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The role of the dorsal lip in the induction of heart mesoderm in *Xenopus laevis*

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Summary

We have examined the tissue interactions responsible for the expression of heart-forming potency during gastrulation. By comparing the specification of different regions of the marginal zone, we show that heart-forming potency is expressed only in explants containing both the dorsal lip of the blastopore and deep mesoderm between 30° and 45° lateral to the dorsal midline. Embryos from which both of these 30°–45° dorsolateral regions have been removed undergo heart formation in two thirds of cases, as long as the dorsal lip is left intact. If the dorsal lip is removed along with the 30°–45° regions, heart formation does not occur. These results indicate that the dorsolateral deep mesoderm must interact with the dorsal lip in order to express heart-forming potency. Transplantation of the dorsal lip into the ventral marginal zone of host embryos results in the formation of a secondary axis; in over half of cases, this secondary axis includes a heart derived from the host mesoderm. These findings suggest that the establishment of heart mesoderm is initiated by a dorsalizing signal from the dorsal lip of the blastopore.

Key words: embryonic induction, heart, dorsal lip, *Xenopus*.

Introduction

In a previous study, we showed that the heart mesoderm becomes specified prior to the end of gastrulation in *Xenopus* embryos (Sater and Jacobson, 1989). Although in urodeles the heart is induced by the pharyngeal endoderm during gastrula and postneurula stages (Balinsky, 1939; Nieuwkoop, 1947; Chuang and Tseng, 1957; Jacobson and Duncan, 1968; for review, see Jacobson and Sater, 1988), the mechanism by which heart mesoderm is established during *Xenopus* development remains unclear. The tissue isolation and recombination experiments traditionally used to examine inductive interactions are precluded in this case, because heart mesoderm is specified before tissue layers have become distinct (Nieuwkoop and Faber, 1967).

The establishment of dorsoventral pattern within the mesoderm may be an early critical step in the specification of heart mesoderm. Several lines of evidence suggest that the heart is one of a complex of dorsal axial structures, including the notochord, neural tube, eyes and otocysts, whose formation is dependent upon the expression of the dorsoventral axis. First, treatments that inhibit the formation of the dorsoventral axis also prevent heart formation. For example, embryos subjected to ultraviolet (UV) irradiation during the first cell cycle exhibit a graded series of dorsal axial defects (Scharf and Gerhart, 1980, 1983); heart formation is inhibited in embryos that show little or no axial development (C. R. Phillips, personal communication). Treatments that promote the development of dorsoanterior structures tend to promote heart formation as well: embryos treated with moderate doses of lithium for brief periods during cleavage stages exhibit exaggerated dorsoanterior features, few if any dorsoposterior features, and large, radial hearts (Kao and Elinson, 1988; Sater, unpublished observations; but *cf.* Cooke and Smith, 1988). In addition, Black and Gerhart (1986) have demonstrated that embryos subjected to centrifugation during the cell cycle form 'twins', which develop two equal body axes that are joined in the ventral region. Each twin forms a complete set of dorsoanterior features, including a heart.

Circumstantial evidence for a relationship between heart mesoderm and dorsal axial structures arises from fate maps of the 32-cell-stage *Xenopus* embryo (Dale and Slack, 1987*a*), which demonstrates that the descendants of the dorsal-most blastomeres of the third tier contribute to both the notochord and the heart. Fate maps of the *Xenopus* gastrula (Keller, 1976) also assign the prospective heart mesoderm to a dorsolateral position.

The dorsal lip of the blastopore can alter dorsoventral pattern within the marginal zone under experimen-

tal conditions. The dorsal lip grafts of Spemann and Mangold (1924) demonstrated that this region, often referred to as the Organizer, is able to induce the ventral mesoderm of the host to contribute to dorsal mesodermal structures such as somites. In recent years, these findings have been confirmed in *Xenopus* embryos by Smith and Slack (1983). In related experiments, Gimlich and Cooke (1983) grafted the dorsal lip into the marginal zone of embryos whose ventral submarginal cells had been labelled with lineage tracer to show that the neural structures of the secondary axis arise from the ventral tissues of the host.

Slack and his colleagues (Smith and Slack, 1983; Dale and Slack, 1987*b*) have developed a model for the establishment of dorsoventral pattern within the marginal zone of the amphibian embryo. This model proposes that distinct inductive signals are responsible for the formation of dorsal mesoderm and ventral mesoderm, and that dorsal mesoderm is induced in a narrow region centered around the dorsal midline, while 'ventral' mesoderm is induced in lateral and ventral regions of the marginal zone. Signals emanating from the dorsal mesoderm and spreading into the lateral marginal zone would cause the dorsalization of this essentially ventral-type mesoderm, resulting in the formation of dorsolateral and intermediate mesodermal structures, such as somites or pronephros. This model is supported by specification maps of the marginal zone of the *Xenopus* blastula, which are based on the differentiation in culture of explants of the lateral marginal zone (Dale and Slack, 1987*b*). These specification maps show that explants of the lateral marginal zone of the early blastula exhibit ventral differentiation, e.g. mesenchyme, mesothelium and blood, suggesting that some dorsalizing interaction brings about a change in the potency of this region. We define 'potency' as the ability to give rise to a specific organ or structure under a range of experimental conditions (Slack, 1983).

In this paper, we demonstrate that interactions between the prospective heart mesoderm and the dorsal lip occurring early in gastrulation are necessary for the expression of heart-forming potency. Specifically, the establishment of heart mesoderm appears to be dependent upon the Organizer activity of the dorsal lip. In addition, the expression of heart-forming potency is correlated with the appearance of dorsoanterior characters, suggesting that the acquisition of anterior specification is also necessary for the establishment of heart-forming potency.

Materials and methods

Adult *Xenopus* were maintained in 10% Holtfreter's solution on a diet of frog brittle (Nasco, Ft. Atkinson, WI) twice weekly. Embryos were obtained either by natural matings, as described previously (Sater and Jacobson, 1989), or by *in vitro* fertilization. For *in vitro* fertilizations, females were induced to ovulate by an injection of 500 international units (i.u.) human chorionic gonadotropin (hCG; Sigma, St. Louis, MO). Approximately 12 h later, oocytes were stripped into a Petri dish containing minced testis tissue in a minimal volume of 33% modified amphibian Ringer (MR; 100% MR: 0.1 M-NaCl, 2 mM-KCl, 2 mM-$CaCl_2$, 1 mM-$MgCl_2$, buffered to pH 7.4 with $NaHCO_3$; Gimlich and Gerhart, 1984). Following fertilization, embryos were dejellied with cysteine HCl and washed extensively. Embryos obtained by *in vitro* fertilization were maintained in 33% MR at 17°C. Embryos were staged according to Nieuwkoop and Faber (1967).

Microsurgery

Microsurgical operations were performed with sharpened watchmaker's forceps and eyebrow hair knives (Keller and Danilchik, 1988) in Niu–Twitty solution (Jacobson, 1967) on a bed of 2% agar. Explants were cultured either in hanging drops of Niu–Twitty solution plus 50 i.u. ml^{-1} penicillin and 50 $\mu g\,ml^{-1}$ streptomycin (pen/strep) or in 24-well culture dishes containing the same medium. Embryos subjected to operations were maintained in 24-well culture dishes containing Niu–Twitty solution plus pen/strep.

Microinjection of lineage tracer

Embryos were pressure-injected during the first cell cycle with approximately 3 nl of 50 $mg\,ml^{-1}$ fluoresceinated dextran-amine (FDA; Sigma) in distilled water, for a final concentration of approximately 150 $ng\,embryo^{-1}$. Micropipettes were prepared for microinjection by pulling borosilicate glass microcapillary tubes on a Brown–Flaming electrode puller (Sutter Instruments; San Francisco, CA) to produce an external tip diameter of 20–25 μm. Micropipettes were calibrated by expelling FDA into a drop of mineral oil and measuring the diameter of the expelled FDA. During the injections, embryos were maintained in 5% ficoll in 33% MR; embryos were allowed to heal in the same medium. Embryos that leaked cytoplasm from wounds resulting from microinjection were discarded.

Histology

Embryos containing grafts of FDA-labelled tissue were fixed in freshly prepared 2% paraformaldehyde in 0.1 M-sodium cacodylate, pH 7.4, for approximately 20 h at 6°C. Embryos were dehydrated through an ethanol–butanol series, embedded in paraplast, and sectioned at 8 μm. Sections were collected on gelatin-coated slides, dewaxed through a xylene–ethanol series, and mounted in 80% glycerol containing 4% *N*-propyl gallate (Sigma) (Gimlich and Braun, 1985). Sections were viewed using epifluorescence optics (Zeiss).

Results

The location of heart-forming potency in the early gastrula

The first experiments ascertain the location of heart-forming potency within the marginal zone of the early gastrula. A fate map of the *Xenopus* embryo at the onset of gastrulation indicates that the two sites of prospective heart mesoderm are located within the dorsolateral regions of the deep mesoderm, to either side of the prospective head mesoderm and subjacent to the chordamesoderm (Keller, 1976). However, this fate map does not indicate the lateral extent of the prospective heart mesoderm, nor does it identify the region of heart-forming potency, which may extend beyond the region fated to give rise to the heart.

A series of explants was prepared, encompassing different regions of the marginal zone along the circum-

ference of the early gastrula (stages 10 to 10.25). A diagram showing the region of tissue included in each explant is shown in Fig. 1. Each explant contained mesoderm, some of the underlying deep endoderm and the external epithelium. Explants were maintained in either hanging drops or culture dishes for at least two weeks at 17°C. All explants were scored for the formation of a beating heart and the development of dorsoanterior characteristics. Dorsoanterior axial features included axial extension of the chordamesoderm and eyes, which were easily visible once the pigmented retinal epithelium was present. Other axial characteristics included melanocytes and the development of axial muscular contractions.

The results are shown in Table 1. Explants of the dorsal lip that encompassed 60° of the marginal zone (30° to either side of the dorsal midline; Fig. 1A) underwent heart formation in 4% of cases. Dorsal explants that included 90° of the marginal zone (45° to either side of the dorsal midline; Fig. 1B) formed beating hearts in 30% of cases. A dorsal 90° explant in culture is shown in Fig. 2. Both dorsal 60° explants and dorsal 90° explants displayed dorsoanterior axial features in nearly every case, although the extent of dorsoanterior axial development varied somewhat. The frequency of dorsoanterior structures in these explants is shown in Table 2.

The high frequency of dorsoanterior development in explants of the dorsal marginal zone contrasted sharply with the course of development observed in explants of the dorsolateral marginal zone (Fig. 1C), which included tissue extending from the lateral midline through approximately 60° toward the dorsal midline. These dorsolateral 60° explants generally exhibited dorsal posterior characteristics, such as the formation of a tailfin; some cultures formed pronephric tubules, a characteristic of lateral mesoderm. In addition, a number of cultures developed some dorsoanterior axial features. Heart formation was observed in only 5% of cases.

Ventrolateral explants, which included a region of the marginal zone extending from the ventral midline through 90° to the lateral midline, displayed either ventral or dorsal posterior characteristics. Heart formation was not observed in ventrolateral explants.

These results demonstrate that a region of mesoderm, located approximately 30° to 45° from the dorsal midline at the beginning of gastrulation, participates in the establishment of heart mesoderm. The ability of this mesoderm to express heart-forming potency autonomously was examined by isolating the 30° to 45° areas from the rest of the marginal zone and observing them in hanging drop cultures. Heart formation was observed only once in 48 cultures, indicating that this 30° to 45°

Table 1. *Heart-forming potency in different regions of the marginal zone of the* Xenopus *embryo at the onset of gastrulation*

Explant	No. of cases that form hearts	Total no. of cases	% cases that form hearts
Dorsal 60°	1	24	4%
Dorsal 90°	17	57	30%
Ventrolateral 90°	0	12	0%
Dorsolateral 60°	3	59	5%
30°–45°	1	48	2%

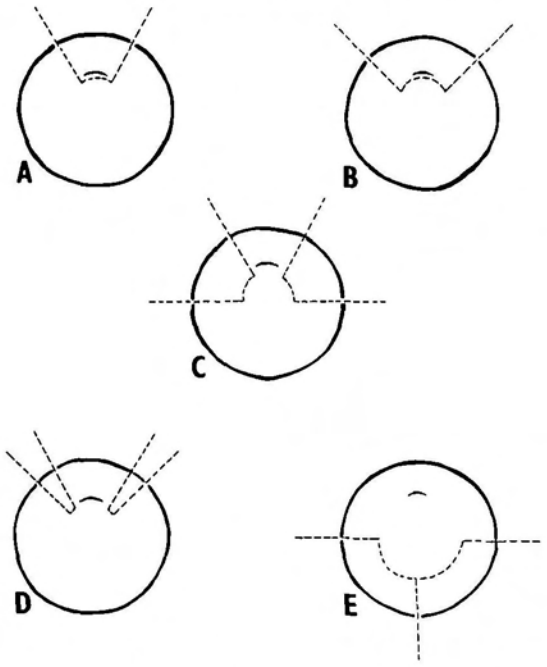

Fig. 1. Explants of the marginal zone. All embryos are viewed from the vegetal pole. (A) Dorsal 60° explants. (B) Dorsal 90° explants. (C) Dorsolateral 60° explants. (D) 30°–45° explants. (E) Ventrolateral explants.

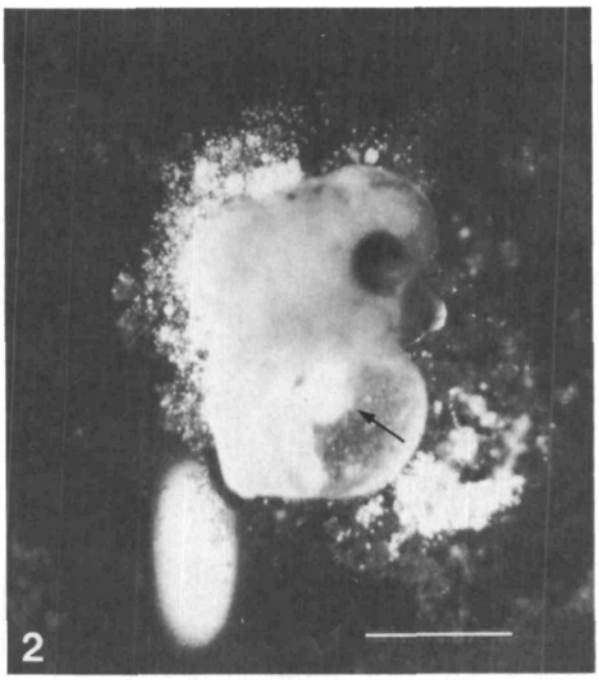

Fig. 2. Dorsal 90° explant after seven days in culture. Explant was removed at stage 10.25. Arrow points to heart; bar, 0.5 mm.

Table 2. *Appearance of dorsoanterior characteristics in explants of different regions of the marginal zone*

Explant	No. of cases	AE	CG	PRE	MEL	MC	FIN
D60°	24	18	20	7	7	7	0
D90°	57	54	52	43	18	11	0
VL90°	12	10	0	0	6	0	0
DL60°	59	32	16	13	27	3	17
30°–45°	48	2	9	8	11	2	0

AE, axial extension; CG, cement gland; PRE, pigmented retinal epithelium; MEL, melanocytes; MC, muscular contractions; FIN, tailfin.

region, which apparently gives rise to the heart *in vivo*, will not do so in isolation.

Regulative replacement of heart mesoderm is dependent upon the presence of dorsal mesoderm

The ability of the marginal zone to undergo regulative replacement of the heart mesoderm in the absence of the pair of 30° to 45° regions was investigated by removing both of these regions from early gastrula embryos and observing the subsequent development of these embryos. In other cases, either the dorsal 90° region or the dorsal 60° region was removed; embryos subjected to these operations were also observed in culture. Embryos were examined for heart formation and the appearance of dorsoanterior axial features.

The results are summarized in Tables 3 and 4. Embryos from which only the paired 30–45° regions had been removed showed dorsoanterior axial features, including eyes, otocysts, axial extension of notochord and somites, and axial musculature. The degree of dorsoanterior axial development varied among embryos to some extent, but all cultures displayed substantial dorsoanterior axial development. Six out of 14 cases formed beating hearts after removal of the 30–45° regions at stage 10, reflecting substantial regulative ability with respect to heart formation in the marginal zone.

Table 3. *Regulative replacement of heart mesoderm by the marginal zone after removal of regions of heart-forming potency*

Embryos after removal of:	No. of cases that form hearts	Total no. of cases	% cases that form hearts
St. 10			
30°–45° region	6	14	43%
Dorsal 90° region	0	22	0%
Dorsolateral 60° region	8	17	47%
Dorsal 60° region	0	17	0%
St. 10.25			
30°–45° region	10	11	91%
Dorsal 90° region	0	5	0%
Dorsal 60° region	9	15	60%
St. 10.5			
30°–45° region	N/D		
Dorsal 90° region	1	9	11%

Embryos from which the entire dorsal 90° region had been removed at stages 10 or 10.25 exhibited a radically different pattern of development. Most of these embryos developed primarily ventral features, in some cases showing no axial characteristics whatsoever. Other embryos subjected to the same operation developed dorsoposterior features, including extensive tailfins, as well as axial musculature and melanocytes. Neither dorsoanterior characteristics nor heart formation were ever observed in these cultures.

Embryos from which the dorsal 90° region had been removed at stage 10.5 usually developed dorsoposterior characteristics. Although dorsoanterior axial features were not observed in these embryos, 1 out of 9 cases did form a small beating heart. In total, 35 out of 36 embryos lacking the dorsal 90° region failed to undergo heart formation. These results indicate that early gastrula embryos from which the dorsal 90° region has been removed are generally incapable of regulative replacement of heart mesoderm.

Embryos lacking the dorsal 60° region were unable to undergo heart formation, despite the presence of the prospective heart mesodermal regions themselves. While the majority of these embryos displayed dorsal characteristics, such as axial musculature or melanocytes, dorsoanterior characteristics such as pigmented retinal epithelia formed in only 2 of 17 cases. The frequency of dorsoanterior characteristics is presented in Table 4. Both blood, a ventral characteristic, and tailfin, a dorsoposterior characteristic, were commonly observed. Thus, the dorsal 60° region seems to be required for the expression of heart-forming potency and the appearance of dorsoanterior characteristics.

Table 4. *Appearance of dorsoanterior characteristics after removal of heart-forming regions at the beginning of gastrulation (stage 10)*

	No. of cases	AE	CG	PRE	MEL	MC	FIN	BL
30°–45° donors	14	14	12	9	12	11	1	1
D90° donors	22	11	0	0	13	0	14	6
D60° donors	17	15	2	2	14	5	11	8
DL60° donors	17	17	16	15	16	17	14	13

BL, blood; for others, see Table 2.

Interaction with the dorsal lip is sufficient to induce heart formation in the ventral marginal zone of the early gastrula

The results of the preceding experiments indicate that the expression of heart-forming potency is dependent upon interactions between the dorsolateral deep mesoderm and the dorsal lip: explants of the region fated to give rise to the heart will not undergo heart formation unless they are in contact with the dorsal lip, and removal of this region does not prevent heart formation unless the dorsal lip is removed as well. Moreover, removal of the dorsal 60° region at stage 10 is sufficient to block heart formation. To determine whether interactions with the dorsal lip are *sufficient* for the establishment of heart-forming potency, experiments were performed in which dorsal lip material was grafted into the ventral marginal zone of early gastrula embryos (stage 10–10.25). As Spemann and Mangold (1924) and others (Gimlich and Cooke, 1983; Smith and Slack, 1983) have shown, such grafts generally result in the formation of a secondary axis in which the grafted tissue gives rise to the notochord and pharyngeal endoderm, with some contribution to the somites. The derivatives of the host mesoderm surrounding the graft include somites and intermediate mesodermal structures such as the pronephros. It was necessary to repeat this classic experiment because previous accounts fail to indicate whether a second heart is formed within the secondary axis.

Grafts were prepared from embryos that had been microinjected with fluoresceinated dextran-amine (FDA) during the first cell cycle. The presence of FDA in the cells of the graft permitted the identification at subsequent stages of cells and tissues derived from the grafted tissue. Grafts of the dorsal lip were prepared as described in Gimlich and Cooke (1983), with some minor variations. A diagram of the operation is shown in Fig. 3. Donor embryos containing FDA and unlabelled host embryos were transferred to 66% MR when they reached stage 10. A region of the dorsal lip encompassing approximately 10° to 15° to either side of the dorsal midline (for a total of 20° to 30° centered at the dorsal midline) was excised and trimmed of most of the deep endoderm. A piece of tissue of approximately the same size was removed from the ventral marginal zone at the ventral midline of an unlabelled host embryo, and the labelled graft was inserted in its place. Several grafts were also prepared using unlabelled donor and host embryos. Grafts were allowed to heal at room temperature for 45–60 min before they were transferred to 33% MR+pen/strep. Grafts were allowed to develop at 17°C.

Regions of the ventral marginal zone were also grafted into the ventral marginal zone to serve as negative controls (Fig. 3). Grafts of ventral tissue were of approximately the same size and were treated in the same manner as the dorsal lip grafts. In addition, embryos that served as donors of dorsal lip grafts were observed in culture to determine whether heart formation could occur in the absence of the dorsal lip.

A summary of the results is shown in Table 5. Out of 36 grafts, 26 developed unambiguous secondary axes. Over half of the embryos with secondary axes formed beating hearts in both axes, as shown in Figs 4 and 5. Of the remainder, half formed one large beating heart that extended into both axes, and half underwent heart formation only in the primary axis. In nearly all of the embryos that did not form a heart in the secondary axis, the secondary axis itself was abnormal: the cranial region of the secondary axis was extremely narrow, and, in some cases, the pigmented retinal epithelia surrounding the outside of the optic vesicles were fused across the dorsal midline of the secondary axis (Fig. 4). These abnormalities were not observed in secondary

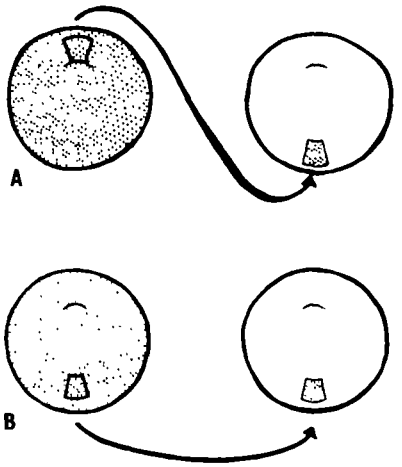

Fig. 3. Grafts of the marginal zone at stage 10. All embryos are viewed from the vegetal pole. (A) The dorsal lip of the blastopore is grafted into the ventral marginal zone. (B) A region of the marginal zone surrounding the ventral midline is grafted into the ventral marginal zone.

Table 5. *Heart formation in embryos containing dorsal lip grafts transplanted into the ventral marginal zone*

Graft	No. attempted	No. with 2 axes	No. with 2 hearts	No. with 1 shared heart	No. without hearts in 2° axis
Dorsal graft into ventral marginal zone	36	26	14	6	6
Ventral graft into ventral marginal zone	11	0	0	–	–

Note: donors of dorsal grafts (Dorsal 20°–30° region) formed hearts in 3 of 13 cases.

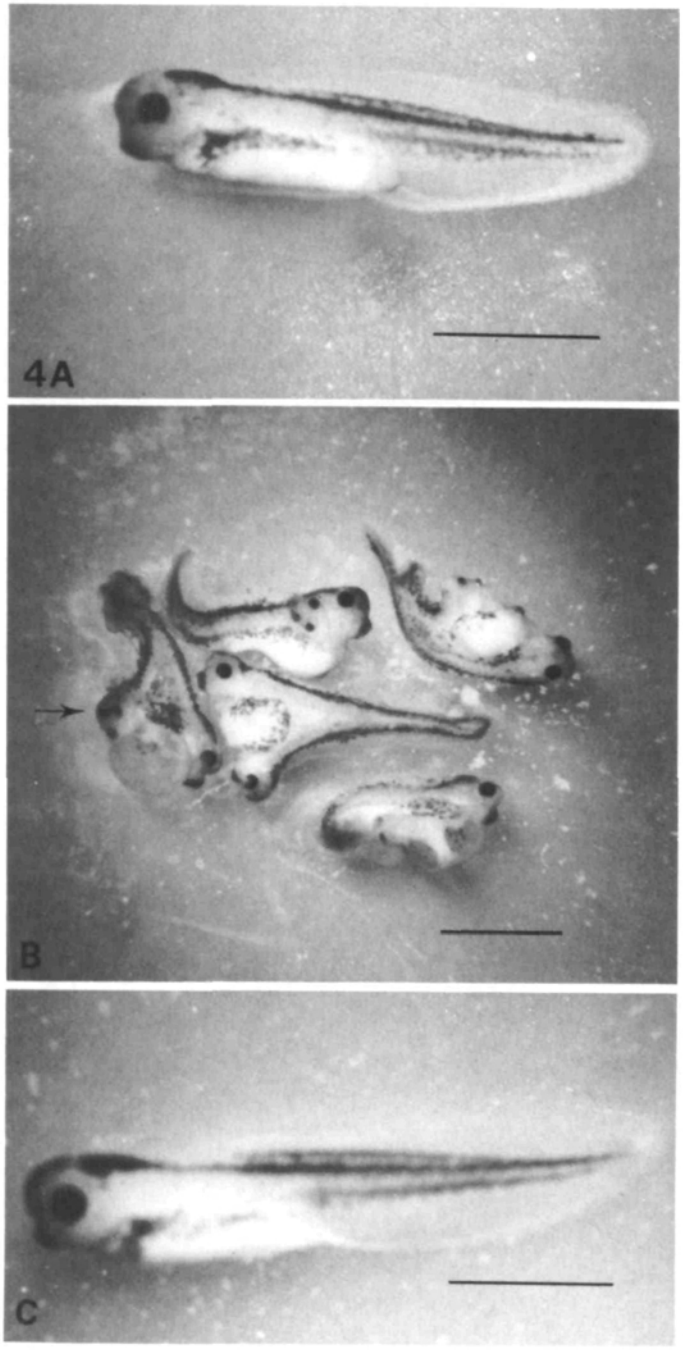

Fig. 4. (A) Normal embryo at stage 41; bar, 2.0 mm. (B) Stage 41 embryos resulting from grafts of the dorsal lip into the ventral marginal zone at the beginning of gastrulation. Each embryo has formed a double axis. Arrow points to fused pigmented retinae in secondary axis of embryo at left; bar, 2.5 mm. (C) Stage 41 embryo following a graft of the ventral marginal zone into the ventral marginal zone; bar, 2.0 mm.

axes that underwent heart formation. In one embryo that lacked a heart in the secondary axis, the primary and secondary axes were fused anterior to the heart.

Grafts of ventral tissue into the ventral marginal zone did not result in the formation of a secondary axis

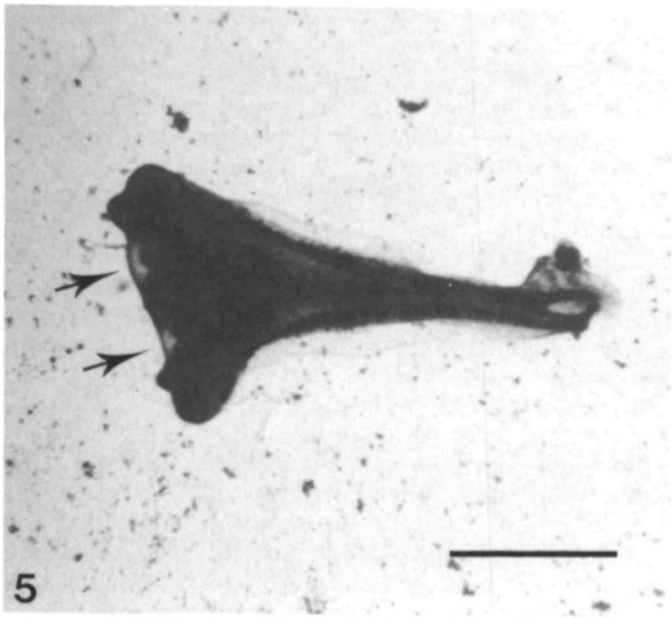

Fig. 5. Double axis embryo at stage 41. Each axis has formed a beating heart (indicated by arrows); bar, 2.0 mm.

(Fig. 4). Embryos subjected to this operation formed a single, apparently normal heart. Donors of dorsal lip grafts formed hearts in less than one-fourth of the cases.

These results demonstrate that heart formation can occur in conjunction with the establishment of a secondary body axis. To determine whether the dorsal lip can induce the formation of a secondary heart from host tissue, it is necessary to know the origin of the cells that constitute the secondary heart. The use of lineage tracers such as FDA in either donor or host tissues makes it easy to establish whether a given structure arises from host tissues or differentiates from the graft itself.

A section through a secondary axis resulting from a graft of FDA-labelled dorsal lip is shown in Fig. 6. The pharyngeal endoderm is fluorescent, indicating that it is derived from the labelled graft. Labelled cells are not visible in the heart, however, indicating that the heart is derived from the unlabelled host tissue. Since the dorsal lip graft was placed at the ventral midline of the marginal zone, the heart is apparently derived from what was originally ventral mesoderm. This result suggests that the grafted dorsal lip is capable of inducing heart formation from ventral mesoderm during the establishment of a secondary axis. An alternative interpretation is that prospective heart mesoderm cells from the original host axis have migrated into the secondary axis. Gimlich and Cooke (1983), however, have shown that cells do not move from the original axis into the neural structures of the secondary axis.

Interactions with the dorsal lip are not essential after early gastrula stages

These results suggest that at the beginning of gastrulation, interactions with the dorsal lip are necessary for the expression of heart-forming potency. To determine

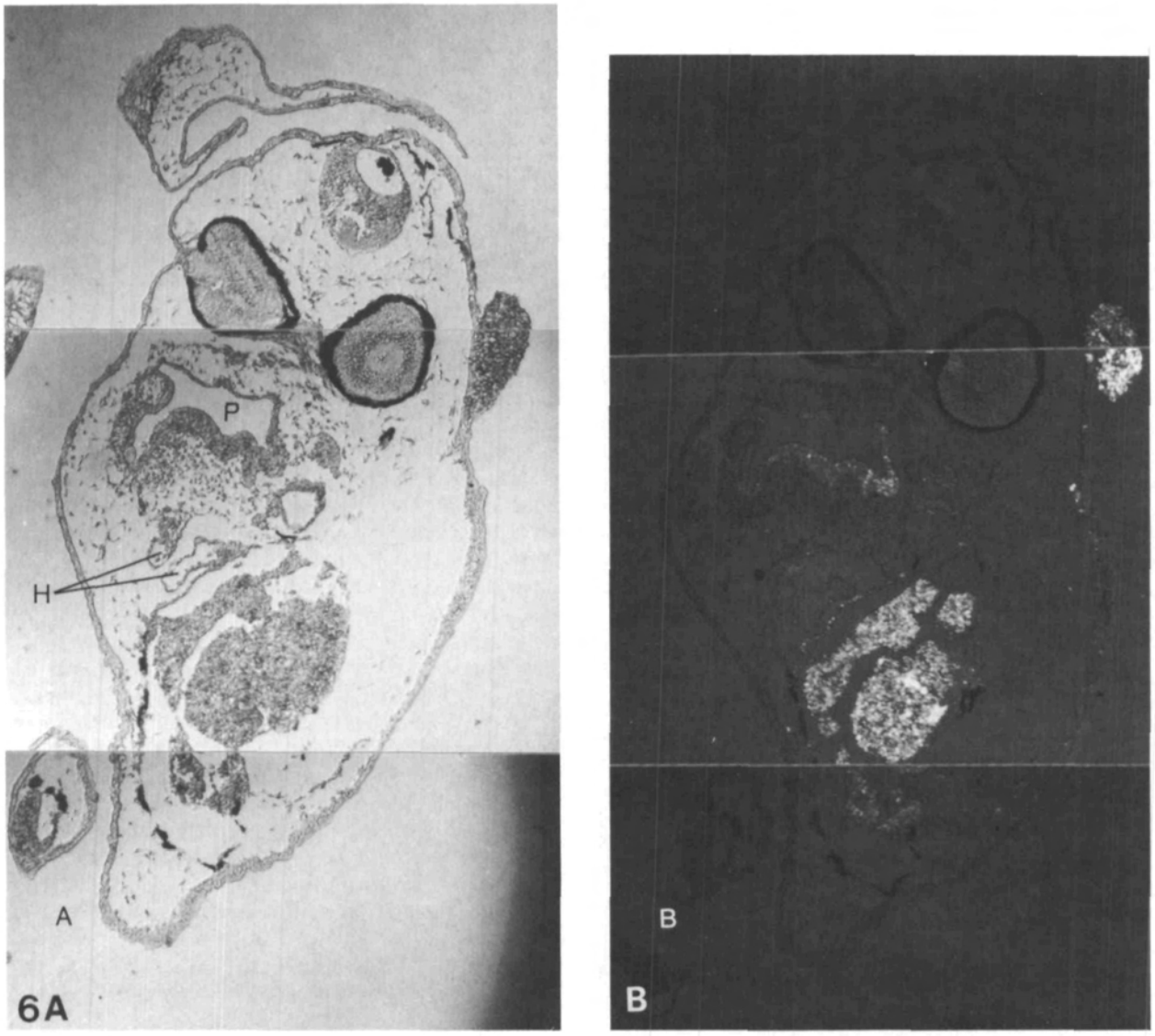

Fig. 6. Sections through secondary axis induced by transplantation of dorsal lip tissue containing FDA into the ventral marginal zone. Embryo is at stage 41. (A) Bright-field image; H, heart; P, pharyngeal cavity. (B) Epifluorescence image to show location of labelled graft cells. Labelled cells are present in pharyngeal endoderm; no labelled cells are visible in the heart.

whether this requirement persists during later stages, we removed the dorsal 60° region from embryos at stages 10, 10.25, 10.5, and 11. The remainder of the embryo was maintained in culture for two weeks and monitored for heart formation and the appearance of dorsoanterior structures.

The results, shown in Table 6, indicate that there is an absolute requirement for the dorsal 60° region at stage 10. When this region is removed at stage 10.25, however, heart formation occurs in over half of the cases. Removal of the dorsal 60° region at stage 11 has no effect on heart formation. Thus, while interactions, with the dorsal lip at the beginning of gastrulation are essential for the specification of heart mesoderm, this interaction is largely complete shortly thereafter.

Table 6. *Frequency of heart formation in the absence of the dorsal lip*

Dorsal 60° region removed at stage	No. that form hearts	Total no. of embryos	% that form hearts
10	0	17	0
10.25	9	15	60%
10.5	20	23	87%
11	10	10	100%

Heart-forming potency increases during the first half of gastrulation

If heart-forming potency is normally expressed in the

Table 7. *Heart-forming potency of dorsal regions throughout gastrulation*

Explant	No. of cases that form hearts	Total no. of cases	% cases that form hearts
Dorsal 90° at			
st. 10	6	28	21%
st. 10.25	11	29	38%
st. 10.5	20	21	95%
st. 11	5	5	100%
st. 11.5	8	8	100%
st. 12	8	8	100%

dorsolateral deep mesoderm of the early gastrula, as these results suggest, then it is surprising that most explants of the dorsal 90° region from stage 10 embryos fail to undergo heart formation. To determine whether heart-forming potency is expressed more frequently in explants from later stages, explants of the dorsal 90° region were isolated from embryos at stages throughout gastrulation. These explants were monitored for the formation of beating hearts and the expression of dorsoanterior axial characteristics.

The results are summarized in Table 7. While explants of the dorsal 90° region removed from embryos at stage 10 undergo heart formation in approximately one-fifth of cases, the frequency of heart formation increases to nearly two-fifths of cases for explants removed from embryos at stages 10.25. Explants removed from embryos at later stages of gastrulation form beating hearts in virtually all cases. These results indicate that the ability to express heart-forming potency is acquired during the early stages of gastrulation prior to stage 10.5.

Discussion

The principal conclusion that emerges from these results is that interactions with the dorsal lip of the blastopore are essential for the expression of heart-forming potency. Although heart-forming potency is normally expressed in a restricted area of the marginal zone, the dorsolateral deep mesoderm, other regions of the marginal zone are capable of heart formation when brought into close proximity with the dorsal lip. Thus, the dorsal lip initiates the induction of heart mesoderm, presumably *via* the dorsalizing 'Organizer' activity that characterizes the dorsal lip. In addition, heart-forming potency increases during the first half of gastrulation, an increase that may be linked to further regional specification events occurring throughout the embryo at this time.

Heart-forming potency is normally expressed in deep mesoderm located at least 30° lateral to the dorsal midline at the onset of gastrulation. This dorsal boundary is demonstrated by the absence of heart formation in explants of the dorsal 60° region. A lateral boundary is considerably harder to identify. Since over 25% of dorsal 90° explants are capable of heart formation, the paired regions 30°–45° lateral to the dorsal midline serve as a 'minimal estimate' of the prospective heart mesoderm within the marginal zone. The assignment of heart-forming potency to these regions is roughly in agreement with the fate map prepared by Keller (1976), which indicates that the prospective heart mesoderm is located in two areas of the deep mesoderm, one to either side of the dorsal midline. These results also concur with the specification map of the urodele gastrula, derived from the differentiation of explants in culture (Holtfreter, 1938).

The role of the dorsal lip of the blastopore

Two hypotheses may be proposed to account for the circumstantial evidence linking heart formation and the expression of dorsoventral pattern. First, the prospective heart mesoderm may arise as part of an hypothetical region of dorsal axial mesoderm, which would eventually give rise to chordamesoderm, somites and head mesoderm, as well as heart mesoderm. Presumably, the prospective heart mesoderm would segregate from the rest of this region at or before the beginning of gastrulation. In this case, the heart would be induced directly by the dorsovegetal cells relatively early in development. A second hypothesis suggests that the heart mesoderm is induced by the dorsal axial mesoderm itself during late blastula or very early gastrula stages. In theory, it is also possible that heart-forming potency requires an initial interaction with the dorsovegetal cells, and a subsequent interaction with the dorsal lip. Our results, however, demonstrate that interaction with the dorsal lip of the blastopore is the earliest required step in the establishment of heart mesoderm. The formation of a heart within the secondary axis induced by a grafted dorsal lip indicates that the dorsovegetal region need not be directly involved in early steps of heart induction.

The following results demonstrate that the expression of heart-forming potency is dependent upon interactions with the dorsal lip, the site of Spemann's Organizer. First, explants of the dorsal 90° region, which include both the dorsolateral deep mesoderm and the dorsal lip, are frequently capable of heart formation; explants that include the dorsolateral deep mesoderm fail to undergo heart formation in the absence of the dorsal lip. Second, removal of the dorsolateral deep mesoderm at the beginning of gastrulation does not prevent heart formation, while heart formation does not occur in embryos lacking both the dorsolateral deep mesoderm and the dorsal 60° region. Finally, removal of the dorsal 60° region alone at the beginning of gastrulation prevents heart formation. This dorsal 60° region corresponds with the region of the marginal zone that is capable of behaving as Spemann's Organizer (R. Stewart, personal communication). Presumably, the role of the dorsal lip in heart induction represents a previously unidentified function of Spemann's Organizer.

Presumably, the heart-inducing activity resides primarily in the prospective chordamesoderm. In *Xenopus*, the dorsal lip of the blastopore consists of an internal layer of prospective chordamesoderm and a

superficial layer of prospective pharyngeal endoderm. In a previous paper (Sater and Jacobson, 1989), we demonstrated that removal of the superficial layer does not inhibit heart formation; therefore, the prospective chordamesoderm can sustain this inductive interaction in the absence of the superficial layer *in vivo*.

These results, as well as the induction of heart formation in the ventral marginal zone by a grafted dorsal lip, imply that Spemann's Organizer is both necessary and sufficient for the expression of heart-forming potency. However, tissue interactions responsible for the specification of heart mesoderm occur over the course of gastrulation, presumably involving the dorsoanterior endoderm (Sater and Jacobson, 1989). The importance of interactions with the dorsal lip decreases substantially after the beginning of gastrulation: removal of the dorsal 60° region at subsequent stages usually does not prevent heart formation.

Heart-forming potency and the expression of the anterior–posterior axis

Our results present a paradox: the expression of heart-forming potency in explants of the dorsal 90° region increases during the first half of gastrulation. Since the dorsal 90° explants contain the tissues that participate in the induction of heart mesoderm, the dorsal lip, the dorsoanterior endoderm and the prospective heart mesoderm itself, it is unclear why these explants do not undergo heart formation more frequently when isolated at the beginning of gastrulation. Heart-forming potency may be more sensitive to the experimental manipulation at earlier stages than at later stages. Alternatively, the increase in heart-forming potency might result from movements of convergence toward the dorsal midline, which occur during gastrulation (Keller, 1976). Convergence may bring more lateral tissues into contact with the prospective heart mesoderm, leading to new interactions that contribute to heart-forming potency.

Conceivably, another answer may lie in the relationship between heart-forming potency and the expression of the anterior–posterior axis within the mesoderm. Several lines of evidence suggest that the anterior–posterior axis is established during gastrulation. First, Dale and Slack (1987*a*) claim that the fate map of the 32-cell stage *Xenopus* embryo is inconsistent with the idea that the anterior–posterior axis is specified prior to the onset of gastrulation. A second line of evidence is provided by embryos arrested during gastrulation by the injection into the blastocoel of either trypan blue (Danilchik, 1986) or antibodies directed against fibronectin (Boucaut *et al.* 1984, 1985). In embryos arrested early in gastrulation, anterior structures are reduced or absent, while arrest at later stages produces progressively milder defects. Finally, Kanéda and Hama (1979) have demonstrated that the differentiative and inductive capacities of the 'trunk organizer' (posterior chordamesoderm) of the urodele *Cynops pyrrhogaster* are acquired over the first half of gastrulation.

While these observations do not constitute a critical test of the hypothesis that regional specification along the anterior–posterior axis occurs during gastrulation, they do suggest that events occurring during gastrulation permit the expression of anterior–posterior characteristics. Such putative events may also contribute to the expression of heart-forming potency, in that the degree of anterior specification necessary for the expression of heart-forming potency may be acquired within the deep mesoderm between stages 10–10.5.

The following speculations about the origin of heart-forming potency in the *Xenopus* embryo emerge from our results. The dorsal lip of the blastopore dorsalizes the mesoderm immediately lateral to it, both in the deep zone, which will give rise to the heart, and in the involuting marginal zone, which will give rise to the somites. This dorsalizing interaction is not complete until after the onset of gastrulation. A second regional specification event, the establishment of the anterior–posterior axis, may also participate in the acquisition of heart-forming potency during gastrulation. Finally, the prospective heart mesoderm also interacts with the deep dorsoanterior endoderm, presumably throughout gastrulation (Sater and Jacobson, 1989).

These findings emphasize the importance of regional specification events in the acquisition of commitment to a developmental pathway. Previous studies of heart induction have focussed on interactions between the prospective heart mesoderm and the pharyngeal endoderm during later developmental stages. As we begin to recognize the importance of early tissue interactions in organogenesis, we are forced to recognize the role of regional specification, to which these early tissue interactions are coupled. The establishment of embryonic axes may lead to spatial heterogeneity in the competence to participate in subsequent tissue interactions.

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