used interchangeably and can mean different things to different people. Although it might be assumed that 'biologic' refers to any pharmaceutical product produced by biotechnological endeavour, its definition is more limited. In pharmaceutical circles, 'biologic' generally refers to medicinal products derived from blood, as well as vaccines, toxins and allergen products. 'Biotechnology' has a much broader and long-established meaning. Essentially, it refers to the use of biological systems (*e.g.*, cells or tissues) or biological molecules (*e.g.*, enzymes or antibodies) for/in the manufacture of commercial products.

The term 'biopharmaceutical' was first used in the 1980s and came to describe a class of therapeutic proteins produced by modern biotechnological techniques, specifically via genetic engineering or, in the case of monoclonal antibodies, by hybridoma technology. Although the majority of biopharmaceuticals or biotechnology products now approved or in development are proteins produced via genetic engineering, these terms now also encompass nucleic-acid-based, *i.e.*, deoxyribonucleic acid (DNA)- or ribonucleic acid (RNA)-based products, and whole-cell-based products.

Table 1.2 Some traditional pharmaceutical substances that are generally produced by direct chemical synthesis

Drug	*Molecular formula*	*Molecular mass*	*Therapeutic indication*
Acetaminophen (paracetamol)	$C_8H_9NO_2$	151.16	Analgesic
Ketamine	$C_{13}H_{16}C/NO$	237.74	Anaesthetic
Levamisole	$C_{11}H_{12}N_2S$	204.31	Anthelmintic
Diazoxide	C_8H_7C/N_2O_2S	230.7	Antihypertensive
Acyclovir	$C_8H_{11}N_5O_3$	225.2	Antiviral agent
Zidovudine	$C_{10}H_{13}N_5O_4$	267.2	Antiviral agent
Dexamethasone	$C_{22}H_{29}FO_5$	392.5	Anti-inflammatory and immunosuppressive agent
Misoprostol	$C_{22}H_{38}O_5$	382.5	Anti-ulcer agent
Cimetidine	$C_{10}H_{16}N_6$	252.3	Anti-ulcer agent

History of the Pharmaceutical Industry

The pharmaceutical industry, as we now know it, is barely 60 years old. From very modest beginnings, it has grown rapidly, reaching an estimated value of US$100

billion by the mid 1980s. Its current value is likely double or more this figure. There are well in excess of 10 000 pharmaceutical companies in existence, although only about 100 of these can claim to be of true international significance. These companies manufacture in excess of 5000 individual pharmaceutical substances used routinely in medicine.

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Table 1.3 Some pharmaceuticals that were traditionally obtained by direct extraction from biological source material. Many of the protein-based pharmaceuticals mentioned are now also produced by genetic engineering

Substance	*Medical application*
Blood products (*e.g.*, coagulation factors)	Treatment of blood disorders such as hemophilia A or B
Vaccines	Vaccination against various diseases
Antibodies	Passive immunization against various diseases
Insulin	Treatment of diabetes mellitus
Enzymes	Thrombolytic agents, digestive aids, debriding agents (*i.e.*, cleansing of wounds)
Antibiotics	Treatment against various infectious agents
Plant extracts (*e.g.*, alkaloids)	Various, including pain relief

The first stages of development of the modern pharmaceutical industry can be traced back to the turn of the twentieth century. At that time (apart from folk cures), the medical community had at their disposal only four drugs that were effective in treating specific diseases:

- Digitalis (extracted from foxglove) was known to stimulate heart muscle and, hence, was used to treat various heart conditions.
- Quinine, obtained from the barks/roots of a plant (*Cinchona* genus), was used to treat malaria.
- Pecacuanha (active ingredient is a mixture of alkaloids), used for treating dysentery, was obtained from the bark/roots of the plant genus *Cephaelis*.
- Mercury, for the treatment of syphilis.

This lack of appropriate, safe and effective medicines contributed in no small way to the low life expectancy characteristic of those times. Developments in biology (particularly the growing realization of the microbiological basis of many diseases), as well as a developing appreciation of the principles of organic chemistry, helped underpin future innovation in the fledgling pharmaceutical industry. The successful synthesis of various artificial dyes, which proved to be therapeutically useful, led to the formation of pharmaceutical/ chemical companies such as Bayer and Hoechst in the late 1800s. Scientists at Bayer, for example, succeeded in synthesizing aspirin in 1895.

Despite these early advances, it was not until the 1930s that the pharmaceutical industry began to develop in earnest. The initial landmark discovery of this era was probably the discovery, and chemical synthesis, of the sulfa drugs. These are a group of related molecules derived from the red dye *prontosil rubrum*. These drugs proved effective in the treatment of a wide variety of bacterial infections (Figure 1.1). Although it was first used therapeutically in the early 1920s, large-scale industrial production of insulin also commenced in the 1930s.

The medical success of these drugs gave new emphasis to the pharmaceutical industry, which was boosted further by the commencement of industrial-scale penicillin manufacture in the early 1940s. Around this time, many of the current leading pharmaceutical companies (or their forerunners) were founded. Examples include Ciba Geigy, Eli Lilly, Wellcome, Glaxo and Roche. Over the next two to three decades, these companies developed drugs such as tetracyclines, corticosteroids, oral contraceptives, antidepressants and many more. Most of these pharmaceutical substances are manufactured by direct chemical synthesis.

The Age of Biopharmaceuticals

Biomedical research continues to broaden our understanding of the molecular mechanisms underlining both health and disease. Research undertaken since the 1950s has pinpointed a host of proteins produced naturally in the body that have obvious therapeutic applications. Examples include the interferons and interleukins (which regulate the immune response), growth factors, such as erythropoietin (EPO; which stimulates red blood cell production), and neurotrophic factors (which regulate the development and maintenance of neural tissue).

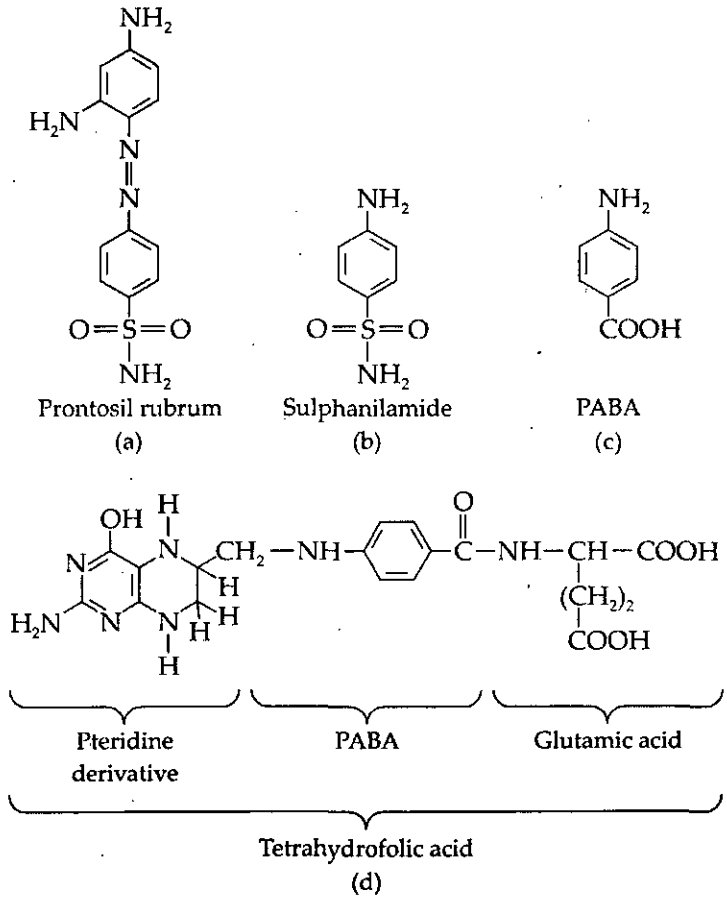

Fig. 1.1 Sulfa drugs and their mode of action

Although the pharmaceutical potential of these regulatory molecules was generally appreciated, their widespread medical application was in most cases rendered impractical due to the tiny quantities in which they were naturally produced. The advent of recombinant DNA technology (genetic engineering) and monoclonal antibody technology (hybridoma technology) overcame many

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such difficulties, and marked the beginning of a new era of the pharmaceutical sciences. Recombinant DNA technology has had a fourfold positive impact upon the production of pharmaceutically important proteins:

- *It overcomes the problem of source availability.* Many proteins of therapeutic potential are produced naturally in the body in minute quantities. Examples include interferons, interleukins and colony-stimulating factors. This rendered impractical their direct extraction from native source material in quantities sufficient to meet likely clinical demand. Recombinant production units 3 and 5 allows the manufacture of any protein in whatever quantity it is required.
- *It overcomes problems of product safety.* Direct extraction of product from some native biological sources has, in the past, led to the unwitting transmission of disease. Examples include the transmission of blood-borne pathogens such as hepatitis B and C and human immunodeficiency virus (HIV) via infected blood products and the transmission of Creutzfeldt-Jakob disease to persons receiving human growth hormone (GH) preparations derived from human pituitaries. *It provides an alternative to direct extraction from inappropriate/dangerous source material.* A number of therapeutic proteins have traditionally been extracted from human urine. Folliclestimulating hormone (FSH), the fertility hormone, for example, is obtained from the urine of postmenopausal women, and a related hormone, human chorionic gonadotrophin (hCG), is extracted from the urine of pregnant women. Urine is not considered a particularly desirable source of pharmaceutical products. Although several products obtained from this source remain on the market, recombinant forms have now also been approved. Other potential biopharmaceuticals are produced naturally in downright dangerous sources. Ancrod, for example, is a protein displaying anti-coagulant activity and, hence, is of potential clinical use. It is, however, produced naturally by the Malaysian pit viper. Although retrieval by milking snake venom is possible, and indeed may be quite an exciting procedure, recombinant production in less dangerous organisms, such as *Escherichia coli* or *Saccharomycese cerevisiae,* would be considered preferable by most.
- *It facilitates the generation of engineered therapeutic proteins displaying some clinical advantage over the native protein product.* Techniques such as site-directed mutagenesis facilitate the logical introduction of predefined changes in a protein's amino acid sequence. Such changes can be as minimal as the insertion, deletion or alteration of a single amino acid residue, or can be more substantial (*e.g.,* the alteration/deletion of an entire domain, or the generation of a novel hybrid protein). Such changes can be made for a number of reasons, and several engineered products have now gained marketing approval. An overview summary of some engineered product types now on the market is provided in Table 1.4. These and other examples will be discussed in subsequent chapters.

Despite the undoubted advantages of recombinant production, it remains the case that many protein-based products extracted directly from native source material remain on the market. In certain circumstances, direct extraction of native source material can prove equally/more attractive than recombinant production. This may be for an economic reason if, for example, the protein is produced in very large quantities by the native source and is easy to extract/purify, *e.g.,* human serum

albumin. Also, some blood factor preparations purified from donor blood actually contain several different blood factors and, hence, can be used to treat several haemophilia patient types. Recombinant blood factor preparations, on the other hand, contain but a single blood factor and, hence, can be used to treat only one haemophilia type. The advent of genetic engineering and monoclonal antibody technology underpinned the establishment of literally hundreds of start-up biopharmaceutical (biotechnology) companies in the late 1970s and early 1980s. The bulk of these companies were founded in the USA, with smaller numbers of start-ups emanating from Europe and other world regions.

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Table 1.4 Selected engineered biopharmaceutical types/products that have now gained marketing approval.

Product description/type	*Alteration introduced*	*Rationale*
Faster acting insulins	Modified amino acid sequence	Generation of faster acting insulin
Slow acting insulins	Modified amino acid sequence	Generation of slow acting insulin
Modified tissue plasminogen activator	Removal of three of the five native domains of tPA	Generation of a faster acting thrombolytic (clot degrading agent)
Modified blood factor VIII	Deletion of 1 domain of native factor VIII	Production of a lower molecular mass product
Chimaeric/humanized antibodies	Replacement of most/ virtually all of the murine amino acid sequences with sequences found in human antibodies	Greatly reduced/eliminated immunogenicity. Ability to activate human effector functions
'Ontak', a fusion protein	Fusion protein consisting of the diphtheria toxin linked to interleukin-2 (IL-2)	Targets toxin selectively to cells expressing an IL-2 receptor

SUMMARY

- Bioinformatics, the subject of the current review, is often defined as the application of computational techniques to understand and organise the information associated with biological macromolecules.
- Most bioinformatics analyses focus on three primary sources of data: DNA or protein sequences, macromolecular structures and the results of functional genomics experiments.
- A concept that underpins most research methods in bioinformatics is that much of the data can be grouped together based on biologically meaningful similarities.
- Sequence Retrieval System, SRS, which allows flat-file databases to be indexed to each other.
- 'Biologic' refers to any pharmaceutical product produced by biotechnological endeavour.
- It refers to the use of biological systems (*e.g.*, cells or tissues) or biological molecules (*e.g.*, enzymes or antibodies) for/in the manufacture of commercial products.

NOTES

REVIEW QUESTIONS

1. Explain the meaning of bioinformatics.
2. What are different sources associated with bioinformatics?
3. What are the aims of bioinformatics?
4. Define the following terms:
   (*i*) Biologic (*ii*) Biotechnology (*iii*) Biopharmaceutical
5. Discuss the fourfold positive impact of recombinant DNA technology upon the production of pharmaceutically important proteins.

UNIT II: EXPERIMENTAL DESIGNS

NOTES

★ STRUCTURE ★

2.1 LEARNING OBJECTIVES

After going through this unit, you will be able to:

- discuss the use of factorial designs
- define the terms like factors, levels, effect and interaction
- perform factorial experiments
- analyse data by the method of Yates

2.2 INTRODUCTION

In this unit, you will come to know about factorial design, terms related to central composite design, how to perform factorial experiments, how to analyse data by the Method of Yates and fractional factorial designs.

2.3 INTRODUCTION TO FULL AND FACTORIAL DESIGN

Factorial designs are used in experiments where the effects of different factors, or conditions, on experimental results are to be elucidated. Some practical examples where factorial designs are optimal are experiments to determine the effect of pressure and lubricant on the hardness of a tablet formulation, to determine the effect of disintegrant and lubricant concentration on tablet dissolution, or to determine the efficacy of a combination of two active ingredients in an over-the-counter cough preparation. Factorial designs are the designs of choice for simultaneous determination of the effects of several factors and their interactions. This chapter introduces some elementary concepts of the design and analysis of factorial designs.

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2.4 DEFINITIONS (VOCABULARY): CENTRAL COMPOSITE DESIGN

Factor

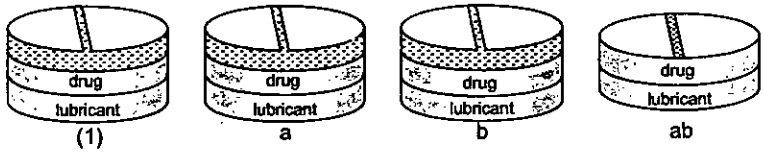
A *factor* is an *assigned variable* such as concentration, temperature, lubricating agent, drug treatment, or diet. The choice of factors to be included in an experiment depends on experimental objectives and is predetermined by the experimenter. A factor can be qualitative or quantitative. A *quantitative factor* has a numerical value assigned to it. For example, the factor "concentration" may be given the values 1%, 2%, and 3%. Some examples of *qualitative factors* are treatment, diets, batches of material, laboratories, analysts, and tablet diluent. Qualitative factors are assigned names rather than numbers. Although factorial designs may have one or many factors, only experiments with two factors will be considered in this chapter. Single-factor designs fit the category of one-way ANOVA designs. For example, an experiment designed to compare three drug substances using different patients in each drug group is a one-way design with the single factor "drugs."

Levels

The levels of a factor are the values or designations assigned to the factor. Examples of levels are 30° and 50° for the factor "temperature," 0.1 molar and 0.3 molar for the factor "concentration," and "drug" and "placebo" for the factor "drug treatment." The *runs* or *trials* that comprise factorial experiments consist of all combinations of all levels of all factors. As an example, a two-factor experiment would be appropriate for the investigation of the effects of drug concentration and lubricant concentration on dissolution time of a tablet. If both factors were at two levels (two concentrations for each factor), four runs (dissolution determinations for four formulations) would be required, as follows:

Symbol	*Formulation*
(1)	Low drug and low lubricant concentration
a	Low drug and high lubricant concentration
b	High drug and low lubricant concentration
ab	High drug and high lubricant concentration

"Low" and "high" refer to the low and high concentrations pre-selected for the drug and lubricant. (Of course, the actual values selected for the low and high concentrations of drug will probably be different from those chosen for the lubricant.) The notation (symbol) for the various combinations of the factors, (1), *a*, *b*, *ab*, is standard. When both factors are at their low levels, we denote the combination as (1). When factor A is at its high level and factor B is at its low level, the combination is called a. b means that only factor B is at the high level, and ab means that both factors A and B are at their high levels.



Effects

The *effect* of a factor is the change in response caused by varying the level(s) of the factor. The *main effect* is the *effect* of a factor *averaged over all levels of the other factors.*

In the previous example, a two-factor experiment with two levels each of drug and lubricant, the main effect due to drug would be the difference between the average response when drug is at the high level (runs b and ab) and the average response when drug is at the low level [runs (1) and a]. For this example the main effect can be characterized as a linear response, since the effect is the difference between the two points shown in Fig. 2.1.

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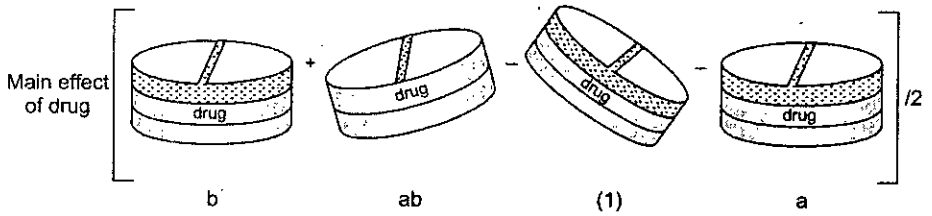

More than two points would be needed to define more clearly the nature of the response as a function of the factor drug concentration. For example, if the response plotted against the levels of a quantitative factor is not linear, the definition of the main effect is less clear. Figure 2.2 shows an example of a curved (quadratic) response based on experimental results with a factor at three levels. In many cases, an important objective of a factorial experiment is to characterize the effect of changing levels of a factor or combinations of factors on the response variable.

Interaction

Interaction may be thought of as a lack of "additivity of factor effects." For example, in a two-factor experiment, if factor A has an effect equal to 5 and factor B has an effect of 10, additivity would be evident if an effect of 15 (5 + 10) were observed when both

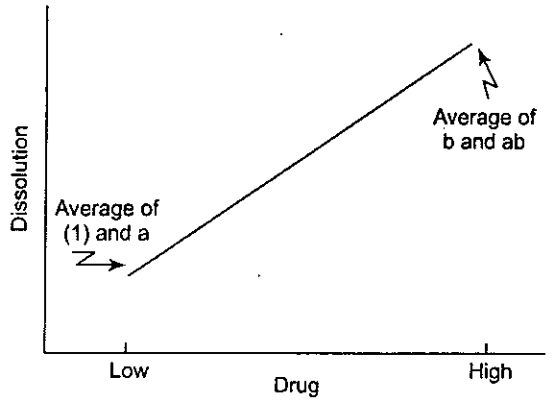

Fig. 2.1 Linear effect of drug. a = lubricant; b = drug

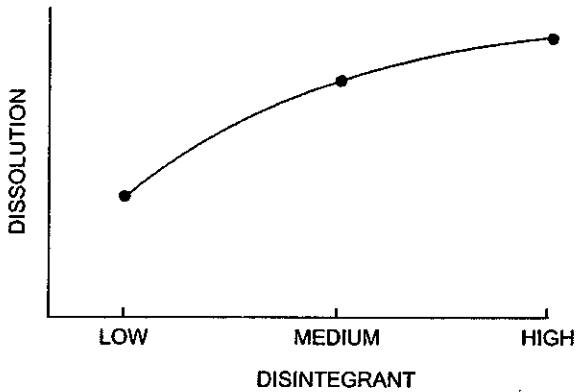

Fig. 2.2 Nonlinear (quadratic) effect

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A and B are at their high levels (in a two-level experiment). (It is well worth the extra effort to examine and understand this concept as illustrated in Fig. 2.3.) If the effect is greater than 15 when both factors are at their high levels, the result is *synergistic* (in biological notation) with respect to the two factors. If the effect is less than 15 when A and B are at their high levels, an *antagonistic* effect is said to exist. In statistical terminology, the lack of additivity is known as *interaction*. In the example above (two factors each at two levels), interaction can be described as the difference between the effects of drug concentration at the two lubricant levels. Equivalently, interaction is also the difference between the effects of lubricant at the two drug levels. More specifically,

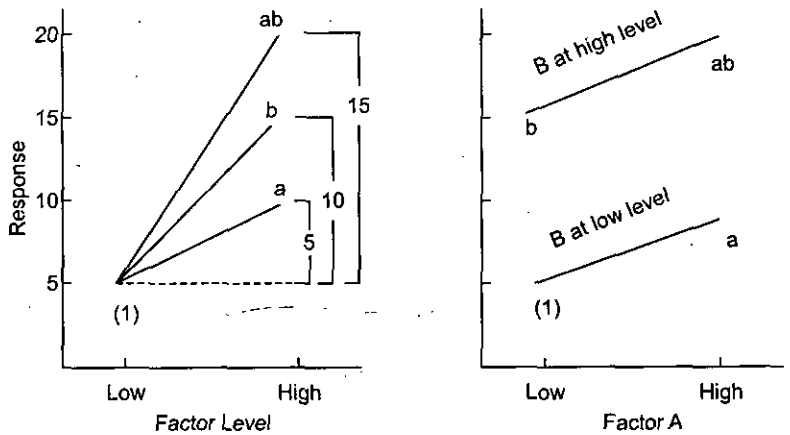

Fig. 2.3 Additivity of effects: Lack of interaction

Factorial Designs

This means that the drug effect measured when the lubricant is at the low level [*a*- (1)] is *different* from the drug effect measured when the lubricant is at the high level (*ab-b*). If the drug effects are the same in the presence of both high and low levels of lubricant, the system is additive, and no interaction exists. Interaction is conveniently shown graphically as depicted in Fig. 2.4. If the lines representing the effect of drug concentration at each level of lubricant are "parallel," there is no interaction. Lack of parallelism, as shown in Fig. 2.4B, suggests interaction. Examination of the lines in Fig. 2.4B reveals that the effect of drug concentration on dissolution is dependent on the concentration of lubricant. The effects of drug and lubricant are not additive.

Factorial designs have many advantages:

1. In the absence of interaction, factorial designs have maximum efficiency in estimating main effects.
2. If interactions exist, factorial designs are necessary to reveal and identify the interactions.
3. Since factor effects are measured over varying levels of other factors, conclusions apply to a wide range of conditions.
4. Maximum use is made of the data since all main effects and interactions are calculated from all of the data (as will be demonstrated below).
5. Factorial designs are orthogonal; all estimated effects and interactions are independent of effects of other factors. Independence, in this context, means that when we estimate a main effect, for example, the result we obtain is due only to the main effect of interest, and is not influenced by other factors in the experiment.

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In non-orthogonal designs (as is the case in many multiple-regression-type "fits"—) effects are not independent. *Confounding* is a result of lack of independence. When an effect is confounded, one cannot assess how much of the observed effect is due to the factor under consideration. The effect is influenced by other factors in a manner that often cannot be easily unraveled, if at all. Suppose, for example, that two drugs are to be compared, with patients from a New York clinic taking drug A and patients from a Los Angeles clinic taking drug B. Clearly, the difference observed between the two drugs is confounded with the different locations. The two locations reflect differences in patients, methods of treatment, and disease state, which can affect the observed difference in therapeutic effects of the two drugs. A simple factorial design where both drugs are tested in both locations will result in an "unconfounded," clear estimate of the drug effect if designed correctly, *e.g.*, equal or proportional number of patients in each treatment group at each treatment site.

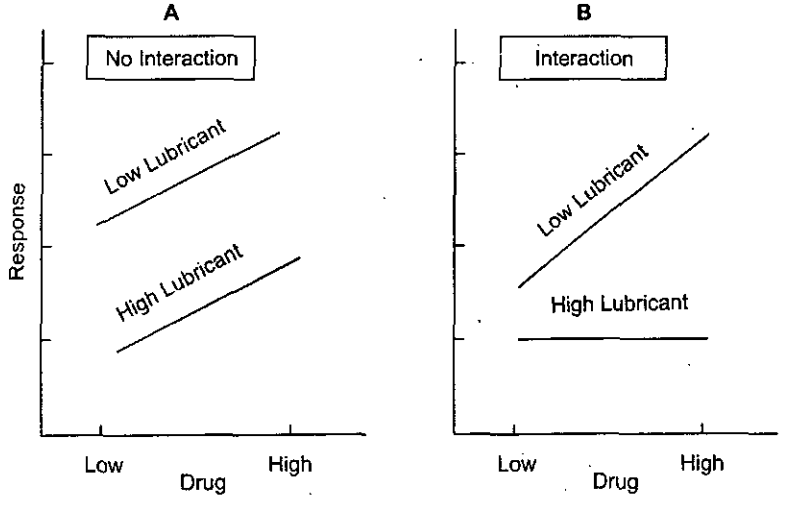

Fig. 2.4 Illustration of interaction

2.5 TWO SIMPLE HYPOTHETICAL EXPERIMENTS TO ILLUSTRATE THE ADVANTAGES OF FACTORIAL DESIGNS

The following hypothetical experiment illustrates the advantage of the factorial approach to experimentation when the effects of multiple factors are to be assessed. The problem is to determine the effects of a special diet and a drug on serum cholesterol levels. To this end, an experiment was conducted in which cholesterol changes were measured in three groups of patients. Group A received the drug, group B received the diet, and group C received both the diet and drug. The results are shown below. The experimenter concluded that there was no interaction between drug and diet (*i.e.*, their effects are additive).

Drug alone: decrease of 10 mg %

Diet alone: decrease of 20 mg %

Diet + drug: decrease of 30 mg %

However, suppose that patients given *neither* drug nor diet would have shown a decrease of serum cholesterol of 10 mg % had they been included in the experiment. (Such a result could occur because of "psychological effects" or seasonal changes, for example.) Under these circumstances, we would conclude that drug alone has

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no effect, that diet results in a cholesterol lowering of 10 mg %, and that the combination of drug and diet is synergistic.

The combination of drug and diet results in a decrease of cholesterol equal to 20 mg %. This concept is shown in Fig. 2.5. Thus, without a fourth group, the control group (low level of diet and drug), we have no way of assessing the presence of interaction. This example illustrates how estimates of effects can be incorrect when pieces of the design are missing. Inclusion of a control group would have completed the factorial design, two factors at two levels. Drug and diet are the factors, each at two levels, either present or absent. The complete factorial design consists of the following four groups:

(1) Group on normal diet without drug (drug and special diet at low level)

a Group on drug only (high level of drug, low level of diet)

b Group on diet only (high level of diet, low level of drug)

ab Group on diet and drug (high level of drug and high level of diet)

The effects and interaction can be clearly calculated based on the results of these four groups (see Fig. 2.5). Incomplete factorial designs such as those described above are known as the *one-ata-time* approach to experimentation. Such an approach is usually very *inefficient*. By performing the entire factorial, we usually have to do *less work*, and we get *more* information. This is a consequence of an important attribute of factorial designs: effects are measured with maximum precision.

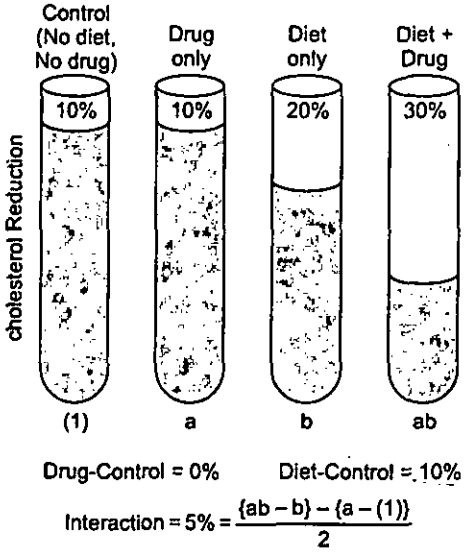

Fig. 2.5 Synergism in cholesterol lowering as a result of drug and diet

To demonstrate this property of factorial designs, consider the following hypothetical example. The objective of this experiment is to weigh two objects on an insensitive balance. Because of the lack of reproducibility, we will weigh the items in duplicate. The balance is in such poor condition that the zero point (balance reading with no weights) is in doubt. A typical one-at-a-time experiment is to weigh each object separately (in duplicate) in addition to a duplicate reading with no weights on the balance. The weight of item A is taken as the average of the readings with A on the balance minus the average of the readings with the pans empty. Under the assumption that the variance is the same for all weighings, regardless of the amount of material being weighed, the variance of the weight of A is the sum of the variances of the average weight of A and the average weight with the pans empty.

$$\frac{\sigma^2}{2}+\frac{\sigma^2}{2}=\sigma^2$$

Note that the variance of the *difference* of the average of two weighings is the *sum of the variances* of each weighing. (The variance of the average of *two* weighings is $\sigma^2/2$.) Similarly, the variance of the weight of B is $\sigma^2 = \sigma^2/2 + \sigma^2/2$. Thus, based on six readings (two weighings each with the balance empty, with A on the balance and with B on the balance), we have estimated the weights of A and B with variance equal to σ^2, where σ^2 is the variance of a single weighing.

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In a factorial design, an extra reading(s) would be made, a reading with both A and B on the balance. In the following example, using a full factorial design, we can estimate the weight of A with the same precision as above using only 4 weighings (instead of 6).

In this case the weighings are made without replication. That is, four weighings are made as follows:

(1) Reading with balance empty 0.5 kg

a Reading with item A on balance 38.6 kg

b Reading with item B on balance 42.1 kg

ab Reading with both items A and B on balance 80.5 kg

With a full factorial design, as illustrated above, the *weight of A* is estimated as (the main effect of A)

$$\frac{a-(1)+ab-b}{2}$$

Above expression says that the estimate of the weight of A is the average of the weight of A alone minus the reading of the empty balance [a - (1)] and the weight of both items A and B minus the weight of B. According to the weights recorded above, the weight of A would be estimated as

$$\frac{38.6-0.5+80.5-42.1}{2}=38.25 \text{ kg}$$

Similarly, the weight of B is estimated as

$$\frac{42.1-0.5+80.5-38.6}{2}=41.75 \text{ kg}$$

Note how we use *all the data* to estimate the weights of A and B; the weight of B alone is used to help estimate the weight of A, and vice versa! *Interaction* is measured as the average difference of the weights of A in the presence and absence of B as follows:

$$\frac{(ab-b)-\left[a-(1)\right]}{2}$$

We can assume that there is no interaction, a very reasonable assumption in the present example. (The weights of the combined items should be the sum of the individual weights.) The estimate of interaction in this example is

$$\frac{(80.5-42.1)-(38.6-0.5)}{2}=0.3$$

The estimate of interaction is not zero because of the presence of random errors made on this insensitive balance.

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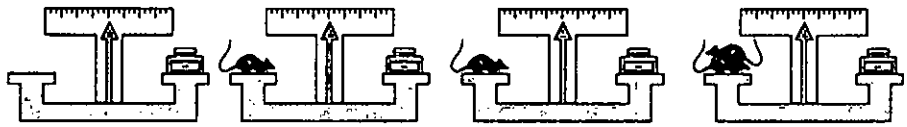
Table 2.1 Eight Experiments for a 2^3 Factorial Design[a]

Combination	*A*	*B*	*C*
(1)	–	–	–
a	+	–	–
b	–	+	–
ab	+	+	–
c	–	–	+
ac	+	–	+
bc	–	+	+
abc	+	+	+

[a] –, factor at low level; +, factor at high level



In this example, we have made *four* weighings. The variance of the main effects (*i.e.*, the average weights of A and B) is _2, *exactly the same variance as was obtained using six weightings in the one-at-a-time experiment!* We obtain the same precision with two thirds of the work: four readings instead of six. In addition to the advantage of greater precision, if interaction were present, we would have had the opportunity to estimate the interaction effect in the full factorial design. *It is not possible to estimate interaction in the one-at-a-time experiment.*

2.6 PERFORMING FACTORIAL EXPERIMENTS: RECOMMENDATIONS AND NOTATION

The simplest factorial experiment, as illustrated above, consists of four trials, two factors each at two levels. If three factors, A, B, and C, each at two levels, are to be investigated, eight trials are necessary for a full factorial design, as shown in Table 2.1. This is also called a 23 experiment, three factors each at two levels.

As shown in Table 2.1, in experiments with factors at two levels, the low and high levels of factors in a particular run are denoted by the absence or presence of the letter, respectively. For example, if all factors are at their low levels, the run is denoted as (1). If factor A is at its high level, and B and C are at their low levels, we use the notation a. If factors A and B are at their high levels, and C is at its low level, we use the notation ab; and so on. Before implementing a factorial experiment, the researcher should carefully consider the experimental objectives vis-a'-vis the appropriateness of the design. The results of a factorial experiment may be used (*a*) to help interpret the mechanism of an experimental system; (*b*) to recommend or implement a practical procedure or set of conditions in an industrial manufacturing situation; or (*c*) as guidance for further experimentation. In most situations where one is interested in the effect of various factors or conditions on some experimental outcome, factorial designs will be optimal.

The choice of factors to be included in the experimental design should be considered carefully. Those factors not relevant to the experiment, but which could influence the results, should be carefully controlled or kept constant. For example, if the use of different technicians, different pieces of equipment, or different excipients can affect experimental outcomes, but are not variables of interest, they should not be allowed to vary randomly, if possible. Consider an example of the comparison of

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two analytical methods. We may wish to have a single analyst perform both methods on the same spectrophotometer to reduce the variability that would be present if different analysts used different instruments. However, there will be circumstances where the effects due to different analysts and different spectrophotometers are of interest. In these cases, different analysts and instruments may be designed into the experiment as additional factors. On the other hand, we may be interested in the effect of a particular factor, but because of time limitations, cost, or other problems, the factor is held constant, retaining the option of further investigation of the factor at some future time. In the example above, one may wish to look into possible differences among analysts with regard to the comparison of the two methods (an analyst × method interaction). However, time and cost limitations may restrict the extent of the experiment. One analyst may be used for the experiment, but testing may continue at some other time using more analysts to confirm the results. The more extraneous variables that can be controlled, the smaller will be the residual variation. The residual variation is the random error remaining after the ANOVA removes the variability due to factors and their interactions. If factors known to influence the experimental results, but of no interest in the experiment, are allowed to vary "willynilly," the effects caused by the random variation of these factors will become part of the residual error. Suppose the temperature influences the analytical results in the example above. If the temperature is not controlled, the experimental error will be greater than if the experiment is carried out under constant-temperature conditions. The smaller the residual error, the more sensitive the experiment will be in detecting effects or changes in response due to the factors under investigation.

The choice of levels is usually well defined if factors are qualitative. For example, in an experiment where a product supplied by several manufacturers is under investigation, the levels of the factor "product" could be denoted by the name of the manufacturer: company X, company Y, and so on. If factors are quantitative, we can choose two or more levels, the choice being dependent on the size of the experiment (the number of trials and the amount of replication) and the nature of the anticipated response. If a response is known to be a linear function of a factor, two levels would be sufficient to define the response. If the response is "curved" (a quadratic response), at least three levels of the quantitative factor would be needed to characterize the response. Two levels are often used for the sake of economy, but a third level or more can be used to meet experimental objectives as noted above. A rule of thumb used for the choice of levels in two-level experiments is to divide extreme ranges of a factor into four equal parts and take the one-fourth (1/4) and three-fourths (3/4) values as the choice of levels. For example, if the minimum and maximum concentrations for a factor are 1% and 5%, respectively, the choice of levels would be 2% and 4% according to this empirical rule.

The trials comprising the factorial experiment should be done in random order if at all possible. This helps ensure that the results will be unbiased (as is true for many statistical procedures). The fact that all effects are averaged over all runs in the analysis of factorial experiments is also a protection against bias.

2.7 A WORKED EXAMPLE OF A FACTORIAL EXPERIMENT

The data in Table 2.2 were obtained from an experiment with three factors each at two levels. There is no replication in this experiment. Replication would consist

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of repeating each of the eight runs one or more times. The results in Table 2.2 are presented in standard order. Recording the results in this order is useful when analyzing the data by hand (see below) or for input into computers where software packages require data to be entered in a specified or standard order. The standard order for a 22 experiment consists of the first four factor combinations in Table 2.2. For experiments with more than three factors, see Davies for tables and an explanation of the ordering.

The experiment that we will analyze is designed to investigate the effects of three components (factors)—stearate, drug, and starch-on the thickness of a tablet formulation. In this example, two levels were chosen for each factor. Because of budgetary constraints, use of more than two levels would result in too large an experiment. For example, if one of the three factors were to be studied at three levels, 12 formulations would have to be tested for a $2 \times 2 \times 3$ factorial design. Because only two levels are being investigated, nonlinear responses cannot be elucidated. However, the pharmaceutical scientist felt that the information from this two-level experiment would be sufficient to identify effects that would be helpful in designing and formulating the final product. The levels of the factors in this experiment were as follows:

Table 2.2 Results of 2^3 Factorial Experiment: Effect of Stearate, Drug, and Starch Concentration on Tablet Thickness[a]

Factor combination	*Stearate*	*Drug*	*Starch*	*Response (thickness) (cm $\times 10^3$)*
(1)	–	–	–	475
a	+	–	–	487
b	–	+	–	421
ab	+	+	–	426
c	–	–	+	525
ac	+	–	+	546
bc	–	+	+	472
abc	+	+	+	522

* –, factor at low level; +, factor at high level.

Factor	*Low level (mg)*	*High level (mg)*
A: Stearate	0.5	1.5
B: Drug	60.0	120.0
C: Starch	30.0	50.0

The computation of the main effects and interactions as well as the ANOVA may be done by hand in simple designs such as this one. Readily available computer programs are usually used for more complex analyses. (For *n* factors, an *n*-way analysis of variance is appropriate. In typical factorial designs, the factors are usually considered to be fixed.) For two-level experiments, the effects can be calculated by applying the signs (+ or –) arithmetically for each of the eight responses as shown in Table 2.3. This table is constructed by placing *a* + or – in columns A, B, and C depending on whether or not the appropriate factor is at the high or low level in the particular run. If the letter appears in the factor combination, *a* + appears in the column corresponding to that letter. For example, for the product combination *ab*, *a* + appears in columns A and B, and *a* – appears in column C. Thus for column A, runs *a*, *ab*, *ac*, and *abc* have *a* + because in these runs, A is at the high level. Similarly, for runs (1), *b*, *c*, and *bc*, *a* – appears in column A since these runs have A at the low level.

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Columns denoted by AB, AC, BC, and ABC in Table 2.3 represent the indicated interactions (*i.e.*, AB is the interaction of factors A and B, etc.). The signs in these columns are obtained by multiplying the signs of the individual components. For example, to obtain the signs in column AB we refer to the signs in column A and column B. For run (1), the + sign in column AB is obtained by multiplying the – sign in column A times the – sign in column B. For run a, the – sign in column AB is obtained by multiplying the sign in column A (–) times the sign in column B (–). Similarly, for column ABC, we multiply the signs in columns A, B, and C to obtain the appropriate sign. Thus run ab has a – sign in column ABC as a result of multiplying the three signs in columns A, B, and C: (+) × (+) × (–).

The average effects can be calculated using these signs as follows. To obtain the average effect, multiply the response times the sign for each of the eight runs in a column, and divide the result by 2^{n-1}, where n is the number of factors (for three factors, 2^{n-1} is equal to 4). This will be illustrated for the calculation of the main effect of A (stearate). The main effect for factor A is

$$\frac{\left[-(1)+a-b+ab-c+ac-bc+abc\right]\times 10^{-3}}{4}$$

Note that the main effect of A is the average of all results at the high level of A minus the average of all results at the low level of A. This is more easily seen if above expression is rewritten as follows:

$$\text{Main effect of A} = \frac{a+ab+ac+abc}{4} - \frac{(1)+b+c+bc}{4}$$

Table 2.3 Signs to Calculate Effects in a 2^3 Factorial Experiment[a]

Factor combination	*Level of factor in experiment*			*Interaction*[b]			
	A	*B*	*C*	*AB*	*AC*	*BC*	*ABC*
(1)	–	–	–	+	+	+	–
a	+	–	–	–	–	+	+
b	–	+	–	–	+	–	+
ab	+	+	–	+	–	–	–
c	–	–	+	+	–	–	+
ac	+	–	+	–	+	–	–
bc	–	+	+	–	–	+	–
abc	+	+	+	+	+	+	+

[a] –. Factor at low level: +, factor at high level

[b] Multiply signs of factors to obtain signs for interaction terms in combination [*e.g.*, AB at (1) = (–) × (–) = (+)]

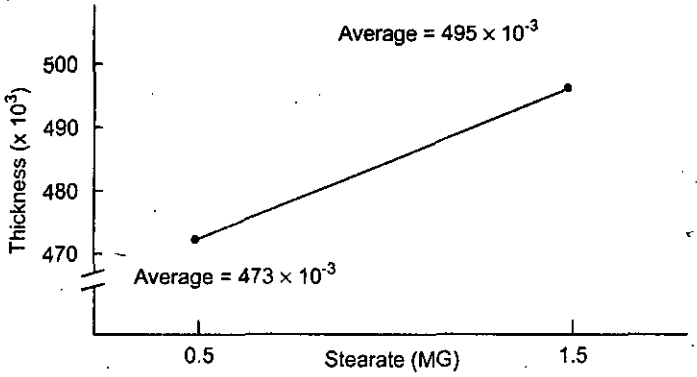

Fig. 2.6 Main effect of the factor "stearate"

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"Plugging in" the results of the experiment for each of the eight runs in above equation, we obtain

$$\frac{\left[487 + 426 + 546 + 522 - (475 + 421 + 525 + 472)\right] \times 10^{-3}}{4}$$

$$= 0.022 \text{ cm}$$

The *main effect of A is interpreted* to mean that the net effect of increasing the stearate concentration from the low to the high level (averaged over all other factor levels) is to increase the tablet thickness by 0.022 cm. This result is illustrated in Fig. 2.6. The interaction effects are estimated in a manner similar to the estimation of the main effects. The signs in the column representing the interaction (*e.g.*, AC) are applied to the eight responses, and as before the total divided by 2^{n-1}, where n is the number of factors. The interaction AC, for example, is defined as one-half the difference between the effect of A when C is at the high level and the effect of A when C is at the low level (see Fig. 2.7). Applying the signs as noted above, the AC interaction is estimated as

$$\text{AC interaction} = \frac{1}{4}\left\{(abc + ac - bc - c) - \left[ab + a - b - (1)\right]\right\}$$

The interaction is shown in Fig. 2.7. With starch (factor C) at the high level, 50 mg, increasing the stearate concentration from the low to the high level (from 0.5 mg to 1.5 mg) results in an increased thickness of 0.0355 cm. At the low level of starch, 30 mg, increasing stearate concentration from 0.5 mg to 1.5 mg results in an increased thickness of 0.0085 cm. Thus stearate has a greater effect at the higher starch concentration, a possible starch × stearate interaction.

Lack of interaction would be evidenced by the same effect of stearate at both low and high starch concentrations. In a real experiment, the effect of stearate would not be identical at both levels of starch concentration in the absence of interaction because of the presence of experimental error. The statistical tests described below show how to determine the significance of observed nonzero effects.

The description of interaction is "symmetrical." The AC interaction can be described in two equivalent ways: (*a*) the effect of stearate is greater at high starch concentrations, or (*b*) the effect of starch concentration is greater at the high stearate concentration (1.5 mg) compared to its effect at low stearate concentration (0.5 mg). The effect of starch at low stearate concentration is 0.051. The effect of starch at high stearate concentration is 0.078. (Also see Fig. 2.7.)

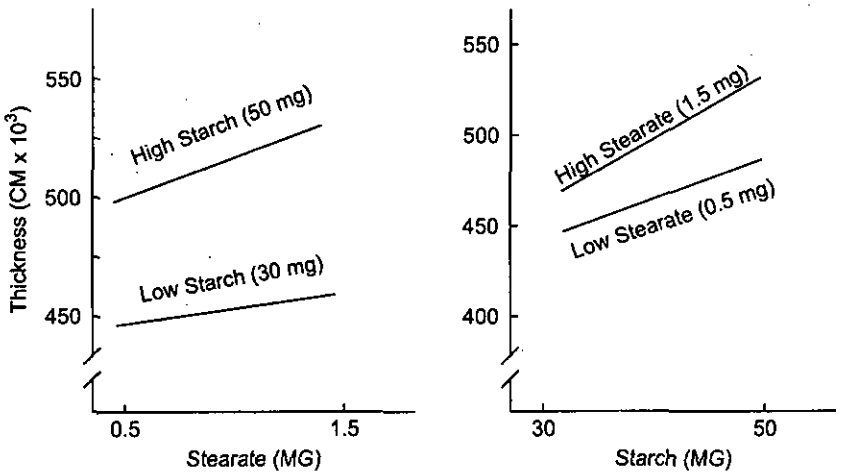

Fig. 2.7 Starch × stearate interaction.

2.8 DATA ANALYSIS

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Method of Yates

Computers are usually used to analyze factorial experiments. However, hand analysis of simple experiments can give insight into the properties of this important class of experimental designs. A method devised by Yates for systematically analyzing data from 2^n factorial experiments (n factors each at two levels) is demonstrated in Table 2.4. The data are first tabulated in standard order (see Ref. 1 for experiments with more than two levels). The data are first added in pairs, followed by taking differences in pairs as shown in column (1) in Table 2.4.

475 + 487 = 962
421 + 426 = 847
525 + 546 = 1071
472 + 522 = 994

487 – 475 = 12
426 – 421 = 5
546 – 525 = 21
522 – 472 = 50

This, addition and subtraction process is repeated sequentially on the n columns. (Remember that n is the number of factors, three columns for three factors.) Thus the process is repeated in column (2), operating on the results in column (1) of Table 2.4. Note, for example, that 1809 in column (2) is 962 + 847 from column (1). Finally, the process is repeated, operating on column (2) to form column (3). Column (3) is divided by 2^{n-1} ($2^{n-1} = 4$ for 3 factors) to obtain the average effect. The mean squares for the ANOVA (described below) are obtained by dividing the square of column (n) by 2^n. For example, the mean square attributable to factor A is

$$\text{Mean square for A} = \frac{(88)^2}{8} = 968$$

Table 2.4 Yates Analysis of the Factorial Tableting Experiment for Analysis Variance

Combination	Thickness ($\times 10^1$)	(1)	(2)	(3)	Effect ($\times 10^3$) (3)/4	Mean square ($\times 10^6$) $(3)^2/8$
(1)	475	962	1809	3874	—	—
a	487	847	2065	88	22.0	968
b	421	1071	17	– 192	– 48.0	4608
ab	426	994	71	22	5.5	60.5
c	525	12	– 1152	256	64.0	8192
ac	546	5	– 77	54	13.5	364.5
bc	472	21	– 7	38	9.5	180.5
abc	522	50	29	36	9.0	162

The mean squares are presented in an ANOVA table, as discussed ahead.

Analysis of Variance

The results of a factorial experiment are typically presented in an ANOVA table, as shown in Table 2.5. In a 2^n factorial, each effect and interaction has 1 degree of

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freedom. The error mean square (for statistical tests and estimation) can be estimated in several ways for a factorial experiment. Running the experiment with replicates is best. Duplicates are usually sufficient. However, replication may result in an inordinately large number of runs. Remember that replicates do not usually consist of replicate analyses or observations on the same run. A true replicate usually is obtained by repeating the run, from "scratch." For example, in the 2^3 experiment described above, determining the thickness of several tablets from a single run [*e.g.*, the run denoted by a (A at the high level)] would probably not be sufficient to estimate the experimental error in this system. The proper replicate would be obtained by preparing a new mix with the same ingredients, retableting, and measuring the thickness of tablets in this new batch. In the absence of replication, experimental error may be estimated from prior experience in systems similar to that used in the factorial experiment. To obtain the error estimate from the experiment itself is always most desirable. Environmental conditions in prior experiments are apt to be different from those in the current experiment. In a large experiment, the experimental error can be estimated without replication by pooling the mean squares from higher-order interactions (*e.g.*, three-way and higher-order interactions) as well as other interactions known to be absent, a priori. For example, in the tableting experiment, we might average the mean squares corresponding to the two-way interactions, AB and BC, and the three-way ABC interaction, if these interactions were known to be zero from prior considerations. The error estimated from the average of the AB, BC, and ABC interactions is with 3 degrees of freedom (assuming that these interactions do not exist).

$$(60.5 + 180.5 + 162) \times \frac{10^{-6}}{3} = 134.2 \times 10^{-6}$$

Table 2.5 Analysis of Variance for the Factorial Tableting Experiment

Factor	*Source*	*d.f.*	*Mean square (×10⁶)*	*F*[a]
A	Stearate	1	968	7.2[b]
B	Drug	1	4608	34.3[c]
C	Starch	1	8192	61.0[c]
AB	Stearate × drug	1	60.5	
AC	Stearate × starch	1	364.5	2.7
BC	Drug × starch	1	130.5	
ABC	Stearate × drug × starch	1	152	

[a] Error mean square based on AB, BC, and ABC interactions, 3 d.f.
[b] $P < 0.1$
[c] $P < 0.01$.

Interpretation

In the absence of interaction, the main effect of a factor describes the change in response when going from one level of a factor to another. If a large interaction exists, the main effects corresponding to the interaction do not have much meaning as such. Specifically, an AC interaction suggests that the effect of A depends on the level of C and a description of the results should specify the change due to A at each level of C. Based on the mean squares in Table 2.5, the effects which are of interest are A, B, C, and AC. Although not statistically significant, stearate and

starch interact to a small extent, and examination of the data is necessary to describe this effect (see Fig. 2.7). Since B does not interact with A or C, it is sufficient to calculate the effect of drug (B), averaged over all levels of A and C, to explain its effect. The effect of drug is to *decrease* the thickness by 0.048 mm when the drug concentration is raised from 60 mg to 120 mg [Table 2.4, column (3)/4].

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2.9 FRACTIONAL FACTORIAL DESIGNS

In an experiment with a large number of factors and/or a large number of levels for the factors, the number of experiments needed to complete a factorial design may be inordinately large. For example, a factorial design with 5 factors each at 2 levels requires 32 experiments; a 3-factor experiment each at 3 levels requires 27 experiments. If the cost and time considerations make the implementation of a full factorial design impractical, fractional factorial experiments can be used in which a fraction (*e.g.*, 1/2, 1/4, etc.) of the original number of experiments can be run. Of course, something must be sacrificed for the reduced work. If the experiments are judiciously chosen, it may be possible to design an experiment so that effects which we believe are negligible are confounded with important effects. (The word "confounded" has been noted before in this chapter.) In fractional factorial designs, the negligible and important effects are indistinguishable, and thus confounded.

This will become clearer in the first example. To illustrate some of the principles of fractional factorial designs, we will discuss and present an example of a fractional design based on a factorial design where each of 3 factors is at 2 levels, a 2^3 design. Table 2.3 shows the 8 experiments required for the full design. With the full factorial design, we can estimate 7 effects from the 8 experiments, the 3 main effects (A, B, and C), and the 4 interactions (AB, AC, BC, and ABC). In a 1/2 replicate fractional design, we perform 4 experiments, but we can only estimate 3 effects. With 3 factors, a 1/2 replicate can be used to estimate the main effects, A, B, and C. The following procedure is used to choose the 4 experiments. Table 2.6 shows the 4 experiments that define a 2^2 factorial design using the notation described in previous Section.

Table 2.6 2^2 Factorial Design

Experiment	*A level*	*B level*	*AB*
(1)	–	–	+
a	+	–	–
b	–	+	–
ab	+	+	+

To construct the 1/2 replicate with 3 factors, we equate one of the effects to the third factor. In the 2^2 factorial, the interaction, AB is equated to the third factor, C. Table 2.7 describes the 1/2 replicate design for 3 factors. The 4 experiments consist of (1) c at the high level (*a*, *b* at the low level); (2) a at the high level (*b*, *c* at the low level); (3) b at the high level (*a*, *c* at the low level); and (4) *a*, *b*, *c* all at the high level. From Table 2.7, we can define the confounded effects, also known as aliases. An effect is defined by the signs in the columns of Table 2.7. For example, the effect of A is

$$(a + abc) - (c + b)$$

Note that the effect of A is exactly equal to BC. Therefore, BC and A are confounded (they are aliases). Also note that C = AB (by definition) and B = AC. Thus, in this design the main effects are confounded with the two factor interactions. This means that the main effects cannot be clearly interpreted if interactions are not absent or negligible. If interactions are negligible, this design will give fair estimates of the main effects. If interactions are significant, this design is not recommended.

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SUMMARY

- Factorial designs are used in experiments where the effects of different factors, or conditions, on experimental results are to be elucidated.
- A *factor* is an *assigned variable* such as concentration, temperature, lubricating agent, drug treatment, or diet.
- *Interaction* may be thought of as a lack of "additivity of factor effects."
- *Confounding* is a result of lack of independence. When an effect is confounded, one cannot assess how much of the observed effect is due to the factor under consideration.
- Computers are usually used to analyze factorial experiments.

REVIEW QUESTIONS

1. Discuss the factorial method for the pharmaceuticals.
2. Discuss the use of factorial designs.
3. Define the following:
   (a) Factor (b) Levels (c) Effects (d) Interaction
4. What are the advantages of factorial design?
5. Discuss the two simple hypothetical experiments to illustrate the advantages of factorial designs.

UNIT III: STUDY OF MATHEMATICAL MODEL AND CONTOUR DESIGN

NOTES

★ STRUCTURE ★

3.1 LEARNING OBJECTIVES

After going through this unit, you will be able to:

- discuss the concept of transformation
- explain the use of logarithmic transformation
- discuss about the problems that arise as a consequence of using the log transformation
- illustrate that why the antilog of the mean of the logs is not equal to the mean of the untransformed values

3.2 INTRODUCTION

In this unit, you will come to know about transformation and its use, the logarithmic transformation, the problems that arise as a consequence of using the log transformation, lognormal distribution.

3.3 TRANSFORMATIONS AND OUTLIERS

Critical examination of the data is an important step in statistical analyses. Often, we observe either what seem to be unusual observations (outliers) or observations that appear to violate the assumptions of the analysis. When such problems occur, several courses of action are available depending on the nature of the problem and statistical judgement. Most of the analyses described in previous chapters are appropriate for groups in which data are normally distributed with equal variance. As a result of the Central Limit theorem, these analyses perform well for data that

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are not normal provided the deviation from normality is not large and/or the data sets are not very small. (If necessary and appropriate, nonparametric analyses can be used in these instances.) However, lack of equality of variance (heteroscedascity) in *t* tests, analysis of variance and regression, for example, is more problematic. The Fisher-Behrens test is an example of a modified analysis that is used in the comparison of means from two independent groups with unequal variances in the two groups. Often, variance heterogeneity and/or lack of normality can be corrected by a data transformation, such as the logarithmic or square root transformation.

Bioequivalence parameters such as AUC and CMAX currently require a log transformation prior to statistical analysis. Transformations of data may also be appropriate to help linearize data. For example, a plot of log potency vs. time is linear for stability data showing first-order kinetics.

Variance heterogeneity may also be corrected using an analysis in which each observation is weighted appropriately, *i.e.*, a weighted analysis. In regression analysis of kinetic data, if the variances at each time point differ, depending on the magnitude of drug concentration, for example, a weighted regression would be appropriate. For an example of the analysis of a regression problem requiring a weighted analysis for its solution. Data resulting from gross errors in observations or overt mistakes such as recording errors should clearly be omitted from the statistical treatment. However, upon examining experimental data, we often find unusual values that are not easily explained. The prudent experimenter will make every effort to find a cause for such aberrant data and modify the data or analysis appropriately. If no cause is found, one should use scientific judgement with regard to the disposition of these results. In such cases, a statistical test may be used to detect an outlying value. An outlier may be defined as an observation that is extreme and appears not to belong to the bulk of data. Many tests to identify outliers have been proposed and several of these are presented in this chapter.

3.4 TRANSFORMATIONS

A transformation applied to a variable changes each value of the variable as described by the transformation. In a *logarithmic* (*log*) *transformation*, each data point is changed to its logarithm prior to the statistical analysis. Thus the value 10 is transformed to 1 (*i.e.*, log 10 =1). The log transformation may be in terms of logs to the base 10 or logs to the base *e* (*e* = 2.718 ...), known as natural logs (ln). For example, using natural logs, 10 would be transformed to 2.303 (ln 10 = 2.303). The *square-root* transformation would change the number 9 to 3.

Parametric analyses such as the *t* test and analysis of variance are the methods of choice in most situations where experimental data are continuous. For these methods to be valid, data is assumed to have a normal distribution with constant variance within treatment groups. Under appropriate circumstances, a transformation can change a data distribution which is not normal into a distribution that is approximately normal and/or can transform data with heterogeneous variance into a distribution with approximately homogeneous variance.

Thus, data transformations can be used in cases where (1) the variance in regression and analysis of variance is not constant and/or (2) data are clearly not normally distributed (highly skewed to the left or right).

Another application of transformations is to linearize relationships such as may occur when fitting a least squares line (not all relationships can be linearized). Table 3.1 shows some examples of such linearizing transformations. When making linearizing transformations, if statistical tests are to be made on the transformed data, one should take care that the normality and variance homogeneity assumptions are not invalidated by the transformation.

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3.5 THE LOGARITHMIC TRANSFORMATION

Probably the most common transformation used in scientific research is the log transformation. Either logs to the base 10 ($\log_{10}$) or the base e, $\log_e$ (ln) can be used. Data skewed to the right as shown in Fig. 3.1 can often be shown to have an approximately lognormal distribution. A lognormal distribution is a distribution that would be normal following a log transformation, as illustrated in Fig. 3.2. When statistically analyzing data with a distribution similar to that shown in Fig. 3.1, a log transformation should be considered. One should understand that a reasonably large data set or prior knowledge is needed in order to know the form of the distri-bution. Table 3.2 shows examples of two data sets, listed in ascending order of magnitude. Data set A would be too small to conclude that the underlying distribution is not normal in the absence of prior information. Data set B, an approximately lognormal distribution, is strongly suggestive of nonnormality.

Table 3.1 Some Transformations Used to Linearize Relationships Between Two Variables, *X* and *Y*

Function	*Transformation*	*Linear form*
$Y = Ae^{-BX}$	Logarithm of Y	$\ln Y = A - BX$
$Y = 1/(A + BX)$	Reciprocal of Y	$1/Y = A + BX$
$Y = X/(AX + B)$	Reciprocal of Y	$1/Y = A + B(1/X)^a$

[a]A plot of 1/Y vs. 1/X is linear.

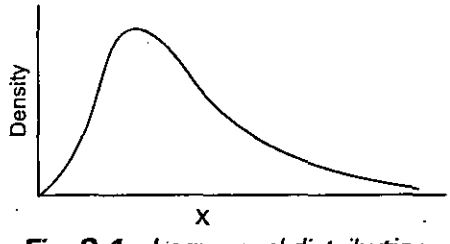


Fig. 3.1 Lognormal distribution

Two problems may arise as a consequence of using the log transformation.

1. Many people have trouble interpreting data reported in logarithmic form. Therefore, when reporting experimental results, such as means for example, a back transformation (the antilog) may be needed. For example, if the mean of the logarithms of a data set is 1.00, the antilog, 10, might be more meaningful in a formal report of the experimental results. The mean of a set of untransformed numbers is not, in general, equal to the antilog of the mean of the logs of these numbers. If the data are relatively nonvariable, the means calculated by these two methods will be close. The mean of the logs and the log of the mean will be identical only if each observation is the same, a highly unlikely circumstance.

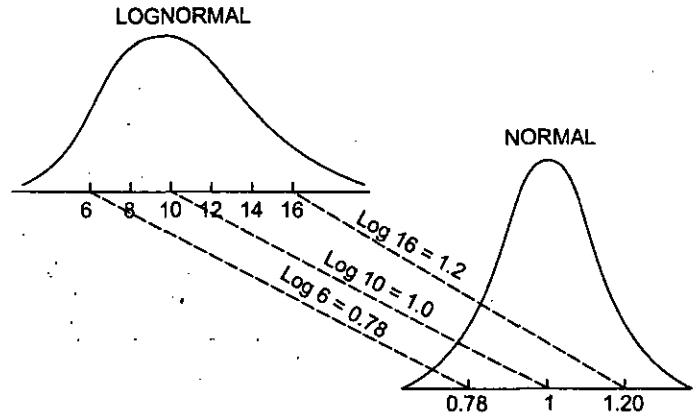


Fig. 3.2 Transformation of a lognormal distribution to a normal distribution via the log transformation

Table 3.2 Two Data Sets that May Be Considered Lognormal

Data set A:	2, 17, 23, 43, 55, 125, 135
Data set B:	10, 13, 40, 44, 55, 63, 115, 145, 199, 218, 231, 370, 501, 790, 795, 980, 1260, 1312, 1500, 4520

Table 3.3 illustrates this concept. Note that the antilog of the mean of a set of log transformed variables is the geometric mean. This lack of "equivalence" can raise questions when someone reviewing the data is unaware of this divergence, "the nature of the beast," so to speak.

2. The second problem to be considered when making log transformations is that the log transformation which "normalizes" log-normal data also changes the variance. If the variance is not very large, the variance of the ln transformed values will have a variance approximately equal to S^2/X^{-2}. That is, the standard deviation of the data after the transformation will be approximately equal to the coefficient of variation (C.V.), S/X^-. For example, consider the following data:

	X	$ln\ X$
	105	4.654
	102	4.625
	100	4.605
	110	4.700
	112	4.718
Mean	105.8	4.6606
s.d.	5.12	0.0483

The coefficient of variation of the original data is 5.12/105.8 = 0.0484. The standard deviation of the ln transformed values is 0.0483, very close to the C.V. of the transformed data. This property of the transformed variance can be advantageous when working with data groups that are both *lognormal* and have a *constant coefficient of variation*. If the standard deviation within treatment groups, for example, is not homogeneous but is proportional to the magnitude of the measurement, the coefficient of variation (C.V.) will be constant. In analytical procedures, one often observes that the s.d. is proportional to the quantity of material being assayed. In these circumstances, the ln transformation will result in data with homogeneous s.d. equal to C.V. (The s.d. of the transformed data is approximately equal to C.V.).

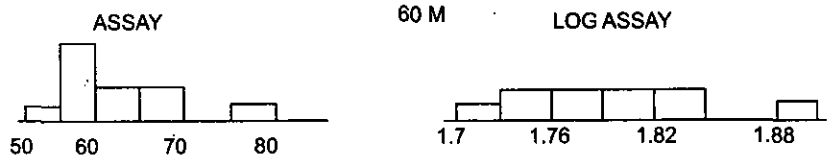
Fortunately, in many situations, data that are approximately lognormal also have a constant C.V. In these cases, the log transformation results in normal data with approximately homogeneous variance. The transformed data can be analyzed using techniques that depend on normality and homogeneous variance for their validity (*e.g.*, ANOVA).

Table 3.3 Illustration of Why the Antilog of the Mean of the Logs is Not Equal to the Mean of the Untrasformed Values

	Case I			Case II	
	Original data	*Log transform*		*Original data*	*Log transform*
	5	0.699		4	0.603
	5	0.699		6	0.778
	5	0.699		8	0.903
Mean	5	0.699		10	1.000
	5		Mean	7	
	Antilog (0.699) = 5			Antilog (0.821) = 6.62	

Table 3.4 Results of an Assay at Three Different Levels of Drug

	At 40 mg		At 60 mg		At 80 mg	
	Assay	*Log assay*	*Assay*	*Log assay*	*Assay*	*Log assay*
	37	1.568	63	1.799	82	1.914
	43	1.633	77	1.886	68	1.833
	42	1.623	56	1.748	75	1.875
	40	1.602	64	1.806	97	1.987
	30	1.477	66	1.820	71	1.851
	35	1.544	58	1.763	86	1.934
	38	1.580	67	1.826	71	1.851
	40	1.602	52	1.716	81	1.908
	39	1.591	55	1.740	91	1.959
	36	1.556	58	1.763	72	1.857
Average	38	1.578	61.6	1.783	79.4	1.897
s.d.	3.77	0.045	7.35	0.050	9.67	0.052
C.V.	0.10		0.12		0.12	



SUMMARY

- The Fisher-Behrens test is an example of a modified analysis that is used in the comparison of means from two independent groups with unequal variances in the two groups.
- Bioequivalence parameters such as AUC and CMAX currently require a log transformation prior to statistical analysis.
- An outlier may be defined as an observation that is extreme and appears not to belong to the bulk of data.

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- The log transformation may be in terms of logs to the base 10 or logs to the base e (e = 2.718 ...), known as natural logs (ln).
- Probably the most common transformation used in scientific research is the log transformation.
- A lognormal distribution is a distribution that would be normal following a log transformation.
- The mean of the logs and the log of the mean will be identical only if each observation is the same.
- If the standard deviation within treatment groups, for example, is not homogeneous but is proportional to the magnitude of the measurement, the coefficient of variation (C.V.) will be constant.

REVIEW QUESTIONS

1. *Write a short note on transformation.*
2. What are the applications of transformation?
3. What do you understand by outlier?
4. Discuss the concept of logarithmic transformation.
5. Discuss the two problems that may arise as a consequence of using the log transformation.

UNIT IV: PATENTS

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★ STRUCTURE ★

4.1 LEARNING OBJECTIVES

After going through this unit, you will be able to:

- discuss about the needs of patents
- explain different types of patents
- discuss about the guidelines to prepare a laboratory note book
- know about the applications of provisional patent
- discuss about the patents rules, 2003

4.2 INTRODUCTION

In this unit, you will learn about needs of patents, types of patents like utility patents, design patents and plant patents, the terms related to product patent like device patent, apparatus patent, drafting of protect claims, policies used to achieve protectionist goals and the patent rules, 2003.

4.3 DEFINITION AND NEEDS OF PATENTS

America has a rich history of patents. The U.S. patent system was created in 1790 by an act of President George Washington. His intention was to spur innovation and industrial development in a burgeoning country. Little did he know that he was building the foundation for America's future economic strength.America was destined to become the most dynamic, inventive country in the world. This very patent system, established more than two centuries ago, is the foundation of our country's dynamic prosperity leading into the twenty-first century.

Entire industries have been created based on the granting of patents. Edison, Westinghouse, Singer Sewing Machines, Levi Jeans, and General Electric are only

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a fraction of those companies that came into existence based on the security of patent protection. Even today, the Pullman brakes used in trains are the original units developed by Westinghouse more than 100 years ago. Chances are that your company and its jobs can be directly linked back to the creation of new patented products.

Today, new products and new high-performance variations on old ones are being invented. Yesterday's high-volume generic product line has been splintered into many innovative niches. The best way to protect these niches is with patent protection. Because of their importance to commerce, patents today have more respect than ever before. In the past 15 years, record judgments of $100 million and more—a few approaching the $1 billion mark (*Polaroid Corporation* v. *Eastman Kodak Company,* 16 USPQ2d 1481, 1483 (1990); Polaroid awarded damages of $909,457,567.00)—have been awarded to patent holders as a result of patent infringement suits. In many emerging industries and technologies, patent values have soared 20- to 50-fold in just the past several years. Much of the fluctuation in share value of these companies is linked to the increase or decrease of these patent values.

Simply stated, developing and licensing new patented ideas can be a fast and economical way for companies to protect new product launches, gain new profits, and secure their future. If either you or your company are part of the patent revolution in America, this can be good news for you. Patent ownership also brings along with it a corresponding liability. There is an old saying that rings true: "Nobody wants a worthless patent, but everyone wants a piece of a valuable patent."This means that valuable patents may be almost as much a liability as they are assets. If your company's patents have particularly high value, chances are they will ultimately wind up in the court system—either used offensively against alleged infringers or in a defensive campaign to prove their novelty and validity.

Patents have become the driving force behind the computer industry and the Internet. Patents protect America's technological revolution, and they can secure our prosperity far into the future. In light of the now infamous Enron meltdown, heated global competition, and changes in world intellectual property policy, the changing business landscape is demanding an ever-higher level of responsibility by corporate managers in every functional department in the organization—a responsibility to manage, develop, and exploit patents to the maximum benefit of the shareholders.

Now it's time for you to learn about patents, the invention process, and how to contribute to Patent Quality Management (PQM). Corporate or outside legal or patent counsel will most likely handle patent legal work for your company, so use this book to familiarize yourself with the terminology, processes, and some of the intricacies of patents.Thus you will be able to more effectively contribute to your company's objectives of creating valuable patents to protect the sales of new product releases and new improvements.

4.4 INTRODUCTION TO SEARCH PATENT

A patent is, in essence, a monopoly granted by the U.S. government to an inventor in exchange for full public disclosure of the invention. When a patent is granted to an inventor, it becomes a public document that fully discloses the details of the

invention so that others skilled in the technology can duplicate the results achieved by the patented invention; however, the invention owner retains the sole right to exclude others from making, selling, using, or importing the invention. This concept was so important to America's founding fathers that they made a provision granting rights to inventors in the U.S. Constitution: The Congress shall have Power. To promote the Progress of Science and useful Arts, by securing for limited Times to Authors and Inventors the exclusive Right to their respective Writings and Discoveries. Over the decades, the period for which the monopolistic right was granted to an inventor has varied. Today, it is for a period of 20 years from the date the application for patent is filed. At the end of the patent life, the patent owner loses the monopolistic right and the invention falls into the public domain for anyone to make, sell, or import. Many requirements must be met in order for an engineering development or technical discovery to be considered patentable, but the most fundamental requirements are that the invention is (1) novel, (2) useful, and (3) not obvious to one skilled in the art.

This chapter provides a brief overview to the invention process, but we wish to emphasize the business of invention-that strategic thinking and tactical implementation used by a patent owner to properly exploit an invention for maximized profit and increased shareholder value. Negative Rights When a patent is granted by the U.S. government, it gives the inventor the right to exclude others from manufacturing, using, offering for sale, or importing the invention into the United States. In other words, patent ownership does not give the owner the right to make, use, sell, or import the invention, but instead gives the invention owner the right to *exclude* others from practicing these activities for the entire term of the patent.

Patents are sometimes referred to as a legal monopoly because they can be used to prevent others from practicing the invention.

4.5 TYPES OF PATENTS, DURATION

There are three basic types of patents:

1. *Utility patents* may be granted to anyone who invents or discovers any new and useful process, machine, article of manufacture, system (or method of use), software and Internet methodologies, composition of matter, or any new, useful improvement thereof. Utility patents are granted for the term, which begins on the date of the grant and ends 20 years from the date the patent application was first filed.
2. *Design patents* may be granted to anyone who invents a new, original, ornamental design for an article of manufacture. Design patents are granted for a term of 14 years from the date of the grant.
3. *Plant patents* may be granted to anyone who invents or discovers and asexually reproduces any distinct and new variety of plant. Plant patents are granted for the term, which begins on the date of the grant and ends 20 years from the date the patent application was first filed.

4.6 GUIDELINE TO PREPARE A LABORATORY NOTE BOOK

Most people think of patents in terms of a product, but utility patent protection can take on many other forms. It is important that those individuals, especially

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those on the PQM team, who have had little previous contact with patents, know these various forms of protection. A heightened awareness to the various forms of patents can help team members identify patenting opportunities, as well as improve the company's ability to protect its product line against infringement. The language we use to describe the various forms of patent protection is not based on statute, but are general terms used throughout the patent trade, whether that be an inventor, scientist, engineer, patent attorney, or patent examiner. Become familiar with these terms and you and your company's inventive potential will be substantially broadened.

Product Patents

These patents are usually easy to identify because they refer to the physical product itself. For instance, the lightbulb, the paper clip, and the mousetrap are all fairly famous patents. Product patents may also encompass devices, apparatuses, or an entire group of associated products.

When a specific unique, novel, useful element is used with a product, that too defines the product as unique and would be considered a product patent. The terms *device patent* or *apparatus patent* are also commonly used, but for ease of explanation, they are best grouped into the single category of *product patents.*

Method of Use or System Patents

These patents usually relate to making products people friendly. They should be of particular interest to every company that sells products in virtually every facet of business. Unfortunately, many companies and their engineers are not familiar with this form of patent protection even though they may be creating superior methodologies and products. Systems patents can also reduce handling time and improve productivity. When you think about productivity, keep in mind the classic economic principle of "productivity produces income." From this perspective, systems patents can be valuable assets to protect the sale of commercial products as well as consumer products. Simply put, systems patents refer to two methodologies:

1. *A method in which a product is used.* For instance, scanning bar codes over laser reading devices. Or, self-opening plastic grocery sacks that automatically open on a dispensing rack when the previous bag is removed from the rack.
2. *A method related to employees' business operations.* For example, a methodology in which machine operators employ computerized statistical process controls to the operation of a piece of equipment. Or, even a method of new employee training that maximizes the time investment. Both applications save time.They can increase output, improve customer satisfaction, improve quality, increase profits, and so on. Developing superior systems can represent the central focus of a company's product line and result in an endless number of future opportunities as the company strives to make its product line 100 percent automatic and intuitively people friendly. From this perspective, systems patents can be the single most important asset a company owns.

We know that patents may not be obtained on commonly used products and components, but when they are used in a novel, useful, and unique method, patentability then becomes possible. One or all of the components may be prior art as long as the outcome of the combined use is novel and unique.Think in terms of efficiency, effectiveness, and convenience for the end user, and you're thinking in terms of systems patents.

If your PQM team starts thinking more in terms of systems patents, it will be improving the company's market position and giving the company a competitive edge. Ensuring that all departments understand the importance and the impact of developing and patenting systems, not only for the product line, but also internally within the department, should be a central focus of the PQM team in the twenty-first century.

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Process Patents

Process patents generally refer to manufacturing processes. They would typically improve productivity, reduce defects, or offer some value-added quality. These patents are of primary importance to the manufacturing department as well as the engineering department. One of the best examples of a process patent would be U.S. Patent No. 135,245, patented by Louis Pasteur of France, in 1873. It revealed the fundamentals of the food sterilization process now known as pasteurization. It is easy to understand the economic impact of such an important process patent.

If the development of internal processes makes a product line so generic with such a narrow focus that it cannot be modified and improved, it will continue to lose market share to those product lines that are more adaptable to change and can satisfy emerging trends.

Process patents can also be a valuable tool to overcome another emerging danger. At times, companies maintain certain manufacturing processes as closely guarded trade secrets; however, if an outside entity files a patent application that covers that trade secret, the company can lose the rights to the trade secret. In other words, the company holding the trade secret would be forced to license its own manufacturing process from the new patent holder, regardless of how long the process had been in prior use. There have been several court case precedents of this kind of action. The negative impact such a scenario could have on the corporation, its management, and the shareholders could be disastrous.

The best way to keep this from happening is by filing process patents on your trade secrets before others do. Then you will be in the enviable position of licensing out to them instead. A shift in focus to being a more customer-driven, innovation oriented corporation must be accompanied by cost-effective manufacturing processes.

Improvement Patents

The term *improvement patent* may refer to any number of new incremental improvements made to an existing product, system, or process. This can be something as simple as a new tread design for a tire that displaces more water than existing designs or as sophisticated as a method to improve the optical magnification or resolution of the Hubbell Space.

Telescope

Improvement patents can also be systems patents, just like the selfopening grocery sack of the late 1980s was an improvement over the prior art "T-shirt bag" invented in 1966. Improvement patents can also be called product patents, if the improvement creates a new, improved product that replaces a prior art product. In a way, it really does not matter how you categorize an improvement patent; what is important is whether your PQM Team takes action when these opportunities arise and you protect these company and shareholder assets with patents.

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A customer-oriented company should always be striving to innovate based on customer needs. With total quality management (TQM), making incremental improvements in manufacturing processes of existing products leads to higher quality and improved output. With superior quality, incremental changes in existing processes, systems, and products can then be made to advance the company's profitability as it strives to improve output, sales (via customer satisfaction), and quality. It is simply not acceptable to be complacent with a present market position. If you don't improve your products and processes, your competitors will. If you don't protect them, your competitors will patent them out from underneath you. Ask yourself: Who will be paying royalties to whom in order to stay in business?

Machine Patents

When several elements are used in combination that have some sort of productive output, it is referred to as a *machine patent.* An example is the machine that rolls dough over a mandrel to form a bagel with a round hole in the middle. Or, any number of high-speed bottling machines used in the beverage industry.

Machine patents are usually generated by companies that are in the machinery manufacturing business. These patents often have accompanying process patents. It is also common to include several of the individual inventive aspects of the machinery as part of the overall patented machine. Some people refer to machine patents as *apparatus patents,* thus further blurring the terminology used in the field.

Composition of Matter

Chemical composition patents are scientific by nature, such as those granted for various types of plastics. A burgeoning field of new compositions is in the field of genetic and biological engineering. Composition of matter is sometimes referred to as chemical compositions. If you are developing new patented compositions, you are probably a scientist working for any number of chemical- or medical-oriented companies or a major university.

Software Patents

This type of patent is more of a catchall term referring to any number of computer- or Internet-related patents. It can include software itself, computer applications such as the one-touch screen, and Internet applications and methods, such as those used for secure credit card transactions. One of the more famous software patents is the one invented by Xerox that was used by Apple to create its mouse applications. It could be considered an improvement patent as well and was instrumental in turning the PC into a multibillion-dollar breakthrough opportunity. Today, the mouse is one of the standard input devices for all desktop PCs. One area of concern to companies is software that is developed and used internally for business operations. If the company does not research the concept to verify that it is not subject to an existing patent, the company may be infringing. Or, if the company does not pursue patenting the subject matter-maintaining it either as an internal trade secret or just neglecting to consider patenting-the concept may be subject to someone else's subsequent patent.

Patent Monopoly versus Antitrust

Patent laws were established to grant monopolistic rights to the patent owner. Once a patent issues, the patent owner can prevent others from selling, making,

importing, or using a product that infringes that patent. Conversely, antitrust laws were established to prevent one company from unfairly monopolizing a particular market or industry segment. Antitrust laws conflict in some cases with patent laws. With the growing number of antitrust claims being levied against patent owners (for their electing to "unfairly" shut down infringers rather than license the patent rights to them), this dilemma is becoming a hotly contested issue in the Federal Trade Commission and the U.S. Department of Commerce.

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In the past, patent owners have sought to find companies that infringed their invention. After some posturing and negotiation, the result of the skirmish was that the infringing company would usually execute a license to the patent and pay the patent owner an agreed-to royalty against manufacturers' sales.

With the recent case of *CSU* v. *Xerox*, it has become clear that a company's assertion of its patent rights, and its refusal to license its patents or technology, does not constitute antitrust.

Nevertheless, the debate continues, and by mid-2002 the Federal Trade Commission (FTC) and the Department of Justice Antitrust Division cosponsored hearings, called "Competition and Intellectual Property Law and Policy in the Knowledge-Based Economy." These hearings included debates ranging from "Patents Should Not Be a Defense to Antitrust," to "The Monopolistic Right of Patent Owners Is Absolute, and the U.S. Constitution Lays the Groundwork for Patent Owners to Exploit Their Rights," to "The Monopolistic Advantage against Competitors."

So while patents confer monopolistic rights on the patent owner, it is important for key managers and intellectual property counsel to keep an eye on new legislation being developed in the patents versus antitrust arena.

4.7 DRAFTING OF PROTECT CLAIMS

In order for the U.S. government to grant a patent, patent laws say that the subject matter must be (1) novel, (2) useful, and (3) not obvious to one skilled in the art.

In retrospect, it's rather easy to identify the usefulness of many inventions: The cost of a pair of Levi's jeans would be outrageous had it not been for Whitney's cotton gin. We take for granted many of the useful features of inventions, such as the improved lightbulb, Velcro, and ZipLoc bags-all of which have proven their usefulness. Products or processes in patent applications that are not provably useful are rejected by the Patent Office. For instance, perpetual motion machines have not been proven to the Patent Office to work and are therefore not patentable.

A patented invention must be *operative.* This means it must work according to the claims in the application. For instance, square tires would be considered neither useful nor operative. A patent on a process whose claims are based on improved output, but that does not perform as indicated, is not valid.

The invention must be *new* or *novel.* An invention cannot be patented if:

- It was previously known or patented in any part of the world at any given time.
- It was previously described in an article and published anywhere in the world.
- The difference between your invention and a previous patent (or publicly known product, process, etc.) is such that it would have been obvious to

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any person skilled in the art. For instance, simply changing size or color for the sake of making your invention different would probably not be patentable.

- It was offered for sale or put into use more than one year prior to filing for a U.S. patent.

In this last scenario, the United States (Canada and Mexico, too) has what is referred to as the one-year rule, or the one-year-on-sale bar. It means that the first, true inventor may file a patent application up to one year after the first public disclosure or first public offering of the invention or product for sale.

First to Invent

The United States is the only country in the world that recognizes the rightful inventor to be the one who is first to invent rather than the inventor who is the first to file a patent application. This means that only the first, true inventor will be acknowledged as the patent grantee. Any invention or discovery an inventor is working on that has not been abandoned has precedence over subsequent discoveries that are the same or similar in scope. If two persons are granted patents on the same subject matter, the inventor who can prove his or her discovery has precedence over the other will have the valid patent. This is true regardless of who filed first or which patent was granted first.

Proof of Inventorship

Three criteria are used to verify legal inventorship:

1. The *date of original conception* may be established by executing an invention disclosure that clearly reveals the inventive matter. This is usually accomplished with drawings depicting the subject matter and adequate specifications explaining how the invention works. Once completed, the disclosure should be signed by a qualified party who can verify the content and the date signed. This would not be a spouse or business partner but a third party who has nothing to gain from the invention's development. Inventor Journals, sometimes called Scientific Journals, are commonly used to establish the date of original conception.
2. Next, the invention must be *reduced to practice.* In other words, it must be shown to work the way you say it works. This is usually accomplished by providing a sufficient explanation in a journal, by producing computer-assisted drafting (CAD) drawings, or by building a functional prototype. If none of these steps is done or documented, then the filing of the patent application is considered the date of reduction to practice.
3. An invention must not have been *abandoned* during its development. If so, it could void the date of original conception and/or the date of reduction to practice. In other words, an inventor must use *diligence* when developing new concepts and inventions.

Filing Patent Applications

Your corporate counsel or patent attorney will be filing most of your patent applications, so this section is more informative than instructive. Several legal and business strategies are involved with the timing and completeness of a patent application, so it is important for all members of the PQM Team to discuss and agree on each new application filed.

U.S. patent applications are applied for in writing to the Commissioner of Patents and Trademarks using one of two types of applications: a provisional patent application or the permanent, nonprovisional patent application. Once either application is received by the U.S. Patent Office, the words *patent pending* or *patent applied for* may be marked on products, brochures, and so on.To use these terms falsely may subject the inventor or individual claiming a pending patent to a substantial fine.

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The most commonly used method today to establish that a patent application has been received by the U.S. Patent Office is to send it via Express Mail. The U.S. Postal Service is a U.S. government agency, which effectively acts as a receiving agent for the U.S. Patent Office. Once a patent application is mailed in person using Express Mail Service, it is legally considered received by the Patent Office on the date it was deposited, and the patent-pending notice may be applied.

Nonprovisional Patent Applications

The permanent, nonprovisional application begins the examination process that may lead to the granting of a patent. Some refer to the nonprovisional patent application as a regular patent application. It must at a minimum include the following:

- *Complete specifications*, which is considered a satisfactory description, or explanation, of the invention and at least one claim.
- *Drawings* as required to sufficiently illustrate how the invention works. Photographs are rarely used other than with plant patent applications.
- An *Inventors' Declaration* stating that he or she is the first and sole inventor. If more than one inventor was involved in the creation of the present invention, then all of them will need to execute a declaration.
- The appropriate *filing fee*.

Provisional Patent Applications

A provisional patent application, or PPA, is a simplified version of the permanent nonprovisional patent application. Although the name may imply that this is an application for a provisional patent, in fact, the provisional patent application is more accurately described as a provisional application for a patent. The PPA will never turn or mature into a regular patent. The PPA establishes a filing date but does not begin the examination process. It is held by the Patent Office for one year. If it is not followed up with a corresponding permanent nonprovisional application, it is discarded.

The use of provisional applications is becoming more popular because it preserves international filing rights if filed before a first public disclosure. The PPA has some additional strategic and tactical uses, which we will cover in more depth in later chapters. Provisional patent applications must include the following:

- A *cover sheet* identifying the application as a provisional application, the name of the inventor, and other bibliographic data
- At least a partial *specification* that satisfactorily describes the inventive matter, but without the legal claims
- *Drawings* if necessary (they almost always are).
- The required *filing fee*.

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Who Can File and Who Owns the Patent?

Only the true inventor can own, sell, or assign his or her interest in a patent application or patent. Any individual, firm, corporation, or partnership can own it. An inventor automatically owns his or her patent when granted, unless it is assigned to another entity. The transfer of an inventor's rights is by way of a patent assignment. The assignment of a patent (or application) may be recorded at the U.S. Patent Office, although this is not a legal requirement.

When you work for a company and invent something related to your company's business, on company time and for its use, you cannot ask for compensation for two reasons: (1) your continued employment is considered fair compensation for the invention, and (2) the discovery probably occurred in the job environment and would not have been made otherwise.

If an invention was conceived before joining a company, and it is being pursued and patented for the company's use, asking for compensation from the new employer is a valid request; however, it would be wise to agree upon the compensation before beginning employment and not afterward. Federal laws state that if you are employed by a firm and receive a patent on an unrelated idea in another field, and development was not on company time or at company expense, your employer cannot claim any rights.

Patents in the Corporation

New ideas usually start out as a conceptual seed from a single person or a small group of individuals, but because patents affect every department in the corporation, sooner or later other managers in the organization will play major roles in the development and commercialization of the patent. One of the foremost opportunities within a corporate structure is the ability to quickly build teams and take on new projects. Fortunately most modern TQM structures can adapt to a team development effort, especially from a top-down directive. The right team can dramatically speed up the time-to-market effort.

Corporate structure can be a downfall of some corporations as well. The wrong management style (*e.g.*, autocratic or micromanaged) will have a difficult time fostering a true team effort. If everything hinges on the approval of a single person, it will be slow going. Of course, patents are considered intangible assets, and under the new Financial Accounting Standards Board (FASB), increased emphasis is being put on financial reporting and patent value as a reportable intangible corporate asset.

Patents across the Corporate Structure

Patents increasingly contribute to the creation and enhancement of shareholder value, help establish competitive market positioning, and are becoming an important source of licensing revenues. It is no wonder then that managers throughout the organization will increasingly come in contact with patents and will be increasingly required to make management decisions related to patents.

From this position of having provided you with a basic understanding of what patents are, and how they work. Patent value doesn't just happen. It is planned (or at least it should be). Patent strategy, patent tactics, and patent management

throughout the organization are critical factors in achieving Patent Quality Management. Patents are powerful tools based on the right to exclude others from manufacturing, using, and selling products that fall under the scope of your patents. Thus, patents can be the basis to protect your company's sales and assets. This basis also represents a popular means of generating additional revenue—more specifically, through licensing out your company's patents and licensing in others that may expand your company's sales.

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4.8 INTRODUCTION TO TRADE PROTECTION AND WO PATENTS

Protectionism is the economic policy of restraining trade between states, through methods such as tariffs on imported goods, restrictive quotas, and a variety of other government regulations designed to discourage imports, and prevent foreign take-over of domestic markets and companies.

This policy contrasts with free trade, where government barriers to trade and movement of capital are kept to a minimum. In recent years, it has become closely aligned with anti-globalization. The term is mostly used in the context of economics, where protectionism refers to policies or doctrines which protect businesses and workers within a country by restricting or regulating trade with foreign nations. A variety of policies can be used to achieve protectionist goals. These include:

1. *Tariffs*: Typically, tariffs (or taxes) are imposed on imported goods. Tariff rates usually vary according to the type of goods imported. Import tariffs will increase the cost to importers, and increase the price of imported goods in the local markets, thus lowering the quantity of goods imported. Tariffs may also be imposed on exports, and in an economy with floating exchange rates, export tariffs have similar effects as import tariffs. However, since export tariffs are often perceived as 'hurting' local industries, while import tariffs are perceived as 'helping' local industries, export tariffs are seldom implemented.
2. *Import quotas*: To reduce the quantity and therefore increase the market price of imported goods. The economic effects of an import quota is similar to that of a tariff, except that the tax revenue gain from a tariff will instead be distributed to those who receive import licenses. Economists often suggest that import licenses be auctioned to the highest bidder, or that import quotas be replaced by an equivalent tariff.
3. *Administrative barriers*: Countries are sometimes accused of using their various administrative rules (*e.g.*, regarding food safety, environmental standards, electrical safety, etc.) as a way to introduce barriers to imports.
4. *Anti-dumping legislation:* Supporters of anti-dumping laws argue that they prevent "dumping" of cheaper foreign goods that would cause local firms to close down. However, in practice, anti-dumping laws are usually used to impose trade tariffs on foreign exporters.
5. *Direct subsidies*: Government subsidies (in the form of lumpsum payments or cheap loans) are sometimes given to local firms that cannot compete well against foreign imports. These subsidies are purported to "protect" local jobs, and to help local firms adjust to the world markets.
6. *Export subsidies*: Export subsidies are often used by governments to increase exports. Export subsidies are the opposite of export tariffs, exporters are

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paid a percentage of the value of their exports. Export subsidies increase the amount of trade, and in a country with floating exchange rates, have effects similar to import subsidies.

7. *Exchange rate manipulation:* A government may intervene in the foreign exchange market to lower the value of its currency by selling its currency in the foreign exchange market. Doing so will raise the cost of imports and lower the cost of exports, leading to an improvement in its trade balance. However, such a policy is only effective in the short run, as it will most likely lead to inflation in the country, which will in turn raise the cost of exports, and reduce the relative price of imports.
8. *International patent systems:* There is an argument for viewing national patent systems as a cloak for protectionist trade policies at a national level. Two strands of this argument exist: one when patents held by one country form part of a system of exploitable relative advantage in trade negotiations against another, and a second where adhering to a worldwide system of patents confers "good citizenship" status despite 'de facto protectionism'. Peter Drahos explains that "States realized that patent systems could be used to cloak protectionist strategies. There were also reputational advantages for states to be seen to be sticking to intellectual property systems. One could attend the various revisions of the Paris and Berne conventions, participate in the cosmopolitan moral dialogue about the need to protect the fruits of authorial labor and inventive genius...knowing all the while that one's domestic intellectual property system was a handy protectionist weapon."

4.9 PATENT ACT, 1970

What are Not Inventions?

The following are not inventions within the meaning of this Act,—

(*a*) an invention which is frivolous or which claims anything obviously contrary to well established natural laws;

(*b*) an invention the primary or intended use or commercial exploitation of which could be contrary to public order or morality or which causes serious prejudice to human, animal or plant life or health or to the environment;

(*c*) the mere discovery of a scientific principle or the formulation of an abstract theory;

(*d*) the mere discovery of a new form of a known substance which does not result in the enhancement of the known efficacy of that substance or the mere discovery of any new property or new use for a known substance or of the mere use of a known process, machine or apparatus unless such known process results in a new product or employs at least one new reactant;

(*e*) a substance obtained by a mere admixture resulting only in the aggregation of the properties of the components thereof or a process for producing such substance;

(*f*) the mere arrangement or re-arrangement or duplication of known devices each functioning independently of one another in a known way;

(*g*) a method of agriculture or horticulture;

(*h*) any process for the medicinal, surgical, curative, prophylactic 1[diagnostic, therapeutic] or other treatment of human beings or any process for a similar

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treatment of animals to render them free of disease or to increase their economic value or that of their products;

(*i*) plants and animals in whole or any part thereof other than micro-organisms but including seeds, varieties and species and essentially biological processes for production or propagation of plants and animals;

(*j*) a mathematical or business method or a computer program per se or algorithms;

(*k*) a literary, dramatic, musical or artistic work or any other aesthetic creation whatsoever including cinematrographic works and television productions;

(*l*) a mere scheme or rule or method of performing mental act or method of playing game;

(*m*) a presentation of information;

(*n*) topography of integrated circuits;

(*o*) an invention which, in effect, is traditional knowledge or which is an aggregation or duplication of known properties of traditionally known component or components.

Persons Entitled to Apply for Patents

Subject to the provisions contained in Section 134, an application for a patent for an invention may be made by any of the following persons, that is to say,-

(*a*) by any person claiming to be the true and first investor of the invention;

(*b*) by any person being the assignee of the person claiming to be the true and first inventor in respect of the right to make such an application;

(*c*) by the legal representative of any deceased person who immediately before his death was entitled to make such an application;

(*d*) An application of the persons referred to therein either alone or jointly with any other person.

4.10 THE PATENTS RULES, 2003

(*a*) An application for grant of a patent, other than a Convention application, by the true and first inventor shall be made, Provided that if the true and first inventor is not a party to the application, the applicant shall produce the original deed of assignment or other document from such true and first inventor, under which he is entitled to apply for a patent, unless such an application made itself is endorsed by the true and first inventor(s) in the presence of two witnesses with a statement that the application shall be made without his name as an applicant for the patent.

(*b*) A convention application by the true and first inventor shall be made provided that if the true and first inventor is not a party to the application, the applicant shall produce the original deed of assignment or other document from such true and first inventor, under which he is entitled to apply for a patent, unless such application made itself is endorsed by the true and first inventor in the presence of two witnesses with a statement that the application shall be made without his name as an applicant for a patent.

(*c*) In the case of an application, other than a Convention application, by the personal representative of a deceased person, who, immediately before his death, was entitled to make such an application, the probate of the will of the deceased, or the letter of administration of his estate, or an official copy of

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the probate or letter of administration, shall be produced at the Patent Office in proof of the applicant's title to act as the personal representative.

(*d*) Where in pursuance of sub-section (3) of Section 14, the Controller allows a single complete specification to be proceeded with in respect of two or more applications in respect of which two or more provisional specifications have been filed, the single complete specification may include any matter disclosed in any of the said specifications and shall be deemed to have been filed on such date, not earlier than the earliest date on which all the matter disclosed in the said single complete specification has been disclosed to the Patent Office in or in connection with the applications, as the Controller may direct.

(*e*) Where an applicant has made an application for a patent and, before the acceptance of the complete specification, makes a divisional application for a patent for matter included in the first mentioned application or in any specification filed in pursuance thereof, the Controller may direct that the fresh application or any specification filed in pursuance thereof shall be ante-dated to a date not earlier than the date of filing of the first mentioned application or specification if the applicant includes in the fresh application a request to that effect. Provided that the Controller may require such amendment of the complete specification filed in pursuance of either of the said applications as may be necessary to ensure that neither of the said complete specification includes a claim for matter claimed in the other.

(*f*) Where a complete specification has been filed pursuant to two or more applications accompanied by provisional specifications for inventions which the applicant believes to be cognate or modifications one of another, and the Controller is of opinion that such inventions are not cognate or modifications one of another, the Controller may allow the complete specification to be divided into such number of complete specifications as may be necessary to enable that applications to be proceeded with as two or more separate applications for patents.

(*g*) Where a single Convention application has been made in respect of all or part of the inventions in respect of which two or more applications for protection have been made in one or more Convention countries, and the Examiner reports that the claims of the specification filed in the said Convention application relate to more than one invention, the Controller may allow one or more further applications to be filed and the specification to be divided into such number of specifications as may be necessary to enable two or more separate Convention applications to be proceeded with and may direct that the said applications be deemed to have been filed on the date of filing of the original application.

(*h*) Where in pursuance of sub-section (1) of Section 88 the Controller allows more than three months to file a copy or copies of the specification or specifications, and drawings or documents filed or deposited by the applicant or his predecessor in title, as the case may be, in respect of the Conventional application, a request for such extension of time shall be made provided, however that such extension shall not exceed maximum 3 periods of 3 months each from the date of filing of the Convention application.

(*i*) Save as aforesaid, all proceedings in connection with a Convention application shall be taken within the time and in the manner required by the Ordinance or prescribed by these rules for ordinary applications.

(*j*) Applications shall, on receipt by the Controller, be numbered and dated in the order of their receipt.

SUMMARY

- A patent is, in essence, a monopoly granted by the U.S. government to an inventor in exchange for full public disclosure of the invention.
- Patents are sometimes referred to as a legal monopoly because they can be used to prevent others from practicing the invention.
- *Utility patents* may be granted to anyone who invents or discovers any new and useful process, machine, article of manufacture, system (or method of use), software and Internet methodologies.
- *Design patents* may be granted to anyone who invents a new, original, ornamental design for an article of manufacture.
- *Plant patents* may be granted to anyone who invents or discovers and asexually reproduces any distinct and new variety of plant.
- When a specific unique, novel, useful element is used with a product, that too defines the product as unique and would be considered a product patent. The terms *device patent* or *apparatus patent* are also commonly used, but for ease of explanation, they are best grouped into the single category of *product patents.*
- Process patents generally refer to manufacturing processes. They would typically improve productivity, reduce defects, or offer some value-added quality.
- The term *improvement patent* may refer to any number of new incremental improvements made to an existing product, system, or process.
- When several elements are used in combination that have some sort of productive output, it is referred to as a *machine patent.*
- The *date of original conception* may be established by executing an invention disclosure that clearly reveals the inventive matter.
- A provisional patent application, or PPA, is a simplified version of the permanent nonprovisional patent application.
- Protectionism is the economic policy of restraining trade between states, through methods such as tariffs on imported goods, restrictive quotas, and a variety of other government regulations designed to discourage imports, and prevent foreign take-over of domestic markets and companies.

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REVIEW QUESTIONS

1. Define the patents, and its different types.
2. Explain the following:
   (*a*) Process patents (*b*) Improvement patents
3. What are the applications of provisional patents?
4. Discuss different patent laws.
5. What do you understand by protectionism?

UNIT V: BIOTECHNOLOGY IN DRUGS

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★ STRUCTURE ★

5.1 LEARNING OBJECTIVES

After going through this unit, you will be able to:

- define terms like molecular biology, genetic engineering and r DNA technology
- discuss the importance of nucleic acids
- describe the recombinant production of therapeutic proteins
- understand the concept of cDNA cloning

5.2 INTRODUCTION

In this unit, students will come to know about the terms related to biotechnology, the structure and functions of nucleic acids, how to purify nucleic acid, the cloning of classical gene and its identification, the concept of cDNA cloning, and the concept of cloning via polymerase chain reaction.

5.3 INTRODUCTION TO BIOTECHNOLOGY

The biopharmaceutical sector is largely based upon the application of techniques of molecular biology and genetic engineering for the manipulation and production of therapeutic macromolecules. The majority of approved biopharmaceuticals are proteins produced in engineered cell lines by recombinant means. Examples include the production of insulin in recombinant *E. coli* and recombinant *S. cerevisiae*, as well as the production of EPO in an engineered (Chinese hamster ovary) animal cell line.

Terms such as 'molecular biology', 'genetic engineering' and 'recombinant DNA (rDNA) technology' are sometimes used interchangeably and often mean slightly different things to different people. Molecular biology, in its broadest sense, describes the study of biology at a molecular level, but focuses in particular upon the structure, function and interaction/relationship between DNA, RNA and proteins. Genetic engineering, on the other hand, describes the process of manipulating genes (outside of a cell's/organism's normal reproductive process). It generally involves the isolation, manipulation and subsequent reintroduction of stretches of DNA into cells and is usually undertaken in order to confer on the recipient cell the ability to produce a specific protein, such as a biopharmaceutical. 'rDNA technology' is a term used interchangeably with 'genetic engineering'. rDNA is a piece of DNA artificially created *in vitro* which contains DNA (natural or synthetic) obtained from two or more sources.

When developing a new protein biopharmaceutical, one of the earliest actions undertaken entails identifying and isolating the gene (or complementary DNA (cDNA); see later) coding for the target protein, the generation of an appropriate piece of rDNA containing the protein's coding sequence and the introduction of this rDNA into an appropriate host cell such that the target protein is made in large quantities by that engineered cell. Before we look at these techniques, however, we will briefly review the basic biology and structure of nucleic acids.

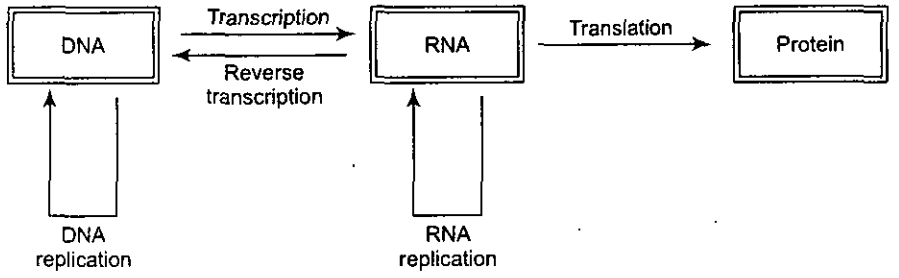

Fig. 5.1 Schematic representation of the so-called central dogma of molecular biology.

5.4 NUCLEIC ACIDS: FUNCTION AND STRUCTURE

Nucleic acids represent a prominent category of biomolecule present in living cells. The term incorporates both DNA and RNA. DNA represents the repository of genetic information (the genome) of most life forms. RNA replaces DNA as the repository of genetic information in some viruses. In most life forms, however, RNA plays a role in mediating the conversion of genetic information stored in specific DNA sequences (genes) into polypeptides. There are three subcategories of RNA, each playing a different role in the conversion of gene sequences into the amino acid sequence of polypeptides. Messenger RNA (mRNA) carries the genetic coding information from the gene to the ribosome, where the polypeptide is actually synthesized. Ribosomal RNA (rRNA), along with a number of proteins, forms the ribosome itself, and transfer RNA (tRNA) functions as an adaptor molecule, transferring a specific amino acid to a growing polypeptide chain on the ribosomal site of polypeptide synthesis. Therefore, nucleic acids, between them all, mediate the flow of genetic information via the processes of replication, transcription and translation as outlined in what has become known as the central dogma of molecular biology (Fig. 5.1).

Structurally, nucleic acids are polymers in which the basic recurring monomer is a nucleotide (*i.e.*, nucleic acids are polynucleotides). Nucleotides themselves consist

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of three components: a phosphate group, a pentose (five-carbon sugar) and a nitrogenous-containing cyclic structure known as a base (Fig. 5.2). The nucleotide sugar associated with RNA is ribose, whereas that found in DNA is deoxyribose (Fig. 5.3). In total, five different bases are found in nucleic acids. They are categorized as either purines (adenine and guanine, or A and G, found in both RNA and DNA) or pyrimidines (cytosine, thymine and uracil, or C, T and U). Cytosine is found in both RNA and DNA, whereas thymine is unique to DNA and uracil is unique to RNA (Fig. 5.4). The DNA or RNA polymer consists of a chain of nucleotides of specific base sequence, linked via phosphodiester bonds (Fig. 5.5).

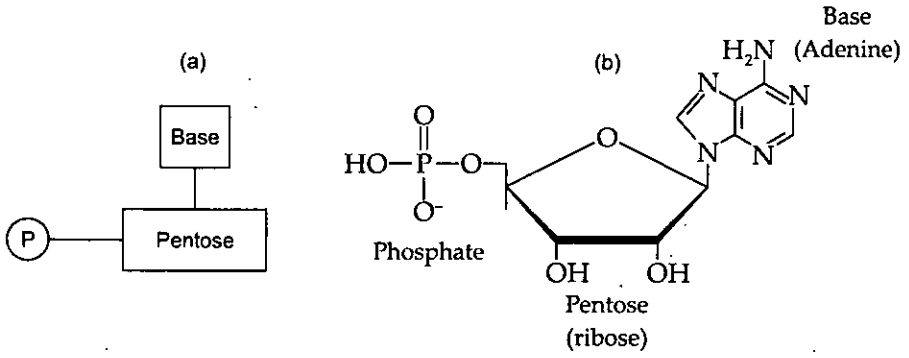

Fig. 5.2 (a) The basic structure of a nucleotide. (b) The actual chemical structure of one representative nucleotide (adenylate, i.e., adenosine 5'-monophosphate)

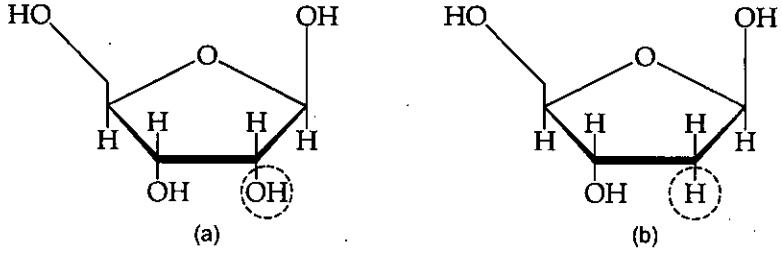

Fig. 5.3 Chemical structure of (a) ribose and (b) 2'-deoxyribose, the nucleotide pentoses found in RNA and DNA respectively. The differences in chemical structure are highlighted by the dotted circles

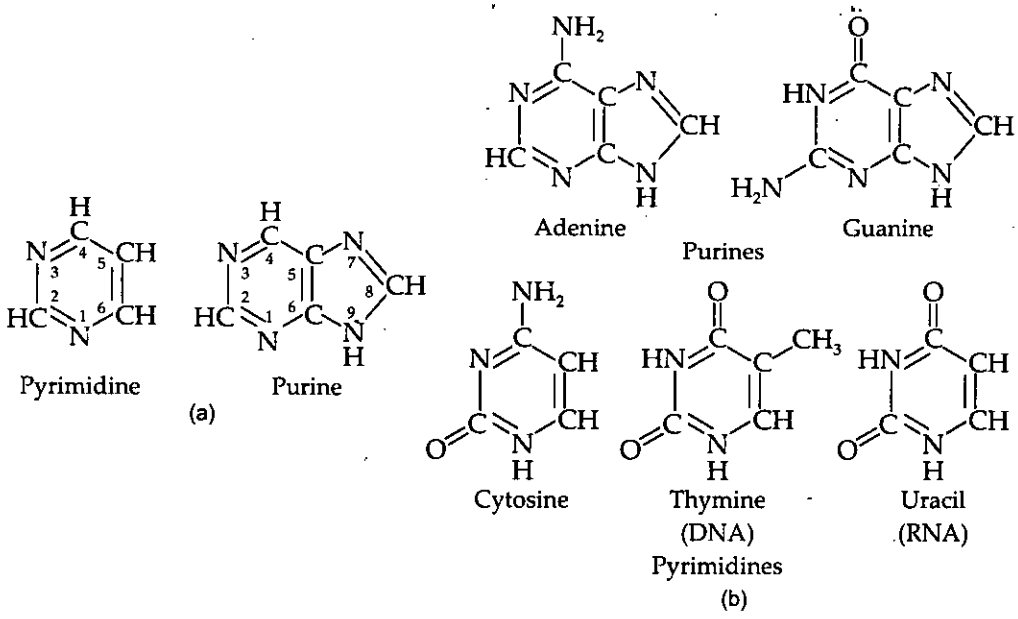

Fig. 5.4 The five bases found in nucleic acids may be categorized as either pyrimidines or purines.

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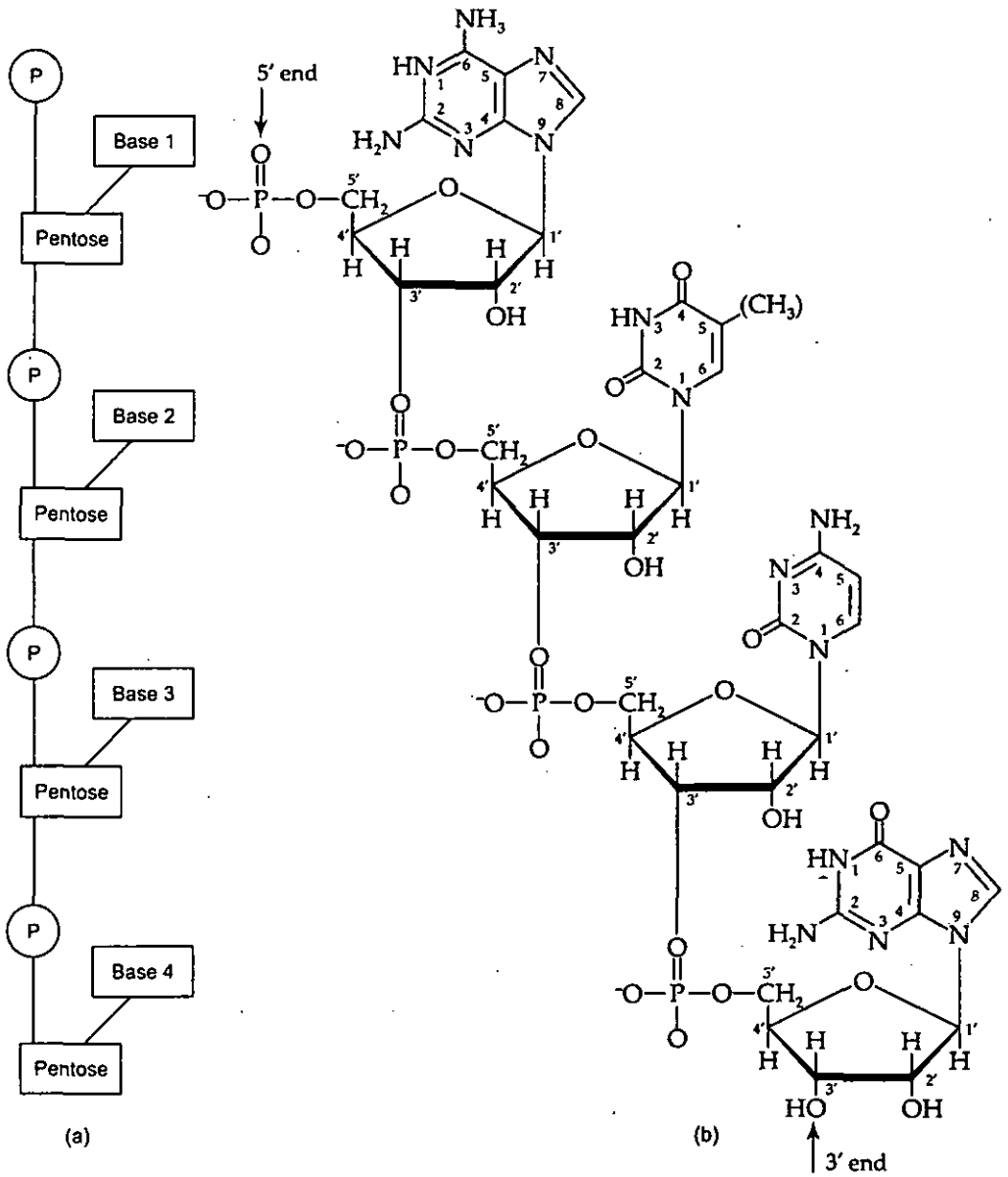

Fig. 5.5 The basic polynucleotide structure as shown in (a) outline form and (b) in chemical detail. The 5' end of the chain is defined by lacking a nucleotide attached to the first sugar's carbon number 5; the 3' end lacks a nucleotide attached to the carbon number 3 of the last sugar in the backbone

RNA is a single-stranded polynucleotide, although RNA molecules tend to adopt higher order three-dimensional shapes. DNA, on the other hand, is a double-stranded molecule (Fig. 5.6) that assumes a double helical structure. The two polynucleotide strands face each other in an antiparallel manner (Fig. 5.6), with the hydrophilic sugar and phosphate residues facing outwards, towards the surrounding aqueous-based environment, and the more hydrophobic bases point inwards. The base sequence of each chain displays complementarity. Wherever thymine is found in one chain, adenine is found positioned opposite it in the other. Wherever guanine is found in one chain, cytosine is found positioned opposite it in the second chain.

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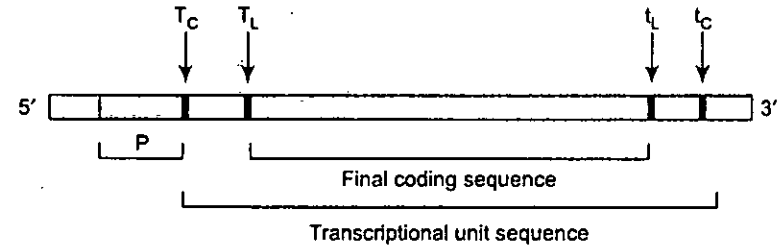

Fig. 5.7 Generalized gene organization within the genome

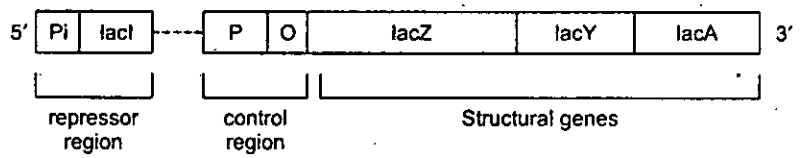

Fig. 5.8 The lac operon houses three structural genes: lac Z, lac Y and lac A

5.6 NUCLEIC ACID PURIFICATION

A prerequisite step to any rDNA work is the initial isolation of DNA or RNA from the source material (which can be microbial, plant, animal or viral). Numerous methodologies have been developed to achieve nucleic acid purification, and some of these methodologies have been adapted for use in a variety of commercially available purification kits. Although details vary, the general approach adopted entails initial liberation of the nucleic acid by disruption of any cell wall present (or viral capsid) and of the cellular plasma membrane, followed by selective precipitation and often chromatography. In the context of plants and some microorganisms, initial disruption of the cell wall may require application of physical or other vigorous disruptive influences. This can potentially complicate DNA purification, particularly as it can cause physical shearing (fragmentation) of the extremely long DNA chromosome. The gentlest method of cell lysis usually involves incubation with cell-wall-degrading enzymes, and the addition of detergent will solubilize the plasma membrane. Following cellular disruption, initial purification steps normally entail solvent-based extraction/precipitation. For example, shaking in the presence of phenol (or a mixture of phenol and chloroform), followed by standing or centrifugation (to achieve phase separation) results in extraction of the (now denatured) proteins into the phenol phase and/or accumulation at the interphase, with nucleic acids remaining in the upper, aqueous phase. Further purification may be achieved by selective precipitation of the nucleic acids using ethanol or isopropanol as precipitant. If DNA is required, then the RNA present may now be removed by the addition of the enzyme ribonuclease, which selectively degrades RNA. On the other hand, if (eukaryotic) mRNA is required, then affinity-based purification may be undertaken using an oligo (dT) column (Fig. 5.10). Nucleic acids absorb UV light maximally at 260 nm (compared with 280 nm in the case of proteins); thus, absorbance at 260 nm can be used to quantify the amount of nucleic acid present and to follow the purification protocol. The ratio of absorbance at 260 nm versus 280 nm can also be used to determine how contaminated the nucleic acid preparation is with protein. The ratio $A260/A280 \approx 1.8$ for pure DNA and 2.0 for pure RNA preparations; lower ratios usually indicate the presence of contaminant protein. DNA can also be detected and quantified by the addition of the chemical ethidium bromide. Ethidium bromide molecules intercalate (bind) in between DNA bases and fluoresce when illuminated with UV light.

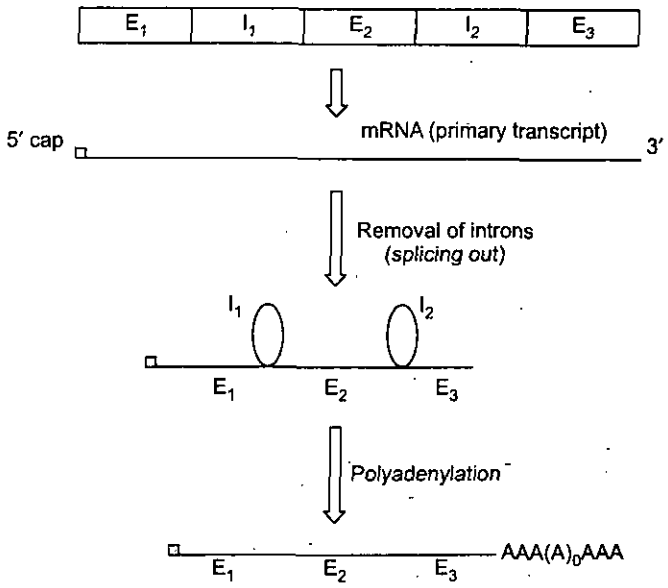

Fig. 5.9 Overview of the transcription of eukaryote genes and subsequent mRNA editing

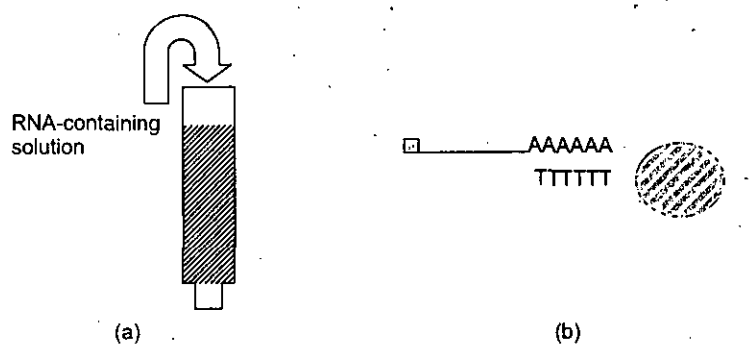

Fig. 5.10 Affinity-based purification of mRNA

5.7 NUCLEIC ACID SEQUENCING

The determination of the exact base sequence present in a stretch of nucleic acid (particularly in DNA) underpins much of modern molecular biology. Sequencing plays a central role in rDNA cloning experiments, as well as in determining genome data. Two approaches have been developed to sequence DNA: the Maxam-Gilbert chemical sequencing method and the Sanger-Coulson enzymatic sequencing method. Both involve the ultimate generation of a full set of fragments of the DNA strand to be sequenced, as illustrated in Figure 5.11. The methodologies employed ensure that the identity of the final (3′) base in each fragment is known. The fragments are then separated on the basis of their size by electrophoresis and, because the identity of the end base in each fragment is already known, the full sequence can simply be read from the ladder of fragments generated. Full details of sequencing methodologies are outside the scope of this book, but they are included in all core molecular biology and biochemistry student textbooks. RNA is sequenced by an enzyme-based method somewhat similar to the enzyme-based DNA method.

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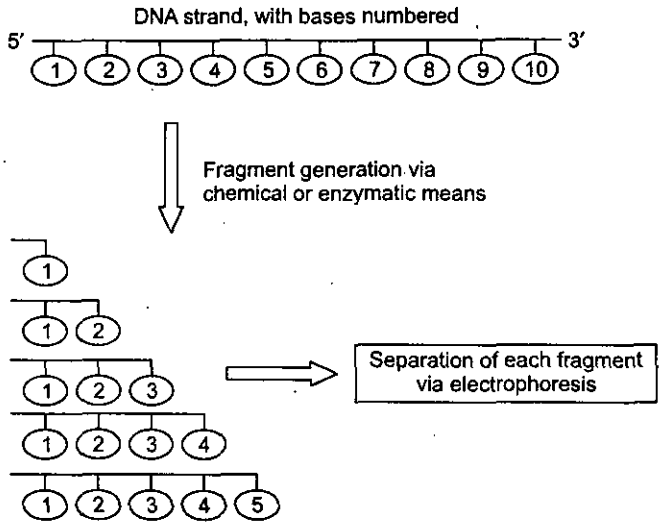

Fig. 5.11 A simplified overview of the approaches adopted to both chemical and enzyme-based DNA sequencing.

5.8 RECOMBINANT PRODUCTION OF THERAPEUTIC PROTEINS

The evaluation of any protein as a potential biopharmaceutical and its subsequent routine medical use are dependent upon the availability of sufficient quantities of the target protein. In most instances this is best achieved via production by recombinant means (*i.e.*, via genetic engineering). In addition to facilitating the production of any protein in substantial quantities, recombinant based production can have a number of additional advantages over direct extraction from a naturally producing source, as described in unit 1. Production of any protein via rDNA technology entails the initial identification and isolation of a DNA sequence coding for the target protein.

This sequence can be direct genomic DNA, but mRNA coding for the protein of interest can also act as a starting point. In the latter approach, the mRNA is enzymatically 'reverse transcribed' into cDNA. If the target therapeutic protein is eukaryotic (which is invariably the case) then the genomic DNA will contain both coding (exon) and non-coding (intron) sequences (Fig. 5.9), whereas the cDNA will be a reflection of the exons only. The desired gene/cDNA is normally amplified, sequenced and then introduced into an expression vector that facilitates its introduction and expression (transcription and translation) in an appropriate producer cell type. All recombinant therapeutic proteins approved to date are produced in *E. coli, S. cerevisiae* or in animal cell lines (mainly CHO or BHK cells).

5.9 CLASSICAL GENE CLONING AND IDENTIFICATION

The basic approach to cloning a segment of DNA entails: Initial enzyme-based fragmentation of intact genomic DNA (usually chromosomes isolated as described earlier in this unit) so that it is broken down into manageable fragment sizes for further manipulation. Ideally all/most fragments will contain one gene.

Integration of the various fragments generated into cloning vectors, which are themselves small DNA molecules capable of self-replication. Typically, these are

plasmids or viral DNAs and the composite or engineered DNA molecules generated are called rDNA. Introduction of the vectors housing the DNA fragments into host cells. Growing these cells on agar plates. Screening/identification of the host cell colonies containing the rDNA molecules (*i.e.*, screening the 'library' of clones generated) in order to identify the specific colony containing the target DNA fragment, *i.e.*, the target gene (Fig. 5.12). We will now look at each of these stages separately. The initial fragmentation of genomic DNA is undertaken using enzymes known as restriction endonucleases (REs). Some 800 different REs have been identified thus far. These enzymes recognize, bind and cut DNA sequences which exhibit a defined base sequence (Table 5.2). These sequences normally exhibit a twofold symmetry around a specific point and are usually 4, 6 or 8 bp in length. Such areas are often termed palindromes. In general, the larger the recognition sequence the fewer such sequences present in a given DNA molecule and, hence, the smaller the number of DNA fragments that will be generated. Depending upon the specific RE utilized, DNA cleavage may yield blunt ends (*e.g.*, BsaAI and EcoRV in Table 5.2) or staggered ends - the latter are often referred to as sticky ends.

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An essential feature of the cloning vector used is that it must be capable of self-replication in the cell into which it is introduced, which is usually *E. coli*. Two of the most commonly used types of vector in conjunction with *E. coli* are plasmids and bacteriophage λ. Plasmids are circular extra-chromosomal DNA molecules, generally between 5000 and 3500000 bp in length, that are found naturally in a wide range of bacteria. They generally house several genes, often including one or more genes whose product renders the plasmid-containing cell resistant to specific antibiotic(s). One plasmid often used in cloning experiments with *E. coli* is pUC18 (Fig. 5.13). Bacteriophage ('phage') are viruses capable of infecting and replicating inside bacteria. Bacteriophage λ DNA is approximately 48500 bp in length. Another vector type sometimes used are the bacterial artificial chromosomes (BACs), which are effectively very large plasmids used to clone very large stretches of DNA (usually DNA fragments above 100000 bp).

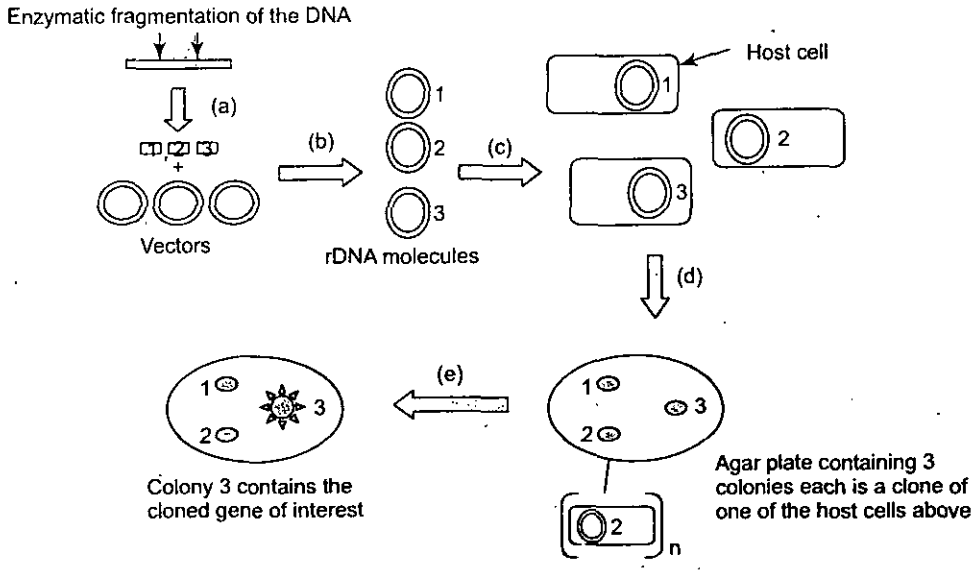

Fig. 5.12 A basic overview of the DNA cloning process

Integration of the DNA fragments into the chosen vector is undertaken by 'opening up' the circular vector via treatment with the same RE as used to generate

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the DNA fragments for cloning, followed by co-incubation of the cleaved vector and the fragments under conditions that promote the annealing of complementary sticky ends. Some vectors may simply recircularize to reform their original structure, but pretreatment of the vector in various ways can prevent this from happening. Most of the recircularized plasmids will have incorporated a fragment of DNA to be cloned. The plasmids are then incubated with another enzyme, a DNA ligase, which catalyses the formation of phosphodiester bonds in the DNA backbone and thus will seal or 'ligate' the plasmid.

The next stage of the cloning process entails the introduction of the engineered vector into *E. coli* cells. This can be achieved by a number of different means. One approach (called transformation) involves co-incubation of the plasmids and cells in a solution of calcium chloride, initially at 0°C, with subsequent increase in temperature to 42°C. This temperature shock facilitates entry of plasmids into some cells.

Table 5.2 Some commercially available REs, their sources, DNA recognition sites and cleavage points

Restriction enzyme	*Source*	*DNA recognition sequence and cleavage site*[a]
BclI	Bacillus caldolyticus	5′-T↓GATCA-3′ 3′-ACTAG↑T-5′
Bg1II	Recombinant E. coli carrying Bg1II gene from Bacillus globigii	5′-A↓GATCT-3′ 3′-TCTAG↑A-5′
BsaAI	Recombinant E. coli carrying BsaAI gene from Bacillus stearothermophilus A	5′-PyAC↓GTPu-3′ 3′-PuTG↑CAPy-5′
BsaJI	B. stearothermophilus J	5′-C↓CNNGG-3′ 3′-GGNNC↑C-5′
BsiEI	B. stearothermophilus	5′-CGPuPy↓CG-3′ 3′-GC↑PyPuGC-5′
EcoRV	Recombinant E. coli carrying EcoRV gene from the plasmid J62 plg 74	5′-GAT↓ATC-3′ 3′-CTA↑TAG-5′
MwoI	Recombinant E. coli carrying cloned MwoI gene from Methanobacterium wolfeii	5′-GCNNNNN↓NNGC-3′ 3′-CGNN↑NNNNNCG-5′
Tsp509I	Thermus sp.	5′-↓AATT-3′ 3′-TTAA↑-5′
XbaI	Recombinant E. coli carrying XbaI gene from Xanthomanas badvii	5′-T↓CTAGA-3′ 3′-AGATC↑T-5′
XhoI	Recombinant E. coli carrying XhoI gene from X. holcicola	5′-C↓TCGAG-3′ 3′-GAGCT↑C-5′

[a]G: guanine; C: cytosine; A: adenine; T: thymine; Pu: any purine; Py: any pyrimidine; N: either a purine or pyrimidine. Arrow indicates site of cleavage.

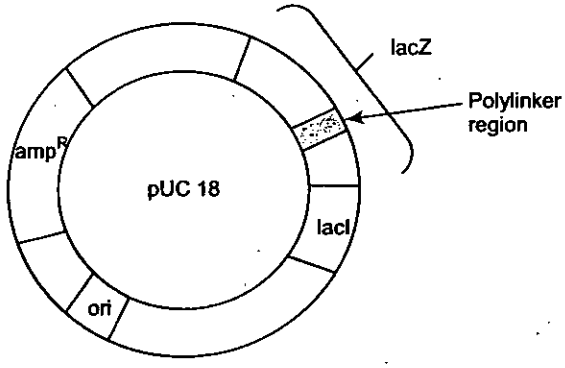

Fig. 5.13 The plasmid pUC18 is often used for cloning purposes

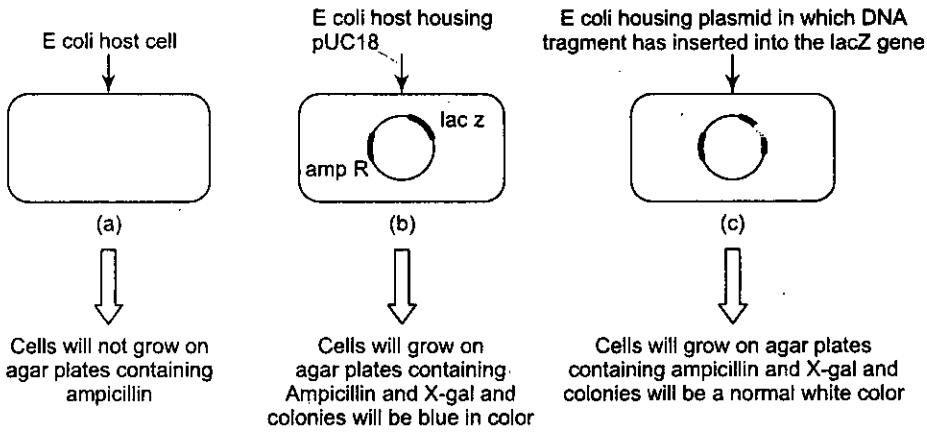

Fig. 5.14 Identification of E. coli host cell clones containing rDNA using pUC18 vectors

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The *E. coli* cells are next spread out on the surface of an agar plate and incubated under appropriate conditions in order to kill cells that have not taken up plasmid. Each individual cell will thus form a colony (clone of cells). Three main types of cell will be initially transferred onto these agar plates: (a) some cells will have failed to take up any plasmid; (b) some transformed cells may have a plasmid in which no foreign DNA had been inserted; (c) some cells will house a plasmid that does carry a fragment of the target DNA. These latter cells are the only ones of interest, and various strategies may be adopted to identify them. One such strategy is outlined in Figure 5.14.

Once the various *E. coli* clones (colonies) containing vector into which DNA fragments have been successfully integrated (*i.e.*, clones containing rDNA) have been identified, all that remains to be achieved is to pinpoint which colony harbours the rDNA fragment containing the gene of interest (see Figure 5.12).

Assuming you started off with whole genomic DNA, the procedure thus far has effectively generated a library of clones containing different genomic DNA fragments. The final task remaining, therefore, is to identify which specific clone/clones harbour the actual DNA fragment of interest (in our context this would be the fragment containing the gene coding for the desired therapeutic protein). This can be a major task, as libraries often consist of 109 or more clones. The most common means of achieving this is via sequence-based hybridization studies. The basic approach taken entails the use of a labelled (*e.g.*, radioactive) probe that is a single-strand DNA fragment or an RNA fragment synthesized to have a base sequence complementary to a sequence within the gene of interest. Genome projects now mean that such sequence information is known for many proteins. Alternatively, likely base sequences can be deduced if a partial amino acid sequence of the protein is known. Hybridization studies are usually initiated by physically pressing a nitrocellulose paper onto the agar plates containing the recombinant colonies. A replica of the plate is thus created on the paper, as some cells from each colony adhere to it. Subsequent treatment of the paper with alkali lyses the cells, releasing and denaturing the DNA within. The DNA adsorbs tightly to the paper. The paper is then exposed to a solution containing the labelled DNA probe under conditions that allow it to anneal to the target DNA, if it is present. After washing (to remove unbound probe), any probe retained on the paper surface can de detected by an appropriate visualization technique (*e.g.*, autoradiography if the

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probe is radiolabelled), and the positioning of the label on the paper surface pinpoints which colony on the agar surface houses the desired DNA fragment. Cells from the appropriate colony can then be grown up in larger amounts by submerged fermentation in order to produce larger amounts of the desired (now cloned) gene. The cells can be collected, lysed and the vector therein recovered by standard microbiological techniques. The cloned gene can then be excised from the vector via treatment with an appropriate RE and purified by standard molecular techniques.

5.10 CDNA CLONING

An alternative to cloning genomic DNA, as outlined in the sections above, entails beginning the process not with chromosomal fragments but with mRNA. This is often an approach taken when cloning eukaryotic genes in particular. As described in Figure 5.10, total eukaryotic cellular mRNA can be purified from the cell via an affinity-based mechanism. The mRNAs recovered in this way reflect only the polypeptide-encoding genes that are expressed in the cells at the time of their extraction. Incubation of the mRNA with the enzyme reverse transcriptase results in the conversion of the single-stranded mRNA into double-stranded DNA known as cDNA (Fig. 5.1). These cDNA fragments can then be cloned to generate a cDNA library, and the desired cDNA clone can be identified by means similar to those already described in the context of cloning genomic DNA. cDNA libraries are smaller than genomic libraries as they are derived only from expressed genes. Non-coding regions in the genome (as well as quiescent genes and genes coding for rRNA and tRNA) are not represented in the library. Therefore, cDNA libraries are more manageable to work with, assuming that the gene of interest is being expressed.

5.11 CLONING VIA POLYMERASE CHAIN REACTION

An enormous number of individual genes have been sequenced over the last two decades or more. Of latter years in particular, genome projects have also begun to make available the sequence of the entire complement of genes present in many species. The bulk of this sequence information has been made publicly available by its deposition in sequence databases. As a result, scientists who now wish to clone a particular gene will usually have prior access to partial/entire sequence information from the relevant organism or a closely related species. This sequence information allows them to obtain large amounts of the gene of interest by using the PCR technique (Fig. 5.15). This approach to cloning has now come to the fore, as it is faster and more convenient than the more classical methods described above. The process begins by extraction of total genomic DNA from the source of interest (*e.g.*, human cells if you wish to clone a specific human gene). Oligonucleotide primers whose sequences flank the target gene/DNA segment are synthesized and used to amplify that portion of DNA selectively. Recognition sites for REs can be incorporated into the oligonucleotides to allow cloning of the amplified gene, as outlined earlier. Because the target gene sequence is the only segment of the extracted DNA to be amplified by the prior PCR step, the vast majority of clones in the library now generated should contain the desired gene. This can be

confirmed by direct sequencing of the inserted DNA fragment from several of the colonies. Sequencing is important not only to prove definitively that the cloned DNA is the target gene, but also that its sequence perfectly matches the published sequence. The PCR process is prone to the introduction of sequence errors.

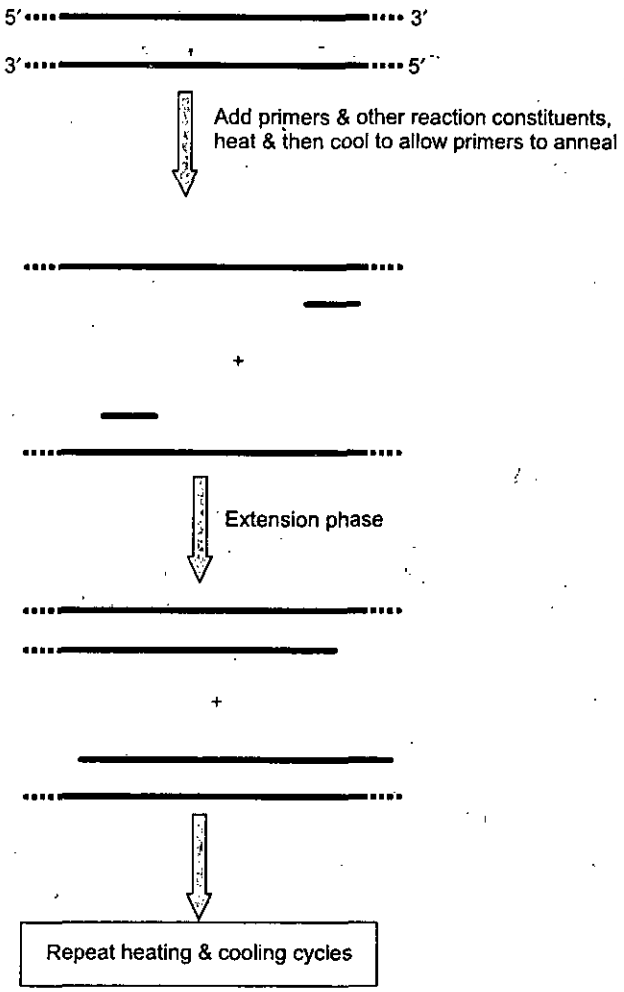

Fig. 5.15 The PCR is initiated by separation of the double-stranded DNA into its two constituent strands

SUMMARY

- Terms such as 'molecular biology', 'genetic engineering' and 'recombinant DNA (rDNA) technology' are sometimes used interchangeably and often mean slightly different things to different people.
- Nucleic acids represent a prominent category of biomolecule present in living cells. The term incorporates both DNA and RNA. DNA represents the repository of genetic information (the genome) of most life forms. RNA replaces DNA as the repository of genetic information in some viruses.
- RNA is a single-stranded polynucleotide, although RNA molecules tend to adopt higher order three-dimensional shapes. DNA, on the other hand, is a double-stranded molecule that assumes a double helical structure.
- The genome refers to the entire hereditary information present in an organism.
- Sequencing plays a central role in rDNA cloning experiments, as well as in determining genome data.

- An essential feature of the cloning vector used is that it must be capable of self-replication in the cell into which it is introduced, which is usually *E. coli*. Two of the most commonly used types of vector in conjunction with *E. coli* are plasmids and bacteriophage λ.

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REVIEW QUESTIONS

1. Describe the structure and functions of nucleic acids.
2. Discuss the concept of genome.
3. Describe the methodologies used for purification of nucleic acid.
4. Discuss the role of sequencing in rDNA cloning.
5. Explain the concept of cDNA cloning.

UNIT VI: THE DRUG DEVELOPMENT PROCESS

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★ STRUCTURE ★

6.1 LEARNING OBJECTIVES

After going through this unit, you will be able to:

- describe the discovery of biopharmaceuticals
- discuss the impact of genomics and related technologies upon drug discovery
- discuss about gene chips and proteomics
- define pharmacogenetics
- discuss about novel drug delivery systems and novel biological agents

6.2 INTRODUCTION

In this unit, you will come to know about the discovery of biopharmaceuticals, impact of genomics and related technologies upon drug discovery, concept of structural genomics, importance of pharmacogenetics, pharmacokinetics and pharmacodynamics, delivery systems of drugs and carrier of drug delivery.

6.3 DISCOVERY OF BIOPHARMACEUTICALS

The discovery of virtually all the biopharmaceuticals discussed in this text was a knowledgebased one. Continuing advances in the molecular sciences have deepened our understanding of the molecular mechanisms that underline health and disease. An understanding at the molecular level of how the body functions in health and of the deviations that characterize the development of a disease often renders obvious potential strategies likely to cure/control that disease. Simple

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examples illustrating this include the use of insulin to treat diabetes and the use of GH to treat certain forms of dwarfism. The underlining causes of these types of disease are relatively straightforward, in that they are essentially promoted by the deficiency/absence of a single regulatory molecule. Other diseases, however, may be multifactorial and, hence, more complex. Examples include cancer and inflammation. Nevertheless, cytokines, such as interferons and interleukins, known to stimulate the immune response/regulate inflammation, have proven to be therapeutically useful in treating several such complex diseases.

An understanding, at the molecular level, of the actions of various regulatory proteins, or the progression of a specific disease does not, however, automatically translate into pinpointing an effective treatment strategy. The physiological responses induced by the potential biopharmaceutical *in vitro* (or in animal models) may not accurately predict the physiological responses seen when the product is administered to a diseased human. For example, many of the most promising biopharmaceutical therapeutic agents (*e.g.*, virtually all the cytokines), display multiple activities on different cell populations. This makes it difficult, if not impossible, to predict what the overall effect administration of any biopharmaceutical will have on the whole body, hence the requirement for clinical trials.

In other cases, the widespread application of a biopharmaceutical may be hindered by the occurrence of relatively toxic side effects (as is the case with tumour necrosis factor α (TNF-α). Finally, some biomolecules have been discovered and purified because of a characteristic biological activity that, subsequently, was found not to be the molecule's primary biological activity. TNF-α again serves as an example. It was first noted because of its cytotoxic effects on some cancer cell types *in vitro*. Subsequently, trials assessing its therapeutic application in cancer proved disappointing due not only to its toxic side effects, but also to its moderate, at best, cytotoxic effect on many cancer cell types *in vivo*. TNF's major biological activity *in vivo* is now known to be as a regulator of the inflammatory response. In summary, the 'discovery' of biopharmaceuticals, in most cases, merely relates to the logical application of our rapidly increasing knowledge of the biochemical basis of how the body functions. These substances could be accurately described as being the body's own pharmaceuticals. Moreover, rapidly expanding areas of research, such as genomics and proteomics, will likely hasten the discovery of many more such products, as discussed below.

The Impact of Genomics and Related Technologies upon Drug Discovery

The term 'genomics' refers to the systematic study of the entire genome of an organism. Its core aim is to sequence the entire DNA complement of the cell and to map the genome arrangement physically (assign exact positions in the genome to the various genes/non-coding regions). Prior to the 1990s, the sequencing and study of a single gene represented a significant task. However, improvements in sequencing technologies and the development of more highly automated hardware systems now render DNA sequencing considerably faster, cheaper and more accurate. Modern sequencing systems can sequence thousands of bases per hour. Such innovations underpin the 'high-throughput' sequencing necessary to evaluate an entire genome sequence within a reasonable time-frame. By early 2006 some 364 genome projects had been completed (297 bacterial, 26 Archaeal and 41 Eucaryal, including the human genome) with in excess of 1000 genome sequencing projects

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ongoing. From a drug discovery/development prospective, the significance of genome data is that they provide full sequence information of every protein the organism can produce. This should result in the identification of previously undiscovered proteins that will have potential therapeutic application, *i.e.*, the process should help identify new potential biopharmaceuticals. The greatest pharmaceutical impact of sequence data, however, will almost certainly be the identification of numerous additional drug targets. It has been estimated that all drugs currently on the market target one (or more) of a maximum of 500 targets. The majority of such targets are proteins (mainly enzymes, hormones, ion channels and nuclear receptors). Hidden in the human genome sequence data is believed to be anywhere between 3000 and 10 000 new protein-based drug targets. Additionally, present in the sequence data of many human pathogens is sequence data of hundreds, perhaps thousands, of pathogen proteins that could serve as drug targets against those pathogens (*e.g.*, gene products essential for pathogen viability or infectivity).

While genome sequence data undoubtedly harbours new drug leads/drug targets, the problem now has become one of specifically identifying such genes. Impeding this process is the fact that the biological function of many sequenced gene products remains unknown. The focus of genome research, therefore, is now shifting towards elucidating the biological function of these gene products, *i.e.*, shifting towards 'functional genomics'. Assessment of function is critical to understanding the relationship between genotype and phenotype and, of course, for the direct identification of drug leads/targets. The term 'function' traditionally has been interpreted in the narrow sense of what isolated biological role/activity the gene product displays (*e.g.*, is it an enzyme and, if so, what specific reaction does it catalyse). In the context of genomics, gene function is assigned a broader meaning, incorporating not only the isolated biological function/activity of the gene product, but also relating to:

- where in the cell that product acts and, in particular, what other cellular elements does it influence/interact with;
- how do such influences/interactions contribute to the overall physiology of the organism.

The assignment of function to the products of sequenced genes can be pursued via various approaches, including:

- sequence homology studies;
- phylogenetic profiling;
- Rosetta stone method;
- gene neighbourhood method;
- knockout animal studies;
- DNA array technology (gene chips);
- proteomics approach;
- structural genomics approach.

With the exception of knockout animals, these approaches employ, in part at least, sequence structure/data interrogation/comparison. The availability of appropriate highly powerful computer programs renders these approaches 'high throughput'. However, even by applying these methodologies, it will not prove possible to identify immediately the function of all gene products sequenced.

Sequence homology studies depend upon computer-based (bioinformatic) sequence comparison between a gene of unknown function (or, more accurately, of unknown gene product function) and genes whose product has previously been assigned a

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function. High homology suggests likely related functional attributes. Sequence homology studies can assist in assigning a putative function to 40-60 per cent of all new gene sequences.

Phylogenetic profiling entails establishing a pattern of the presence or absence of the particular gene coding for a protein of unknown function across a range of different organisms whose genomes have been sequenced. If it displays an identical presence/absence pattern to an already characterized gene, then in many instances it can be inferred that both gene products have a related function.

The Rosetta stone approach is dependent upon the observation that sometimes two separate polypeptides (*i.e.*, gene products X and Y) found in one organism occur in a different organism as a single fused protein XY. In such circumstances, the two protein parts (domains), X and Y, often display linked functions. Therefore, if gene X is recently discovered in a newly sequenced genome and is of unknown function but gene XY of known function has been previously discovered in a different genome, then the function of the unknown X can be deduced.

The gene neighbourhood method is yet another computation-based method. It depends upon the observation that two genes are likely to be functionally linked if they are consistently found side by side in the genome of several different organisms. Knockout animal studies, in contrast to the above methods, are dependent upon phenotype observation. The approach entails the generation and study of mice in which a specific gene has been deleted. Phenotypic studies can sometimes yield clues as to the function of the gene knocked out.

Gene Chips

Although sequence data provide a profile of all the genes present in a genome, they give no information as to which genes are switched on (transcribed) and, hence, which are functionally active at any given time/under any given circumstances. Gene transcription results in the production of RNA, either mRNA (usually subsequently translated into a polypeptide) or rRNA or tRNA (which have catalytic or structural functions; Unit 3). The study of under which circumstances an RNA species is expressed/not expressed in the cell/organism can provide clues as to the biological function of the RNA (or, in the case of mRNA, the function of the final polypeptide product). Furthermore, in the context of drug lead/target discovery, the conditions under which a specific mRNA is produced can also point to putative biopharmaceuticals/drug targets. For example, if a particular mRNA is only produced by a cancer cell, that mRNA (or, more commonly, its polypeptide product) may represent a good target for a novel anti-cancer drug. Levels of RNA (usually specific mRNAs) in a cell can be measured by well-established techniques such as northern blot analysis or by PCR analysis. However, the recent advent of DNA microarray technology has converted the identification and measurement of specific mRNAs (or other RNAs if required) into a 'high-throughput' process. DNA arrays are also termed oligonucleotide arrays, gene chip arrays or, simply, chips.

The technique is based upon the ability to anchor nucleic acid sequences (usually DNA based) on plastic/glass surfaces at very high density. Standard gridding robots can put on up to 250 000 different short oligonucleotide probes or 10 000 full-length cDNA sequences per square centimeter of surface. Probe sequences are generally produced/designed from genome sequence data; hence, chip production is often

referred to as 'downloading the genome on a chip'. RNA can be extracted from a cell and probed with the chip. Any complementary RNA sequences present will hybridize with the appropriate immobilized chip sequence (Fig. 6.1). Hybridization is detectable as the RNA species are first labelled. Hybridization patterns obviously yield critical information regarding gene expression.

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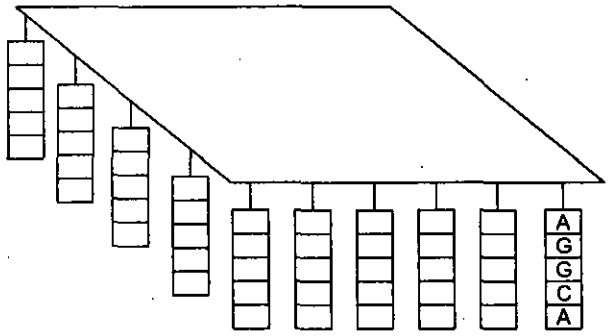

Fig. 6.1 Generalized outline of a gene chip

Proteomics

Although virtually all drug targets are protein based, the inference that protein expression levels can be accurately (if indirectly) detected/measured via DNA array technology is a false one, as: mRNA concentrations do not always directly correlate with the concentration of the mRNA encoded polypeptide; a significant proportion of eukaryote mRNAs undergo differential splicing and, therefore, can yield more than one polypeptide product (Fig. 6.2).

Additionally, the cellular location at which the resultant polypeptide will function often cannot be predicted from RNA delection/sequences nor can detailed information regarding how the polypeptide product's functional activity will be regulated (*e.g.*, via post-translational mechanisms such as phosphorylation, partial proteolysis, etc.). Therefore, protein-based drug leads/targets are often more successfully identified by direct examination of the expressed protein complement of the cell, *i.e.*, its proteome. Like the transcriptome (total cellular RNA content), and in contrast to the genome, the proteome is not static, with changes in cellular conditions triggering changes in cellular protein profiles/concentrations. This field of study is termed proteomics.

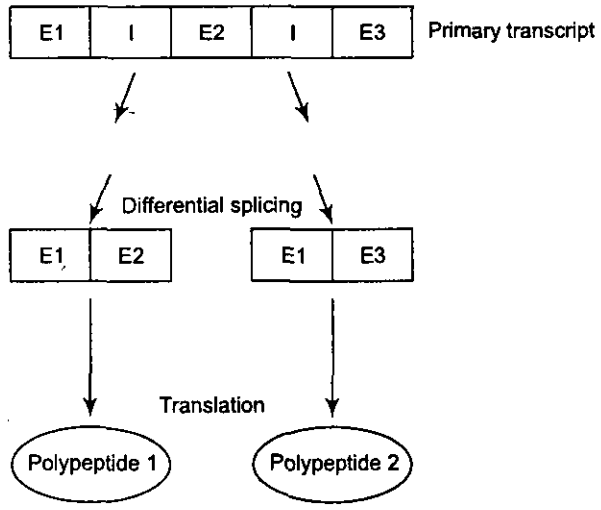

Fig. 6.2 Differential splicing of mRNA can yield different polypeptide products

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Proteomics, therefore, is closely aligned to functional genomics and entails the systematic and comprehensive analysis of the proteins expressed in the cell and their function. Classical proteomic studies generally entailed initial extraction of the total protein content from the target cell/tissue, followed by separation of the proteins therein using two-dimensional electrophoresis.

Isolated protein 'spots' could then be eluted from the electrophoretic gel and subjected to further analysis; mainly to Edman degradation, in order to generate partial amino acid sequence data. The sequence data could then be used to interrogate protein sequence databanks in order to, for example, assign putative function by sequence homology searches (Fig. 6.3). Two-dimensional electrophoresis, however, is generally capable of resolving no more than 2000 different proteins, and proteins expressed at low levels may not be detected at all if their gel concentration is below the (protein) staining threshold. The latter point can be particularly significant in the context of drug/target identification, as most such targets are likely to be kinases and other regulatory proteins that are generally expressed within cells at very low levels. More recently, high-resolution chromatographic techniques (particularly reverse-phase and ion exchanged-based high-performance liquid chromatography (HPLC)) have been applied in the separation of proteome proteins and high-resolution mass spectrometry is being employed to aid high-throughput sequence determination.

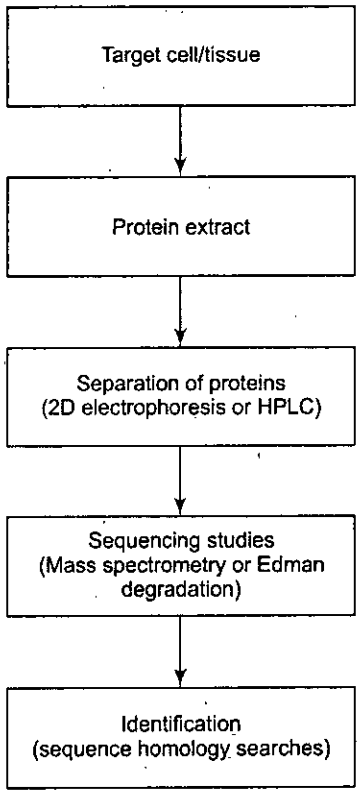

Fig. 6.3 The proteomics approach

Structural genomics

Related to the discipline of proteomics is that of structural genomics. The latter focuses upon the large-scale systematic study of gene product structure. While this embraces rRNA and tRNA, in practice the field focuses upon protein structure.

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The basic approach to structural genomics entails the cloning and recombinant expression of cellular proteins, followed by their purification and three-dimensional structural analysis. High-resolution determination of a protein's structure is amongst the most challenging of molecular investigations. By the year 2000, protein structure databanks housed in the region of 12 000 entries. However, such databanks are highly redundant, often containing multiple entries describing variants of the same molecule. For example, in excess of 50 different structures of 'insulin' have been deposited (*e.g.*, both native and mutated/engineered forms from various species, as well as insulins in various polymeric forms and in the presence of various stabilizers and other chemicals). In reality, by the year 2000, the three-dimensional structure of approximately 2000 truly different proteins had been resolved.

Until quite recently, X-ray crystallography was the technique used almost exclusively to resolve the three-dimensional structure of proteins. As well as itself being technically challenging, a major limitation of X-ray crystallography is the requirement for the target protein to be in crystalline form. It has thus far proven difficult/impossible to induce the majority of proteins to crystallize. NMR is an analytical technique that can also be used to determine the three-dimensional structure of a molecule, and without the necessity for crystallization. For many years, even the most powerful NMR machines could resolve the three-dimensional structure of only relatively small proteins (less than 20-25 kDa). However, recent analytical advances now render it possible to analyse much larger proteins by this technique successfully. The ultimate goal of structural genomics is to provide a complete three-dimensional description of any gene product. Also, as the structures of more and more proteins of known function are elucidated, it should become increasingly possible to link specific functional attributes to specific structural attributes. As such, it may prove ultimately feasible to predict protein function if its structure is known, and vice versa.

6.4 DNA CLONING: PHARMACOGENETICS

Pharmacogenetics relates to the emerging discipline of correlating specific gene DNA sequence information (specifically sequence variations) to drug response. As such, the pursuit will ultimately impinge directly upon the drug development process and should allow doctors to make better-informed decisions regarding what exact drug to prescribe to individual patients. Different people respond differently to any given drug, even if they present with essentially identical disease symptoms. Optimum dose requirements, for example, can vary significantly. Furthermore, not all patients respond positively to a specific drug (*e.g.*, IFN-â is of clinical benefit to only one in three multiple sclerosis patients). The range and severity of adverse effects induced by a drug can also vary significantly within a patient population base. While the basis of such differential responses can sometimes be non-genetic (*e.g.*, general state of health, etc.), genetic variation amongst individuals remains the predominant factor. Although all humans display almost identical genome sequences, some differences are evident. The most prominent widespread-type variations amongst individuals are known as single nucleotide polymorphisms (SNPs, sometimes pronounced 'snips'). SNPs occur in the general population at an average incidence of 1 in every 1000 nucleotide bases; hence, the entire human genome harbours 3 million or so. SNPs are not mutations; the latter arise more infrequently, are more diverse and are generally caused by spontaneous/mutagen-induced mistakes in DNA repair/replication.

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SNPs occurring in structural genes/gene regulatory sequences can alter amino acid sequence/expression levels of a protein and, hence, affect its functional attributes. SNPs largely account for natural physical variations evident in the human population (*e.g.*, height, colour of eyes, etc.).

The presence of an SNP within the regulatory or structural regions of a gene coding for a protein that interacts with a drug could obviously influence the effect of the drug on the body. In this context, the protein product could, for example, be the drug target or perhaps an enzyme involved in metabolizing the drug.

The identification and characterization of SNPs within the human genomes is, therefore, of both academic and applied interest. Several research groups continue to map human SNPs, and over 1.5 million have thus far been identified.

By identifying and comparing SNP patterns from a group of patients responsive to a particular drug with patterns displayed by a group of unresponsive patients, it may be possible to identify specific SNP characteristics linked to drug efficacy. In the same way, SNP patterns/characteristics associated with adverse reactions (or even a predisposition to a disease) may be uncovered. This could usher a new era of drug therapy where drug treatment could be tailored to the individual patient. Furthermore, different drugs could be developed with the foreknowledge that each would be efficacious when administered to specific (SNP-determined) patient sub-types. A (distant) futuristic scenario could be visualized where all individuals could carry chips encoded with SNP details relating to their specific genome, allowing medical staff to choose the most appropriate drugs to prescribe in any given circumstance.

Linking specific genetic determinants to many diseases, however, is unlikely to be as straightforward as implied thus far. The progress of most diseases, and the relative effectiveness of allied drug treatment, is dependent upon many factors, including the interplay of multiple gene products. 'Environmental' factors such as patient age, sex and general health also play a prominent role.

The term 'pharmacogenomics' is one that has entered the 'genomic' vocabulary. Although sometimes used almost interchangeably with pharmacogenetics, it more specifically refers to studying the pattern of expression of gene products involved in a drug response.

Initial Product Characterization

The physicochemical and other properties of any newly identified drug must be extensively characterized prior to its entry into clinical trials. As the vast bulk of biopharmaceuticals are proteins, a summary overview of the approach taken to initial characterization of these biomolecules is presented. A prerequisite to such characterization is initial purification of the protein. Purification to homogeneity usually requires a combination of three or more high-resolution chromatographic steps. The purification protocol is designed carefully, as it usually forms the basis of subsequent pilot- and process-scale purification systems. The purified product is then subjected to a battery of tests that aim to characterize it fully. Moreover, once these characteristics have been defined, they form the basis of many of the QC identity tests routinely performed on the product during its subsequent commercial manufacture. As these identity tests are discussed, only an abbreviated overview is presented here, in the form of Fig. 6.4.

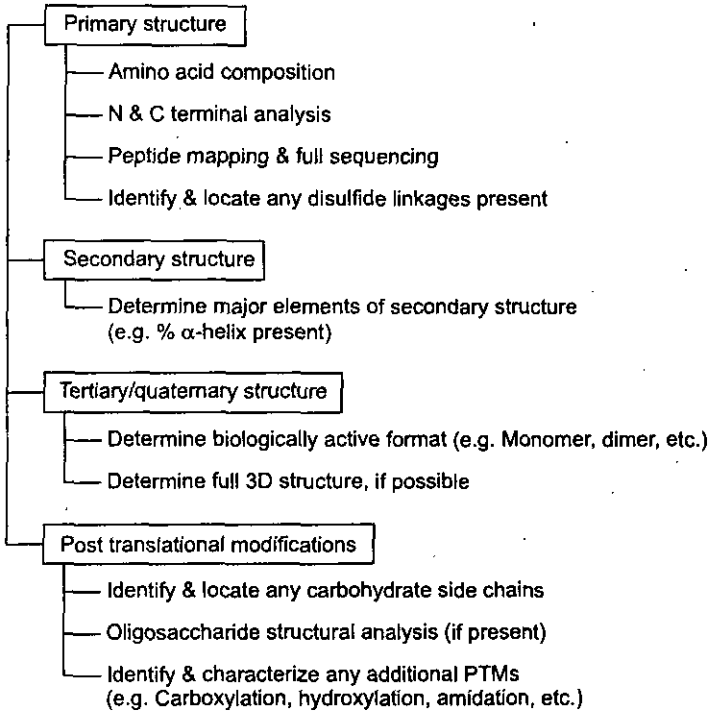

Fig. 6.4 Task tree for the structural characterization of a therapeutic protein

In addition to the studies listed in Fig. 6.4, stability characteristics of the protein with regard to *e.g.*, temperature, pH and incubation with various potential excipients are studied. Such information is required in order to identify a suitable final product formulation, and to give an early indication of the likely useful shelf-life of the product.

Patenting

The discovery and initial characterization of any substance of potential pharmaceutical application is followed by its patenting. The more detail given relevant to the drug's physicochemical characteristics, a method of synthesis and its biological effects, the better the chances of successfully securing a patent. Thus, patenting may not take place until preclinical trials and phase I clinical trials are completed. Patenting, once successfully completed, does not grant the patent holder an automatic right to utilize/sell the patented product; first, it must be proven safe and effective in subsequent clinical trials, and then be approved for general medical use by the relevant regulatoryauthorities.

Delivery of Biopharmaceuticals

An important issue that must be addressed during the preclinical phase of the drug development process relates to the route by which the drug will be delivered/administered. To date, the vast majority of biopharmaceuticals approved for general medical use are administered by direct injection (*i.e.*, parenterally) usually by intravenous (i.v.), subcutaneous (s.c., *i.e.*, directly under the skin) or intramuscular (i.m., *i.e.*, into muscle tissue) routes. Administration via the s.c. or i.m. route is generally followed by slow release of the drug from its depot site into the bloodstream. Amongst the few exceptions to this parenteral route are the enzyme DNase, used to treat cystic fibrosis, and platelet-derived growth factor (PDGF), used to treat certain skin ulcers. However, neither of these products is required to reach the bloodstream in order to achieve its therapeutic effect. In fact, in each case the delivery system delivers the biopharmaceutical directly to its site of action (DNase is delivered directly to

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the lungs via aerosol inhalation, and PDGF is applied topically, *i.e.*, directly on the ulcer surface, as a gel). Parenteral administration is not perceived as a problem in the context of drugs which are administered infrequently, or as a once-off dose to a patient. However, in the case of products administered frequently/daily (*e.g.*, insulin to diabetics), non-parenteral delivery routes would be preferred. Such routes would be more convenient, less invasive, less painful and generally would achieve better patient compliance. Alternative potential delivery routes include oral, nasal, transmucosal, transdermal or pulmonary routes. Although such routes have proven possible in the context of many drugs, routine administration of biopharmaceuticals by such means has proven to be technically challenging. Obstacles encountered include their high molecular mass, their susceptibility to enzymatic inactivation and their potential to aggregate.

Preclinical Studies

In order to gain approval for general medical use, the quality, safety and efficacy of any product must be demonstrated. Demonstration of conformance to these requirements, particularly safety and efficacy, is largely attained by undertaking clinical trials. However, preliminary data, especially safety data, must be obtained prior to the drug's administration to human volunteers. Regulatory authority approval to commence clinical trials is based largely upon preclinical pharmacological and toxicological assessment of the potential new drug in animals. Such preclinical studies can take up to 3 years to complete, and at a cost of anywhere between US$10 million and US$30 million. On average, approximately 10 per cent of potential new drugs survive preclinical trials.

In many instances, there is no strict set of rules governing the range of tests that must be undertaken during preclinical studies. However, guidelines are usually provided by regulatory authorities. The range of studies generally undertaken with regard to traditional chemical-based pharmaceuticals is summarized in Table 6.1. Most of these tests are equally applicable to biopharmaceutical products.

Pharmacokinetics and Pharmacodynamics

Pharmacology may be described as the study of the properties of drugs and how they interact with/affect the body. Within this broad discipline exist (somewhat artificial) subdisciplines, including pharmacokinetics and pharmacodynamics. Pharmacokinetics relates to the fate of a drug in the body, particularly its ADME, *i.e.*, its absorption into the body, its distribution within the body, its metabolism by the body, and its excretion from the body.

Table 6.1 The range of major tests undertaken on a potential new drug during preclinical trials

Pharmacokinetic profile
Pharmacodynamic profile
Bioequivalence and bioavailability
Acute toxicity
Chronic toxicity
Reproductive toxicity and teratogenicity
Mutagenicity
Carcinogenicity
Immunotoxicity
Local tolerance

The results of such studies not only help to identify any toxic effects, but also point to the most appropriate method of drug administration, as well as the most likely effective dosage regime to employ. Generally, ADME studies are undertaken in two species, usually rats and dogs, and studies are repeated at various different dosage levels. All studies are undertaken in both males and females. If initial clinical trials reveal differences in human versus animal model pharmacokinetic profiles, additional pharmacokinetic studies may be necessary using primates. Pharmacodynamic studies deal more specifically with how the drug brings about its characteristic effects. Emphasis in such studies is often placed upon how a drug interacts with a cell/organ type, the effects and side effects it induces, and observed dose-response curves.

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Bioavailability and bioequivalence are also usually assessed in animals. Such studies are undertaken as part of pharmacokinetic and/or pharmacodynamic studies. Bioavailability relates to the proportion of a drug that actually reaches its site of action after administration. As most biopharmaceuticals are delivered parenterally (*e.g.*, by injection), their bioavailability is virtually 100 per cent. On the other hand, administration of biopharmaceuticals by mouth would, in most instances, yield a bioavailability at or near 0 per cent. Bioavailability studies would be rendered more complex if, for example, a therapeutic peptide was being administered intranasally. Bioequivalence studies come into play if any change in product production/delivery systems was being contemplated. These studies would seek to identify whether such modifications still yield a product equivalent to the original one in terms of safety and efficacy. Modifications could include an altered formulation or method of administration, dosage regimes, etc.

6.5 NOVEL DRUG DELIVERY SYSTEMS

The method by which a drug is delivered can have a significant effect on its efficacy. Some drugs have an optimum concentration range within which maximum benefit is derived, and concentrations above or below this range can be toxic or produce no therapeutic benefit at all. To minimize drug degradation and loss, to prevent harmful side-effects and to increase drug bioavailability and the fraction of the drug accumulated in the required zone, various drug delivery and drug targeting systems are currently under development. Among drug carriers one can name soluble polymers, micro particles made of insoluble or biodegradable natural and synthetic polymers, Microchip, microcapsules, cells, cell ghosts, lipoproteins, liposome's, and micelles. The carriers can be made slowly degradable, stimuli-reactive (*e.g.*, pHor temperature-sensitive), and even targeted (*e.g.*, by conjugating them with specific antibodies against certain characteristic components of the area of interest). Targeting is the ability to direct the drug— Two major mechanisms can be distinguished for addressing the desired sites for drug release: (*i*) passive and (*ii*) active targeting Controlled drug release and subsequent biodegradation are important for developing successful formulations. Potential release mechanisms involve: (*i*) desorption of surface-bound /adsorbed drugs; (*ii*) diffusion through the carrier matrix; (*iii*) diffusion (in the case of nanocapsules) Through the carrier wall; (*iv*) carrier matrix erosion; and (*v*) a combined erosion /diffusion process. The mode of delivery can be the difference between a drug's success and failure, as the choice of a drug is often influenced by the way the medicine is administered. Sustained (or continuous) release of a drug involves polymers that release the drug at a controlled rate due to diffusion out of the polymer or by degradation of the polymer over time. Pulsatile release is often

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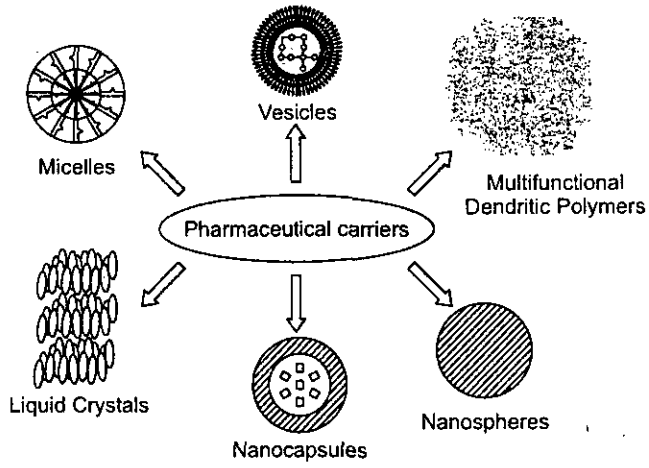
the preferred method of drug delivery, as it closely mimics the way by which the body naturally produces hormones such as insulin.



Drug Delivery Systems

The global market for advanced drug delivery systems was more than €37.9 billion in 2000 and is estimated to grow and reach €75B by 2005 (*i.e.*, controlled release €19.8B, needleless injection €0.8B, injectable/implantable polymer systems €5.4B, transdermal €9.6B, transnasal €12.0B, pulmonary €17.0B, transmucosal €4.9B, rectal €0.9B, liposomal drug delivery €2.5B, cell/gene therapy €3.8B, miscellaneous €1.9B). Developments within this market are continuing at a rapid pace, especially in the area of alternatives to injected macromolecules, as drug formulations seek to cash in on the €6.2B worldwide market for genetically engineered protein and peptide drugs and other biological therapeutics.

Drug Delivery Carriers

Colloidal drug carrier systems such as micellar solutions, vesicle and liquid crystal dispersions, as well as nanoparticle dispersions consisting of small particles of 10-400 nm diameter show great promise as drug delivery systems. When developing these formulations, the goal is to obtain systems with optimized drug loading and release properties, long shelf-life and low toxicity. The incorporated drug participates in the microstructure of the system, and may even influence it due to molecular interactions, especially if the drug possesses amphiphilic and/or mesogenic properties.

Pharmaceutical Carriers

Micelles formed by self-assembly of amphiphilic block copolymers (5-50 nm) in aqueous solutions are of great interest for drug delivery applications. The drugs can be physically entrapped in the core of block copolymer micelles and transported at concentrations that can exceed their intrinsic water-solubility. Moreover, the hydrophilic blocks can form hydrogen bonds with the aqueous surroundings and form a tight shell around the micellar core. As a result, the contents of the hydrophobic core are effectively protected against hydrolysis and enzymatic degradation. In addition, the corona may prevent recognition by the reticuloendothelial system and therefore preliminary elimination of the micelles from the bloodstream. A final feature that makes amphiphilic block copolymers attractive for drug delivery applications is the fact that their chemical composition, total molecular weight and block length ratios can be easily changed, which allows control of the size and

morphology of the micelles. Functionalization of block copolymers with crosslinkable groups can increase the stability of the corresponding micelles and improve their temporal control. Substitution of block copolymer micelles with specific ligands is a very promising strategy to a broader range of sites of activity with a much higher selectivity.

Liposomes are a form of vesicles that consist either of many, few or just one phospholipid bilayers. The polar character of the liposomal core enables polar drug molecules to be encapsulated. Amphiphilic and lipophilic molecules are solubilized within the phospholipid bilayer according to their affinity towards the phospholipids. Participation of nonionic surfactants instead of phospholipids in the bilayer formation results in niosomes. Channel proteins can be incorporated without loss of their activity within the hydrophobic domain of vesicle membranes, acting as a size-selective filter, only allowing passive diffusion of small solutes such as ions, nutrients and antibiotics. Thus, drugs that are encapsulated in a nanocagefunctionalized with channel proteins are effectively protected from premature degradation by proteolytic enzymes. The drug molecule, however, is able to diffuse through the channel, driven by the concentration difference between the interior and the exterior of the nanocage.

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Administration Routes

The choice of a delivery route is driven by patient acceptability, the properties of the drug (such as its solubility), access to a disease location, or effectiveness in dealing with the specific disease. The most important drug delivery route is the peroral route. An increasing number of drugs are protein- and peptide-based. They offer the greatest potential for more effective therapeutics, but they do not easily cross mucosal surfaces and biological membranes; they are easily denatured or degraded, prone to rapid clearance in the liver and other body tissues and require precise dosing. At present, protein drugs are usually administered by injection.

6.6 NOVEL DRUG SCREENING STRATEGIC

High-throughput screening (HTS) has gone through a series of significant changes over the last two decades, with many companies now describing the journey they have taken. It has evolved from an ad hoc set of lightly connected instruments and teams, producing a somewhat unpredictable end product, into a highly integrated, automated process capable of delivering a sustained, high-quality output (5). Not only has HTS managed to deliver on its core promise as a reliable platform for producing lead compounds, it has also been able to expand into academic research institutes, as scientists there seek specific compounds to probe disease models. In several companies, the technology platforms underpinning HTS have also been exported into lead optimization and drug safety teams, again showing the flexibility and maturity of the approach.

Of course, it has not all been smooth sailing, and the initial hype around HTS and its ability to transform R&D productivity has ultimately proven to be a significant hindrance in assessing where HTS can really be impactful. HTS alone was never going to be the answer to what ailed pharma companies in the late 1990s or even today. What it always had the potential to do was to provide a fast, reliable, high-capacity method for finding lead compounds and helping to profile and optimize

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them. Those companies that focused on delivering that type of service from their HTS platforms have probably been more successful and satisfied than those that hoped HTS would be the bedrock for generating more drugs into their late-stage pipelines. The fact that the sister technologies to HTS—genomics, proteomics, and combinatorial chemistry—also largely failed to live up to their early promise, left some observers with a somewhat jaundiced perspective on the drug discovery revolution that was expected, but seemingly failed to materialize.

However, the stuttering performance of most pharmaceutical companies and their R&D engines, over the last decade, cannot be placed solely at the door of discovery organizations; regulatory tightening, pricing control, access, IP, and generics have all played their part in changing the landscape for pharmaceutical companies, making it even more difficult for them to be successful. However, it cannot be denied that R&D productivity has significantly declined over a period of time when investment in R&D has been at a historical high. The answer to why that should be is no doubt complex but several scenarios that most thought would occur over the last 10 years or so have not panned out as expected.

The prediction that the human genome project would greatly improve our understanding of human disease and unleash a tsunami of targets amenable for drug intervention has not yet come to pass. Human biology and our understanding of disease mechanisms are just as complex and difficult today as they were 20 years ago. We may have more technologies and techniques for probing and trying to understand disease pathways of interest, but our ability to fully predict successful outcomes through drug intervention are still highly limited. In fact, the genes of interest that have come out of the genome project are so novel that one could argue that they have added to the burden of optimizing and developing drugs. More resources are needed to develop a deep understanding of the biology underpinning these targets compared to the fast follow on approaches seen with well-validated mechanisms. Although, novel gene targets offer the chance for break through medicines and the opportunity to be first in class, they come with a very high risk of failure. We have also not significantly improved our ability to predict whether a particular drug and molecular target will be effective in the clinic. Using animal disease models as a surrogate for human disease has been an important staple of the drug discovery process over many years. The drive to show correlation between animal data and human clinical data has had some success but not enough to allow you to buy down the risk of a late-stage clinical failure. Predictive biomarkers of efficacy have fared no better outside the well-published impact of biomarkers in clinical oncology; for example, HER2/neu and Herceptin. When you add in the initial failure of combinatorial chemistry to deliver the huge increase in high-quality small molecules, one can begin to see why the R&D new world order has not yet arrived. Nevertheless, this technology did evolve by using parallel array methodologies to start to deliver very useful focused libraries.

So, does HTS deserve to be added to this list of technologies that did not deliver? In reality, for some companies, HTS has proven to be a great success and in others it has been an abject failure (8). So, why do we see such different outcomes for a process and technology platform that is largely similar in most companies? That is the big question and no doubt the answer will not be a simple equation or solution, but I think there are a few good pointers to show the path to success. I believe there are several major factors or observations that can determine the ultimate success or failure of any HTS operation.

6.7 KEEP CUSTOMER FOCUSED AND DON'T PROMISE WHAT YOU CAN'T DELIVER

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One of the fundamental errors any HTS organization can make is to not know or understand who its customers are. This may seem obvious but there are several examples in the industry of HTS and/or technology support teams who have built enterprises that do not deliver what is actually needed by their discovery organizations. An essential step in preventing this is to ensure that HTS goals are completely aligned with the goals of the therapeutic area project teams they are supporting. This can include short-term goals such as the number of targets, timelines, and level of support needed by the discovery projects during any particular year, as well as longer term goals such as ensuring the compound deck has a deep supply of diverse structures against targets and target classes that are of current and future interest to therapeutic area teams. It is also very important to understand, up front, the major milestones for the project being supported.

Those projects that are in backup mode can have a very different set of priorities and expectations than a program just starting out. Making sure that high priority targets are screened with speed and quality almost always aligns with the goals of the therapeutic area customer.

Standardize, Integrate, and Eliminate Waste

Cost-disciplined science has become a major reality for most HTS organizations over the last few years. As corporate compound collections have continued to increase along side the demand for screening, the cost burden of running a large HTS infrastructure has grown significantly. By aggressive implementation of automation, miniaturized screening formats, and waste management processes, several HTS groups have been able to increase their overall productivity while keeping their costs flat. Automation of the HTS process has also allowed the fulltime employees (FTE) burden to be reduced considerably compared to 10 years ago. Modular functionality, parallel processes, and standard user interfaces along side the general standardization of work flows have greatly increased the flexibility of HTS. Once this type of flexible, standardized functionality has been put in place, the ability to offer customized services is greatly increased and can be done in a nondisruptive, cost-managed way. A fully integrated work flow from lead discovery through profiling and optimization is the best way to ensure success. Ensuring that work streams and capacity flows are matched in the lead discovery phase is a really important factor for integration and streamlined operations. Keeping HTS capacity aligned with the growth in the compound deck, or vice versa, is a basic example of this impedance matching and integration. However, global scalability and seamless integration of a process do not naturally go hand in hand and can be incredibly difficult, if not impossible to achieve. In this type of scenario, it is critical to have strongly, aligned leadership around the accountability and role of the HTS function.

For those large global companies that have tried to centralize and standardize their HTS operations, they have hit problems of scalability and lack of integration. In these situations, trying to deliver a rapid, high-quality service that fits the needs of every therapeutic area and project team is challenging at best. This has led several large companies to look at how they operate their R&D processes and to find ways of becoming more innovative and flexible. Breaking down large organizations into smaller, more nimble, and entrepreneurial units is one strategy being employed to reduce the burden of keeping large discovery units. Another approach,

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employed at Bristol-Myers Squibb is to use a centralized, fully accountable base organization that is able to standardize all the lead discovery and optimization platforms and have them "exported" to the other sites in a federated fashion. This has the benefit of local therapeutic area proximity and decision making plus global standardization and elimination of duplicated efforts.

Use Technologies That Work: Track Your Impact, Learn, and Evolve

It is always tempting to use the latest and greatest new technologies to try and enhance your HTS capabilities or to solve a problem. However, in the transition from using state-of-the-art technologies to those that are cutting, or bleeding edge, one can run the risk of experiencing technology failures. Not all the technologies developed for HTS over the years have been able to deliver what they promised and not all have provided a clear return on investment. When bringing new technology platforms or services into play, it can be a huge help to have these systems run "off line" for a period of time to see if they actually can deliver what is promised. Don't implement a new service until it can.

Once implemented the only way to know if your HTS operation is truly having an impact is by asking, measuring, and tracking it over time. Comprehensive metrics showing operational efficiency gains and program impact are the most definitive way of finding out whether your discovery and optimization process are delivering. Using these metrics to assess whether a particular piece of technology or process change actually improved outcomes is a very useful learning tool. Being able to drop an ineffective technology platform or process can be a powerful method for improving efficiency and overall impact.

Hire and Develop Cross-Functional Lead Discovery Specialists

A critical factor in the success of several HTS operations has been the presence of cross-disciplined scientists such as biologist, chemists, informatics specialists, discovery technologists, and engineers housed in the same organization. This cross-pollination of backgrounds and ideas creates a real environment for innovation and problem solving. It encourages collaboration with external vendors to come up with technology solutions that work as well as develop specific customization of in-house platforms or processes. This type of embedding of highly specialized, cross-disciplined skill sets into the core lead discovery environment has also allowed the evolution of HTS platforms into different settings in biology, chemistry, and drug safety.

These are only a few observations that this author has found to be critical when trying to achieve success in the lead discovery and optimization process. I think they are key elements that will stand the test of time no matter what new technologies and innovations are introduced over the coming years. HTS approaches will continue to evolve and have impact beyond its home base, throughout the R&D process. This will be especially true if the lessons learned from HTS and the type of lean thinking and culture needed to be successful get exported along with the technology platforms.

6.8 NOVEL BIOLOGICAL AGENTS

The use of novel biological therapies is reserved for cases of advanced prostate cancers, primarily those that are resistant to the proven effects of hormonal therapy,

or patients whose prostate cancer becomes androgen-independent (*i.e.*, hormone refractory prostate cancer).

Approaches under investigation include anti-angiogenic, anti-proliferative, and/or anti-invasive, which target various stages of tumor cell growth and metastasis [3]. Enhancing the immune response to target abnormally growing prostate cells using so-called cancer vaccines is also showing promise.

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- Angiogenesis inhibitors
- Endothelin antagonists
- Cytochrome P-17 inhibitors
- Gene therapy

Angiogenesis Inhibitors

Angiogenesis is a major target for novel agents, as it plays an essential role in the development of tumor vasculature and in metastases. Thalidomide is thought to be active against platelet-derived growth factor and in a phase II study, when used in combination with docetaxel, a trend towards improved progression-free survival and overall survival was seen in hormone-refractory prostate cancer.

Vascular endothelial growth factor (VEGF) is involved in this process and the anti-VEGF, humanized, monoclonal antibody bevacizumab has been shown to cause partial radiographic responses, and to reduce prostate-specific antigen (PSA) levels in patients with advanced prostate cancer. Bevacizumab has been combined with docetaxel in a Phase III CALGB study comparing it with docetaxel alone. The results of this study are expected in the near future.

A number of orally active tyrosine kinase inhibitors *e.g.*, gefitinib, sunitinib + sorafenib are currently being investigated in advanced prostate cancer, with Phase II studies identifying encouraging reductions in PSA levels.

Gene Therapy

Gene therapy is an attractive concept to administering anti-angiogenic therapy. Several approaches have been tested in clinical trials, including:

- Incorporating cancer-fighting genes into immune cells to help them attack cancer cells more forcefully;
- Removing cancer cells from the body, changing their genetic make-up, then returning them to the body where they trigger a strong immune response; and
- Injecting genes to make tumor cells vulnerable to treatment with a specific drug, such as an antibiotic. Subsequent treatment with that drug kills the tumor cells that contain the new gene but should not harm any other cells that do not contain the gene.

PROSTAVAC is a prostate specific antigen expressing poxvirus vaccine, which has been genetically engineered to carry a human PSA gene. The encouraging results from Phase I + II studies, need to be verified in Phase III randomised trials.

Oblimersen, an oligonucleotide, is an anti-sense treatment that is designed to reduce the expression of Bcl-2, a protein expressed in many cancers including prostate cancer. However, an EORTC Phase II study has identified that when given prior to docetaxel, the primary end-points of the study *i.e.*, PSA response of >30% + major toxicevent rate of <45%, were not achieved. Therefore, there are some doubts about the future of this agent.

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SUMMARY

- The term 'genomics' refers to the systematic study of the entire genome of an organism. Its core aim is to sequence the entire DNA complement of the cell and to map the genome arrangement physically.
- The recent advent of DNA microarray technology has converted the identification and measurement of specific mRNAs (or other RNAs if required) into a 'high-throughput' process. DNA arrays are also termed oligonucleotide arrays, gene chip arrays or, simply, chips.
- Pharmacogenetics relates to the emerging discipline of correlating specific gene DNA sequence information to drug response.
- The discovery and initial characterization of any substance of potential pharmaceutical application is followed by its patenting.
- Pharmacology may be described as the study of the properties of drugs and how they interact with/affect the body.
- The method by which a drug is delivered can have a significant effect on its efficacy.
- Liposomes are a form of vesicles that consist either of many, few or just one phospholipid bilayers.
- The use of novel biological therapies is reserved for cases of advanced prostate cancers, primarily those that are resistant to the proven effects of hormonal therapy, or patients whose prostate cancer becomes androgen-independent.
- Angiogenesis is a major target for novel agents, as it plays an essential role in the development of tumor vasculature and in metastases.
- Gene therapy is an attractive concept to administering anti-angiogenic therapy.

REVIEW QUESTIONS

1. Explain the discovery of biopharmaceuticals.
2. Discuss DNA cloning.
3. Discuss new novel biological targets for drug development.
4. Discuss biological agents in detail with their applications.
5. Define gene therapy.

UNIT VII: QUALITY SYSTEMS REGULATION

NOTES

★ STRUCTURE ★

7.1 LEARNING OBJECTIVES

After going through this unit, you will be able to:

- discuss the concept of quality assurance
- describe the characteristics of Quality System Regulation
- understand the requirement of ICH for quality
- discuss about the relationship of ICH Q10
- discuss about the New Drug Application

7.2 INTRODUCTION

In this unit, you will learn about the quality assurance, characteristics of quality system regulation, benefits of implementing ISO 9001, objectives of ICHQ10, fundamental points of GLP, application of new drug and the guidance documents for NDA.

7.3 INTRODUCTION TO QUALITY ASSURANCE

Manufacturers must establish and follow quality systems to help ensure that their products consistently meet applicable requirements and specifications. The quality systems for FDA-regulated products (food, drugs, biologics, and devices) are known as current good manufacturing practices (CGMP's). CGMP requirements for devices in part 820 (21 CFR part 820) were first authorized by section 520(f) of

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the Federal Food, Drug, and Cosmetic Act (the act). Under section 520(f) of the act, FDA issued a final rule in the Federal Register of July 21, 1978 (43 FR 31 508), prescribing CGMP requirements for medical devices. This regulation became effective on December 18, 1978, and was codified under part 820.

In 1990, FDA undertook the start of the revision of the CGMP regulation to add the design controls authorized by the Safe Medical Devices Act. Also, the agency believed that it would be beneficial to the public and the medical device industry for the CGMP regulation to be consistent, to the extent possible, with the requirements for quality systems contained in applicable international standards, primarily, the International Organization for Standards (ISO) 9001:1994 "Quality Systems—Model for Quality Assurance in Design, Development, Production, Installation, and Servicing," and at the time the ISO committee draft (CD) revision of ISO/CD 13485 "Quality Systems-Medical Devices-Supplementary Requirements to ISO 9001." After an extensive effort, the part 820 revision was published on October 7, 1996 (61 FR 52602) and went into effect June 1, 1997. For additional information on the history and international harmonization of the revised regulation, with international standards and the Global Harmonization Task Force (GHTF), see the preamble (pages 52602-52654) to the Quality System regulation (61 FR 52602).

The preamble describes the public comments received during the development of the QS regulation and describes the FDA Commissioner's resolution of the comments. Thus, the preamble contains valuable insight into the meaning and intent of the QS regulation.

7.4 CHARACTERISTICS OF QUALITY SYSTEM REGULATION

Flexibility of the QS Regulation

The QS regulation embraces the same "umbrella" approach to the CGMP regulation that was the underpinning of the original CGMP regulation. Because the regulation must apply to so many different types of devices, the regulation does not prescribe in detail how a manufacturer must produce a specific device. Rather, the regulation provides the framework that all manufacturers must follow by requiring that manufacturers develop and follow procedures and fill in the details that are appropriate to a given device according to the current state-of-the-art manufacturing for that specific device.

Manufacturers should use good judgement when developing their quality system and apply those sections of the QS regulation that are applicable to their specific products and operations, 21 CFR 820.5 of the QS regulation. Operating within this flexibility, it is the responsibility of each manufacturer to establish requirements for each type or family of devices that will result in devices that are safe and effective, and to establish methods and procedures to design, produce, distribute, etc. devices that meet the quality system requirements. The responsibility for meeting these requirements and for having objective evidence of meeting these requirements may not be delegated even though the actual work may be delegated.

FDA has identified in the QS regulation the essential elements that a quality system shall embody, without prescribing specific ways to establish these elements. Because the QS regulation covers a broad spectrum of devices, production processes, etc., it allows some leeway in the details of quality system elements. It is left to

manufacturers to determine the necessity for, or extent of, some quality elements and to develop and implement specific procedures tailored to their particular processes and devices.

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Applicability of the QS Regulation

The QS regulation applies to finished device manufacturers who intend to commercially distribute medical devices. A finished device is defined in 21 CFR 820.3(l) as any device or accessory to any device that is suitable for use or capable of functioning, whether or not it is packaged, labeled, or sterilized.

Certain components such as blood tubing and diagnostic x-ray components are considered by FDA to be finished devices because they are accessories to finished devices. A manufacturer of accessories is subject to the QS regulation.

cGMP Exemptions

FDA has determined that certain types of medical devices are exempt from GMP requirements. These devices are exempted by FDA classification regulations published in the Federal Register and codified in 21 CFR 862 to 892. Exemption from the GMP requirements does not exempt manufacturers of finished devices from keeping complaint files (21 CFR 820.198) or from general requirements concerning records (21 CFR 820.180).

Medical devices manufactured under an investigational device exemption (IDE) are not exempt from design control requirements under 21 CFR 820.30 of the QS regulation.

7.5 ISO 9001 - QUALITY MANAGEMENT STANDARD

ISO 9001 is the internationally recognised standard for the quality management of businesses. It

- applies to the processes that create and control the products and services an organisation supplies
- prescribes systematic control of activities to ensure that the needs and expectations of customers are met
- is designed and intended to apply to virtually any product or service, made by any process anywhere in the world

ISO 9001 is one of the standards in the ISO 9000 family.

The Benefits of Implementing ISO 9001

Implementing a Quality Management System will motivate staff by defining their key roles and responsibilities. Cost savings can be made through improved efficiency and productivity, as product or service deficiencies will be highlighted. From this, improvements can be developed, resulting in less waste, inappropriate or rejected work and fewer complaints. Customers will notice that orders are met consistently, on time and to the correct specification. This can open up the market place to increased opportunities.

Why Seek Certification to ISO 9001?

- Registration to ISO 9001 by an accredited certification body shows committed to quality, customers, and a willingness to work towards improving efficiency.

- It demonstrates the existence of an effective quality management system that satisfies the rigours of an independent, external audit.
- An ISO 9001 certificate enhances company image in the eyes of customers, employees and shareholders alike.

It also gives a competitive edge to an organisation's marketing.

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7.6 ICH REQUIREMENT FOR QUALITY

This sement establishes a new ICH tripartite guideline describing a model for an effective *quality* management system for the pharmaceutical industry, referred to as the *Pharmaceutical Quality System*. Throughout this guideline, the term "pharmaceutical quality system" refers to the ICH Q10 model.

ICH Q10 describes one comprehensive model for an effective pharmaceutical quality system that is based on International Standards Organisation (ISO) quality concepts, includes applicable Good Manufacturing Practice (GMP) regulations and complements ICH Q8 "Pharmaceutical Development" and ICH Q9 "Quality Risk Management". ICH Q10 is a model for a pharmaceutical quality system that can be implemented throughout the different stages of a product lifecycle. Much of the content of ICH Q10 applicable to manufacturing sites is currently specified by regional GMP requirements. ICH Q10 is not intended to create any new expectations beyond current regulatory requirements. Consequently, the content of ICH Q10 that is additional to current regional GMP requirements is optional.

ICH Q10 demonstrates industry and regulatory authorities' support of an effective pharmaceutical quality system to enhance the quality and availability of medicines around the world in the interest of public health. Implementation of ICH Q10 throughout the product lifecycle should facilitate *innovation* and *continual improvement* and strengthen the link between pharmaceutical development and manufacturing activities.

This guideline applies to the systems supporting the development and manufacture of pharmaceutical drug substances (*i.e.*, API) and drug products, including biotechnology and biological products, throughout the product lifecycle. The elements of ICH Q10 should be applied in a manner that is appropriate and proportionate to each of the product lifecycle stages, recognising the differences among, and the different goals of each stage.

For the purposes of this guideline, the product lifecycle includes the following technical activities for new and existing products:

- Pharmaceutical Development:
  - Drug substance development;
  - Formulation development (including container/closure system);
  - Manufacture of investigational products;
  - Delivery system development (where relevant);
  - Manufacturing process development and scale-up;
  - Analytical method development.
- Technology Transfer:
  - New product transfers during Development through Manufacturing;

- Transfers within or between manufacturing and testing sites for marketed products.

- Commercial Manufacturing:
  - Acquisition and control of materials;
  - Provision of facilities, utilities, and equipment;
  - Production (including packaging and labelling);
  - Quality control and assurance;
  - Release;
  - Storage;
  - Distribution (excluding wholesaler activities).
- Product Discontinuation:
  - Retention of documentation;
  - Sample retention;
  - Continued product assessment and reporting.

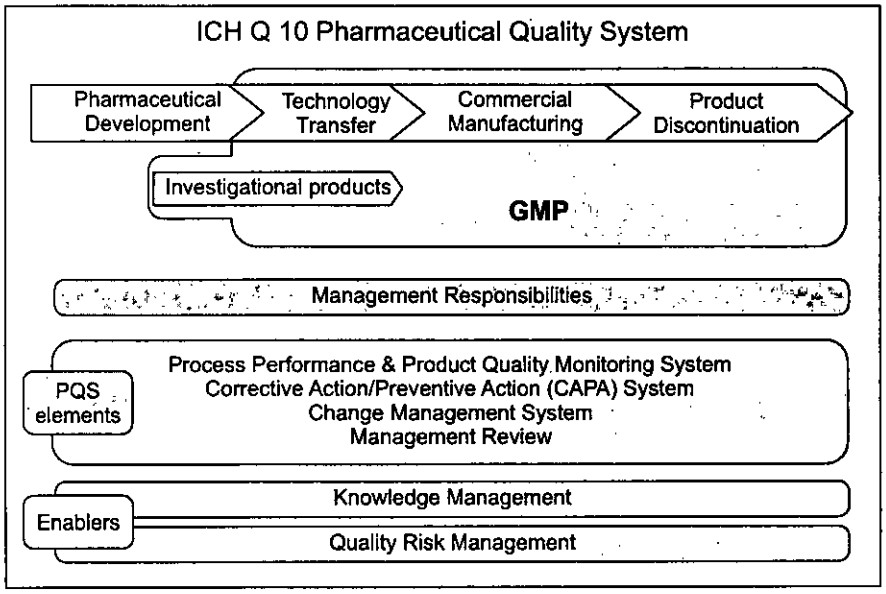

Fig. 7.1 Diagram of the ICH Q10 Pharmaceutical Quality System Model

This diagram illustrates the major features of the ICH Q10 Pharmaceutical Quality System (PQS) model. The PQS covers the entire lifecycle of a product including pharmaceutical development, technology transfer, commercial manufacturing, and product discontinuation as illustrated by the upper portion of the diagram. The PQS augments regional GMPs as illustrated in the diagram. The diagram also illustrates that regional GMPs apply to the manufacture of investigational products.

The next horizontal bar illustrates the importance of management responsibilities explained in Section 2 to all stages of the product lifecycle. The following horizontal bar lists the PQS elements which serve as the major pillars under the PQS model. These elements should be applied appropriately and proportionally to each lifecycle stage recognising opportunities to identify areas for continual improvement.

The bottom set of horizontal bars illustrates the enablers: knowledge management and quality risk management, which are applicable throughout the lifecycle stages. These enablers support the PQS goals of achieving product realisation, establishing and maintaining a state of control, and facilitating continual improvement.

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7.7 RELATIONSHIP OF ICH Q10

Relationship of ICH Q10 to Regional GMP Requirements, ISO Standards and ICH Q7

Regional GMP requirements, the ICH Q7 Guideline, "Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients", and ISO quality management system guidelines form the foundation for ICH Q10. To meet the objectives described below, ICH Q10 augments GMPs by describing specific quality system elements and management responsibilities. ICH Q10 provides a harmonised model for a pharmaceutical quality system throughout the lifecycle of a product and is intended to be used together with regional GMP requirements.

The regional GMPs do not explicitly address all stages of the product lifecycle (*e.g.*, Development). The quality system elements and management responsibilities described in this guideline are intended to encourage the use of science and risk based approaches at each lifecycle stage, thereby promoting continual improvement across the entire product lifecycle.

Relationship of ICH Q10 to Regulatory Approaches

Regulatory approaches for a specific product or manufacturing facility should be commensurate with the level of product and process understanding, the results of *quality risk management*, and the effectiveness of the pharmaceutical quality system. When implemented, the effectiveness of the pharmaceutical quality system can normally be evaluated during a regulatory inspection at the manufacturing site. Potential opportunities to enhance science and risk based regulatory approaches are identified in Annex 1. Regulatory processes will be determined by region.

ICH Q10 Objectives

Implementation of the Q10 model should result in achievement of three main objectives which complement or enhance regional GMP requirements.

Achieve Product Realisation

To establish, implement and maintain a system that allows the delivery of products with the quality attributes appropriate to meet the needs of patients, health care professionals, regulatory authorities (including compliance with approved regulatory filings) and other internal and external customers.

Establish and Maintain a State of Control

To develop and use effective monitoring and control systems for process performance and product quality, thereby providing assurance of continued suitability and *capability of processes*. Quality risk management can be useful in identifying the monitoring and control systems.

Facilitate Continual Improvement

To identify and implement appropriate product quality improvements, process improvements, variability reduction, innovations and pharmaceutical quality system

enhancements, thereby increasing the ability to fulfil quality needs consistently. Quality risk management can be useful for identifying and prioritising areas for continual improvement.

7.8 THE FUNDAMENTAL POINTS OF GLP

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The GLP Principles set out the requirements for the appropriate management of nonclinical safety studies. This helps the researcher to perform his/her work in compliance with his/her own pre-established scientific design. GLP Principles help to define and standardise the planning, performance, recording, reporting, monitoring and archiving processes within research institutions. The regulations are not concerned with the scientific or technical content of the studies *per se*. The regulations do not aim to evaluate the scientific value of the studies: this task is reserved first for senior scientists working on the research programme, then for the Registration Authorities, and eventually for the international scientific community as a whole. The GLP requirements for proper planning, for controlled performance of techniques, for faithful recording of all observations, for appropriate monitoring of activities and for complete archiving of all raw data obtained, serve to eliminate many sources of error.

Whatever the industry targeted, GLP stresses the importance of the following main points:

1. Resources: Organisation, personnel, facilities and equipment;
2. Characterisation: Test items and test systems;
3. Rules: Protocols, standard operating procedures (SOPs);
4. Results: Raw data, final report and archives;
5. Quality Assurance: Independent monitoring of research processes.

The WHO/TDR training programme takes each of these 5 fundamental points in turn and explains the requirements of GLP in each case. The major points addressed are summarized below and then dealt with in detail in the sections which follow.

Organisation and Personnel

GLP regulations require clear definitions of the structure of the research organisation and the responsibilities of the research personnel. This means that the organisational chart should reflect the reality of the institution and should be kept up to date. Organisational charts and job descriptions give an immediate idea of the way in which the laboratory functions and the relationships between the different departments and posts.

GLP also stresses that the number of personnel available must be sufficient to perform the tasks required in a timely and GLP-compliant way. The responsibilities of all personne should be defined and recorded in job descriptions and their qualifications and competence defined in education and training records. To maintain adequate levels of competence, GLP attaches considerable importance to the qualifications of staff, and to both internal and external training given to personnel.

A point of major importance in GLP is the position of the Study Director who is the pivotal point of control for the whole study. This person is appointed by the test facility management and will assume full responsibility for the GLP compliance

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of all activities within the study. He/she is responsible for the adequacy of the study protocol and for the GLP compliant conduct of the study. He/she will assert this at the end of the study in his/her dated and signed GLP Compliance Statement which is included in the study report. The Study Director must therefore be aware of all events that may influence the quality and integrity of the study, evaluate their impact and institute corrective actions as necessary. Even when certain phases or parts of the study are delegated to other test sites (as in the case of multisite studies), the Study Director retains overall responsibility for the entire study, including the parts delegated, and for the global interpretation of the study data. (The OECD has produced a guidance document on the roles and responsibilities of the Study Director which is in the annexe to this Handbook. A specific training module on the Study Director is included in the WHO/TDR GLP Training Manuals.)

Facilities and Equipment

The GLP Principles emphasise that facilities and equipment must be sufficient and adequate to perform the studies. The facilities should be spacious enough to avoid problems such as overcrowding, cross contamination or confusion between projects. Utilities (water, electricity etc.) must be adequate and stable.

All equipment must be in working order; a programme of validation/qualification, calibration and maintenance attains this. Keeping records of use and maintenance is essential in order to know, at any point in time, the precise status of the equipment and its history.

Characterisation

In order to perform a study correctly, it is essential to know as much as possible about the materials used during the study. For non-clinical studies intended to evaluate the safetyelated properties of pharmaceutical compounds, it is a prerequisite to have detailed knowledge about the properties of the test item, and of the test system (often an animal or isolated part thereof) to which it is administered.

Characteristics such as identity, potency, composition, stability, impurity profile, etc. should be known for the test item, for the vehicle and for any reference material. If the test system is an animal (which is very often the case) it is essential to know such details as its strain, health status, normal biological values, etc.

Protocol or Study Plan

The study plan or protocol outlines the design and conduct of the study and provides evidence that the study has been properly thought through and planned: the principal steps of studies conducted in compliance with GLP are thus described in the study protocol. The protocol must be approved by the Study Director, by dated signature, before the study starts. Alterations to the study design can only be made through formal amendment procedures. All this will ensure that the study can be reconstructed at a later point in time. The GLP Principles list the essential elements to be included in a study protocol.

Written Procedures

It is not reasonable to include all the technical details of study conduct in the protocol. The details of all routine procedures are described in Standard Operating

Procedures (SOPs) which are part of the documentation system of the institution. SOPs contribute to reducing bias in studies by standardising frequently performed techniques. Laboratories also need to standardise certain techniques to facilitate comparison of results between studies; here again written SOPs are an invaluable tool. To be able to exactly reconstruct a study is *a sine qua non* for the mutual acceptance of data; another reason why routine procedures are described in written SOPs, used throughout the institution.

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But procedures cannot be fixed for all time, since this would stifle technical progress and lead to the use of out-dated methods and processes. Consequently, they have to be adapted to developments in knowledge. They must, therefore, be reviewed regularly, and they may be modified so that they reflect actual "state of the art". Finally, for ease of consultation, it is important that SOPs are available directly at the work place, and in their current version only.

Raw Data

All studies generate raw data, sometimes called source data. Raw data are the original data collected during the conduct of a procedure. But, raw data also document the procedures and circumstances under which the study was conducted. They are, therefore, essential for the reconstruction of studies and contribute to the traceability of the events of a study. Raw data are the results of the experiment upon which the conclusions of the study will be based. Some of the raw data will be treated statistically, while others may be used directly. Whatever the case, the results and their interpretations provided by the scientist in the study report must be a true and accurate reflection of the raw data.

Study Report

The study report, like all the other scientific aspects of the study, is the responsibility of the Study Director. He/she must ensure that it describes the study accurately. The Study Director is responsible for the scientific interpretation included in the study report and is also responsible for declaring to what extent the study was conducted in compliance with the GLP Principles. The GLP Principles list the essential elements to be included in a final study report.

Archives

A study may have to be reconstructed many years after it has ended. Thus the storage of records must enable their safekeeping for long periods of time without loss or deterioration and, preferably, in a way which allows quick retrieval. In order to promote safe storage of precious data, it is usual practice to restrict access to archive facilities to a limited number of staff and to record the documents logged in and out. Even if the access is restricted to certain staff, records are also kept of the people entering and leaving the archives.

Quality Assurance

Quality Assurance (QA) — sometimes also known as the Quality Assurance Unit (QAU) as defined by GLP is a team of persons charged with assuring management that GLP compliance has been attained in the test facility as a whole and in each individual study. QA must be independent of the operational conduct of the studies, and functions as a "witness" to the whole preclinical research process.

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7.9 REGISTRATION OF NEW DRUGS FOR IMPORTING AND MANUFACTURING IN INDIA AND LAWS AND INVESTIGATION

In the beginning of the current century Drug Industry was practically non-existent in India and pharmaceuticals were being important from abroad. The first world war changed the situation and not only were finished and cheap drugs imported in increasing volume, the demand for indigenous products also was voiced from all sides. With the clamour for swadeshi goods manufacturing concerns, both Indian and Foreign, sprang up to produce pharmaceuticals at cheaper rates to compete with imported products. Naturally some of these were of inferior quality and harmful for public health. The Government was, therefore, called upon to take notice of the situation and consider the matter of introducing legislation to control the manufacture, distribution and sale of drugs and medicines.

Two of the laws, The Poisons Act and the Dangerous Drugs Act were passed in 1919 and 1930 respectively. The Opium Act was quite old having being adopted as early as 1878. But to have a comprehensive legislation, which the rapid expansion of the pharmaceutical production and drug market required by the end of the second decade for its control, the Indian Government appointed, in 1931, a Drugs Enquiry Committee under the Chairmanship Lt. Col. R. N. Chopra which was asked to make shifting enquiries into the whole matter of drug production, distribution and sale by inviting opinions and meeting concerned people. The Committee was asked to make recommendations about the ways and means of controlling the production and sale of drugs and pharmaceuticals in the interest of public health. The Chopra Committee toured all over the country and after carefully examining the data placed before it, submitted a voluminous report to government suggesting creation of drug control machinery at the centre with branches in all provinces. For an efficient and speedy working of the controlling department the committee also recommended the establishment of a well-equipped Central Drugs Laboratory with competent staff and experts in various branches for data standardization work. Under the guidance of the Central Laboratory, it was suggested, small laboratories would work, in the provinces. For the training of young men and women, the Committee recommended the permission of Central Pharmacy Council, and the Provincial Pharmacy Councils, with registrars who would maintain the lists containing names and addresses of the licensed pharmacists.

The outbreak of the second world war in 1939 delayed the introduction of legislation on the lines suggested by the Chopra Committee which the Indian government contemplated and considered as urgent. However, the Drugs Act was passed in 1940 partly implementing the Chopra recommendations. With the achievement of independence in 1947 the rest of the required laws were put on the Statute Book. In 1985, the Narcotic Drugs and Psychotropic Substances Act was enacted repealing the Dangerous Drugs Act, 1930 and the Opium Act of 1878.

At present the following Acts and Rules made thereunder that govern the manufacture, sale, import, export and clinical research of drugs and cosmetics in India.

- The Drugs and Cosmetics Act, 1940
- The Pharmacy Act, 1948
- The Drugs and Magic Remedies (Objectionable Advertisement) Act, 1954
- The Narcotic Drugs and Psychotropic Substances Act, 1985

- The Medicinal and Toilet Preparations (Excise Duties) Act, 1956
- The Drugs (Prices Control) Order, 1995 (under the Essential Commodities Act)

Some Other Laws

There are some other laws which have a bearing on pharmaceutical manufacture, distribution and sale in India. The important ones being:

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- The Industries (Development and Regulation) Act, 1951
- The Trade and Merchandise Marks Act, 1958
- The Indian Patent and Design Act, 1970
- Factories Act

Current Federal law requires that a drug be the subject of an approved marketing application before it is transported or distributed across state lines. Because a sponsor will probably want to ship the investigational drug to clinical investigators in many states, it must seek an exemption from that legal requirement. The IND is the means through which the sponsor technically obtains this exemption from the FDA.

During a new drug's early preclinical development, the sponsor's primary goal is to determine if the product is reasonably safe for initial use in humans, and if the compound exhibits pharmacological activity that justifies commercial development. When a product is identified as a viable candidate for further development, the sponsor then focuses on collecting the data and information necessary to establish that the product will not expose humans to unreasonable risks when used in limited, early-stage clinical studies.

FDA's role in the development of a new drug begins when the drug's sponsor (usually the manufacturer or potential marketer) having screened the new molecule for pharmacological activity and acute toxicity potential in animals, wants to test its diagnostic or therapeutic potential in humans. At that point, the molecule changes in legal status under the Federal Food, Drug, and Cosmetic Act and becomes a new drug subject to specific requirements of the drug regulatory system.

There are three IND types:

- An Investigator IND is submitted by a physician who both initiates and conducts an investigation, and under whose immediate direction the investigational drug is administered or dispensed. A physician might submit a research IND to propose studying an unapproved drug, or an approved product for a new indication or in a new patient population.
- Emergency Use IND allows the FDA to authorize use of an experimental drug in an emergency situation that does not allow time for submission of an IND in accordance with 21CFR, Sec. 312.23 or Sec. 312.34. It is also used for patients who do not meet the criteria of an existing study protocol, or if an approved study protocol does not exist.
- Treatment IND is submitted for experimental drugs showing promise in clinical testing for serious or immediately life-threatening conditions while the final clinical work is conducted and the FDA review takes place.

There are two IND categories:

- Commercial
- Research (non-commercial)

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The IND application must contain information in three broad areas:

- Animal Pharmacology and Toxicology Studies—Preclinical data to permit an assessment as to whether the product is reasonably safe for initial testing in humans. Also included are any previous experiences with the drug in humans (often foreign use).
- Manufacturing Information—Information pertaining to the composition, manufacturer, stability, and controls used for manufacturing the drug substance and the drug product. This information is assessed to ensure that the company can adequately produce and supply consistent batches of the drug.
- Clinical Protocols and Investigator Information—Detailed protocols for proposed clinical studies to assess whether the initial-phase trials will expose subjects to unnecessary risks. Also, information on the qualifications of clinical investigators—professionals (generally physicians) who oversee the adminisration of the experimental compound—to assess whether they are qualified to fulfill their clinical trial duties. Finally, commitments to obtain informed consent from the research subjects, to obtain review of the study by an institutional review board (IRB), and to adhere to the investigational new drug regulations.

Once the IND is submitted, the sponsor must wait 30 calendar days before initiating any clinical trials. During this time, FDA has an opportunity to review the IND for safety to assure that research subjects will not be subjected to unreasonable risk.

7.10 NEW DRUG APPLICATION (NDA) AND ITS RESOURCES

For decades, the regulation and control of new drugs in the United States has been based on the New Drug Application (NDA). Since 1938, every new drug has been the subject of an approved NDA before U.S. commercialization. The NDA application is the vehicle through which drug sponsors formally propose that the FDA approve a new pharmaceutical for sale and marketing in the U.S. The data gathered during the animal studies and human clinical trials of an Investigational New Drug (IND) become part of the NDA.

The goals of the NDA are to provide enough information to permit FDA reviewer to reach the following key decisions:

- Whether the drug is safe and effective in its proposed use(s), and whether the benefits of the drug outweigh the risks.
- Whether the drug's proposed labeling (package insert) is appropriate, and what it should contain.
- Whether the methods used in manufacturing the drug and the controls used to maintain the drug's quality are adequate to preserve the drug's identity, strength, quality, and purity.

The documentation required in an NDA is supposed to tell the drug's whole story, including what happened during the clinical tests, what the ingredients of the drug are, the results of the animal studies, how the drug behaves in the body, and how it is manufactured, processed and packaged.

Resources for NDA Submissions

The following resources have been gathered to provide you with the legal requirements of a new drug application, assistance from CDER to help you meet those requirements, and internal NDA review principles, policies and procedures.

7.11 GUIDANCE DOCUMENTS FOR NDAs

NOTES

Guidance documents represent the Agency's current thinking on a particular subject. These documents are prepared for FDA review staff and applicants/sponsors to provide guidelines to the processing, content, and evaluation/approval of applications and also to the design, production, manufacturing, and testing of regulated products. They also establish policies intended to achieve consistency in the Agency's regulatory approach and establish inspection and enforcement procedures. Because guidances are not regulations or laws, they are not enforceable, either through administrative actions or through the courts. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both. For information on a specific guidance document, please contact the originating office.

7.12 ABBREVIATED NEW DRUG APPLICATION (ANDA)

An Abbreviated New Drug Application (ANDA) contains data which when submitted to FDA's Center for Drug Evaluation and Research, Office of Generic Drugs, provides for the review and ultimate approval of a generic drug product. Once approved, an applicant may manufacture and market the generic drug product to provide a safe, effective, low cost alternative to the American public.

A generic drug product is one that is comparable to an innovator drug product in dosage form, strength, route of administration, quality, performance characteristics and intended use. All approved products, both innovator and generic, are listed in FDA's Approved Drug Products with Therapeutic Equivalence Evaluations (Orange Book).

Generic drug applications are termed "abbreviated" because they are generally not required to include preclinical (animal) and clinical (human) data to establish safety and effectiveness. Instead, generic applicants must scientifically demonstrate that their product is bioequivalent (*i.e.*, performs in the same manner as the innovator drug). One way scientists demonstrate bioequivalence is to measure the time it takes the generic drug to reach the bloodstream in 24 to 36 healthy, volunteers. This gives them the rate of absorption, or bioavailability, of the generic drug, which they can then compare to that of the innovator drug. The generic version must deliver the same amount of active ingredients into a patient's bloodstream in the same amount of time as the innovator drug.

Using bioequivalence as the basis for approving generic copies of drug products was established by the "Drug Price Competition and Patent Term Restoration Act of 1984," also known as the Waxman-Hatch Act. This Act expedites the availability of less costly generic drugs by permitting FDA to approve applications to market generic versions of brand-name drugs without conducting costly and duplicative clinical trials. At the same time, the brand-name companies can apply for up to five additional years longer patent protection for the new medicines they developed to make up for time lost while their products were going through FDA's approval process. Brand-name drugs are subject to the same bioequivalence tests as generics upon reformulation.

The Office of Generic Drugs home page provides additional information to generic drug developers, focusing on how CDER determines the safety and bioequivalence

of generic drug products prior to approval for marketing. Generic drug application reviewers focus on bioequivalence data, chemistry and microbiology data, requests for plant inspection, and drug labeling information.

SUMMARY

NOTES

- The QS regulation embraces the same "umbrella" approach to the CGMP regulation that was the underpinning of the original CGMP regulation.
- ISO 9001 is the internationally recognised standard for the quality management of businesses.
- ICH Q10 provides a harmonised model for a pharmaceutical quality system throughout the lifecycle of a product and is intended to be used together with regional GMP requirements.
- GLP regulations require clear definitions of the structure of the research organisation and the responsibilities of the research personnel.
- Quality Assurance (QA) - sometimes also known as the Quality Assurance Unit (QAU) as defined by GLP is a team of persons charged with assuring management that GLP compliance has been attained in the test facility as a whole and in each individual study.
- An Abbreviated New Drug Application (ANDA) contains data which when submitted to FDA's Center for Drug Evaluation and Research, Office of Generic Drugs, provides for the review and ultimate approval of a generic drug product.

REVIEW QUESTIONS

1. Discuss the quality assurance and characteristics of quality system regulation.
2. Explain why it is required to seek ISO 9001 certificate and the benefits of implementing ISO 9001.
3. Discuss the ICH requirement for quality and illustrate the diagram of the ICH Q10 pharmaceutical quality system model.
4. Discuss about relationship of ICH Q10 and its objectives.
5. What are the main points of GLP?