Cell movements and cell fate during zebrafish gastrulation

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Summary

The early lineages of the zebrafish are indeterminate and a single cell labeled before the late blastula period will contribute progeny to a variety of tissues. Therefore, early cell lineages in the zebrafish do not establish future cell fates and early blastomeres must necessarily remain pluripotent. Eventually, after a period of random cell mixing, individual cells do become tissue restricted according to their later position within the blastoderm. The elucidation of a fate map for the zebrafish gastrula (Kimmel et al., 1990), has made it possible to study the processes by which cellular identity is conferred and maintained in the zebrafish. In this chapter, I describe single cell transplantation experiments designed to test for the irreversible restriction or 'commitment' of embryonic blastomeres in the zebrafish embryo. These experiments support the hypothesis that cell fate in the vertebrate embryo is determined by cell position. Work on the spadetail mutation will also be reviewed; this mutation causes a subset of mesodermal precursors to mismigrate during gastrulation thereby leading to a change in their eventual cell identity.

Key words: Brachydanio rerio, commitment, extraembryonic, gastrulation, mesoderm, mutation, zebrafish.

Introduction

Although, the zebrafish has attracted a great deal of recent interest, fish have been around for a very long time, probably about 500 million years, which is considerably longer than any other vertebrate group. As a system for developmental studies, fish embryos have a varied and interesting history deeply rooted in classical embryology (Clapp, 1891; Morgan, 1893; Wilson, 1889). The fish has been a favored organism for observational and experimental studies, in part because egg-laying fish can often produce hundreds of embryos, which develop rapidly and are easy to culture. Despite this background of study, there is still much about early fish development that remains to be studied or reexamined using modern techniques. For instance, until just a few years ago, an active controversy existed as to whether mesodermal precursors in fish embryos involuted during gastrulation or delaminated from the ectoderm to form the separate germ layers. This particular issue has since been resolved by the use of video time lapse and fluorescent lineage tracer techniques, which showed that embryonic fish cells do, in fact, involute during gastrulation (Wood and Timmermans, 1988; Warga and Kimmel, 1990). However, such confusions may be understandable when one considers that the superclass of Pisces is not only the oldest but also, by far, the most diverse vertebrate group. This diversity is partly reflected in the different organizations of various fish embryos ranging from those of the sturgeon, which are opaque and form a 'grey crescent' much like amphibian embryos (Devillers, 1961; Clavert, 1962; Delaf and Ginsberg, 1954), to those of teleosts, such as the zebrafish, which have evolved unique structures such as the 'yolk syncytial layer' (YSL) (Long, 1983). However, minor differences aside, gastrulation in fish embryos follows the basic vertebrate pattern. In this chapter, I will outline the cell movements that occur during gastrulation in the zebrafish and how the detailed knowledge of these cell movements has led to the elucidation of a fate map for zebrafish development. I will also describe experiments designed to test for the commitment of cell fate in the zebrafish embryo and how the isolation and characterization of interesting early mutations have given us insights into the conferral of cell identity in the vertebrate embryo.

Gastrulation movements in the zebrafish

The development of the zebrafish for the first embryonic day is shown in Fig. 1. In this section, the cellular movements that occur during gastrulation, namely epiboly, involu- lution, convergence and extension, will be briefly described. One of the earliest movements in the zebrafish is epiboly, which begins at 4h (where h=hours of development at 28.5°C). At this time, the zebrafish embryo has already undergone its 'mid-blastula transition' (Newport and Kri- shner, 1982a,b; Kane, 1992) an hour previous and has formed the YSL or yolk syncytial layer of nuclei within the yolk cell underlying the blastoderm (Long, 1980; Kimmel and Law, 1985). Epiboly starts as the blastoderm begins to flatten and expand. This flattening is partly driven by the directed radial intercalations of deeper lying cells into more superficial positions within the blastoderm (Keller et al.,
Fig. 1. Life history of the zebrafish. (A) Zygotic stage, (0-0.7h) where h=hours of development at 28.5°C one-cellled embryo. In this and all following drawings, the animal pole is towards the top of the page. (B) Cleavage period (1.5h). (C) 1000-cell stage (3h). Beginning of the midblastula transition and the formation of the YSL from marginal blastomeres. (D) Sphere stage (4h). Late blastula period just prior to the onset of epiboly. (E) Shield stage (6h). Cells around the margin have first involuted around the margin to form the germ ring and then converged towards the dorsal side to produce the embryonic shield. In this and all following drawings, the dorsal side of the embryo is to the right. (F) 70%-epiboly stage (7.5h). Epiboly movements continue to draw the blastoderm vegetally around the yolk cell and convergent-extension movements rearrange cells to elongate the shield along the anterior-posterior axis. (G) 14-somite stage (16h). At this time, the optic vesicle and the otic placode are clearly visible. A differentiated notochord is present and the somitic mesoderm organizes into characteristic V-shaped segments of lateral muscle. The tail bud begins to elongate and extend away from the yolk cell. (H) 24h stage. The body plan of the fish is relatively complete and the major organs are clearly visible. Hatching occurs at 48h. In all drawings, the diameter of the yolk cell is approximately 500 μm. The adult zebrafish is approximately 4 cm in length.

1989; Warga and Kimmel, 1990). These cell rearrangements, along with cell shape changes occurring in the yolk cell, cause the blastoderm to thin and spread around the yolk cell in a vegetal direction. The actin-based actions of the YSL also contribute in a major way to this movement as the YSL constricts and 'drags' the margin of the blastoderm down to the vegetal pole (Trinkaus, 1951, 1984). These epiboly movements continue until the end of gastrulation when the blastoderm has entirely engulfed the yolk cell.

As epiboly movements bring the margin of the blastoderm to the equator of the yolk cell, the morphogenetic movements of gastrulation begin. Gastrulation in the zebrafish occurs as the vegetal deep layer cells all around the margin of the blastoderm involute underneath the margin towards the underlying yolk cell surface. After cells involute, they migrate away from the margin region using either the surface of the yolk cell or the overlying cells as substrata. This concerted involution movement forms a bilayered ‘germ ring’ of an inner ‘hypoblast’ layer and an overlying ‘epiblast’ layer of cells.

While epiboly and involution are occurring, both involuting and non-involuting cells of the blastoderm ‘converge’ to the dorsal side of the embryo to form the embryonic shield. As cells enter the shield, they also intercalate between other cells leading to the lengthening or ‘extension’ of the embryonic axis in the anteroposterior direction. The convergent extension movements are similar to the processes that have been described in the Xenopus embryo. (Keller and Danilchik, 1988; Keller and Tibbets, 1989; Keller et al., 1989 and, as in Xenopus, tissues from different areas of the zebrafish embryo undergo convergent extensions to varying degrees, with the dorsal axial tissues undergoing the greatest amount of lengthening.
The zebrafish fate map

Before the onset of gastrulation, the movements of individual cells are somewhat random and unpredictable. This is due, in part, to the radial intercalations of cells that occur during the early stages of epiboly and also to the cryptic orientation of the dorsoventral axis. An early zebrafish blastomere, labeled with lineage tracer dye, will contribute progeny to many diverse tissues of the embryo. Therefore the early lineages of the zebrafish are indeterminate with respect to the future fates of cells (Kimmel and Warga, 1987). For these reasons, it has not been possible to construct an accurate fate map for the zebrafish embryo before gastrulation.

As described in the section above, the movements of cells become more patterned by the onset of gastrulation. Even though the processes of epiboly, involution, convergence and extension are occurring simultaneously, these are directed, non-random morphogenetic movements making the migrations of cells at this stage somewhat more predictable. By cataloguing the positions and later fates of fluorescently labeled cells at gastrulation, Kimmel et al. (1990) elucidated the fate map for the zebrafish shown in Fig. 2. This is the first time in development, just prior to the onset of gastrulation (5.2h, approximately 8000-cell stage) that the fates of individual deep cells can be accurately predicted, (in comparison, a fate map has been reported as early as the 32-cell stage in Xenopus, (Dale and Slack, 1987), also the YSL and the enveloping layer cells (EVL) in the zebrafish become lineally restricted around 3.5h and 4h respectively, see below).

To summarize a few points about the zebrafish fate map:

1. Though not shown in Fig. 2, the YSL and the EVL involute during gastrulation to form the ‘hypoblast’ germ layer, which gives rise to the mesoderm and endoderm, whereas the non-involuting deep cells form the outer ‘epiblast’ layer, which gives rise exclusively to ectodermal derivatives.

Cell fate commitment

In many organisms, the earliest precursor cells are thought to be pluripotent, that is, developmentally undefined and capable of expressing a large number of possible phenotypes. This is likely the case for early zebrafish cells, especially considering that before gastrulation the movements of cells appear random and the lineages are indeterminant (Kimmel and Warga, 1987). Eventually, however, individual cells do come to express separate and specific phenotypes. As already described, the elucidation of a fate map for the zebrafish allows one to make an accurate prediction about the future phenotype of an individual cell. However, we have to ask if this lineage restriction is the result of some fundamental change in the developmental program of that particular cell. In other words, simply because a cell during normal development always gives rise to a particular phenotype, does it necessarily follow that the cell is actually restricted to expressing only that phenotype? In fact, it does not. It is possible that, if placed into a different environment, the cell in question could choose to follow a new developmental pathway and express a different fate, where fate is defined as the definitive set of phenotypic characteristics that a cell will eventually come to express.

One of the major problems of developmental biology is knowing when and how an initially pluripotent cell becomes “committed” to expressing a particular fate. Here
Commitment to an extraembryonic fate

The enveloping layer, or EVL, is the second tissue-restricted lineage to become separate in the zebrafish (YSL being the first). The EVL cells have features distinct from the deep cells, such as a flattened epithelial appearance, shared tight junctions (Betchaku and Trinkaus, 1978) and a slower cell cycle (Kane, 1992). Also, whereas the deep cells give rise to future embryonic tissues, the EVL cells develop into an extraembryonic 'periderm', which has been reported to be sloughed off in other fish embryos (Bouvet, 1976). The EVL layer is first formed at the 64-cell stage by the most superficial cells of the blastoderm which surround the internal deep cells. The deep cells always remain interior to the EVL cells and do not contribute progeny to the EVL-derived epithelium. However, EVL cells can contribute progeny to the deep cell layer; in the early blastula, an EVL cell either divides in the plane of the EVL epithelium to form a pair of new EVL cells or it divides perpendicularly to the plane of the epithelium to form one EVL cell and one deep cell.

By the late blastula stage, EVL precursors cease to generate deep cells and become tissue-restricted to generating only EVL cells (Kimmel et al., 1990). At this time, the EVL epithelium is an extraembryonic lineage separate from the deep cell layer tissues of the embryo. Does this lineage restriction represent the irreversible commitment of these cells to an EVL fate? This is an obvious question, as previous to this time the EVL cells were able to generate both EVL cells and deep cells. To test for commitment to the EVL fate, single labeled EVL cells were transplanted into the deep cell layers of an unlabeled host embryo and assayed according to their position and morphology within the embryo after 24h.

Results from these types of experiments are shown in Fig. 3. Fig. 3A shows the result obtained when single late blastula (4h) EVL cells are transplanted into the deep cell layer. Under these conditions the transplanted EVL cells later expressed a typical deep layer fate such as the group of spinal interneurons shown in Fig. 3A. These results showed that when the EVL layer can be first defined as a tissue restricted lineage, individual EVL cells are still pluripotent and therefore not yet committed to expressing only the EVL fate. Interestingly, EVL cells at this time do show a "community effect" (Gurdon, 1988) as a group of four transplanted EVL cells do not transfate when placed into the deep cell layer but retain their EVL phenotype (Fig. 3B). The very different results obtained when a cell is transplanted either singly or in a group, point out the importance of studying the responses of single cells in the absence of all their normal neighbors.

Eventually, EVL cells do become committed to an EVL fate as shown by the single cell transplants of older EVL cells. EVL cells taken from an embryo at the onset of gastrulation (5.2h) did not transfate to a deep cell phenotype when placed into the deep cell layer. These older transplanted EVL cells either retained an EVL phenotype, indicating that this cell was committed to an EVL fate (Fig. 3C) or formed an isolated epithelial vesicle (Fig. 3D). The formation of an epithelial vesicle, a very abnormal phenotype, is not direct evidence for commitment to an EVL fate but suggests that cells have undergone a restriction in potential and have lost the ability to express a normal deep cell fate.

These experiments show that, when the EVL cells can be first described as lineally restricted, they are uncommitted and able to assume the fate of their closest neighboring cells. However, an hour later in the late blastula, these cells lose their potential to express every type of fate. Just what types of changes occur during this process remains one of the most important and long-lived questions in developmental biology.

**Margin cells are uncommitted before gastrulation**

As stated previously, the fates of deep cells at the blastula stage cannot be accurately predicted. However, just prior to the onset of gastrulation (5.2h, 50% epiboly), individual deep cells will give rise to progeny that are tissue restricted. For example, cells near the margin have been fate mapped to give rise to mesodermal and endodermal derivatives, and it is only these marginal cells that involute at gastrulation to form the hypoblast germ-layer which comes to lie internal to and underneath the non-involuting, ectodermal epiblast layer (Warga and Kimmel, 1990).

Regions of the embryo now also express specific gene products. One of the earliest expression patterns is exhibited by the zebrafish homologue of the murine T-gene. In the mouse, the T-gene is initially expressed in all mesodermal precursors (Herrmann et al., 1990; Wilkinson et al., 1990) and the pattern of expression in the zebrafish is very similar. By gastrulation, the zebrafish homologue of the T-
Fig. 3. Results from the transplantation of EVL cells into the deep cell layer. For these experiments, a single early EVL cell is labeled by the intracellular injection of rhodaminated-dextran (ca M, 40,000). After one or two cell divisions, a single labeled EVL cell is gently drawn up into a tooled microcapillary and expelled directly into the deep cell layer of an unlabeled host embryo, (see Ho and Kane, 1991 for transplantation techniques). (A) A single EVL cell transplanted just after the time of tissue restriction (4h) into the deep cell layer is still pluripotent, uncommitted and able to express a deep cell fate, such as this group of spinal interneurons. (B) In this panel, four labeled EVL cells at the same age as the cell in A had been transplanted into the deep cell layer. These cells exhibited a mass or ‘community’ effect as they retained an EVL fate. Single EVL cells transplanted from older embryos (after 5h) into the deep cell layers either (C) retained an EVL fate, showing that at this time the transplanted cell was committed to an EVL fate, or (D) expressed an abnormal phenotype, such as this epithelial ball of cells (solid arrow) located near the ear (open arrow). Scale bars = 100μm.

gene is expressed in the marginal cells of the blastoderm (Shulte-Merker, 1992), most of which will involute at gastrulation to form the hypoblast.

So just prior to the onset of gastrulation at 50% epiboly, the margin region of the blastoderm has characteristics that distinguish it from other areas of the embryo. The careful reader should be able to anticipate the next question. Does the acquisition of these traits denote the commitment of the margin region cells to a hypoblast germ-layer fate, or are these cells still developmentally pluripotent? To approach this question, single margin region cells were transplanted into the animal pole region, which gives rise exclusively to ectodermal derivatives. These transplantations were performed between donor and host embryos at 50% epiboly, which is the first time that the zebrafish fate map can be described. The purpose of these manipulations is to see if the transplanted margin cell later expressed the fate of its new position, namely ectoderm, in which case you would conclude that the margin cell was still pluripotent and therefore not committed to a germ-layer fate. Conversely, if the cell retained its old fate though moved to a new environment, you would conclude that under these experimental conditions the transplanted cell was committed to a mesodermal germ-layer fate.

Under these conditions, most of the cells transplanted at 50% epiboly took on the fate of their new position and formed ectodermal progeny such as epidermis, neurons and retinal tissues (Fig. 4). None of the transplanted cells retained a mesodermal fate though a small percentage of cells (<10%) were characterized as mesenchymal, (i.e. residing in a mesenchymal area of the embryo and undifferentiated at 48h). Therefore, margin cells just prior to gastrulation are not yet committed to a mesodermal germ-layer fate but are pluripotent and still able to express an ectodermal phenotype.

In the preceding section, the EVL cells were shown to become committed to an extraembryonic fate but only after a period in which they were a tissue-restricted, but still
uncommitted, lineage. A similar phenomenon apparently occurs in the deep layer margin cells. Even though their position within the blastoderm at 50% epiboly is predicative of their future fate, the margin cells were shown to be still uncommitted to a particular phenotype. Thus, by the onset of gastrulation, different cells of the embryo are distinct with respect to their cell potential; the EVL cells are committed at this time, whereas the marginal deep cells are still pluripotent. Thus, the time at which commitment occurs does not appear to be a global feature of the embryo, but different populations may undergo this process at separate times. By analogy to the EVL cells, the deep layer cells may eventually become committed to a specific fate, but this issue remains the focus of future studies.

These experiments showed that transplanting a cell before gastrulation into a different region of the embryo caused that cell to express the phenotype of its new position. Therefore, in this vertebrate embryo, cell position plays a very important role in the determination of future cell fate. Having stressed this point, it seems likely that mechanisms should have evolved to ensure the orderly and even dispersal of cells into the various tissue anlagen. As already described, the majority of these cell movements occur during gastrulation, and presumably, any defact that interferes with the normal pattern of gastrulation would have drastic effects upon cell fate and the later patterning of the embryo.

The spadetail mutation affects cell movements during gastrulation

One of the advantages of working with the zebrafish is its amenability to genetic analyses (Streisinger, 1981; Walker and Streisinger, 1983) and screens for zygotic lethal mutations that affect the patterning of the embryo are underway. The zygotic lethal spadetail mutation is the first identified zebrafish mutant that affects the movements of cells at gastrulation (Kimmel et al., 1989). At 24h, spadetail mutants lack sufficient mesoderm to make somitic segments in the trunk region and also have a very characteristic bulge of excess cells at the end of the tail (Fig. 5).

In wild-type embryos, the precursors of trunk somitic tissue are located within the lateral margin of the blastoderm. Lateral margin precursors normally involute during gastrulation and then converge towards the dorsal side of the embryo. In the spadetail mutant, labeled lateral margin cells involute but then fail to converge properly and instead move abnormally towards the vegetal pole which becomes the tail bud. These labeled cells later gave rise to a variety of cell fates including mesenchyme, notochord and muscle cells within the tail; however, the majority of the cells at the end of the tail die after formation of the terminal body segment.

The spadetail mutation appears to affect a very specific morphogenetic movement, namely, the convergence of cells into the dorsal axis during gastrulation. To confirm this hypothesis, Don Kane and I used cell transplantation techniques to create genetic mosaics in which we could study the differences between wild-type and mutant cells (Ho and Kane, 1990). We were interested to know which cells of the embryo were being affected by the spadetail mutation.
and, secondly, to describe the nature of the defect. In regards to the latter point, two possibilities presented themselves: the *spadetail* mutation could either be affecting the environmental cues that normally guide cells into their correct pathways or alternatively, the mutation could be directly affecting some function of the cells in a cell-autonomous fashion.

To answer these questions, mixtures of wild-type and mutant cells from differently labeled donor embryos were co-transplanted into the lateral marginal region of an unlabeled host embryo before the onset of gastrulation (Fig. 6A). Regardless of the host embryo genotype, the wild-type cells always converged dorsally to form trunk somitic mesoderm whereas the mutant cells failed to migrate with the wild-type cells and instead ended up in the tail region where they gave rise to tail mesenchyme (Fig. 6B,C).

Such extensive separations of neighboring cells do not occur during normal development. In control animals, in which both groups of transplanted cells were of the same genotype (i.e. either wild-type & wild-type or *spadetail* & *spadetail*) both groups of cells migrated together and formed the same derivatives located in the same area of the 24h embryo (Fig. 6D,E). Therefore, our results shown in Fig. 6A-C are due to differences in the behaviors of mutant and wild-type cells. Also, because the mutant phenotype of the *spadetail* cells was not rescued by being transplanted into a wild-type environment, we concluded that the *spadetail* mutation was acting autonomously in the lateral margin precursors.

Furthermore, we were able to delineate the functional boundaries of the *spadetail* gene action. By transplanting cells into various areas of the zebrafish fate map, we determined that the large-scale separation of wild-type and *spadetail* cells only occurred if the transplantation site was the lateral margin region of the blastoderm; *spadetail* cells placed into other areas of the blastoderm always migrated with the co-transplanted wild-type cells and formed the same types of derivatives. Fig. 6F,G shows the results obtained when wild-type and *spadetail* cells were transplanted into the fate map regions that give rise to notochord and spinal cord, respectively. In these host animals, the *spadetail* cells migrated with the co-transplanted wild-type cells and formed the same types of derivatives within the same position.

The functional boundaries of the *spadetail* gene action, as assayed by cell transplantsations, (Fig. 2) correlate well with our knowledge of cell fate and cell movements in the embryo at gastrulation. These findings have revealed a surprisingly delicate genetic control of vertebrate gastrulation, as the *spadetail* gene appears to be necessary for the migrations of only those cells located in the lateral margin of the blastoderm, i.e. those cells that both involute and converge during gastrulation to form trunk somitic mesoderm. The *spadetail* gene function does not appear to be necessary in other precursor cells and one important prediction is that these other cells may be using different mechanisms, other than the *spadetail* gene, to migrate and differentiate. The isolation of this early acting, tissue-specific mutation has alerted us to the possible existence of a system of migration-specific gene functions. The study and characterization of the *spadetail* mutation has also opened up a path for a molecular analysis of early cell movements during vertebrate gastrulation, which is a topic about which we still know very little.

**Concluding remarks**

The morphogenetic movements that occur during zebrafish gastrulation appear similar to the cellular movements described in other vertebrates, especially amphibians. Because fish embryos are optically transparent, one can observe the movements of even the very deepest cells within the intact embryo. The detailed knowledge of these cell movements, coupled with the ability to label early blas-
tomereres with lineage tracing molecules, has led to the elucidation of a detailed fate map for the zebrafish just prior to gastrulation.

The organization of this fate map emphasizes the important role that cell position plays in the determination of cell fate. By 4h, cells around the outside of the blastoderm become lineage restricted to forming EVL cells, whereas the deep layer cells can be fate mapped to germ-layer and tissue-specific fates by 5.2h. However, at the times when the EVL and the deep layer cells can be first described as separate, tissue restricted lineages, they are apparently not yet committed to expressing specific fates but are still pluripotent. Experiments, in which an early single cell is transplanted from one area of the blastoderm into another area, show that changing the position of a cell also changes the identity of that cell. Early EVL cells are able to express a deep cell phenotype if transplanted into the deep cell layers, and early marginal deep cells, fate mapped at the beginning of gastrulation to give rise to mesodermal precursors, can form ectodermal progeny if transplanted into an ectodermal producing region of the blastoderm. Presumably, cells of the embryo eventually become irreversibly restricted to expressing only a single fate, and it was experimentally shown that the EVL cells do become committed to forming only an extraembryonic EVL fate, though somewhat after the time when they can be first fate-mapped as a separate lineage.

The different fate map territories of the blastoderm also represent areas of region-specific morphogenetic movements that will occur during gastrulation. For instance, lateral margin cells, which are shown in Fig. 2 to be muscle precursors, have been shown to first involute and then converge dorsally during gastrulation. This stereotyped pattern of movements places these particular cells into the lateral trunk region of the embryonic axis where they contribute to somitic mesoderm. Cell labeling and cell transplantation experiments have shown that the spadetail mutation exclusively and autonomously affects the convergence of these lateral margin precursors, causing them to move abnormally towards the vegetal pole. Consequently, mutant embryos have an excess of precursors in the tail and are very deficient in trunk somitic muscle cells.

Although the nature of the spadetail gene function is presently unknown, there are at least two related explanations for why mutant cells move incorrectly. (1) The spadetail mutation may have directly changed the identity of the lateral margin cells into tail bud precursors and as a consequence these cells migrated to the vegetal pole, or (2) The spadetail mutation may have directly affected some aspect of the lateral margin cells' ability to migrate correctly and, as a consequence, the misdirected cells expressed a fate appropriate for their new position. The first possibility seems somewhat less likely, in light of the single cell transplantation experiments described earlier in this paper. Margin cells were shown to be uncommitted to any particular fate at the beginning of gastrulation and the spadetail gene appears to first exert its effects at this same time. Also, control experiments in which spadetail cells were transplanted into various non-marginal areas of the blastoderm showed that mutant cells were capable of expressing a variety of cell fates, including muscle cells in non-trunk regions. Thus, it does not seem likely that the spadetail mutation is directly causing cells to become committed to a specific fate; however, the action of the spadetail gene appears to be a very important step in the process of assigning identities to the lateral margin precursors. As described in the preceding section, the spadetail mutation interferes with the migrations of these cells, but a description of the actual cellular defect awaits a molecular characterization of the spadetail mutant. The study of the actions of the spadetail gene has emphasized the importance of cell position upon cell fate in the vertebrate embryo, which has formed the theme of this paper. Changing the position of early cells, either by physical transplantations or through genetic means, leads to a change in their expressed cell fate.

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References


