

Table 1. Stages of Early Embryonic Development in *Ciona intestinalis*

Hotta et. al., (2007) Dev.Dyn.

Hotta's Stage	Characteristics		Measurement of embryos				
			T	% hatch	Head Length (μm)	Tail Length (μm)	Ratio Tail/Head
I. Zygote period (0-1.0hr)							
St. 1	One cell	Zygote, fertilized egg	24min (0.4hpf)	3%			
II. Cleavage period (1.0-4.5hr)							
St. 2	2-cell	Two cell-stage embryo	55min (0.9hpf)	6%			
St. 3	4-cell	Four cell-stage embryo	1hr 27min (1.45hpf)	8%			
St. 4	8-cell	Eight cell-stage embryo	1hr 54min (1.9hpf)	11%			
St. 5a	early 16-cell	Early sixteen-cell stage embryo	2hr 21min (2.35hpf)	13%			
St. 5b	late 16-cell	Late sixteen-cell stage embryo	2hr 39min (2.65hpf)	15%			
St. 6a	early 32 cell	Early thirty two-cell stage embryo	3hr (3hpf)	17%			
St. 6b	late 32 cell	Late thirty two-cell stage embryo	3hr 12min (3.2hpf)	18%			
St. 7	44-cell	Fourty four-cell stage embryo. The vegetal side of the embryo is very round.	3hr 21min (3.35hpf)	19%			
St. 8	64-cell	Sixty four-cell stage embryo. Embryo has a square shape seen from the top, with bulging B7.4 cells.	4hr (4hpf)	23%			
St. 9	76-cell	Seventy six cell stage embryo. The vegetal side of the embryo is very flat	4hr 12min (4.2hpf)	24%			
III. Gastrula Period (4.5-6.3hr)							
St. 10	110-cell, initial gastrula	Gastrulation starts with the apical constriction of A7.1 blastomeres.	4hr 33min (4.5hpf)	26%			
St. 11	early gastrula	The notochord has invaginated. The vegetal side of the embryo has a horseshoe shape.	4hr 54min (4.9hpf)	28%			
St. 12	mid gastrula	Six-row neural plate stage. The blastopore is still central and open.	5hr 39min (5.65hpf)	32%			
St. 13	late gastrula	The blastopore is in posterior position and nearly closed. The embryo elongates anteriorly. The neural plate has more than 6 rows and the A-line neural rows (I and II) start to curve (neurulation begin). The large b6.5 progeny are coming together at the midline.	5hr 55min (5.9hpf)	34%			
VI. Neurula Period (6.3-8.5hr)							
St. 14	early neurula	A-line neural plate forms a gutter lined by b6.5 descendants. The embryo has a diamond shape. The gutter is not closed.	6hr 21min (6.35hpf)	36%			
St. 15	mid neurula	The neural tube has formed on most of its length. The embryo has an oval shape. The a-line neural plate also forms a gutter.	6hr 48min (6.8hpf)	39%	83.1	83.0	1.0
St. 16	late neurula	The neural tube starts to form in the posterior territories. The embryo elongates.	7hr 24min (7.4hpf)	42%	85.3	84.2	1.0
V. Tailbud Period (8.5-17.5hr)							
St. 17	initial tailbud I	First indication of a separation between tail and trunk territories. The tail is not bent and has the same length as the trunk. Any notochord cells not finished intercalation.	8hr 27min (8.45hpf)	48%	89.9	87.8	1.0
St. 18	initial tailbud II	The tail is clearly separated from the trunk. Tail and trunk have same length. Neuropore still open, a-line neurulation.	8hr 50min (8.8hpf)	50%	97.6	111.9	1.1
St. 19	early tailbud I	The tail bends about 40° and is slightly longer than the trunk. A few anterior most notochord cells begin to intercalate and linear.	9hr 19min (9.3hpf)	53%	103.0	120.3	1.2
St. 20	early tailbud II	Neuropore closed, tail bent by 60°, neurulation complete.	9hr 30min (9.5hpf)	54%	112.0	146.7	1.3
St. 21	mid tailbud I	Tail 1 1/2 times longer than trunk and curve ventrally (90°). Intercalation of notochord cells just finished.	10hr 2min (10hpf)	57%	114.3	180.7	1.6
St. 22	mid tailbud II	The body adopts a half circle shape. Tail twice as long as trunk.	10hr 54min (10.9hpf)	62%	118.0	221.2	1.9
St. 23	late tailbud I	Initiation of the pigmentation of the otolith. Tail strongly curved with tip close to the anterior end of the trunk.	11hr 54min (11.9hpf)	68%	118.9	255.1	2.1
St. 24	late tailbud II	Notochord vacuolation begins, palps start to be visible at the front end of the embryo. Tail straightens.	13hr 27min (13.5hpf)	77%	130.8	442.6	3.4
St. 25	late tailbud III	Ocellus melanization. All notochord cells have vacuoles. Tail bent dorsally.	15hr 54min (15.9hpf)	91%	143.7	558.6	3.9
IV. Larva Period (17.5hr-)							
St. 26	hatching larva	Hatching. head adopts an elongated rectangular shape.	17hr 30min (17.5hpf)	100%	159.3	661.6	4.2

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From the left column, "Hotta's stage" is the staging criteria defined in this manuscript. A total of 26 stages are divided into six periods. Parentheses in each period mean the start-time and end-time of each period at 18°C. "Characteristics" is mainly based on the observation under dissecting microscopy. "Measurement of embryos": T, Time after fertilization (average at 18°C, n=3), % hatch = rate of T (min) / 1050 (min), head length, tail length and ratio of tail / head length