

# A flipbook of zebrafish embryogenesis

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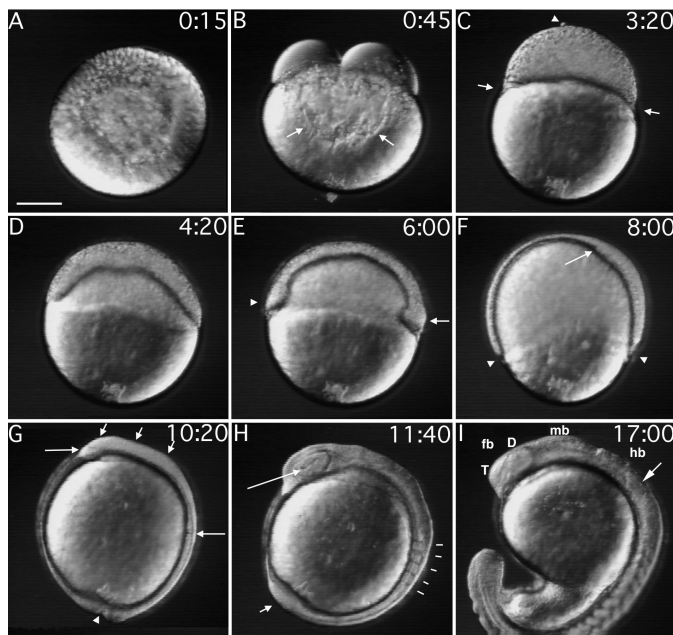
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We modified a time-lapse recording to make a ‘flipbook’ of zebrafish embryogenesis. By flipping through the pages of this issue and watching the pictures in the upper corner of each even-numbered page, the dynamic cellular movements that form the zebrafish embryo can be visualized. The time-lapse record starts at the 2-cell stage (page 2 and Fig. 1B) and ends at the 16-somite stage (Fig. 1I), approximately 17 hours later.



**Fig. 1.** Selected samples from the time-lapse record. (A) Fertilized egg, dorsal view, at 0:15 (hours:minutes postfertilization). The cytoplasm clears from the yolk and moves towards the animal pole during early cleavage. (B) 2-cell stage, side view. Cytoplasm in the yolk (arrows) continues to stream into the meroblastic cells at the animal pole. (C) High blastula stage, early in the zebrafish midblastula transition. Occasionally, dividing cells protrude from the blastoderm (arrowhead). The yolk syncytial layer forms at the margin of the blastoderm (arrows). (D) Dome stage. The yolk protrudes into the blastoderm. The blastoderm begins its spread over the yolk toward the vegetal pole, the movement called epiboly. (E) Shield stage in the early gastrula. The thickening hypoblast forms the germ ring (arrow and arrowhead). The shield (arrow) forms at the dorsal side of the embryo. (F) 70% epiboly. The margin of the blastoderm (arrowheads) has moved to 70% of the distance from the top to the bottom of the embryo. Hypoblast cells of the shield region migrate toward the future head; the leading portion (arrow) will form the prechordal plate. (G) 1-somite stage. The first somite border (arrow at right) forms posterior to the first somite. Cells from the dorsal and ventral side of the gastrula aggregate to form the tail bud, here still separated by a cleft (arrowhead). The brain neural ectoderm thickens anteriorly (short arrows). The polster (long arrow), the anterior edge of the prechordal plate, is visible ventral and anterior to the future forebrain. (H) 5-somite stage (somite borders marked by lines). The eye primordium appears (long arrow). The tailbud (short arrow) moves ventrally. (I) 16-somite stage. Brain regionalization is apparent as the forebrain (fb): telencephalon (T) and diencephalon (D); midbrain (mb) and hindbrain (hb) become distinguishable. The otic placode (arrow) forms lateral to rhombomere 5 of the hindbrain. Scale bar, 200  $\mu$ m.

Table 1 lists the normalized times when the major embryonic stages occur (see Kimmel et al., 1995; *Developmental Dynamics* **203**, 253-310), the corresponding page numbers in this issue, and a brief explanation of events clearly visible in the time-lapse. Several time points from the time-lapse are shown in Fig. 1. This flip-book will serve as an aid in embryonic staging and should help with the interpretation of mutant phenotypes described in this issue.

The embryo was mounted and photographed as described by Kane et al. (this issue, pp. 47-55). Selected images were digitized and processed in NIH Image; for the exact times of the developmental stages refer to Table 1.

This movie is available in a QuickTime format on the internet at: <http://wwwweb.mpib-tuebingen.mpg.de>. We thank Dr Frederick Bonhoeffer who suggested this project and Dr Christiane Nüsslein-Volhard in whose laboratory we did the recording.

**Table 1. Zebrafish developmental stages**

Period Stage	Time after fertilization*	Page	Minutes per frame†	Major developmental events visible
<b>Cleavage</b>				
2-cell	0:45	2	1:00	Movement of the cytoplasm toward the animal pole
16-cell	1:30	60	1:45	Synchronous divisions every 15 minutes
128-cell	2:15	140	2:30	Right to left metasynchronous cleavages
<b>Blastula</b>				
High blastula	3:20	218	3:20	Slowing of the cell cycle; loss of cell synchrony
Sphere	4:00	232	4:00	Shimmering of blastoderm caused by cell motility
dome	4:20	242	4:20	Beginning of epiboly
40% epiboly	5:10	252	5:00	Thinning of the blastoderm
<b>Gastrula</b>				
Germ ring	5:40	266	5:00	Beginning of hypoblast formation
Shield	6:00	274	6:00	Thickening of the dorsal hypoblast
90% epiboly	9:00	330	6:40	Dorsal hypoblast reaches animal pole
Tailbud	10:00	340	7:00	Tailbud formation at the vegetal pole
<b>Segmentation</b>				
1-somite	10:20	346	7:30	Thickening of the anterior neural ectoderm
5-somite	11:40	374	7:30	Eye primordium formation
14-somite	16:00	448	8:00	Segmentation of the brain

\*Hours:minutes postfertilization at 28.5°C.

†Minutes:seconds between each sequential frame.