Developmental Biology
Topic: Development of Fish

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Normal development of Zebrafish

- Zebrafish eggs, **telolecithal**, are about 0.7 mm in diameter and surrounded by **a transparent chorion**.
- The eggs and sperm are shed by the parents into the water and fertilization is external. As in Xenopus, the **animal hemisphere is cytoplasm-rich** and the **vegetal hemisphere is yolk-rich**.
- Cleavage is **meroblastic**, **discoidal** as it involves **only the animal pole** region. The first three cleavages are all vertical, generating an eight-cell stage composed of two rows of four blastomeres.
- The **outer parts of the cell contacts** are enriched in the **proteins vasa and nanos** which may be determinants for germ cell formation. During this **early phase the blastomeres** remain **connected to the main yolk mass** by **cytoplasmic bridges**. This yolk mass becomes known as the yolk cell.
- Succeeding cleavages occur about every 15 minutes, until **after about 10 divisions (approximately 3 hours) the midblastula transition (MBT)** occurs.

Some developmental genes in the zebrafish

Gene	Homolog	Developmental function	Gene product*
headless	*tcf3*	early dorsalization	HMG TF
cyclops	*nodal*	mes-endoderm induction	IF
squint	*nodal*	mes-endoderm induction	IF
one-eyed pinhead	*cripto*	needed for nodal action	EGF-CFC factor
dharma/nieuwkoid/bozozok		defines organizer	paired homeo TF
notail	*brachyury/T*	defines posterior mesoderm	T-box TF
spadetail	*tbx6/vegT*	defines trunk mesoderm	T-box TF
acerebellar	*fgf8*	posteriorising	IF
swirl	*bmp2b*	ventralizing	IF
snailhouse	*bmp7*	ventralizing	IF
vent	*vent1/PV1*	defines ventral	homeodomain TF
vox	*vent2/xom*	defines ventral	homeodomain TF
bonnie-and-clyde	*mixer*	defines endoderm	T-box TF
faust	*gata5*	heart/endoderm	Zn finger TF

*TF, transcription factor; IF, inducing factor.

Developmental stages of zebrafish

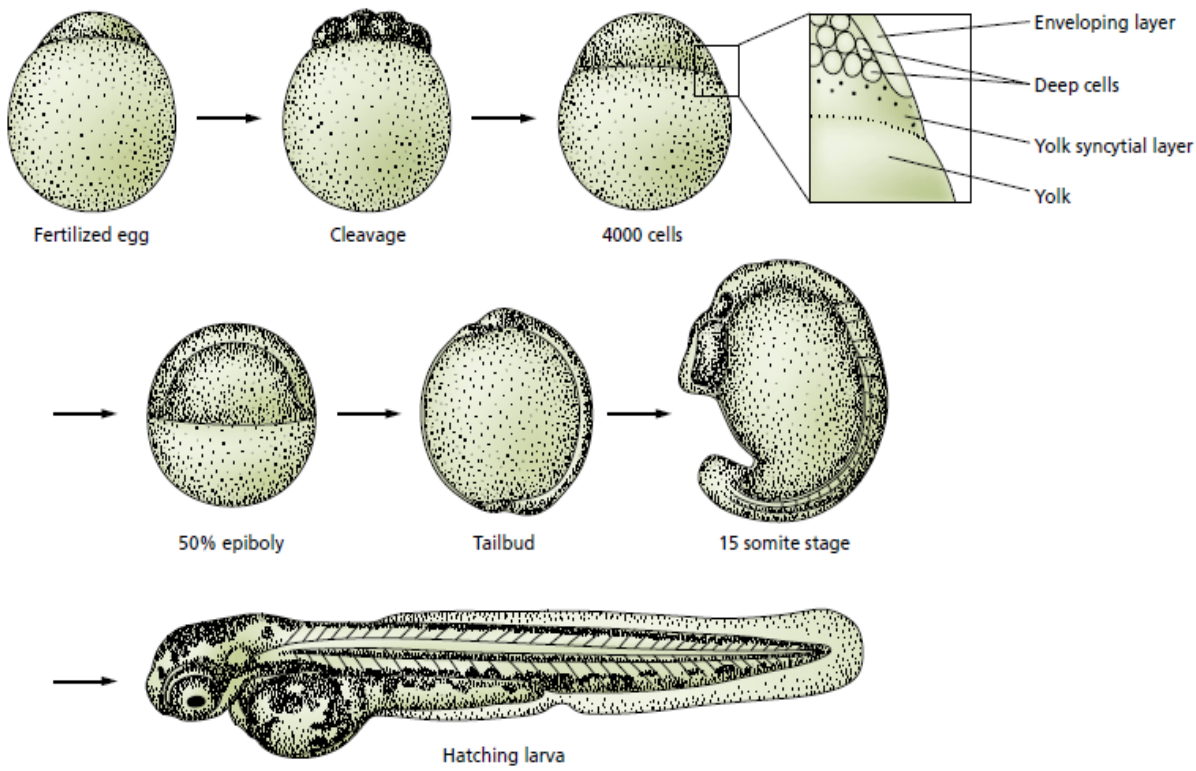
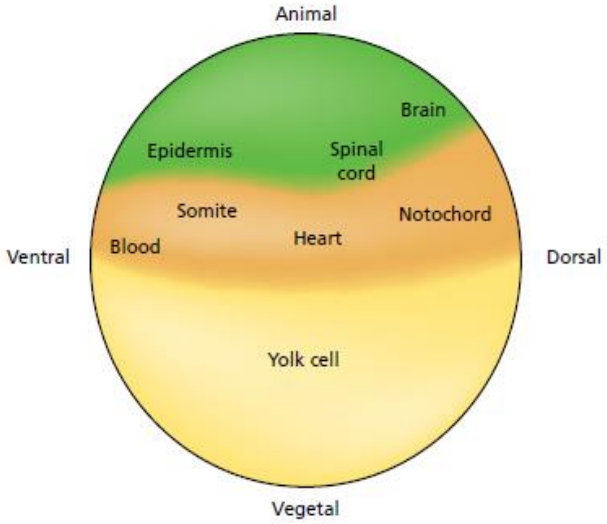


Fig. 8.1 Normal development of zebrafish.

- By this stage the **embryo consists of four regions**. The majority of cells form a mass in the animal pole region and are called **deep cells**, which are covered by a thin **epithelial enveloping layer** (**EVL**).
- The vegetal part of the embryo is occupied by the yolk cell, and the region near the blastoderm contains a number of nuclei and is called the **yolk syncytial layer**. These **nuclei arise from the cells at the edge of the blastoderm** after **MBT**, which fuse with the **adjacent yolk cell** to form the **syncytium**.
- Starting shortly after MBT, the blastoderm commences **an active expansion** called **epiboly** such that the **margin moves down progressively** to cover the yolk cell.
- **Stages of zebrafish development** are **identified as percentage of epiboly**, depending on **how much of the yolk cell has been surrounded**. This movement is driven by the **yolk syncytial layer** and depends on the **activity of microtubules** within the yolk cell. At **50% epiboly** (approximately 5.5 hours) the **margin of the blastoderm begins to involute.**

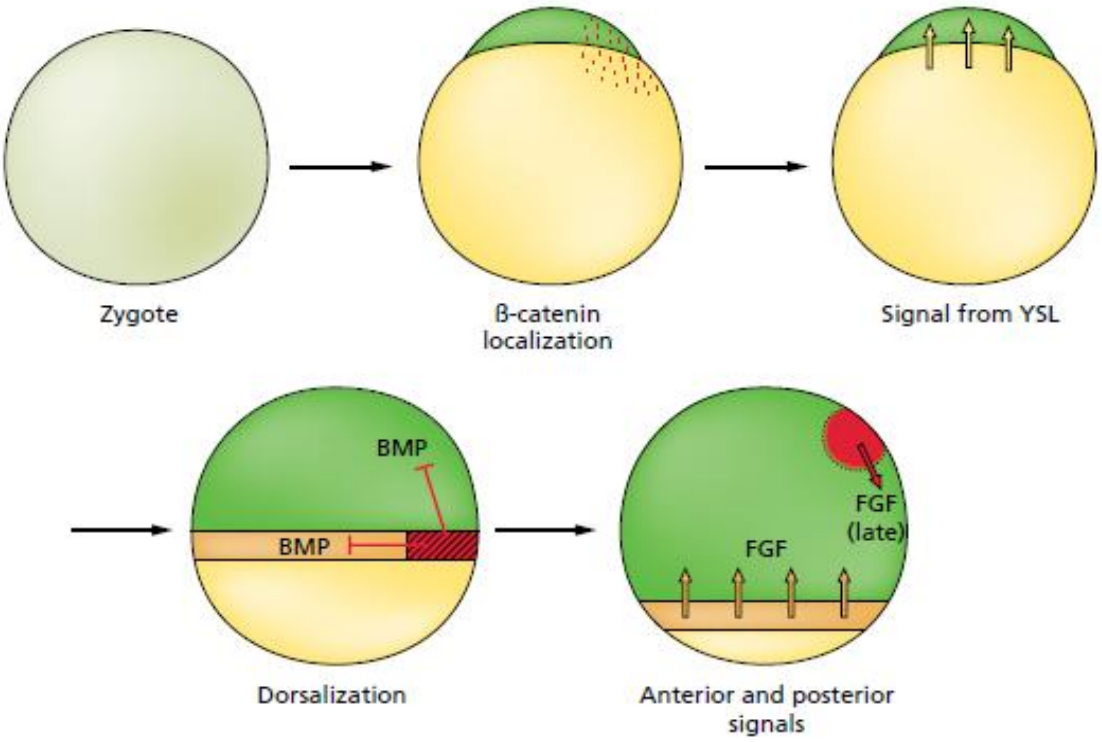
- In the zebrafish the term **gastrulation** is normally reserved specifically for the **involution movement of the mesoderm** as distinct from the epibolic spreading process.
- Involution takes place all around the blastoderm margin, but, as in Xenopus, **the dorsal involution** is much more pronounced than the ventral. The involution means that the blastoderm is thicker around the margin than elsewhere and **this thickening is called the germ ring.**
- The **involution movement** is carried out **only by the deep cells**. **The outer enveloping layer cells do not participate** and go on to become the outer layer of the larval epidermis, or periderm/epiblast.
- Simultaneous with the involution, the **dorsal region starts to elongate** in an **anterior-posterior sense by convergent extension**, drawing in cells from more lateral regions. This process causes the **dorsal marginal zone to thicken** relative to the remainder of the circumference and it then becomes known as the **embryonic shield**.
- **Epiboly** reaches completion at about **9.5 h** when **the yolk cell is completely covered by the blastoderm.** The outer layer of the shield becomes the neural plate. This sinks into the interior as **a solid mass of cells**, and **a lumen forms by cavitation** to create a **neural tube**.
- The mesodermal layer forms a midline **notochord** and **paraxial somites**, segmenting from anterior to posterior.
- The **basic body plan structures** becomes visible by about **14 h**, and the **axis straightens** by **24 h**. **Hatching** occurs after about **48 h**, and **feeding** commences about **5 d** after fertilization.

Fate map



- Each individual blastomere contributes to a very wide region of the later embryo. By the beginning of gastrulation the range of random cell movement is much reduced and a fate map can be produced with similar resolution to that of Xenopus.
- The fate map shown is for the **beginning of gastrulation** and **concerns just the deep cells**. Individual cells may populate more than one tissue type or even more than one germ layer if labeled in the early stages of **epiboly**.

Regional specification



The organizer

- The **organizer region in zebrafish** is called the **embryonic shield**. Grafting of the shield to **a ventral position** can induce **a secondary axis** containing somites and a neural tube. As in Xenopus, **dorsalization of the mesoderm** by **signals from the organizer** involves inhibitors of BMPs.
- **Two BMP genes**, ***swirl*** (BMP2b) and ***snailhouse*** (BMP7), are expressed in the **ventral part** of the gastrula. Their loss of-function mutants show **dorsalization**, showing that **BMP signaling is needed for ventral development**.
- The transcription factor **dharma**, **expressed in the organizer region**, normally **suppresses expression of *swirl***.
- The loss of function mutant of dharma is **ventralized**, and this can be rescued by injection of mRNA for the **EnR domain swap** version of **dharma**, showing that the factor normally acts as a transcriptional repressor.
- The action of **dharma** also leads to **activation of the chordin gene**, presumably indirectly since dharma itself is a repressor. The **zebrafish chordin** homolog of **chordin**

is called **dino or chordino**, and, as expected, the loss-of-function mutant of chordino causes ventralization.

- The ventral state is defined by expression of **two homeodomain transcription** factors, **Vent** and **Vox**, which are the homologs of Vent 1 and 2 in Xenopus. These have a redundant action, but loss-of-function mutants of both genes together produce a dorsalized phenotype.
- Their normal expression is dependent on **BMP signaling**, and overexpression of **Vent or Vox** has a **ventralizing effect**.
- Thus, as in Xenopus, it seems that the **dorsalizing action** of the **organizer** arises from inhibition of BMPs by direct transcriptional inhibition in the organizer itself and by secreted BMP inhibitors in the surrounding regions.

Dorso-ventral polarity:

- There is no obvious cortical rotation in the zebrafish, but treatment with the **microtubule depolymerizing drug nocodazole** does suppress axis formation, suggesting a role for microtubules in the establishment of the dorsal center.
- The **Tcf3 transcription factor** is encoded by the **gene headless**, whose loss of function phenotype is anterior reduction, suggestive of a role for the **Wnt pathway**.
- Formation of the **dorsal axis** can be suppressed by overexpression of **gsk3**, and **a dominant negative gsk3** can induce **a secondary axis**. There is a migration of ***β-catenin*** to **the nuclei on the dorsal side**, visible by immune-staining.
- Overexpression of ***β-catenin*** will activate transcription of a gene of the paired-homeobox class, ***dharma*** (also called ***nieuwkoid*** or ***bozozok***).
- ***dharma*** is necessary for **formation of the organizer** as the loss-of function mutant lacks notochord, prechordal plate, and neural tube.

Antero-posterior patterning

- The antero-posterior patterning mechanism shows some similarity and some difference to that in Xenopus.
- During gastrulation, the **blastopore region** emits **a posteriorizing signal** in a similar way. There is good evidence that **FGFs** are an important constituent of the signal. **Overexpression of FGFs** causes **anterior truncation**, or induction of posterior

markers in anterior explants. Conversely **overexpression of a dominant negative FGF** receptor causes posterior truncation.

- In terms of loss-of function mutation, if the ***fgf8*** (a cerebellar) mutant is combined with injection of **a morpholino oligo** directed against ***fgf24***, then formation of posterior structures is inhibited. This suggests that these two FGFs make up most of the normal posteriorizing signal.
- As in Xenopus, there is also some evidence for a role for **Wnt** and **retinoic acid signaling** since **both types of factor will posteriorize on overexpression**. It has been argued that the **ventral marginal zone** is a "**tail organizer**," as it will form a tail if combined with an animal cap, much of the tail arising from the animal cap cells.
- This effect can be mimicked by injection of a combination of mRNAs encoding **nodal + BMP + Wnt,** and since the combination of **nodal + BMP** might be expected to generate **ventral mesoderm**, this is further evidence for the **posteriorizing activity of Wnt**.
- There is also a group of anterior ectoderm cells, fated to form anterior telencephalon, pituitary, and nasal placodes, which has an anteriorizing influence when grafted to other parts of the neural plate.
- Removal of the embryonic shield ablates the notochord and prechordal plate, together with the ventral midline structures of the central nervous system, but it does not greatly affect the overall antero-posterior pattern of forebrain–midbrain–hindbrain–spinal cord. This is now known to be a source of FGF at a later stage.

Meso-endoderm induction

- The **sequence of inductive events** that build up the body pattern also seems very similar to those in Xenopus with a few variations. There is an **initial mesoderm** induction, involving the **formation of a ring of mesoderm**, with **an organizer region** (**the embryonic shield**) on the **dorsal side**.
- The **organizer region** then emits signals leading to **neural induction of the ectoderm** and to dorsalization of the lateral mesoderm. One variation concerns the action of **veg T**.
- In zebrafish this is encoded by the gene ***spadetail***, which is expressed zygotically in the **mesoderm** and whose mutant phenotype is a defect in the trunk rather than loss of all mesoderm and endoderm.

- **Gene *spadetail*** is not expressed **maternally**. However the yolk cell, which is the zebrafish equivalent of the Xenopus vegetal hemisphere, does emit a meso-endodermal inducing signal. If the yolk cell is removed from the embryo before the 16-cell stage, then formation of the mesoderm is prevented. If the yolk cell, with its associated yolk syncytial layer, is recombined with **an animal cap**, then mesoderm is induced around the junction, as shown by **expression of markers like *notail*** (the zebrafish homolog of ***brachyury***) in the responding tissue.
- There are **two zebrafish homologs of nodal**: ***cyclops*** and ***squint***. The double loss-of-function mutant has **no germ ring** and forms little mesoderm. A similar phenotype is shown by loss-of-function mutants of the gene **one-eyed-pinhead**. This encodes the zebrafish homolog of ***cripto***, an extracellular factor whose action is required for nodal signaling.
- Furthermore, a similar loss of mesoderm follows overexpression of **Cerberus-**short, the fragment of the Xenopus Cerberus factor that **antagonizes nodal**. Conversely, the overexpression of **nodal mRNA or of a mRNA** for a constitutive nodal receptor, will induce mesoderm in zebrafish animal caps.
- These experiments make up good evidence that **mesoderm induction** is carried out by **nodal signals**.
- Transcription factor genes characteristic of the early endoderm can be also activated by **high concentration of nodal**. These include **bonnie** and **clyde** (=mixer), **faust** (=**GATA5**), and **casanova** (**sox** related).