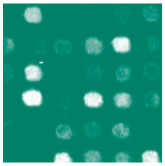

Principles of Genome Analysis and Genomics

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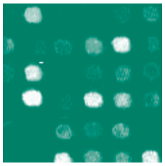


Preface

For most of the 20th century, a central problem in genetics was the creation of maps of entire chromosomes. These maps were crucial for the understanding of the structure of genes, their function and their evolution. For a long time these maps were created by genetic means, i.e. as a result of sexual crosses. Starting about 15 years ago, recombinant DNA technology was used to generate molecular or physical maps, defined here as the ordering of distinguishable DNA fragments by their position along the chromosome. Two key features of physical maps are that they can be generated much more quickly than genetic maps, and they usually have a much denser array of markers. The existence of physical maps now is greatly facilitating the analysis of a number of key questions in genetics such as the molecular basis of polygenic disorders and quantitative traits.

Physical mapping embraces a wide range of manipulative and analytical techniques which are detailed in specialist journals using a specialist language. This means that it is difficult for even the experienced biologist entering the field to comprehend the latest developments or even what has been achieved. The first edition of this book was written to provide these new entrants with an overview of the methodologies employed.

The ultimate physical map is a complete genome sequence. Shortly after the first edition of this book was published in 1995, the complete sequence of a bacterial genome was reported for the first time. From a technical point of view this was a particularly noteworthy achievement because the genome sequenced had a size of 1.8 million base pairs, yet the longest individual piece of DNA that can be sequenced is only 600–800 nucleotides. Soon thereafter, the sequences of several mammalian chromosomes were reported as well as the entire genome of the yeast *Saccharomyces cerevisiae*. The key issue was no longer how to sequence a genome but how to handle the sequence data. These changes were reflected in the second edition published in 1998.

As we entered the 21st century, the list of sequenced genomes included over 60 bacteria plus those of yeast, the nematode, the fruit fly, a flowering plant and humans. The size of the human genome (3 billion base pairs) indicates the progress made since the first edition was published. Sequencing of whole genomes is progressing at a rapid rate but the emphasis is now shifting back to biological questions. For example, how do the different components of the genome and the different gene products interact? Answers to questions such as this are being provided using yet another new set of tools in combination with the established mapping and sequencing methodologies. This branch of biology is known as genomics and its importance is such that half of this third edition is devoted to it.

We would like to thank our friends and colleagues who supplied material for this book and took the time to read and make comments on individual chapters, in particular Drs Phillip Gardner, Dylan Sweetman, Ajay Kohli, Gavin Craig, Eva Stoger and Professor Christine Godson. Thanks also to Sue Goddard and her staff in the CAMR library who cheerfully found references and papers at short notice. Richard Twyman would like to thank Kathryn Parry and family (Cain, Evan, Tate and Imogen) for their kindness and hospitality during the summer of 2001 when this project began. He would like to dedicate this book to his parents, Peter and Irene, his children, Emily and Lucy, and to Hannah, Joshua and Dylan.

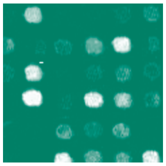
Abbreviations

2DE	two-dimensional gel electrophoresis
Ac	*Activator*
ADME	adsorption, distribution, metabolism and excretion
AFBAC	affected family-based control
AFLP	amplified fragment length polymorphism
ALL	acute lymphoblastic leukaemia
AML	acute myeloid leukaemia
APL	acute promyelocytic leukaemia
ARS	autonomously replicating sequence
ATRA	all-*trans*-retinoic acid
BAC	bacterial artificial chromosome
BCG	Bacille Calmette–Guérin
bFGF	basic fibroblast growth factor
BIND	Biomolecular Interaction Network Database
BLAST	Basic Local Alignment Search Tool
BLOSUM	Blocks Substitution Matrix
BMP	bone morphogenetic protein
bp	base pair
BRET	bioluminescence resonance energy transfer
CAPS	cleaved amplified polymorphic sequences
CASP	Critical Assessment of Structural Prediction
CATH	Class, Architecture, Topology and Homologous superfamily (database)
CCD	charge couple device
CD	circular dichroism
cDNA	complementary DNA
CEPH	Centre d'Etude du Polymorphisme Humain
CHEF	contour-clamped homogeneous electrical field
CID	collision-induced dissociation
cM	centimorgan
COG	cluster of orthologous groups
cR	centiRay
cRNA	complementary RNA
CSSL	chromosome segment substitution line
ct	chloroplast
DDBJ	DNA Databank of Japan
DIP	Database of Interacting Proteins
DMD	Duchenne muscular dystrophy
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
Ds	*Dissociation*
dsDNA	double-stranded DNA
dsRNA	double-stranded RNA
EGF	epidermal growth factor
ELISA	enzyme-linked immunosorbent sandwich assay
EMBL	European Molecular Biology Laboratory
ENU	ethylnitrosourea
ES	embryonic stem (cells)
ESI	electrospray ionization
EST	expressed sequence tag
EUROFAN	European Functional Analysis Network (consortium)
FACS	fluorescence-activated cell sorting
FEN	flap endonuclease
FIAU	Fialuridine (1-2′-deoxy-2′-fluoro-β-D-arabinofuranosyl-5-iodouracil)
FIGE	field-inversion gel electrophoresis
FISH	fluorescence *in situ* hybridization
FPC	fingerprinted contigs
FRET	fluorescence resonance energy transfer
FSSP	Fold classification based on Structure–Structure alignment of Proteins (database)
GASP	Genome Annotation Assessment Project
G-CSF	granulocyte colony stimulating factor
GeneEMAC	gene external marker-based automatic congruencing
GGTC	German Gene Trap Consortium

GST	gene trap sequence tag
GST	glutathione-*S*-transferase
HAT	hypoxanthine, aminopterin and thymidine
HDL	high-density lipoprotein
HERV	human endogenous retrovirus
HPRT	hypoxanthine phosphoribosyl-transferase
HTF	*Hpa*II tiny fragment
htSNP	haplotype tag single nucleotide polymorphism
IDA	interaction defective allele
Ihh	Indian hedgehog
IPTG	isopropylthio-β-D-galactopyranoside
IST	interaction sequence tag
IVET	*in vivo* expression technology
kb	kilobase
LCR	low complexity region
LD	linkage disequilibrium
LINE	long interspersed nuclear element
LOD	logarithm$_{10}$ of odds
LTR	long terminal repeat
m : z	mass : charge ratio
MAD	multiwavelength anomalous diffraction
MAGE	microarray and gene expression
MAGE-ML	microarray and gene expression mark-up language
MAGE-OM	microarray and gene expression object model
MALDI	matrix assisted laser desorption ionization
Mb	megabase
MGED	Microarray Gene Expression Database
MIAME	minimum information about a microarray experiment
MIP	molecularly imprinted polymer
MIPS	Munich Information Center for Protein Sequences
MM	'mismatch' oligonucleotide
MPSS	massively parallel signature sequencing
mRNA	messenger RNA
MS	mass spectrometry
MS/MS	tandem mass spectroscopy
mt	mitochondrial
MTM	Maize Targeted Mutagenesis project
Mu	*Mutator*

MudPIT	multidimensional protein identification technology
NGF	nerve growth factor
NIL	near isogenic line
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
NOESY	NOE spectroscopy
nt	nucleotide
OFAGE	orthogonal-field-alternation gel electrophoresis
ORF	open-reading frame
ORFan	orphan open-reading frame
P/A	presence/absence polymorphism
PAC	P1-derived artificial chromosome
PAGE	polyacrylaminde gel electrophoresis
PAI	pathogenicity island
PAM	percentage of accepted point mutations
PCR	polymerase chain reaction
PDB	Protein Databank (database)
Pfam	Protein families database of alignments
PFGE	pulsed field gel electrophoresis
PM	'perfect match' oligonucleotide
poly(A)$^+$	polyadenylated
PQL	protein quantity loci
PRINS	primed *in situ*
PS	position shift polymorphism
PSI-BLAST	Position-Specific Iterated BLAST (software)
PVDF	polyvinylidine difluoride
QTL	quantitative trait loci
RACE	rapid amplification of cDNA ends
RAPD	randomly amplified polymorphic DNA
RARE	RecA-assisted restriction endonuclease
RC	recombinant congenic (strains)
RCA	rolling circle amplification
rDNA/RNA	ribosomal DNA/RNA
RFLP	restriction fragment length polymorphism
RIL	recombinant inbred line
R-M	restriction-modification
RNA	ribonucleic acid
RNAi	RNA interference
RNase	ribonuclease
RPMLC	reverse phase microcapillary liquid chromatography

RT-PCR	reverse transcriptase polymerase chain reaction
RTX	repeats in toxins
SAGE	serial analysis of gene expression
SCOP	Structural Classification of Proteins (database)
SDS	sodium dodecylsulphate
SELDI	surface-enhanced laser desorption and ionization
SGDP	*Saccharomyces* Gene Deletion Project
Shh	sonic hedgehog
SINE	short interspersed nuclear element
SINS	sequenced insertion sites
SNP	single nucleotide polymorphism
SPIN	Surface Properties of protein–protein Interfaces (database)
Spm	*Suppressor–mutator*
SPR	surface plasmon resonance
SRCD	synchrotron radiation circular dichroism
SSLP	simple sequence length polymorphism
SSR	simple sequence repeat
STC	sequence-tagged connector
STM	signature-tagged mutagenesis
STS	sequence-tagged site
TAC	transformation-competent artificial chromosome
TAFE	transversely alternating-field electrophoresis
TAR	transformation-associated recombination
T-DNA	*Agrobacterium* transfer DNA
TIGR	The Institute for Genomic Research
TIM	triose phosphate isomerase
TOF	time of flight
tRNA	transfer RNA
TUSC	Trait Utility System for Corn
UPA	universal protein array
UTR	untranslated region
VDA	variant detector array
VIGS	virus-induced gene silencing
Y2H	yeast two-hybrid
YAC	yeast artificial chromosome



CHAPTER 1

Setting the scene: the new science of genomics

Introduction

Genetics is the study of the inheritance of traits from one generation to another. As such, it examines the phenotypes of the offspring of sexual crosses. Useful as these data may be, they cannot provide an explanation for the biological basis of a phenotype for that requires biochemical information. In some cases the jump from phenotype to biochemical explanation was relatively simple. Good examples are amino acid auxotrophy and antibiotic resistance in microorganisms and phenylketonuria and sickle cell disease in humans. However, until recently, it was almost impossible to determine the biochemical basis for most of the traits in most organisms.

The first major advance in understanding phenotypes came in the mid-1970s with the development of methods for manipulating genes *in vitro* ('genetic engineering'). This permitted genes to be cloned and sequenced which in turn provided data on the amino acid sequence of the gene product. It also became possible to overexpress the gene product, thereby facilitating its purification and characterization. As the number of characterized gene products has grown, the determination of gene function has become easier, as it is possible to search databases for closely related proteins whose properties are known. Other techniques that have facilitated the analysis of phenotypes are site-directed mutagenesis, where specific base changes or deletions can be made in genes, and gene replacement. All of these techniques, and many others, are described in our companion volume *Principles of Gene Manipulation* (Primrose *et al*. 2001).

Over the past 25 years a vast amount of data has been generated for thousands of different gene products from many different organisms, most of it as a direct result of the ability to manipulate genes. Impressive as this is, gene manipulation on its own cannot meet all the needs of biologists. First, in many instances, a gene needs to be mapped close to a convenient marker before it can be cloned. While this may be easy in an organism such as *Drosophila* where many mutants are available, it is much more difficult in humans or in organisms whose genetics have been poorly studied. Secondly, understanding the phenotype of one or a few genes gives little information about the whole organism and how all its components interact, e.g. its metabolic capabilities or how it controls its development. Thirdly, the analysis of a few genes does not enable us to answer the big questions in biology. For example, how did speech and memory evolve, what changes at the DNA level occurred as the primates evolved, etc.? However, these needs now are being met as a result of efforts to sequence the entire genomes of a number of organisms.

Physical mapping of genomes

In the mid-1980s, scientists began to discuss seriously how the entire human genome might be sequenced. To put these discussions in context, the largest stretch of DNA that can be sequenced in a single pass is 600–800 nucleotides and the largest genome that had been sequenced was the 172 kb Epstein–Barr virus DNA (Baer *et al*. 1984). By comparison, the human genome has a size of 3000 Mb. One school of thought was that completely new sequencing methodology would be required and a number of different technologies were explored but with little success. Early on, it was realized that in order to sequence a large genome it would be necessary to break the genome down into more manageable pieces for sequencing and then join the pieces together again. The problem here was that there were not enough markers on the human genome. It should be noted that humans represent an extreme case of difficulty in creating a genetic map. Not only are directed matings not possible, but the length of the breeding cycle (15–20 years) makes conventional

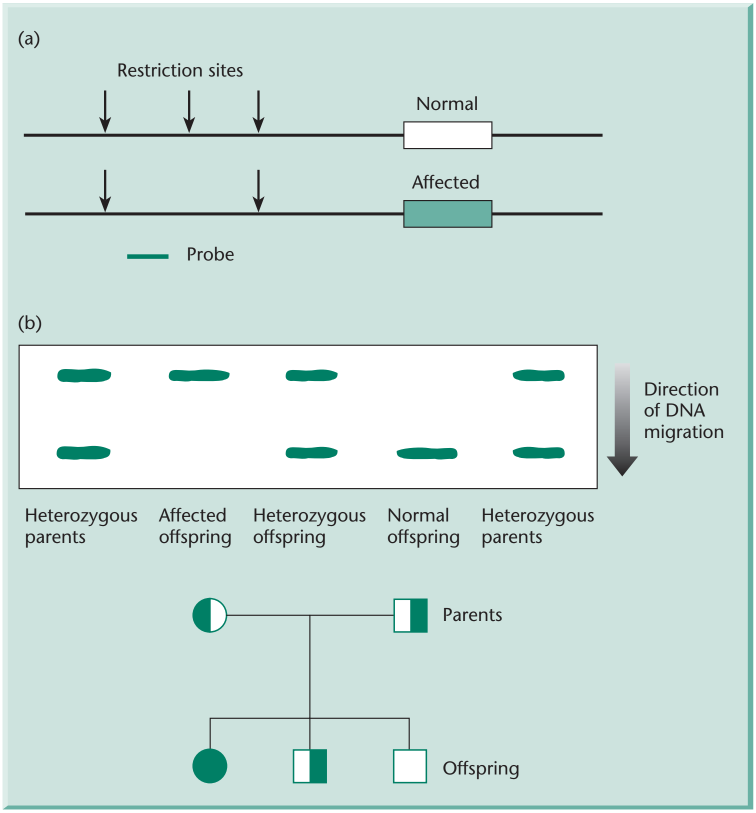


Fig. 1.1 Example of a RFLP and its use for gene mapping. (a) A polymorphic restriction site is present in the DNA close to the gene of interest. In the example shown, the polymorphic site is present in normal individuals but absent in affected individuals. (b) Use of the probe shown in Southern blotting experiments with DNA from parents and progeny for the detection of affected offspring.

analysis impossible. A major breakthrough was the development of methods for using DNA probes to identify polymorphic sequences (Botstein *et al.* 1980). The first such DNA polymorphisms to be detected were differences in the length of DNA fragments after digestion with sequence-specific restriction endonucleases, i.e. restriction fragment length polymorphisms (RFLPs; Fig. 1.1).

To generate an RFLP map the probes must be highly informative. This means that the locus must not only be polymorphic, it must be *very* polymorphic. If enough individuals are studied, any randomly selected probe will eventually discover a polymorphism. However, a polymorphism in which one allele exists in 99.9% of the population and the other in 0.1% is of little utility because it seldom will be informative. Thus, as a general rule, the RFLPs used to construct the genetic map should have two, or perhaps three, alleles with equivalent frequencies.

The first RFLP map of an entire genome (Fig. 1.2) was that described for the human genome by Donis-Keller *et al.* (1987). They tested 1680 clones from a phage library of human genomic DNA to see whether they detected RFLPs by hybridization to Southern blots of DNA from five unrelated individuals. DNA from each individual was digested with 6–9 restriction enzymes. Over 500 probes were identified that detected variable banding patterns indicative of polymorphism. From this collection, a subset of 180 probes detecting the highest degree of polymorphism was selected for inheritance studies in 21 three-generation human families (Fig. 1.3). Additional probes were generated from chromosome-specific libraries such that ultimately 393 RFLPs were selected. The various loci were arranged into linkage groups representing the 23 human chromosomes by a combination of mathematical linkage analysis and physical location of selected clones. The latter was achieved by hybridizing probes to panels of rodent–human hybrid cells containing varying human chromosomal complements (see p. 38). RFLP maps have not been restricted to the human genome. For example, RFLP maps have

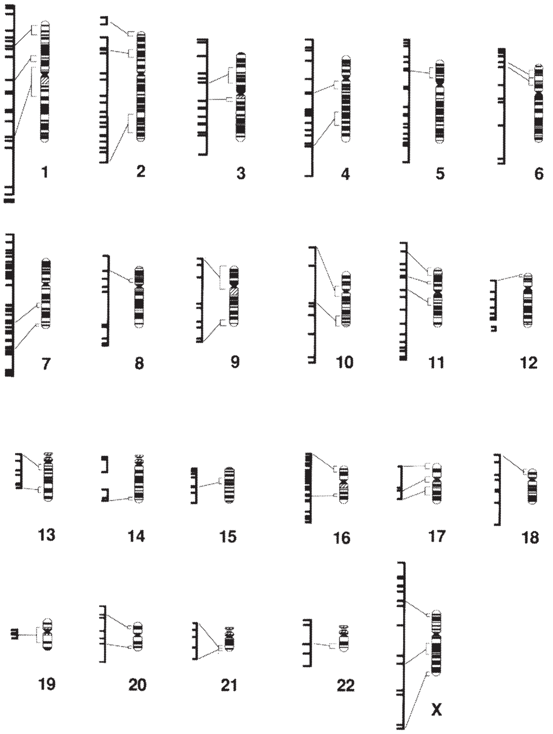


Fig. 1.2 The first RFLP genetic linkage map of the entire human genome. (Reproduced from Donis-Keller *et al.* 1987, with permission from Elsevier Science.)

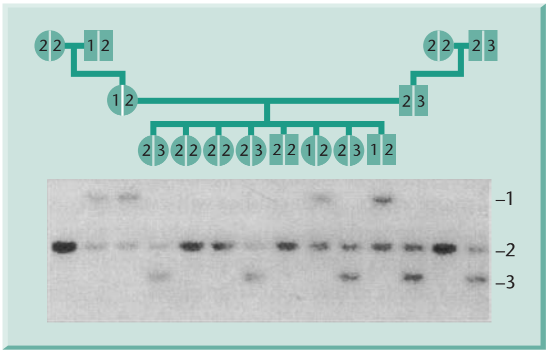


Fig. 1.3 Inheritance of a RFLP in three generations of a family. The RFLP probe used detects a single locus on human chromosome 5. In the family shown, three alleles are detected on Southern blotting after digestion with *TaqI*. For each of the parents it can be inferred which allele was inherited from the grandmother and which from the grandfather. For each child the grandparental origin of the two alleles can then be inferred. (Redrawn from Donis-Keller *et al.* 1987, with permission from Elsevier Science.)

been published for most of the major crops (see for example Moore *et al.* 1995).

The human genome map produced by Donis-Keller *et al.* (1987) was a landmark publication. However, it identified RFLP loci with an average spacing of 10 centimorgans (cM). That is, the loci had a 10% chance of recombining at meiosis. Given that the human genome is 4000 cM in length, the distance between the RFLPs is 10 Mb on average. This is too great to be of use for gene isolation. However, if the methodology of Donis-Keller *et al.* (1987) was used to construct a 1 cM map, then 100 times the effort would be required! This is because 10 times as many probes would be required and 10 times more families studied. The solution has been to use more informative polymorphic markers and other mapping techniques and these are described in detail in Chapter 4. Use of these techniques has led to the generation of a human map with the desired density of markers. More important, these advances in gene mapping were not restricted to the human genome. Rather, the methodology is generic and now has been applied to a wide range of animal and plant genomes.

Sequencing whole genomes

The late 1980s and early 1990s saw much debate about the desirability of sequencing the human genome. This debate often strayed from rationale scientific debate into the realms of politics, personalities and egos. Among the genuine issues raised were questions such as: Is the sequencing of the human genome an intellectually appropriate project for biologists?; Is sequencing the human genome feasible?; What benefits might arise from the project?; Will these benefits justify the cost and are there alternative ways of achieving the same benefits?; Will the project compete with other areas of biology for funding and intellectual resources? Behind the debate was a fear that sequencing the human genome was an end in itself, much like a mountaineer who climbs a new peak just because it is there.

In early 2001 two different groups (International Human Genome Sequencing Consortium 2001; Venter *et al.* 2001) reported the draft sequence of the

Table 1.1 Increases in sizes of genomes sequenced.

Genome sequenced	Year	Genome size	Comment
Bacteriophage φX174	1977	5.38 kb	First genome sequenced
Plasmid pBR322	1979	4.3 kb	First plasmid sequenced
Bacteriophage λ	1982	48.5 kb	
Epstein–Barr virus	1984	172 kb	
Yeast chromosome III	1992	315 kb	First chromosome sequenced
Haemophilus influenzae	1995	1.8 Mb	First genome of cellular organism to be sequenced
Saccharomyces cerevisiae	1996	12 Mb	First eukaryotic genome to be sequenced
Ceanorhabditis elegans	1998	97 Mb	First genome of multicellular organism to be sequenced
Drosophila melanogaster	2000	165 Mb	
Arabidopsis thaliana	2000	125 Mb	First plant genome to be sequenced
Homo sapiens	2001	3000 Mb	First mammalian genome to be sequenced
Rice (*Oryza sativa*)	2002	430 Mb	First crop plant to be sequenced
Pufferfish (*Fugu rubripes*)	2002	400 Mb	Smallest known vertebrate genome
Mouse (*Mus musculis*)	2002/3	2700 Mb	Closest model organism to man

human genome. An analysis of this achievement provides clear answers to the questions raised above. Those opposed to the idea of sequencing the human genome had cited the resources (thousands of scientists and billions of dollars) and time that would be required to accomplish the task. Furthermore, they believed that once the human genome was sequenced there would be a major logistical problem in handling the sequence data. What happened was that the scientific community developed new strategies for sequencing genomes, rather than new methods for sequencing DNA, and complemented these with the development of highly automated methodologies. The net effect was that by the time the human genome had been sequenced, the complete sequence was already known for over 30 bacterial genomes plus that of a yeast (*Saccharomyces cerevisiae*), the fruit fly (*Drosophila melanogaster*), a nematode (*Caenorhabditis elegans*) and a plant (*Arabidopsis thaliana*) (Table 1.1). Furthermore, a whole new science, *bioinformatics*, had been developed to handle and analyse the vast amounts of information being generated by these sequencing projects. Fortuitously, the global development of the Internet occurred at the same time and this enabled scientists around the world to have access to the bioinformatics tools developed in global centres of excellence.

The development of bioinformatics not only facilitated the handling and analysis of sequence data but the development of sequencing strategies as well. For example, when a European consortium set themselves the goal of sequencing the entire genome of the budding yeast *Saccharomyces* (15 Mb), they segmented the task by allocating the sequencing of each chromosome to different groups. That is, they subdivided the genome into more manageable parts. At the time this project was initiated there was no other way of achieving the objective and when the resulting genomic sequence was published (Goffeau *et al*. 1996), it was the result of a unique multicentre collaboration. While the *Saccharomyces* sequencing project was underway, a new genomic sequencing strategy was unveiled: shotgun sequencing. In this approach, large numbers of genomic fragments are sequenced and sophisticated bioinformatics algorithms used to construct the finished sequence. In contrast to the consortium approach used with *Saccharomyces*, a single laboratory set up as a sequencing factory undertook shotgun sequencing.

The first success with shotgun sequencing was the complete sequence of the bacterium *Haemophilus influenzae* (Fleischmann *et al*. 1995) and this was quickly followed with the sequences of *Mycoplasma genitalium* (Fraser *et al*. 1995), *Mycoplasma pneumoniae* (Himmelreich *et al*. 1996) and *Methanococcus jannaschii* (Bult *et al*. 1996). It should be noted that *H. influenzae* was selected for sequencing because so little was known about it: there was no genetic map and not much biochemical data either. By contrast, *S. cerevisiae* was a well-mapped and well-characterized organism. As will be seen in Chapter 5, the relative merits of shotgun sequencing vs. ordered, map-based sequencing still are being debated today. Nevertheless, the fact that a major sequencing laboratory can turn out the entire sequence of a bacterium in 1–2 months shows the power of shotgun sequencing.

Benefits of genome sequencing

Fears that sequencing the human genome would be an end in itself have proved groundless. Because so many different genomes have been sequenced it now is possible to undertake comparative analyses, a topic known as *comparative genomics*. By comparing genomes from distantly related species we can begin to decipher the major stages in evolution. By comparing more closely related species we can begin to uncover more recent events such as genome rearrangement and mutation processes. Currently, the most fertile area of comparative genomics is the analysis of bacterial genomes because so many have been sequenced. Already this analysis is throwing up some interesting questions. For example, over 25% of the genes in any one bacterial genome have no analogues in any other sequenced genome. Is this an artefact resulting from limited sequence data or does it reflect the unique evolutionary events that have shaped the genomes of these organisms? Again, comparative analysis of the genomes of a wide range of thermophiles has revealed numerous interesting features, including strong evidence of extensive horizontal gene transfer. However, what is the genomic basis for thermophily? We still do not know. Comparative genomics also is of value with higher organisms. Selective breeding is not acceptable with humans so we use other mammals as surrogates. Which is the best species to select as a surrogate? Comparative genomics can answer this question.

One of the fascinating aspects of the classic paper of Fleischmann *et al.* (1995) was their analysis of the metabolic capabilities of *H. influenzae* which they deduced from sequence information alone. This analysis (*metabolomics*) has been extended to every other sequenced genome and is providing tremendous insight into the physiology and ecological adaptibility of different organisms. For example, obligate parasitism in bacteria is linked to the absence of genes for certain enzymes involved in central metabolic pathways. Another example is the correlation between genome size and the diversity of ecological niches that can be colonized. The larger the bacterial genome, the greater are the metabolic capabilities of the host organism and this means that the organism can be found in a greater number of habitats.

Analysis of genomes has enabled us to identify most of the genes that are present. However, we still do not know what functions many of these genes perform and how important these genes are to the life of the cell. Nor do we know how the different gene products interact with each other. Because most gene products are proteins, the study of these interactions is known as *proteomics*. It is a discipline which is rooted in experiments rather than computer analysis. For example, the principal way of determining the function of a gene is to delete it and then monitor the fitness of the deletion strain under a variety of selective conditions. This is an enormous task but strategies have been developed for simplifying it. Of course, such a methodology cannot be used with humans, hence the need for comparative genomics.

The biochemistry and genetics of many human diseases have been elucidated but most of these are simple single-gene disorders. Unfortunately, most of the common diseases of humans, and those that put most financial burden on health provision, are polygenic disorders, e.g. hypertension and cancer. Currently we know very little about the *causes* of these diseases and hence we are reduced to treating the *symptoms*. One consequence of this is that many drugs have undesirable side-effects or else only work with certain individuals. Advances in genomics are enabling us to get a better handle on the causes of disease and this will lead to new therapies. Advances in physical mapping of the human genome are enabling us to better predict the effectiveness of a drug and the likely side-effects. Another advance in human genetics is the beginning of an understanding of complex social traits. For example, the first gene controlling speech has just been identified (Lai *et al.* 2001). Polygenic traits are of equal importance in agronomy. Many different characteristics such as weight gain, size, yield, etc. are controlled by many different loci. Again, physical mapping is enabling us to identify the different loci involved and this will facilitate more rational breeding programmes.

Another benefit of genome mapping and sequencing that deserves mention is international scientific collaboration. In magnitude, the goal of sequencing the human genome was equivalent to putting a man on the moon. However, putting a man on the moon was a race between two nations and was driven by global political ambitions as much as by scientific challenge. By contrast, genome sequencing truly has been an international effort requiring laboratories in Europe, North America and Japan to collaborate in a way never seen before. The extent of this collaboration can be seen from an analysis of the affiliations of the authors of the papers on the sequencing of the genomes of *Arabidopsis* (The *Arabidopsis* Genome Initiative 2000) and humans (International Human Genome Sequencing Consortium 2001), for example. The fact that one US company, Celera, has successfully undertaken many sequencing projects in no way diminishes this collaborative effort. Rather, they have constantly challenged the accepted way of doing things and have increased the efficiency with which key tasks have been undertaken.

Three other aspects of genome sequencing and genomics deserve mention. First, in other branches of science such as nuclear physics and space exploration, the concept of 'superfacilities' is well established. With the advent of whole genome sequencing, biology is moving into the superfacility league and a number of sequencing 'factories' have been established. Secondly, high throughput methodologies have become commonplace and this has meant a partnering of biology with automation, instrumentation and data management. Thirdly, many biologists have eschewed chemistry, physics and mathematics but progress in genomics demands that biologists have a much greater understanding of these subjects. For example, methodologies such as mass spectrometry, X-ray crystallography and protein structure modelling are now fundamental to the identification of gene function. The impact that this has on undergraduate recruitment in the sciences remains to be seen.

Outline of the rest of the book

The remainder of the book is divided into three parts. The first part concentrates on the methods developed for mapping and sequencing genomes and on the basics of bioinformatics (Fig. 1.4). The second part deals with genomics; i.e. the analysis of genome data and the use of map and sequence data to locate genes of interest and to understand phenotypes and fundamental biological phenomena (Fig. 1.5). Thus in the second part of the book, we provide a solution to the problem of understanding the phenotype as outlined earlier in this chapter. Finally, in the last part (Chapter 12) we review some of the applications of the methodologies discussed in the preceding two sections.

The genomes of free-living cellular organisms range in size from less than 1 Mb for some bacteria to millions, or tens of millions, of megabases for some plants. It may even come as a surprise to some to know that a protozoan, never mind a plant, can have a larger genome than that of humans. However, size does not necessarily equal gene content, a phenomenon first elaborated as the C-value paradox (p. 11). Rather, size is often a reflection of genome structure and organization, particularly that of repetitive DNA. This topic is covered in Chapter 2.

The sheer size of the genome of even a simple bacterium is such that to handle it in the laboratory we need to break it down into smaller pieces that are handled as clones. The methods for doing this are covered in Chapter 3. The process of putting the pieces back together again involves mapping. Many different sequence markers are used to do this, as well as some novel mapping methods, and these are described in Chapter 4. DNA sequencing technology is such that only short stretches (~600 bp) can be analysed in a single reaction. Consequently, the genome has to be fragmented and the sequence of each fragment determined and the total sequence reassembled (Chapter 5). Fortunately, the tools and techniques used for mapping also can be applied to genome sequencing. Finally, all the sequence data generated need to be stored and the information that they contain extracted. An introduction to this topic of bioinformatics is provided in Chapter 6.

Sequencing a genome is not an end in itself. Rather, it is just the first stage in a long journey whose goal is a detailed understanding of all the biological functions encoded in that genome and their evolution. To achieve this goal it is necessary to define all the genes in the genome and the functions that they encode. There are a number of different ways of doing this and these are covered in the second part of the book (Chapters 7–11; Fig. 1.5). One such technique is comparative genomics (Chap-

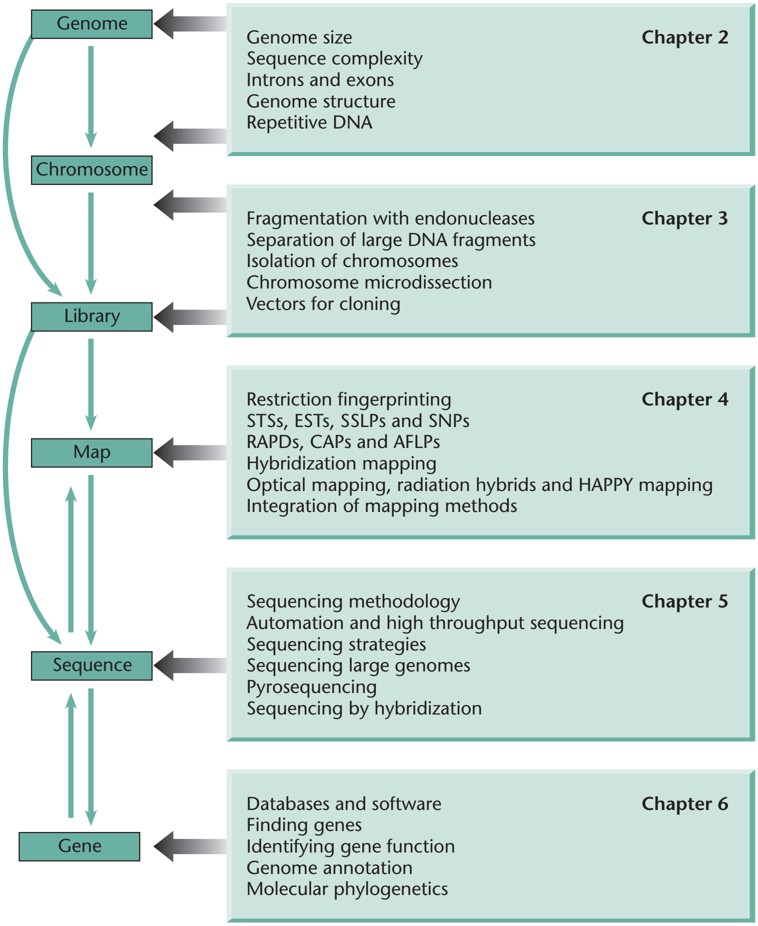


Fig. 1.4 'Road map' outlining the different methodologies used for mapping and sequencing genomes.

ter 7). The premise here is that DNA sequences encoding important cellular functions are likely to be conserved whereas dispensable or non-coding sequences will not. However, comparative genomics only gives a broad overview of the capabilities of different organisms. For a more detailed view one needs to identify each gene in the genome and its function. Such whole genome annotation involves a combination of computer and experimental analysis and is described in Chapters 8–11.

In classical biochemistry one starts by purifying a protein of known function and then determining its structure. In structural genomics, as described in Chapter 8, one does the opposite on the basis that peptide sequences with a similar primary or secondary structure are likely to have similar functions. Chapter 9 is devoted to the burgeoning field of high throughput expression analysis which is being used with great success to determine the function of anonymous genes. The methodologies used also give qualitative and quantitative information about gene expression as it relates to the biology of the whole organism. For example, it is possible to identify all the genes being transcribed in any cell or tissue at any time and the extent to which these RNAs are translated into proteins. Chapter 10 explores the idea of determining gene function by mutation. Whereas this is carried out on a gene-by-gene basis in classical genetics, in genomics it is performed on a genome-wide scale. Chapter 11 describes the investigation of protein–protein interactions and how these interactions are being mapped and assembled into databases in an attempt to link all proteins in the cell into a functional network.

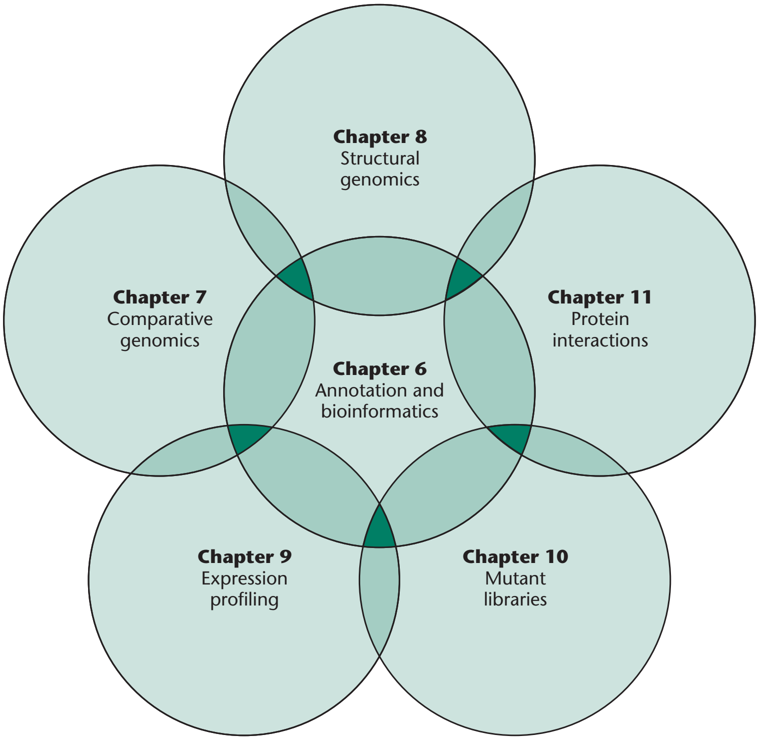


Fig. 1.5 Organization of the second part of the book. Note that Chapter 6, which discusses bioinformatics and the structural annotation of genomes, is the central underpinning theme. This links genome maps and sequences (Chapters 1–5) with comparative genomics (Chapter 7) and functional genomics (Chapters 8–11). Chapters 8–11 consider different aspects of functional genomics but, as shown by the overlapping circles, none is a totally isolated field. This figure conveys how a holistic approach is the best way to mine the genome for functional information.

Terminology

Workers in the field of genomics have coined a whole series of '-omics' terms to describe sub-disciplines of what they do. Confusingly, not everyone uses these terms in the same way. Our use of these terms is defined in Box 1.1.

Keeping up to date

The science of genomics is moving forward at an incredible pace and significant new advances are being reported weekly. This in turn has led to the publication of a plethora of new journals with '-omics' in their titles and which many hard-pressed libraries will be unable to afford. Fortunately, much of this material can now be accessed through the Internet and in the chapters that follow reference is made to relevant websites whenever possible. Any reader not familiar with the PubMed website (http://www.ncbi.nlm.nih.gov/PubMed/) is strongly advised to spend some time browsing it as it provides very useful access to a wide range of literature. It also has links to the contents pages of many journals.

Suggested reading

Donis-Keller H. *et al.* (1987) A genetic linkage map of the human genome. *Cell* **51**, 319–337. *This is a classic paper and describes the first comprehensive human genetic map to be constructed using DNA-based markers.*

Fleischmann R.D. *et al.* (1995) Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. *Science* **269**, 496–512. *This is another classic paper and the wealth of information about the biology of the bacterium that was inferred from the sequence data provided the justification, if one was needed, for whole genome sequencing.*

Primrose S.B., Twyman R.M. & Old R.W. (2001) *Principles of Gene Manipulation* (6th edn.) Blackwell Science, Oxford. *This textbook is widely used around the world and provides a detailed introduction to the many different techniques of gene manipulation that form the basis of the methods for genome analysis.*

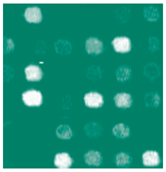
Box 1.1 Genomics definitions used throughout this book

Term	Definition
Genomics	The study of the structure and function of the genome
Functional genomics	The high throughput determination of the function of a gene product. Included within this definition is the expression of the gene, the relationship of the sequence and structure of the gene product to other gene products in the same or other organisms, and the molecular interactions of the gene product
Structural genomics	The high throughput determination of structural motifs and complete protein structures and the relationship between these and function
Comparative genomics	The use of sequence similarity and comparative gene order (synteny) to determine gene function and phylogeny
Proteomics	The study of the proteome, i.e. the full complement of proteins made by a cell. The term includes protein–protein and protein–small molecule interactions as well as expression profiling
Transcriptomics	The study of the transcriptome, i.e. all the RNA molecules made by a cell, tissue or organism
Metabolomics	The use of genome sequence analysis to determine the capability of a cell, tissue or organism to synthesize small molecules
Bioinformatics	The branch of biology that deals with *in silico* processing and analysis of DNA, RNA and protein sequence data
Annotation	The derivation of structural or functional information from unprocessed genomic DNA sequence data

Useful websites

http://www3.ncbi.nlm.nih.gov/
This is the website of the National Center for Biotechnology Information. It contains links to many other useful websites. The OMIM pages on this site contain a wealth of information on Mendelian inheritance in humans. This site also is the entry point to PubMed which enables researchers to access abstracts and journal articles on-line.

http://www.sciencemag.org/feature/plus/sfg/resources/
This is the website on functional genomics resources hosted by *Science* magazine. It contains many useful pages and the ones on model organisms are well worth visiting. There also are features pages which are revised regularly.



CHAPTER 2

The organization and structure of genomes

Introduction

There is no such thing as a common genome structure. Rather, there are major differences between the genomes of bacteria, viruses and organelles on the one hand and the nuclear genomes of eukaryotes on the other. Within the eukaryotes there are major differences in the types of sequences found, the amounts of DNA and the number of chromosomes. This wide variability means that the mapping and sequencing strategies involved depend on the individual genome being studied.

Genome size

Because the different cells within a single organism can be of different ploidy, e.g. germ cells are usually haploid and somatic cells diploid, genome sizes always relate to the haploid genome. The size of the haploid genome also is known as the C-value. Measured C-values range from 3.5×10^3 bp for the smallest viruses, e.g. coliphage MS2, to 10^{11} bp for some amphibians and plants (Fig. 2.1). The largest viral genomes are $1–2 \times 10^5$ bp and are just a little smaller than the smallest cellular genomes, those of some mycoplasmas (5×10^5 bp). Simple unicellular eukaryotes have a genome size ($1–2 \times 10^7$ bp) that is not much larger than that of the largest bacterial genomes. Primitive multicellular organisms such as nematodes have a genome size about four times larger. Not surprisingly, an examination of the genome sizes of a wide range of organisms has shown that the *minimum* C-value found in a particular phylum is related to the structural and organizational complexity of the members of that phylum. Thus the minimum genome size is greater in organisms that evolutionarily are more complex (Fig. 2.2).

A particularly interesting aspect of the data shown in Fig. 2.1 is the range of genome sizes found within

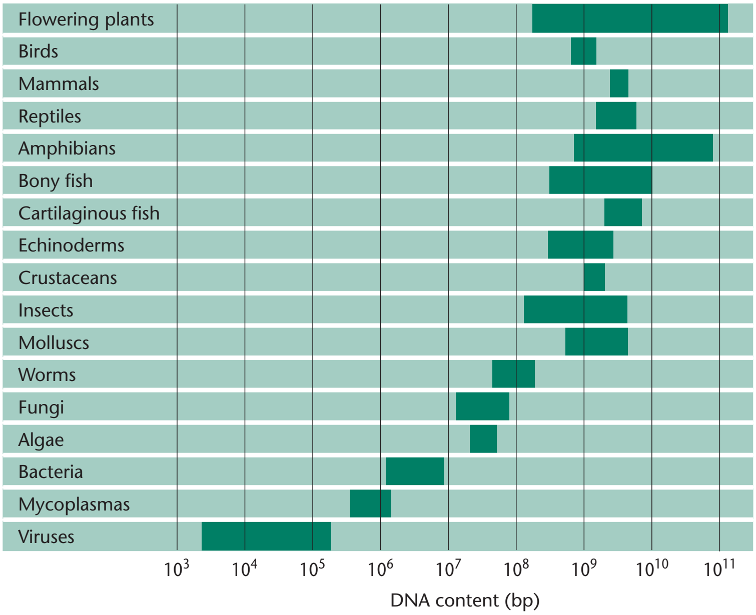


Fig. 2.1 The DNA content of the haploid genome of a range of phyla. The range of values within a phylum is indicated by the shaded area. (Redrawn from Lewin 1994 by permission of Oxford University Press.)

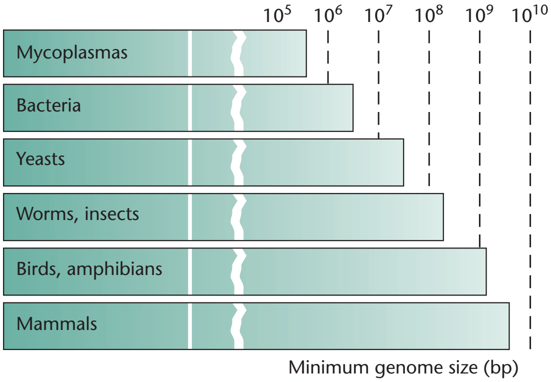


Fig. 2.2 The minimum genome size found in a range of organisms. (Redrawn from Lewin 1994 by permission of Oxford University Press.)

each phylum. Within some phyla, e.g. mammals, there is only a twofold difference between the largest and smallest C-value. Within others, e.g. insects and plants, there is a 10- to 100-fold variation in size. Is there really a 100-fold variation in the number of genes needed to specify different flowering plants? Are some plants really more organizationally complex than humans, as these data imply? Although there is evidence that birds with smaller genomes are better flyers (Hughes & Hughes 1995) and that plants are more responsive to elevated carbon dioxide concentrations (Jasienski & Bazzaz 1995) as their genomes increase in size, this is not sufficient to explain the size differential. The resolution of this apparent C-value paradox was provided by the analysis of sequence complexity by means of reassociation kinetics.

Sequence complexity

When double-stranded DNA in solution is heated, it denatures ('melts') releasing the complementary single strands. If the solution is cooled quickly the DNA remains in a single-stranded state. However, if the solution is cooled slowly reassociation will occur. The conditions for efficient reassociation of DNA were determined originally by Marmur *et al.* (1963) and since then have been extensively studied by others (for a review, see Tijssen 1993). The key parameters are as follows. First, there must be an adequate concentration of cations and below 0.01 M sodium ion there is effectively no reassociation. Secondly, the temperature of incubation must be high enough to weaken intrastrand secondary structure. In practice, the optimum temperature for reassociation is 25°C below the melting temperature (T_m), that is, the temperature required to dissociate 50% of the duplex. Thirdly, the incubation time and the DNA concentration must be sufficient to permit an adequate number of collisions so that the DNA can reassociate. Finally, the size of the DNA fragments also affects the rate of reassociation and is conveniently controlled if the DNA is sheared to small fragments.

The reassociation of a pair of complementary sequences results from their collision and therefore the rate depends on their concentration. As two strands are involved the process follows second-order kinetics. Thus, if *C* is the concentration of DNA that is single stranded at time *t*, then

$$\frac{\mathrm{d}C}{\mathrm{d}t} = -\mathrm{k}C^2$$

where k is the reassociation rate constant. If C_0 is the initial concentration of single-stranded DNA at time $t = 0$, integrating the above equation gives

$$\frac{C}{C_0} = \frac{1}{1 + \mathrm{k}\cdot C_0 t}.$$

When the reassociation is half complete, $C/C_0 = 0.5$ and the above equation simplifies to

$$C_0 t_{1/2} = \frac{1}{\mathrm{k}}.$$

Thus the greater the $C_0 t_{1/2}$ value, the slower the reaction time at a given DNA concentration. More important, for a given DNA concentration the half-period for reassociation is proportional to the number of different types of fragments (sequences) present and thus to the genome size (Britten & Kohne 1968). This can best be seen from the data in Table 2.1. Because the rate of reassociation depends on the concentration of complementary sequences, the $C_0 t_{1/2}$ for organism B will be 200 times greater than for organism A.

Experimentally it has been shown that the rate of reassociation is indeed dependent on genome size (Fig. 2.3). However, this proportionality is only true in the absence of repeated sequences. When the

Table 2.1 Comparison of sequence copy number for two organisms with different genome sizes.

	Organism A	**Organism B**
Starting DNA concentration (C_0)	10 pg ml^{-1}	10 pg ml^{-1}
Genome size	0.01 pg	2 pg
No. of copies of genome per ml	1000	5
Relative concentration (A vs. B)	200	1

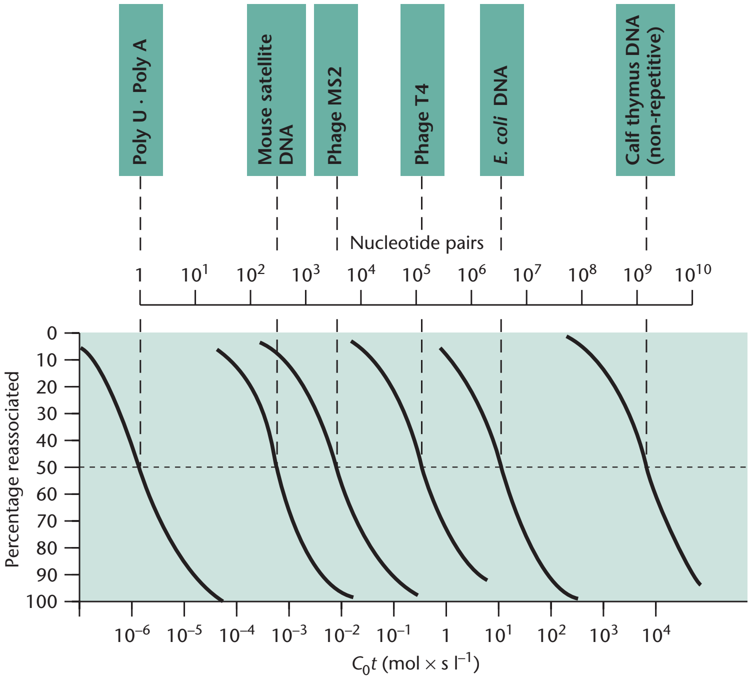


Fig. 2.3 Reassociation of double-stranded nucleic acids from various sources. (Redrawn from Lewin 1994 by permission of Oxford University Press.)

reassociation of calf thymus DNA was first studied, kinetic analysis indicated the presence of two components (Fig. 2.4). About 40% of the DNA had a $C_0t_{1/2}$ of 0.03, whereas the remaining 60% had a $C_0t_{1/2}$ of 3000. Thus the concentration of DNA sequences that reassociate rapidly is 100 000 times, the concentration of those sequences that reassociate slowly. If the slow fraction is made up of unique sequences, each of which occurs only once in the calf genome, then the sequences of the rapid fraction must be repeated 100 000 times, on average. Thus the $C_0t_{1/2}$ value can be used to determine the sequence complexity of a DNA preparation. A comparative analysis of DNA from different sources has shown that repetitive DNA occurs widely in eukaryotes (Davidson & Britten 1973) and that different

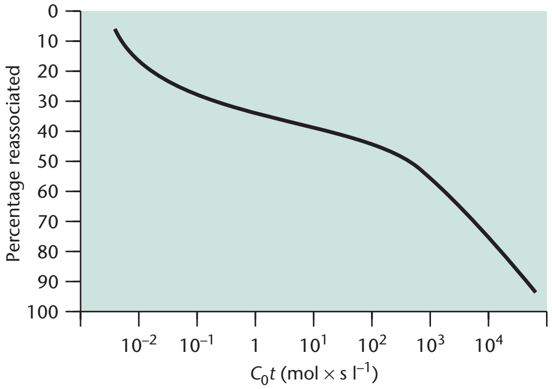
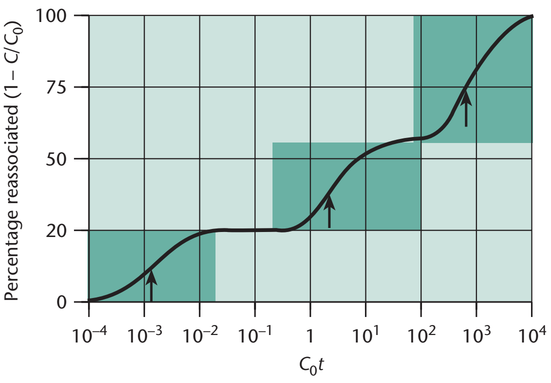


Fig. 2.4 The kinetics of reassociation of calf thymus DNA. Compare the shape of the curve with those shown in Fig. 2.3.

types of repeat are present. In the example shown in Fig. 2.5 a fast-renaturing and an intermediate-renaturing component can be recognized and are present in different copy numbers (500 000 and 350, respectively) relative to the slow component which is unique or non-repetitive DNA. The complexities of each of these components are 340 bp, 6 × 10^5 bp and 3 × 10^8 bp, respectively. The proportion of the genome that is occupied by non-repetitive DNA versus repetitive DNA varies in different organisms (Fig. 2.6), thus resolving the C-value paradox. In general, the length of the non-repetitive DNA component tends to increase as we go up the evolutionary tree to a maximum of 2 × 10^9 bp in mammals. The fact that many plants and animals have a much higher C-value is a reflection of the presence of large amounts of repetitive DNA. Analysis of messenger RNA (mRNA) hybridization to DNA shows that most of it anneals to non-repetitive DNA, i.e. most genes are present in non-repetitive DNA. Thus genetic complexity is proportional to the content of non-repetitive DNA and not to genome size.



	Fast component	Intermediate component	Slow component
Per cent of genome	25	30	45
$C_0t_{1/2}$	0.0013	1.9	630
Complexity (bp)	340	6.0 × 10^5	3.0 × 10^8
Repetition frequency	500 000	350	1

Fig. 2.5 The reassociation kinetics of a eukaryotic DNA sample showing the presence of two types of repeated DNA. The arrows indicate the $C_0t_{1/2}$ values for the three components. (Redrawn from Lewin 1994 by permission of Oxford University Press.)

Introns and exons

Introns were initially discovered in the chicken ovalbumin and rabbit and mouse β-globin genes (Breatnach *et al.* 1977; Jeffreys & Flavell 1977). Both these genes had been cloned by isolating the mRNA from expressing cells and converting it to complementary DNA (cDNA). The next step was to use the cloned cDNA to investigate possible

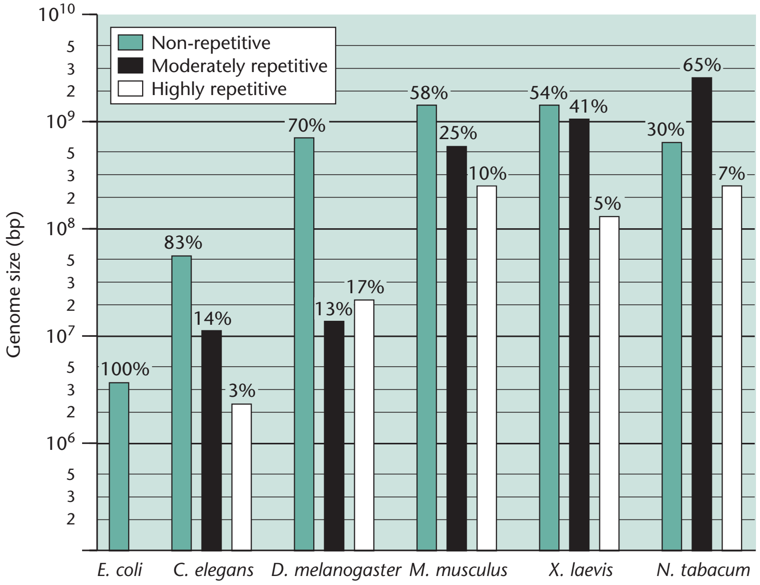


Fig. 2.6 The proportions of different sequence components in representative eukaryotic genomes. (Redrawn from Lewin 1994 by permission of Oxford University Press.)

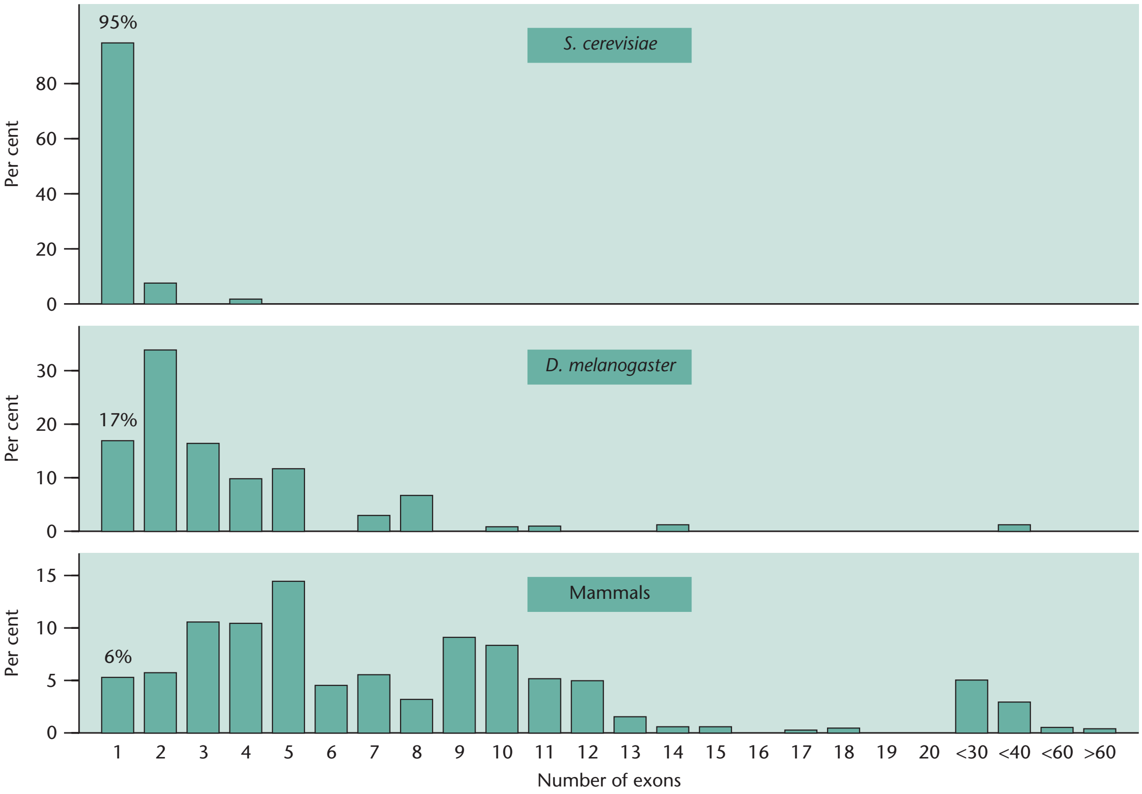


Fig. 2.7 The number of exons in three representative eukaryotes. Uninterrupted genes have only one exon and are totalled in the left-hand column. (Redrawn from Lewin 1994 by permission of Oxford University Press.)

differences in the structure of the gene from expressing and non-expressing cells. Here the Southern blot hybridizations revealed a totally unanticipated situation. It was expected that the analysis of genomic restriction fragments generated by enzymes that did not cut the cDNA would reveal only a single band corresponding to the entire gene. Instead several bands were detected in the hybridized blots. The data could be explained only by assuming the existence of interruptions in the middle of the protein-coding sequences. Furthermore, these insertions appeared to be present in both expressing and non-expressing cells. The gene insertions that are not translated into protein were termed *introns* and the sequences that are translated were called *exons*.

Since the original discovery of introns, a large number of split genes has been identified in a wide variety of organisms. These introns are not restricted to protein-coding genes for they have been found in rRNA and tRNA genes as well. Split genes are rare in prokaryotes (Edgell *et al*. 2000; Martinez-Abarca & Toro 2000). They also are not particularly common in lower eukaryotes (see below) but the mitochondrial DNA of *Euglena* is an exception with 38% of the genome consisting of intron DNA (Hallick *et al*. 1993).

In *Saccharomyces cerevisiae*, sequencing of the complete genome suggests that there are 235 introns compared with over 6000 open-reading frames and that introns account for less than 1% of the genome (Goffeau *et al*. 1996). Those genes which do have introns usually have only one small one and the longest intron is only 1 kb in size.

However, proceeding up the evolutionary tree, the number of split genes, and the number and size of introns per gene, increases (Fig. 2.7 and Table 2.2). More important, genes that are related by evolution have exons of similar size, i.e. the introns are in the same position. However, the introns may vary in length, giving rise to variation in the length of

Table 2.2 Intron statistics for genes from different species.

Species	Average exon number	Average intron number	Average length (kb)	Average mRNA length (kb)	% Exon per gene
Yeast	1	0	1.6	1.6	100
Nematode	4	3	4.0	3.0	75
Fruit fly	4	3	11.3	2.7	24
Chicken	9	8	13.9	2.4	17
Mammals	7	6	16.6	2.2	13

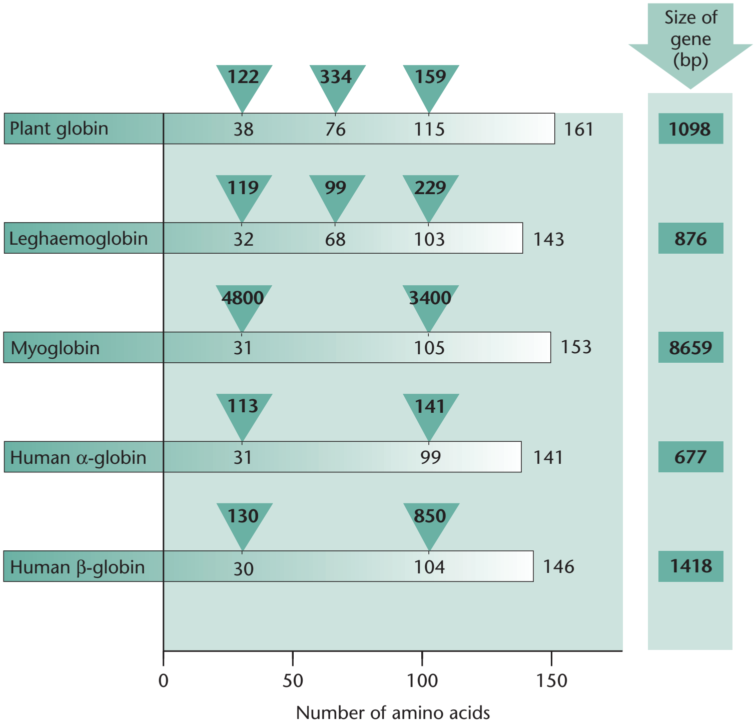


Fig. 2.8 The placement of introns in different members of the globin superfamily. The size of the introns in base pairs is indicated inside the inverted triangles. Note that the size of each polypeptide and the location of the different introns are relatively consistent.

the genes (Fig. 2.8). Note also that introns are much longer than exons, particularly in higher eukaryotes.

If a split gene has been cloned, it is possible to sub-clone either the exon or the intron sequences. If these sub-clones are used as probes in genomic Southern blots, it is possible to determine if these same sequences are present elsewhere in the genome. Often, the exon sequences of one gene are found to be related to sequences in one or more other genes. Some examples of such *gene families* are given in Table 2.3. In some instances the duplicated genes are clustered, whereas in others they are dispersed. Also, the members may have related, or even identical, functions, although they may be expressed at different times or in different cell types. Thus different globin proteins are found in embryonic and adult red blood cells, while different actins are found in muscle and non-muscle cells.

Functional divergence between members of a multigene family may extend to the loss of gene function by some members. Such *pseudogenes* come in two types. In the first type they retain the usual intron and exon structure but are functionless or

Table 2.3 Some examples of multigene families.

Gene family	Organism	Approximate no. of genes	Clustered (L) or dispersed (D)
Actin	Yeast	1	–
	Slime mould	17	L, D
	Drosophila	6	D
	Chicken	8–10	D
	Human	20–30	D
Tubulin	Yeast	3	D
	Trypanosome	30	L
	Sea urchin	15	L, D
	Mammals	25	D
α-Amylase	Mouse	3	L
	Rat	9	?
	Barley	7	?
β-Globin	Human	6	L
	Lemur	4	L
	Mouse	7	L
	Chicken	4	L

they lack one or more exons. In the second type, found in dispersed gene families, processed pseudogenes are found which lack any sequences corresponding to the introns or promoters of the functional gene members. Multiple copies of an exon also may be found because the same exons occur in several apparently unrelated genes. Exons that are shared by several genes are likely to encode polypeptide regions that endow the disparate proteins with related properties, e.g. adenosine triphosphate (ATP) or DNA binding. Some genes appear to be mosaics that were constructed by patching together copies of individual exons recruited from different genes, a phenomenon known as *exon shuffling* (see pp. 31 and 114).

By contrast with exons, introns are not related to other sequences in the genome, although they contain the majority of dispersed, highly repetitive sequences. Thus, for some genes the exons constitute slightly repetitive sequences embedded in a unique context of introns. It should be noted that introns are not necessarily junk because there now is evidence that some of them encode functional RNA (Moore 1996).

Two intron databases have been constructed (Schisler & Palmer 2000). The Intron DataBase (IDB) contains detailed information about introns and the other, the Intron Evolution DataBase, provides a statistical analysis of the intron and exon sequences catalogued in the IDB.

Genome structure in viruses and prokaryotes

The genomes of viruses and prokaryotes are very simple structures, although those of viruses show remarkable diversity (for a review see Dimmock *et al.* 2001). Most viruses have a single linear or circular genome but a few, such as reoviruses, bacteriophage ϕ6 and some plant viruses, have segmented RNA genomes. For a long time it was believed that all eubacterial genomes consisted of a single circular chromosome. However, linear chromosomes have been found in *Borrelia* sp., *Streptomyces* sp. and *Rhodococcus fascians* and mapping suggests that *Coxiella burnetii* also has a linear genome. Two chromosomes have been found in a number of bacteria including *Rhodobacter spheroides*, *Brucella melitensis*, *Leptospira interrogans* and *Agrobacterium tumefaciens* (Cole & Saint Girons 1994). In the case of *Agrobacterium*, there is one circular chromosome and one non-homologous linear chromosome (Goodner *et al.* 1999). Linear plasmids have been found in *Borrelia*

Box 2.1 The need for telomeres

The ends of eukaryotic chromosomes are also the ends of linear duplex DNA and are known as *telomeres*. That these must have a special structure has been known for a long time. For example, if breaks in DNA duplexes are not rapidly repaired by ligation they undergo recombination or exonuclease digestion, yet, the ends of chromosomes are stable and chromosomes are not ligated together. Also, DNA replication is initiated in a 5′→3′ direction with the aid of an RNA primer. After removal of this primer there is no way of completing the 5′ end of the molecule (Fig. B2.1). Thus, in the absence of a method for completing the ends of the molecules, chromosomes would become shorter after each cell division.

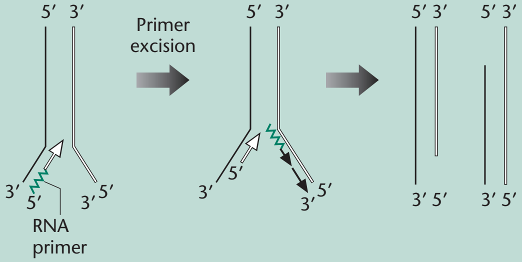


Fig. B2.1 Formation of two daughter molecules with complementary single-stranded 3′ tails after primer excision.

sp. and *Streptomyces* sp. as well as a number of bacteria with circular chromosomes (Hinnebush & Tilley 1993). *Borrelia* has a very complex plasmid content with 12 linear molecules and nine circular molecules (Casjens *et al*. 2000).

Bacterial genomes lack the centromeres found in eukaryotic chromosomes although there may be a partitioning system based on membrane adherence. Duplication of the genomes is initiated at an origin of replication and may proceed unidirectionally. The structure of the origin of replication, the *ori*C locus, has been extensively studied in a range of bacteria and found to consist essentially of the same group of genes in a nearly identical order (Cole & Saint Girons 1994). The *ori*C locus is defined as a region harbouring the *dna*A (DNA initiation) or *gyr*B (B subunit of DNA gyrase) genes linked to a ribosomal RNA operon.

Many bacterial and viral genomes are circular or can adopt a circular conformation for the purposes of replication. However, those viral and bacterial genomes which retain a linear configuration need a special mechanism to replicate the ends of the chromosome (see Box 2.1). A number of different strategies for replicating the ends of linear molecules have been adopted by viruses (see Dimmock *et al*. 2001) but in bacteria there are two basic mechanisms (Volff & Altenbuchner 2000). In *Borrelia*, the chromosomes have covalently closed hairpin structures at their termini. Such structures are also found in *Borrelia* plasmids, *Escherichia coli* phage N15, poxviruses and linear mitochondrial DNA molecules in the yeasts *Williopsis* and *Pichia*. Exactly how these hairpin structures facilitate replication of the ends of the molecule is not known. By contrast, in *Streptomyces*, the linear molecules have proteins bound to the 5′ ends of the DNA and such proteins are also found in adenoviruses, and a number of bacteriophages and fungal and plant mitochondrial plasmids. These terminal proteins probably are involved in the completion of replication. In addition, *Streptomyces* linear replicons have palindromic sequences and inverted repeats at their termini.

The bacterial genomes that have been completely sequenced have sizes ranging from 0.6 to 7.6 Mb. The difference in size between the smallest and the largest is not a result of introns for these are rare in prokaryotes (Edgell *et al*. 2000; Martinez-Abarca & Toro 2000). Nor is it a result of repeated DNA. Analysis of the kinetics of reassociation of denatured bacterial DNA did not indicate the presence of repeated DNA in *E. coli* (Britten & Kohne 1968) and only small amounts have been detected in all of the bacterial genomes that have been sequenced. In both *Mycoplasma genitalium* (0.58 Mb) and *E. coli* (4.6 Mb) about 90% of the genome is dedicated to protein-coding genes. Therefore the differences in size reflect the number of genes carried. This begs the question as to the minimal genome size possible for a bacterium. The current view is about 300 genes (Mushegian 1999; see also p. 114). In *Borrelia* the small genome size (1.5 Mb) may be complemented by the high plasmid content (see above) which constitutes 0.67 Mb. These plasmids carry 535 genes, many of which have no counterparts in other organisms, suggesting that they perform specialized functions (Casjens *et al*. 2000).

The organization of organelle genomes

Mitochondria and chloroplasts both possess DNA genomes that code for all of the RNA species and some of the proteins involved in the functions of the organelle. In some lower eukaryotes the mitochondrial (mt) DNA is linear but more usually organelle genomes take the form of a single circular molecule of DNA. Because each organelle contains several copies of the genome and because there are multiple organelles per cell, organelle DNA constitutes a repetitive sequence. Whereas chloroplast (ct) DNA falls in the range 120–200 kb, mtDNA varies enormously in size. In animals it is relatively small, usually less than 20 kb, but in plants it can be as big as 2000 kb.

Organization of the chloroplast genome

The complete sequence of ctDNA has been reported for over a dozen plants including the single-celled protist, *Euglena* (Hallick *et al*. 1993), a liverwort (Ohyama *et al*. 1986), and angiosperms such as *Arabidopsis*, spinach, tobacco and rice (Shinozaki *et al*. 1986; Hiratsuka *et al*. 1989; Sato *et al*. 1999; Schmitz-Linneweber *et al*. 2001). Overall, there is a remarkable similarity in size and organization (Fig. 2.9 and Table 2.4). The differences in size are accounted for by differences in length of introns and intergenic regions and the number of genes. A general feature of ctDNA is a 10–24 kb sequence that is present in two identical copies as an inverted repeat. The proportion of the genome that is represented by introns can be very high, e.g. in *Euglena* it is 38%.

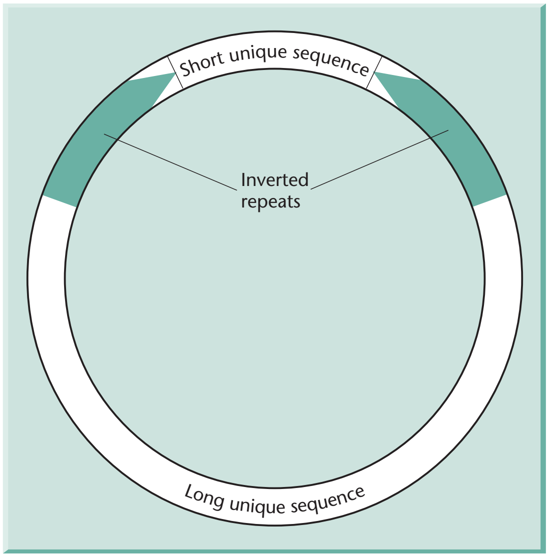


Fig. 2.9 Generalized structure of ctDNA.

The chloroplast genome encodes 70–90 proteins, including those involved in photosynthesis, four rRNA genes and 30 or more tRNA genes. Chloroplast mRNAs are translated with the standard genetic code (cf. mitochondrial mRNA). However, editing events cause the primary structures of several

Table 2.4 Key features of chloroplast DNA.

Feature	*Arabidopsis*	Spinach	Maize
Inverted repeats	26 264 bp	25 073 bp	22 748 bp
Short unique sequence	17 780 bp	17 860 bp	12 536 bp
Long unique sequence	84 170 bp	82 719 bp	82 355 bp
Length of total genome	154 478 bp	150 725 bp	140 387 bp
Number of genes	108	108	104
rRNA genes	4	4	4
tRNA genes	37	30	30
Protein-encoding genes	87	74	70

transcripts to deviate from the corresponding genomic sequences by C to U transitions with a strong bias for changes at the second codon position (Maier *et al*. 1995). This editing makes it difficult to convert chloroplast nucleotide sequences into amino acid sequences for the corresponding gene products.

Astasia longa is a colourless heterotrophic flagellate which is closely related to *Euglena gracilis*. It contains a plastid DNA that is 73 kb in length, about half the size of the ctDNA from *Euglena*. Sequencing of this plastid DNA has shown that all chloroplast genes for photosynthesis-related proteins, except that encoding the large subunit of ribulose-1, 5-bisphosphate carboxylase, are missing (Gockel & Hachtel 2000).

Organization of the mitochondrial genome

Mitochondrial DNA (mtDNA) is an essential component of all eukaryotic cells. It ensures consistency of function (cellular respiration and oxidative phosphorylation) despite the great diversity of genome organization. However, many of the genes for mitochondrial proteins are found in the nucleus. Some organisms use the standard genetic code to translate nuclear mRNAs and a different code for their mitochondrial mRNAs.

The mtDNA from animals is about 15–17 kb in size, nearly always circular and very compact. It encodes 37 genes: 13 for proteins, 22 for tRNAs and two for rRNAs. There are no introns and very little intergenic space. It has been found that mtDNA can survive in museum specimens and palaeontological remains and so is proving useful for the study of the genetic relationships of extinct animals (Hofreiter *et al*. 2001).

Plant mtDNA is much larger than that from animals and in angiosperms ranges from 200 kb to 2 Mb (i.e. larger than some bacterial genomes). Plant mtDNAs rival the eukaryotic nucleus in terms of the C-value paradox they present. That is, larger plant mt genomes do not contain more genes than smaller ones but simply have more spacer DNA. Plant mtDNAs do have introns but there is little variation in intron content and size across the range of angiosperms. As an example of the C-value paradox, *Arabidopsis* mtDNA is 367 kb in size whereas human mtDNA is 16.6 kb in size but the former has only 14 more genes (Marienfeld *et al*. 1999). Angiosperm mtDNAs are larger than their animal counterparts partly because of frequent duplications and partly because of the frequent capture of DNA sequences from the chloroplast and the nucleus. Plant mtDNAs also can lose sequences to the nucleus (Palmer *et al*. 2000).

Fungal mtDNAs resemble plant mtDNAs in that they are larger than those from animals and more heterogeneous in size, e.g. *Saccharomyces* mtDNA is 86 kb in size whereas that from *Mucor* is 34 kb (Foury *et al*. 1998; Papp *et al*. 1999). Fungal mtDNA also contains introns.

Plant mtDNAs differ from those of animals and lower eukaryotes in more than just size. At the sequence level they have an exceptionally low rate of point mutations that is 50–100 times lower than that seen in vertebrate mitochondria. At the structural level, plant mtDNAs have a high rate of genomic rearrangement and duplication.

The organization of nuclear DNA in eukaryotes

Gross anatomy

Each eukaryotic nucleus encloses a fixed number of chromosomes which contain the nuclear DNA. During most of a cell's life, its chromosomes exist in a highly extended linear form. Prior to cell division, however, they condense into much more compact bodies which can be examined microscopically after staining. The duplication of chromosomes occurs chiefly when they are in the extended stage (interphase). One part of the chromosome, however, always duplicates during the contracted metaphase state. This is the *centromere*, a body that controls the movement of the chromosome during mitosis. Its structure is discussed later (p. 30).

The ends of eukaryotic chromosomes are also the ends of linear duplex DNA and are known as *telomeres*. That these must have a special structure has been known for a long time (see Box 2.1). In most eukaryotes the telomere consists of a short repeat of TTAGGG many hundreds of units long but in *Saccharomyces cerevisiae* the repeat unit is TG_{1-3}. These repeats vary considerably in length between species (Table 2.5) but each species maintains a fixed average telomere length in its germline. The enzyme

Table 2.5 Length of the telomere repeat in different eukaryotic species.

Species	Length of telomere repeat
S. cerevisiae (yeast)	300 bp
Mouse	50 kb
Human	10 kb
Arabidopsis	2–5 kb
Cereals	12–15 kb
Tobacco	60–160 kb

telomerase is responsible for maintaining the integrity of telomeres (see Box 2.2).

The fruit fly *Drosophila* differs from most other eukaryotes in having an unusually elaborate method for forming chromosome ends. Instead of telomere repeats it has telomere-specific transposable elements (Pardue *et al*. 1996) that resemble long interspersed nuclear elements (LINEs) (see p. 28).

In many eukaryotes, a variety of treatments will cause chromosomes in dividing cells to appear as a series of light- and dark-staining bands (Fig. 2.10). In G-banding, for example, the chromosomes are subjected to controlled digestion with trypsin before Giemsa staining which reveals alternating positively (dark G-bands) and negatively (R-bands or pale G-bands) staining regions. As many as 2000 light and dark bands can be seen along some mammalian chromosomes. An identical banding pattern (Q-banding) can be seen if the Giemsa stain is replaced with a fluorescent dye such as quinacrine which intercalates between the bases of DNA. A structural basis for metaphase bands has been proposed that is based on the differential size and packing of DNA loops and matrix-attachment sites in G- vs. R-bands

Box 2.2 Telomerase, immortality and cancer

Telomeres are found at the ends of chromosomes and their role is to protect the ends of chromosomes. They also stop chromosomes from fusing to each other. Telomeres consist of repeating units of TTAGGG that can be up to 15 000 bp in length. The very ends of the chromosomes are not blunt-ended but have 3′ single-stranded overhangs of 12 or more nucleotides. The enzyme telomerase, also called telomere terminal transferase, is a ribonucleoprotein enzyme whose RNA component binds to the single-stranded end of the telomere. An associated reverse transcriptase activity is able to maintain the length and structure of telomeres by the mechanism shown in Fig. B2.2. For a detailed review of the mechanism of telomere maintenance the reader should consult the paper by Shore (2001).

Telomerase is found in fetal tissues, adult germ cells and cancer cells. In normal somatic cells the activity of telomerase is very low and each time the cells divide some of the telomere (25–200 bp) is lost. When the telomere becomes too short the chromosome no longer divides and the host cell dies by a process known as apoptosis. Thus, normal somatic cells are mortal and in tissue culture they will undergo 50–60 divisions (Hayflick limit) before they senesce. In contrast to mammals, indeterminately growing multicellular organisms, such as fish and crustaceae, maintain unlimited growth potential throughout their entire life and retain telomerase activity (for a review see Krupp *et al*. 2000).

Cancer cells can divide indefinitely in tissue culture and thus are immortal. Telomerase has been found in cancer cells at activities 10- to 20-fold greater than in normal cells. This presence of telomerase confers a selective growth advantage on cancer cells and allows them to grow uncontrollably. Telomerase is an ideal target for chemotherapy because this enzyme is active in most tumours but inactive in most normal cells.

If recombinant DNA technology is used to express telomerase in human somatic cells maintained in culture, senescence is avoided and the cells become immortal. This immortalization usually is accompanied by an increased expression of the *c-myc* oncogene to the levels seen in many cancer cell lines.

Chromosomal rearrangements involving telomeres are emerging as an important cause of human

continued