Formation and Patterning Roles of the Yolk Syncytial Layer

Takuya Sakaguchi[1], Toshiro Mizuno[2], and Hiroyuki Takeda[1,3]

1 Introduction

Zebrafish develop in a typical manner of teleosts (for review, see Driever 1995). The animal/vegetal axis is specified during oogenesis and sperm can enter the egg through a single entry site (micropyle) located at the animal pole. When eggs are laid, yolk and cytoplasm are intermixed. After fertilization, cytoplasm streams to the animal pole as it segregates from the yolk. About 30 min after fertilization, the cytoplasm forms the blastodisc at the animal pole and surrounds the vegetal yolk mass as a thin cytoplasmic layer (Fig. 1A,B). The cleavages take place atop a large yolk mass. During mid-blastula transition, activation of zygotic transcription (10th cleavage in zebrafish) coincides with the generation of the first three separate lineages of the embryo (Fig. 1C,D). Two of these lineages are extra-embryonic, the enveloping layer (EVL) forming the outer surface of the blastoderm, and the yolk syncytial layer (YSL) that covers the animal part of the yolk cell, respectively. The third lineage, termed the deep cell layer, will form the embryo proper.

One unique and fascinating feature of early teleost development is the formation of the YSL at the surface of the yolk, peripheral to and underneath the blastoderm. The syncytium, which was originally known as the "periblast", was renamed "yolk syncytial layer" by Trinkaus (1992). The YSL has great importance for teleost development. Since it separates the yolk from the embryo (deep cell layer), all nutrients from the yolk must pass through it to reach the embryo. Indeed, analyses with the zebrafish anemia mutant, *weissherbst*, revealed that Ferroportin1 expressed in the YSL plays a crucial role in transport of iron from the yolk to the embryo (Donovan et al. 2000). In addition to the role in nutrient transport, the YSL has been implicated in epiboly movement (Trinkaus 1993). More recently, the YSL has been found to play an important role in induction and patterning of the mesoderm and endoderm (Mizuno et al. 1996 1999b; Ober and Schulte-Merker 1999; Rodaway et al. 1999). Thus,

[1] Division of Early Embryogenesis, National Institute of Genetics, Yata, Mishima 411-8540, Japan
[2] Graduate School of Science, Osaka City University, Sumiyoshi, Osaka, 558-8585, Japan
[3] Department of Biological Sciences, Graduate School of Science, University of Tokyo, Bunkyo, Tokyo 113-0033, Japan
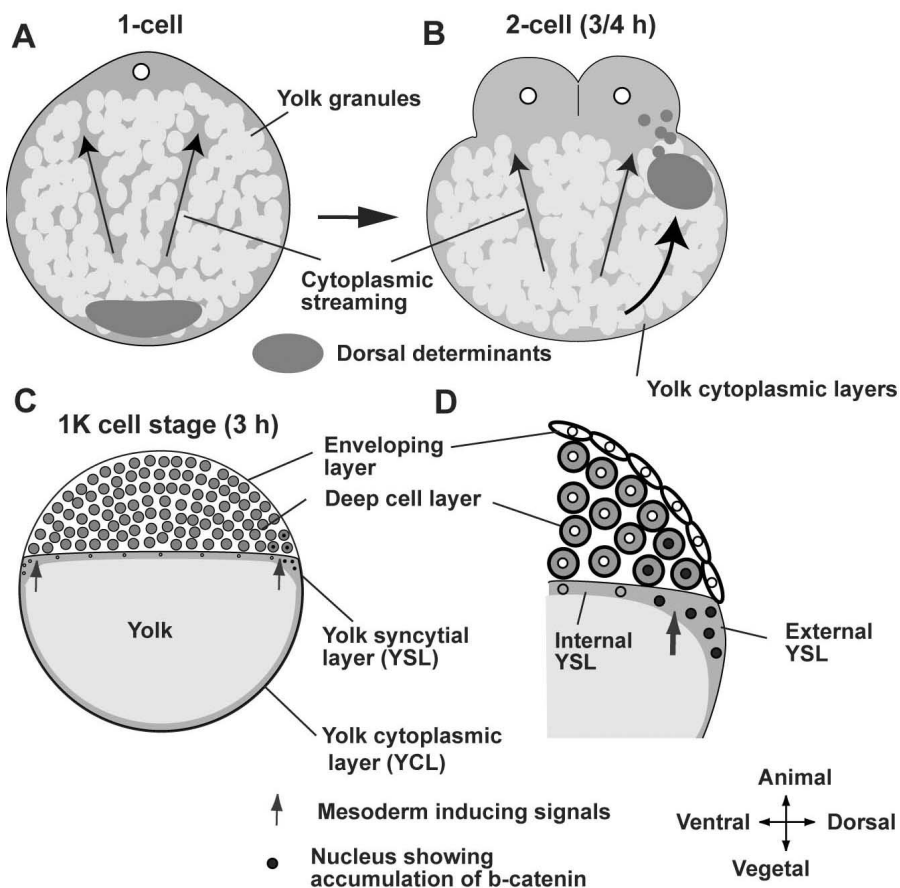
Fig. 1A–D. Early development of zebrafish. The development of zebrafish embryos from 1-cell to mid-blastula stages is represented by schematic drawings of mid-sagittal sections of the embryos. The embryos are oriented with future dorsal to the right and developmental times are in hours postfertilization at 28 °C. **A, B** In the zygote, yolk (*white*) and cytoplasm (*gray*) are mixed, but separate during the first 2 h of development by cytoplasmic streaming to the animal pole (*arrows*). Cytoplasmic determinants that are required for dorsal development (*dorsal determinants*) may be present in the vegetal region at the time of fertilization. They are then translocated to the future dorsal sides in a microtubule-dependent manner during early cleavage stages. **C, D** The 1000(1k)-cell embryo represents the mid-blastula. The different embryonic and extra-embryonic lineages can be clearly distinguished at this stage: deep cell layer (*embryo proper*), enveloping layer (*EVL*), yolk syncytial layer (*YSL*) and yolk cytoplasmic layer (*YCL*). The YSL is further divided into the internal and external YSLs. The *arrows* in **C** represent mesoderm- and endoderm-inducing signals from the YSL. High magnification of the dorsal region is shown in **D.** Nuclear localization of β-catenin (*solid circle*) is detected in dorsal blastomeres as well as the external YSL

the YSL has attracted the attention of more fish biologists than ever before and has become the target of various studies. In this chapter, we will discuss the formation and the activities of the zebrafish YSL, focusing on its roles in germ layer formation and its patterning.
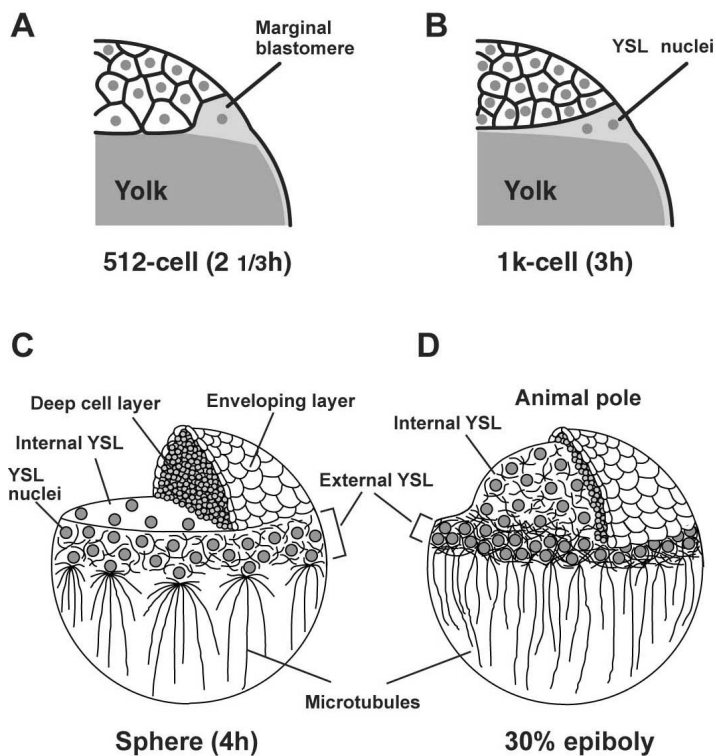
2 Formation of the YSL

In zebrafish, the initial nine cleavages occur synchronously at 15-min intervals (Kimmel et al. 1995). The first five cleavage planes are usually vertical in orientation and alternate at right angles to one another. The sixth cleavage plane is horizontal, thus producing two tiers of blastomeres. These early cleavages are meroblastic and the marginal vegetal blastomeres maintain large cytoplasmic bridges with the yolk cell (Fig. 2A,B). Formation of the YSL begins by fusion of the marginal blastomeres with the yolk cell at mid-blastula stage.
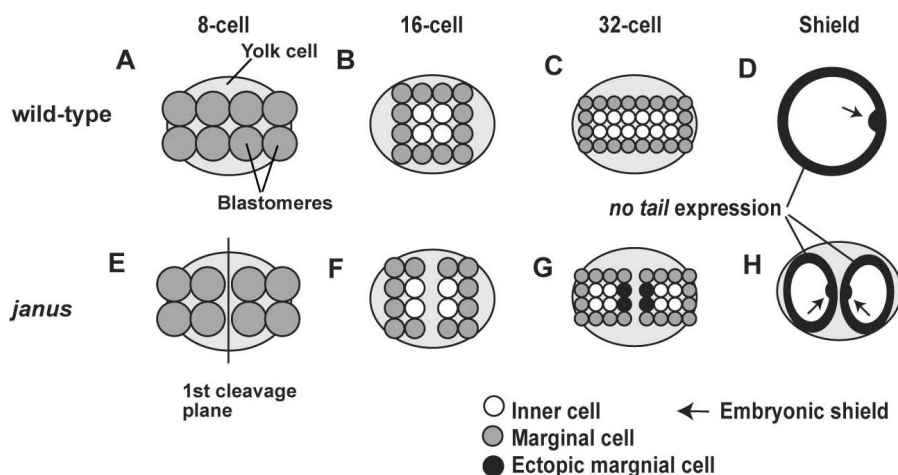
Fig. 2. Cleavage pattern and possible marginal zone formation in wild-type (**A–D**) and *janus*-mutant embryos (**E–H**). Schematic drawings viewed from the animal pole are shown. **A–D** The cleavages are meroblastic so that initially all blastomeres (2–8-cell stages) and later the marginal blastomeres (16–512-cell stages) maintain cytoplasmic connections with yolk cell (marked as *gray circles*). From the 16-cell stage on, the central blastomeres (*open circles*) lose cytoplasmic connections and are completely separated from the yolk (**B, C**). At the shield stage, *no tail*, a pan-mesodermal marker, is expressed in the blastoderm margin (indicated by the *solid black zone*; **D**). Most of the marginal zone is mainly constituted by cells that stem from the lineages of the early marginal cells. **E–H** In *janus*-mutant embryos, a separation of the blastoderm into two halves frequently occurs within the first four cleavages and each half separately develops until fusion occurs at the end of gastrulation (Abdelila and Driever 1997). The separation results in ectopic formation of marginal cells that would normally contribute mainly to the animal-pole region (*solid circle* in **G**). At the shield stage, *no tail* expression is detected in the entire margin of each half including the ectopic region. (After Solnica-Krezel et al. 1995; Abdelila and Driever 1997)

Interestingly, YSL formation appears to be correlated with the number of cell divisions and a loss of cytoplasmic bridges.

Using both dye injections into blastomeres and direct examination of blastomere cleavages in live embryos, Kimmel and Law (1985b) described in detail the process of YSL formation in zebrafish. Between the 9th and 10th cleavages (512- to 1024-cell stages), the lower cell borders adjacent to the yolk cell fade and disappear as the marginal cells collapse. When the cells complete 10th mitosis, the nuclei are detected within the YSL and are lined up in a single ring around the cellular blastoderm (Fig. 3A,B). Once incorporated, YSL nuclei

Fig. 3. Schematic drawings of YSL formation (**A, B**) and changes in organization of the blastoderm and the yolk cell at the onset of epiboly (**C,D**). At the 512-cell stage (complete of 9th cleavage) in zebrafish, dividing blastomeres at the margin of the blastoderm are still in cytoplasmic confluence with the yolk cell (**A**). As the 10th cell cycle begins, the cytoplasm and nuclei of marginal blastomeres collapse into the yolk cell, thus forming the YSL (**B**). Just before the onset of epiboly (**C**, *sphere*), the yolk cell is furnished with two types of microtubular arrays: a network in the microtubules present in the YSL, and animal-vegetal-oriented microtubules in the YCL originating in the YSL. Epiboly starts with the yolk cell bulging towards the animal pole, a process called "doming" (**D**). The external YSL has contracted and exhibits densely packed YSL nuclei. As epiboly proceeds, the YSL with the nuclei and intercrossed microtubule network will spread vegetally, while the YCL with the animal-vegetal microtubule system will decrease in size (see text). **C** and **D** were adapted from Solnica-Krezel and Driever (1994)

divide more rapidly than their deep cell cousins and undergo a series of metachronous divisions eventually becoming postmitotic at the 12th cycle (about 4 h of development; Kane et al. 1992). After 10th cleavage, usually no further contribution of blastomeres to the YSL is observed.

YSL formation is accompanied by a loss of cytoplasmic bridges between the yolk cell and blastoderm. Until the formation of the YSL, marginal blastomeres remain joined to the yolk cell through the cytoplasmic bridges. However, the bridges are disconnected as soon as the YSL is formed: injection of the fluorescent tracer fluorescein-dextran (FD) into the yolk cell after the YSL has been formed usually labels the yolk cell specifically. FD is expected to diffuse through cytoplasm but to be too large to pass through gap junctions.

With respect to the lineage that contributes to the YSL, whether a blastomere will participate in YSL formation appears to depend on its marginal position within the embryo rather than on its lineage. At the 64-cell stage, usually 20 marginal blastomeres remain connected to the yolk cell (Fig. 2A,B; Kimmel and Law 1985a). It could be that all of the progeny of the marginal cells participate in YSL formation. However, this possibility is unlikely because injection of FD into marginal cells at 64- to 256-cell stages produces both a labeled YSL and many labeled cells within the blastoderm (Kimmel and Law 1985b), suggesting that not all, but only a subset of the progeny of early marginal cells contribute to the YSL. Direct microscope observation supports the result of the lineage-tracing experiment (Kimmel and Law 1985b).

When YSL formation completes at mid-blastula stage, three compartments of continuous cortical cytoplasm can be distinguished in the yolk cell (Fig. 1C,D). A thin, anuclear yolk cytoplasmic layer (YCL) surrounds the bulk of the yolk mass with the vegetal pole. The YSL can be further divided into two parts. One is the external YSL, located between the YCL and the blastoderm rim, a relatively thick belt of cytoplasm populated by the YSL nuclei. The other is internal YSL that is located beneath the blastoderm, comprising a thinner cytoplasm. The internal YSL is finally populated by nuclei derived from the external YSL.

3 Epibolic Movement and the YSL

During epiboly, the deep cells, the enveloping layer (EVL) and the YSL lineages vegetally expand eventually to cover the entire yolk cell. The beginning of epiboly is marked by a major morphological change in the yolk cell, which bulges toward the animal pole in a process called "doming" (Fig. 3C, D; Warga and Kimmel 1990). Subsequently, the YSL spreads toward the vegetal pole, with the YCL being diminished.

Studies of another teleost, *Fundulus heteroclitus*, suggested a crucial role of the YSL in epiboly (for details, see Kane and Adams, this Vol.). The blastoderm isolated from a *Fundulus* embryo does not undergo epiboly unless it is attached to the YSL. The YSL, in contrast, undergoes epiboly in the absence of the