
Contents

I. Germ Layer Formation and Early Patterning

Formation and Patterning Roles of the Yolk Syncytial Layer

Takuya Sakaguchi, Toshiro Mizuno, Hiroyuki Takeda

1	Introduction	1
2	Formation of the YSL	3
3	Epibolic Movement and the YSL	5
4	Dorsal Determinants in Teleost Yolk Cell	6
5	Determinants Function in the YSL and Dorsal Blastomeres	8
6	The Role of the YSL in Mesoderm and Endoderm Formation	11
7	Localized Inducing Activities Within the YSL	12
8	Is Visceral Endoderm in Mammal Equivalent to Teleost YSL?	12
9	Searching for Genes Specifically Expressed in the YSL	14

Mesoderm Induction and Patterning

David Kimelman, Alexander F. Schier

1	The Origin of Mesoderm	15
2	Induction of Mesoderm by Intercellular Signals	17
3	Nodal Signaling	19
4	Dorsal-Ventral Patterning of the Mesoderm	21
5	Anterior-Posterior Patterning of the Mesoderm	24
6	Wnt Signals in Mesoderm Patterning	26
7	Future Directions	27

The Guts of Endoderm Formation

Rachel M. Warga, Didier Y. R. Stainier

1	Endoderm Formation During the Blastula Period	28
1.1	Location of Endodermal Progenitors	28
1.2	Early Topographic Map of Endodermal Organs	29
1.3	Organs of Ambiguity: the Hypochord and Forerunner Cells	30
1.4	Cell Behavior of Endodermal Progenitors	31
1.5	Genes Involved in Endoderm Formation	31
1.6	The Nodal Factors and Cofactors: Cyclops, Squint, and One-Eyed Pinhead	31

1.7	Nodal Versus Bmp Activity	32
1.8	Nodal-Independent, Oep-Dependent Cell Motility	33
1.8.1	Oep as a Component of EGF Signaling	33
1.9	Effectors of Nodal Signaling: Casanova, Bonnie and Clyde, and Faust	34
1.9.1	The Endodermal Phenotypes of <i>cas</i> , <i>bon</i> and <i>fau</i> Mutants and the Expression of These Genes in the Blastula	34
1.9.2	Cas Is Sufficient To Convert Mesoderm into Endoderm	36
1.10	A Molecular Pathway Leading to Endoderm Formation	37
2	Endoderm Formation During the Gastrula Period	39
2.1	Formation of the Endodermal Layer	39
2.2	Spatial Allocation of Endodermal Precursors	39
2.3	Genes Involved in Endoderm Formation: <i>cas</i> , <i>bon</i> , and <i>fau</i> Revisited	41
2.3.1	Cas May Regulate <i>sox17</i> Directly	42
2.3.2	The Fox/Forkhead Transcription Factors	42
2.3.3	Other Genes Expressed in the Endoderm	43
2.4	Regional Expression in the Endoderm at the End of Gastrulation	43
2.5	Further Thoughts on Endodermal Patterning	45
3	Pharyngeal Pouch Endoderm Versus Digestive Tract Endoderm ..	46
4	Conclusions and Prospects	47

Organizer Formation and Function

Masahiko Hibi, Toshio Hirano, Igor B. Dawid

1	Introduction	48
2	Dorsal Determinants and the Maternal Wnt Signal	50
2.1	Dorsal Determinants	50
2.2	A Maternally Derived Signal Activating the Wnt Pathway	51
3	The Nieuwkoop Center and Organizer Induction	54
3.1	Non-cell-autonomous Induction of the Organizer	54
3.2	<i>bozozok/dharma</i>	55
3.3	Nodal-Related Genes, <i>squint</i> and <i>cyclops</i>	57
3.4	Cooperative Roles of <i>boz/dha</i> and <i>sqt</i> in the Induction of the Organizer	60
3.5	<i>vega/vox/vent</i>	61
3.6	Induction by the Nieuwkoop Center Versus Cell-Autonomous Establishment of the Organizer	61
4	The Organizer	63
4.1	Is the Embryonic Shield the Fish Organizer?	63
4.2	Organizer Genes	64
4.3	<i>chordino</i> , <i>noggin1</i> , <i>twisted gastrulation</i> , and <i>ogon</i>	65

4.4 <i>dickkopf1</i>	66
4.5. Fibroblast Growth Factors	66
4.6 Role of the Organizer in AP Patterning	67
5 Cell Movements and the Organizer	68
6 Summary and Prospects	70

**Dorsoventral Patterning in the Zebrafish:
Bone Morphogenetic Proteins and Beyond**
Matthias Hammerschmidt, Mary C. Mullins.

1 Dorsoventral Patterning in Frog, Fish and Fly	72
2 Mutant Analyses of Vertebrate DV Regulators	74
3 Zebrafish DV Mutants	75
4 Different Phases of DV Pattern Formation	79
4.1 Phase 1: Establishment of the Spemann-Mangold Organizer ..	79
4.2 Phase 2: Establishment of the Morphogenetic Bmp Gradient ..	83
4.3 Phase 3: Morphogenetic Interpretation of the Gradient by Target Cells	86
5 Implications of DV Patterning on the Anteroposterior Axis	90
6 Role of Chordin and Tolloid During Ventral Tail Development	91
7 Perspectives	93

Specification of Left-Right Asymmetry
Christopher V.E. Wright, Marnie E. Halpern

1 Introduction	96
2 Mechanisms Underlying Left-Right Patterning	97
3 Breaking Symmetry	97
4 Stabilizing, Propagating and Reinforcing Left-Right Asymmetry ..	102
5 Transferring Left-Right Information to the Organ Precursors	104
6 Effector Programs of Left-Right Asymmetric Morphogenesis	107
7 Cardiac Left-Right Asymmetry	108
8 Asymmetry of the Zebrafish Forebrain	110
9 Summary and Future Perspectives	113

II. Gastrulation Movements

Life at the Edge: Epiboly and Involution in the Zebrafish
Don Kane, Richard Adams

1 Introduction	117
2 Mid-Blastula Transition and the Beginning of Cell Motility	119
3 Epiboly	120
4 The Epiboly Mutants	125
5 Towards a Unification of Vertebrate Epiboly	127

6	Hypoblast Formation	129
7	How Do Cells Internalize at the Margin: Involution or Ingression?	133
8	Conclusions and Prospects?	135

Cellular and Genetic Mechanisms of Convergence and Extension

Lilianna Solnica-Krezel, Mark S. Cooper

1	Introduction	136
2	Compaction at Blastula Stages	138
3	Distinct Domains of Convergence and Extension Movements in the Zebrafish Gastrula	139
4	Cellular Behaviors Effecting Convergence and Extension Movements	143
4.1	Epiboly and Anteriorward Mesendoderm Migration Contribute to Convergence and Extension	144
4.2	Directed Migration Is a Key Cell Behavior Underlying Convergence and Extension in Lateral Regions of the Gastrula	146
4.3	Mediolateral Cell Intercalation Is a Key Cell Behavior Underlying Convergence and Extension in Dorsal Regions of the Gastrula	147
4.4	Cellular Segregation, Directed Migration and Mediolateral Intercalation Underlie Dorsal Hypoblast Formation	148
5	Molecular Genetic Basis of Convergence and Extension Movements	153
5.1	Wnt Planar Cell Polarity Pathway	154
5.2	Cell Adhesion Molecules	157
5.3	Slit	158
5.4	Eph Receptors and Ephrins	158
5.5	Calcium	159
5.6	Ethanol	159
6	Molecular Genetic Coordination of Convergence and Extension Movements with Cell Fate Specification	160
6.1	<i>Spadetail</i>	160
6.2	<i>Nodal</i>	160
6.3	Bone Morphogenetic Proteins	161
6.4	Fibroblast Growth Factor	162
7	Role of C&E Movements in Generating Embryonic Morphology ..	163
8	Future Directions	164

Primordial Germ Cell Development in Zebrafish

Erez Raz, Nancy Hopkins

1	Introduction	166
2	Specification of Germ Cells in Fish	167

2.1 The Premolecular Markers Era	167
2.2 The Molecular Markers Era	169
3 PGC Migration in Zebrafish	172
4 Maintenance of the Fate of Migrating PGCs	176
5 PGC Development in Zebrafish as Compared with That in Other Organisms	178
6 Conclusions and Future Directions	179

III. Neural Development

Patterning the Zebrafish Central Nervous System

Steve W. Wilson, Michael Brand, Judith S. Eisen

1 Introduction	181
2 Nervous System Morphogenesis	181
3 The Spinal Cord	182
3.1 Bmp Signaling Establishes DV Pattern in the Spinal Cord	184
3.2 Hedgehog and Nodal Pathways Pattern the Ventral Spinal Cord	186
3.3 Delta/Notch Signaling Segregates Neural Fates Within Neural Plate Domains	188
3.4 Later Signals May Refine Cell Identity	189
4 The Forebrain	190
4.1 DV Patterning of the Zebrafish Forebrain	193
4.1.1 Formation of the Hypothalamus	193
4.1.2 Establishment of the Optic Stalks	195
4.1.3 Establishment of Ventral Telencephalic Fates	197
4.1.4 Specification of Dorsal Forebrain Fates	197
4.2 Left/Right Patterning in the Brain	198
4.3 AP Patterning of the Prospective Brain	199
4.3.1 Establishment of Early AP Pattern in the Neural Plate ..	199
4.3.2 Local Induction of the Telencephalon and Eyes	201
5 The Midbrain and Hindbrain	202
5.1 Midbrain and Hindbrain Development Starts in Gastrulation	203
5.1.1 Initial AP Subdivision of the Neural Plate	205
5.1.2 Wnt8 Signaling Positions the Midbrain and Hindbrain	206
5.2 Wnts and Fgfs Maintain and Pattern the Midbrain and Hindbrain	206
5.2.1 Polarization of the Midbrain	207
5.2.2 Fgf Signaling in the Rostral Hindbrain	208
5.2.3 Feedback Control of Fgf Signaling	208
5.2.4 Controlling Competence to Respond to Fgf8 Signaling	209

5.3 DV Patterning of the Midbrain and Isthmus	210
5.4 Later Steps of Patterning the Hindbrain	211
5.4.1 Dorsorostral Patterning	211
5.4.2 Forming and Maintaining Rhombomeres	212
5.4.3 Extrinsic Signals Controlling Segmentation	212
5.5 Secondary Modification of the Ground Plan by Neuronal Migration	214
6 Summary	214

Specification of the Zebrafish Neural Crest

Robert N. Kelsh, David W. Raible

1 Introduction	216
1.1 Markers and Their Specificity	217
1.2 Zebrafish Neural Crest Mutants	222
2 Neural Crest Induction	222
3 Cell Fate Specification	223
3.1 When Does Specification Occur?	225
3.2 Progressive Fate Restriction	226
3.3 Pigment Cell Specification as a Model for Cell Fate Choice	227
4 Regional Specification	231
4.1 Pharyngeal Arch Specification	234
5 Summary	235

Neurogenesis and Specification of Neuronal Identity

Bruce Appel, Ajay Chitnis

1 Introduction	237
2 Zebrafish Spinal Cord Anatomy	237
2.1 Roof Plate	239
2.2 Rohon-Beard Sensory Neurons	239
2.3 Interneurons	239
2.4 Motor Neurons	240
2.5 Floor Plate	240
2.6 Glia	241
3 Neurulation and the Early Pattern of Neurons	241
4 Regulation of Neurogenesis in the Zebrafish Neural Plate	242
5 Creating Proneuronal Domains: Regulation of <i>ngn1</i> Expression	244
6 Dorsal Spinal Cord Development	246
7 Ventral Spinal Cord Development	247
8 Elaboration of Cell Fate Specification by Cell-Cell Signaling	248
9 Neuronal Specification and Transcriptional Codes	249
10 Perspectives	251

**Cellular, Genetic and Molecular Mechanisms
of Axon Guidance in the Zebrafish**

Christine E. Beattie, Michael Granato, John Y. Kuwada

1	Introduction: Pathfinding Is Precise and Cell-Specific	252
2	Axon Pathfinding in the Hindbrain and Spinal Cord	253
2.1	Redundant Cues Guide Growth Cones in the Spinal Cord	253
2.2	Molecules That Guide Spinal and Hindbrain Growth Cones	256
2.3	Mutations That Affect the Development of Neural Circuits in the Hindbrain and Spinal Cord	259
3	Axonal Pathfinding by Spinal Motoneurons	260
3.1	Zebrafish Motor Axons Follow a Common Pathway and Then Make Divergent Choices	260
3.2	Molecules That Guide Motor Growth Cones	261
3.2.1	Semaphorins	261
3.2.2	GDNF	262
3.2.3	Neurolin	262
3.3	Mutations That Disrupt the Formation of Stereotyped Motor Projections	263
3.3.1	Diwanka Mutants	263
3.3.2	Unplugged Mutants	264
3.3.3	Stumpy Mutants	264
4	Axonal Pathfinding in the Visual System	265
5	Conclusions	268

IV. Aspects of Organogenesis**Somitogenesis**

Caroline Brennan, Sharon L. Amacher, Peter D. Currie

1	Introduction	271
1.1	Generalized Overview of Somitogenesis	271
1.2	Morphological Aspects of Zebrafish Somitogenesis	272
2	General Anterior/Posterior Pattern and Specification of Paraxial Mesoderm	273
2.1	<i>Hox</i> Gene Expression Patterns and Overall A/P Pattern	273
2.2	The Origin of Somatic Cells	274
2.3	<i>spadetail</i> , a Gene Controlling Paraxial Mesoderm Formation	275
3	Establishing a Segmental Pattern	276
3.1	Somitic Periodicity and the Cell Cycle	277
3.2	Existence of a Molecular Oscillator	277
3.2.1	The Notch Pathway and Establishment of Segmental Pattern	278

3.2.2 Insights from Zebrafish	280
3.3 The Fused-Somite Mutant and Operation of a Wavefront	281
3.4 Establishment of Anterior/Posterior Somite Polarity	282
4 Formation of the Somite Boundary	284
5 Induction and Patterning the Presomitic and Somitic Mesoderm	286
5.1 Embryonic Myotome Formation and the Initiation of Myogenesis	286
5.1.1 Formation of the “Adaxial” Cell Compartment and Presomitic Myogenic Induction	286
5.1.2 Muscle Pioneer Cells and Myotomal Architecture	288
5.2 Fiber Type and Myotome Morphogenesis	289
5.2.1 Fiber Type Formation in Separate Myotomal Compartments	289
5.2.2 Myotomal Patterning Mutants and the Molecular Mechanisms Controlling Slow Muscle Cell Specification	291
5.3 Migratory or Hypaxial Muscle Formation in Zebrafish Embryos	292
5.4 Sclerotome Formation	294
5.5 Other Somite-Derived Cell Types	295
6 Questions for the Future	296

Cardiovascular System

Deborah Yelon, Brant M. Weinstein, Mark C. Fishman

1 Introduction	298
1.1 Background in Classical Embryology: Some of the Questions	299
2 Zebrafish: a Propitious Embryo for Cardiovascular Studies	300
3 Patterning the Heart	301
3.1 Formation of the Myocardium in Zebrafish	301
3.2 Genetic Regulation of Myocardial Development in Zebrafish	307
3.2.1 Requirements for <i>nkx2.5</i> Induction	308
3.2.2 Requirements for Myocardial Differentiation	311
3.2.3 Requirements for Chamber-Specific Differentiation	312
3.2.4 Requirements for Heart Tube Assembly	313
4 Pattern and Orientation to the Onset of Function	314
5 Vascular Pattern in the Zebrafish	315
5.1 Formation of Blood Vessels in the Zebrafish	316
5.2 Molecular Analysis of Blood Vessel Formation in the Zebrafish	317
5.3 Genetic Analysis of Blood Vessel Formation in the Zebrafish	318

5.4 Experimental Analysis of Vascular Form and Function: Imaging Blood Vessels In Situ	320
6 Prospects for Future Zebrafish Cardiovascular Research	320

The Pronephros

Iain Drummond

1 Introduction	322
1.1 Variation and Evolution of the Kidney	322
1.2 A Brief History of the Kidney	326
2 Morphogenesis and Patterning of the Zebrafish Pronephros	326
2.1 Patterning of the Mesoderm and Formation of the Pronephric Primordium	326
2.2 Mediolateral Patterning of the Intermediate Mesoderm and Induction of the Pronephros	329
2.3 Development of the Pronephric Duct	334
2.4 Nephron Formation	336
2.5 Cell Interactions in the Vascularization of the Glomerulus	337
3 Summary and Perspectives	344

The Zebrafish Eye: Developmental and Genetic Analysis

Stephen S. Easter, Jr., Jarema J. Malicki

1 Introduction	346
2 Morphogenesis	347
2.1 Optic Vesicle	347
2.2 Eye Cup	348
2.3 Lens	351
3 Neurogenesis	352
3.1 The Fan Gradient	352
3.2 Ganglion Cell Layer	353
3.3 Inner Nuclear Layer	355
3.4 Outer Nuclear Layer	357
3.5 Prolonged Neurogenesis and Regeneration	358
3.6 Modulation of the Rate of Proliferation	361
3.7 Pulsatile Production of Neurons	361
4 Pattern and Patterning of Cellular Architecture in the Retina	362
4.1 Pattern of Differentiated Retina	362
4.2 Formation of Retinal Architecture	363
5 Terminal Differentiation of Cellular Morphology	366
5.1 Ganglion Cell Axogenesis	366
5.2 Photoreceptor Differentiation	367
6 Summary	370
References	371
Subject Index	431