

Pattern formation in 8-cell composite embryos of *Xenopus laevis*

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SUMMARY

We have shown in defect experiments that an 8-cell embryo of *Xenopus laevis* consists of three kinds of cells, that is, animal, vegetal dorsal and vegetal ventral cells, and that cells of different kinds are distinctly different in their developmental capacity. Complete pattern formation occurs in any defect embryo which contains at least two animal, one vegetal dorsal and one vegetal ventral cell. In the present transplantation experiments, we replaced one or two cells of one 8-cell embryo by those of another to obtain 29 series of composite embryos, in which the cell composition of an embryo and/or the dorsoventral orientation of individual cells differed from those of a normal 8-cell embryo. The resulting embryos were examined macroscopically when controls reached stage 26 (tailbud stage) and later.

The results showed that both the two animal dorsal cells or one vegetal dorsal cell could be replaced by animal ventral cells or a vegetal ventral cell, respectively, without any detectable effect on pattern formation, irrespective of the ventrodorsal direction of the ventral cells. On the other hand, replacement of an animal ventral or a vegetal ventral cell by an animal dorsal or a vegetal dorsal cell, respectively, made most composite embryos twins. Twins were also formed when a left-handed vegetal dorsal cell was replaced by a right-handed counterpart and *vice versa*. In these composite embryos, the dorsoventral orientation of the transplanted cell was different from that of a resident dorsal cell or cells of a recipient, and several lines of evidence show that the dorsal cell transplanted in an off-axis orientation is responsible for twin formation. Thus, dorsal cells have the capacity to form dorsal axial structures at later stages and this capacity is localized on the dorsal side, and endows the cells with polarity. On the other hand, ventral cells did not have this capacity or polarity, judging from the fact that their orientation had no effect on pattern formation. One vegetal dorsal or ventral cell could be replaced by an animal dorsal or ventral cell, respectively, without any marked effect. However, replacement of two vegetal cells by animal ones and of one or two animal cells by vegetal ones resulted in deficiency of vegetal cells and oedema and in deficiency of animal cells and incomplete invagination, respectively.

Twin formation in composite embryos with animal dorsal cells in place of animal ventral ones is discussed in consideration of findings in recombination experiments by Nieuwkoop.

INTRODUCTION

We have shown that most lateral halves of 8-cell *Xenopus* embryos give rise to normal larvae, whereas almost all dorsal halves and all ventral, animal and vegetal halves develop abnormally (Kageura & Yamana, 1983). This suggests that the cells of an embryo differ from one another in their developmental capacity and that a

Key words: pattern formation, composite embryos, *Xenopus laevis*.

certain combination of them is required for complete pattern formation. Defect experiments showed that the eight cells of an embryo are of three kinds and that the combination of cells required for complete pattern formation is one vegetal dorsal, one vegetal ventral and two animal cells (Kageura & Yamana, 1984).

The purpose of the present transplantation experiments is to study the effect of the orientation of individual cells in an embryo as well as the cell composition of an embryo on pattern formation. We have made 29 series of composite embryos by transplanting a cell or cells from one 8-cell embryo into another in place of a cell or cells of a different kind and with or without rotation through 90° or 180° with respect to the dorsoventral orientation. Embryos derived from these composite embryos were examined macroscopically when control composite embryos and unoperated embryos reached stage 26 (tailbud stage) and later.

Results show that some of the composite embryos developed into normal embryos, despite their abnormal cell composition and/or orientation, whereas others became abnormal embryos, their morphology depending on their cell composition and cell orientation. A vegetal dorsal or ventral cell could be replaced by an animal dorsal or ventral cell, respectively, without any effect on pattern formation, although replacement of two vegetal cells by two animal ones caused almost all composite embryos to become abnormal. When one or two animal cells were replaced by vegetal ones, almost all composite embryos underwent incomplete invagination. Vegetal and animal dorsal cells could also be replaced by vegetal and animal ventral cells, respectively, regardless of the orientation of the ventral cells. On the other hand, the replacement of vegetal ventral cells by vegetal dorsal ones and the replacement of one of the two vegetal dorsal cells by the other gave rise to joined twins. In these replacements, dorsal cells were transplanted in an off-axis orientation, which was responsible for twin formation. We interpret these results to mean that vegetal dorsal cells of an 8-cell embryo have acquired the capacity to form mesodermal cells and to induce animal cells to make them mesodermalized later, and that the capacity is localized on their dorsal side and so they have distinct polarity. The replacement of animal ventral cells by animal dorsal cells also results in formation of joined twins. Cell lineage analysis in chimaeric embryos shows that the secondary embryo axis consisted mainly of the progeny of the animal dorsal cells transplanted in place of the animal ventral cells. Furthermore, most composite embryos with no vegetal dorsal cells developed dorsal axial structures when they had animal dorsal cells. The simplest interpretation of these results is that as early as the 8-cell stage, animal dorsal cells have acquired the capacity to become dorsal axial structures later independently of vegetal dorsal cells. The implication of this interpretation to the findings of Nieuwkoop (1969*a,b*) is discussed.

This is the first report of systematic studies on the development of composite embryos at the 8-cell stage. The results obtained are important in understanding the relation between pattern formation and cell arrangement, that is, cell composition and cell orientation.

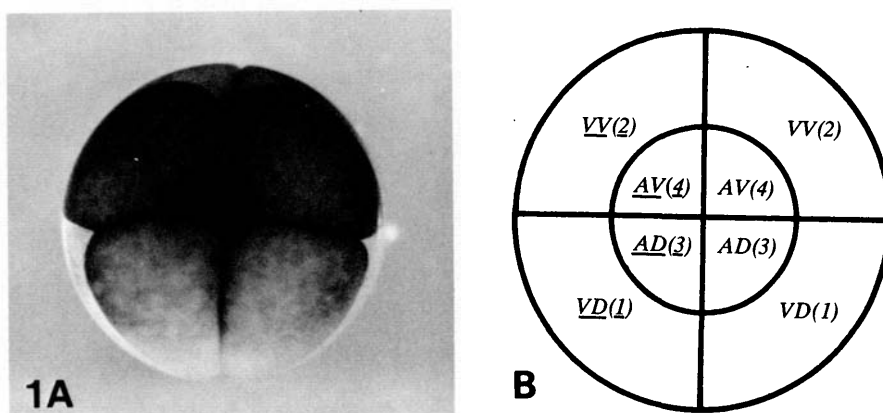


Fig. 1. An 8-cell embryo, viewed from the animal pole. The upper blastomeres are ventral and the lower ones are dorsal. (A) A photograph of an embryo. The shape of animal dorsal cells is quite different from that of ventral cells, and the latter are much darker than the former. (B) Schematic representation of an embryo. The vertical and horizontal lines and the inner circle represent the first, second and third cleavage planes, respectively. Vegetal dorsal (VD), vegetal ventral (VV), animal dorsal (AD) and animal ventral (AV) cells. These cells are represented as 1, 2, 3 and 4, respectively, in Tables. Underlines indicate left-handed cells.

MATERIALS AND METHODS

Fertilized eggs were obtained from *Xenopus laevis* reared in our laboratory. Embryos with regular and symmetrical patterns of cleavage and pigmentation were selected and sterilized. The vitelline membrane was then removed manually. The dorsal side of an 8-cell embryo was easily identified by the difference in shape and pigmentation between animal dorsal and ventral cells (Fig. 1A). The embryos were transferred to 50% Leibovitz (L-15) medium supplemented with 10% foetal calf serum in a Petri dish coated with 2% agar.

The procedure for removing a cell from an embryo was as described previously (Kageura & Yamana, 1983). One or more cells removed from a donor were transferred to a tissue-culture plate (Falcon-3034) coated with 2% agar. Another 8-cell embryo, the recipient, was also transferred to the plate. From the recipient one or more cells were removed to make a cavity, into which the cell or cells from the donor were then implanted, placing the newly exposed surface of the transplant cell or cells in contact with those of the resident cells of the recipient. A hair loop was used for transfer of cells. Transplants soon healed in place.

The cells of an 8-cell embryo are right and left vegetal dorsal, vegetal ventral, animal dorsal and animal ventral cells (Fig. 1B). These are represented as 1, 2, 3 and 4, respectively, in Tables.

An embryo to which a cell or cells had been transplanted is referred to as a composite embryo. Four groups of composite embryos were made (Fig. 2). The first group of composite embryos were controls, in which one or more cells of the same side and same kind were transplanted (orthotopic transplantation) (Fig. 2A). Composite embryos of the second group were made by replacing a vegetal cell of a recipient by an animal cell of a donor or *vice versa* (Fig. 2B). The cell composition of these composite embryos was abnormal, but the dorsoventral or ventrodorsal orientation of the transplanted cell was kept normal. The third group consisted of composite embryos, in which a right-handed cell was substituted for a left-handed one of the same kind (Fig. 2C). In some composite embryos of this group, the right-handed cell was also replaced by a left-handed one, the two transplant cells being obtained from different embryos. In the composite embryos of this group, the cell composition was 'normal', but the orientation of the transplanted cell was 90° different from normal. In the fourth group, a dorsal animal or vegetal cell was replaced by a ventral one and *vice versa* (Fig. 2D,E). Not only the cell composition of these composite embryos, but also the orientation of the transplanted cells was abnormal, being 90° or 180° different from normal orientation.

Composite embryos were cultured in 50% Leibovitz (L-15) medium supplemented with 10% foetal calf serum, which was gradually changed to 10% Steinberg solution. In each series, 50 composite embryos were used and were allowed to develop until control composite embryos and unoperated embryos derived from the same batch of eggs had reached stage 26 (tailbud stage, about 30 h after fertilization under the present conditions) (Nieuwkoop & Faber, 1967). The embryos were examined macroscopically at this stage and later.

RESULTS AND DISCUSSION

An animal cell soon became round when isolated, and a cavity made in the animal half soon became smaller. Therefore, transplantation of an animal cell into the animal half was difficult. Some abnormalities appearing at later stages could be ascribed to this technical difficulty, and the frequency with which normal embryos developed was much less when an animal cell was transplanted into the animal half.

(A) Controls (Table 1)

One or more cells of a recipient were replaced by an equivalent cell or cells of a donor. 70 to 80% of the composite embryos formed in this way developed normally. However, in series 4-2, the frequency of normal embryos was markedly lower for the reason described above.

The composite embryos of series 0-1 were made by putting an animal half on a vegetal half obtained from a different embryo. The dorsal side of the animal half coincided with that of the vegetal one.

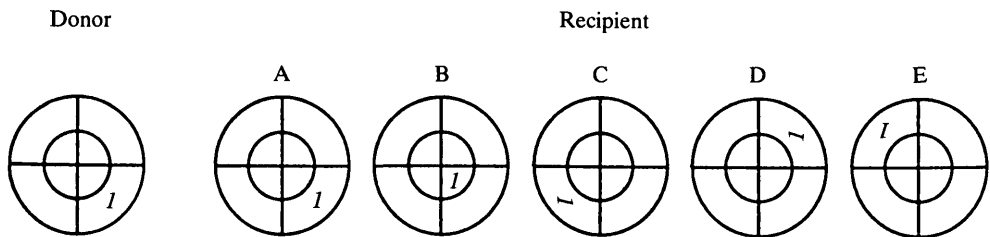


Fig. 2. Schematic representation of cell transplantations. As an example the transplantation of a vegetal dorsal cell is represented. The dorsoventral direction of the transplanted cell is shown by the direction of the number. (A) (Table 1, Controls) A vegetal dorsal cell of a recipient is substituted by a vegetal dorsal cell of a donor. The position and orientation of the transplanted cell is normal. (B) (Table 2) An animal dorsal cell of a recipient is replaced by a vegetal dorsal cell of a donor. The cell composition of the resulting composite embryo is abnormal, but the orientation of the transplanted cell is normal. (C) (Table 3) The right-handed vegetal dorsal cell of a donor is introduced in place of the left-handed vegetal dorsal one of a recipient. The cell composition of the composite embryo is 'normal', but the dorsoventral direction of the transplanted cell is rotated 90° from normal. (D,E) (Table 4) A right- and left-handed vegetal ventral cell of the recipient, respectively, is replaced by a right-handed vegetal dorsal cell of a donor. The dorsoventral direction of the transplanted cell is rotated 90° and 180° , respectively, from normal. Therefore, both the cell composition of the composite embryo and the orientation of the transplanted cell are abnormal.

(B) *Replacement of animal and vegetal cells (Table 2)*

On replacing a vegetal cell or cells by an animal cell or cells, or *vice versa*, the cell composition of the composite embryo differed from that of a normal embryo. However, the original dorsoventral or ventrodorsal orientation of a transplanted cell was maintained in the recipient.

(1) *Replacement of an animal cell by a vegetal one*

The composite embryos of series 1-3 had one vegetal dorsal cell instead of an animal dorsal cell. 14 composite embryos developed into normal embryos, and 28 became abnormal, showing incomplete invagination and at later stages exposed endodermal and mesodermal cells (cf. Fig. 3A). These embryos are referred to as 'incompletely invaginated embryos' in Tables. The remaining composite embryos showed some defects of the head or their development was arrested soon after the operation. The replacement of two animal dorsal cells by vegetal dorsal cells caused incomplete invagination in all cases (series 1-4).

34 and 33 controls, in which an animal dorsal cell or cells were derived from a donor, gave rise to normal embryos (series 3-1 and 3-2), while most of the rest showed head abnormalities.

Thus pattern formation was markedly disturbed in more than half the composite embryos which had a vegetal dorsal cell instead of an animal dorsal cell and consisted of three animal and five vegetal cells. Composite embryos in which the other animal dorsal cell was also replaced by a vegetal dorsal cell all failed to invaginate normally.

One or two vegetal ventral cells were substituted for an animal ventral cell or cells (series 2-3 and 2-4). 29 composite embryos in series 2-3 developed into normal embryos. 10 composite embryos became 'flat back' embryos, i.e. their back was flat and wide (cf. Fig. 6D), and microscopic examination of transverse sections revealed that the neural tube was duplicated anteriorly (the two neural tubes sometimes not being separated) and in some typical cases there were two rows of notochord. 48 composite embryos in series 2-4 underwent incomplete invagination.



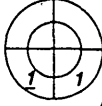
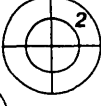
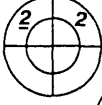





Of the embryos derived from control composite embryos of series 4-1 and 4-2, which had a transplanted animal ventral cell or cells in the normal place, 37 and 24, respectively, were normal, while 15 showed head defects (series 4-2).

The replacement of one animal ventral cell by one vegetal ventral cell only slightly disturbed pattern formation and about 60% (about 80% as corrected for values of controls) of these composite embryos developed normally (series 2-3). When the two animal ventral cells were both replaced, however, scarcely any of the composite embryos invaginated normally (series 2-4). The possibility that incomplete invagination is due to deficiency of animal cells will be discussed later.

(2) *Replacement of a vegetal cell by an animal one*

41 normal embryos were obtained from composite embryos of series 3-3, in which one vegetal dorsal cell was replaced by an animal dorsal cell; the other two

Table 1. *Controls*

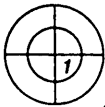

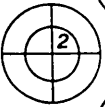

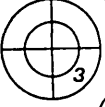
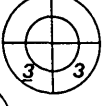

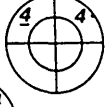
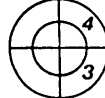
| Series number | Composite embryo | Normal embryos | Embryos with secondary axial structures | | | | Other abnormal embryos | Incompletely invaginated embryos | Early deaths |
|---------------|---|----------------|---|---------------|-------------|--|------------------------|----------------------------------|--------------|
| | | | Flat back | Unequal twins | Equal twins | | | | |
| 1-1 |  | 47 | 0 | 0 | 0 | 0 | 3 | 0 | |
| 1-2 |  | 36 | 0 | 0 | 1 | Head deformity (5) Small head (2) | 2 | 4 | |
| 1-2* |  | 40 | 0 | 0 | 0 | Head deformity (6) | 3 | 1 | |
| 2-1 |  | 42 | 0 | 2 | 1 | Head deformity (4) | 1 | 0 | |
| 2-2 |  | 35 | 0 | 4 | 1 | Head deformity (4) Large head-small tail (1) Small head (2) | 3 | 0 | |
| 3-1 |  | 34 | 0 | 0 | 0 | Head deformity (8) Small head (4) Twisted body (1) | 2 | 1 | |
| 3-2 |  | 33 | 0 | 0 | 0 | Head deformity (10) Small head (3) | 4 | 0 | |
| 4-1 |  | 37 | 0 | 2 | 0 | Head deformity (3) Curved or twisted body (3) | 2 | 3 | |
| 4-2 |  | 24 | 8 | 0 | 0 | Head deformity (12) Small head (3) Curved body (1) | 2 | 0 | |
| 0-1 |  | 31 | 0 | 0 | 5 | Head deformity (3) Small head (1) No head (1), curved body (5) | 1 | 3 | |

See Figs 1 and 2 for explanation of procedures. The dorsoventral direction of the transplanted cell is shown by the direction of the numbers: 1, vegetal dorsal; 2, vegetal ventral; 3, animal dorsal; 4, animal ventral.

*Two transplanted vegetal dorsal cells derived from two different donor embryos.

had oedema of the belly, but were otherwise normal. When both the two vegetal dorsal cells of a recipient were replaced with animal dorsal cells (series 3-4), the resulting composite embryos were expected to develop abnormally owing to the absence of a vegetal dorsal cell (Kageura & Yamana, 1984) and, in fact, all but one became abnormal. They lacked a head region but their trunk and tail appeared normal. These abnormal embryos resembled those derived from ventral halves at the 4-cell stage (cf. fig. 3F in Kageura & Yamana, 1983) and defect embryos having no vegetal dorsal cells (Kageura & Yamana, 1984). However, it is noteworthy that these composite embryos with four animal dorsal cells but no vegetal dorsal cells

Table 2. Replacement by animal and vegetal cells

| Series number | Composite embryo | Normal embryos | Embryos with secondary axial structures | | | | Other abnormal embryos | Incompletely invaginated embryos | Early deaths |
|---------------|---|----------------|---|---------------|-------------|---|------------------------|----------------------------------|--------------|
| | | | Flat back | Unequal twins | Equal twins | | | | |
| 1-3 |  | 14 | 0 | 0 | 0 | Small head (4) Large head-small tail (1) | 28 | 3 | |
| 1-4 |  | 0 | 0 | 0 | 0 | 0 | 50 | 0 | |
| 2-3 |  | 29 | 10 | 0 | 0 | Head deformity (4) Small head (1) | 2 | 4 | |
| 2-4 |  | 0 | 0 | 0 | 0 | 0 | 48 | 2 | |
| 3-3 |  | 41 | 0 | 0 | 0 | Nearly normal (2) Head deformity (4) | 1 | 2 | |
| 3-4 |  | 0 | 0 | 0 | 0 | No head (49) | 0 | 1 | |
| 4-3 |  | 48 | 0 | 0 | 0 | 0 | 0 | 2 | |
| 4-4 |  | 4 | 0 | 0 | 0 | Large head-small tail (46) | 0 | 0 | |
| 0-2 |  | 3 | 0 | 0 | 0 | Nearly normal (34) Head deformity (2) Large head-small tail (9) | 0 | 2 | |

See Figs 1 and 2 for explanation of procedures. The dorsoventral direction of the transplanted cell is shown by the direction of the number. 1, vegetal dorsal; 2, vegetal ventral; 3, animal dorsal; 4, animal ventral.

formed dorsal axial structures, though these were underdeveloped. This will be referred to again later.

47 and 36 composite controls gave rise to normal embryos (series 1-1 and 1-2). The others showed some defect of the head, invaginated incompletely, or showed some other abnormality. The frequency of normal embryos was about 20% lower when the two vegetal dorsal cells were transplanted together or separately (series 1-2 and 1-2*).

The above results show that the replacement of one of the two vegetal dorsal cells by an animal dorsal cell had no effect on pattern formation in these composite embryos, but that replacement of both the two vegetal dorsal cells resulted in marked disturbance of development.

One vegetal ventral cell was replaced by one animal ventral cell (series 4-3). All the resulting composite embryos were normal except two, in which development was arrested soon after the operation. However, when one more vegetal ventral cell was replaced (series 4-4), only four normal embryos were obtained; the other 46 became 'large head–small tail' embryos, which are the characteristic type derived from dorsal halves at the 4-cell stage (see fig. 3A in Kageura & Yamana, 1983) and defect embryos lacking vegetal ventral cells (Kageura & Yamana, 1984).

Most control composite embryos were normal (series 2-1 and 2-2), although a few were abnormal. Some of the latter will be described in detail later.

The results obtained in this section indicate the following four points. First, it is much easier for an animal cell to replace a vegetal one than *vice versa*. On replacement of one vegetal cell by one animal one, about 90% (95% when corrected for values of controls) of composite embryos developed normally, whereas after reciprocal replacement about 30–60% (40–80% when corrected for values of controls) developed normally. This fact suggests that at the 8-cell stage a vegetal cell has become more specialized than an animal one. This conclusion is consistent with the fact that in our defect experiments the dorsal and ventral cells were clearly distinguished in the vegetal half, but not in the animal half (Kageura & Yamana, 1984). Second, the composite embryos that were deprived of either vegetal dorsal cell or vegetal ventral cell gave rise to characteristically abnormal embryos, such as 'no head' or 'large head–small tail' embryos. This is also compatible with the finding in defect experiments that at least one vegetal dorsal cell and one vegetal ventral cell, in addition to two animal cells, are necessary for complete pattern formation (Kageura & Yamana, 1984). Third, some animal-cell deficiency causes incomplete invagination. All, or almost all, the composite embryos in which two animal dorsal or two animal ventral cells were

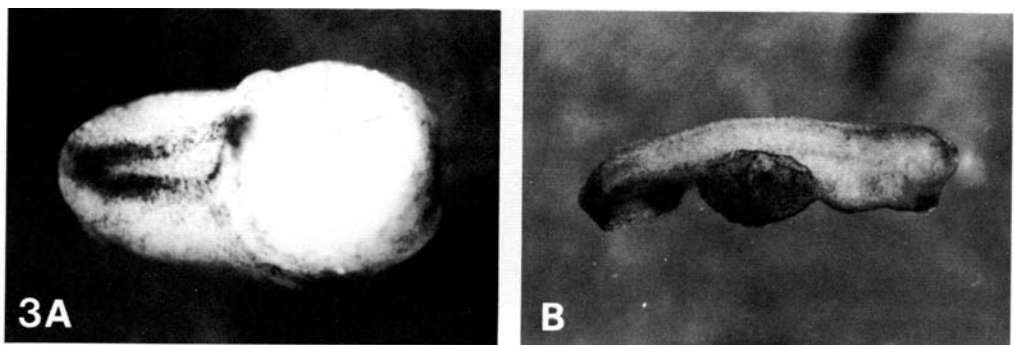


Fig. 3. Abnormalities due to deficiency or excess of animal cells. (A) An incompletely invaginating embryo (stage 26), viewed from the dorsal side, derived from a composite embryo with two vegetal ventral cells instead of two animal ventral cells (series 2-4). Endodermal and mesodermal cells are exposed. (B) An embryo with oedema of the belly (stage 26), derived from a composite embryo consisting of one vegetal dorsal, one vegetal ventral, three animal dorsal and three animal ventral cells (series 0-2).

replaced by vegetal dorsal or vegetal ventral cells, respectively, failed to invaginate normally. Furthermore, it seems that an animal dorsal cell is more important for invagination. Invagination was incomplete in 28 composite embryos that had lost one animal dorsal cell (series 1-3), but in only two of these that had lost one animal ventral cell (series 2-3). Similar results were obtained in defect experiments: more defect embryos failed to undergo complete invagination when an animal dorsal cell or cells were absent than when an animal ventral cell or cells were absent (table 1, series 1, 2, 5 and 6, in Kageura & Yamana, 1984). However, the frequency of incomplete invagination was much lower in these defect embryos, ranging from 18% to 32%. This implies that the frequency of incomplete invagination was increased not only by the absence of an animal cell, but also by the presence of an extra vegetal cell. Fourth, excess animal cells caused oedema formation. This was clearly seen in the composite embryos of series 0-2. The composite embryos of these series all consisted of six animal cells, one vegetal dorsal cell and one vegetal ventral cell. 34 composite embryos developed into normally proportioned embryos, but had an oedema of the belly or the side of the body (Fig. 3B). In the composite embryos of series 3-4 and 4-4, the possible effect of excess animal cells could be overcome by the absence of a vegetal dorsal or ventral cell.

(C) *Replacement by left-handed and right-handed cells of the same kind (Table 3)*

The cell composition of these composite embryos was normal, that is, the same as that of a normal 8-cell embryos, but the dorsoventral or ventrodorsal orientation of the transplanted cells was rotated 90° from normal.

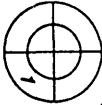

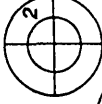



(1) *Replacement of left-handed and right-handed vegetal dorsal cells*

In series 1-5, the left vegetal dorsal cell was off-axis. 23 normal embryos and 15 joined twins appeared. These twins were duplicated anteriorly and their secondary embryos were much smaller than the primary embryos (cf. Fig. 6F). This type is referred to as 'unequal twins'. Seven composite embryos developed into 'flat back' embryos. In controls (series 1-1), as many as 47 composite embryos developed normally, but three underwent incomplete invagination.

In series 1-6 the two transplanted vegetal dorsal cells were off-axis and had opposite orientation. Only three composite embryos developed normally, while 35 gave rise to joined twins of a different type, in which the primary and secondary embryos were almost the same size (Fig. 4). This type is referred to as 'equal twins'. Seven composite embryos gave rise to 'flat back' embryos. Of the controls (series 1-2*), 40 developed normally and 6 showed head defects.

These results show that the orientation of a vegetal dorsal cell is critical for pattern formation. More than half the composite embryos, in which this cell was rotated 90° from the normal orientation showed markedly abnormal development. This is the only case of replacement of two cells of the same kind that had a marked effect on pattern formation. The predominant abnormality was unequal twins, which depended on duplication of dorsal axial structures. Upon rotation of one

Table 3. *Replacement by left-handed and right-handed cells of the same kind*

| Series number | Composite embryo | Normal embryos | Embryos with secondary axial structures | | | Other abnormal embryos | Incompletely invaginated embryos | Early deaths |
|---------------|---|----------------|---|---------------|-------------|--|----------------------------------|--------------|
| | | | Flat back | Unequal twins | Equal twins | | | |
| 1-5 |  | 23 | 7 | 15 | 0 | Small head (4) | 0 | 1 |
| 1-6 |  | 3 | 7 | 0 | 35 | Head deformity (3) | 2 | 0 |
| 2-5 |  | 43 | 0 | 0 | 0 | Head deformity (1) Curved body (3) | 1 | 2 |
| 2-6 |  | 39 | 0 | 3 | 0 | Head deformity (4) | 3 | 1 |
| 3-5 |  | 37 | 3 | 3 | 0 | Head deformity (4) Twisted body (1) | 2 | 0 |
| 4-5 |  | 38 | 3 | 2 | 0 | Head deformity (1) Small head (5) | 1 | 0 |

See Figs 1 and 2 for explanation of procedures. The dorsoventral direction of the transplanted cell is shown by the direction of the number. 1, vegetal dorsal; 2, vegetal ventral; 3, animal dorsal; 4, animal ventral.

more vegetal dorsal cell, the frequency of normal embryos decreased and equal twins appeared, the decrease in the former being equal to the increase in the latter. The frequencies of other abnormal embryos did not change. Thus, it is likely that twin formation is characteristic of vegetal dorsal cell transplantation in an off-axis orientation and is indeed caused by it. It is known that the progeny of the vegetal dorsal cell induces the progeny of animal cells to form dorsal axial structures (Nieuwkoop, 1969*a,b*). This will be discussed later.

(2) *Replacement of left-handed and right-handed vegetal ventral cells*

The composite embryos of series 2-5 had one vegetal ventral cell in the normal orientation and position, and one vegetal ventral cell in an off-axis orientation. 43 composite embryos developed normally and no predominant abnormalities were found. Essentially the same results were obtained in series 2-6, where the ventrodorsal axis of one vegetal ventral cell was reversed with respect to the other vegetal ventral cell. In this series, 39 composite embryos gave rise to normal embryos (Fig. 5), 4 displayed slight head deformities, and 3 became unequal twins.

These results show that the orientation of a vegetal ventral cell has little effect on pattern formation, although the presence of at least one vegetal ventral cell in a

composite embryo is absolutely essential for complete pattern formation, as will be shown later.

(3) *Replacement of left-handed and right-handed animal dorsal cells*

Of 50 composite embryos with an animal dorsal cell rotated 90° from normal, 37 became normal (series 3-5), while some showed defects of the head or trunk, and three became unequal twins. In controls (series 3-1), normal embryos developed from 34 composite embryos. The frequency of head defects was rather higher, but the frequencies of other abnormalities were similar to those in the experimental series.

Thus, the orientation of an animal dorsal cell does not greatly interfere with pattern formation under the present conditions. However, as described later, this cell has some effect when transplanted into the ventral region with rotation through 90° or 180°.

(4) *Replacement of left-handed and right-handed animal ventral cells*

When the right animal ventral cell was rotated 90° (series 5-5), 38 composite embryos developed normally. The abnormal embryos included five 'small head'

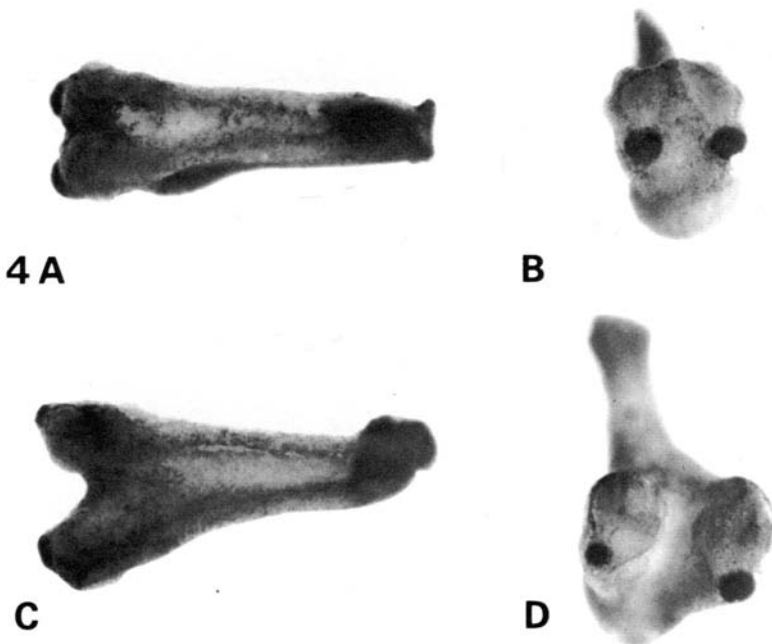


Fig. 4. Equal twins (series 1-6). (A) Dorsal view (stage 26). (B) Frontal view of the same embryos as A. (C) Dorsal view (stage 26). (D) Frontal view of the same embryo as C.

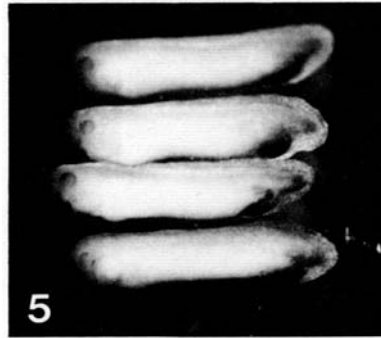


Fig. 5. Normal embryos at the tailbud stage, lateral view (series 2-6). They are derived from composite embryos in which the ventrodorsal orientations of the two transplanted vegetal ventral cells were rotated 90°, clockwise or counter-clockwise from normal; therefore, the orientation of one was the opposite of that of the other.

and three 'flat back' embryos. Essentially similar results were obtained in controls (series 4-1).

Pattern formation was not affected by the orientation of an animal ventral cell. Furthermore, unlike an animal dorsal cell, an animal ventral cell did not interfere with development even when transplanted into the dorsal region.

(D) *Replacement of dorsal and ventral cells of the same hemisphere (Table 4)*

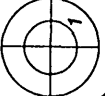

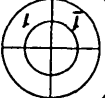
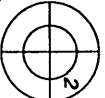
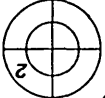


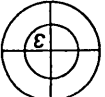

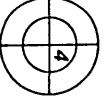



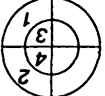
Dorsal animal or vegetal cells were replaced by ventral cells and *vice versa*. These composite embryos had an extra dorsal or ventral cell or cells and, furthermore, the dorsoventral or ventrodorsal orientation of the transplanted cell was rotated 90° or 180° from normal.

Composite embryos of series 0-3 and 0-4 are also included in Table 4. In series 0-3 an animal half of an embryo was put on a vegetal half of another with the dorsal side of the former at the ventral side of the latter. In series 0-4, two right lateral halves were fused so that the orientation of the cells of one half was the opposite of that of the other half.

(1) *Replacement of a vegetal ventral cell by a vegetal dorsal one*

One vegetal dorsal cell was transplanted in place of a right or left vegetal ventral cell, with its dorsoventral axis rotated 90° and 180°, respectively, from normal (series 1-7 and 1-8). 80 to 90% of the composite embryos became equal (Fig. 6A) or unequal (Fig. 6F) twins. In series 1-7, a few gave rise to normal embryos, but in series 1-8, no normal embryos were found. In series 1-9, two vegetal ventral cells were replaced by vegetal dorsal cells with the reverse dorsoventral orientations to those of the two resident vegetal dorsal cells. It was expected that almost all these composite embryos would develop abnormally, as they contained no vegetal ventral cell. In fact, none of them became normal, and as many as 31 composite

Table 4. Replacement by dorsal and ventral cells of the same hemisphere

| Series number | Composite embryo | Normal embryos | Embryos with secondary axial structures | | | | Other abnormal embryos | Incompletely invaginated embryos | Early deaths |
|---------------|---|----------------|---|---------------|-------------|---|------------------------|----------------------------------|--------------|
| | | | Flat back | Unequal twins | Equal twins | | | | |
| 1-7 |  | 6 | 5 | 27 | 11 | 0 | | 1 | 0 |
| 1-8 |  | 0 | 0 | 39 | 7 | 0 | | 0 | 4 |
| 1-9 |  | 0 | 0 | 9 | 31 | 0 | | 3 | 7 |
| 2-7 |  | 37 | 0 | 0 | 0 | Head deformity (7) Small head (3) | 1 | 2 | |
| 2-8 |  | 38 | 0 | 0 | 0 | Head deformity (4) Small head (5) | 0 | 3 | |
| 2-9 |  | 1 | 0 | 0 | 0 | Head deformity (1) Small head (13), No head (22) Vesicle with a small axis (13) | 0 | 0 | |
| 3-7 |  | 17 | 19 | 12 | 0 | Head deformity (1) | 1 | 0 | |
| 3-8 |  | 9 | 6 | 27 | 7 | 0 | 1 | 0 | |
| 3-9 |  | 9 | 2 | 32 | 1 | 0 | 5 | 1 | |
| 4-7 |  | 36 | 0 | 0 | 0 | Large head-small tail (1) Head deformity (8) Small head (1) | 3 | 1 | |
| 4-8 |  | 38 | 0 | 0 | 0 | Small head (1) Twisted body (1) | 8 | 2 | |
| 4-9 |  | 35 | 0 | 0 | 0 | Head deformity (5) | 9 | 1 | |
| 0-3 |  | 12 | 3 | 24 | 4 | 0 | 4 | 3 | |
| 0-4 |  | 0 | 0 | 2 | 43 | 0 | 0 | 5 | |

See Figs 1 and 2 for explanation of procedures. The dorsoventral direction of the transplanted cell is shown by the direction of the number. 1, vegetal dorsal; 2, vegetal ventral; 3, animal dorsal; 4, animal ventral.

embryos developed into equal twins (Fig. 6B). This high frequency of equal twins was due to the orientation of the transplanted vegetal dorsal cells, rather than to the absence of vegetal ventral cells. The frequency of equal twins was the highest when two right or left halves of 8-cell embryos, each containing one vegetal ventral cell, were fused in such a way that their dorsoventral orientation was reversed (series 0-4 (Fig. 6C)).

In the controls (series 2-1 and 2-2), 70–80% of the composite embryos developed normally. Several types of abnormal embryos were found, and equal and unequal twins also appeared at a frequency of 6–10% for some unknown reason.

As shown in series 1-5, the replacement of one of the two vegetal dorsal cells by the other caused the formation of second axial structures in about half the embryos. The present replacement of a vegetal ventral cell by a vegetal dorsal cell with rotation of the latter resulted in development of equal or unequal twins in almost all cases (series 1-7 and 1-8). These results show that the orientation of a vegetal dorsal cell is intimately related with twin formation.

Comparison of series 1-7 to 1-9 reveals an interesting relation between the frequency of equal twins and the ratio of the two resident vegetal dorsal cells to the number of transplanted vegetal dorsal cells. In the series 1-7 and 1-8, in which one vegetal dorsal cell was transplanted in an off-axis orientation, unequal twins were much more frequent than equal twins, whereas in series 1-9, in which two vegetal dorsal cells were transplanted, the reverse was the case. This suggests that a secondary embryo formed by two vegetal dorsal cells is larger than that formed by one.

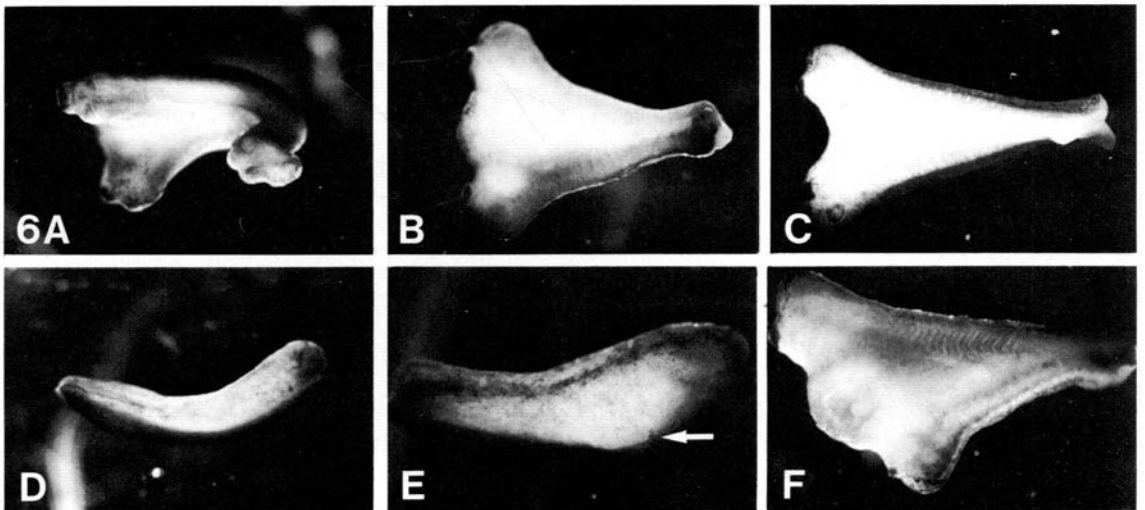


Fig. 6. Various types of embryos with secondary axial structures (stage 33/34). (A) Equal twins, dorsal view (series 1-8). (B) Equal twins, lateral view (series 1-9). (C) Equal twins, lateral view (series 0-4). (D) 'Flat back' embryos, dorsal view (series 3-7). (E) Unequal twins, dorsal view (series 3-7). The secondary embryo (arrow) is underdeveloped. (F) Unequal twins, lateral view (series 1-8).

(2) *Replacement of a vegetal dorsal cell by a vegetal ventral one*

When one vegetal cell was implanted in place of a right or left vegetal dorsal cell (series 2-7 and 2-8), 37 and 38, respectively, of the composite embryos developed normally. The head regions of some embryos were abnormal. The present observation that most of composite embryos with only vegetal dorsal cell could develop normally is consistent with the results of defect experiments (Kageura & Yamana, 1984). Of the composite embryos in which two vegetal dorsal cells were replaced by vegetal ventral cells, only one became normal (series 2-9), the others all showing marked defects in the head region, giving rise to 'no head' or 'vesicles with a small axis'. These abnormal embryos have been shown to be formed by the absence of vegetal dorsal cells (Kageura & Yamana, 1984). Of the controls (series 1-1 and 1-2), 70-90% developed into normal embryos, a few showed head defects, and only one became equal twins.

Thus, replacement of one vegetal dorsal cell by one vegetal ventral cell does not interfere with pattern formation, irrespective of the orientation of the latter.

(3) *Replacement of an animal ventral cell by an animal dorsal one*

It has been shown that the orientation of an animal dorsal cell has no effect, provided that it is rotated in the animal dorsal region (series 3-5). In the present series, however, different results were obtained (series 3-7). When an animal dorsal cell was introduced with 90° rotation instead of a right animal ventral cell, 17 normal embryos, 12 unequal twins (Fig. 6E) and 19 'flat back' embryos resulted (Fig. 6D). When an animal dorsal cell was introduced with 180° rotation instead of a left animal ventral cell (series 3-8), 9 normal embryos, 6 'flat back' embryos and 27 unequal twins were obtained. 7 equal twins also appeared. When both animal ventral cells were replaced by animal dorsal cells in such a way that the dorso-ventral axis of the transplanted animal dorsal cells was the reverse of that of the resident animal dorsal cells (series 3-9), only 9 composite embryos developed into normal embryos, and as many as 32 became unequal twins. In controls (series 4-1 and 4-2), 37 and 24 composite embryos, respectively, developed normally.

These results show that pattern formation is greatly influenced by the orientation of an animal dorsal cell. Probably, the discrepancy between the previous and present results reflects the greater deviation of the animal dorsal cell from its normal position and orientation in the present series.

When the animal half was separated from its vegetal half and put on the vegetal half of another embryo making the dorsal side of the animal half diametrically opposite to the dorsal side of the vegetal half (series 0-3), the results were essentially the same as those obtained when only the animal dorsal cells were rotated and introduced in place of animal ventral cells (series 3-9). In addition to 12 normal embryos, 24 unequal twins and 4 equal twins developed. Controls (series 0-1) gave rise to 31 normal embryos and 5 equal twins.

An important finding was that the secondary dorsal axial structures are derived from the animal dorsal cells transplanted in place of animal ventral cells. This was

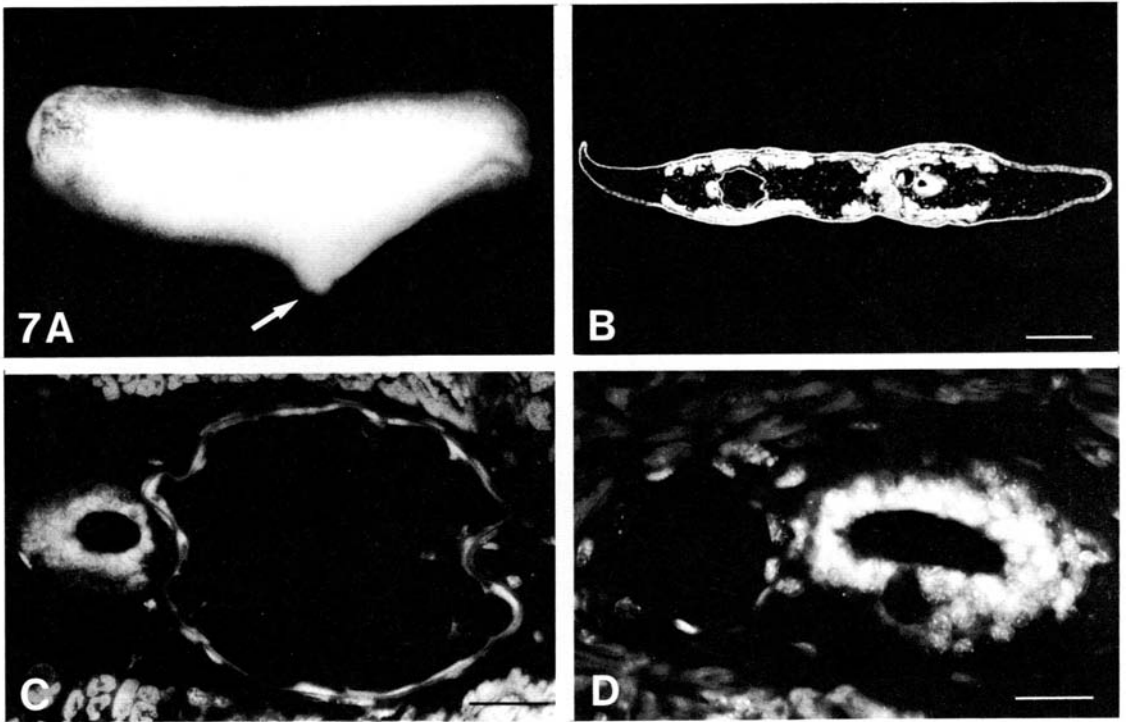


Fig. 7. Twins derived from a composite embryo made of an albino 8-cell *X. laevis* embryo (lacking two animal ventral cells) and two animal dorsal cells of an 8-cell *X. borealis* embryo. This composite embryo was essentially the same as that in series 3-9. It was cultured until stage 47. (A) Lateral view (stage 28). A secondary embryo is seen (arrow). Pigmented cells cover all the surface of the body, except the face. (B) Transverse section (stage 47). The right half is the secondary embryo and the left half the host. Bar equals 0.2 mm. (C) Axial structures of the host. No cells are identified as those of *X. borealis*, which have bright spots in their nuclei. Bar equals 30 μ m. (D) Axial structures of the secondary embryo. Cells of *X. borealis* are seen in the neural tube, in the periphery of the notochord and in the somite. Bar equals 30 μ m.

shown by cell lineage analysis in composite embryos made by transplanting two animal dorsal cells of an *X. borealis* 8-cell embryo into the animal ventral region of an *X. laevis* embryo at the same stage. These composite embryos were allowed to develop until stage 47, and the unequal twins that developed were fixed and sectioned. The sections were then stained with quinacrine to distinguish between the cell nuclei of the two species (Thiébaud, 1983). The notochord at this stage was vacuolated and a few nuclei were observed in a thin layer of cytoplasm at the periphery. Most of these cells and those in the neural tube of secondary embryos were cells of *X. borealis*, where cells of this species were excluded from the primary embryo axis (Fig. 7).

(4) Replacement of an animal dorsal cell by an animal ventral cell

Normal embryos arose from 70% or more (100% when corrected for values of controls) of composite embryos in which a right or left animal dorsal cell was

replaced by an animal ventral cell rotated in the dorsal axis 90° or 180° from normal (series 4-7 and 4-8). The embryos that arose from the remaining composite embryos showed various defects of the head, or incomplete invagination. These results are very similar to those in series 4-9, where the two animal dorsal cells were replaced by animal ventral cells, and those of controls (series 3-1 and 3-2), where one or two animal dorsal cells of a recipient were replaced by an animal dorsal cell or cells of a donor.

These series revealed that an animal ventral cell or cells can be substituted for an animal dorsal cell or cells without any interference with pattern formation, irrespective of the orientation of the animal ventral cell(s) in the animal dorsal region. The unique feature of this replacement is that both the two animal dorsal cells can be replaced by two animal ventral cells: except for this replacement only one of the two cells of one kind can be replaced by a cell of another.

An animal ventral cell compensates for the animal dorsal cell it replaces (Koga, Kageura & Yamana, 1986). A lateral half of dorsal axial structures is formed by the ventral cell, the other half being formed by the remaining dorsal cell.

CONCLUDING REMARKS

We have shown that the cells of an 8-cell embryo differ distinctly from each other in ability to adjust their development in accordance with new locations and then to participate in complete pattern formation in developing composite embryos. An animal ventral cell has the greatest ability to replace a cell of a different kind, whereas a vegetal dorsal cell cannot replace even another vegetal dorsal cell without severe disturbance of pattern formation. The ability of an animal dorsal cell and a vegetal ventral cell are rather similar to those of a vegetal dorsal cell and an animal ventral cell, respectively. Thus, the ability of a cell to replace another decreases in the order of animal ventral, vegetal ventral, animal dorsal and vegetal dorsal cells. This is shown in Fig. 8.

(A) *Cell compositions and orientations that allow complete pattern formation*

We have shown that complete pattern formation occurs even in composite embryos whose cell compositions and/or orientations differ from those of a normal 8-cell embryo. One vegetal dorsal cell is necessary and sufficient for an 8-cell composite embryo. All the other vegetal cells can be vegetal ventral cells and, furthermore, the orientation of these cells has no effect on the morphology of embryos that arise from composite embryos. The dorsoventral axis of a developing embryo depends on the one-dimensional arrangement of the vegetal dorsal cell and vegetal ventral cells. If two vegetal dorsal cells are present in a composite embryo, their arrangement should be the same as that in a normal embryo, that is, unless the two vegetal dorsal cells are side by side and their dorsoventral axes coincide, one of them forms a secondary embryo axis independently of the other, and twins develop. The presence of three or more vegetal dorsal cells leads to

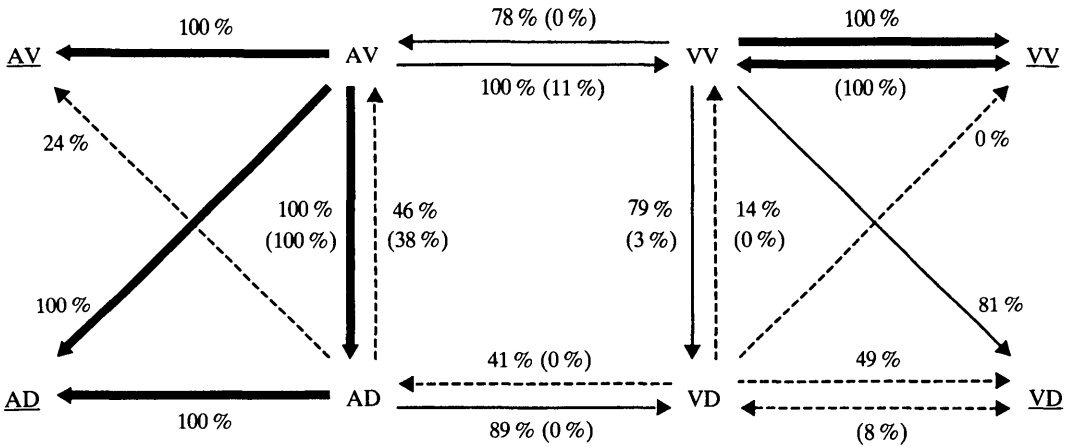


Fig. 8. Ease of replacement of a cell by another of the same or a different kind. *AD*, animal dorsal; *AV*, animal ventral; *VD*, vegetal dorsal; *VV*, vegetal ventral cell. Underlines indicate left-handed cells. AV (animal ventral cell) ← *AV* (animal ventral cell), for example, represents the replacement of a left-handed animal ventral cell by a right-handed animal ventral cell. **→** 100% of the composite embryos developed normally. **→** 78–89% of the composite embryos developed normally. **- - - - -** → 0–49% of the composite embryos developed normally. Figures represent the percentages of normal embryos that developed on replacement of one of the two cells of a kind, and values in parentheses represent percentages of normal embryos on replacement of both cells of the same kind. The percentages are corrected for those of controls.

formation of twins, because the dorsoventral axis of an extra vegetal dorsal cell or cells cannot coincide with those of the others.

Not more than two animal dorsal cells should be placed on vegetal dorsal cells, although their orientation is not important. Furthermore, not only one but also two animal dorsal cells can be replaced by animal ventral cells, irrespective of the orientation of the latter.

From these results, the extreme example of a composite embryo that can be expected to develop into a normal embryo is that consisting of one vegetal dorsal, three vegetal ventral and four animal ventral cells. Among these eight cells, the vegetal dorsal cell is the only cell whose orientation influences pattern formation in the embryo.

(B) Comparison of the results of defect and replacement experiments

In our previous defect experiments on 8-cell embryos, the cell composition of defect embryos was changed only by removing a particular cell or cells (Kageura & Yamana, 1984). In the present transplantation experiments, we changed the cell composition of composite embryos within a wider range by replacing a cell or cells of one kind by a cell or cells of another between a donor and a recipient. Furthermore, the dorsoventral orientation of transplanted cells was also changed in many series of composite embryos. It is therefore interesting to compare the results of the present experiments with those of previous defect experiments.

First, in the present study we found an animal dorsal cell is replaceable by an animal ventral cell, but the latter is not replaceable by the former. Furthermore, the left and right vegetal dorsal cells are not interchangeable. In the defect experiments, however, the four animal cells were equivalent and the two vegetal dorsal cells were indistinguishable. This discrepancy is due to the fact that the dorsoventral orientation of a dorsal cell is changed in the present experiments, but not in the defect experiments, and will be discussed later. Second, each one of the two vegetal dorsal and vegetal ventral cells was found to be replaceable by a different cell, but at least one vegetal dorsal and one vegetal ventral cell were necessary for complete pattern formation. This finding is consistent with results of defect experiments. Third, two animal cells were sufficient in a defect embryo consisting of not more than four vegetal cells, but not in a composite embryo consisting of five or more vegetal cells. This implies that more animal cells are required with increase in vegetal cells in a composite embryo. As has been shown, deficiency in animal cells often leads to incomplete invagination.

(C) *Twin formation*

We found that many or most composite embryos become twins or 'flat back' embryos when a vegetal dorsal cell or cells were introduced in an off-axis orientation, regardless of the region into which the cell or cells were transplanted. This finding is essentially the same as that of Gimlich and Gerhart (1984) that vegetal dorsal cells of a 64-cell embryo cause formation of a secondary body axis when transplanted among ventral vegetal cells. In addition, the introduction of an animal dorsal cell or cells in an off-axis orientation into the ventral region has also been shown to cause secondary axis formation in most composite embryos. The formation of twins and 'flat back' embryos is ascribed to the partial duplication of dorsal axial structures. Several lines of evidence show that the transplanted dorsal cell is responsible for this duplication. First, secondary embryos were formed on the side of the composite embryo into which the dorsal cell had been transplanted. Second, the type of embryo that arose depended on the transplanted cell: unequal twins and 'flat back' embryos were predominant when an animal dorsal cell was introduced in an off-axis orientation into the ventral region, whereas equal twins were often formed when a vegetal dorsal cell was introduced in this orientation. This implies that an animal dorsal and a vegetal dorsal cell are responsible for a smaller and a larger embryo axis, respectively. Third, both the number of twins that arose and the type of twins varied depending on the angle of rotation at which a dorsal cell was transplanted. Finally, most convincing evidence was obtained by cell lineage analysis in chimaeras made by introducing two animal dorsal cells of an *X. borealis* embryo into an *X. laevis* 8-cell embryo in place of two animal ventral cells: as described above, the results showed that the dorsal axial structures of the secondary embryos consisted mainly of *X. borealis* cells.

Secondary embryo formation by a dorsal cell transplanted in an off-axis orientation implies that a dorsal cell has the capacity to form dorsal axial structures and this capacity is confined so much to the dorsal side that the dorsal cell is

'polarized'. In contrast, ventral cells show no sign of this capacity or polarity. This difference between dorsal and ventral cells explains the difficulty of replacing a ventral cell by a dorsal one and of replacing a left-handed vegetal dorsal cell by its right-handed counterpart.

The progeny of animal ventral cells can also form dorsal axial structures in the presence of the progeny of vegetal dorsal cells. This was shown in series 4-9, where 70% (100% as corrected for values of controls) of the composite embryos, though deprived of animal dorsal cells, gave rise to normal embryos. These results confirm some of the results of Nieuwkoop and Sudarwati, who defined inductive interaction in their recombination experiments on axolotl and *Xenopus* mid-blastulae (Nieuwkoop, 1969*a,b*; Sudarwati & Nieuwkoop, 1971). They combined the animal and vegetal halves of midblastulae, from which the marginal zone had been removed, and showed that dorsal axial structures always formed from cells of the animal half on the prospective dorsal side with reference to the vegetal half. From these results, it is obvious that animal cells that come to reside directly above the vegetal dorsal cells can be induced by the latter and form axial structures, irrespective of their normal fate.

(D) *Twin formation by animal dorsal cells*

The present experiments showed that dorsal axial structures, though not well developed, formed in almost all the composite embryos in series 3-4. This axis formation could not be the consequence of inductive interaction with the progeny of vegetal dorsal cells, as these composite embryos had no vegetal dorsal cell. Furthermore, 60-80% of composite embryos with an animal dorsal cell or cells in the animal ventral region developed into twins or 'flat back' embryos (series 3-7 to 3-9 and 0-3). It has also been shown that secondary embryos of these twins were produced by the progeny of the implanted animal dorsal cells. We found previously that about half the isolated animal halves of 8-cell embryos became 'vesicles with a long projection', which contained muscle as well as other structures (Kageura & Yamana, 1983). Essentially similar results were reported by others. Some animal halves at the 8-cell stage developed a notochord and somites when cultured in isolation (Nakamura, 1978). These findings strongly suggest that the capacity of animal dorsal cells to form dorsal axial structures is inherent in these cells and is not acquired at a later stage by interacting with vegetal dorsal cells or their progeny, because animal cells are first separated from vegetal ones in the 8-cell stage, and no interaction between them occurs at an earlier stage.

Our present proposal that the progeny of animal dorsal cells have the capacity to become mesodermalized by themselves to form dorsal axial structures in the absence of vegetal dorsal cells does not necessarily contradict the previous findings of Nieuwkoop and Sudarwati (Nieuwkoop, 1969*a,b*; Sudarwati & Nieuwkoop, 1971). The reason for this is that their recombinates lacked the marginal zone, which contained a large part of the progeny of animal dorsal cells. It is, therefore,

possible that their recombinates would have developed into twins like our composite embryos of series 3-7 to 3-9 and 0-3, if the cells of the marginal zone had been retained.

As described above, a vegetal dorsal cell and an animal dorsal cell form a larger and a smaller embryo axis, respectively. Now, it can be expected that the frequencies of equal twins (in which secondary embryo axes are larger than in unequal twins) is the highest in the composite embryos having a vegetal dorsal and an animal dorsal cell (series 0-4), intermediate in those having a vegetal dorsal cell (series 1-7 to 1-9), and the lowest in those having animal dorsal cell only (series 3-7 to 3-9 and 0-3). The obtained results agree well with this expectation.

Our defect experiments showed that the cells of an 8-cell embryo are of three kinds and that four cells of the three kinds are necessary for complete pattern formation (Kageura & Yamana, 1984). Almost all the defect embryos lacking any one of the necessary cells developed abnormally, the missing or underdeveloped part being that which would have been formed by the missing cell. In addition, the present experiments showed that the cell composition of a composite embryo and the dorsoventral orientation of individual cells are very important for pattern formation, and that certain abnormal cell arrangements caused characteristic defects. Cells of an early embryo are not regulative in their development. From these observations, we conclude that the very early phase of development of Amphibia depends more on the combination and orientation of cells within which particular regions of egg cytoplasm have become localized, than on the interaction among cells of different kinds, and the latter interaction becomes more important as development proceeds. Essentially the same conclusion was recently drawn by Gurdon and his co-workers on the basis of a different line of evidence (Gurdon, Mohun, Fairman & Brennan, 1985).

We are grateful to Dr J. B. Gurdon for providing *X. borealis* and to M. Yuge for his assistance. This study was supported by a Grant-in-Aid for Special Project Research (No. 59480023) from the Ministry of Education, Science, and Culture of Japan.

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(Accepted 20 August 1985)