CELL FATE OF SUPERFICIAL CELLS IN THE MARGINAL ZONE OF THE *PLEURODELES WALTL* EMBRYO

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During embryogenesis, the commitment of a single cell to different fates results from interactions with neighboring groups of cells. Recently, we have show that descendants of superficial cells in the dorso marginal zone (DMZ) of a blastula give rise in the tail bud stage to mesodermal and endodermal derivatives distributed in three or four different embryonic structures (Delarue et al., 1992). By the early gastrula stage, however, individual superficial cells in the same region (IIA region) now contribute to a more restricted set of embryonic structures. In *Xenopus* embryo, slow but continuous intermixing of cells occurs during embryogenesis that lead to new cell-cell contacts and new cellular environments (Wetts and Fraser, 1989), but cells with mesodermal fates are restricted to the deep cell layers of the early *Xenopus* gastrula (Smith and Malacinski, 1983). For most amphibians other than *Xenopus*, little is know about the developmental potential of single cells and their progenie. Consequently, we have performed an analysis of the lineage history of individual cells in the marginal zone, and we have compared the fates and lineage restrictions of indentified groups of cells at the late blastula and early gastrula stages of *Pleurodeles* embryos.

Embryos of *Pleurodeles waltl* were obtained from natural mating in the laboratory, reared as previously described (Delarue et al., 1992) and staged according Shi and Boucaut (1995). Single cells in the late blastula (stage 7) and early gastrula (stage 8a) were microinjected with 0.5 nl of rhodamine-lysine-dextran (RLDx) tracer (50mg/ml) in distilled water. Embryos at stage 8a were positioned under a square ocular reticle in a Leitz stereomicroscope and single cells were injected according to their location with respect to the nascent blastopore at a defined location in the ocular reticle as previously described (Delarue et al., 1992). In embryos at stage 7 the presumptive dorsal lip of the blastopore was marked with a small crystal of Nile blue sulfate prior to its appearance. We then injected a single cell at a defined location in the ocular reticle and allowed embryos to develop until the dorsal lip of the blastopore became visible. When the vital dye mark was inappropriately placed (i.e., did not correspond to the actual dorsal lip) we discarded embryos.

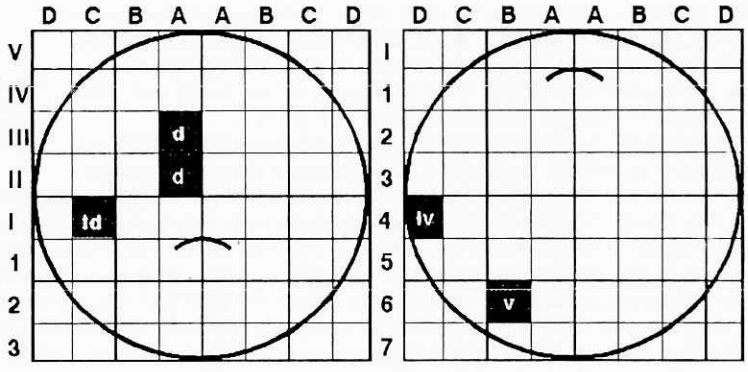

Figure 1. Diagrams illustrating the location of injected cells at the early gastrula estage (stage 8a) (shaded squares) in the DMZ (d), DLMZ (ld), VLMZ (lv) and VMZ (v).

Single cell injections were made into the IIA, IIIA, IC, 4D and 6B squares in the reticle in 140 blastula (stage 7) and 343 gastrula (stage 8a) (Fig. 1). At the blastula stage, injections were made in the IIA, IIIA (DMZ), IC (dorsolateral marginal zone, DLMZ), 4D (ventrolateral marginal zone, VLMZ) and 6B (ventral lateral zone, VMZ). At the gastrula stage, similar injections were made. After injection, embryos were allowed to develop until the taild bud stage (stage 30), fixed in 3.7% formaldehyde, dehydrated in graded ethanol, embedded in polyethylene glycol distearate 400 and sectioned at 10 µm. Sections were mounted in Mowiol and observed with a Leitz Laborlux microscope with epifluorescence. Each injected cell gave rise to a clone of cells located in as little as one organ or as many as four organs.

To assess the validity of our single cell tracing procedure, we injected RLDx into two different blastomeres separated by three or four cells in the dorsal lip region of a stage 7 embryo and then followed the movement of the cells through stages 7 and 8. Results show that injected pairs of cells are essentially stationary.

Table 1. Distribution of injected cell progeny in a single mesodermal derivative in taild bud stage *P. waltl* embryos

Location of injected cell	Stages of injection	No. of cases	Single mesodermal derivatives	Head mesenchyme	Notochord	Myotomes	Pronephros	Lateral Plate
IIA	Blastula (7)	18	2 (11%)	-	2 (11%)	-	-	-
	Gastrula (8a)	36	26 (72%)	7 (20%)	11 (30%)	8 (22%)	-	-
IIIA	Blastula (7)	9	4 (44%)	-	3 (33%)	1 (11%)	-	-
	Gastrula (8a)	37	24 (65%)	-	19 (51%)	5 (14%)	-	-
IC	Blastula (7)	13	1 (7%)	-	-	1 (7%)	-	-
	Gastrula (8a)	63	42 (67%)	31 (49%)	-	11 (18%)	-	-
4D	Blastula (7)	10	0	-	-	-	-	-
	Gastrula (8a)	31	6 (19%)	-	-	2 (6%)	1 (3%)	3 (10%)
6B	Blastula (7)	10	0	-	-	-	-	-
	Gastrula (8a)	57	5 (8%)	-	-	-	-	5 (8%)

There is a variation in the number of cells derived from a single injected cell in different regions of the blastula stage. The smaller blastomeres (DMZ and DLMZ) form fewer progeny than the larger cells (VLMZ and VMZ). However, at the gastrula stage, the numbe of progeny is relativley constant in all regions studied. Probably, blastula cells undergo 5 or 6 mitotic cycles, while early gastrula cells undergo only 4 mitotic cycles. Thus, there is approximately one cell division between the blastula and early gastrula stage.

Results show a temporal and spatial restriction of cell distribution in the embrionic organs at the tail bud stage (table 1, figure 2). There is a spatial restriction because a single cell injected in the DMZ or DLMZ at the blastula stage embryo give rise to a single derivative in a higher percentage than a single cell injected in the VLMZ or VMZ. At the early gastrula stage, there are still spatial restrictions between DMZ, DLMZ, VLMZ and VMZ. Restricted cell lineages are more numerous, especially in the dorsal zone. In summary, in both the blastula and early gastrula stages, spatial restriction is more pronunced in the DMZ than in the other regions studied.

At the blastula stage, cell distribution in one or several derivatives is different for dorsal, dorsolateral, ventrolateral or ventral origins of cell lineages. For example, in the DMZ (IIIA) region, about half of the cell lineages distribute in only one derivative (44%) such as truncal or posterior notochord or myotomes. The other lineages contribute to two derivatives but none contributes to more than two mesodermal derivatives. In contrast, most of the cell lineages coming from DMZ (IIA), contribute to two, three or four derivatives simultaneosly. Only a few lineages contribute to only one mesodermal derivative (11%), typically notochord. Most of the cell lineages in the lateral and ventral regions contribute to two or three derivatives. Only a few lineages in the DLMZ (7%) and none in the VLMZ and VMZ contribute to a single derivative (table 1, Fig. 2).

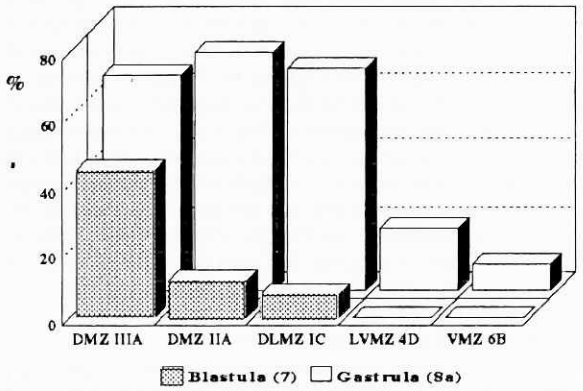

Figure 2. Diagram illustrating the restriction of cell lineage to one mesosermal derivative in the tail bud stage embryo at the blastula and early gastrula stages. The height of the bars is proportional to the percentages of number of cases with a single fate.

Moreover, there is a temporal restriction because a single cell injected in a defined region of a early gastrula stage embryo give rise to only one derivative in a higher percentage than a blastula stage blastomere at the same zone.

The fates of single injected blastula stage cells are considerably more diverse than the fates of a gastrula stage cell from the same region. For example, a single cell injected in the DMZ (IIA) of a blastula give rise to only one derivative in 11% of cases, while a single blastomere injected in the same region at the early gastrula stage give rise to a single derivative in 72% of cases. This temporal restriction of fates occurs first in the DMZ but next in the DLMZ and finally in the VLMZ and VMZ.

Our studies of the distribution of the daughter cells of defined injected cells are consistent with the results obtained with orthotopic grafting at the beginning of gastrulation (Delarue et al., 1992). For example, a double labeled DMZ (IIA) graft forms head mesenchyme, anterior notochord and anterior myotomes. A single injected cell at the same region (IIA region, near the IA region) forms head mesenchyme, anterior notochord, anterior myotomes and pharyngeal endoderm. In orthotopic grafting, IA superficial cells also formed pharyngeal endoderm. A similar congruence is noted in other regions of the embryo.

The spatial and temporal restriction of cell fate observed in the present experiments can be compared to the cell lineage restriction observed in the chick embryo at the level of Hensen's node (Selleck and Stern, 1981). In this respect, the possibility that molecular mechanisms controlling cell interactions and cell lineage history can be common to the various vertebrate embryos is an intriguing one.

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