Table 1-1.
Some Genomes That Have Been Completely Sequenced

SPECIES	SPECIAL FEATURES	HABITAT	GENOME SIZE (1000s OF NUCLEOTIDE PAIRS PER HAPLOID GENOME)	NUMBER OF GENES (PROTEINS)
EUBACTERIA				
Mycoplasma genitalium	smallest genome of any known cell	human genital tract	580	468
Synechocystis sp.	photosynthetic, oxygen-generating (cyanobacterium)	lakes and streams	3573	3168
Escherichia coli	laboratory favorite	human gut	4639	4289
Helicobacter pylori	causes stomach ulcers and predisposes to stomach cancer	human stomach	1667	1590
Bacillus subtilis	bacterium	soil	4214	4099
Aquifex aeolicus	lithotrophic; lives at high temperatures	hydrothermal vents	1551	1544
Mycobacterium tuberculosis	causes tuberculosis	human tissues	4447	4402
Treponema pallidum	spirochaete; causes syphilis	human tissues	1138	1041
Rickettsia prowazekii	bacterium most closely related to mitochondria; causes typhus	lice and humans (intracellular parasite)	1111	834
Thermotoga maritima	organotrophic; lives at high temperatures	hydrothermal vents	1860	1877
ARCHAEA				
Methanococcus jannaschii	lithotrophic, anaerobic, methane-producing	hydrothermal vents	1664	1750
Archaeoglobus fulgidus	lithotrophic or organotrophic, anaerobic, sulfate-reducing	hydrothermal vents	2178	2493
Aeropyrum pernix	aerobic, organotrophic hot-steam vents	coastal volcanic	669	2620
EUCARYOTES				
Saccharomyces cerevisiae (budding yeast)	minimal model eucaryote	grape skins, beer	12,069	~6300
Arabidopsis thaliana (wall cress)	model organism for flowering plants	soil and air	~142,000	~26,000
Caenorhabditis elegans (nematode worm)	simple animal with perfectly predictable development	soil	~97,000	~19,000
Drosophila melanogaster (fruit fly)	key to the genetics of animal development	rotting fruit	~137,000	~14,000

Homo sapiens (human)	most intensively studied mammal	houses	~3,200,000	~30,000

Table 1-2.
The Numbers of Gene Families, Classified by Function, That Are Common to All Three Domains of the Living World

GENE FAMILY FUNCTION	NUMBER OF "UNIVERSAL" FAMILIES
Translation, ribosomal structure and biogenesis	61
Transcription	5
Replication, repair, recombination	13
Cell division and chromosome partitioning	1
Molecule chaperones	9
Outer membrane, cell-wall biogenesis	3
Secretion	4
Inorganic ion transport	9
Signal transduction	1
Energy production and conversion	18
Carbohydrate metabolism and transport	14
Amino acid metabolism and transport	40
Nucleotide metabolism and transport	15
Coenzyme metabolism	23
Lipid metabolism	8
General biochemical function predicted; specific biological role unknown	33
Function unknown	1

For the purpose of this analysis, gene families are defined as "universal" if they are represented in the genomes of at least two diverse archaeans *(Archaeoglobus fulgidus* and *Aeropyrum pernix),* two evolutionarily distant bacteria *(Escherichia coli* and *Bacillus subtilis)* and one eucaryote (yeast, *Saccharomyces cerevisiae).* (Data from R.L. Tatusov, E.V. Koonin, and D.J. Lipman, *Science* 278:631637, 1997; R.L. Tatusov, M.Y. Galperin, D.A. Natale, and E.V. Koonin, *Nucleic Acids Res.* 28:3336, 2000; and E.V. Koonin, personal communication.)

Table 2-1.
Atomic Characteristics of the Most Abundant Elements in Living Tissues

COMMON ELEMENTS IN LIVING ORGANISMS						
element		protons	neutrons	electrons	**atomic number**	atomic weight
Hydrogen	H	1	0	1	1	1
Carbon	C	6	6	6	6	12
Nitrogen	N	7	7	7	7	14
Oxygen	O	8	8	8	8	16
LESS COMMON ELEMENTS						

element		protons	neutrons	electrons	**atomic number**	atomic weight
Sodium	Na	11	12	11	11	23
Magnesium	Mg	12	12	12	12	24
Phosphorus	P	15	16	15	15	31
Sulfur	S	16	16	16	16	32
Chlorine	Cl	17	18	17	17	35
Potassium	K	19	20	19	19	39
Calcium	Ca	20	20	20	20	40

Table 2-2.
Covalent and Noncovalent Chemical Bonds

		STRENGTH (kcal/mole)	
BOND TYPE	LENGTH (nm)	IN VACUUM	IN WATER
Covalent	0.15	90	90
Noncovalent: ionic	0.25	80	3
hydrogen	0.30	4	1
van der Waals attraction (per atom)	0.35	0.1	0.1

Table 2-3.
The Approximate Chemical Composition of a Bacterial Cell

	PERCENT OF TOTAL CELL WEIGHT	NUMBER OF TYPES OF EACH MOLECULE
Water	70	1
Inorganic ions	1	20
Sugars and precursors	1	250
Amino acids and precursors	0.4	100
Nucleotides and precursors	0.4	100
Fatty acids and precursors	1	50
Other small molecules	0.2	~300
Macromolecules (proteins, nucleic acids, and polysaccharides)	26	~3000

Table 2-4.
Approximate Chemical Compositions of a Typical Bacterium and a Typical Mammalian Cell

	PERCENT OF TOTAL CELL WEIGHT	
COMPONENT	*E. COLI* BACTERIUM	MAMMALIAN CELL
H_2O	70	70

Inorganic ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, etc.)	1	1
Miscellaneous small metabolites	3	3
Proteins	15	18
RNA	6	1.1
DNA	1	0.25
Phospholipids	2	3
Other lipids	-	2
Polysaccharides	2	2
Total cell volume	2×10^{-12} cm^3	4×10^{-9} cm^3
Relative cell volume	1	2000

Proteins, polysaccharides, DNA, and RNA are macromolecules. Lipids are not generally classed as macromolecules even though they share some of their features; for example, most are synthesized as linear polymers of a smaller molecule (the acetyl group on acetyl CoA), and they self-assemble into larger structures (membranes). Note that water and protein comprise most of the mass of both mammalian and bacterial cells.

Table 2-5.
Relationship Between the Standard Free- Energy Change, ΔG°, and Equilibrium Constant

EQUILIBRIUM CONSTANT (liters/mole)	FREE ENERGY OF B MINUS FREE ENERGY OF A (kcal/mole)
10^5	-7.1
10^4	-5.7
10^3	-4.3
10^2	-2.8
10	-1.4
1	0
10^{-1}	1.4
10^{-2}	2.8
10^{-3}	4.3
10^{-4}	5.7
10^{-5}	7.1

Values of the equilibrium constant were calculated for the simple chemical reaction A B using the equation given in the text.The $\Delta G°$ given here is in kilocalories per mole at 37°C (1 kilocalorie is equal to 4.184 kilojoules). As explained in the text, $\Delta G°$ represents the free-energy difference under standard conditions (where all components are present at a concentration of 1.0 mole/liter).From this table, we see that if there is a favorable free-energy change of -4.3 kcal/mole for the transition A B, there will be 1000 times more molecules in state B than in state A.

Table 2-6.
Some Activated Carrier Molecules Widely Used in Metabolism

ACTIVATED CARRIER	GROUP CARRIED IN HIGH-ENERGY LINKAGE

ATP	phosphate
NADH, NADPH, $FADH_2$	electrons and hydrogens
Acetyl CoA	acetyl group
Carboxylated biotin	carboxyl group
S-Adenosylmethionine	methyl group
Uridine diphosphate glucose	glucose

Table 3-1.
Some Common Types of Enzymes

ENZYME	REACTION CATALYZED
Hydrolases	general term for enzymes that catalyze a hydrolytic cleavage reaction.
Nucleases	break down nucleic acids by hydrolyzing bonds between nucleotides.
Proteases	break down proteins by hydrolyzing bonds between amino acids.
Synthases	general name used for enzymes that synthesize molecules in anabolic reactions by condensing two smaller molecules together.
Isomerases	catalyze the rearrangement of bonds within a single molecule.
Polymerases	catalyze polymerization reactions such as the synthesis of DNA and RNA.
Kinases	catalyze the addition of phosphate groups to molecules. Protein kinases are an important group of kinases that attach phosphate groups to proteins.
Phosphatases	catalyze the hydrolytic removal of a phosphate group from a molecule.
Oxido-Reductases	general name for enzymes that catalyze reactions in which one molecule is oxidized while the other is reduced. Enzymes of this type are often called *oxidases*, *reductases*, and *dehydrogenases.*
ATPases	hydrolyze ATP. Many proteins with a wide range of roles have an energy-harnessing ATPase activity as part of their function, for example, motor proteins such as *myosin* and membrane transport proteins such as the *sodiumpotassium pump.*

Enzyme names typically end in "-ase," with the exception of some enzymes, such as pepsin, trypsin, thrombin and lysozyme that were discovered and named before the convention became generally accepted at the end of the nineteenth century. The common name of an enzyme usually indicates the substrate and the nature of the reaction catalyzed. For example, citrate synthase catalyzes the synthesis of citrate by a reaction between acetyl CoA and oxaloacetate.

Table 3-2.
Many Vitamins Provide Critical Coenzymes for Human Cells

VITAMIN	COENZYME	ENZYME-CATALYZED REACTIONS REQUIRING THESE COENZYMES
Thiamine (vitamin B_1)	thiamine pyrophosphate	activation and transfer of aldehydes
Riboflavin (vitamin B_2)	FADH	oxidationreduction
Niacin	NADH, NADPH	oxidationreduction
Pantothenic acid	coenzyme A	acyl group activation and transfer

Pyridoxine	pyridoxal phosphate	reactions involving amino acid activation
Biotin	biotin	CO_2 activation and transfer
Lipoic acid	lipoamide	acyl group activation; oxidationreduction
Folic acid	tetrahydrofolate	activation and transfer of single carbon groups
Vitamin B_{12}	cobalamin coenzymes	isomerization and methyl group transfers

Table 4-1.
Vital Statistics of Human Chromosome 22 and the Entire Human Genome

	CHROMOSOME 22	HUMAN GENOME
DNA length	48×10^6 nucleotide pairs*	3.2×10^9
Number of genes	approximately 700	approximately 30,000
Smallest protein-coding gene	1000 nucleotide pairs	not analyzed
Largest gene	583,000 nucleotide pairs	2.4×10^6 nucleotide pairs
Mean gene size	19,000 nucleotide pairs	27,000 nucleotide pairs
Smallest number of exons per gene	1	1
Largest number of exons per gene	54	178
Mean number of exons per gene	5.4	8.8
Smallest exon size	8 nucleotide pairs	not analyzed
Largest exon size	7600 nucleotide pairs	17,106 nucleotide pairs
Mean exon size	266 nucleotide pairs	145 nucleotide pairs
Number of pseudogenes**	more than 134	not analyzed
Percentage of DNA sequence in exons (protein coding sequences)	3%	1.5%
Percentage of DNA in high-copy repetitive elements	42%	approximately 50%
Percentage of total human genome	1.5%	100%

*** The nucleotide sequence of 33.8×10^6 nucleotides is known; the rest of the chromosome consists primarily of very short repeated sequences that do not code for proteins or RNA.**

**** A pseudogene is a nucleotide sequence of DNA closely resembling that of a functional gene, but containing numerous deletion mutations that prevent its proper expression. Most pseudogenes arise from the duplication of a functional gene followed by the accumulation of damaging mutations in one copy.**

Table 5-1.
The Three Steps That Give Rise to High-Fidelity DNA Synthesis

REPLICATION STEP	ERRORS PER NUCLEOTIDE POLYMERIZED
53 polymerization	1×10^5
35 exonucleolytic proofreading	1×10^2
Strand-directed mismatch repair	1×10^2
Total	1×10^9

The third step, strand-directed mismatch repair, is described later in this chapter.

Table 5-2.
Inherited Syndromes with Defects in DNA Repair

NAME	PHENOTYPE	ENZYME OR PROCESS AFFECTED
MSH2, 3, 6, MLH1, PMS2	colon cancer	mismatch repair
Xeroderma pigmentosum (XP) groups AG	skin cancer, cellular UV sensitivity, neurological abnormalities	nucleotide excision-repair
XP variant	cellular UV sensitivity	translesion synthesis by DNA polymerase δ
Ataxiatelangiectasia (AT)	leukemia, lymphoma, cellular γ-ray sensitivity, genome instability	ATM protein, a protein kinase activated by double-strand breaks
BRCA-2	breast and ovarian cancer	repair by homologous recombination
Werner syndrome	premature aging, cancer at several sites, genome instability	accessory 3-exonuclease and DNA helicase
Bloom syndrome	cancer at several sites, stunted growth, genome instability	accessory DNA helicase for replication
Fanconi anemia groups AG	congenital abnormalities, leukemia, genome instability	DNA interstrand cross-link repair
46 BR patient	hypersensitivity to DNA-damaging agents, genome instability	DNA ligase I

Table 5-3.
Three Major Classes of Transposable Elements

Table 6-1.
Principal Types of RNAs Produced in Cells

TYPE OF RNA	FUNCTION
mRNAs	messenger RNAs, code for proteins
rRNAs	ribosomal RNAs, form the basic structure of the ribosome and catalyze protein synthesis
tRNAs	transfer RNAs, central to protein synthesis as adaptors between mRNA and amino acids
snRNAs	small nuclear RNAs, function in a variety of nuclear processes, including the splicing of pre-mRNA
snoRNAs	small nucleolar RNAs, used to process and chemically modify rRNAs
Other noncoding RNAs	function in diverse cellular processes, including telomere synthesis, X-chromosome inactivation, and the transport of proteins into the ER

Table 6-2.
The Three RNA Polymerases in Eucaryotic Cells

TYPE OF POLYMERASE	GENES TRANSCRIBED
RNA polymerase I	5.8S, 18S, and 28S rRNA genes
RNA polymerase II	all protein-coding genes, plus snoRNA genes and some snRNA genes
RNA polymerase III	tRNA genes, 5S rRNA genes, some snRNA genes and genes for other small RNAs

Table 6-3.
Inhibitors of Protein or RNA Synthesis

INHIBITOR	SPECIFIC EFFECT
Acting only on bacteria	
Tetracycline	blocks binding of aminoacyl-tRNA to A-site of ribosome
Streptomycin	prevents the transition from initiation complex to chain-elongating ribosome and also causes miscoding
Chloramphenicol	blocks the peptidyl transferase reaction on ribosomes (step 2 in Figure 6-65)
Erythromycin	blocks the translocation reaction on ribosomes (step 3 in Figure 6-65)
Rifamycin	blocks initiation of RNA chains by binding to RNA polymerase (prevents RNA synthesis)
Acting on bacteria and eucaryotes	
Puromycin	causes the premature release of nascent polypeptide chains by its addition to growing chain end
Actinomycin D	binds to DNA and blocks the movement of RNA polymerase (prevents RNA synthesis)
Acting on eucaryotes but not bacteria	
Cycloheximide	blocks the translocation reaction on ribosomes (step 3 in Figure 6-65)
Anisomycin	blocks the peptidyl transferase reaction on ribosomes (step 2 in Figure 6-65)
α-Amanitin	blocks mRNA synthesis by binding preferentially to RNA polymerase II

The ribosomes of eucaryotic mitochondria (and chloroplasts) often resemble those of bacteria in their sensitivity to inhibitors. Therefore, some of these antibiotics can have a deleterious effect on human mitochondria.

Table 6-4.
Some Biochemical Reactions That Can Be Catalyzed by Ribozymes

ACTIVITY	RIBOZYMES
Peptide bond formation in protein synthesis	ribosomal RNA
RNA cleavage, RNA ligation	self-splicing RNAs; also *in vitro* selected RNA
DNA cleavage	self-splicing RNAs
RNA splicing	self-splicing RNAs, perhaps RNAs of the spliceosome
RNA polymerizaton	*in vitro* selected RNA
RNA and DNA phosphorylation	*in vitro* selected RNA
RNA aminoacylation	*in vitro* selected RNA
RNA alkylation	*in vitro* selected RNA
Amide bond formation	*in vitro* selected RNA
Amide bond cleavage	*in vitro* selected RNA
Glycosidic bond formation	*in vitro* selected RNA
Porphyrin metalation	*in vitro* selected RNA

Table 7-1.
Some Gene Regulatory Proteins and the DNA Sequences That They Recognize

	NAME	DNA SEQUENCE RECOGNIZED*
Bacteria	lac repressor	5 AATTGTGAGCGGATAACAATT
		3 TTAACACTCGCCTATTGTTAA
	CAP	TGTGAGTTAGCTCACT
		ACACTCAATCGAGTGA
	lambda repressor	TATCACCGCCAGAGGTA
		ATAGTGGCGGTCTCCAT
Yeast	Gal4	CGGAGGACTGTCCTCCG
		GCCTCCTGACAGGAGGC
	Matα2	CATGTAATT
		GTACATTAA
	Gcn4	ATGACTCAT
		TACTGAGTA
Drosophila	Kruppel	AACGGGTTAA
		TTGCCCAATT
	Bicoid	GGGATTAGA
		CCCTAATCT
Mammals	Sp1	GGGCGG
		CCCGCC
	Oct-1 Pou domain	ATGCAAAT
		TACGTTTA
	GATA-1	TGATAG
		ACTATC
	MyoD	CAAATG
		GTTTAC
	p53	GGGCAAGTCT
		CCCGTTCAGA

*** Each protein in this table can recognize a set of closely related DNA sequences (see Figure 6-12); for convenience, only one recognition sequence, rather than a consensus sequence, is given for each protein.**

Table 7-2.
Sigma Factors of *E. coli*

SIGMA FACTOR	PROMOTERS RECOGNIZED
σ^{70}	most genes
σ^{32}	genes induced by heat shock
σ^{28}	genes for stationary phase and stress response
σ^{28}	genes involved in motility and chemotaxis
σ^{54}	genes for nitrogen metabolism

The sigma factor designations refer to their approximate molecular weights, in kilodaltons.

Table 8-1.
Composition of a Typical Medium Suitable for the Cultivation of Mammalian Cells

AMINO ACIDS	VITAMINS	SALTS	MISCELLANEOUS	PROTEINS (REQUIRED IN SERUM-FREE, CHEMICALLY DEFINED MEDIA)
Arginine	biotin	NaCl	glucose	insulin
Cystine	choline	KCl	penicillin	transferrin
Glutamine	folate	NaH_2PO_4	streptomycin	specific growth factors
Histidine	nicotinamide	$NaHCO_3$	phenol red	
Isoleucine	pantothenate	$CaCl_2$	whole serum	
Leucine	pyridoxal	$MgCl_2$		
Lysine	thiamine			
Methionine	riboflavin			
Phenylalanine				
Threonine				
Trytophan				
Tyrosine				
Valine				

Glucose is used at a concentration of 510 mM. The amino acids are all in the L form and, with one or two exceptions, are used at concentrations of 0.1 or 0.2 mM; vitamins are used at a 100-fold lower concentration, that is, about 1 μM. Serum, which is usually from horse or calf, is added to make up 10% of the total volume. Penicillin and streptomycin are antibiotics added to suppress the growth of bacteria. Phenol red is a pH indicator dye whose color is monitored to ensure a pH of about 7.4. Cultures are usually grown in a plastic or glass container with a suitably prepared surface that allows the attachment of cells. The containers are kept in an incubator at 37°C in an atmosphere of 5% CO_2, 95% air.

Table 8-2.
Some Commonly Used Cell Lines

CELL LINE*	CELL TYPE AND ORIGIN
3T3	fibroblast (mouse)
BHK21	fibroblast (Syrian hamster)
MDCK	epithelial cell (dog)
HeLa	epithelial cell (human)
PtK1	epithelial cell (rat kangaroo)
L6	myoblast (rat)
PC12	chromaffin cell (rat)
SP2	plasma cell (mouse)
COS	kidney (monkey)
293	kidney (human); transformed with adenovirus

CHO	ovary (chinese hamster)
DT40	lymphoma cell for efficient targeted recombination (chick)
R1	embryonic stem cells (mouse)
E14.1	embryonic stem cells (mouse)
H1, H9	embryonic stem cells (human)
S2	macrophage-like cells *(Drosophila)*
BY2	undifferentiated meristematic cells (tobacco)

*** Many of these cell lines were derived from tumors. All of them are capable of indefinite replication in culture and express at least some of the special characteristics of their cell of origin. BHK21 cells, HeLa cells, and SP2 cells are capable of efficient growth in suspension; most of the other cell lines require a solid culture substratum in order to multiply.**

Table 8-3.
Some Landmarks in the Development of Tissue and Cell Culture

1885 **Roux** shows that embryonic chick cells can be maintained alive in a saline solution outside the animal body.

1907 **Harrison** cultivates amphibian spinal cord in a lymph clot, thereby demonstrating that axons are produced as extensions of single nerve cells.

1910 **Rous** induces a tumor by using a filtered extract of chicken tumor cells, later shown to contain an RNA virus (Rous sarcoma virus).

1913 **Carrel** shows that cells can grow for long periods in culture provided they are fed regularly under aseptic conditions.

1948 **Earle** and colleagues isolate single cells of the L cell line and show that they form clones of cells in tissue culture.

1952 **Gey** and colleagues establish a continuous line of cells derived from a human cervical carcinoma, which later become the well-known HeLa cell line.

1954 **Levi-Montalcini** and associates show that nerve growth factor (NGF) stimulates the growth of axons in tissue culture.

1955 **Eagle** makes the first systematic investigation of the essential nutritional requirements of cells in culture and finds that animal cells can propagate in a defined mixture of small molecules supplemented with a small proportion of serum proteins.

1956 **Puck** and associates select mutants with altered growth requirements from cultures of HeLa cells.

1958 **Temin and Rubin** develop a quantitative assay for the infection of chick cells in culture by purified Rous sarcoma virus. In the following decade the characteristics of this and other types of viral transformation are established by **Stoker**, **Dulbecco**, **Green**, and other virologists.

1961 **Hayflick and Moorhead** show that human fibroblasts die after a finite number of divisions in culture.

1964 **Littlefield** introduces HAT medium for the selective growth of somatic cell hybrids. Together with the technique of cell fusion, this makes somatic-cell genetics accessible.

Kato and Takeuchi obtain a complete carrot plant from a single carrot root cell in tissue culture.

1965 **Ham** introduces a defined, serum-free medium able to support the clonal growth of certain mammalian cells.

Harris and Watkins produce the first heterocaryons of mammalian cells by the virus-induced fusion of human and mouse cells.

1968 **Augusti-Tocco and Sato** adapt a mouse nerve cell tumor (neuroblastoma) to tissue culture and isolate clones that are electrically excitable and that extend nerve processes. A number of other differentiated cell lines are isolated at about this time, including skeletal muscle and liver cell lines.

1975 **Köhler and Milstein** produce the first monoclonal antibody-secreting hybridoma cell lines.

1976 **Sato** and associates publish the first of a series of papers showing that different cell lines require different mixtures of hormones and growth factors to grow in serum-free medium.

1977 **Wigler and Axel** and their associates develop an efficient method for introducing single-copy mammalian genes into cultured cells, adapting an earlier method developed by **Graham and van der Eb**.

1986 **Martin** and **Evans** and colleagues isolate and culture pluripotent embryonic stem cells from mouse.

1998 **Thomson** and **Gearhart** and their associates isolate human embryonic stem cells.

Table 8-4.
Some Major Events in the Development of Cell-Free Systems

1897	**Buchner** shows that cell-free extracts of yeast can ferment sugars to form carbon dioxide and ethanol, laying the foundations of enzymology.
1926	**Svedberg** develops the first analytical ultracentrifuge and uses it to estimate the mass of hemoglobin as 68,000 daltons.
1935	**Pickels and Beams** introduce several new features of centrifuge design that lead to its use as a preparative instrument.
1938	**Behrens** employs differential centrifugation to separate nuclei and cytoplasm from liver cells, a technique further developed for the fractionation of cell organelles by **Claude**, **Brachet**, **Hogeboom**, and others in the 1940s and early 1950s.
1939	**Hill** shows that isolated chloroplasts, when illuminated, can perform the reactions of photosynthesis.
1949	**Szent-Györgyi** shows that isolated myofibrils from skeletal muscle cells contract upon the addition of ATP. In 1955 a similar cell-free system was developed for ciliary beating by **Hofmann-Berling**.
1951	**Brakke** uses density-gradient centrifugation in sucrose solutions to purify a plant virus.
1954	**de Duve** isolates lysosomes and, later, peroxisomes by centrifugation.
1954	**Zamecnik** and colleagues develop the first cell-free system to perform protein synthesis. A decade of intense research activity, during which the genetic code is elucidated, follows.
1957	**Meselson**, **Stahl**, **and Vinograd** develop equilibrium density-gradient centrifugation in cesium chloride solutions for separating nucleic acids.
1975	**Dobberstein and Blobel** demonstrate protein translocation across membranes in a cell-free system.
1976	**Neher and Sakmann** develop patch-clamp recording to measure the activity of single ion channels.
1983	**Lohka and Masui** makes concentrated extracts from frog eggs that performs the entire cell cycle *in vitro.*
1984	**Rothman** and colleagues reconstitute Golgi vesicle trafficking *in vitro* with a cell-free system.

Table 8-5.
Landmarks in the Development of Chromatography and Electrophoresis and their Applications to Protein Molecules

1833	**Faraday** describes the fundamental laws concerning the passage of electricity through ionic solutions.
1850	**Runge** separates inorganic chemicals by their differential adsorption to paper, a forerunner of later chromatographic separations.
1906	**Tswett** invents column chromatography, passing petroleum extracts of plant leaves through columns of powdered chalk.
1933	**Tiselius** introduces electrophoresis for separating proteins in solution.
1942	**Martin and Synge** develop partition chromatography, leading to paper chromatography on ion-exchange resins.
1946	**Stein and Moore** determine for the first time the amino acid composition of a protein, initially using column chromatography on starch and later developing chromatography on ion-exchange resins.
1955	**Smithies** uses gels made of starch to separate proteins by electrophoresis.
	Sanger completes the analysis of the amino acid sequence of bovine insulin, the first protein to be sequenced.
1956	**Ingram** produces the first protein fingerprints, showing that the difference between sickle-cell and normal hemoglobin is due to a change in a single amino acid.
1959	**Raymond** introduces polyacrylamide gels, which are superior to starch gels for separating proteins by electrophoresis; improved buffer systems allowing high-resolution separations are developed in the next few years by **Ornstein and Davis**.
1966	**Maizel** introduces the use of sodium dodecyl sulfate (SDS) for improving the polyacrylamide-gel electrophoresis of proteins.
1975	**O'Farrell** devises a two-dimensional gel system for analyzing protein mixtures in which SDS polyacrylamide-gel electrophoresis is combined with separation according to isoelectric point.

Table 8-6.
Some Reagents Commonly Used to Cleave Peptide Bonds in Proteins

	AMINO ACID 1	AMINO ACID 2
Enzyme		
Trypsin	Lys or Arg	any
Chymotrypsin	Phe, Trp, or Tyr	any
V8 protease	Glu	any
Chemical		
Cyanogen bromide	Met	any
2-Nitro-5-thiocyanobenzoate	any	Cys

The specificity for the amino acids on either side of the cleaved bond is indicated; amino acid 2 is linked to the C-terminus of amino acid 1.

Table 8-7.
Some Major Steps in the Development of Recombinant DNA and Transgenic Technology

1869	**Miescher** first isolates DNA from white blood cells harvested from pus-soaked bandages obtained from a nearby hospital.
1944	**Avery** provides evidence that DNA, rather than protein, carries the genetic information during bacterial transformation.
1953	**Watson and Crick** propose the double-helix model for DNA structure based on x-ray results of **Franklin and Wilkins**.
1955	**Kornberg** discovers DNA polymerase, the enzyme now used to produce labeled DNA probes.
1961	**Marmur and Doty** discover DNA renaturation, establishing the specificity and feasibility of nucleic acid hydridization reactions.
1962	**Arber** provides the first evidence for the existence of DNA restriction nucleases, leading to their purification and use in DNA sequence characterization by **Nathans and H**. **Smith**.
1966	**Nirenberg**, **Ochoa**, and **Khorana** elucidate the genetic code.
1967	**Gellert** discovers DNA ligase, the enzyme used to join DNA fragments together.
1972-1973	DNA cloning techniques are developed by the laboratories of **Boyer**, **Cohen**, **Berg**, and their colleagues at Stanford University and the University of California at San Francisco.
1975	**Southern** develops gel-transfer hybridization for the detection of specific DNA sequences.
1975-1977	**Sanger and Barrell** and **Maxam and Gilbert** develop rapid DNA-sequencing methods.
1981-1982	**Palmiter and Brinster** produce transgenic mice; **Spradling and Rubin** produce transgenic fruit flies.
1982	**GenBank**, NIH's public genetic sequence database, is established at Los Alamos National Laboratory.
1985	**Mullis** and co-workers invent the polymerase chain reaction (PCR).
1987	**Capecchi** and **Smithies** introduce methods for performing targeted gene replacement in mouse embryonic stem cells.
1989	**Fields and Song** develop the yeast two-hybrid system for identifying and studying protein interactions
1989	**Olson** and colleagues describe sequence-tagged sites, unique stretches of DNA that are used to make physical maps of human chromosomes.
1990	**Lipman** and colleagues release BLAST, an algorithm used to search for homology between DNA and protein sequences.
1990	**Simon** and colleagues study how to efficiently use bacterial artificial chromosomes, BACs, to carry large pieces of cloned human DNA for sequencing.

1991	**Hood and Hunkapillar** introduce new automated DNA sequence technology.
1995	**Venter** and colleagues sequence the first complete genome, that of the bacterium *Haemophilus influenzae.*
1996	**Goffeau** and an international consortium of researchers announce the completion of the first genome sequence of a eucaryote, the yeast *Saccharomyces cerevisiae.*
1996-1997	**Lockhart** and colleagues and **Brown and DeRisi** produce DNA microarrays, which allow the simultaneous monitoring of thousands of genes.
1998	**Sulston and Waterston** and colleagues produce the first complete sequence of a multicellular organism, the nematode worm *Caenorhabditis elegans.*
2001	Consortia of researchers announce the completion of the draft human genome sequence.

Table 8-8.
Landmarks in the Development of X-ray Crystallography and NMR and Their Application to Biological Molecules

1864	**Hoppe-Seyler** crystallizes, and names, the protein hemoglobin.
1895	**Röntgen** observes that a new form of penetrating radiation, which he names x-rays, is produced when cathode rays (electrons) hit a metal target.
1912	**Von Laue** obtains the first x-ray diffraction patterns by passing x-rays through a crystal of zinc sulfide.
	W.L. Bragg proposes a simple relationship between an x-ray diffraction pattern and the arrangement of atoms in a crystal that produce the pattern.
1926	**Summer** obtains crystals of the enzyme urease from extracts of jack beans and demonstrates that proteins possess catalytic activity.
1931	**Pauling** publishes his first essays on 'The Nature of the Chemical Bond,' detailing the rules of covalent bonding.
1934	**Bernal and Crowfoot** present the first detailed x-ray diffraction patterns of a protein obtained from crystals of the enzyme pepsin.
1935	**Patterson** develops an analytical method for determining interatomic spacings from x-ray data.
1941	**Astbury** obtains the first x-ray diffraction pattern of DNA.
1951	**Pauling and Corey** propose the structure of a helical conformation of a chain of L-amino acids - the α helix - and the structure of the β sheet, both of which were later found in many proteins.
1953	**Watson and Crick** propose the double-helix model of DNA, based on x-ray diffraction patterns obtained by **Franklin and Wilkins**.
1954	**Perutz** and colleagues develop heavy-atom methods to solve the phase problem in protein crystallography.
1960	**Kendrew** describes the first detailed structure of a protein (sperm whale myoglobin) to a resolution of 0.2 nm, and **Perutz** presents a lower-resolution structure of the larger protein hemoglobin.
1966	**Phillips** describes the structure of lysozyme, the first enzyme to have its structure analyzed in detail.
1971	**Jeener** proposes the use of two-dimensional NMR, and **Wuthrich** and colleagues first use the method to solve a protein structure in the early 1980s.
1976	**Kim and Rich** and **Klug** and colleagues describe the detailed three-dimensional structure of tRNA determined by x-ray diffraction.
1977-1978	**Holmes and Klug** determine the structure of tobacco mosaic virus (TMV), and **Harrison** and **Rossman** determine the structure of two small spherical viruses.
1985	**Michel**, **Deisenhofer** and colleagues determine the first structure of a transmembrane protein (a bacterial reaction center) by x-ray crystallography. **Henderson** and colleagues obtain the structure of bacteriorhodopsin, a transmembrane protein, by high-resolution electron-microscopy methods between 1975 and 1990.

Table 9-1.
Some Important Discoveries in the History of Light Microscopy

1611	**Kepler** suggests a way of making a compound microscope.

1655 **Hooke** uses a compound microscope to describe small pores in sections of cork he calls "cells".

1674 **Leeuwenhoek** reports his discovery of protozoa. He sees bacteria for the first time nine years later.

1833 **Brown** publishes his microscopic observations of orchids, clearly describing the cell nucleus.

1838 **Schleiden** and **Schwann** propose the cell theory, stating that the nucleated cell is the unit of structure and function in plants and animals.

1857 **Kolliker** describes mitrochondria in muscle cells.

1876 **Abbé** analyzes the effects of diffraction on image formation in the microscope and shows how to optimize microscope design.

1879 **Flemming** describes with great clarity chromosome behavior during mitosis in animals.

1881 **Retzius** describes many animal tissues with a detail that has not been surpassed by any other light microscopist. During the next two decades, he, Cajal, and other histologists develop staining methods and lay the foundations of microscopic anatomy.

1882 **Koch** uses aniline dyes to stain microorganisms and identifies the bacteria that cause tuberculosis and cholera. In the following two decades, other bacteriologists, such as **Klebs** and **Pasteur**, identify the causative agents of many other diseases by examining stained preparations under the microscope.

1886 **Zeiss** makes a series of lenses, to the design of **Abbé**, that enable microscopists to resolve structures at the theoretical limits of visible light.

1898 **Golgi** first sees and describes the Golgi apparatus by staining cells with silver nitrate.

1924 **Lacassagne** and collaborators develop the first autoradiographic method to localize radiographic polonium in biological specimens.

1930 **Lebedeff** designs and builds the first inference microscope. In 1932, **Zernicke** invents the phase-contrast microscope. These two developments allow unstained living cells to be seen in detail for the first time.

1941 **Coons** uses antibiotics coupled to fluorescent dyes to detect cellular antigens.

1952 **Nomarski** devises and patents the system of differential interference contrast for the light microscope that still bears his name.

1968 **Petran** and collaborators make the first confocal microscope.

1981 **Allen** and **Inoué** perfect video-enhanced light microscopy.

1984 **Agard and Sedat** use computer deconvolution to reconstruct *Drosophilia* polytene nuclei.

1988 Commercial confocal microscopes come into widespread use.

1994 **Chalfie** and collaborators introduce green fluorescent protein (GFP) as a marker in microscopy.

Table 9-2.
Major Events in the Development of the Electron Microscope and Its Application to Cell Biology

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1897 **Thomson** announces the existence of negatively charged particles, later termed electrons.

1924 **de Broglie** proposes that a moving electron has wavelike properties.

1926 **Busch** proves that it is possible to focus a beam of electrons with a cylindrical magnetic lens, laying the foundation of electron optics.

1931 **Ruska** and colleagues build the first transmission electron microscope.

1935 **Knoll** demonstrates the feasibility of the scanning electron microscope; three years later, a prototype instrument is built by **Von Ardenne**.

1939 **Siemens** produces the first commercial transmission electron microscope.

1944 **Williams** and **Wyckoff** introduce the metal shadowing technique.

1945 **Porter**, **Claude**, and **Fullam** use the electron microscope to examine cells in tissue culture, fixing and staining them with OsO_4.

1948 **Pease** and **Baker** reliably prepare thin sections (0.10.2 μm thick) of biological material.

1952 **Palade**, **Porter**, and **Sjöstrand** develop methods of fixation and thin sectioning that enable many intracellular structures to be seen for the first time. In one of the first applications of these techniques, **Huxley** shows that skeletal muscle contains overlapping arrays or protein filaments, supporting the sliding-filament hypothesis of muscle contraction.

1953 **Porter and Blum** develop the first widely accepted ultramicrotome, incorporating many features previously introduced by **Claude** and **Sjöstrand**.

1956 **Glauert** and colleagues show that the epoxy resin Araldite is a highly effective embedding agent for electron microscopy. **Luft** introduces another embedding resin, Epon, five years later.

1957 **Robertson** describes the trilaminar structure of the cell membrane, seen for the first time in the electron microscope.

1957 Freeze-fracture techniques, initially developed by **Steere**, are perfected by **Moor and Mühlethaler**. Later (1966), **Branton** demonstrates that freeze-fracture allows the interior of the membrane to be visualized.

1959 **Singer** uses antibodies coupled to ferritin to detect cellular molecules in the electron microscope.

1959 **Brenner and Horne** develop the negative staining technique, invented four years previously by **Hall**, into a generally useful technique for visualizing viruses, bacteria, and protein filaments.

1963 **Sabatini**, **Bensch**, **and Barrnett** introduce glutaraldeyhde (usually followed by OsO_4) as a fixative for electron microscopy.

1965 Cambridge Instruments produces the first commercial scanning electron microscope.

1968 **de Rosier and Klug** describe techniques for the reconstruction of three-dimensional structures from electron micrographs.

1975 **Henderson and Unwin** determine the first structure of a membrane protein by computer-based reconstruction from electron micrographs of unstained samples.

1979 **Heuser**, **Reese**, and colleagues develop a high-resolution, deep-etching technique using very rapidly frozen specimens.

1980s **Dubochet** and colleagues introduce rapid freezing in thin films of vitreous ice for high-resolution electron microscopy.

1997- **Crowther**, **Fuller**, **Frank**, and colleagues use single-particle reconstruction to determine structures of viruses and the ribosome at high resolution (810 Å).

Table 9-3.
Some Radioisotopes in Common Use in Biological Research

ISOTOPE	HALF-LIFE
^{32}P	14 days
^{131}I	8.1 days
^{35}S	87 days
^{14}C	5570 years
^{45}Ca	164 days
^{3}H	12.3 years

The isotopes are arranged in decreasing order of the energy of the β radiation (electrons) they emit. ^{131}I also emits γ radiation. The half-life is the time required for 50% of the atoms of an isotope to disintegrate.

Table 10-1.
Approximate Lipid Compositions of Different Cell Membranes

	PERCENTAGE OF TOTAL LIPID BY WEIGHT					
LIPID	LIVER CELL PLASMA MEMBRANE	RED BLOOD CELL PLASMA MEMBRANE	MYELIN	MITOCHONDRION (INNER AND OUTER MEMBRANES)	ENDOPLASMIC RETICULUM	*E. COLI* BACTERIUM

Cholesterol	17	23	22	3	6	0
Phosphatidylethanolamine	7	18	15	25	17	70
Phosphatidylserine	4	7	9	2	5	trace
Phosphatidylcholine	24	17	10	39	40	0
Sphingomyelin	19	18	8	0	5	0
Glycolipids	7	3	28	trace	trace	0
Others	22	13	8	21	27	30

Table 11-2.
Some Ion Channel Families

Table 12-1.
Relative Volumes Occupied by the Major Intracellular Compartments in a Liver Cell (Hepatocyte)

INTRACELLULAR COMPARTMENT	PERCENTAGE OF TOTAL CELL VOLUME
Cytosol	54
Mitochondria	22
Rough ER cisternae	9
Smooth ER cisternae plus Golgi cisternae	6
Nucleus	6
Peroxisomes	1
Lysosomes	1
Endosomes	1

Table 12-2.
Relative Amounts of Membrane Types in Two Kinds of Eucaryotic Cells

MEMBRANE TYPE	PERCENTAGE OF TOTAL CELL MEMBRANE	
	LIVER HEPATOCYTE*	PANCREATIC EXOCRINE CELL*
Plasma membrane	2	5
Rough ER membrane	35	60
Smooth ER membrane	16	<1
Golgi apparatus membrane	7	10
Mitochondria		
Outer membrane	7	4
Inner membrane	32	17
Nucleus		
Inner membrane	0.2	0.7
Secretory vesicle membrane	not determined	3

Lysosome membrane	0.4	not determined
Peroxisome membrane	0.4	not determined
Endosome membrane	0.4	not determined

*** These two cells are of very different sizes: the average hepatocyte has a volume of about 5000 μm^3 compared with 1000 μm^3 for the pancreatic exocrine cell. Total cell membrane areas are estimated at about 110,000 μm^2 and 13,000 μm^2, respectively.**

Table 12-3.
Some Typical Signal Sequences

Table 13-1.
Subcellular Locations of Some Rab Proteins

PROTEIN	ORGANELLE
Rab1	ER and Golgi complex
Rab2	*cis* Golgi network
Rab3A	synaptic vesicles, secretory granules
Rab4	early endosomes
Rab5A	plasma membrane, clathrin-coated vesicles
Rab5C	early endosomes
Rab6	*medial* and *trans* Golgi cisternae
Rab7	late endosomes
Rab8	secretory vesicles (basolateral)
Rab9	late endosomes, *trans* Golgi network

Table 14-1.
Product Yields from the Oxidation of Sugars and Fats

Table 14-2.
Relative Amounts of Organelle DNA in Some Cells and Tissues

ORGANISM	TISSUE OR CELL TYPE	DNA MOLECULES PER ORGANELLE	ORGANELLES PER CELL	ORGANELLE DNA AS PERCENTAGE OF TOTAL CELLULAR DNA
MITOCHONDRIAL DNA				
Rat	liver	510	1000	1
Yeast*	vegetative	250	150	15
Frog	egg	510	10^7	99

CHLOROPLAST DNA				
Chlamydomonas	vegetative	80	1	7
Maize	leaves	2040	2040	15

*** The large variation in the number and size of mitochondria per cell in yeasts is due to mitochondrial fusion and fission.**

Table 14-3.
Some Differences Between the "Universal" Code and Mitochondrial Genetic Codes*

Table 15-1.
Some Hormone-induced Cell Responses Mediated by Cyclic AMP

TARGET TISSUE	HORMONE	MAJOR RESPONSE
Thyroid gland	thyroid-stimulating hormone (TSH)	thyroid hormone synthesis and secretion
Adrenal cortex	adrenocorticotrophic hormone (ACTH)	cortisol secretion
Ovary	luteinizing hormone (LH)	progesterone secretion
Muscle	adrenaline	glycogen breakdown
Bone	parathormone	bone resorption
Heart	adrenaline	increase in heart rate and force of contraction
Liver	glucagon	glycogen breakdown
Kidney	vasopressin	water resorption
Fat	adrenaline, ACTH, glucagon, TSH	triglyceride breakdown

Table 15-2.
Some Cell Responses in Which G-Protein-linked Receptors Activate the Inositol-Phospholipid Signaling Pathway

TARGET TISSUE	SIGNALING MOLECULE	MAJOR RESPONSE
Liver	vasopressin	glycogen breakdown
Pancreas	acetylcholine	amylase secretion
Smooth muscle	acetylcholine	contraction
Blood platelets	thrombin	aggregation

Table 15-3.
Three Major Families of Trimeric G Proteins*

FAMILY	SOME FAMILY MEMBERS	ACTION MEDIATED BY	FUNCTIONS

I	G_s	α	activates adenylyl cyclase; activates Ca^{2+} channels
	G_{olf}	α	activates adenylyl cyclase in olfactory sensory neurons
II	G_i	α	inhibits adenylyl cyclase
		βγ	activates K^+ channels
	G_o	βγ	activates K^+ channels; inactivates Ca^{2+} channels
		α and βγ	activates phospholipase C-β
	G_t (transducin)	α	activates cyclic GMP phosphodiesterase in vertebrate rod photoreceptors
III	G_q	α	activates phospholipase C-β

*** Families are determined by amino acid sequence relatedness of the α subunits. Only selected examples are shown. About 20 α subunits and at least 4 β subunits and 7 γ subunits have been described in mammals.**

Table 15-4.
Some Signaling Proteins That Act Via Receptor Tyrosine Kinases

SIGNALING LIGAND	RECEPTORS	SOME RESPONSES
Epidermal growth factor (EGF)	EGF receptor	stimulates proliferation of various cell types
Insulin	insulin receptor	stimulates carbohydrate utilization and protein synthesis
Insulin-like growth factors (IGF-1 and IGF-2)	IGF receptor-1	stimulate cell growth and survival
Nerve growth factor (NGF)	Trk A	stimulates survival and growth of some neurons
Platelet-derived growth factors (PDGF AA, BB, AB)	PDGF receptors (α and β)	stimulate survival, growth, and proliferation of various cell types
Macrophage-colony-stimulating (M-CSF)	M-CSF receptor factor	stimulates monocyte/macrophage proliferation and differentiation
Fibroblast growth factors (FGF-1 to FGF-24)	FGF receptors (FGF-R1-FGF- R4, plus multiple isoforms of each)	stimulate proliferation of various cell types; inhibit differentiation of some precursor cells; inductive signals in development
Vascular endothelial growth factor (VEGF)	VEGF receptor	stimulates angiogenesis
Ephrins (A and B types)	Eph receptors (A and B types)	stimulate angiogenesis; guide cell and axon migration

Table 15-5.
Some Signaling Proteins That Act Through Cytokine Receptors and the Jak-STAT Signaling Pathway

SIGNALING LIGAND	RECEPTOR-ASSOCIATED JAKS	STATS ACTIVATED	SOME RESPONSES

γ-interferon	Jak1 and Jak2	STAT1	activates macrophages; increases MHC protein expression
α-interferon	Tyk2 and Jak2	STAT1 and STAT2	increases cell resistance to viral infection
Erythropoietin	Jak2	STAT5	stimulates production of erythrocytes
Prolactin	Jak1 and Jak2	STAT5	stimulates milk production
Growth hormone	Jak2	STAT1 and STAT5	stimulates growth by inducing IGF-1 production
GM-CSF	Jak2	STAT5	stimulates production of granulocytes and macrophages
IL-3	Jak2	STAT5	stimulates early blood cell production

Table 16-1.
Major Types of Intermediate Filament Proteins in Vertebrate Cells

TYPES OF IF	COMPONENT POLYPEPTIDES	CELLULAR LOCATION
Nuclear	lamins A, B, and C	nuclear lamina (inner lining of nuclear envelope)
Vimentin-like	vimentin	many cells of mesenchymal origin
	desmin	muscle
	glial fibrillary acidic protein	glial cells (astrocytes and some Schwann cells)
	peripherin	some neurons
Epithelial	type I keratins (acidic)	epithelial cells and their
	type II keratins (basic)	derivatives (e.g., hair and nails)
Axonal	neurofilament proteins (NF-L, NF-M, and NF-H)	neurons

Table 16-2.
Drugs That Affect Actin Filaments and Microtubules

ACTIN-SPECIFIC DRUGS	
Phalloidin	binds and stabilizes filaments
Cytochalasin	caps filament plus ends
Swinholide	severs filaments
Latrunculin	binds subunits and prevents their polymerization
MICROTUBULE-SPECIFIC DRUGS	
Taxol	binds and stabilizes microtubules
Colchicine, colcemid	binds subunits and prevents their polymerization
Vinblastine, vincristine	binds subunits and prevents their polymerization
Nocodazole	binds subunits and prevents their polymerization

Table 17-1.
The Major Cyclins and Cdks of Vertebrates and Budding Yeast

CYCLIN-CDK COMPLEX	VERTEBRATES		BUDDING YEAST	
	CYCLIN	CDK PARTNER	CYCLIN	CDK PARTNER
G_1-Cdk	cyclin D*	Cdk4, Cdk6	Cln3	Cdk1**
G_1/S-Cdk	cyclin E	Cdk2	Cln1, 2	Cdk1
S-Cdk	cyclin A	Cdk2	Clb5, 6	Cdk1
M-Cdk	cyclin B	Cdk1**	Clb1, 2, 3, 4	Cdk1

*** There are three D cyclins in mammals (cyclins D1, D2, and D3).**

**** The original name of Cdk1 was Cdc2 in both vertebrates and fission yeast, and Cdc28 in budding yeast.**

Table 17-2.
Summary of the Major Cell-cycle Regulatory Proteins

GENERAL NAME	FUNCTIONS AND COMMENTS
Protein kinases and protein phosphatases that modify Cdks	
Cdk-activating kinase (CAK)	phosphorylates an activating site in Cdks
Wee1 kinase	phosphorylates inhibitory sites in Cdks; primarily involved in controlling entry into mitosis
Cdc25 phosphatase	removes inhibitory phosphates from Cdks; three family members (Cdc25A, B, C) in mammals; Cdc25C is the activator of Cdk1 at the onset of mitosis
Cdk inhibitory proteins (CKIs)	
Sic1 (budding yeast)	suppresses Cdk activity in G_1; phosphorylation by Cdk1 triggers its destruction
p27 (mammals)	suppresses G_1/S-Cdk and S-Cdk activities in G_1; helps cells to withdraw from cell cycle when they terminally differentiate; phosphorylation by Cdk2 triggers its ubiquitylation by SCF
p21 (mammals)	suppresses G_1/S-Cdk and S-Cdk activities following DNA damage in G_1; transcriptionally activated by p53
p16 (mammals)	suppresses G_1-Cdk activity in G_1; frequently inactivated in cancer
Ubiquitin ligases and their activators	
SCF	catalyzes ubiquitylation of regulatory proteins involved in G_1 control, including CKIs (Sic1 in budding yeast, p27 in mammals); phosphorylation of target protein usually required for this activity
APC	catalyzes ubiquitylation of regulatory proteins involved primarily in exit from mitosis, including Securin and M-cyclins; regulated by association with activating subunits
Cdc20	APC-activating subunit in all cells; triggers initial activation of APC at metaphase-to-anaphase transition; stimulated by M-Cdk activity
Hct1	maintains APC activity after anaphase and throughout G_1; inhibited by Cdk activity
Gene regulatory proteins	
E2F	promotes transcription of genes required for G_1/S progression, including genes encoding G_1/S cyclins, S-cyclins, and proteins required for DNA synthesis; stimulated when G_1-Cdk phosphorylates Rb in response to extracellular mitogens
p53	promotes transcription of genes that induce cell cycle arrest (especially p21) or apoptosis in response to DNA damage or other cell stress; regulated by association with Mdm2, which promotes p53 degradation

Table 19-1.
A Functional Classification of Cell Junctions

OCCLUDING JUNCTIONS
1. tight junctions (vertebrates only)
2. septate junctions (invertebrates mainly)
ANCHORING JUNCTIONS
Actin filament attachment sites
1. cell-cell junctions (adherens junctions)
2. cell-matrix junctions (focal adhesions)
Intermediate filament attachment sites
1. cell-cell junctions (desmosomes)
2. cell-matrix junctions (hemidesmosomes)
COMMUNICATING JUNCTIONS
1. gap junctions
2. chemical synapses
3. plasmodesmata (plants only)

Table 19-2.
Anchoring Junctions

JUNCTION	TRANSMEMBRANE ADHESION PROTEIN	EXTRACELLULAR LIGAND	INTRACELLULAR CYTOSKELETAL ATTACHMENT	INTRACELLULAR ANCHOR PROTEINS
Cell-Cell				
Adherens junction	cadherin (E-cadherin)	cadherin in neighboring cell	actin filaments	α- and β-catenins, vinculin, α-actinin, plakoglobin (γ-catenin)
Desmosome	cadherin (desmoglein, desmocollin)	desmogleins and desmocollins in neighboring cell	intermediate filaments	desmoplakins, plakoglobin (γ-catenin)
Cell-Matrix				
Focal adhesion	integrin	extracellular matrix proteins	actin filaments	talin, vinculin, α-actinin, filamin
Hemidesmosome	integrin $\alpha_6\beta_4$, BP180	extracellular matrix proteins	intermediate filaments	plectin, BP230

Table 19-3.
Some Members of the Cadherin Superfamily

NAME	MAIN LOCATION	JUNCTION ASSOCIATION	PHENOTYPE WHEN INACTIVATED IN MICE
Classical cadherins			
E-cadherin	epithelia	adherens junctions	die at blastocyst stage; embryos fail to undergo compaction
N-cadherin	neurons, heart, skeletal muscle, lens, and fibroblasts	adherens junctions and chemical synapses	embryos die from heart defects
P-cadherin	placenta, epidermis, breast epithelium	adherens junctions	abnormal mammary gland development
VE-cadherin	endothelial cells	adherens junctions	abnormal vascular development (apoptosis of endothelial cells)
Nonclassical cadherins			
Desmocollin	skin	desmosomes	unknown
Desmoglein	skin	desmosomes	blistering skin disease due to loss of keratinocyte cell-cell adhesion
T-cadherin	neurons, muscle	none	unknown
Fat (in *Drosophila*)	epithelia and CNS	none	enlarged imaginal discs and tumors
Protocadherins	neurons	chemical synapses	unknown

Table 19-4.
Some Common Proteoglycans

PROTEOGLYCAN	APPROXIMATE MOLECULAR WEIGHT OF CORE PROTEIN	TYPE OF GAG CHAINS	NUMBER OF GAG CHAINS	LOCATION	FUNCTIONS
Aggrecan	210,000	chondroitin sulfate + keratan sulfate	~130	cartilage	mechanical support; forms large aggregates with hyaluronan
Betaglycan	36,000	chondroitin sulfate/ dermatan sulfate	1	cell surface and matrix	binds TGF-β
Decorin	40,000	chondroitin sulfate/ dermatan sulfate	1	widespread in connective tissues	binds to type I collagen fibrils and TGF-β
Perlecan	600,000	heparan sulfate	215	basal laminae	structural and filtering function in basal lamina
Syndecan-1	32,000	chondroitin sulfate + heparan sulfate	13	epithelial cell surface	cell adhesion; binds FGF and other growth factors
Dally (in *Drosophila*)	60,000	heparan sulfate	13	cell surface	co-receptor for Wingless and Decapentaplegic signaling proteins

Table 19-5.
Some Types of Collagen and Their Properties

	TYPE	MOLECULAR FORMULA	POLYMERIZED FORM	TISSUE DISTRIBUTION
Fibril-forming (fibrillar)	I	$[\alpha1(I)]_2\alpha2$ (I)	fibril	bone, skin, tendons, ligaments, cornea, internal organs (accounts for 90% of body collagen)
	II	$[\alpha1(II)]_3$	fibril	cartilage, invertebral disc, notochord, vitreous humor of the eye
	III	$[\alpha1(III)]_3$	fibril	skin, blood vessels, internal organs
	V	$[\alpha1(V)]_2\alpha2(V)$ and $\alpha1(V)\alpha2(V)\alpha3(V)$	fibril (with type I)	as for type I
	XI	$\alpha1(XI)\alpha2(IX)\alpha3(XI)$	fibril (with type II)	as for type II
Fibril-associated	IX	$\alpha1(IX)\alpha2(IX)\alpha3(IX)$	lateral association with type II fibrils	cartilage
	XII	$[\alpha1(XII)]_3$	lateral association with some type I fibrils	tendons, ligaments, some other tissues
Network-forming	IV	$[\alpha1(IV)]_2\alpha2(IV)$	sheetlike network	basal lamina
	VII	$[\alpha1(VII)]_3$	anchoring fibrils	beneath stratified squamous epithelia
Transmembrane	XVII	$[\alpha1(XVII)]_3$	not known	hemidesmosomes
Others	XVIII	$[\alpha1(XVIII)]_3$	not known	basal lamina around blood vessels

Note that types I, IV, V, IX, and XI are each composed of two or three types of α chains, whereas types II, III, VII, XII, XVII, and XVIII are composed of only one type of α chain each. Only 11 types of collagen are shown, but about 20 types of collagen and about 25 types of α chains have been identified so far.

Table 19-6.
Some Types of Integrins

INTEGRIN	LIGAND*	DISTRIBUTION
$\alpha_5\beta_1$	fibronectin	ubiquitous
$\alpha_6\beta_1$	laminin	ubiquitous
$\alpha_7\beta_1$	laminin	muscle
$\alpha_L\beta_2$ (LFA-1, see p. 1411)	Ig superfamily counterreceptors	white blood cells
$\alpha_2\beta_3$	fibrinogen	platelets
$\alpha_6\beta_4$	laminin	epithelial hemidesmosomes

* **Not all ligands are listed.**

Table 19-7.
Cell Adhesion Molecule Families

	SOME FAMILY MEMBERS	Ca^{2+} OR Mg^{2+} DEPENDENCE	HOMOPHILIC OR HETEROPHILIC	CYTOSKELETON ASSOCIATIONS	CELL JUNCTION ASSOCIATIONS
Cell-Cell Adhesion					
Classical cadherins	E, N, P, VE	yes	homophilic	actin filaments (via catenins)	adherens junctions
Desmosomal cadherins	desmoglein	yes	homophilic	intermediate filaments (via desmoplakin, plakoglobin, and other proteins)	desmosomes
Ig family members	N-CAM	no	both	unknown	no
Selectins (blood cells and endothelial cells only)	L-, E-, and P-selectins	yes	heterophilic	actin filaments	no
Integrins on blood cells	$\alpha_L\beta_2$ (LFA-1)	yes	heterophilic	actin filaments	no
Cell-Matrix Adhesion					
Integrins	many types	yes	heterophilic	actin filaments (via talin, filamin, α-actinin, and vinculin)	focal adhesions
	$\alpha_6\beta_4$	yes	heterophilic	intermediate filaments (via plectin)	hemidesmosomes
Transmembrane proteoglycans	syndecans	no	heterophilic	actin filaments	no

Table 19-8.
The Polymers of the Plant Cell Wall

POLYMER	COMPOSITION	FUNCTIONS
Cellulose	linear polymer of glucose	fibrils confer tensile strength to all walls
Cross-linking glycans	xyloglucan, glucuronoarabinoxylan, and mannans	cross-link cellulose fibrils into robust network
Pectin	homogalacturonans and rhamnogalacturonans	forms negatively charged, hydrophilic network that gives compressive strength to primary walls; cell-cell adhesion
Lignin	cross-linked coumaryl, coniferyl, and sinapyl alcohols	forms strong waterproof polymer that reinforces secondary cell walls
Proteins and glycoproteins	enzymes, hydroxyproline-rich proteins	responsible for wall turnover and remodeling helps defend against pathogens

Table 21-1.
Some Signal Proteins That Are Used Over and Over Again as Inducers in Animal Development

SIGNALING PATHWAY	LIGAND FAMIILY	RECEPTOR FAMILY	EXTRACELLULAR INHIBITORS/ MODULATORS
Receptor tyrosine kinase (RTK)	EGF	EGF receptors	Argos
	FGF (Branchless)	FGF receptors (Breathless)	
	ephrins	Eph receptors	
TGFβ superfamily	TGFβ	TGFβ receptors	chordin (Sog), noggin
	BMP (Dpp)	BMP receptors	
	Nodal		
Wnt	Wnt (Wingless)	Frizzled	Dickkopf, Cerberus
Hedgehog	Hedgehog	Patched, Smoothened	
Notch	Delta	Notch	Fringe

Only a few representatives of each class of proteins are listed - mainly those mentioned in this chapter. Names peculiar to *Drosophila* are shown in parentheses. Many of the listed components have several homologs distinguished by numbers (FGF1, FGF2, etc.) or by forenames (Sonic hedgehog, Lunatic fringe). For further details, see Chapter 15.

Table 21-2.
Some Major Families of Gene Regulatory Proteins in Arabidopsis, Drosophila, C. elegans, and the Yeast *Saccharomyces cerevisiae*

FAMILY	NUMBER OF FAMILY MEMBERS PREDICTED FROM GENOME ANALYSIS			
	ARABIDOPSIS	*DROSOPHILA*	*C. ELEGANS*	YEAST
Myb	190	6	3	10
AP2/EREBP (Apetala2/ethylene-responsive-element binding protein)	144	0	0	0
bHLH (basic helix-loop-helix)	139	46	25	8
NAC	109	0	0	0
C2H2 (Zn finger)	105	291	139	53
Homeobox	89	103	84	9
MADS box	82	2	2	4
bZIP	81	21	25	21
WRKY (Zn finger)	72	0	0	0
GARP	56	0	0	0
C2C2 (Zn finger)/GATA	104	6	9	10
Nuclear hormone receptor	0	21	25	0
C6 (Zn finger)	0	0	0	52
Estimated total (including many not listed above)	1,533	635	669	209
% of genes in genome	5.9	4.5	3.5	3.5

The Table lists only those families that have at least 50 members in at least one organism. (Data from J.L. Riechmann et al., *Science* 290:21052110, 2000.)

Table 22-1.
Blood Cells

TYPE OF CELL	MAIN FUNCTIONS	TYPICAL CONCENTRATION IN HUMAN BLOOD (CELLS/LITER)
Red blood cells (erythrocytes)	transport O_2 and CO_2	5×10^{12}
White blood cells (leucocytes)		
Granulocytes		
Neutrophils (polymorphonuclear leucocytes)	phagocytose and destroy invading bacteria	5×10^9
Eosinophils	destroy larger parasites and modulate allergic inflammatory responses	2×10^8
Basophils	release histamine (and in some species serotonin) in certain immune reactions	4×10^7
Monocytes	become tissue macrophages, which phagocytose and digest invading microorganisms and foreign bodies as well as damaged senescent cells	4×10^8
Lymphocytes		
B cells	make antibodies	2×10^9
T cells	kill virus-infected cells and regulate activities of other leucocytes	1×10^9
Natural killer (NK) cells	kill virus-infected cells and some tumor cells	1×10^8
Platelets (cell fragments arising from *megakaryocytes* in bone marrow)	initiate blood clotting	3×10^{11}

Humans contain about 5 liters of blood, accounting for 7% of body weight. Red blood cells constitute about 45% of this volume and white blood cells about 1%, the rest being the liquid blood plasma.

Table 22-2.
Some Colony-stimulating Factors (CSFs) That Influence Blood Cell Formation

FACTOR	TARGET CELLS	PRODUCING CELLS	RECEPTORS
Erythropoietin	CFC-E	kidney cells	cytokine family
Interleukin 3 (IL-3)	multipotent stem cell, most progenitor cells, many terminally differentiated cells	T lymphocytes, epidermal cells	cytokine family
Granulocyte/ macrophage CSF (GM-CSF)	GM progenitor cells	T lymphocytes, endothelial cells, fibroblasts	cytokine family

Granulocyte CSF (G-CSF)	GM progenitor cells and neutrophils	macrophages, fibroblasts	cytokine family
Macrophage CSF (M-CSF)	GM progenitor cells and macrophages	fibroblasts, macrophages, endothelial cells	receptor tyrosine kinase family
Steel factor (stem cell factor)	hemopoietic stem cells	stromal cells in bone marrow and many other cells	receptor tyrosine kinase family

Table 23-1.
Variation Between Countries in the Incidence of Some Common Cancers

SITE OF ORIGIN OF CANCER	HIGH-INCIDENCE POPULATION		LOW-INCIDENCE POPULATION	
	LOCATION	INCIDENCE*	LOCATION	INCIDENCE*
Lung	USA (New Orleans, blacks)	110	India (Madras)	5.8
Breast	Hawaii (Hawaiians)	94	Israel (non-Jews)	14.0
Prostate	USA (Atlanta, blacks)	91	China (Tianjin)	1.3
Uterine cervix	Brazil (Recife)	83	Israel (non-Jews)	3.0
Stomach	Japan (Nagasaki)	82	Kuwait (Kuwaitis)	3.7
Liver	China (Shanghai)	34	Canada (Nova Scotia)	0.7
Colon	USA (Connecticut, whites)	34	India (Madras)	1.8
Melanoma	Australia (Queensland)	31	Japan (Osaka)	0.2
Nasopharynx	Hong Kong	30	UK (Southwestern)	0.3
Esophagus	France (Calvados)	30	Romania (urban Cluj)	1.1
Bladder	Switzerland (Basal)	28	India (Nagpur)	1.7
Uterus	USA (San Francisco Bay Area, whites)	26	India (Nagpur)	1.2
Ovary	New Zealand (Polynesian Islanders)	26	Kuwait (Kuwaitis)	3.3
Rectum	Israel (European and USA born)	23	Kuwait (Kuwaitis)	3.0
Larynx	Brazil (São Paulo)	18	Japan (rural Miyagi)	2.1
Pancreas	USA (Los Angeles, Koreans)	16	India (Poona)	1.5
Lip	Canada (Newfoundland)	15	Japan (Osaka)	0.1
Kidney	Canada (NWT and Yukon)	15	India (Poona)	0.7
Oral cavity	France (Bas-Rhin)	14	India (Poona)	0.4
Leukemia	Canada (Ontario)	12	India (Nagpur)	2.2
Testis	Switzerland (urban Vaud)	10	China (Tianjin)	0.6

*** Incidence = number of new cases per year per 100,000 population, adjusted for standardized population age distribution (so as to eliminate effects due merely to differences of population age distribution). Figures for cancers of breast, uterine cervix, uterus, and ovary are for women; other figures are for men. (Adapted from V.T. DeVita, S. Hellman, and S.A. Rosenberg (eds.), Cancer: Principles and Practice of Oncology, 4th edn. Philadelphia: Lippincott, 1993; based on data from C. Muir et al., Cancer Incidence in Five Continents, Vol. 5. Lyon: International Agency for Research on Cancer, 1987.)**

Table 23-2.
Viruses Associated with Human Cancers

VIRUS	ASSOCIATED TUMORS	AREAS OF HIGH INCIDENCE
DNA Viruses		
Papovavirus family		
Papillomavirus	warts (benign)	worldwide
(many distinct strains)	carcinoma of the uterine cervix	worldwide
Hepadnavirus family		
Hepatitis-B virus	liver cancer (hepatocellular carcinoma)	Southeast Asia, tropical Africa
Herpesvirus family		
Epstein-Barr virus	Burkitt's lymphoma (cancer of B lymphocytes)	West Africa, Papua New Guinea
	nasopharyngeal carcinoma	southern China, Greenland
RNA viruses		
Retrovirus family		
Human T-cell leukemia virus type I (HTLV-1)	adult T-cell leukemia/lymphoma	Japan, West Indies
Human immuno-deficiency virus (HIV, the AIDS virus)	Kaposi's sarcoma	Central and Southern Africa

For all the above viruses, the number of people infected is much larger than the numbers who develop cancer: the viruses must act in conjunction with other factors. Moreover, some of the viruses contribute to cancer only indirectly; for example, HIV, by upsetting normal cell- mediated immune defenses, allows endothelial cells to be transformed by another virus (a type of herpesvirus) and thrive as a tumor instead of being destroyed by the immune system.

Table 23-3.
Some Genetic Abnormalities Detected in Colorectal Cancer Cells

GENE	CLASS	PATHWAY AFFECTED	TUMORS WITH MUTATIONS (%)
K-Ras	oncogene	receptor tyrosine-kinase signaling	40
β-*catenin*	oncogene	Wnt signaling	510
p53	tumor suppressor	stress/genetic-damage response	60
APC	tumor suppressor	Wnt signaling	> 60
Smad4	tumor suppressor	TGFβ signaling	30
*TGF*β *receptor II*	tumor suppressor	TGFβ signaling	10
MLH1 and other DNA	tumor suppressor	DNA mismatch repair	15
mismatch repair genes			(often silenced by methylation)

Table 24-1.
Properties of the Major Classes of Antibodies in Humans

	CLASS OF ANTIBODY				
PROPERTIES	IgM	IgD	IgG	IgA	IgE
Heavy chains	μ	δ	γ	α	ε
Light chains	κ or λ	κ or λ	κ or λ	κ or λ	κ or λ

Number of four-chain units	5	1	1	1 or 2	1
Percentage of total Ig in blood	10	<1	75	15	<1
Activates complement	++++	-	++	-	-
Crosses placenta	-	-	+	-	-
Binds to macrophages and neutrophils	-	-	+	-	-
Binds to mast cells and basophils	-	-	-	-	+

Table 24-2.
Properties of Human Class I and Class II MHC Proteins

	CLASS I	CLASS II
Genetic loci	*HLA-A, HLA-B, HLA-C*	*DP, DQ, DR*
Chain structure	α chain + β_2-microglobulin	α chain + β chain
Cell distribution	most nucleated cells	antigen-presenting cells (including B cells), thymus epithelial cells, some others
Involved in presenting antigen to	cytotoxic T cells	helper T cells
Source of peptide fragments	proteins made in cytoplasm	endocytosed plasma membrane and extracellular proteins
Polymorphic domains	$\alpha_1 + \alpha_2$	$\alpha_1 + \beta_1$
Recognition by co-receptor	CD8	CD4

Table 24-3.
Some Accessory Proteins on the Surface of T Cells

PROTEIN*	SUPERFAMILY	EXPRESSED ON	LIGAND ON TARGET CELL	FUNCTIONS
CD3 complex	Ig (except for ζ)	all T cells	-	helps transduce signal when antigen-MHC complexes bind to T cell receptors; helps transport T cell receptors to cell surface
CD4	Ig	helper T cells	class II MHC	promotes adhesion to antigen-presenting cells and to target cells; signals T cell
CD8	Ig	cytotoxic T cells	class I MHC	promotes adhesion to antigen-presenting cells and infected target cells; signals T cell
CD28	Ig	most T cells	B7 proteins (CD80 and CD86)	provides signal 2 to some T cells
CTLA	Ig	activated T cells	B7 proteins (CD80 and CD86)	inhibits T cell activation
CD40 ligand	Fas ligand family	effector helper T cells	CD40	costimulatory protein that helps activate macrophages and B cells

LFA-1	integrin	most white blood cells, including all T cells	ICAM-1	promotes cell-cell adhesion

*** CD stands for cluster of differentiation, as each of the CD proteins was originally defined as a blood cell "differentiation antigen" recognized by multiple monoclonal antibodies. Their identification depended on large-scale collaborative studies in which hundreds of such antibodies, generated in many laboratories, were compared and found to consist of relatively few groups (or "clusters"), each recognizing a single cell-surface protein. Since these initial studies, however, more than 150 CD proteins have been identified.**

Table 24-4.
Properties of Some Interleukins

CYTOKINE	SOME SOURCES	SOME TARGETS	SOME ACTIONS
IL-2	all helper T cells; some cytotoxic T cells; activated mast cells	all activated T cells and B cells	stimulates proliferation and differentiation
IL-4	T_H2 cells and mast cells	B cells and T_H cells	stimulates B cell proliferation, maturation, and class switching to IgE and IgG1; inhibits T_H1 cell development
IL-5	T_H2 cells and mast cells	B cells, eosinophils	promotes proliferation and maturation
IL-10	T_H2 cells, macrophages, and dendritic cells	macrophages and T_H1 cells	inhibits macrophages and T_H1 cell development
IL-12	B cells, macrophages, and dendritic cells	naïve T cells	induces T_H2 cell development and inhibits T_H1 cell development
IFN-γ	T_H1 cells	B cells, macrophages, endothelial cells	activates various MHC genes and macrophages; increases MHC expression in many cell types
TNF-α	T_H1 cells and macrophages	endothelial cells	activates