HOKKAIDO UNIVERSITY

Title	Dissociated Blastomeres with High Concentration of Activin Contribute Endoderm Lineage When Transplanted into Blastula in Zebrafish (Danio rerio), and Goldfish (Carassius auratus)
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Citation	北海道大学水産科学研究彙報, 75, 11-26
Issue Date	2025-10-20
DOI	https://doi.org/10.14943/bull.fish.75.11
Doc URL	https://hdl.handle.net/2115/96108
Type	departmental bulletin paper
File Information	SuppTable1-6.pdf, Table S1-S6

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Table S1. Effect of injetion on survival in zebrafish embryos.

	No. of eggs	Survival rate after 24 hpf	Abnormal embryos
	n	%	%
Dechorionated control	24	91.7	8.3
Rhodamine	24	83.3	16.7
Rhodamine + rhAct A	31	0	100
Rhodamine + rhAct B	30	0	100
Rhodamine + rhAct AB	30	0	100

Table S2. Frequencies of elongated embryos developed from animal caps treated with rhAct B in goldfish.

	No. of eggs	Elongated embryos
	n	%
Blastodisc	21	100
Animal cap	20	0
Animal cap （1 ng/mL, 3 h）	6	33.3
Animal cap （10 ng/mL, 3 h）	6	100
Animal cap （50 ng/mL, 3 h）	18	100
Animal cap （100 ng/mL, 3 h）	12	100
Animal cap （200 ng/mL, 3 h）	14	100
Animal cap （50 ng/mL, 1 min）	13	69.2
Animal cap （50 ng/mL, 5 min）	13	100
Animal cap （50 ng/ mL, 10 min）	12	100
Animal cap （50 ng/mL, 30 min）	13	100
Animal cap （50 ng/mL, 1 h）	15	100
Animal cap （50 ng/mL, 24 h）	19	100

Table S3. Frequencies of elongated embryos developed from animal caps treated with rhAct B in zebrafish.

	No. of eggs	Elongated embryos
	n	%
Blastodisc	64	100
Animal cap	61	0
Animal cap （1 ng/ mL, 10 min）	55	5.5
Animal cap （10 ng/ mL, 10 min）	52	15.4
Animal cap （100 ng/ mL, 10 min）	57	89.5

Table S4. Survival and abnormal rates of resultant embryos which developed from transplantation of dissociated blastomeres treated with several concentration of rhAct B at the blastula stage in goldfish.

Host	Donors treatment	Number of transplant	Survival embryo after 2 day		Normal embryos		Abnormal embryos	
			Number	(%)	Number	(%)	Number	(%)
Exp. 1								
-	-	36	33	91.7%	26	78.8%	7	21.2%
-	0 ng/mL	15	13	86.7%	10	76.9%	3	23.1%
Intact blastula	500 ng/mL	30	28	93.3%	21	75.0%	7	25.0%
Intact blastula	1 μg/mL	53	41	77.4%	21	51.2%	20	48.8%
Exp, 2								
-	-	34	34	100.0%	30	88.2%	4	11.8%
-	0 ng/mL	23	22	95.7%	17	77.3%	5	22.7%
Intact blastula	500 ng/mL	96	90	93.8%	63	70.0%	27	30.0%
Intact blastula	1 μg/mL	48	47	97.9%	39	83.0%	8	17.0%

Table S5. Survival and abnormal rates of resultant embryos which developed from transplantation of dissociated blastomeres treated with 50 ng/mL rhAct B at the blastula stage in goldfish.

Treatment	No. of eggs	Survived embryos		Abnormal embryos	
	n	n	(%)	n	(%)
Control embryo without transplantation	12	12	(100.0)	0	(0.0)
Embryos transplanted with non-treated donor cells	12	11	(91.7)	1	(8.3)
Embryos transplanted with treated donor cells with rhAct B	12	2	(16.7)	10	(91.7)

Table S6. Survival, normality, and distribution of donor cells in control and resulting embryos developed after transfer of zebrafish rhAct B-treated blastomeres. Donor cells were transferred at the blastomere stage, and at the embryo shield stage, the resulting embryos were classified into three categories according to their distribution: dorsal, ventral, and lateral. Then, at the high thoracic stage, the resulting embryos were examined to see where the donor cells were distributed along the anterior-posterior regions of the endoderm.

Donor	Host	Distributed position at the shield stage	Number of transplant	Dead embryos		Normal in survived embryos		Main distribution region of donor cells in endoderm of normal embryos									
								Front part		Most endoderm		Pharynx		Tail		other	
				n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
control zebrafish			24	0	(0.0)	24	(100.0)										
FITC control			12	0	(0.0)	12	(100.0)										
Dissociated blastomeres with rhAct B	zebrafish	dorsal	29	1	(3.6)	23	(85.2)	0	(0.0)	4	(17.4)	19	(82.6)	0	(0.0)	0	(0.0)
Dissociated blastomeres with rhAct B	zebrafish	lateral	24	0	(0.0)	19	(79.2)	0	(0.0)	8	(41.2)	11	(57.9)	0	(0.0)	0	(0.0)
Dissociated blastomeres with rhAct B	zebrafish	ventral	19	1	(5.3)	8	(44.4)	1	(12.5)	4	(50.0)	1	(12.5)	1	(12.5)	1	(12.5)
Dissociated blastomeres with rhAct B	zebrafish	No distinction	32	9	(28.1)	16	(69.6)	1	(12.5)	5	(31.3)	9	(56.3)	0	(0.0)	1	(6.3)