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Xenopus Development

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CONTENTS

- Cover
- Title page
- Copyright page
- Contributors
- Preface
- Section I: Oocyte and Early Embryo
  - 1 Transcription in the Xenopus Oocyte Nucleus
    - Introduction
    - LBC structure: The standard model
    - Chromomeres and loops
    - Transcription on LBC loops
    - Transcripts produced during oogenesis
    - In situ hybridization of nascent transcripts on individual LBC loops
    - Appendix
    - Acknowledgments
    - References
  - 2 RNA Localization during Oogenesis in Xenopus laevis
    - Xenopus oocytes as a model system for exploring RNA localization
    - Cis-elements and the role of short repeated motifs
    - Proteins, RNAs, and the endoplasmic reticulum
    - Mechanism(s) for RNA localization to the vegetal cortex
    - Looking toward the future

References
3 From Oocyte to Fertilizable Egg
Mechanisms of mRNA translational control: Global versus selective targeting
Sequestration of maternal mRNA contributes to control of gene expression during Xenopus oogenesis
Future perspectives
Acknowledgments
References
4 Polarity of Xenopus Oocytes and Early Embryos
Oocyte polarity and embryonic axes
Development of A–V polarity during oogenesis
Vegetal hemisphere maternal factors
Vegetal cortex
Animal hemisphere maternal factors
Asymmetry of inorganic maternal factors
Maternal determination of planar and basolateral polarity and L–R asymmetry
Conclusions
References
5 Germ-Cell Specification in Xenopus
Background
Formation of the Xenopus germline
Molecular components of germ plasm
Do chromatin modifications play a role in Xenopus PGC specification?
Concluding remarks
Acknowledgments

References
Section II: Midblastula Transition, Gastrulation, and Neurulation
6 The Xenopus Embryo as a Model System to Study Asymmetric Furrowing in Vertebrate Epithelial Cells
Introduction
MELK is a cell cycle-regulated kinase involved in development and cancer
MELK in Xenopus laevis embryo cytokinesis
Asymmetric furrowing is a mode of cytokinesis conserved throughout evolution
The Xenopus embryo as a model system to analyze asymmetric furrowing
Conclusions
Acknowledgments
References
7 Induction and Differentiation of the Xenopus Ciliated Embryonic Epidermis
Introduction
Nonneural ectoderm specification
Ontogeny of the mucociliary epithelium
Perspectives and outstanding questions
Concluding remarks
References
8 Wnt Signaling during Early Xenopus Development
Introduction
Wnt “canonical” and “noncanonical” pathways: Complexity and uncertainties
Major processes regulated by Wnts during early Xenopus development

Wnt signaling at postgastrula stages
References
9 Neural Tube Closure in Xenopus
Introduction
Narrowing and elongation of the neural plate
Cell-shape changes causing neural tube morphogenesis
Complete tube closure assisted by nonneural ectoderm
References
Section III: Metamorphosis and Organogenesis
10 Primordial Germ Cell Migration
References
11 Development of Gonads, Sex Determination, and Sex Reversal in Xenopus
Origin and structure of undifferentiated gonad
Sexual differentiation of the gonads
Development of testis
Spermatogenesis
Development of ovary
Sex determination
Sex reversal
Conclusions
References
12 The Xenopus Pronephros
Introduction
Xenopus embryonic kidney development
Xenopus as a model of disease and regeneration
Modulation of gene expression

Imaging
Screens
Conclusions
Acknowledgments
References
13 Development of Neural Tissues in Xenopus laevis
Introduction
Xenopus as a model system of neural development
Neural specification
Formation of the anterior–posterior axis
Neurulation and dorsal–ventral patterning
Neural plate border specification and neural crest induction
Neural crest delamination and migration
Molecular regulation of neurogenesis
Conclusions
Acknowledgments
References
14 The Development of the Immune System in Xenopus
Introduction
The establishment of innate immunity components during Xenopus ontogeny
The establishment of the adaptive immune system components
Selection of T and B cell repertoires
Immunity during ontogeny
Immunological issues at metamorphosis
Conclusion

- Acknowledgments
- References

15 Neural Regeneration in Xenopus Tadpoles during Metamorphosis
- Spinal cord regeneration
- Lens regeneration
- Neural retina regeneration
- Optic nerve regeneration
- Role of neural regeneration during tail regeneration
- Role of neural regeneration during limb regeneration
- Telencephalon and mesencephalon regeneration
- Summary and future perspectives
- Acknowledgments
- References

Section IV: Novel Techniques and Approaches

16 Atomic Force Microscopy Imaging of Xenopus laevis Oocyte Plasma Membrane
- Introduction
- Atomic force microscopy
- Sample preparation protocols for AFM imaging of Xenopus laevis oocyte plasma membrane
- AFM imaging of Xenopus laevis oocyte plasma membrane
- Conclusions and future perspectives
- References

17 Size Scaling of Subcellular Organelles and Structures in Xenopus laevis and Xenopus tropicalis
- Introduction to organelle scaling

Xenopus interspecies scaling
Advantages of studying two closely related Xenopus species
Interspecies nuclear scaling
Interspecies mitotic spindle scaling
Interspecies mitotic chromosome scaling
Techniques: X. tropicalis egg extracts
Xenopus developmental scaling
Developmental nuclear scaling
Developmental mitotic spindle scaling
Developmental mitotic chromosome scaling
Techniques: Xenopus embryo extracts
Functions of organelle scaling
Future directions
Acknowledgments
References
18 A Model for Retinal Regeneration in Xenopus
Xenopus as a model animal for the study of retinal regeneration
X. laevis: A new animal model of retinal regeneration
Culture models for the study of X. laevis retinal regeneration
A transgenic approach to retinal regeneration
X. tropicalis: A novel animal model for retinal regeneration
A hypothetical model for retinal regeneration and future perspectives on retinal regeneration studies
Acknowledgments
References

19 The Xenopus Model for Regeneration Research
Introduction
Xenopus tadpole tail regeneration
Xenopus limb as a model for stimulating regeneration
Lens regeneration
Conclusions
Acknowledgments
References
20 Genomics and Genome Engineering in Xenopus
Introduction
Xenopus genomics
Xenopus genome engineering
Acknowledgments
References
Index
Eula

List of Tables

Chapter 03
Table 3.1 RBP influence the fate of an mRNA.
Table 3.2 Summary of regulatory elements controlling mRNA translational recruitment during oocyte maturation. Source: Adapted from MacNicol and MacNicol (2010).
Chapter 05
Table 5.1 Germ plasm RNAs.
Table 5.2 Germ plasm proteins.

Table 5.3 Proteins in common with P-bodies and GG.

Chapter 08

Table 8.1 Maternal and zygotic Wnts during early Xenopus development.

Table 8.2 Depletions of maternal components.

Table 8.3 Morpholino depletions and other interferences.

Table 8.4 Wnt signaling in postgastrula developmental processes.

Chapter 11

Table 11.1 Compounds disrupting sexual development in anurans.

Chapter 13

Table 13.1 Primers for quantitative RT-PCR of neural specification and differentiation genes.

Chapter 14

Table 14.1 Age-related unidirectional MLR. Source: Summarized from Du Pasquier et al. (1979).

Chapter 15

Table 15.1 Regeneration ability of X. laevis.

Chapter 17

Table 17.1 Comparisons between X. laevis and X. tropicalis.

Chapter 19

Table 19.1 Signaling pathways in Xenopus tail regeneration.

Chapter 20

Table 20.1 Parameters of the X. tropicalis genome.

Table 20.2 Nomenclature conventions for Xenopus genes and proteins.

Table 20.3 Web resources for Xenopus genome data mining.

List of Illustrations

Chapter 01

Figure 1.1 Oocytes of X. tropicalis. The top panel shows the range of oocyte sizes found in an ovary from an immature frog (3.5 cm snout to vent). At this stage, most oocytes have diameters under 100 µm. The lower panel shows oocytes of different sizes, obtained from the ovary of a mature female. Such ovaries also contain smaller oocytes like those shown in the upper panel.

Figure 1.2 LBCs of the newt Notophthalmus viridescens (A and B) and X. tropicalis (C and D). (A) A short segment of an LBC stained with antibodies against pol II (green) and the RNA-binding protein CELF1 (red) (Morgan 2007). The axes of all loops appear as diffraction-limited green lines, because they are covered with closely spaced pol II molecules. One pair of sister chromatids is preferentially stained with CELF1, revealing the prominent thin-to-thick orientation of the associated loop matrix (RNP transcripts). (B) The same segment of LBC stained with the DNA-specific dye DAPI reveals the axis of transcriptionally inactive chromomeres. (C) Bivalent No. 2 of X. tropicalis stained with antibodies against pol II (green) and pol III (red). The vast majority of loops are transcribed by pol II. The loops of X. tropicalis are much shorter than those of the newt, and only a few are recognizable as loops in this image

(arrow). (D) The same bivalent showing strong staining of the chromomere axes with DAPI. DAPI also reveals two amplified rDNA cores (arrowheads) in each of two extrachromosomal nucleoli. Regions of high protein concentration in the nucleoli also bind DAPI to a lesser extent. The same is true of two moderately stained structures near the middle of this bivalent (arrows), which represent loop pairs whose matrix has fused into a single large mass (lumpy loops).

Figure 1.3 Highly stylized diagram of LBC structure. Transcriptionally active sister chromatids extend laterally from the main axis of the chromosome, which consists of regions where transcriptionally inactive sisters are closely paired and associated with cohesins (Austin et al. 2009). Loops can consist of one or more TUs, which may have either the same or opposite polarities on the same loop. RNA polymerase II molecules are packed closely along the DNA axis of each loop and elongating RNA transcripts are attached to them. The transcripts are associated with various proteins, including splicing factors (not shown here). It is not known what holds the bases of the loops together. One possibility is that insulators or similar molecules that define transcriptionally active regions of chromatin are involved.

Figure 1.4 An LBC consisting of a single unpaired chromatid. This LBC was formed when a sperm head of X. laevis was injected into the GV of the newt N. viridescens. Individual chromatids derived from the sperm begin transcribing shortly after injection, eventually forming giant chromosomes similar to the endogenous LBCs. Because the X. laevis chromatids do not replicate in the GV, the LBCs formed from

them consist of single chromatids and the transcription loops are unpaired.

Figure 1.5 A dlb in a chromosome of the newt N. viridescens. Such bridges can be formed by stretching a chromosome with microneedles, but they also occur by accident when LBCs are prepared for microscopical examination. Note the polarity of the loops, which allows one to determine the direction of transcription (arrow) relative to the chromosome as a whole.

Figure 1.6 Images of a loop from the newt N. viridescens. (A) The entire loop imaged by phase contrast microscopy. The pronounced thin-to-thick polarity of the RNP matrix signifies the direction of transcription (arrows). (B) A confocal image of the same loop after immunostaining with mAb H14 against phosphorylated pol II (green) and mAb Y12 against symmetrical dimethylarginine, an epitope found on several splicing snRNPs (red). Green pol II stain is evident at the thin end of the loop but is obscured by the heavy mAb Y12 stain along most of the loop. (C) Image of the same loop taken by structured illumination superresolution microscopy. (D) Confocal image of the thin end of the loop at higher magnification. (E) The same loop imaged by structured illumination microscopy. Pol II now appears as a green line of nearly uniform width along the length of the loop. The red RNP matrix is resolved into a series of small particles about 50 nm in diameter. The superresolution images were taken on a DeltaVision OMX structured illumination microscope by Sidney Shaw and James Powers, Department of Biology, Indiana University.

Figure 1.7 Similarity of transcriptomes from X. tropicalis oocytes less than 100 μm diameter and oocytes that have reached 350–400 μm diameter, approximately half their final size. Shown here are the log 2 FPKM scores for approximately 9700 different transcripts. The slope of approximately 1.0 and the high correlation (R = 0.84) show that transcripts are stored at similar relative concentrations from the earliest to midstages of oogenesis. Transcripts from fully mature oocytes are similar (not shown here).

Figure A1.1 Cytological maps of the 18 LBCs of X. laevis, based on the analysis of 41 complete or nearly complete spread preparations. Lengths are given as fraction of the total length of all chromosomes. The numbering system is the same as that given in Murphy et al. (2002), differing slightly from the original maps in Callan et al. (1987). Centromere positions (large solid circles) were determined from a subset of 15 preparations in which the oocytes had been injected with a myc-tagged transcript of the centromere-specific protein CENP-C, and centromeres detected with an antibody against the tag. Pol III sites are shown as elongated ovals at positions described earlier in Murphy et al. (2002). Three chromosomes (Nos. 8, 11, and 16) bear histone locus bodies (HLB) at the histone gene loci (Callan et al. 1991). The nucleolus organizer is located near the centromere of chromosome No. 3 (Callan et al. 1988), although a nucleolus is only rarely seen at this locus. Oocyte-specific 5S genes are located at or near the end of the long arm of all chromosomes except Nos. 10, 17, and 18 (Callan et al. 1988). These regions are recognizable by the presence of a small terminal granule (solid circle) and pol III-labeled loops. Bodies

identical in morphology and immunostaining properties to extrachromosomal speckles (B-snurposomes) are regularly seen at specific chromosome termini and at a few interstitial sites (small open circles). A dlb near the nucleolus organizer of chromosome No. 3 is associated with the RNA-editing enzyme ADAR1 (Eckmann and Jantsch 1999). Double-axis regions of unknown significance occur near the centromeres of chromosome Nos. 8 and 9.

Figure A1.2 Cytological maps of the 10 LBCs of X. tropicalis, based on the analysis of 29 complete or nearly complete spread preparations. Lengths are given as fraction of the total length of all chromosomes. Centromere positions (large solid circles) were determined from a subset of 10 preparations in which the oocytes had been injected with a myc-tagged transcript of the centromere-specific protein CENP-C, and centromeres detected with an antibody against the tag. Terminal spheres of unknown nature are present on 15 of the 20 telomeres. These stain with an antibody against pol III, as do multiple internal sites (small solid circles). Four pol III sites on chromosome Nos 3, No. 4, and No. 10 frequently have pearls (P) associated with them (Nizami and Gall 2012). Three chromosomes (Nos. 3, 7, and 9) bear HLBs, presumably at the histone loci (not independently verified). The single nucleolus is located near the middle of chromosome No. 8, and the position of the nucleolus organizer (NO) has been verified by in situ hybridization. The large gray masses on several chromosomes are presumed to be “lumpy loops” as described originally by Callan in the newt Triturus (Callan and Lloyd 1960).

Chapter 02

Figure 2.1 Distribution of early- and late-pathway RNAs in stage I–IV oocytes. On the left is a stage I oocyte showing the nucleus (N), the Vg1 mRNA distributed throughout the cytoplasm (blue), and the Xcat-2 localized to the Balbiani body or mitochondrial cloud adjacent to the nucleus (red). By stage II, the mitochondrial cloud and early-pathway RNAs have moved to the vegetal cortex, whereas late-pathway RNAs, such as Vg1 (blue), begin to localize to a wedge-shaped structure between the nucleus (N) and the early-pathway RNAs at the vegetal pole. A stage IV oocyte is shown on the right with a pigmented animal hemisphere at the top and Vg1 (blue) distributed through most of the vegetal cortex. Xcat-2 (red) and other early RNAs remain in the vegetal cortex but mostly at the vegetal pole. The oocytes are drawn to relative scale with the stage I oocyte being approximately 100 μM in diameter. The process of growing from a stage I to stage IV oocyte takes months in an adult female. For a comprehensive book of protocols and high-quality photos of different-staged oocytes, the reader is referred to volume 36 of Methods in Cell Biology (O’Keefe et al. 1991).

Figure 2.2 Intermolecular base pairing potential of the Xcat-2 RNA LE. Two tandem copies of the Xcat-2 MCLE connected with 10 N were analyzed with MFOLD. The resulting structure is shown that has extensive intermolecular base-pairing potential that would support the formation of dimers or multimers in vivo. Evidence for this structure comes from the fact that mutations that reduce intermolecular base pairing impair localization but are rescued by compensatory mutations in trans (data not shown). For Xcat-2, nucleotides 403–610 were used, but we

resequenced the DNA since a predicted restriction enzyme site from the NCBI sequence (Acc#X72340) was absent, and we identified a sequencing error that significantly affected the predicted extent of intermolecular base pairs. The sequence in this figure is the corrected sequence.

Figure 2.3 REPFIND output of the Xpat 3′-UTR. REPFIND was used to identify the most significant cluster of any repeat greater than 5 nt in length. The scale bar at the top represents the length of the 3′-UTR in nt. The colored bars represent UGCAC motifs (different colors are used to help visual counting only) considered in the calculated p-value shown at the right. The two grey bars to the right represent UGCAC motifs that were not included in the calculation. The p-value is the probability of finding this particular cluster. The first approximately 550-nt fragment localizes when injected into oocytes, whereas the remaining approximately 2.5-kb fragment does not (Betley et al. 2002).

Figure 2.4 The association of Vera/Vg1RBP with the ER depends upon RNA. Concentrated cytoplasmic extracts were prepared from stage IV to VI oocytes and subjected to sucrose density gradient centrifugation as previously described (Deshler et al. 1997). Fractions were collected and analyzed by Western blotting using antibodies to TRAPα and actin to identify fractions that contain the ER and soluble proteins, respectively. An anti-Vera antibody shows that Vera cofractionates with ER membranes (left panel). The same experiment was repeated, but the extracts were first diluted about twofold with buffer and treated with RNase. As can be seen, Vera is released from the ER and sediments with soluble proteins. This indicates that Vera is associated with

ER membranes through its association with RNAs and RNP particles in the undiluted extracts.

Figure 2.5 Four hypothetical phases to RNA localization in Xenopus oocytes. Features of the cis-elements that specify vegetal localization and the proteins involved in the localization process are described in the text. In this figure, sequence-specific RNA binding proteins and RNP-associated proteins, including the double-stranded RNA binding protein Staufen, are depicted as the indicated symbols at the upper right of the figure. A generic RNA LE that directs a particular mRNA to the vegetal cortex is shown as a squiggly line. During the first phase, the RNA is mostly single-stranded in the nucleus. Phases II and II occur throughout the cytoplasm, while the fourth phase, anchoring, occurs in the mitochondrial cloud of stage I oocytes (not depicted) or the vegetal cortex of stage II–IV oocytes.

Chapter 03

Figure 3.1 Translation initiation. Translation initiation in eukaryotes is a complex, multistep process. Of central importance is the formation of two major complexes: the 43S preinitiation complex and the eIF4F cap-binding complex. Formation of both complexes can be regulated to control gene expression. ORF, open reading frame; PABP, poly[A] binding protein; eIF, eukaryotic initiation factor; Met, methionine; PI3K, phosphoinositide 3-kinase; mTOR, mammalian target of rapamycin; 4E-BP, eIF4E binding protein; GTP, guanine triphosphate; GDP, guanine diphosphate; PERK, PRK-like ER kinase; PKR, protein kinase double-stranded RNA dependent; GCN2, general control nonderepressible-2; HRI, heme-regulated inhibitor.

Figure 3.2 A series of positive feedback loops mediate amplification of a weak progesterone “trigger” signal to trigger activation of MPF. The sequential action of specific RBP regulates the ordered activation of signal transduction pathways and temporal recruitment of maternal mRNAs during meiotic cell cycle progression. A number of nested positive feedback loops contribute to the amplification of the initial progesterone stimulus and all-or-none transition through the cell cycle. For the purposes of focus on mRNA translational control and clarity, a number of negative feedback loops have been omitted. See text for details.

Chapter 04

Figure 4.1 Three-dimensional reconstruction of interphase cysts. Two different eight-cell cysts with six nuclei visible. Cyst 1 (A) and four different views of cyst 2 (B–E). Cytoplasm is gray, nuclei are red, mitochondria of PMC are green, centrioles are blue, and ring canals are yellow. In cyst 1, five ring canals and four centrioles near the PMC and ring canals are visible. Spatial relationships between mitochondria, centrioles, and ring canals are visible in all reconstructions. Also note the constant distance (2 μm) between the centrioles and ring canals in all cystocytes (see text). PMC, ring canals, and centrioles face each other and are located centripetally in “the rosette” conformation (see text). These reconstructions were from 38 serial ultrathin sections similar to the section shown in Figure 1B in Kloc et al. (2004a).

Figure 4.2 Three-dimensional ultrastructural reconstruction of mitochondrial cloud and germinal granules in stage I oocyte. (A) The mitochondrial

cloud was reconstructed from 21 serial electron microscopy (EM) sections. The cloud is a sphere composed of thousands of mitochondria (green speckles) and germinal granules (red spheres). Germinal granules are concentrated in the form of a ring in the METRO region that is the part of the cloud facing the vegetal pole and are excluded from the center of the cloud. The oocyte nucleus, not visible in the picture, is above, and the vegetal pole of the oocyte is below the plane of the picture. (B and D) The half sections of three germinal granules from a mitochondrial cloud similar to the one shown in (A). The images were reconstructed from four serial sections of oocytes hybridized in situ with Xpat (B and D) and Xcat2 (C and D) antisense RNA probes. The Xpat RNA (green dots) is predominantly on the granule periphery with a small portion localized internally, while the majority of Xcat2 (black dots) is sequestered internally in the granule. For better clarity of the image, the original silver-enhanced gold label was replaced (using proper logarithm and computational programs; see Materials and Methods in Kloc et al. 2002) with uniform-sized dots. The bar is equal to 4.5 mm in (A) and 250 nm in (B–D).

Figure 4.3 Polarity and axes formation during oogenesis, fertilization, and early cleavage. (A) During oogonial divisions, the PMC (the aggregate of mitochondria and mitochondrial cement containing various early pathway-localized RNAs) is located around the centrioles (black rods), which in all oogonia are invariably facing the cytoplasmic bridges that mark the presumptive vegetal pole of the oocyte. There are four synchronous oogonial divisions (for simplicity, only the first division is shown here) resulting in the formation of a nest of 16 oocytes

connected by cytoplasmic bridges. During early (stage I to II) oogenesis, the early pathway-localized RNAs and germinal granules are concentrated at the vegetal tip of the mitochondrial cloud (Bb) that is always facing the vegetal pole of the oocyte. In contrast, at this stage of oogenesis, the late pathway RNAs, such as Vg1, are dispersed within the ooplasm. Between stages III and V of oogenesis, fragmentation of the mitochondrial cloud delivers germinal granules and early pathway RNAs to the vegetal cortex, while the late pathways RNAs (shown as clouds) are vegetally mobilized via microtubules (shown as rods). In full-grown stage VI oocytes, germinal granules and their resident early pathway RNAs are anchored at the vegetal cortex, and the late pathway RNAs are concentrated in the vegetal hemisphere. During cleavage, germinal granules and their resident RNAs segregate to vegetal blastomeres, which are the progenitors of PGCs. (B) After fertilization, the Xenopus egg contains a dense central cytoplasm surrounded by a shear zone of less dense cytoplasm containing the vegetally localized late pathway RNAs and dorsalizing activity (shown as crescent). The cortex rotates 30° relative to the core cytoplasm, displacing dorsal determinants and dorsalizing activity toward the presumptive dorsal region where they interact (see Weaver and Kimelman, 2004, for review) to specify the dorsal part of the embryo. The D–V axis-determining process acts in conjunction with the chiral properties of the egg cortex to form the L–R axis (see Danilchik et al. 2006).

Chapter 05

Figure 5.1 Schematic of germline formation in Xenopus laevis. (A) Stage I oocyte: germ plasm (green) assembles in MC (red) in close association

with the germinal vesicle (GV). Stage II/III oocyte: MC fragments and moves toward the vegetal cortex. Stage VI: germ plasm within the vegetal cortical area. Eight-cell embryo: germ plasm is inherited by vegetal blastomeres shown from the lateral and vegetal pole perspective. Blastula: germ plasm lies near the plasma membrane of four to six cells, the pPGCs. Gastrulation: germ plasm translocates by a microtubule-based mechanism to a perinuclear position. The germline (PGCs) is now segregated from endoderm lineage (yellow, endoderm; red, mesoderm; blue, ectoderm). Tail bud stages 24–34: PGCs begin migration steps clustering, dispersing laterally, directionally migrating dorsally, and, at tadpole 41, reaggregating at the dorsal tip of the endoderm (adapted from Figure 1e′–h′ in Terayama K, Kataoka K, Morichika K, Orii H, Watanabe K, Mochii M. Developmental regulation of locomotive activity in Xenopus primordial germ cells. Dev Growth Differ 2013;55(2):217–228.) Tadpole: PGCs migrate along the dorsal mesentery to reach the presumptive gonads. (B) Tadpole: PGCs enter somatic gonads where they pass through a mitotic proliferative stage (germ stem cell, cystoblast). Female germline cyst formation: at some point, cytoblast will undergo incomplete cytokinesis, remaining connected through four divisions (M1–M4) by cytoplasmic bridges (ring canals) to form the germline cyst. Note the polarity that is maintained throughout the divisions. Mitochondrial aggregate, the synaptonemal complex, the centriole, the ring canal, and the fusome that likely indicate the future vegetal pole of the oocyte. Primary oocyte: oogonia enter meiosis and transition into primary oocyte within cyst. During prophase, follicle cells move between oocytes and the interconnections are lost. Pre-stage I oocyte:

mitochondria aggregates surround the nucleus (GV) with the aggregate containing the centriole becoming the major site of germ plasm formation. Stage I oocyte: mature MC with germ plasm assembled toward the vegetal pole. Germ plasm or PGCs (green), mitochondria (red), centriole (black dot), nucleus (purple), nuage (light blue), synaptonemal complex (black bars), ring canal (black line), and fusome (black triangle). (Adapted from Figs. 8 and 10 in Kloc M, Bilinski S, Dougherty MT, Brey EM, Etkin LD. Formation, architecture and polarity of female germline cyst in Xenopus. Dev Biol 2004a;266(1):43–61.) (All staging is according to the Normal Table of Xenopus laevis (Nieuwkoop and Faber 1967) (Daudin), Amsterdam: North-Holland Publishing Co.).

Figure 5.2 Isolation of MC with germ plasm. (A) Isolated stage I/II oocytes with MC. (B) Lysed oocytes with free MC. (C and D) MC material taken from Percoll gradient. This material was run on an SDS gel and used for mass spectrometry (see text and Table 5.2).

Chapter 06

Figure 6.1 Asymmetric division vs. asymmetric furrowing. (A) Asymmetric division is achieved here by displacement of the cytokinetic apparatus, including the mitotic chromosomes (ovals). The division plane (dashed line) is not centered but laterally displaced. Consequently, the mother cell divides into two daughter cells of different sizes and fates. (B) Asymmetric furrowing in a polarized cell presenting an apical and a basolateral membrane. The division plane is centered and the two daughter cells will inherit similar size and fate. The cytokinetic furrow (dashed line) progresses asymmetrically: it

starts to ingress basolaterally and progresses toward the apical membrane.

Figure 6.2 Localization of MELK in a Xenopus blastula stage embryo. MELK (green) is localized at cell–cell contacts. In dividing cells, MELK is also concentrated at the division site (empty arrowhead) between mitotic chromosomes. DNA is in blue.

Figure 6.3 Localization of anillin and MELK in dividing gastrula stage embryo. (A) Localization of anillin (green) in the epithelium of a gastrula stage embryo. Diagrams on the left: yellow lines mark the confocal planes relative to the embryo surface. Grey dashed lines were drawn to indicate the limits of the dividing cell. White dashed line symbolizes the plane used for orthogonal projection of the confocal planes shown on the right. The green circle corresponds to the closing cytokinetic furrow. The arrow points to the asymmetric cytokinetic furrow. (B) Anillin localizes as a ring between the two daughter cells as shown on the orthogonal projection of the confocal planes. (C) Localization of MELK (red) in the epithelium of a gastrula stage embryo. Diagrams on the left: yellow lines mark the confocal planes relative to the embryo surface. White dashed line symbolizes the plane used for orthogonal projection of the confocal planes shown on the right. In interphase cells, MELK is localized at the apical junctional complexes (white arrow). In cells undergoing cytokinesis, MELK is localized all around the cell cortex as shown by the orthogonal projection.

Chapter 07

Figure 7.1 Cellular composition of the mature Xenopus embryonic epidermis. At the tail bud stage, the epidermis exhibits its final aspect. MCCs (green)

and ionocytes (brown) are inserted in the superficial epithelial layer among mucus-secreting cells (red). Cells in the inner layer (blue) display a flattened morphology and rest on a basal lamina (not represented). Note the production of small secretory vesicles by mucus-secreting cells and of larger vesicles by ionocytes.

Figure 7.2 Molecular control of germ-layer positioning and transition from pluripotent to committed states of animal cells. At the MBT (st 8.5), the maternal determinants VegT and β-catenin in the vegetal hemisphere and FoxI2 in the animal hemisphere contribute to position endoderm and ectoderm, respectively. VegT induces endodermal regulators directly and through Nodal-related proteins Xnr5 and 6. Nodal signaling also induces mesoderm fates in equatorial cells. Pluripotency regulators that include Pou5f1, Sox3, and Ventx antagonize VegT, β-catenin, and Nodals to prevent mesendoderm induction in animal cells. They also activate together with FoxI2 the ectoderm regulator FoxI1e that in turn authorizes the neural and epidermal programs to unfold. The choice between these two programs is controlled by the BMP genetic system that receives multiple regulatory inputs, particularly from antagonists produced by Spemann's organizer and by FGF/ERK signals. Note that cellular commitment is progressive over time and is linked to the depletion of pluripotency regulators. Only a selection of the known key players is represented for simplicity. Please refer to the text for a more complete description.

Figure 7.3 The four steps of MCC biogenesis. Step 1. From cleavage to blastula stages, divisions along the apical–basal axis generate distinct daughter cells,

through asymmetric segregation of maternal determinants; outer cells inherit the apical protein aPKC, which opposes basolateral factors PAR1 and LGL2 to position tight junctions; inner cells do not inherit aPKC and remain loosely packed. Step 2. During gastrulation, CCPs are born in the inner epidermal layer. They express Delta1, which activates the Notch1 receptor both in neighboring inner cells that go on to express the markers α-DG and P63 and in outer cells that in turn express the goblet marker Intelectin2. CCPs express the transcription factors MCI and FoxJ1, as well as the microRNA miR-449. Note that in CCPs, Delta1 appears to inhibit Notch1 in cis. Step 3. During neurulation, CCPs go through two key events, radial intercalation and centrioles multiplication. Intercalation involves two steps: first, CCPs wedge in between the basal domain of outer cells and send protrusions apically. Second, CCPs migrate apically through vertices formed between three or more outer cells. Centriole multiplication is required to produce dozens of BBs necessary for ciliary growth. Two pathways may be mobilized downstream of MCI: the centriole-dependent duplication pathway and the acentriolar pathway that implicates deuterosomes. This early step of CCP differentiation requires inhibition of Delta1 expression by miR-449. Note that nonintercalating cells make contact with the basal lamina that supports the bilayered epidermis, which allows the interaction of α-DG with extracellular matrix components. Step 4. At tail bud stages, ciliogenesis proceeds through the migration and anchoring of BBs at the apical cortex of the cell, a process under the control of FoxJ1 and PCP components, such as Dvl. PCP factors are also important for the assembly of a cortical actin web necessary for BB anchoring.

Finally, cilium elongation can occur through the control of IFT machinery by RFX2, while FoxJ1 controls the expression of motility factors, such as Dynein arms. Septins act as gatekeepers to control the flow of molecules towards the base of the cilium. Rostrocaudal ciliary beating is coordinated between independent MCCs and within each MCC by PCP signaling that fine-tunes the rotation of BBs. Only a selection of the known key players is represented for simplicity. Please refer to the text for a more complete description.

Chapter 08

Figure 8.1 Major branches of Wnt signaling. Solid arrows represent established connections, dashed arrows and question marks indicate hypothetical connections awaiting experimental validation. In addition to the so-called canonical, which utilizes β-catenin as signal transducer, Wnts can activate several noncanonical pathways. Following the recent suggestion by Houliston and colleagues (Lapebie et al. 2011), we set the classical planar cell polarity pathway (PCP) aside. PKC, Rho, and JNK are typically involved in other branches of the pathway. The conditions that favor one or the other branch are poorly understood. One potential specificity factor is the formation of heterodimer between Frizzled receptor (Fz) and different coreceptors, including LRP6 for β-catenin activation, Ryk and Ror1,2 for noncanonical branches. Several other potential coreceptors have been identified, not depicted here. Heterodimerization however does not fully account for specific activation, since both Ryk and Ror1,2 appear to be able to activate more than one branch. Note also that the many components are shared by the various pathways, which suggest large

overlaps/cross-talks. A series of regulatory mechanisms have been identified, including by soluble inhibitors that direct compete for Wnt-receptors binding, such as members of the Wnt-inhibitor factor (Wif) or soluble Frizzled-related protein, Sfrp, families. Other regulations include systems involving soluble regulators interacting with additional transmembrane proteins that regulate receptor stability (Kremen/Dickkopf, ZNRF3/R-spondins).

Figure 8.2 Maternal Wnt-β-catenin. (A) Distribution of Wnt ligands in the fertilized embryo. Two major maternal ligands are present in the Xenopus egg: Wnt11mRNA is vegetally localized in the oocyte. After fertilization, Wnt11 protein is relocalized to the side opposite to sperm entry, due to a movement of the egg cortex called cortical rotation (Schroeder et al. 1999). Wnt5a mRNA is not localized. The shallow gradient represents the default distribution common to most Xenopus transcripts, with lower levels in the yolk-rich vegetal pole. (A′) Cortical rotation of maternal dorsal determinant. Relocalization of the dorsal determinant has been shown to depend on microtubules. A subpopulation of microtubules is organized in parallel arrays on the prospective dorsal side, and vesicles have been observed to move toward the equator (Houliston 1994). These vesicles most likely transport Wnt11, either ready to be secreted, or possibly already interacting with its receptors Fz7 and LRP6. Note that the global microtubule distribution has not yet been established. The sparser tracks of microtubules on the ventral side represent a hypothetical broader gradient of upward relocalization yielding to a graded Wnt11 distribution around the equator (panel B), which

would explain the presence of nuclear β-catenin all around the equator in the blastula (panel C, see Schohl and Fagotto 2002, 2003). (B) Hypothetical activation of the early maternal Wnt pathway. The β-catenin-activating determinant is here assumed to consist of Wnt11-containing secretory vesicles. Several lines of evidence indicate that the pathway is already activated at early cleavage stages, presumably through both paracrine and autocrine signaling (arrows). (C) Nuclear β-catenin localization (dots) in the early blastula. The diagram compares Wnt5a/Wnt11 distribution (after cortical rotation) and nuclear β-catenin localization in the blastula, is based on Schohl and Fagotto (2002). (C′) Corresponding heat map of predicted β-catenin signaling activity. (D) Prospective regions under the influence of maternal Wnt-β-catenin signaling. Maternal β-catenin takes part in the determination of several different regions that correspond to the future neuroderm, trunk and head mesoderm, and anterior mesoderm. (E) Distribution of the four major inducing signals in the Xenopus blastula. Nuclear activated MAPK, Smad2 and β-catenin distributions have been established by Schohl and Fagotto (2002). NFkB/Rel activity has been detected using a reporter gene (Armstrong et al. 2012). (E′) Correspondence between nuclear signals and soluble ligands. A maternal FGF contribution has been suspected but not demonstrated (light blue). However, most of the FGF activity is probably due to early zygotic FGF ligands induced by maternal β-catenin and Xnrs (dark blue). Indirect evidence argues that the NFkB/Rel pathway is controlled by maternal extracellular ligands (Armstrong et al. 1998). (F–F‴) Interplay between the four signaling pathways. (F) Siamois (and closely related Twin) account for the

transcriptional dorsalizing activity. They are direct targets of maternal β-catenin, with also a contribution from Xnr/Smad2 signaling. (F′) Head mesoderm transcription factor Goosecois (Gsc) is activated by the joined activities of Siamois and Xnrs. (F″) Posterior and ventral mesoderm induction (Xbra) requires cooperation of Wnt, Xnr and FGF signaling. Note that the animal and vegetal boundaries of Xbra expression are further constrained by additional mechanisms (reviewed in Heasman 2006) (F‴) Xnr3 is an active component of the dorsalizing center, required in particular for formation of the neuroderm. Xnr3 is a direct target of Wnt-β-catenin and NFkB/Rel pathways. Note the repressive action of ventral NFkB/Rel (Armstrong et al. 1998).

Figure 8.3 Regulatory circuits involving early Wnt signaling. (A) Posterior-ventral mesoderm induction (Xbra): The network is controlled by maternal Wnts and VegT (and possibly FGF). Xnrs expression is activated by both VegT and Wnts. FGF expression is induced by Wnts and Xnrs, and at later during gastrulation by a positive feedback loop with its target Xbra. Xbra is controlled directly by FGF and Xnrs, and probably also Wnt. Additional inhibitory controls of Xbra, e.g., by Gsc, are not shown. (B) Induction of dorsal anterior mesoderm. Siamois/Twin and Xnrs induce the major dorsal components responsible for patterning of gastrula (Spemann organizer), including the transcription factor Gsc and soluble inhibitors of BMP, Xnr and Wnt pathways, such as Chordin and Cerberus. (C) Control of zygotic Wnt8 expression (ventral-lateral mesoderm) in the gastrula by zygotic Xnrs and FGFs (Heasman 2006). Hedgehog signaling, now also active, also contributes (Mullor et al. 2001), while Gsc repressed Wnt8 in the