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The Restriction of the Heart Morphogenetic Field in *Xenopus laevis*

AMY K. SATER[1] AND ANTONE G. JACOBSON

Department of Zoology, Center for Developmental Biology, University of Texas, Austin, Texas 78712

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We have examined the spatial restriction of heart-forming potency in *Xenopus laevis* embryos, using an assay system in which explants or explant recombinates are cultured in hanging drops and scored for the formation of a beating heart. At the end of neurulation at stage 20, the heart morphogenetic field, i.e., the area that is capable of heart formation when cultured in isolation, includes anterior ventral and ventrolateral mesoderm. This area of developmental potency does not extend into more posterior regions. Between postneurula stage 23 and the onset of heart morphogenesis at stage 28, the heart morphogenetic field becomes spatially restricted to the anterior ventral region. The restriction of the heart morphogenetic field during postneurula stages results from a loss of developmental potency in the lateral mesoderm, rather than from ventrally directed morphogenetic movements of the lateral mesoderm. This loss of potency is not due to the inhibition of heart formation by migrating neural crest cells. During postneurula stages, tissue interactions between the lateral mesoderm and the underlying anterior endoderm support the heart-forming potency in the lateral mesoderm. The lateral mesoderm loses the ability to respond to this tissue interaction by stages 27-28. We speculate that either formation of the third pharyngeal pouch during stages 23-27 or lateral inhibition by ventral mesoderm may contribute to the spatial restriction of the heart morphogenetic field.

INTRODUCTION

Many events of pattern formation and organogenesis are mediated by inductive interactions that establish regions of developmental potency, known as morphogenetic fields. A morphogenetic field generally comprises both the area of tissue that is fated to develop into a given structure during normal development and the tissue immediately surrounding this area, which is capable of developing into the given structure under experimental conditions (Jacobson and Sater, 1988). Extensive experimental analysis by a number of workers has shown that the spatial extent of a morphogenetic field decreases with time, as the developmental potency required to make a given organ or structure is lost from peripheral regions of the field. While the inductive interactions responsible for the establishment of morphogenetic fields have received considerable attention, the mechanisms underlying the spatial and temporal regulation of developmental potency during subsequent development are completely unknown.

We have previously shown that the inductive interactions responsible for the specification of heart mesoderm occur during gastrulation in *Xenopus laevis* (Sater and Jacobson, 1989). A number of findings suggest that tissue interactions during neurula and postneurula stages also contribute to heart formation (Ekman, 1921; Humphrey, 1972). These earlier studies included heterotopic transplantations (Copenhaver, 1924; Ekman, 1925) to measure the spatial extent of heart-forming potency during postneurula stages. In these experiments, the presumptive heart mesoderm was removed from the anterior ventral region and replaced with grafts of mesoderm from more lateral or more posterior regions. Heart formation by the grafted tissue was interpreted as an indicator that the grafted tissue was able to respond to continued inductive signals. In addition, extirpation experiments measured the ability of the surrounding tissues to undergo regulative replacement in the absence of the presumptive heart mesoderm (Copenhaver, 1924). Again, cases of heart formation by the surrounding tissues were thought to result from continued induction by the underlying endoderm. Thus, earlier investigators often regarded tissue interactions during postneurula stages as responsible for the initial establishment of heart-forming potency; the induction of heart formation during gastrula or neurula stages went unrecognized.

Tissue interactions during postneurula stages may regulate the spatial extent of heart-forming potency, i.e., the heart morphogenetic field, which is induced earlier in development. In the following experiments, we have used heart formation in explants as an assay to measure the spatial extent of the heart morphogenetic field in postneurula-stage *Xenopus* embryos. These studies were undertaken in order to examine the tissue in-

[1] Present address: Department of Molecular and Cell Biology, Division of Cell and Developmental Biology, University of California, Berkeley, CA 94720.

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teractions governing the maintenance and restriction of the heart morphogenetic field.

MATERIALS AND METHODS

Embryos

Embryos were obtained from both natural matings and *in vitro* fertilization. Adult *Xenopus* were induced to mate by a series of injections of human chorionic gonadotropin (HCG; Sigma Chemicals, St. Louis, MO), according to the following schedule: males were given two injections of 150 International Units (IU) of HCG 32 and 8 hr before they were placed in the mating tank; females were given one injection of 250 IU HCG 8 hr before and one injection of 500 IU HCG immediately before they were placed in the mating tank. Frogs were allowed to mate overnight. The following morning, embryos were collected and dejellied for 5 min in 2% cysteine HCl, pH 7.4. Dejellied embryos were washed extensively in 10% Holtfreter's solution (Jacobson, 1966) and maintained in 10% Holtfreter's solution at 17°C. All embryos were staged according to Nieuwkoop and Faber (1967).

In vitro fertilizations were carried out by the methods of Gimlich and Gerhart (1984). Ovulation was induced by an injection of 500 IU HCG (Sigma). Approximately 12 hr 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 m*M* KCl, 2 m*M* $CaCl_2$-$2H_2O$, 1 m*M* $MgCl_2$-$6H_2O$, buffered to pH 7.4 with $NaHCO_3$) (Gimlich and Gerhart, 1984). Oocytes and sperm were mixed gently by rotating the dish and then allowed to stand for several minutes so that fertilization could occur. Oocytes were then dejellied as described earlier and maintained in 33% MR at 17°C.

Microsurgery and Culture of Explants and Explant Recombinates

For microsurgery, embryos were transferred to petri dishes containing sterile, freshly prepared Niu-Twitty solution over a cushion of 2% agar. All embryonic operations were performed using electrolytically sharpened tungsten needles, sharpened watchmaker's forceps, and eyebrow hair knives. Operations involving the separation of mesoderm and endoderm were performed in Niu-Twitty solution containing 0.01% trypsin (Sigma Type IX), as described by Slack (1984). Explants isolated in the presence of trypsin were subsequently incubated in 0.02% soybean trypsin inhibitor (SBTI; Sigma Type II-S) in Niu-Twitty solution for several minutes before they were placed in culture. Explant recombinates were allowed to heal in Niu-Twitty solution containing SBTI for up to 1 hr at room temperature (approximately 23°C). Explants and explant recombinates were cultured in hanging drops of Niu-Twitty solution containing 50 IU/ml penicillin and 50 mg/ml streptomycin (Niu-Twitty plus pen/strep) at 17°C, as described in Sater and Jacobson (1989). Grafts of embryonic tissue were allowed to heal in Niu-Twitty solution plus pen/strep for up to 1 hr at 23°C; they were subsequently maintained at 17°C.

Microinjection of Lineage Tracer

Prior to first cleavage, embryos were transferred to 5% Ficoll (Sigma) in 33% MR; embryos were then pressure-injected with approximately 3 nl of 50 mg/ml fluoresceinated dextran-amine (FDA) (Sigma) in distilled water, resulting in a final concentration of approximately 150 ng/embryo. Micropipets with an external tip diameter of 20–25 μm were prepared by pulling borosilicate glass microcapillary tubes on a Brown-Flaming electrode puller (Sutter Instruments, San Francisco, CA). Micropipets were calibrated by expelling FDA into a drop of mineral oil and measuring the diameter of the expelled FDA. Following microinjection, embryos were allowed to heal in 33% MR containing 5% Ficoll. Embryos that leaked cytoplasm were discarded.

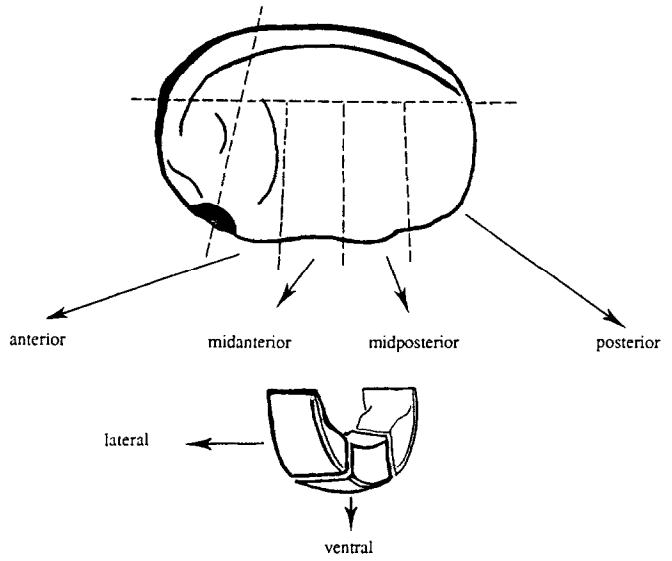
Histology

Embryos containing grafts of FDA-labeled tissue were fixed in freshly prepared 2% paraformaldehyde in 0.1 M Na cacodylate, pH 7.4, for approximately 20 hr at 6°C. Embryos were then washed 15-24 hr in 0.1 *M* Na cacodylate, pH 7.4, 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 Heart Morphogenetic Field at the End of Neurulation

At the end of neurulation (stage 20), the two regions of prospective heart mesoderm, which originally lay at the anterior lateral edge of the mesodermal mantle, have migrated ventrally to fuse at the ventral midline in the anterior region (Nieuwkoop and Faber, 1967). Thus, by stage 20, the prospective heart mesoderm has arrived at the site of heart morphogenesis and undergoes no further large-scale movements. A crude map of the heart morphogenetic field at this stage was constructed by culturing ventral and lateral explants from anterior,



	anterior	midanterior	midposterior	posterior
lateral explants:				
# of cases forming hearts	13	0	0	0
total # of cases	17	15	16	12
ventral explants:				
# of cases forming hearts	8	0	0	0
total # of cases	8	8	7	7

FIG. 1. The heart morphogenetic field at the end of neurulation (stage 20). Lateral and ventral explants were isolated from four regions along the anterior–posterior axis. Beating hearts formed only in lateral and ventral explants from the anterior region.

midanterior, midposterior, and posterior regions of stage 20 embryos and scoring for heart formation; these explants included mesoderm, endoderm, and epidermis. A diagram of the experiment and a summary of the results are shown in Fig. 1. Hearts formed only in anterior ventral and anterior lateral explants; no hearts were observed in explants removed from more posterior regions. Explants of anterior ventral tissue, which included the mesoderm fated to give rise to the heart, formed beating hearts in 100% of cases, while explants of anterior lateral tissue formed beating hearts in 69% of cases. These results demonstrate that at the end of neurulation, the heart morphogenetic field includes both anterior lateral and anterior ventral mesoderm.

Description of Differentiation and Development of Explants

Explants were first examined 2 to 3 days after explantation and daily or every other day thereafter for at least 2 weeks. Initially, explants healed into opaque vesicles, which were examined with a dissecting microscope. Within 3 to 4 days after explantation, explants became inflated and translucent; explants were subsequently examined with a compound microscope and transmitted light. Explants isolated from ventral regions always exhibited large, well-developed hearts, which appeared within 5 days after explantation.

In contrast, the timing of heart formation in explants of lateral tissues varied considerably. Hearts formed from explants of lateral tissues exhibited much greater range of variation in the degree of heart development, from narrow strands or knots of cells contracting in a coordinated "heartbeat" pattern to well-developed hearts with the typical "looping and bending" morphology that delineates the different regions of the heart. Foci of beating tissue were easily distinguishable from strands of skeletal muscle that were occasionally observed in larger explants, since the former exhibited a regular peristaltic or metachronal beat pattern, in contrast to the erratic simple contractions of skeletal muscle fibers. Generally, hearts developing from lateral explants were smaller than those developing from ventral explants.

Other organs or tissues observed in these explant cultures included mesenchyme and mesentery, which were present in nearly all explants, and the cement gland, which appeared only in ventral explants. Lateral explants often contained pronephric tubules, visible as coiled tubes containing beating cilia. Pronephric tubules were not observed in ventral explants.

Spatial Restriction of the Heart Morphogenetic Field during Postneurula Stages

Changes in the spatial distribution of heart-forming potency during postneurula stages were investigated by isolating anterior lateral and anterior ventral regions at successive stages between the end of neurulation at stage 20 and the onset of heart morphogenesis at stage 28. Each explant contained mesoderm, endoderm, and epidermis. Explants were maintained in hanging drop cultures and scored for heart formation.

The frequency of beating heart formation in lateral and ventral explants from postneurula stages is shown in Fig. 2. With the exception of a single case, explants of ventral regions always formed beating hearts.

During early postneurula stages (stages 20 to 22), the frequency of heart formation in lateral explants is high, starting at 69% at stage 20 and increasing to 83% by stage 22. After stage 22, however, the frequency of heart formation in lateral explants begins to decrease. In lateral explants removed at stage 23, the frequency of heart formation is approximately equal to that observed in lateral explants removed at stage 20. The frequency of

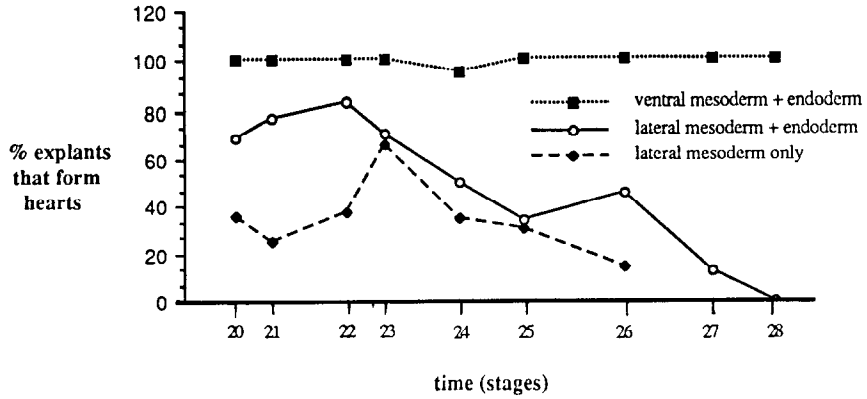


FIG. 2. The frequency of heart formation in mesodermal explants from postneurula stages. Sample sizes for explants of ventral mesoderm plus endoderm: st. 20, $n = 24$; st. 21, $n = 19$; st. 22, $n = 13$; st. 23, $n = 24$; st. 25, $n = 26$; st. 26, $n = 24$; st. 27, $n = 8$; st. 28, $n = 8$. Sample sizes for explants of lateral mesoderm plus endoderm: st. 20, $n = 29$; st. 21, $n = 26$; st. 22, $n = 23$; st. 23, $n = 24$; st. 24, $n = 20$; st. 25, $n = 29$; st. 26, $n = 24$; st. 27, $n = 16$; st. 28, $n = 15$. Sample sizes for explants of lateral mesoderm only: st. 20, $n = 22$; st. 21, $n = 28$ st. 22, $n = 24$; st. 23, $n = 32$; st. 24, $n = 31$; st. 25, $n = 20$; st. 26, $n = 14$.

heart formation in lateral explants removed at late postneurula stages is very low: explants removed at stage 27 form beating hearts in only 12.5% of cases. Lateral explants removed from embryos at stage 28 never form beating hearts.

Restriction of the Heart Morphogenetic Field Is Not Due to Cell Migration

The preceding experiment demonstrates that the heart morphogenetic field becomes spatially restricted to the ventral mesoderm during postneurula stages. Two hypotheses may account for this. First, the lateral mesoderm may lose its ability to participate in heart formation. Second, the lateral mesoderm may migrate ventrally during postneurula stages; in this case, the restriction of the heart morphogenetic field results from morphogenetic movements, rather than from a loss of developmental potency.

This latter possibility was tested by grafting anterior lateral mesoderm labeled with the lineage tracer FDA into the anterior lateral regions of unlabeled host embryos at stage 20. These embryos were fixed at stage 28, sectioned, and examined by fluorescence microscopy.

Labeled embryos to serve as transplant donors were prepared by injecting embryos prior to first cleavage with FDA, as described above. At stage 20, anterior lateral mesoderm plus the overlying epidermis was removed from the labeled embryo and grafted into the anterior lateral region of an unlabeled host. The operations were performed so that the transplanted tissue represented as nearly as possible the area included in explants of anterior lateral mesoderm; the ventral margin of each graft was placed immediately lateral and posterior to the lateral margin of the host cement gland. Approximately 50% of the transplants healed properly, and only these were analyzed. Operated embryos were allowed to heal in Niu-Twitty solution and fixed at stage 28.

A section from a transplant containing a labeled graft is shown in Fig. 3. The labeled mesoderm remains in the lateral region; none of the labeled mesoderm appears in the region surrounding the ventral midline. This region, containing the presumptive heart mesoderm, lies imme-

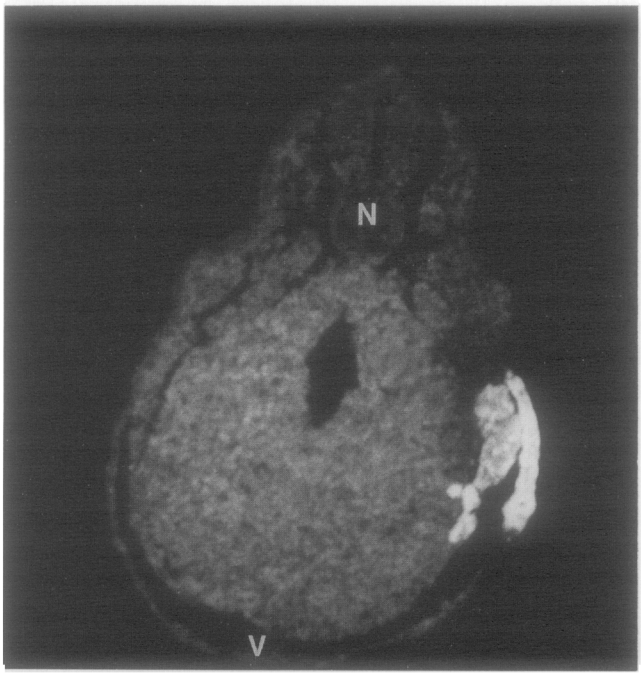


FIG. 3. Section through embryo containing a graft of anterior lateral mesoderm labeled with FDA. Embryo is at stage 28. N, notochord; V, ventral midline.

diately posterior to the cement gland, and explants of this ventral region are as wide as the cement gland. At stage 20, the cement gland is considerably wider than it is at stage 28; this narrowing is probably due to changes in the shape of the cement gland cells and cell movements related to the anterior–posterior elongation occurring during these stages. Thus, explants of ventral regions taken from stage 20 embryos are wider than ventral region explants taken from stage 28 embryos. Since the labeled mesoderm does not enter the ventral region, one can exclude the possibility that ventralward migration of lateral mesoderm is principally responsible for the apparent restriction of the heart morphogenetic field. Moreover, in transplant embryos allowed to develop until stage 34, none of the labeled graft cells appear in the heart itself (data not shown). Therefore, the spatial restriction of the heart morphogenetic field is not due to morphogenetic movements, but rather to a loss of heart-forming potency from lateral mesoderm.

Interactions between Mesoderm and Endoderm during Postneurula Stages

To determine whether interactions between the lateral mesoderm and the underlying anterior lateral endoderm are involved in the maintenance of heart-forming potency in lateral mesoderm during postneurula stages, explants of lateral mesoderm were removed from postneurula stage embryos and cultured in the absence of anterior endoderm. The frequency of heart formation in explants of lateral mesoderm from postneurula embryos is shown in Fig. 2. Explants of lateral mesoderm removed from embryos at stage 20 undergo heart formation in 36% of cases. For explants removed at stage 23, the frequency of heart formation increases to 66%, dropping to 14% for explants removed at stage 26. At early postneurula stages (stages 20 to 22), the frequency of heart formation in explants of lateral mesoderm is considerably lower than the frequency of heart formation in lateral explants containing both mesoderm and endoderm. This result indicates that interactions between the lateral mesoderm and the endoderm support heart-forming potency during these stages. However, during subsequent stages, the frequency of heart formation in explants of lateral mesoderm plus endoderm is only slightly higher than that observed in explants of lateral mesoderm alone, suggesting that this supportive interaction is not effective after stage 23.

POSSIBLE CAUSES OF THE RESTRICTION OF THE HEART MORPHOGENETIC FIELD

Restriction of the Heart Morphogenetic Field Is Not Due to Local Suppression of Heart Formation by Migrating Neural Crest Cells

Neural tissues are known to inhibit heart formation during neurula stages in urodele embryos (Jacobson and Duncan, 1968). Therefore, neural crest cells might suppress heart-forming potency in the lateral mesoderm while migrating through it. To test this hypothesis, the neural crest and neural tube were removed from embryos at stage 20, when the neural crest itself had formed above the neural tube along its entire length (Nieuwkoop and Faber, 1967). Neural crest migration progresses in a cranial-to-caudal fashion during stages 19 to 31 (Nieuwkoop and Faber, 1967). Because cranial neural crest cells begin migrating almost immediately after the appearance of the neural crest itself, the neural tube was removed in addition to the crest, in order to remove any neural crest cells that had begun to move out along the surface of the neural tube. After surgery, the embryos were cultured until intact controls had reached mid- to late postneurula stages (stages 23 to 26). Lateral and ventral explants of embryos subjected to the operation were then prepared as described earlier; each explant contained mesoderm, endoderm, and epidermis. These explants were scored for the frequency of beating heart formation.

If migrating neural crest cells inhibit heart-forming potency in the lateral mesoderm, then removal of the neural crest should result in an increase in the frequency of heart formation in lateral explants at later stages, relative to the frequency observed in lateral explants from unoperated embryos. As the results shown in Table 1 indicate, this prediction is not born out: at nearly all stages tested, the frequency of heart formation in lateral explants from operated embryos is lower than that observed in lateral explants from intact em-

TABLE 1
FREQUENCY OF HEART FORMATION IN EXPLANTS FROM EMBRYOS LACKING THE NEURAL CREST

Stage at explant: Explants	23	24	25	26	27
Ventral mesoderm + endoderm					
No. forming hearts	16	9	9	12	5
Total no. of cases	16	9	9	12	5
% forming hearts	100	100	100	100	100
% heart formation in explants from intact embryos	100	95	100	100	100
Lateral mesoderm ± endoderm					
No. forming hearts	15	6	7	4	1
Total no. of cases	26	13	18	21	10
% forming hearts	58	46	39	19	10
% heart formation in explants from intact embryos	71	50	34	46	12.5

Note. The neural crest and neural tube were removed from donor embryos at stage 20–21.

TABLE 2
FREQUENCY OF HEART FORMATION IN HETEROCHRONIC EXPLANT RECOMBINATIONS OF LATERAL MESODERM AND LATERAL ENDODERM

Stage of mesoderm at explant	Stage of endoderm at explant	No. of recombinates forming hearts	Total no. of recombinates	% of recombinates forming hearts
27-28	20-23	1	14	7
20	20	8	9	89

bryos. Thus, inhibition of heart formation by migrating neural crest cells does not account for the restriction of the heart morphogenetic field.

Loss of Responsiveness in Lateral Mesoderm Contributes to the Restriction of the Heart Morphogenetic Field

Since interactions between the anterior endoderm and the anterior lateral mesoderm serve to maintain the heart morphogenetic field in the lateral mesoderm during early postneurula stages (stages 20 to 23), the perturbation of these interactions may bring about the subsequent restriction of the heart morphogenetic field. These interactions would be disrupted if either the anterior endoderm failed to produce the appropriate signal after early postneurula stages, or the anterior lateral mesoderm lost the ability to respond to signals produced by the anterior endoderm. Experiments to determine whether the endoderm continues to produce the appropriate signal are infeasible because of the difficulties of designing an appropriate assay. In contrast, it is relatively easy to determine whether the responsiveness of the lateral mesoderm persists into late postneurula stages by examining the interactions between late postneurula lateral mesoderm and early postneurula endoderm.

Heterochronic explant recombinations were prepared to determine whether interactions with endoderm from early postneurula embryos could restore heart-forming potency in late postneurula lateral mesoderm. Explants of anterior lateral mesoderm from late postneurula embryos (stages 27 to 28) were recombined with anterior lateral endoderm from embryos at stages 20 to 23. As a control, explant recombinates were prepared in which both the anterior lateral endoderm and the anterior lateral mesoderm were isolated at stage 20. Recombinates were cultured in hanging drops and scored for the frequency of heart formation. The results are shown in Table 2. Out of 14 successful cases, only one heterochronic recombinate formed a beating heart, indicating that interaction with early postneurula endoderm does not restore heart-forming potency in late postneurula lateral mesoderm. In contrast, 89% of the control recombinates formed beating hearts, indicating that the operation itself does not reduce heart-forming potency. These results demonstrate that the lateral mesoderm loses the competence to respond to signals from the anterior endoderm during late postneurula stages; this loss may contribute to the spatial restriction of the heart morphogenetic field.

DISCUSSION

Our results lead to three major conclusions. First, the heart morphogenetic field becomes restricted to the anterior ventral mesoderm during postneurula stages. Second, this spatial restriction is due to a change in the developmental potency of the lateral mesoderm. Third, heart-forming potency in peripheral regions of the morphogenetic field is supported by the underlying endoderm.

The Heart Morphogenetic Field Becomes Spatially Restricted following Neurulation

A specification map of the lateroventral regions of the *Xenopus* embryo at stage 20 indicates that explants of anterior lateral and anterior ventral regions will undergo heart formation in culture. By the onset of heart morphogenesis at stage 28, this heart-forming capacity is restricted to the anterior ventral region. At early postneurula stages, it is unclear whether heart-forming potency is retained by all cells throughout the lateral region. It seems more likely that heart formation in lateral explants results from the retention of heart-forming potency by cells at or near the ventral edge of the lateral region. Cells in more dorsal areas of the lateral region may not participate in heart formation in these explants, instead contributing to the formation of mesenchyme, mesothelium, or pronephros.

Our description of the heart morphogenetic field during postneurula stages is in general agreement with the results of heterotopic transplantation and tissue removal experiments by Copenhaver (1924) and Ekman (1925), which show that the distribution of heart-forming potency is limited to anterior ventral and ventrolateral mesoderm in amphibian embryos. The concurrence of findings on the heart morphogenetic field of amphibians is in marked contrast to studies of the heart morphogenetic field in chick embryos, in which different experimental approaches have yielded conflicting results. Heart-forming potency is expressed by explants removed from large areas of the peripheral blastoderm of chick embryos at the regressing primitive streak stage (Rawles, 1936). However, at the same stage, removal of the prospective heart mesoderm from one side of the embryo prevents the expression of heart-forming potency by any tissue on the operated side (DeHaan, 1965). Heart formation does not occur in chick embryos

from which both regions of prospective heart mesoderm have been removed.

These differences underscore the importance of the choice of experimental manipulation used to define the spatial extent of a morphogenetic field. Observation of the developmental potency expressed by explanted tissues that are cultured in isolation, operationally defined as the "state of specification" of the explanted tissue (Slack, 1983), may be the most easily interpretable way to examine the spatial extent of a morphogenetic field. Such observations reflect only those tissue interactions that occurred prior to the removal of the tissue from the embryo; further inductive or inhibitory signals that might alter the expression of developmental potency following a heterotopic graft or an extirpation experiment are avoided. However, in amphibians, different experimental manipulations have produced similar maps of the heart morphogenetic field. This common result lends support to the idea of the heart morphogenetic field as a "dynamic region of developmental potency" (Jacobson and Sater, 1988), including both the anterior ventral mesoderm, fated to become the heart, and the adjacent lateral mesoderm, fated to contribute to the vascular and skeletal elements of the branchial arches. Moreover, it suggests that inhibitory signals arising in tissues outside the morphogenetic field do not delimit or restrict the heart morphogenetic field. If the heart morphogenetic field were restricted by inhibitory signals arising outside the field itself, one would expect the region of heart-forming potency on a map of the heart morphogenetic field produced by grafting experiments to be smaller than that observed on a map produced by explant experiments, since only the grafted tissues would be subjected to such signals.

Our previous work has shown that the inductive interactions responsible for the specification of heart mesoderm are complete well before the end of neurulation at stage 20 (Sater and Jacobson, 1989). At this stage, the heart morphogenetic field comprises lateral mesoderm as well as the ventral mesoderm fated to give rise to the heart. The work of Fullilove (1970) on heart-inducing tissues in urodele neurulae shows that the areas capable of inducing heart formation include anterior dorsolateral and ventral endoderm, a substantial portion of the total endoderm. Thus, during the establishment of heart mesoderm, the regions of inductive capacity and heart-forming potency are considerably larger than the regions destined to participate in heart formation.

Interactions between Lateral Mesoderm and Lateral Endoderm in Postneurula Embryos

Ekman (1921, 1925) viewed the region of heart-forming potency described by his heterotopic transplantation and tissue removal experiments as a region that possesses the competence to respond to the signals inducing heart formation. Alternatively, this region may represent an induced morphogenetic field, capable of heart formation even in the absence of continued inductive signals. To distinguish between these possibilities, we cultured explants of anterior lateral mesoderm both with and without the underlying endoderm. Our results indicate that anterior lateral endoderm supports heart-forming potency in anterior lateral mesoderm during early postneurula stages: explants containing both mesoderm and endoderm undergo heart formation more frequently than do explants containing mesoderm alone. The specificity of the interaction between the lateral mesoderm and the underlying endoderm has not been examined. Our results suggest, however, that the ability to respond to continued signals from the anterior endoderm may be a critical characteristic of mesoderm in peripheral regions of the heart morphogenetic field.

Two lines of evidence suggest that this supportive interaction does not persist during late postneurula stages. First, the difference in the frequency of heart formation between late postneurula mesodermal explants with and without endoderm is statistically insignificant. In addition, anterior lateral endoderm from early postneurula stages, which supports heart-forming potency in early postneurula lateral mesoderm, does not restore heart-forming potency in late postneurula lateral mesoderm. This finding suggests that by late postneurula stages, the lateral mesoderm has lost its ability to respond to anterior endoderm. The loss of responsive ability during late postneurula stages may contribute to the decline in heart-forming potency in the lateral mesoderm, and thus to the spatial restriction of the heart morphogenetic field.

Similar supportive signals, traditionally referred to as "formative influences" (Holtfreter and Hamburger, 1955), may be the primary target of the *cardiac lethal* mutation in axolotls. This mutation is characterized by the formation of a heart tube that fails to beat; heart mesoderm will, however, develop normally when placed in a wild-type host (Humphrey, 1972). The mutation causes a defect in the endoderm that prevents it from supporting the later stages of heart differentiation (Lemanski *et al.*, 1979). The spatial pattern of supportive interactions may help to delimit morphogenetic fields in a variety of instances.

The Restriction of the Heart Morphogenetic Field Represents a Change in Developmental Potency

Analyses of changes in the spatial distribution of any field over time must examine the possibility that morphogenetic movements may be responsible for such changes. Therefore, spatial restriction of the morphogenetic field may reflect cell movements, rather than a restriction of developmental potency. Our results indicate that the decline in heart-forming potency in explants of anterior lateral mesoderm during postneurula

stages is not due to ventralward migration of lateral mesodermal cells that retain heart-forming potency, because no such migration occurs. Thus, the spatial restriction of the heart morphogenetic field is caused by a loss of heart-forming potency within the lateral mesoderm itself.

Possible Causes of the Restriction of the Heart Morphogenetic Field

Our results also exclude the possibility that neural crest cells suppress heart-forming potency in the lateral mesoderm during their migration into the branchial region, since embryos lacking the neural crest and neural tube do not exhibit greater heart-forming potency within the lateral mesoderm than do intact embryos. Thus, restriction of the heart morphogenetic field is not due to a local inhibition of heart-forming potency by migrating neural crest cells. This is in contrast to the findings of Jacobson and Duncan (1968), who reported that urodele neural tissue, including the neural plate and, later, the neural crest, inhibited heart formation when combined with explants of prospective heart mesoderm in culture. In addition, it has more recently been suggested that neural crest cells participate in the restriction of the lens morphogenetic field by locally inhibiting lens-forming potency in the epidermis (Henry and Grainger, 1987). The differences between the results of Jacobson and Duncan and those reported here may reflect either differences in the tissue interactions of urodele and anuran embryos, or changes in the sensitivity of the heart mesoderm to inhibitory tissue interactions over the course of development.

The restriction of the heart morphogenetic field may result from interactions within the field itself. During postneurula stages, the anterior ventral mesoderm, which is fated to give rise to the heart, may act to suppress heart-forming potency in the anterior lateral mesoderm. This type of local inhibition is analogous to the suppression of bristle formation within a small radius of a bristle organ primordium in the epidermis of *Drosophila* and other insects (Stern, 1954). One model suggests that short-range inhibition of bristle formation by the newly committed bristle organ primordium results in the ordered spatial array of bristles that is characteristic of insect epidermis (Wigglesworth, 1940; Held and Bryant, 1984). Such tissue interactions serve to organize developmental processes within morphogenetic fields and may play a large and as yet unrecognized role in the assignment of cell fate in embryos.

A more proximate cause of the loss of heart-forming potency from the anterior lateral mesoderm during postneurula stages may rest in the formation of the third pharyngeal pouch. During pharyngeal pouch formation, the endoderm lining the lateral walls of the archenteron in the pharyngeal region evaginates toward the ectoderm, moving through the intervening mesoderm. A corresponding invagination of the pharyngeal ectoderm occurs at the same time. Eventually, the endoderm at the tip of the pharyngeal pouch comes into contact and fuses with the invaginating ectoderm. Six pharyngeal pouches form in a cranial-to-caudal sequence in vertebrate embryos, dividing the anterior ventrolateral mesoderm into separate areas.

The third pharyngeal pouches first appear as a dimpling in the ventrolateral pharyngeal endoderm lateral and slightly dorsal to the prospective heart mesoderm at stage 23 (Nieuwkoop and Faber, 1967). The evaginating endoderm of the third pouch makes initial contact with the ectoderm at stage 27 (Nieuwkoop and Faber, 1967). Thus, formation of the third pharyngeal pouch occurs during the period when the anterior lateral mesoderm is losing heart-forming potency. In addition, the third pharyngeal pouch divides the anterior lateral mesoderm, perhaps preventing communication between the regions of lateral mesoderm anterior and posterior to the pouch. The observation that formation of the third pharyngeal pouch coincides spatially and temporally with the loss of heart-forming potency in the anterior lateral mesoderm suggests that pouch formation may contribute to the loss of heart-forming potency. Specifically, the third pharyngeal pouch may divide the anterior lateral mesoderm into isolated regions of tissue that are too small to sustain heart-forming potency, leaving them susceptible to incorporation into the branchial arch skeleton and vascular elements.

The loss of developmental potency as a result of the subdivision of a morphogenetic field beyond a critical point has substantial precedent. Holtfreter and Hamburger (1955) described a number of studies demonstrating that the range of differentiated cell types exhibited by an isolated region of developmental potency is greatly reduced when the isolated tissue is divided into fragments which are then cultured individually. This phenomenon has been exhaustively studied by Ban-Holtfreter (1965), who examined the differentiative capability of isolated regions of the dorsal lip of the blastopore in urodele embryos. She found that explants of the entire dorsal lip exhibited a wide range of differentiated cell types, including cell types to which the dorsal lip does not contribute *in vivo*. Smaller fractions of the dorsal lip produced a much more limited diversity of differentiated tissues. Differentiation in the smallest pieces produced very few cell types: in some of these explants, recognizable differentiation did not occur. The smaller explants were unable to give rise to the full range of cell types which the explanted regions would generate *in vivo*. The limited differentiative capability of very small explants has been attributed to "mass effects": because of small cell number, cell-cell interactions occur at levels insufficient to support a wide range

of differentiative capabilities (Muchmore, 1951). An alternative explanation is that very small explants are incapable of "conditioning" the culture medium with secreted products that are necessary to support the formation of diverse cell types (Niu and Twitty, 1953). Recently Gurdon (1988, 1989) has reinvestigated these phenomena, showing that *Xenopus* animal cap cells are unable to express cardiac actin in response to mesodermal induction unless they are partially surrounded by other cells that are also responding to the inductive signal. He refers to this requirement for similarly responding cells as a "community effect."

It is clear that events occurring within the lateral mesoderm contribute to the loss of heart-forming potency in this region. By late postneurula stages, the lateral mesoderm has lost the ability to participate in tissue interactions with the anterior lateral endoderm that support heart-forming potency of the lateral mesoderm. It is considerably less clear, however, which cellular processes govern this loss of developmental potency.

Numerous experimental analyses of the role of inductive interactions in embryonic determination indicate that inductive interactions occur gradually, with a cumulative effect on the developmental potency of the responding tissue (Jacobson, 1966). It follows that the acquisition of developmental potency also occurs gradually, and that the induced state of determination is labile for some period of time prior to a final, relatively irreversible commitment to a given developmental pathway. Perhaps the spatial restriction of the heart morphogenetic field and the attendant loss of developmental potency from peripheral regions of the heart morphogenetic field represent a failure of an induced state of determination to become "fixed" within the cells at the periphery of the morphogenetic field. The retention of developmental potency by cells that become committed to a developmental pathway, on the other hand, may result from cellular processes that stabilize the induced state of determination.

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