

Second Edition

ANALYSIS OF
Biological
Development



Klaus Kalthoff

chapter 1

Analysis of Development 4

1.1 The Principle of Epigenesis 5

1.2 Developmental Periods and Stages in the Life Cycle 8

Embryonic Development Begins with Fertilization and Ends with the Completion of Histogenesis 8

Postembryonic Development Can Be Direct or Indirect 11

Adulthood Begins with the Onset of Reproduction and Ends with Death 11

1.3 Classical Analytical Strategies in Developmental Biology 12

Embryonic Cells Have Predictable Fates in Development 12

Analysis of Development Requires a Strategy of Controlled Interference 14

Isolation, Removal, and Transplantation of Embryonic Parts Are Key Strategies of Embryologists 14

1.4 Genetic Analysis of Development 17

1.5 Reductionist and Synthetic Analyses of Development 21

chapter 2

The Role of Cells in Development 23

2.1 The Principle of Cellular Continuity 24

2.2 The Cell and Its Organelles 24

2.3 Cell Shape and the Cytoskeleton 26

Cells Change Their External Shape as Well as Their Internal Order 26

Microtubules Maintain Cell Shape and Mediate Intracellular Transport 27

Microfilaments Generate Contracting Forces and Stabilize the Cell Surface 30

Intermediate Filaments Vary among Different Cell Types 31

2.4 The Cell Cycle and Its Control 32

Chromosomes Are Duplicated during S Phase 32

Chromosome Duplicates Are Split between Daughter Cells during Mitosis 32

The Cell Cytoplasm Is Divided during Cytokinesis 34

The Cyclic Activity of a Protein Complex Controls the Cell Cycle 35

2.5 Cell Membranes 36

2.6 Cellular Movement 38

2.7 Cell Junctions in Epithelia and Mesenchyme 39

2.8 Cellular Signaling 42

Intercellular Signals Vary with Respect to Distance, Speed of Action, and Complexity 42

Membrane Receptors Initiate Different Signaling Pathways 43

Adenylate Cyclase Generates cAMP as a Second Messenger 43

Phospholipase C- β Generates Diacylglycerol, Inositol Trisphosphate, and Calcium Ions as Second Messengers 44

The RTK-Ras-MAPK Pathway Activates Transcription Factors 45

Signal Transduction Pathways Are Linked with One Another and with Cell Adhesion 46

chapter 3

Gametogenesis 48

3.1 The Discovery of the Mammalian Egg 49

3.2 The Germ Line Concept and the Dual Origin of Gonads 50

3.3 Meiosis 51

Homologous Chromosomes Are Separated during Meiosis 51

The Timing of Meiosis Differs between Males and Females 52

Meiosis Promotes Genetic Variation, Helps to Establish Homozygous Mutant Alleles, and Eliminates Bad Genes 54

3.4 Spermatogenesis 55

Male Germ Cells Develop in Seminiferous Tubules 55

Spermiogenesis 57

Spermatogonia Behave as Stem Cells 58

3.5 Oogenesis 59

Oocytes Are Supplied with Large Amounts of RNA 60

Method 3.1 Autoradiography 61

Yolk Proteins for Oocytes Are Synthesized in the Liver or Fat Body 64

The Ovarian Anatomy Imposes Polarity on the Oocyte 66

Maturation Processes Prepare the Oocyte for Ovulation and Fertilization	67
Eggs Are Protected by Elaborate Envelopes	71

chapter 4

Fertilization 75

4.1 Interactions before Sperm-Egg Adhesion	76
Some Sperm Must Undergo Capacitation before They Can Fertilize Eggs	77
Many Sperm Are Attracted to Eggs by Chemical Signals	77
4.2 Fertilization in Sea Urchins	78
Sea Urchin Sperm Undergo the Acrosome Reaction before They Adhere to the Vitelline Envelope	79
Method 4.1 Immunostaining	80
Sea Urchin Sperm Adhere to Eggs with an Acrosomal Protein Called Bindin	81
Gamete Fusion Leads to the Formation of a Fertilization Cone	82
4.3 Fertilization in Mammals	83
Mouse Sperm Undergo the Acrosome Reaction after They Adhere to the Zona Pellucida	83
A Bioassay Is a Powerful Strategy to Reveal a Biologically Active Component	85
Mouse Sperm Adhere to a Specific Zona Pellucida Protein	85
Antibodies to Sperm-Egg Adhesion Proteins Can Act as Contraceptives	87
4.4 Egg Activation	88
Egg Activation May Be Triggered by Different Signaling Mechanisms	88
A Temporary Rise in Ca ²⁺ Concentration Is Followed by Activation of Protein Kinase C	89
Egg Activation Triggers the Completion of Meiosis and the Fusion of the Gametes' Haploid Genomes	90
Activation Accelerates the Egg's Metabolism in Preparation for Cleavage	91
4.5 Blocks to Polyspermy	91
The Fertilization Potential Serves as a Fast Block to Polyspermy	92
The Cortical Reaction Causes a Slow Block to Polyspermy	93
4.6 The Principle of Overlapping Mechanisms	93
4.7 Parthenogenesis	95

chapter 5

Cleavage 97

5.1 Yolk Distribution and Cleavage Pattern	98
5.2 Cleavage Patterns of Representative Animals	100
Sea Urchins Have Isolecithal Eggs and Undergo Holoblastic Cleavage	100
Amphibians Have Mesolecithal Eggs but Still Cleave Holoblastically	101
Snails Have Isolecithal Eggs and Follow a Spiral Cleavage Pattern	102

The Ascidian Cleavage Pattern Is Bilaterally Symmetrical	102
Mammalian Eggs Show Rotational Cleavage	103
Eggs with Variable Cleavage Show Regulative Development	106
Birds, Reptiles, and Many Fishes Have Telolecithal Eggs and Undergo Discoidal Cleavage	107
Insects Have Centrolecithal Eggs and Undergo Superficial Cleavage	109
5.3 Spatial Control of Cleavage: Positioning and Orientation of Mitotic Spindles	111
Actin and Myosin Form the Contractile Ring in Cytokinesis	111
The Mitotic Spindle Axis Determines the Orientation of the Cleavage Plane	111
Mechanical Constraints May Orient Mitotic Spindles	113
Centrosomes Organize Mitotic Spindles in Regular Ways during Cleavage	114
Specific Sites in the Egg Cortex Attract and Anchor Centrosomes	114
Maternal Gene Products May Orient Mitotic Spindles	117
5.4 The Timing of Cleavage Divisions	119
The Cell Cycle Slows Down during the Midblastula Transition (MBT)	119
Stage- and Region-Specific Gene Activities Modulate the Basic Cell Cycle after MBT	120
The Nucleocytoplasmic Ratio May Trigger MBT According to a Titration Model	120

chapter 6

Cell Fate, Potency, and Determination 124

6.1 Fate Mapping	125
6.2 The Strategy of Clonal Analysis	126
Method 6.1 Labeling Cells by Somatic Crossover	128
6.3 Potency of Embryonic Cells	129
6.4 Determination of Embryonic Cells	130
Cell Determination Is Discovered through Operational Criteria	130
<i>Drosophila</i> Blastoderm Cells Are Determined to Form Structures within a Single Segment	130
<i>Drosophila</i> Cells Are Born with a Bias That Is Honed by Subsequent Interactions	132
Prospective Neural Plate Cells of Amphibians Are Determined during Gastrulation	135
Mouse Embryonic Cells Are Not Determined until the Blastocyst Stage	136
6.5 Properties of the Determined State	138
Cell Determination Occurs as Part of Embryonic Pattern Formation	138
Determination Is a Stepwise Process of Instruction and Commitment	139
The Determined State Is (Almost) Stably Passed On during Mitosis	141
6.6 Regulation in Development	143

chapter 7

Genomic Equivalence and the Cytoplasmic Environment 147

7.1 Theories of Cell Differentiation	148
7.2 Observations on Cells	149
Cells May Carry Out Different Functions at Different Times	149
Cells Change Their Differentiated State during Regeneration	150
7.3 Observations on Chromosomes	151
Different Cells from the Same Individual Show the Same Sets of Chromosomes	152
Polytene Chromosomes Show the Same Banding Pattern in Different Tissues	152
Chromosome Elimination Is Associated with the Dichotomy between Germ Line and Somatic Cells	154
7.4 Molecular Data on Genomic Equivalence	154
7.5 Totipotency of Differentiated Plant Cells	157
7.6 Totipotency of Nuclei from Embryonic Animal Cells	157
Newt Blastomeres Develop Normally after Delayed Nucleation	158
Nuclei from Embryonic Cells Are Still Totipotent	158
7.7 Pluripotency of Nuclei from Differentiated Animal Cells	160
Nuclei from Older Donor Cells Show a Decreasing Ability to Promote the Development of a New Organism	160
Nuclei from Mature Cells Are Unprepared for the Fast Mitotic Cycles of Early Frog Embryos	161
Some Differentiated Cells Contain Highly Pluripotent Nuclei	161
Mammals Can Be Cloned by Fusing Fetal or Adult Cells with Enucleated Eggs	162
Nuclear Transfer Experiments with Mammals Have Important Applications in Science, Agriculture, and Medicine	165
Will Humans Be Cloned in the Future?	165
7.8 Control of Nuclear Activities by the Cytoplasmic Environment	166
Gene Expression Changes upon Transplantation of Nuclei to New Cytoplasmic Environments	166
Cell Fusion Exposes Nuclei to New Cytoplasmic Signals	168

chapter 8

Localized Cytoplasmic Determinants 171

8.1 The Principle of Cytoplasmic Localization	172
8.2 Polar Lobe Formation as a Means of Cytoplasmic Localization	173
8.3 Germ Cell Determinants in Insect Eggs	174
Rescue Experiments Can Restore Defective Embryos to Normal Development	175

Heterotopic Transplantation Tests Cytoplasmic Determinants for Activity in Abnormal Locations 176

Localization of Polar Granules Requires RNA Transport Along Microtubules 178

Method 8.1 In Situ Hybridization 179

8.4 Bicoid mRNA in *Drosophila* Eggs 180

8.5 Myoplasm in Ascidian Eggs 181

Cytoplasmic Components of Ascidian Eggs Are Segregated upon Fertilization 182

Myoplasm Is Necessary and Sufficient for Tail Muscle Formation 183

Myoplasm Segregation Involves a Plasma Membrane Lamina 185

Myoplasm Is Associated with Localized Maternal RNAs 186

8.6 Cytoplasmic Localization at Advanced Embryonic Stages 186

8.7 Bioassays for Localized Cytoplasmic Determinants 187

8.8 The Principle of Default Programs 188

8.9 Properties of Localized Cytoplasmic Determinants 190

Cytoplasmic Determinants Control Certain Cell Lineages or Entire Body Regions 190

Localized Cytoplasmic Determinants May Be Activating or Inhibitory 191

Cytoplasmic Determinants May or May Not Be Associated with Visible Markers 192

Most Localized Cytoplasmic Determinants Are Maternal mRNAs 193

Cytoplasmic Localization Occurs at Different Stages of Development Using Various Cellular Mechanisms 193

chapter 9

Axis Formation and Mesoderm Induction 196

9.1 Body Axes and Planes 197

9.2 Generation of Rhizoid-Thallus Axis in *Fucus* 199

9.3 Determination of the Animal-Vegetal Axis in Amphibians 201

The Animal-Vegetal Axis Originates by Oriented Transport during Oogenesis 202

The Animal-Vegetal Polarity Determines the Spatial Order of the Germ Layers 202

Vegetal Blastomeres Induce Their Animal Neighbors to Form Mesoderm 203

9.4 The Principle of Induction 205

9.5 Determination of the Dorsovenral Axis in Amphibians 205

Deep Cytoplasm Undergoes Regular Movements during Egg Activation 206

Cytoplasmic Rearrangements Following Fertilization Involve Cortical Rotation 206

Microtubules Move Cytoplasmic Components Dorsally beyond Cortical Displacement	209
A Dorsalizing Activity Moves from the Vegetal Pole to the Dorsal Side During Cortical Rotation	209
Dorsal Vegetal and Equatorial Blastomeres Rescue Ventralized Embryos	211
9.6 Effect of Dorsoventral Polarity on Mesoderm Induction in <i>Xenopus</i>	213
Mesoderm Is Induced with a Rudimentary Dorsoventral Pattern	213
Dorsal Marginal Cells Induce an Array of Mesodermal Organ Rudiments	214
9.7 Molecular Mechanisms of Dorsoventral Axis Formation and Mesoderm Induction	216
β -Catenin May Specify Dorsoventral Polarity	217
Several Growth Factors Have Mesoderm-Inducing Activity	218
TGF- β Members and β -Catenin May Act Combinatorially in Inducing Spemann's Organizer	219
9.8 Determination of Left-Right Asymmetry	219

chapter 10

Gastrulation	223
10.1 The Analysis of Morphogenesis	224
Morphogenesis Involves Typical Epithelial Movements	225
Morphogenesis Is Based on a Small Repertoire of Cell Activities	225
10.2 Gastrulation in Sea Urchins	227
10.3 Gastrulation in Amphibians	232
Different Gastrula Areas Show Distinct Cellular Behaviors	232
Bottle Cells Generate the Initial Depression of the Blastopore	234
Deep Marginal Zone Cells Are Necessary for Involution	234
Deep Zone Cells and Involuting Marginal Zone Cells Migrate on the Inside of the Blastocoel Roof	236
Convergent Extension Is Especially Strong in the Dorsal Marginal Zone	236
Animal Cap and Noninvoluting Marginal Zone Undergo Epiboly	239
How Are Patterns of Cell Behavior Related to Gene Expression?	239
10.4 Gastrulation in Fishes and Birds	241
Zebrafish Embryos Develop from Two Cell Layers Mostly by Convergent Extension	241
Chicken Embryos Develop from One Cell Layer Mostly by Ingression	241
10.5 Gastrulation in Humans	247

chapter 11

Cell Adhesion and Morphogenesis	251
11.1 Cell Aggregation Studies in Vitro	253
Cells from Different Tissues Adhere to One Another Selectively	253

Tissues Form Hierarchies of Adhesiveness	254
11.2 Cell Adhesion Molecules	256
Immunoglobulin-like CAMs May Allow or Hinder Cell Adhesion	256
Method 11.1 Isolating Cell Adhesion Molecules and Their Genes with Antibodies	257
Cadherins Mediate Ca^{2+} -Dependent Cell Adhesion	258
Lectins Bind Heterotypically to Sugar Residues	259
11.3 ECM Molecules and Their Cellular Receptors	261
Glycosaminoglycans and Proteoglycans Form an Amorphous, Hydrophilic Ground Substance	261
Fibrous Glycoproteins Make Up the Dynamic Meshwork of the ECM	263
Integrins Mediate Cell Adhesion to ECM Molecules	264
11.4 The Role of Cell and Substrate Adhesion Molecules in Morphogenesis	265
CAM Expression Is Correlated with Cell Fates	266
Cell Adhesiveness Changes during Sea Urchin Gastrulation	266
CAMs Facilitate the Formation of Cell Junctions	268
Fibrous ECM Components Provide Contact Guidance to Cells	269
Newt Gastrulation Requires Fibronectin on the Inner Surface of the Blastocoel Roof	271
11.5 Morphoregulatory Roles of Cell and Substrate Adhesion Molecules	272
CAM and SAM Genes Are Controlled by Selector Genes	273
Cell-Cell Adhesion and Cell-Substratum Interactions Affect Gene Activity	275

chapter 12

Neurulation and Axis Induction	278
12.1 Neurulation as an Example of Organogenesis	280
Neurulation Is of Scientific and Medical Interest	280
Neurulation in Amphibians Occurs in Two Phases	281
Neural Tubes of Bird Embryos Have Hinges	282
In Humans, Neural Tube Closure Begins in the Neck Region	283
In Fishes, the Neural Tube Originates as a Solid Rod	283
12.2 Mechanisms of Neurulation in Amphibians	283
Neurulation Depends on Tissues Adjacent to the Neural Plate	285
Neural Plate Cells Undergo Columnarization	285
Intercalation of Neural Plate Cells Causes Convergent Extension	286
Both Columnarization and Cell Intercalation Contribute to Generating the Keyhole Shape	287
Neural Tube Closure Is Associated with Apical Constriction, Rapid Anteroposterior Extension, and Cell Crawling	288
12.3 The Role of Induction in Axis Formation	289
The Dorsal Blastopore Lip Organizes the Formation of an Entire Embryo	290
Does the Organizer Have "Structure"?	292
Axis Induction Shows Regional Specificity	293

12.4 Pathways of Neural Induction	295
There Are Two Signaling Pathways—Planar and Vertical—for Neural Induction	295
Planar Induction Plays a Major Role in <i>Xenopus</i> Embryos	296
Neural Induction Is a Multistep Process	298
12.5 Axis Induction by Disinhibition	299
Dorsal Development Occurs as a Default Program in <i>Xenopus</i>	299
Spemann's Organizer Inactivates a Ventralizing Signal	300
Basic Questions Are Still Unresolved	301

chapter 13

Ectodermal Organs 303

13.1 Neural Tube	306
Nervous Tissue Consists of Neurons and Glial Cells	306
The Spinal Cord Is Patterned by Signals from Adjacent Tissues	308
The Basic Organization of the Spinal Cord Is Modified in the Brain	312
The Peripheral Nervous System Is of Diverse Origin	317
13.2 Neural Crest	318
NC Cells Arise at the Boundary between Neural Plate and Epidermis	319
NC Cells Have Different Migration Routes and a Wide Range of Fates	319
The Strategy of Clonal Analysis Shows That NC Cells Are a Heterogeneous Population of Pluripotent Cells	323
The Strategies of Heterotopic and Heterochronic Transplantation Reveal Spatial and Temporal Restrictions to NC Cell Migration	325
Extracellular Matrix Affects the Determination of NC Cells	327
Region-Specific Growth Factors Are Involved in NC Cell Determination	328
13.3 Ectodermal Placodes	329
The Otic Placode Forms the Inner Ear	329
The Lens Placode Develops Together with the Retina	330
Nasal Placodes Form Olfactory Sensory Epithelia	330
13.4 Epidermis	331

chapter 15

The Use of Mutants and Transgenic Organisms in the Analysis of Development 374

15.1 The Historical Separation of Genetics from Developmental Biology	376
--	------------

chapter 14

Endodermal and Mesodermal Organs 336

14.1 Endodermal Derivatives	337
The Embryonic Pharynx Contains a Series of Arches	338
Embryos Pass through a Phylotypic Stage after Organogenesis	341
The Endoderm Lines the Inside of the Intestine and Its Appendages	344
14.2 Axial and Paraxial Mesoderm	345
Axial Mesoderm Forms the Prechordal Plate and the Notochord	345
Paraxial Mesoderm Forms Presomitic Plates and Somites	346
Somites Are Patterned by Signals from Surrounding Organ Rudiments	349
14.3 Connective Tissue and Skeletal Muscle	351
Connective Tissue Contains Large Amounts of Extracellular Matrix	351
Skeletal Muscle Fibers Arise through Cell Fusion	352
14.4 Intermediate Mesoderm	354
14.5 The Principle of Reciprocal Interaction	357
14.6 Lateral Plates	358
The Lateral Plates Surround the Coelomic Cavities	358
The Cardiovascular System Develops from Various Mesodermal Precursors	359
Cardiovascular System Development Recapitulates the Phylotypic Stage	360
Cardiac Muscle and Smooth Muscle Consists of Single Cells	364
14.7 Extraembryonic Membranes	365
The Amnion and Chorion Are Formed by Layers of Ectoderm and Mesoderm	365
The Yolk Sac and Allantois Are Formed by Layers of Endoderm and Mesoderm	365
The Mammalian Placenta Is Formed from the Embryonic Trophoblast and the Uterine Endometrium	367

15.2 Modern Genetic Analysis of Development 377

Mutants Reveal the Hidden "Logic" of Embryonic Development	378
Method 15.1 The Generation and Maintenance of Mutants	379
<i>Drosophila</i> Mutants Allow the Analysis of a Complex Body Pattern	380
Method 15.2 Saturation Mutagenesis Screens	381

<i>Caenorhabditis elegans</i> Mutants Uncover Gene Activities Controlling Cell Lineages	383	16.5 Chromatin Structure and Transcription	418
Genetic Analysis of the Mouse <i>Mus musculus</i> Uses Embryonic Stem Cells	383	Heterochromatic Chromosome Regions Are Not Transcribed	418
The Zebrafish <i>Danio rerio</i> Is Genetically Tractable and Suitable for Cell Transplantation	384	Puffs in Polytene Chromosomes Are Actively Transcribed	419
Genetic Analysis Reveals Similar Control Circuits in Plant and Animal Development	385	DNA in Transcribed Chromatin Is Sensitive to DNase I Digestion	420
15.3 DNA Cloning and Sequencing	385	Transcriptional Control Depends on Histone Acetylation and Deacetylation	421
DNA Can Be Replicated, Transcribed, and Reverse-Transcribed in Vitro	386	16.6 Transcriptional Control and Cell Determination	422
Nucleic Acid Hybridization Allows the Detection of Specific Nucleotide Sequences	386	Combinatorial Action of Transcription Factors Explains the Stepwise Process of Cell Determination	422
Method 15.3 Polymerase Chain Reaction	387	Cell Determination May Be Based on Bistable Control Circuits of Switch Genes	422
DNA Can Be Cut and Spliced Enzymatically	388	<i>Drosophila</i> Homeotic Genes Show Switch Gene Characteristics	423
Method 15.4 Northern Blotting and Southern Blotting	389	DNA Methylation Maintains Patterns of Gene Expression	425
Method 15.5 Recombinant DNA Techniques	390		
DNA Sequencing May Reveal the Biochemical Activity of a Gene Product	392	chapter 17	
15.4 Transfection and Genetic Transformation of Cells	392	RNA Processing	429
15.5 The Strategies of Gene Overexpression, Dominant Interference, and Gene Knockout	393	17.1 Posttranscriptional Modifications of Pre-Messenger RNA	431
15.6 Germ Line Transformation	395	17.2 Control of Development by Alternative Splicing	434
The Genetic Transformation of <i>Drosophila</i> Utilizes Transposable DNA Elements	396	A Cascade of Alternative Splicing Steps Controls Sex Development in <i>Drosophila</i>	436
Mammals Can Be Transformed by Injecting Transgenes Directly into an Egg Pronucleus	398	Alternative Splicing of Calcitonin and Neuropeptide mRNA Is Regulated by Blockage of the Calcitonin-Specific Splice Acceptor Site	441
Transgenic Mice Can Be Raised from Transformed Embryonic Stem Cells	400	Method 17.1 Ribonuclease Protection Assay	443
DNA Insertion Can Be Used for Mutagenesis and Promoter Trapping	402	17.3 Messenger RNP Transport from Nucleus to Cytoplasm	444
Transgenic Organisms Have Many Uses in Basic and Applied Science	402	Nuclear Pore Complexes Are Controlled Gates for the Transport of RNPs to the Cytoplasm	444
		Experiments with Cloned cDNAs Indicate Differential mRNA Retention	445
chapter 16		17.4 Messenger RNA Degradation	447
Transcriptional Control	405	The Half-Life of Messenger RNAs in Cells Is Regulated Selectively	448
16.1 The Principle of Differential Gene Expression	406	Method 17.2 Pulse-Labeling of Molecules and Their Half-Life in Cells	449
16.2 Evidence for Transcriptional Control	407	The Degradation of mRNAs in Cells Is Controlled by Proteins Binding to Specific RNA Motifs	449
16.3 DNA Sequences Controlling Transcription	408		
Promoters and Enhancers Are Regulatory DNA Regions with Different Properties	409	chapter 18	
Regulatory DNA Sequences Are Studied with Fusion Genes	410	Translational Control and Posttranslational Modifications	452
16.4 Transcription Factors and Their Role in Development	411	18.1 Formation of Polysomes and Nontranslated mRNP Particles	453
General Transcription Factors Bind to All Promoters	412	Most mRNAs Are Immediately Recruited into Polysomes and Translated	454
Transcriptional Activators and Repressors Associate with Restricted Sets of Genes and May Occur Only in Certain Cells	412	Some mRNAs Are Stored as Nontranslated mRNP Particles	456
Transcriptional Activators Have Highly Conserved DNA-Binding Domains	414	18.2 Mechanisms of Translational Control	457
The bicoid Protein Acts as a Transcriptional Activator on the <i>hunchback</i> ⁺ Gene in the <i>Drosophila</i> Embryo	414	Calcium Ions and pH May Regulate the Overall Rate of Protein Synthesis at Fertilization	457
The Activity of Transcription Factors Themselves May Be Regulated	416		

Phosphorylation of Initiation Factors and Associated Proteins Controls Translation	457
Regulatory Proteins or RNAs "Mask" Critical Sequences of Specific mRNAs	458
Polyadenylation and Deadenylation Control the Translation of Specific mRNAs	460
18.3 Translational Control in Oocytes, Eggs, and Embryos	462
Early Embryos Use mRNA Synthesized during Oogenesis	463
Specific mRNAs Shift from Subribosomal mRNP Particles to Polysomes during Oocyte Maturation	464
Translation of Some mRNAs Depends on Their Cytoplasmic Localization	465
18.4 Translational Control during Spermiogenesis	467
Protamine mRNA Is Stored in Subribosomal RNP Particles before Translation	467
Messenger RNA May Be Earmarked for Storage by Sequences in Its 5' UTR or 3' UTR	468
18.5 Posttranslational Polypeptide Modifications	469
Polypeptides Are Directed to Different Cellular Destinations	469
Polypeptides May Undergo Several Posttranslational Modifications	471
Protein Degradation Is Differentially Controlled	473

chapter 19

Genetic and Paragenetic Information	476
19.1 The Principle of Genetic and Paragenetic Information	477

chapter 20

Cell Differentiation	501
20.1 The Principle of Cell Differentiation	502
Each Organism Has a Limited Number of Cell Types	503
The Differentiated State Is Generally Stable	503
The Same Cell Type May Be Formed through Different Developmental Pathways	504
20.2 Cell Differentiation and Cell Division	504
Some Cells Divide in the Differentiated State	504
Other Cell Populations Are Renewed from Stem Cells	506
Stem Cells May Be Unipotent or Pluripotent	506
20.3 Stem Cell Development in <i>Hydra</i>	506
The Organization of <i>Hydra</i> Is Relatively Simple	507
Interstitial Cells Contain Pluripotent and Unipotent Stem Cells	508
20.4 Growth and Differentiation of Blood Cells	509
The Same Universal Stem Cell Forms All Types of Blood Cells as Well as Endothelial Cells	512

19.2 Self-Assembly	478
Self-Assembly Is under Tight Genetic Control	478
The Initiation of Self-Assembly Is Accelerated by Seed Structures	479
The Conformation of Proteins May Change during Self-Assembly	480
19.3 Aided Assembly	482
Bacteriophage Assembly Requires Accessory Proteins and Occurs in a Strict Sequential Order	482
Aided Assembly Is Common in Prokaryotic and Eukaryotic Cells	483
19.4 Directed Assembly	483
One Bacterial Protein Can Assemble into Two Types of Flagella	483
Prion Proteins Occur in Normal and Pathogenic Conformations	484
Tubulin Dimers Assemble into Different Arrays of Microtubules	486
Centrioles and Basal Bodies Multiply Locally and by Directed Assembly	488
Ciliated Protozoa Inherit Accidental Cortical Rearrangements	488
19.5 Global Patterning in Ciliates	491
The Global Pattern in Ciliates Is Inherited during Fission	491
The Global Cell Pattern Is Maintained during Encystment	492
Global Patterning Is Independent of Local Assembly	492
19.6 Paragenetic Information in Metazoan Cells and Organisms	493
Progenitor Cell Commitment May Depend on Stable Control Circuits of Genes and Transcription Factors	514
Blood Cell Development Depends on Colony-Stimulating Factors (CSFs)	516
Erythrocyte Development Depends on Successive Exposure to Different CSFs	517
The Abundance of Blood Cells Is Controlled by Cell Proliferation and Cell Death	518
20.5 Genetic Control of Muscle Cell Differentiation	518
Myogenesis Is Controlled by a Family of Myogenic bHLH Proteins	519
Myogenic bHLH Proteins Have Partially Overlapping Functions in Vivo	520
Inductive Signals Regulate Myogenic bHLH Gene Activity	522
Myogenic bHLH Proteins Interact with Cell Cycle Regulators	522
Myogenic bHLH Proteins Cooperate with MEF2 Factors in Activating Muscle-Specific Target Genes	523
20.6 Unexpected Potency of Stem Cells and Prospects for Medical Uses	523

chapter 21

Pattern Formation and Embryonic Fields 527

- 21.1 Regulation and the Field Concept 528**
- 21.2 Characteristics of Pattern Formation 530**
- Pattern Formation Depends upon Cellular Interactions 530
 - The Response to Patterning Signals Depends on Available Genes 531
 - The Response to Patterning Signals Depends on Developmental History 533
 - Patterning Signals Have a High Degree of Generality 533
- 21.3 The Concept of Receiving and Interpreting Positional Value 534**
- 21.4 Limb Regeneration and the Polar Coordinate Model 535**
- Regeneration Restores the Elements Distal to the Cut Surface 535
 - Intercalary Regeneration Restores Missing Segments between Unlike Parts 536
 - Limb Regeneration Resembles Embryonic Limb Bud Development 536
 - The Polar Coordinate Model Is Based on Two Empirical Rules 537
 - The Polar Coordinate Model Explains Supernumerary Limb Formation from Misaligned Regenerates 538
- 21.5 Morphogen Gradients as Mediators of Positional Value 539**
- A Morphogen Gradient Can Specify a Range of Positional Values and Polarity to a Field 541
 - Positional Values Specified by Morphogen Gradients May Elicit Differential Gene Activities and Cell Behaviors 541
 - Morphogen Gradients Confer Size Invariance on Embryonic Fields 542
 - Activin Can Form a Morphogen Gradient in *Xenopus* Embryos 543
 - Insect Development Has Been Modeled by Morphogen Gradients and Local Interactions 546

chapter 22

Genetic and Molecular Analysis of Pattern Formation in the *Drosophila* Embryo 551

- 22.1 Review of *Drosophila* Oogenesis and Embryogenesis 553**
- 22.2 Cascade of Developmental Gene Regulation 554**
- 22.3 Maternal Genes Affecting the Anteroposterior Body Pattern 557**
- The Anteroposterior and Dorsoventral Axes Originate from the Same Signal 558
 - The Anterior Group Generates an Autonomous Signal 559
 - The Posterior Group Acts by Derepression 561
 - Many Posterior-Group Genes Are Also Required for Germ Line Development 562
 - The Terminal Group Relies on the Local Activation of a Receptor 563

22.4 Segmentation Genes 564

- Gap Genes Are Controlled by Maternal Products and by Interactions among Themselves 565
- Pair-Rule Genes Are Controlled by Gap Genes and by Other Pair-Rule Genes 568
- Segment Polarity Genes Define the Boundaries and Polarity of Parasegments 573
- Midway through Embryogenesis, Parasegmental Boundaries Are Replaced with Segmental Boundaries 575

22.5 Homeotic Genes 576

- Homeotic Genes Are Expressed Regionally and Specify Segmental Characteristics 577
- The Homeotic Genes of *Drosophila* Are Clustered in Two Chromosomal Regions 578
- Homeotic Genes Are Part of a Complex Regulatory Network 581

22.6 The Dorsoventral Body Pattern 581

22.7 The Compartment Hypothesis 588

- Compartments Are Controlled by the Activities of Specific Selector Genes 588
- The *Ultrabithorax*⁺ Expression Domain Coincides with a Compartment 589
- engrailed*⁺ Controls the Anteroposterior Compartment Boundary 589
- apterous*⁺ Controls the Dorsoventral Compartment Boundary 592

22.8 Appendage Formation and Patterning 593

- Compartment Interactions across Boundaries Organize Pattern Formation within Compartments 593
- Decapentaplegic Protein Forms a Morphogen Gradient in the Wing Imaginal Disc 596
- Imaginal Disc Cells May Communicate through Cytotemes 598

22.9 Patterning Genes in Other Insects 598

22.10 Table of Patterning Genes in *Drosophila* 601

chapter 23

The Role of *Hox* Genes in Vertebrate Development 609

23.1 Strategies for Identifying Patterning Genes in Vertebrates 611

- Genes Are Cloned on the Basis of Their Chromosomal Map Position 611
- Promoter Trapping Identifies Patterning Genes 611
- Vertebrate Genes Can Be Isolated with Molecular Probes from *Drosophila* Genes 611

23.2 Homeotic Genes and *Hox* Genes 612

- The Homeodomain Is a Cooperative DNA-Binding Region 612
- Hox* Genes Can Be Divided in Paralogy and Orthology Groups 615
- The *Hox* Complex Is Highly Conserved in Evolution 617
- Pseudo-Orthologous *Hox* Genes May Act Redundantly, Independently or Synergistically 617

23.3 The Role of <i>Hox</i> Genes in the Anteroposterior Body Pattern	617
The Order of <i>Hox</i> Genes on the Chromosome Is Colinear with Their Expression in the Embryo	618
Boundaries of <i>Hox</i> Gene Expression Are Also Clonal Restriction Lines	619
Elimination of <i>Hox</i> Genes May Cause Transformations toward More Anterior Fates	621
Overexpression of <i>Hox</i> Genes May Cause Transformations toward More Posterior Fates	623
Only Some of the Control Systems of <i>Hox</i> Gene Activity Are Evolutionarily Conserved	624
23.4 The Dorsoventral Body Pattern	626
A Lobster May Be Viewed as an Upside-Down Rabbit	626
Dorsoventral Patterning Is Controlled by the Same Genes in Flies and Frogs	627
23.5 Pattern Formation and <i>Hox</i> Gene Expression in Limb Buds	629
Limb Field Initiation Is Affected by FGF Signaling and <i>Hox</i> Gene Expression	630
T-box Genes Control the Difference between Hindlimb and Forelimb	631
The Proximodistal Pattern Is Formed in the Progress Zone	632
Signals from Somites and Lateral Plate Specify the Dorsoventral Limb Bud Axis	632
A Zone of Polarizing Activity Determines the Anteroposterior Pattern	634
Sonic Hedgehog Protein Has Polarizing Activity	635
<i>Hox</i> Gene Expression Mediates Anteroposterior Limb Patterning	636
The Principle of Reciprocal Interaction Revisited	637

chapter 24

Genetic and Molecular Analysis of Pattern Formation in Plants **640**

24.1 Reproduction and Growth of Flowering Plants	641
Spore-Forming Generations Alternate with Gamete-Forming Generations	641
Plant Embryos Develop Inside the Flower and Fruit	642
Meristems at the Tips of Root and Shoot Allow Plants to Grow Continuously	644
24.2 Genetic Analysis of Pattern Formation in Plant Embryos	646
Similar Methods Are Used for the Genetic Analysis of Plant and Animal Development	646
Pattern Formation in Plant Embryos Involves 25 to 50 Specific Gene Functions	647
Groups of Genes Control Distinct Patterning Events	648
24.3 Genetic Control of Flower Development	650
Floral Induction Genes Control the Formation of an Inflorescence	650
Meristem Identity Genes Promote the Transition from Floral Meristems to Inflorescence Meristems	651

24.4 The Role of Homeotic Genes in Flower Patterning	652
<i>Arabidopsis</i> Has Homeotic Genes	652
Three Classes of Homeotic Genes Determine the Morphological Characters of Four Flower Whorls	653
Double and Triple Homeotic Phenotypes Confirm the Genetic ABC Model	654
Homeotic Genes Are Controlled by Regulator Genes, Mutual Interactions, and Feedback Loops	657
Other Plants Have Patterning Genes Similar to Those of <i>Arabidopsis</i>	658
<i>Antirrhinum</i> Has Genes Controlling Organ Variations within the Same Whorl	659
24.5 Molecular Analysis of Homeotic Plant Genes	659
Plant Homeotic Genes Encode Transcription Factors	659
Genetic and Molecular Studies Reveal Orthologous Genes between <i>Arabidopsis</i> and <i>Antirrhinum</i>	661
The Known Plant Homeotic Genes Have a MADS Box Instead of a Homeobox	662
Many Questions about Pattern Formation in Flowers Are Still Unanswered	663

chapter 25

Experimental and Genetic Analysis of *Caenorhabditis elegans* Development **665**

25.1 Normal Development	666
Hermaphrodites and Males	666
Fertilization, Cleavage, and Axis Formation	666
Founder Cells	667
Gastrulation, Organogenesis, and Histogenesis	668
Larval Development	669
25.2 Localization and Induction during Early Cleavage	670
The First Cleavage Generates Blastomeres with Different Potentials	670
P Granules Are Segregated into Germ Line Cells	670
<i>Par</i> Genes Affect Cytoplasmic Localization	671
Three Maternal Effect Genes Generate a Localized Activity that Promotes EMS Identity	671
Determination of Anterior Pharyngeal Muscle Cells Requires Multiple Inductive Interactions	673
P ₂ Polarizes EMS to Form Unequal Daughter Cells	675
25.3 Heterochronic Genes	679
Mutations in the <i>lin-14</i> ⁺ Gene Are Heterochronic	679
The <i>lin-14</i> ⁺ Gene Encodes a Nuclear Protein that Forms a Temporal Concentration Gradient	680
The <i>lin-14</i> ⁺ Gene Encodes Small Regulatory RNAs with Antisense Complementarity to <i>lin-14</i> mRNA	680
Down-Regulation of <i>lin-14</i> ⁺ Expression Is Initiated by a Developmental Cue	681
25.4 Programmed Cell Death	681
Programmed Cell Death in <i>C. elegans</i> Is Controlled by a Genetic Pathway	681

The Strategy of Genetic Mosaic Analysis Shows that <i>ced-3⁺</i> and <i>ced-4⁺</i> Act Cell-Autonomously	681
The <i>egl-1</i> , <i>ced-9</i> , and <i>ced-4</i> Gene Products Control the Initiation of Apoptosis	682
The Genes <i>ces-1⁺</i> and <i>ces-2⁺</i> Control the Programmed Death of Specific Cells in the Pharynx	683
The Product of a Human Gene, <i>bcl-2⁺</i> , Prevents Programmed Cell Death in <i>C. elegans</i>	684

25.5 Vulva Development **684**

The Vulval Precursor Cells Form an Equivalence Group	684
The Gonadal Anchor Cell Induces the Primary VPC Fate	686
A Hypodermal Signal Inhibits Vulva Formation	687
Lateral Signals between VPCs Influence Their Fates	688

chapter 26

Sex Determination **691**

26.1 Environmental Sex Determination **692**

26.2 Genotypic Sex Determination **694**

26.3 Sex Determination in Mammals **695**

Maleness in Mammals Depends on the Y Chromosome	695
Dosage Compensation in Mammals Occurs by X Chromosome Inactivation	695
The First Morphological Sex Difference Appears in the Gonad	697
The Testis-Determining Factor Acts Primarily in Prospective Sertoli Cells	698

26.4 Mapping and Cloning of the Testis-Determining Factor **700**

Translocated Y Chromosome Fragments Causing Sex Reversal Are Used to Map TDF	700
The SRY Function Is Necessary for Testis Determination	701
The Mouse <i>Sry⁺</i> Gene Can Be Sufficient for Testis Determination	703
The <i>SRY⁺</i> Gene Controls Primary Sex Differentiation	705

26.5 Sex Determination and Sex Differentiation in *Drosophila melanogaster*, *C. elegans*, and Mammals **706**

One Primary Signal Controls Multiple Aspects of Sex Differentiation	706
The X:A Ratio Is Measured by Numerator and Denominator Elements	707
<i>Drosophila</i> and <i>C. elegans</i> Have Master Regulatory Genes for Sex Differentiation	708
Dosage Compensation Mechanisms Vary Widely	709
The Somatic Sexual Differentiation Occurs with Different Degrees of Cell Autonomy	711
Germ Line Sex Differentiation Involves Interactions with Somatic Gonadal Cells	713

chapter 27

Hormonal Control of Development **716**

27.1 General Aspects of Hormone Action **718**

27.2 Hormonal Control of Sex Differentiation in Mammals **718**

The Synthetic Pathways for Male and Female Sex Hormones Are Interconnected	718
The Genital Ducts Develop from Parallel Precursors, the Wolffian and Müllerian Ducts	719
Male and Female External Genitalia Develop from the Same Embryonic Primordia	722

27.3 Hormonal Control of Brain Development and Behavior in Vertebrates **724**

Androgen and Estrogen Receptors Are Distributed Differently in the Brain	724
Androgens Cause Seasonal and Sexually Dimorphic Changes in the Brains of Birds	724
Prenatal Exposure to Sex Hormones Affects Adult Reproductive Behavior and Brain Anatomy in Mammals	726

27.4 Hormonal Control of Insect Metamorphosis **727**

Insect Molting, Pupation, and Metamorphosis Are Controlled by Hormones	727
Puffs in Polytene Chromosomes Reveal Genes Responsive to Ecdysone	730
Multiple Ecdysone Receptors Have Tissue-Specific Activities	732
Early Regulatory Genes Diversify and Coordinate the Responses of Target Cells to Ecdysone	734

27.5 Hormonal Control of Amphibian Metamorphosis **736**

The Hormonal Response in Amphibian Metamorphosis Is Organ-Specific	737
Amphibian Metamorphosis Is Controlled by Thyroid Hormone	737
The Production of Thyroid Hormone Is Controlled by the Brain	739
Various Defects in Metamorphosis Cause Paedomorphic Development in Salamanders	739
The Receptors for Ecdysone and Thyroid Hormone Share Many Properties	740

chapter 28

Organismic Growth and Oncogenes **743**

28.1 Measurement and Mechanisms of Growth **745**

Growth Is Defined as Change in Mass	745
Growth May Be Isometric or Allometric	745
Growth Occurs By Different Mechanisms	746

28.2 Growth Analysis by Heterospecific Transplantation **747**

The Growth Potential of a Limb Is a Property of the Limb's Mesoderm	747	29.2 Evolutionary Hypotheses on Senescence	773
The Optic Cup and the Lens of the Eye Adjust Their Growth Rates to Each Other	748	The Mutation Accumulation Hypothesis Focuses on Random Deleterious Mutations	773
28.3 Growth Hormones	750	Senescence Can Be Ascribed to Antagonistic Pleiotropy	774
28.4 Growth Factors	751	According to the Disposable Soma Hypothesis, Every Individual Is the Temporary Carrier of His/Her Germ Line	777
Nerve Growth Factor Promotes the Growth and Differentiation of Certain Neurons	751	29.3 Characterized Genes That Affect Animal Life Span	778
Other Growth Factors Affect Multiple Target Cells in a Variety of Ways	754	Life Span—Extending Effects of Mutations in <i>C. elegans</i> May Depend on the Environment	779
The Effects of a Growth Factor May Depend on the Presence of Other Growth Factors	755	<i>Drosophila</i> Mutants Reveal Correlation between Stress Resistance and Longevity	780
28.5 Mechanical Control of Cell Survival and Division	755	The Gene Mutated in Persons with Werner Syndrome Encodes a Helicase	781
28.6 Cell Cycle Control	756	29.4 Caloric Restriction	782
28.7 Tumor-Related Genes	758	29.5 Oxidative Damage and Organismic Senescence	783
Oncogenes Are Deregulated or Mutated Proto-oncogenes	758	Oxidative Phosphorylation Generates Aggressive Oxidants as Intermediates	800
Proto-oncogenes Encode Growth Factors, Growth Factor Receptors, Signal Proteins, Transcription Factors, and Other Proteins	761	Oxidants Damage Cellular DNA, Lipids, and Proteins	783
Oncogenes Arise from Proto-oncogenes through Various Genetic Events	763	Oxidative Damage Causes Senescence	784
Tumor Suppressor Genes Limit the Frequency of Cell Divisions	763	Superoxide Radical Stimulates Cell Division and Growth	786
28.8 Growth and Pattern Formation	765	29.6 Limited Cell Division and Telomerase	786
How Do Organisms Measure Growth?	765	Somatic Mammalian Cells Show a Limited Capacity for Proliferation	786
Patterning Signals Control Cell Cycling and Growth	766	Chromosomal Ends Are Protected by Telomeres	787
Local Cell Interactions Affect Cell Division and Growth	766	Telomerase Activity Allows Unlimited Cell Proliferation	787
		Loss of Telomerase Activity Is a Safeguard Against Cancer	789
chapter 29			
Senescence	769		
29.1 Statistical Definition of Senescence	770		