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Compensation for the Lost Prospective Central Nervous System by Expansion of the Prospective Epidermis in the '16-Cell' Embryos of *Xenopus laevis* Lacking All Animal Dorsal Blastomeres

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ABSTRACT—In the normal embryo of *Xenopus laevis*, the central nervous system (CNS) and epidermis, a pair of main ectodermal tissues, are derived mainly from the animal dorsal (AD) and animal ventral (AV) blastomeres, respectively. The defect embryo, from which all AD blastomeres have been removed at the 16-cell stage, can develop into a normally proportioned embryo, *i.e.*, a regulated embryo, despite the striking deficiency of the prospective CNS. To compare the contribution of the AV blastomeres to the CNS and epidermis between the normal and regulated embryos, each of the AV blastomeres was labelled by a tracer injection at the 16-cell stage, and a clonal domain originating from the labelled blastomere in these ectodermal tissues was examined. By the removal of the AD blastomeres, the clonal domains of the each AV blastomeres were expanded in a dorsal direction, and covered the regions not only in the epidermis just as in normal embryos but also in the CNS extending from the anterior to the posterior end, respectively. Most of the lost prospective regions of the AD blastomeres in the ectodermal tissues were compensated not by the descendants of all the remained blastomeres in the defect embryo, but by the progeny of the AV blastomeres extending dorsally. These facts suggest that compensation for the lost prospective CNS owes mainly to the regulation in the prospective ectoderm, and spreading of the prospective ectoderm is progressively directed after the 16-cell stage by interaction with other parts of the embryo.

INTRODUCTION

From a century ago, it has been known that amphibian eggs can undergo regulative development. Especially in *Xenopus laevis*, extensive experiments of isolation, defect and recombination of blastomere(s) elucidated the conditions for the combination of blastomeres and/or the dorsoventral orientation of blastomere(s) to accomplish regulative development (Kageura and Yamana, 1983, 1984, 1986; Kageura, 1995). Additionally, many cell lineage studies, in which a single ancestral cell is labelled by tracer injection(s), have been carried out for normal development of *X. laevis*, and much information on the fate, movement and migration of cells has been obtained (Jacobson, 1985; Sheard and Jacobson, 1987; Masho and Kubota, 1986; Masho, 1988; Takasaki, 1987; Dale and Slack, 1987; Moody, 1987a, b, 1989). However, the studies of cell lineage in regulative development are very few, and the objects of the studies have been limited to the regulation of Rohon-beard neurons (Jacobson, 1981), mesodermal tissues (Yuge and Yamana, 1989) and retina (Huang and Moody, 1993).

In the present study, the regulation of ectodermal tissues in defect embryos was examined by tracing the cell lineage. The embryos lacking all animal dorsal blastomeres were selected for the present experiment, for the following reasons. (1) The central nervous system and epidermis are the main ectodermal tissues. Most of the CNS, *i.e.*, 88% of the brain and 54% of the spinal cord volume, is derived from the animal dorsal blastomeres. On the other hand, the major part of the epidermis, *i.e.*, 66% of the volume of the epidermis, originates from the animal ventral blastomeres. In short, the majority of these tissues, *i.e.*, 79% of the volume, originates from the animal blastomeres (Hirose and Jacobson, 1979; Masho and Kubota, 1986; Dale and Slack, 1987; Moody, 1987a, b, 1989). (2) The defect embryos which can develop into harmonic larvae in spite of having the most deficient prospective ectoderm, are the embryos from which half of the animal blastomeres have been removed (Kageura and Yamana, 1984). (3) Spreading of ectoderm to cover other parts of the embryo is called epiboly. The regional differences in movements of the ectodermal cells during epiboly are described in detail (reviewed

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in Hausen and Riebesell, 1991). The spreading of the ectoderm is more extensive at the dorsal side than the ventral (Keller, 1978). (4) The CNS, derived mainly from the AD blastomeres, is the tissue in which the most advanced cell lineage studies were performed. The region in which all of the descendants of a labelled single blastomere are dispersed is called a clonal domain. In the CNS, the clonal domains were examined by means of injecting a tracer into the blastomeres during the 2- to 512-cell stages (Jacobson and Hirose, 1978,1981; Hirose and Jacobson, 1979; Jacobson, 1983; Sheard and Jacobson, 1987).

In the present defect embryos, the AV blastomeres are the chief remains of the prospective ectodermal blastomeres. Therefore, in this study, to compare the contribution of the progeny of the AV blastomeres to the CNSs and the epidermises between the normal and regulated embryos, the clonal domains stemming from AV blastomeres in these tissues of both types of embryos were examined. The 16-cell embryo was selected for the operation of blastomere removal and blastomere labelling because a reconstructed clonal domain in the epidermis, resulting from a blastomere labelled with a tracer injection at this stage, has not been represented as a whole embryo illustration, even in a normal embryo. The examined clonal domains descending from the AV blastomeres in the present regulated embryo showed that the ectodermal tissues originated overwhelmingly from the AV blastomeres. Differences in the patterns of the clonal domains between the normal and regulated embryos indicated that spreading of the progeny of the AV blastomeres in the dorsal direction occurred in the defect embryos, in addition to spreading in the ventral and lateral directions just as in the normal embryos.

MATERIALS AND METHODS

Selection of embryos

Fertilized eggs of *Xenopus laevis* were obtained by gonadotropin-induced mating of adult frogs. Embryos were dejellied with 2.5% sodium thioglycolate (pH 9.0). After being washed with water, the embryos were sterilized with 0.1% Sodium p-toluenesulfone chloramide (chloramine T) for 2 min. The 16-cell embryos with regular and symmetrical patterns of cleavage and pigmentation were selected (Fig. 1A). The nomenclature of blastomeres at the 16-cell stage was that of Hirose and Jacobson (1979) (Fig. 1B, C).

Removal of blastomeres

Dejellied embryos were put into 100% Steinberg solution in a Petri dish coated with 2% agar, and the vitelline membranes were removed with two pairs of forceps. All four AD blastomeres were removed from a 16-cell embryo by flipping the cleavage furrow repeatedly with a glass needle. The defect embryos were put into holes of the agar plate, and cultured in 100% Steinberg solution. The removed blastomeres were represented by the filled ones shown in Fig. 1C.

Injection and visualization of lineage tracer

Injections were given to the AV blastomeres of the normal and defect embryos. For control injection, the dejellied normal embryos were put into holes of a tissue-culture plate (Falcon-3034), the surface of which was coated with 2% agar, and filed with 100% Steinberg solution containing 10% w/v Ficoll (type 400: Pharmacia) to prevent leakage of cytoplasm during and after injection (Newport and Kirschner, 1982). In each embryo, each of the AV blastomeres were injected with 0.6nl of lineage tracer fluorescein-dextran-amine (FDA) at 100 mg/ml in 0.2N KCl (Gimlich and Brawn, 1985). A microinjector (Model IM-1: Narishige Sci. Lab.) connected to a needle, whose external tip diameter was 2–5 μm, was used for the injection. The injected embryos were cultured in the above-mentioned medium until stage 8–9 (Nieuwkoop and Faber, 1967). Then, the culture medium was gradually changed to 10% Steinberg solution.

When control embryos reached stage 32 (Nieuwkoop and Faber, 1967, tailbud stage), the injected embryos with normal proportions

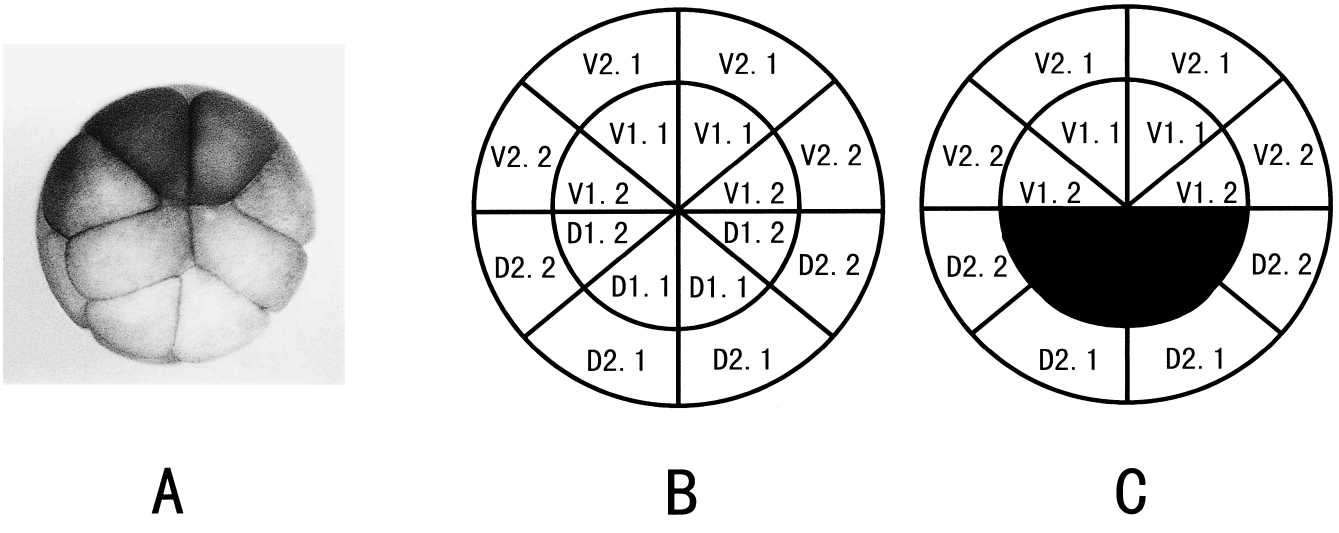

Fig. 1. Animal view of a 16-cell *Xenopus* embryo. The upper side is ventral and the lower one is dorsal. (**A**) A photograph of an embryo with typical pigmentation and cleavage patterns. The blastomere arrangement of the animal dorsal is different from that of animal ventral. Pigmentation is darker in ventral blastomeres than dorsal ones. (**B**) A diagram schematizing the composition of blastomeres of a normal 16-cell embryo. The vertical, horizontal, and oblique lines and the inner circle represent the first, second, fourth and third cleavage planes, respectively. (**C**) A diagram of an embryo lacking all animal dorsal blastomeres. Removed two D1.1 and two D1.2 blastomeres are represented by the filled-in areas.

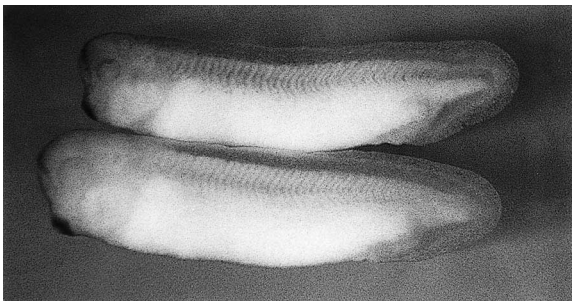

Fig. 2. A regulated embryo, derived from a defect embryo lacking all animal dorsal blastomeres, at Stage 32 (top). The regulated embryo has a complete pattern with a body length leaching 90% or more of a normal one. The lower tadpole is a normal embryo for control.

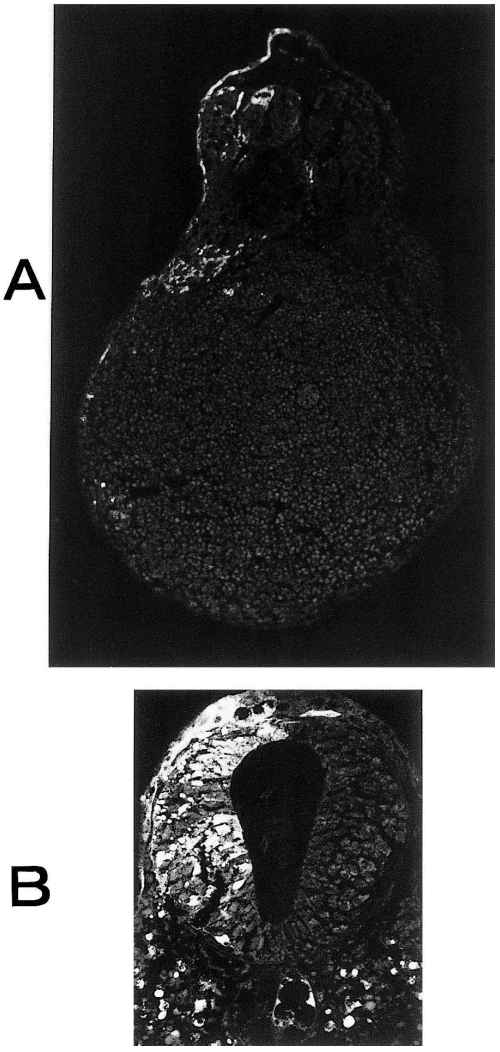

Fig. 3. Photographs of the transverse sections of an embryo, a single blastomere of which had been labelled with fluorescein-dextran-amine (FDA). (**A**) A photograph of a whole section of a normal embryo, whose V1.2 blastomere had been labelled, at the level of the spinal cord. (**B**) A photograph of the mesencephalon of a regulated embryo, whose V1.2 blastomere had been labelled, in a transverse section. The photographs show bright fluorescence of FDA-labelled cells against a faint autofluorescent background. The intermingling between the labelled and unlabelled cells is seen in the dorsal and lateral parts of the mesencephalon.

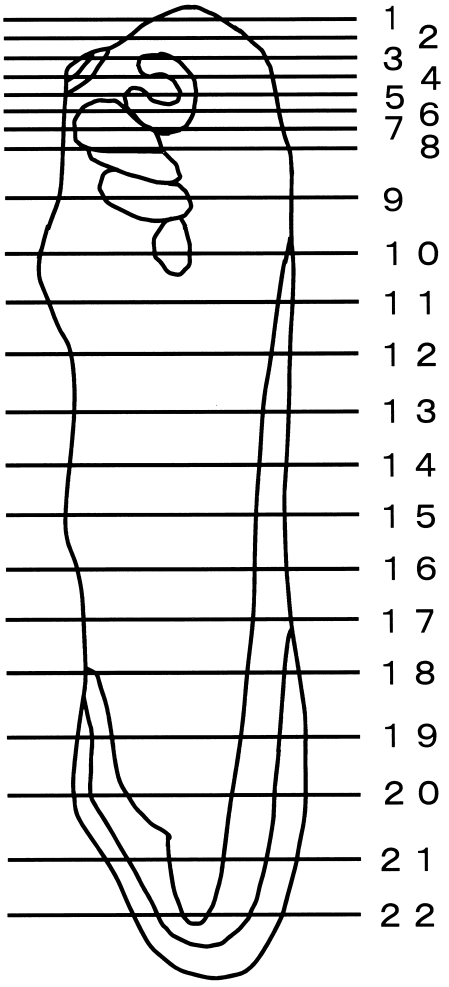

Fig. 4. Illustration of a tailbud embryo (stage 32) and the positions of the analyzed transverse sections in an embryo. No. 8, section through the otocyst; No. 18, section through the anus. Within each of the three regions divided by No. 8 and No. 18, the intervals among the numbered sections were the same.

were selected (Fig. 2), and fixed in 4% formaldehyde in 70% phosphate buffer (pH 7.0) overnight at 4°C. The embryos were washed in 70% phosphate buffer for at least 24 h, dehydrated in an ethanol: butanol series, embedded in paraffin wax (m.p. 51–53°C) and sectioned transversely at 7 μm. After dewaxing in xylene, the rehydrated specimens were stained with 0.5 μg/ml of 4, 6-diamino-2-phenyl indole (DAPI: Wako Pure Chem. Instr. LTD.) for 2 min. All specimens were then dehydrated, cleared in xylene and mounted in MX. (Matsunami Glass Ind., LTD.). The progeny of an FDA-labelled blastomere was examined under a fluorescent microscope. An example of the observed image is shown in Fig. 3.

Analysis

Twenty-two sections per embryo were selected and numbered from head to tail as seen in Fig. 4. Nos. 8 and 18 are the sections through the otocyst and anus, respectively. In each of the three regions of the embryo divided by section Nos. 8 and 18, the intervals among the numbered sections were the same. In each numbered section, spatial distribution of labelled cells was traced on a camera lucida drawing (Fig. 5A).

For the CNS, these 22 drawings and some supplemental drawings were used for a graphical reconstruction procedure known as

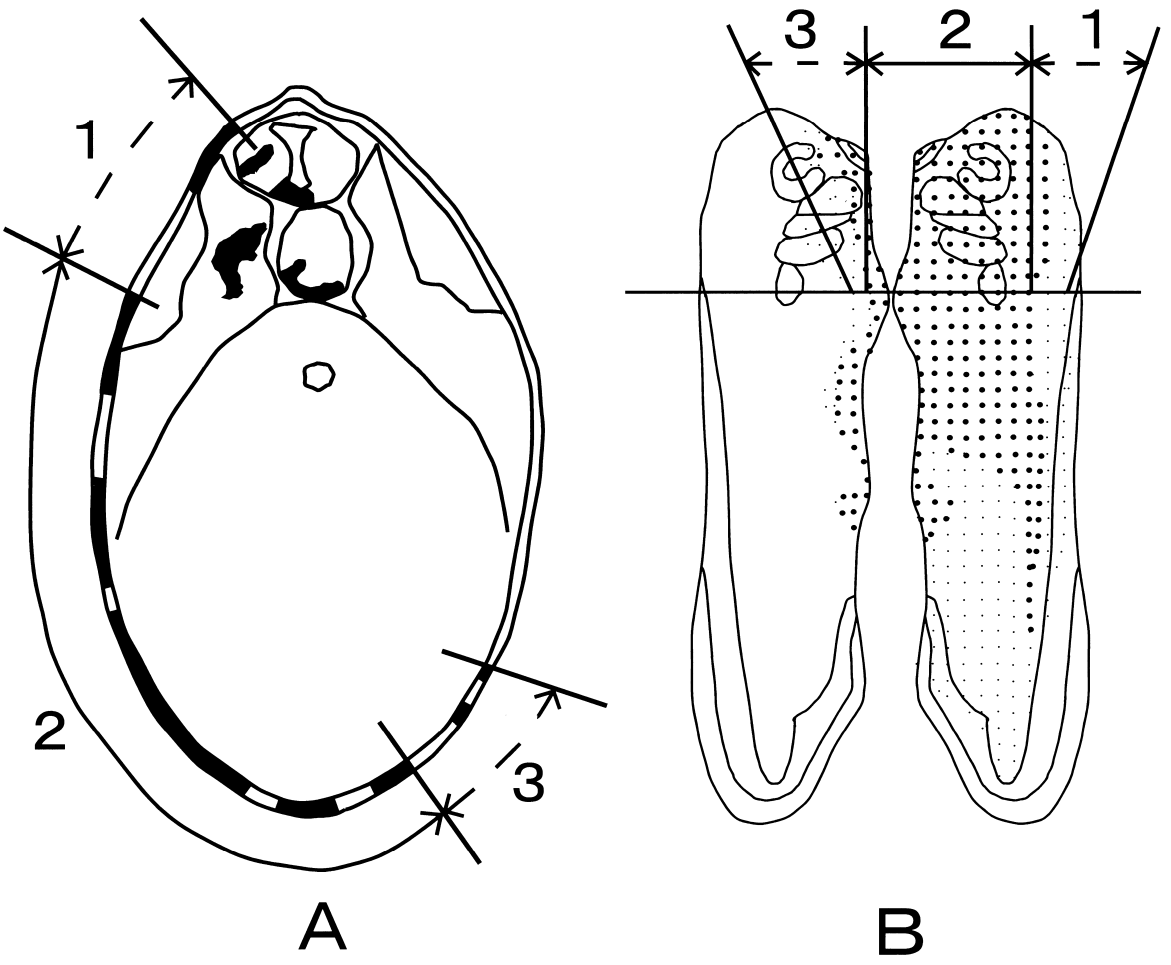

Fig. 5. Relationship between the distribution of labelled cells in the epidermis of a camera lucida drawing from the transverse section of an embryo (**A**) and the reconstructed clonal domain in the epidermis (**B**). The numbered solid and dashed lines with arrowheads in A and B correspond to each other, respectively. The solid line with arrowheads indicates the epidermal region where labelled cells predominate. In **A**, the two outermost stained epidermal parts in this region are shorter than the unstained parts located on the outer side of these stained parts and are longer than the unstained parts located on the inner sides of these stained parts, respectively. In **B**, the line intersects the region in the clonal domain, which is represented by large dots. The dashed lines with arrowheads show the epidermal regions with less incidence of labelled cells. In B, the line intersects the region in the clonal domain, which is represented by small dots.

topological analysis (Nieuwenhuys, 1974; Jacobson and Hirose, 1981). The CNS, which is three dimensional, was represented as a two dimensional graph by this procedure. The region occupied by the major volume of descendants of the labelled blastomere is represented by the area with large dots. On the other hand, the region where a minor volume of labelled cells was dispersed, is indicated by the area having small dots (A and C in Figs. 6 and 7).

Fig. 5A shows a traced transverse section of the embryo in Fig. 5B cut along the solid line. The section also intersects the clonal domain in the epidermis. The stained and unstained parts of the epidermis in the clonal domain of the epidermis alternate in a series. The region in the clonal domain of the epidermis of the section, where stained parts predominate, is indicated with a solid line with two arrow heads (2 in Fig. 5A). However, the two outermost stained epidermal parts in the region, indicated with a solid line, are shorter than the unstained parts located on the outer sides of these stained parts and are longer than the unstained parts located on the inner sides of these stained parts, respectively. The regions with less incidence of stained epidermal parts, located on both sides of the region indicated with the solid line, are represented by the dashed lines with two arrow heads (1 and 3 in Fig. 5A). The regions, in Fig. 5A, indicated with the solid (2) and dashed (1 and 3) lines with arrowheads are reconstructed and represented in the picture of the tailbud embryo in Fig. 5B as the clonal domain with large (2) and small (1 and 3) dots, respectively.

The clonal domains, descended from the same kind of blastomeres, were slightly different among individual embryos. To represent this variation of the clonal domain, the clonal domains were depicted in individual embryos (A and C in Figs. 6–9). In addition, to represent the rate of the observation of the labelled cells in a region of an embryo, the clonal domains were displayed again as the region having the layered dots with four grades (B and D in Figs. 6–9). The overlapped region with both large and small dots is represented by the large dots only.

RESULTS

Sixty five percent of the embryos lacking all AD blastomeres with no injection developed into normally proportioned larvae, that is, regulated embryos, whose body lengths reached 90% or more of the control embryos (Fig. 2). The percentage

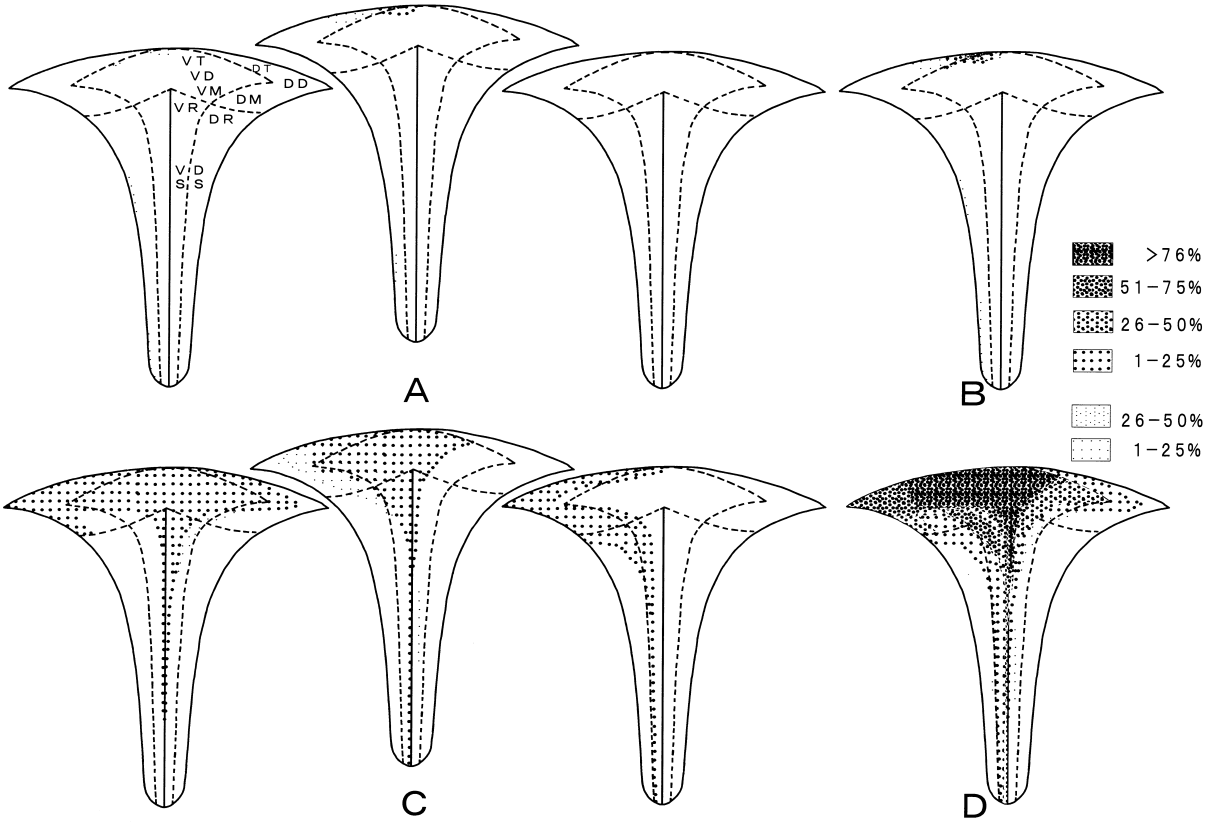

Fig. 6. Diagrams of the position of labelled cells arising from V1.1 in the CNS after Jacobson and Hirose (1981). (**A**) Distribution of labelled cells in individual normal embryos. (**B**) Appearance probability of labelled region in a normal embryo. (**C**) Distribution of labelled cells in individual regulated embryos. (**D**) Appearance probability of labelled region in a regulated embryo. Stippling in the CNS indicates the region in which labelled cells were found. The area with large dots represents the region occupied by a major volume of the descendants of the labelled blastomere. The area with small dots represents the region in which a minor volume of labelled cells was dispersed. The upper four and lower two rectangles in the right of the figure indicate the appearance probabilities of the labelled regions represented with the large and small dots, respectively. The overlapped region with both large and small dots is represented by the large dots only. The upper side is the rostral end and the lower side is the caudal end. The abbreviations: D, diencephalon; DD, dorsal diencephalon; DM, dorsal mesencephalon; DR, dorsal rhombencephalon; DS, dorsal spinal cord; DT, dorsal telencephalon; M, mesencephalon; R, rhombencephalon; S, spinal cord; T, telencephalon; VD, ventral diencephalon; VM, ventral mesencephalon; VR, ventral rhombencephalon; VS, ventral spinal cord; VT, ventral telencephalon.

of regulative development was reduced to 24–26% by the FDA injection. The clonal domains descended from the AV blastomeres of the 16-cell stage in the regulated embryos were compared with those of normal embryos. The results in the CNS are represented in Figs. 6 and 7 and those in the epidermis are shown in Figs. 8 and 9. In the CNS and epidermis respectively, the numbers of the examined embryos are shown in the parentheses of the text, in which the figure numbers are also indicated. The clonal domains only in the CNS and epidermis are described here, although the labelled blastomeres gave rise to cells of many tissues. As no significant difference was found between the clonal domains originating from the right and left blastomeres of the same kind, distinction of right or left is omitted in the following account of the results. The labelled cells mingled with the unlabelled ones in all cases. In the normal embryos, the clonal domains descended from V1.2 were located almost the same regions in the CNS and epidermis, irrespective of the existence of the vitelline membrane (data not shown). Therefore, the following results for the normal embryos are only for cases of embryos with the vitelline membranes.

The CNS

In the normal embryos, progeny of V1.1 was contributed to the anterior-most part of the CNS in very little volume, and the descendants of V1.2 were confined to the dorsal parts of the spinal cord and the brain. On the other hand, in the regulated embryos, the CNS was almost exclusively covered with the clonal domains of V1.1 and V1.2. Progenies of V1.1 and V1.2 in the regulated embryos were distributed throughout the CNS from the anterior to the posterior, and located ventral and dorsal parts of the CNS, respectively. The variation of clonal domains in the CNS among individual regulated embryos was somewhat larger than that among individual normal ones. However, the variation of clonal domains in the CNS of the regulated embryos was not random, and was governed by certain restrictions. Three of the analyzed specimens are shown in A and C in Figs. 6 and 7.

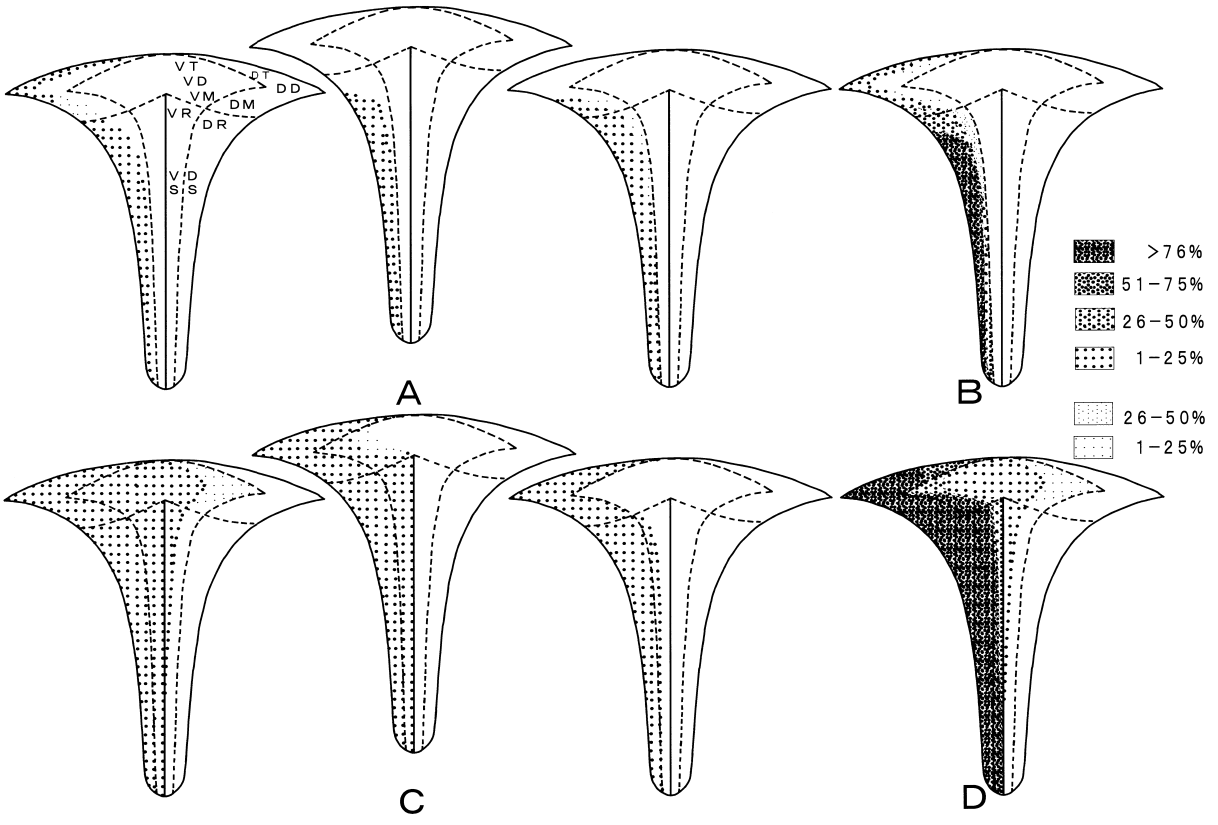

Fig. 7. Diagrams of the position of labelled cells arising from V1.2 in the CNS. (**A**) Distribution of labelled cells in individual normal embryos. (**B**) Appearance probability of labelled region in a normal embryo. (**C**) Distribution of labelled cells in individual regulated embryos. (**D**) Appearance probability of labelled region in a regulated embryo. See the legend of Fig. 6 for details.

V1.1

The normal embryos (n = 5, Fig. 6A, B). In four of five specimens a small volume of labelled cells was seen in the dorsal or lateral telencephalon. Such labeling was not reported by Hirose and Jacobson (1979) or Jacobson and Hirose (1981), but reported by Moody (1987a) in one of nine specimens. In two of these four specimens, a small number of labelled cells was localized in the dorsal spinal cord. Such sparse labelling had also been reported (Jacobson and Hirose, 1981: Moody, 1987a).

The regulated embryos (n = 5, Fig. 6C, D). In all of the regulated embryos, the clonal domain of this blastomere extended from the brain to the spinal cord along the ventral midline. In four embryos, the labelled region was not only on the injected side but also on the uninjected side in the ventral part of the brain, and the labelled regions on the uninjected side were slightly different among individuals. In the forebrain and midbrain on the injection side of the four embryos, labelled cells covered almost the entire regions in three and only the ventral and lateral parts in one. In the remaining one embryo, labelled cells were located only on the ipsilateral side and distributed in the lateral and dorsal regions of the brain. The positions of the streaks of labelled cells along the ventral midline were somewhat different among individual embryos. In three specimens, the streak extended from the brain to the posterior end of the spinal cord. In two specimens, the streaks disappeared at the middle and posterior parts of the spinal cord.

V1.2

The normal embryos (n = 6, Fig. 7A, B). In all cases, labelling was ipsilateral. In four embryos, labelled cells were entirely caudal to the isthmus and confined to the dorsal regions of the rohmbencephalon and spinal cord. In the remaining two embryos, there were a few labelled cells in the dorsal part of the mesencephalon on the injected side only, in addition to the labelled regions in the above cases. In one of these two embryos, there were a few labelled cells also in the dorsal parts of the diencephalon and telencephalon on the injected side. This labelling pattern was consistent with the previous reports (Hirose and Jacobson, 1979; Jacobson and Hirose, 1981; Moody, 1987a).

The regulated embryos (n = 5, Fig. 7C, D). In all of the regulated embryos, extension of the labelled cells from the caudal end of the CNS reached the rostral end, although the extension in the normal embryos came to an end at the rohmbencephalon or mesencephalon in most cases. Also width of the clonal domain of this blastomere in the regulated embryos was wider than in the normal embryos. In four cases, labelling was ipsilateral. In three of the four cases, the entire region of the posterior part of the spinal cord was labelled,

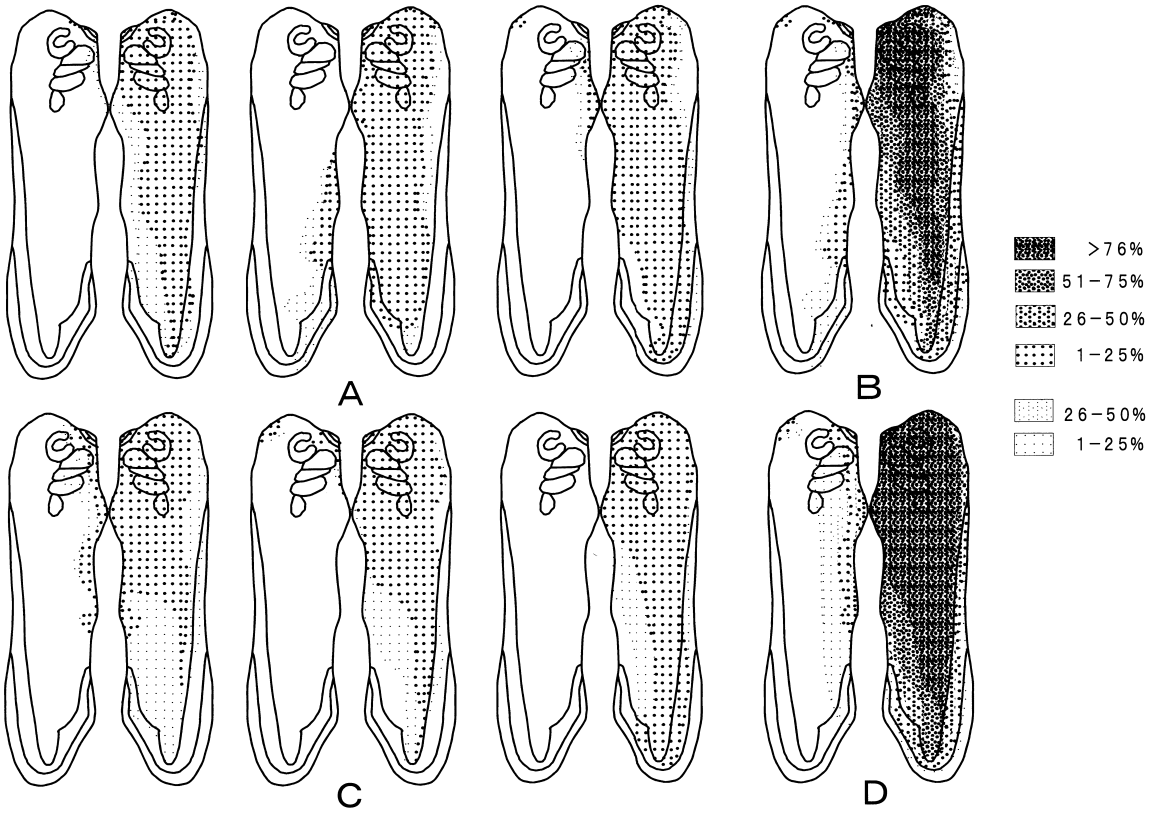

Fig. 8. Diagrams of the position of labelled cells arising from V1.1 in the epidermis. (**A**) Distribution of labelled cells in individual normal embryos. (**B**) Appearance probability of labelled region in a normal embryo. (**C**) Distribution of labelled cells in individual regulated embryos. (D) Appearance probability of labelled region in a regulated embryo. Stippling in the figures of the tailbud larvae indicates the regions in which labelled cells were found. See the explanation at Fig. 5 for the area with large and small dots. The upper four and lower two rectangles in the right of the figure indicate the appearance probabilities of the labelled regions represented with the large and small dots, respectively. The overlapped region with both large and small dots is represented by the large dots only.

and the ventral margin of the labelled region gradually approached the dorsal margin of the brain as it extended to the rostral portion. In the remaining one of the four cases, almost the entire region of the dorsal part of the CNS was labelled. In one case, labelled cells were seen not only in the entire area of the injected side but also in the ventral parts of the brain and spinal cord of the uninjected side. The dorsal margin of the labelled region on the uninjected side gradually approached the ventral midline as it extended to the caudal end.

The Epidermis

In the epidermis, there were little differences in the clonal domains of V1.1 and V1.2 between the normal and regulated embryos. In both types of the embryos, progeny of V1.1 distributed the ventral part of the entire body except for the area surrounding the anus, and the clonal domain of V1.2 were located in more dorsal region than the clonal domain of V1.1.

V1.1

The normal embryos (n = 5, Fig. 8A, B). Three of five embryos are shown in Fig. 8A. In all specimens, the clonal domain of V1.1 occupied the entire region of the body on the injected side except for the dorsal-most part of the body and the area surrounding the anus. The variation in widths of the unstained areas surrounding the anus among individual embryos was large. On the uninjected side, the narrow ventral-most regions were labelled in all specimens. The labelled regions were slightly different individually, and were located in the ventral-most part extending from the sucker to the circumference of the anus.

The regulated embryos (n = 10, Fig. 8C, D). Three of ten specimens are shown in Fig. 8C. The pattern of the clonal domain descending from this blastomere in the regulated embryo quite resembled its counterpart in the normal embryo. In all cases, the labelled cells covered all over the body on the injected side other than the most dorsal region of the body and the area surrounding the anus. Also, the large variation in width of the unlabelled regions surrounding the anus among individual embryos was seen just as in the normal embryos. The small ventral areas extending from the top of the head to the area surrounding the anus on the uninjected side were labelled in eight of ten embryos, although the areas were different among the individuals.

V1.2

The normal embryos (n = 6, Fig. 9A, B). In all cases the

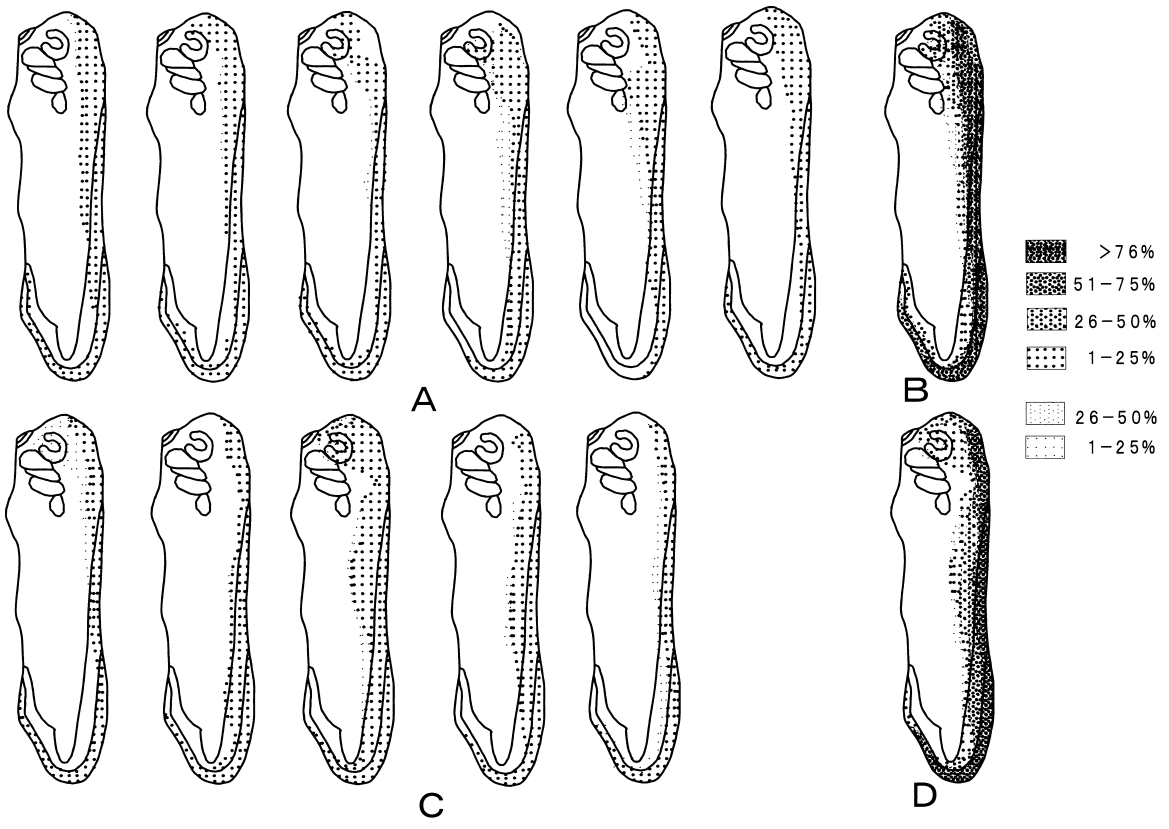

Fig. 9. Diagrams of the position of labelled cells arising from V1.2 in the epidermis. (**A**) Distribution of labelled cells in individual normal embryos. (**B**) Appearance probability of labelled region in a normal embryo. (**C**) Distribution of labelled cells in individual regulated embryos. (**D**) Appearance probability of labelled region in a regulated embryo. See the explanation at Fig. 5 for the area with large and small dots, and the legend of Fig. 8 for details.

clonal domain of V1.2 was ipsilateral, and confined to the dorsal region along the entire body and to the ventral-most part of the tail. In two cases, a small portion of the dorsal part of the head was unlabelled. This observation is consistent with those of Masho and Kubota (1986) and Moody (1987a). In the dorsal region containing the fin fold, extending from the head region to the anus, the labelled area gradually narrowed and the ventral margin of the labelled area was drawn toward the ridge of the fin fold.

The regulated embryos (n = 5, Fig. 9C, D). The distribution pattern of labelled progeny of this blastomere in the regulated embryo was similar to that in the normal embryo, except for the dorsal-most region of the head. In all embryos, the dorsal-most region of the head was covered with the clonal domain of V1.2. This covering was not seen in the clonal domain of V1.2 of the normal embryos in two of six cases. In all cases, the entire clonal domain was ipsilateral and confined to the dorsal region along the entire body and to the ventral-most part of the tail. In the dorsal region including the fin fold, extending from the head to the anus, the labelled area gradually narrowed and the ventral margin of the labelled area was drawn toward the ridge of the fin fold.

DISCUSSION

In the present study, the clonal domains descending from the AV blastomeres in the CNS and the epidermis of regulated embryos were examined and compared with those of normal embryos (Figs. 6–9). To understand the topological relationship between the clonal domains of V1.1 and V1.2 in the CNS and the epidermis, clonal organizations in the ectoderm of the normal and regulated embryos at stage 14 were presumed from the results of the present study and the previous reports (Hirose and Jacobson, 1979; Jacobson and Hirose, 1981; Masho and Kubota, 1986: Kageura *et al*., 1995), and represented in Fig. 10. In this figure, cell mingling among the clonal domains was neglected. The pattern of the clonal domain of V1.1 was remarkably changed by virtue of the removal of AD blastomeres, and the clonal domain was expanded. In the normal embryos, the clonal domain of V1.1 occupied the ventral epidermis, except for the area surrounding the anus, and in some cases a very small portion of the forebrain (A and B in Figs. 6 and 8 and Fig. 10A). In the regulated embryos, the clonal domain of V1.1 substituted for most of the clonal domain of D1.1, and was also positioned in almost the same region as in the normal embryos. Additionally,

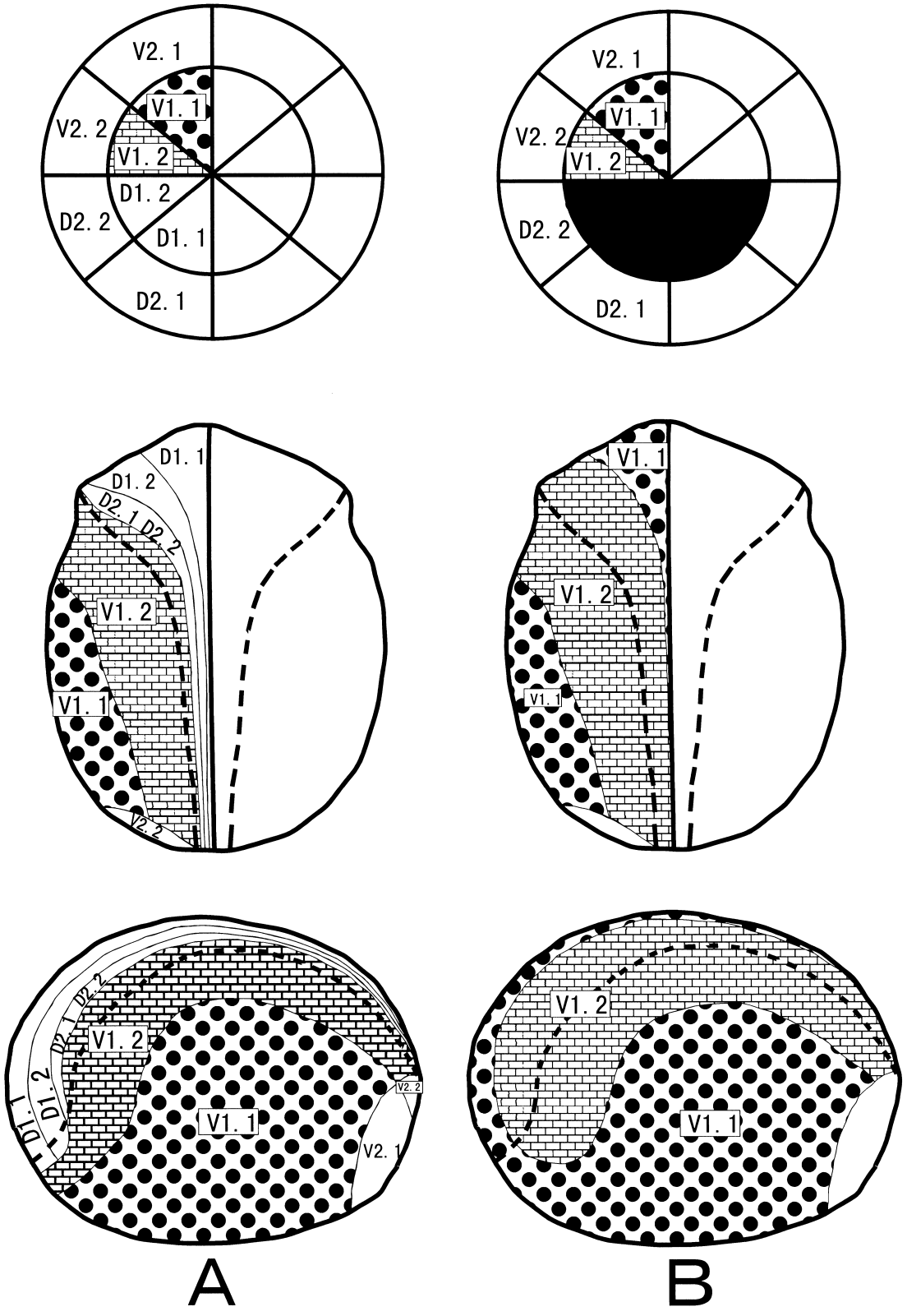

Fig. 10. Presumptive clonal organization of the ectoderm in stage 14 embryos. (**A**) A control embryo. (**B**) A regulated embryo derived from a defect embryo lacking all animal dorsal blastomeres. The upper illustrations show the diagrams schematizing the composition of blastomeres of two types of 16-cell embryos. The middle illustrations are dorsal views and the lower ones are lateral views. The dashed lines show the ridges of the neural folds.

this clonal domain in the CNS was linked with that in the epidermis at the head region, and extended from the anterior to the posterior part (C and D in Figs. 6 and 8 and Fig. 10B). This fact indicates that the convergence and extension, the narrowing and spreading towards the blastopore of the prospective ectoderm, had also occurred in the prospective CNS of the present defect embryos. On the other hand, the difference in the pattern of the clonal domain of V1.2 between the normal and regulated embryos was slight, although this clonal domain also was expanded by removal of the AV blastomeres. In most of the normal embryos, the clonal domain of V1.2 was located in the dorsal parts of the rhombencephalon, spinal cord and epidermis (A and B in Figs. 7 and 9 and Fig. 10A). In the regulated embryos, the clonal domain of V1.2 substituted for the clonal domains of D2.1 and D2.2 and most of the clonal domain of D1.2 in the normal embryos, most parts of which were positioned in the CNS, in addition to being located in almost the same region as in the normal embryos (C and D in Figs. 7 and 9 and Fig. 10B).

It appears that the differences in pattern of the clonal domains in the CNS and epidermis between the normal and regulated embryos largely reflect the difference in ectoderm formation between both types of embryos for the following two reasons. Firstly, in the present defect embryos, the removed AD blastomeres, which contain the prospective dorsal mesoderm and ectoderm (Jacobson, 1985; Masho and Kubota, 1986; Masho, 1988; Takasaki, 1987; Dale and Slack, 1987; Moody, 1987a, b, 1989), were bilaterally symmetrical. This fact indicates that bilaterally symmetrical regulation of mesoderm and ectoderm had occurred in the regulated embryos. So, it is inferred that the difference in the direction of movement of the mesodermal cells between the normal and regulated embryos was little. Secondly, the body length of the regulated tailbuds was more than 90% of the normal tailbuds (Fig. 2), indicating that the difference in the distance of migration for mesodermal cells between the normal and regulated tailbuds was also little. The different location of the clonal domains of V1.1 and V1.2 between both types of embryos (Figs. 6–10) indicates that the prospective ectodermal blastomeres are not determined with respect to the spreading direction in epiboly by the 16-cell stage. Spreading of the prospective ectoderm may be progressively directed after the 16-cell stage by interaction with other parts of the embryo. The clonal domains of V1.1 and V1.2 in the regulated embryos substituted for most of the clonal domains descending from each blastomere, opposite to V1.1 and V1.2 in respect to the right-left axis in the normal embryos, and were also located in nearly the same regions as in the normal embryos (Figs. 6–10). This fact indicates that due to the removal of AD blastomeres, the clonal domains of V1.1 and V1.2 expanded in a dorsal direction, and especially in the case of V1.1, the spreading direction of the descendants of V1.1 was changed from only a ventral direction to a bi-direction along the dorsoventral axis. In addition, this fact also indicates that the AD blastomeres and their descendants are important in the normal embryos not only for spreading towards the dorsal and lateral directions but also for obstructing the spreading of the descendants of AV blastomeres in a dorsal direction.

In the normal embryos, the progeny of the AV blastomeres contributed to the major part of the epidermis and the dorsal part of the CNS (A and B in Figs. 6–9 and Fig. 10A). On the other hand, the descendants of the AD blastomeres in the normal embryos covered the major part of the CNS and a small portion of the head epidermis (Hirose and Jacobson, 1979; Jacobson and Hirose, 1981; Masho and Kubota, 1986; Dale and Slack, 1987; Moody, 1987a, b), and occupied about 40% of the volume in the CNS and the epidermis (Dale and Slack, 1987). In the present regulated embryo, two extreme compensation modes of ectoderm are presupposed. One mode is that the descendants of the remaining prospective ectodermal blastomeres in the defect embryos spread wider than their counterparts in the normal embryos by epibolic movement, and cover the regions where the clonal domains descended from the AD blastomeres ought to be located. Another is that the progeny of the remaining prospective mesodermal or endodermal blastomeres change their fate and become ectodermal tissues. The clonal domains derived from the AV blastomeres in the CNS and epidermis of the regulated embryos were strikingly expanded in contrast to those of the normal embryos and covered not only the major part of the epidermis, but also nearly the entire CNS. In other words, the clonal domains of the AV blastomeres in the regulated embryos compensated for the regions where the clonal domains of the AD blastomeres should be positioned, in addition to covering nearly the same regions where their counterparts in the normal embryos were located. At the same time, the clonal domains of the AV blastomeres in the regulated embryo also covered the clonal domains of D2.1 and D2.2 in the normal embryo (C and D in Figs. 6–9 and Fig. 10B). The difference between both types of the embryos seems to be small for the widths of the areas surrounding the anus, which are covered neither by the clonal domain of V1.1 nor that of V1.2 (Fig. 8, 9 and 10). The descendants of D2.1, which participated in the formation of the CNS in the normal embryos, did not become the ectodermal tissues in the regulated embryos except for a little mixture in the CNS of one of five specimens (unpublished data, Koga). These data indicate that the present compensation mode of ectoderm is closer to the former than the latter.

The present results in the normal embryos are essentially identical with the results of previous reports in which clonal organization of the CNS and the fates of blastomeres had been examined by a tracer injection to a single blastomere (Jacobson, 1985; Masho and Kubota, 1986; Moody, 1987a, b; Dale and Slack, 1987), except that the rate of normal embryos having the descendants of the V1.1 blastomere in the forebrain is somewhat higher than that in these reports. Kageura *et al.* (1995) illustrated the external distribution maps of the progeny of individual blastomeres of 16-cell normal embryos at stage 14 from the results of the analysis in the black-white chimeras of *X. laevis*. In their report, the clonal domain of A3 in the epidermis, corresponding to the present

clonal domain of V1.2, was widest at the middle part of the body in respect to the dorsoventral direction. The width of the clonal domain in the epidermis gradually narrowed as it extended to both sides of the embryo, approaching the ventral margin of the clonal domain to the dorsal ridge of the prospective epidermis. However, in the present analysis of the normal embryos at stage 32, the widest part in the clonal domain of V1.2 in the epidermis was the head region rather than the middle part of the body. It is unclear whether this inconsistency in the results between the two studies is due to differences in techniques for tracing cell lineage, in the examining stage of the embryos, or in the method for appraising the clonal domain. Kageura *et al.* (1995) also reported that the progeny of V1, corresponding to the progeny of present D2.1, made no contribution to the surface of an early neurula. On the other hand, in the studies using a tracer injection, a small contribution of the progeny of D2.1 to the CNS was described (Hirose and Jacobson, 1979; Dale and Slack, 1987; Moody, 1987a). These descriptions are not necessarily contradictory. To represent the contribution of the descendants of the D2.1 to the CNS, the clonal domain of this blastomere is shown in Fig. 10A in combination with the clonal domain of D2.1.

Fig. 6D and Fig. 7D show that the clonal domains of V1.1 and V1.2 in the CNS of the regulated embryos extended from the anterior to the posterior end with ventral and dorsal location, respectively, and overlapped each other in the dorsal and lateral parts of the forebrain and midbrain. Existence of this overlap region between the clonal domains of V1.1 and V1.2 suggests that cell intermingling between the progeny of V1.1 and V1.2 occurred in these regions. This suggestion was supported by the observation of intermingling between labelled and unlabelled cells in these regions (Fig. 3B). On the other hand, in the normal embryos, overlap between the clonal domains of V1.1 and V1.2 in the CNS was quite rare (Fig. 6B and Fig. 7B). From these results, Fig. 10 shows that the boundaries of the clonal domains between V1.1 and V1.2 run through the CNS in the regulated embryo, but not in the normal embryo.

The variation of clonal domains in the CNS among the regulated embryos was somewhat larger than that among the normal ones (Figs. 6 and 7). This may be due to the operation of blastomere removal, because there was no significant difference in the clonal domain of V1.2 in the CNS and epidermis between the normal embryos with and without the vitelline membrane (data not shown). The operation of blastomere removal disturbed the blastomere arrangement, that is, the positions of the blastomeres in the embryo, especially at the dorsal part of the AV blastomeres, because the AD blastomeres, to which the AV blastomeres had adhered at their dorsal parts, had been removed. This variation of the positions of AV blastomeres in the defect embryos may correspond to the variation of clonal domains derived from those blastomeres in the regulated embryos, especially in the CNS. However, this variation was not random and was governed by certain restrictions. The restrictions may be due mainly to adhesion among the remaining blastomeres after the operation.

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