

## Cellular contacts required for neural induction in *Xenopus* embryos: evidence for two signals

JANE E. DIXON and CHRIS R. KINTNER

*Molecular Neurobiology Laboratory, The Salk Institute for Biological Studies, PO Box 85800, San Diego, CA 92138, USA*

### Summary

Neurogenesis begins in amphibian embryos around the time of gastrulation when a portion of the ectoderm receives an inducing signal from dorsal mesoderm. Two different proposals have been made for how ectoderm must come into contact with dorsal mesoderm in order for the inducing signal to pass between the two tissues. Induction in one proposal would require normal gastrulation movements to bring dorsal mesoderm underneath, and into apposition with, the overlying ectoderm. The inducing signal in this case would pass between dorsal mesoderm and ectoderm as apposed tissue layers. The other proposal is that induction requires only a small contact between ectoderm and dorsal mesoderm at the boundary they share before gastrulation. The inducing signal by this proposal would pass laterally across this small area of contact between mesoderm and ectoderm, perhaps before gastrulation, and spread within the ectodermal cell layer. Since it is not known to what extent neurogenesis depends on each of these

proposed contacts between ectoderm and dorsal mesoderm, we have generated explants of embryonic tissue in which one or the other type of contact between mesoderm and ectoderm is favored. The amount of neural tissue formed under these various conditions was then assessed using a quantitative RNase protection assay to measure the levels of two neural-specific RNA transcripts. The results show that neural tissue forms efficiently when ectoderm and dorsal mesoderm only interact laterally within a plane of tissue. In contrast, neural tissue forms extremely poorly when ectoderm is placed experimentally in apposition with involuting, anterior–dorsal mesoderm. Finally, a synergistic effect is obtained when both types of contacts can occur between ectoderm and mesoderm indicating that two signals may be required for neural induction in *Xenopus* embryos.

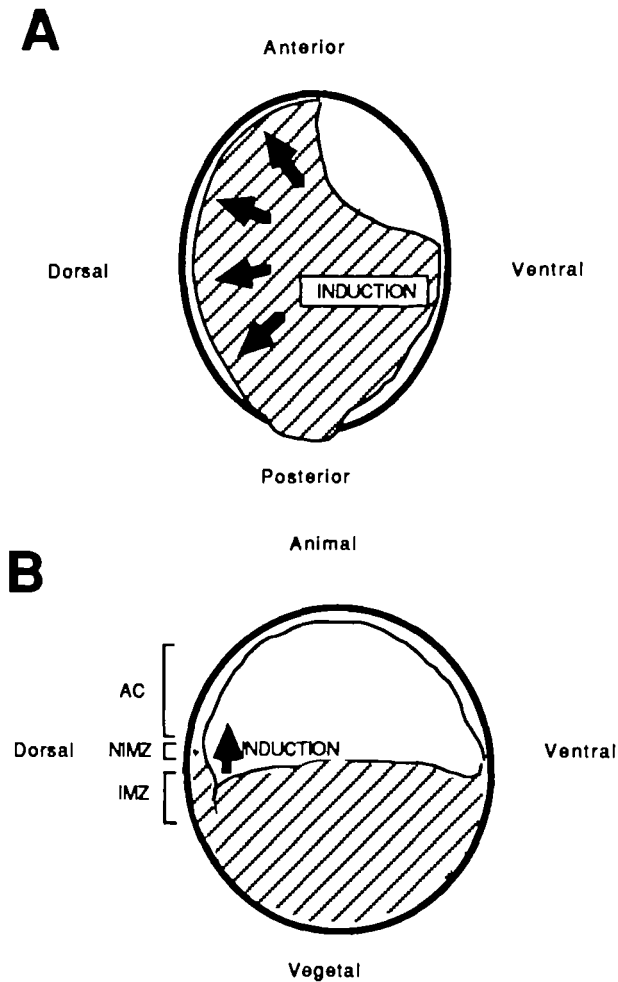
*Key words:* *Xenopus laevis*, cell contact, neural induction.

### Introduction

Embryonic induction is considered a key mechanism underlying cell determination in the vertebrate embryo. One well-known example of this mechanism is the induction of neural tissue around the time of gastrulation in amphibian embryos. During gastrulation, the ectoderm expands and covers the outside of the embryo as mesoderm and endoderm invaginate inward. When gastrulation is complete, most of the ectoderm now lies on the ventral side of the embryo and differentiates into epidermal tissue while one portion of ectoderm lies over dorsal mesoderm and initiates neural development. The division of ectoderm into these two developmental fates is known from a variety of experiments to depend on an inductive interaction. Before gastrulation, all ectoderm is capable of forming either epidermal or neural tissue and, if dissected away and placed in isolation, it will autonomously undergo epidermal differentiation. In contrast, neural development requires an interaction between ectoderm and presumptive dorsal mesoderm. This interaction is thought to be import-

ant both in specifying the region of ectoderm that will form neural tissue and in organizing the early nervous system into distinct regions (Spemann, 1938; also reviewed in Holtfreter & Hamburger, 1955; Gurdon, 1987).

Two proposals have been made for how the inducer might pass between dorsal mesoderm and ectoderm (Fig. 1 and reviewed in Jacobson & Sater, 1988). One proposal (panel A) is that the inducer passes between ectoderm and dorsal mesoderm as they come into contact during gastrulation. The important feature in this case is that an inducing signal passes between two apposed tissue layers; the underlying, invaginating dorsal mesoderm and overlying ectoderm. The other proposal (panel B) is that the inducer passes between ectoderm and dorsal mesoderm laterally across the boundary they share before gastrulation. In this case, the inducer would cross the boundary between ectoderm and mesoderm (the NIMZ–IMZ boundary as defined in Gerhart & Keller, 1986) and perhaps spread within the plane of ectoderm. The spread of a neuralizing signal within ectoderm, sometimes called homo-



**Fig. 1.** Potential interactions between ectoderm and presumptive dorsal mesoderm. A and B diagram two potential routes through which an inducer could pass between dorsal mesoderm and ectoderm. In A, the inducer would pass between the invaginating mesoderm and overlying ectoderm during gastrulation. In B, the inducer would cross laterally the IMZ–NIMZ boundary, perhaps before gastrulation, and then spread within the ectoderm (for nomenclature see Gerhart and Keller, 1986).

genetic induction, would have to be limited by some mechanism to prevent complete neuralization of the ectoderm (Spemann, 1938; Mangold & Spemann, 1927; Slack, 1983).

The extent to which neural induction can occur across the mesoderm/ectoderm contacts diagrammed in Fig. 1 is not known. Holtfreter's early studies on exogastrulated embryos, however, lent considerable support to the idea that induction depends on gastrulation to bring mesoderm into contact with ectoderm (Holtfreter, 1933). Holtfreter showed that embryos cultured in high salt without a vitelline membrane do not gastrulate inward but rather outward to produce an inside-out embryo. These exogastrulae go on to form reasonably normal axial mesoderm and endoderm that is connected by a stalk of tissue to the isolated ectodermal sack. Holtfreter concluded on histological grounds that exo-

gastrulae are neural deficient, indicating that apposition of dorsal mesoderm with ectoderm during gastrulation was necessary for neural induction to occur.

Although Holtfreter's experiment indicated that neural induction requires the normal movements of gastrulation, recent studies suggest that this interpretation needs to be reconsidered, at least for *Xenopus* embryos. In one recent study, exogastrulae were formed with *Xenopus* embryos and examined for the expression of an RNA transcript encoding the neural cell adhesion molecule, N-CAM (Jacobson & Rutishauser, 1986). These studies detected near normal levels of N-CAM RNA in exogastrulae indicating that exogastrulae do form to some extent neural tissue (Kintner & Melton, 1987).

Another analysis that relates gastrulation to neural induction are the experiments of Keller and colleagues (Keller *et al.* 1985). In their experiments, a square sheet of tissue containing both mesoderm and ectoderm is dissected from the dorsal side of a blastula embryo (see Fig. 3). If one of these isolated sheets is cultured alone, the tissue rolls into the shape of a ball and is not very informative. If two such sheets are sandwiched together (referred to here as a Keller sandwich), the tissue remains flat during the gastrulation movements of convergent extension (Keller & Danilchik, 1988). Upon gastrulation, the ectoderm and mesoderm in a Keller sandwich move away from each other in a manner reminiscent of exogastrulae so that mesoderm never involutes and comes into apposition with ectoderm (Keller & Danilchik, 1988). Histological sections of these explants reveal large amounts of neural tissue indicating that neural induction has again taken place without the involution movements associated with normal gastrulation in intact embryos. Keller and Danilchik suggest from their results that the inducer in these explants passes between mesoderm and ectoderm across the boundary they share before gastrulation and that this pathway may also be used in intact embryos.

Since it is not known to what extent neurogenesis depends on each of these proposed contacts between ectoderm and dorsal mesoderm, we have generated explants of embryonic tissue in which one or the other type of contact between mesoderm and ectoderm is favored. Specifically, we have compared the amounts of neural tissue formed when ectoderm and dorsal mesoderm come into contact by apposition (Fig. 1A) or into contact across the IMZ–NIMZ boundary (Fig. 1B). The amount of neural tissue formed under these various conditions was then assessed using a quantitative RNase protection assay to measure the levels of two neural-specific RNA transcripts. The results show that quantitatively, neural tissue forms most efficiently when ectoderm and dorsal mesoderm come in contact laterally within a plane of tissue (as in Fig. 1B). In contrast, little neural tissue is formed when ectoderm and anterior dorsal mesoderm come into contact by apposition (as in Fig. 1A). Both interactions in concert, however, produces a synergistic effect providing strong evidence that two signals may be required for initiating neurogenesis in *Xenopus* embryos.

## Materials and methods

### Animals

*Xenopus laevis* male and female frogs were obtained from NASCO, maintained in charcoal-filtered water and fed liver thrice weekly. Hormone-induced egg laying and *in vitro* fertilization were carried out using standard protocols. Embryos were maintained at 15–18°C overnight in order to slow development. Dissection of embryos and the culture of explanted tissue were done in 0.5 × MMR (Kimelman & Kirschner, 1987) containing penicillin/streptomycin (Gibco).

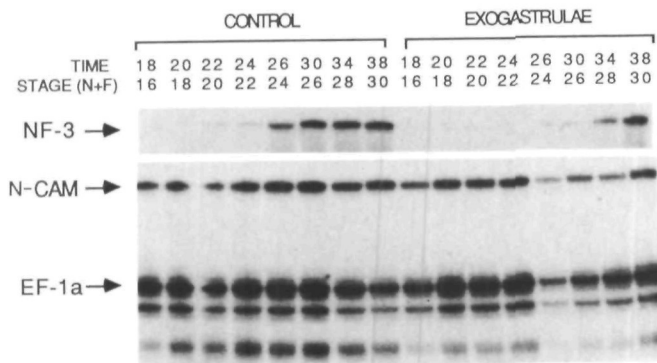
### RNase protection assay

RNA was isolated from embryos or explants and assayed for the expression of N-CAM, NF-3 or EF-1a transcripts using an RNase protection assay as described previously (Kintner & Melton, 1987). The probe for N-CAM was a SP6 polymerase transcript generated from a portion of the N1 cDNA corresponding to the amino terminus of the protein. In the experiment shown in Fig. 3, a second N-CAM probe was also used which detects sequences encoding the large cytoplasmic domain (Krieg *et al.* unpublished data). The probe for NF-3 RNA was a SP6 antisense transcript generated from a cDNA kindly provided by Dr Charnas while the probe for EF-1a RNA was a SP6 antisense transcript generated from an EF-1a cDNA (P. Krieg, personal communication). The assay described in Fig. 2 was carried out by assaying RNA samples separately with each probe. The assays described in Figs 3–5 were carried out by assaying RNA samples simultaneously with all three probes.

## Results

### Assays for neural transcripts

A quantitative assay for neural tissue formation was



**Fig. 2.** Expression of neural transcripts in exogastrulae. RNA was isolated at different developmental stages (Nieuwkoop & Faber, 1967) from normal or exogastrulated embryos (Kintner & Melton, 1987) and assayed for the expression of N-CAM, NF-3 or EF-1a RNA. Equivalent amounts of RNA were assayed in each case as monitored by the levels of EF-1a transcripts. N-CAM RNA has already reached steady-state levels of expression in both exogastrulae and normal embryos by the first time point at stage 16. In contrast, NF-3 RNA undergoes a striking increase in levels at about stage 24 in the normal embryos and about stage 28 in exogastrulae. Note that while the expression of NF-3 RNA is delayed in exogastrulae, eventually the levels reached are comparable to levels in normal embryos.

based on an RNase protection assay that measures the expression levels of two neural RNA transcripts. One of the two neural transcripts encodes the neural cell adhesion molecule, N-CAM, which is expressed in ectoderm soon after induction and is apparently restricted to neural tissue at least in *Xenopus* embryos (Jacobson & Rutishauser, 1986; Kintner & Melton, 1987). N-CAM transcripts serve, therefore, as an early and general marker of neural differentiation. The second neural marker measured in these assays is a cDNA (kindly provided by Dr Lawrence Charnas), which encodes a neurofilament-like protein referred to here as NF-3 (Charnas *et al.* 1987). NF-3 transcripts are first expressed in postmitotic neurones about 4 h after neural tube closure and therefore mark a relatively late stage of neuronal development (L. Charnas, personal communication). Together these two RNA transcripts give an independent assessment of the amount and degree of neural tissue formation. The final probe used in these assays detects a transcript encoding an elongation factor for translation, EF-1a. EF-1a is an ubiquitous protein and thus its transcripts serve as a measure of cell number and RNA recovery (Krieg & Melton, personal communication). An important feature of the RNase protection assay shown here is that the RNA samples are assayed simultaneously with all three probes. This protocol eliminates variability that might otherwise arise during sample processing and electrophoresis.

### Expression of NF-3 RNA in exogastrulae

The expression of N-CAM transcripts in exogastrulae indicated that these altered embryos form neural tissue (Kintner & Melton, 1987). Another argument, however, is that the N-CAM expression under these conditions was an artifact that did not truly reflect neural differentiation. In order to rule out this argument, the expression of another neural marker (called NF-3 here, Charnas *et al.* 1987) was measured in exogastrulae. RNA samples isolated from exogastrulae (Kintner & Melton, 1987) and control embryos at different developmental stages were assayed using probes for N-CAM, NF-3 and EF-1a RNA. The results obtained with the N-CAM probe in this experiment (Fig. 2), as in previous studies (Kintner & Melton, 1987), showed that the level of N-CAM RNA in exogastrulae was similar to the level in control embryos. The results obtained with the NF-3 probe were very similar to the N-CAM results. Exogastrulae expressed NF-3 RNA and the level of these transcripts was similar to the level of expression in control embryos. Comparison of the time course of NF-3 RNA expression, however, revealed a difference between exogastrulae and control embryos. While there was a sharp increase in the level of NF-3 RNA expression in normal embryos at stage 24, a similar level of expression in exogastrulae was delayed until stage 28. Nonetheless, the expression of NF-3 transcripts supports the earlier conclusion based on N-CAM RNA that near normal level of neural tissue can form in exogastrulae.

*Expression of N-CAM and NF-3 in Keller sandwiches*

The expression of NF-3 and N-CAM RNA in exogastrulae supports the notion that normal gastrulation, and therefore apposition between mesoderm and ectoderm, does not have to occur in order for large amounts of neural tissue to form in *Xenopus* embryos. One uncertainty in the interpretation of the results obtained with exogastrulae is determining the extent to which mesoderm and ectoderm interact. For example, gastrulation may begin inward in exogastrulae as it does normally but then reverse and go outward. These truncated inward movements could result in enough apposition of mesoderm and ectoderm to induce neural tissue at reasonable levels. To rule out this argument, we have studied the expression of neural transcripts in the explants described by Keller and colleagues (Keller & Danlichik, 1988). These explants (called Keller sandwiches) also prevent involution movements of dorsal mesoderm in apposition to ectoderm but under more controlled conditions (see Introduction).

Keller sandwiches were made by dissecting a square sheet of tissue from the dorsal side of a blastula that includes a portion of the animal cap extending into the marginal zone just above the blastopore lip (Fig. 3B). Two such tissue sheets were sandwiched together, allowed to develop to the equivalent of stage 30 and then analyzed for the expression of N-CAM, NF-3 and EF-1a RNA. Several conclusions can be drawn from the results of such experiments, one example of which is shown in Fig. 3A.

(1) Keller sandwiches were made from tissue isolated from embryos at the first appearance of the blastopore lip (stage 10, lanes 5,6) or from embryos where the invaginating mesoderm had just turned inward at stage 10.5 (lanes 7 and 8). In both cases, the explant sandwiches made high levels of NF-3 and N-CAM RNA.

(2) The levels of NF-3 and N-CAM RNA expressed in the Keller sandwiches were comparable to the levels found in control embryos. If one takes into account the number of embryos used in the control lane (three embryos, lane 3) and the number of embryos used for the Keller sandwiches (six, lanes 5–8), then one estimates that the latter has formed 50–70% the amount of neural tissue formed in a normal embryo.

(3) The ratio between the level of NF-3 transcript and N-CAM RNA appeared to remain constant regardless of whether their expression occurred in normal embryos or the Keller sandwiches.

(4) In some cases, the blastula explants were not sandwiched together but were left as individuals (separate, lanes 5,7; together, lanes 6,8). This treatment seemed to have a small effect on the expression of N-CAM and NF-3 RNA.

These observations support the results obtained with exogastrulae and indicate that the expression of neural transcripts occurs almost as well as in normal embryos when the only contact between ectoderm and dorsal mesoderm is at the NIMZ–IMZ boundary. The efficient formation of neural tissue does not appear to

depend on the involution of dorsal mesoderm and apposition with ectoderm.

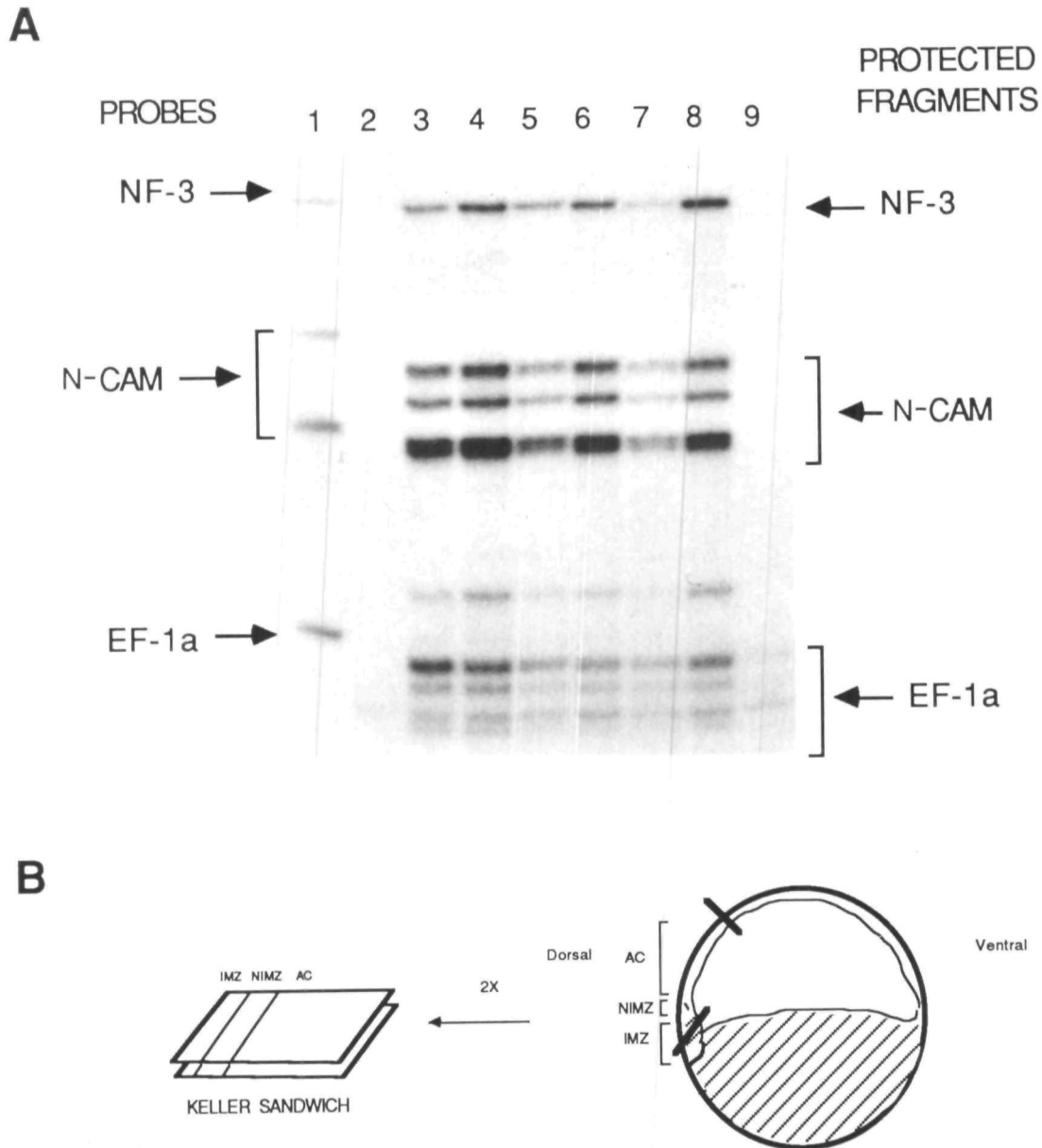
*Expression of N-CAM and NF-3 RNA in recombinants of ectoderm and involuting, anterior–dorsal mesoderm*

We have also examined the expression of N-CAM and NF-3 RNA in explants where dorsal mesoderm is brought experimentally in apposition to ectoderm by making tissue recombinants as shown in Fig. 4B. The purpose of these experiments was to determine how efficiently induction of neural tissue occurs when ectoderm is experimentally apposed to dorsal mesoderm, as it normally does during gastrulation. Ectoderm dissected from stage 9 or 10 blastula was recombined with the anterior half of involuting dorsal mesoderm from stage 12 (midgastrula embryos). An attempt was made during the dissection of the ectoderm used in these studies to isolate ectoderm from the dorsal side (Sharpe *et al.* 1987). These recombined tissues quickly healed together and the ectoderm underwent epiboly during the subsequent few hours to cover the outside of the recombinant. The recombinants were allowed to develop to stage 30 and then assayed for the expression of N-CAM and NF-3 RNA. In the experiment shown in Fig. 4A, the assay was performed on individual recombinants. The results show that recombinants express only very low levels of N-CAM and NF-3 RNA. In the five separate experiments on recombinants, we found variable expression of N-CAM and NF-3 RNA and a correlation between the more posterior regions of the dissected dorsal mesoderm and higher levels of RNA expression. Even in the best recombinants, however, the expression of neural transcripts is found to be extremely low (less than 10% the levels in normal embryos).

In the same experiment, the expression of N-CAM and NF-3 RNA in single Keller sandwiches was also measured. In agreement with the results shown in Fig. 3, these explants express N-CAM and NF-3 RNA at levels approaching the levels (50–70%) found in normal embryos. Again, this should be contrasted to the low (1–10%) levels of NF-3 and N-CAM transcripts obtained in the recombinants between dorsal mesoderm and ectoderm.

*Expression of N-CAM and NF-3 RNA in Keller sandwiches/recombinants*

One interpretation of the results obtained with recombinants is that the involuting, anterior–dorsal mesoderm is only a poor inducer of neural tissue. One argument against this interpretation is that contact between ectoderm and dorsal mesoderm by apposition is sensitive to perturbation and fails to occur when arranged experimentally. As a control to rule out this argument, involuting dorsal mesoderm was placed in apposition with ectoderm of a Keller sandwich as shown in Fig. 5B, allowed to develop 24 h in culture, and assayed for the expression of the neural transcripts. The results from one such experiment are shown in Fig. 5A. The levels of NF-3 and N-CAM RNA in the Keller

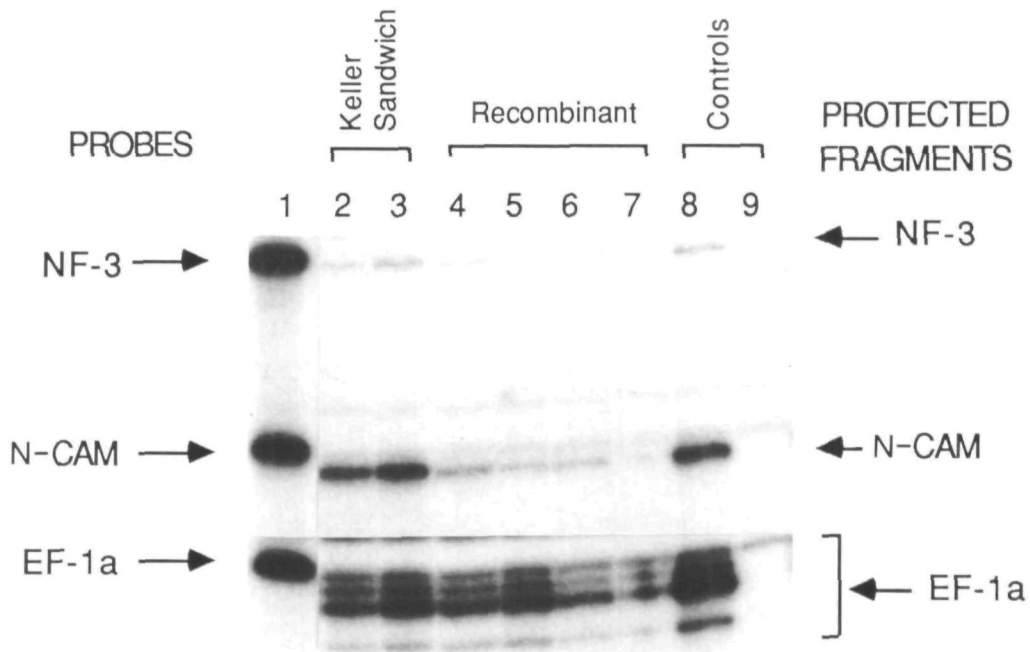


**Fig. 3.** Expression of neural transcripts in Keller sandwiches. A square sheet of tissue was dissected from the dorsal region of a blastula embryo containing presumptive dorsal mesoderm and ectoderm as diagrammed in B. The dissected tissue was either cultured alone (lanes 5 and 7) or two such explants were sandwiched together (Keller Sandwiches, lanes 6, 8). RNA samples isolated from explants after 24 h in culture (stage 30) was assayed for the levels of N-CAM, NF-3 and EF-1a transcripts (A). The left side of A denotes the position of the three probes and the right side denote the position of the fragments protected by the three transcripts. Two probes for N-CAM RNA were used in this experiment as described in Materials and methods. Lane 2 is a negative control where the probes were digested by RNase after hybridization with tRNA. Lane 3 shows the fragments protected from RNase treatment when the probes were hybridized to RNA isolated from stage 30 embryos and lane 4 RNA isolated from ectoderm removed after gastrulation (lane 4). Lanes 5, 6, 7 and 8 show the results obtained when the probes were digested with RNase A after hybridization with RNA from blastula explants. In lanes 5, 6, the tissue used in the explants was isolated as soon as the blastopore lip was evident (stage 9.5–10). In lanes 7, 8, the tissue used in the explants was isolated when the blastopore lip was a small crescent (stage 10–10.5). In lanes 5, 7, the isolated blastula tissue was cultured as individual pieces while in lanes 6, 8, two such explants were sandwiched together. Note that in all cases, the explants express about as much N-CAM and NF-3 RNA transcripts as the control embryo. Finally lane 9 shows the fragments protected from RNase treatment after the probes were hybridized with RNA isolated from blastula ectoderm. As shown previously, isolated ectoderm does not express detectable levels of the neural transcripts (Kintner & Melton, 1987).

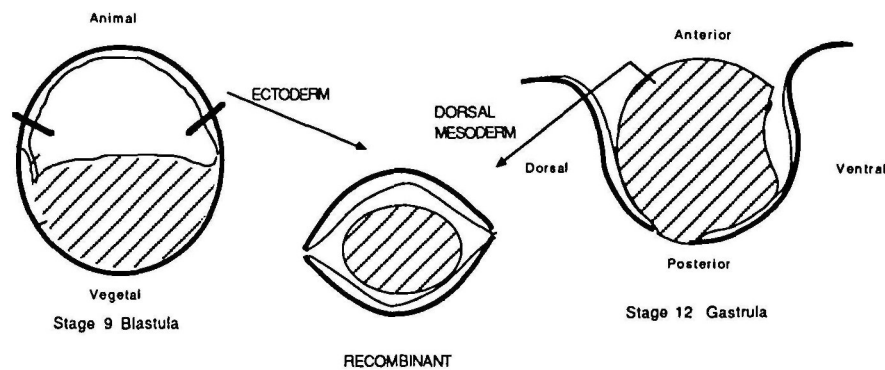
sandwich recombinants are shown in lane 2 (two explants) and should be compared to the levels in control embryos (two embryos, lane 5), Keller sand-

wiches (two explants, lane 3) and recombinants (two explants, lane 4). In accordance with previous results, the Keller sandwiches (lane 3) express 50–70% the

**A**



**B**

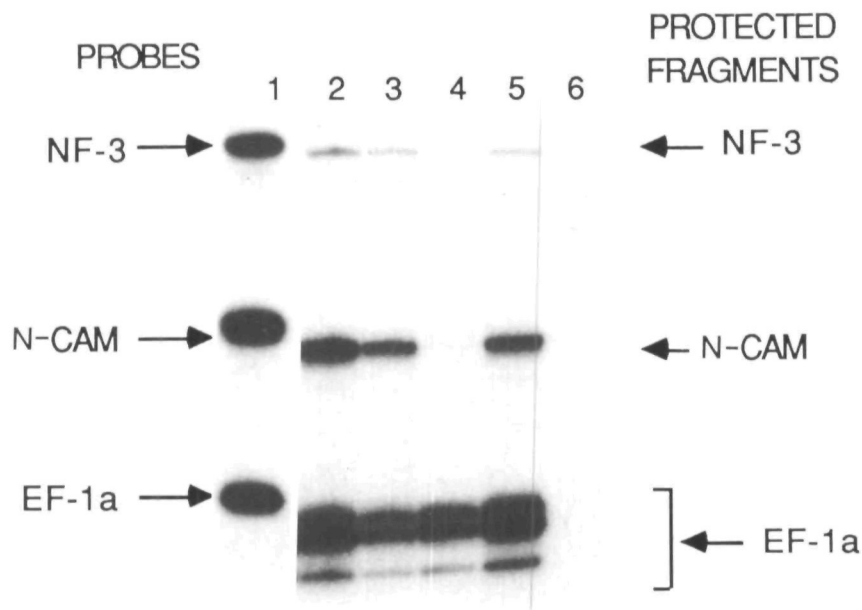
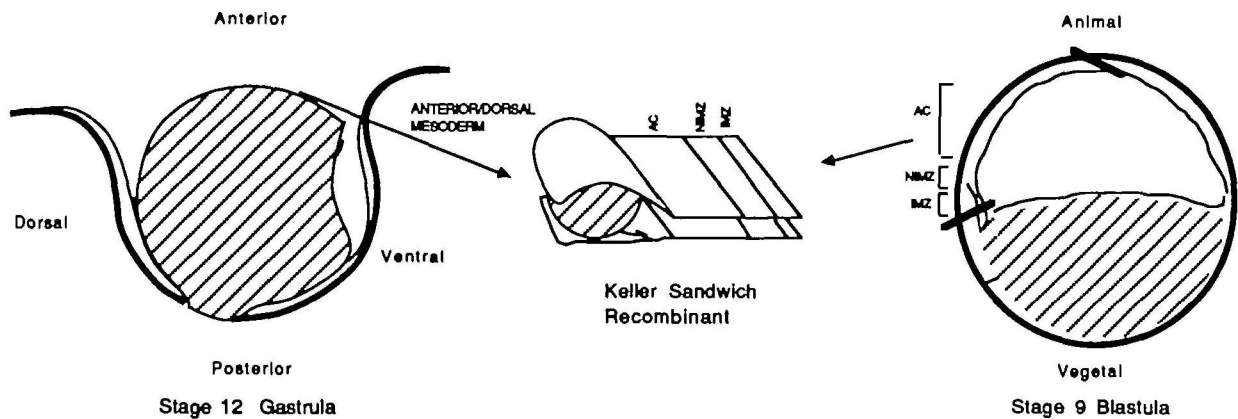


**Fig. 4.** Expression of neural transcripts in recombinants of involuting, anterior-dorsal mesoderm and ectoderm. The anterior half of involuting dorsal mesoderm was dissected from stage 12 gastrulae and recombined with ectoderm dissected from stage 9 blastulae as shown in B. After 24 h in culture (stage 30), RNA was isolated from the recombinants and assayed for the expression of N-CAM, NF-3 and EF-1a RNA (A). The left side of A denotes the position of the three probes and the right side denotes the position of the fragments protected by the three transcripts. Lanes 4-7 show the protected fragments obtained with RNase treatment of the probes after hybridization to RNA isolated from single recombinants. The amounts of NF-3 and N-CAM RNA in these samples should be compared to amounts present in a single embryo (lane 8). For comparison, the amounts of N-CAM and NF-3 RNA present in single Keller sandwiches (as described in Fig. 3B) are shown in lanes 2, 3. Lane 9 is the negative control showing the fragments protected when the probes are treated with RNase digestion after hybridization to tRNA.

levels of N-CAM and NF-3 RNA present in control embryos (lane 5) while recombining the anterior-dorsal mesoderm with ectoderm (recombinant) produces very little NF-3 or N-CAM RNA (lane 4). Importantly, combining the anterior-dorsal mesoderm with the ectoderm of a Keller sandwich has a synergistic effect on the levels of NF-3 and N-CAM RNA. This addition increases the levels of NF-3 and N-CAM RNA twofold or to the same levels as control embryos, indicating that all of the components necessary for generating the full complement of neural tissue are now present. It should be noted that anterior-dorsal mesoderm alone

expresses EF-1a but not N-CAM or NF-3 RNA and this fact explains why the levels of EF-1a are higher in lane 5 relative to lane 3. In other words, the increase in levels of N-CAM and NF-3 RNA that are apparent in lane 5 relative to lane 3 represents a real increase when normalized to a constant amount of ectoderm used in each sample.

More striking after two days of culture, however, was the appearance of the Keller sandwiches that contained involuting, anterior-dorsal mesoderm. The ectodermal surface of a Keller sandwich or a recombinant remains relatively undifferentiated in appearance after several

**A****B**

**Fig. 5.** Expression of neural transcripts in recombinants of involuting, anterior-dorsal mesoderm and ectoderm of a Keller sandwich. Involuting, anterior-dorsal mesoderm was dissected from a stage 12 gastrulae and recombined with the ectoderm of a Keller sandwich as diagrammed in panel B. An effort was made to position the dorsal mesoderm under the ectoderm of the Keller sandwich at a point as far away as possible from the NIMZ-IMZ border. As controls, the dissected mesoderm was also placed in combination with ectoderm from blastula embryos and Keller sandwiches were also constructed without the addition of the involuting dorsal mesoderm. A point was made to use equivalent amounts of ectoderm in each type of explant. The explants were allowed to develop 24 h in culture (stage 30) and then assayed for the expression of N-CAM, NF-3 and EF-1a RNA (A). The left side of A shows the position of the probes and the right side shows the position of the protected fragments. Lane 1 shows the probes without RNase treatment. Lane 2 shows the results obtained when the probes are protected with RNA from Keller sandwiches/Recombinants (B, 2 explants); lane 3 with RNA from Keller sandwiches (Fig. 3B, 2 explants), lane 4 with RNA from Recombinants (Fig. 4B, 2 explants) and lane 5 with RNA from control embryos (2 embryos). Note that combining the involuting dorsal mesoderm with ectoderm (Recombinant, lane 4) produces very little NF-3 or N-CAM RNA although the same mesoderm combined with ectoderm of a Keller sandwich (lane 2) increases the expression of NF-3 and N-CAM RNA twofold relative to their levels in a Keller sandwich alone (lane 3). Although the level of EF-1a RNA is also higher in lane 2 relative to lane 3, the higher level of neural transcripts is considered significant because the involuting dorsal mesoderm expresses EF-1a but not N-CAM or NF-3 RNA. Lane 6 is the negative control where the probes were protected with tRNA.

days in culture. In contrast, the addition of involuting, anterior–dorsal mesoderm to the ectoderm of a Keller sandwich has a profound result in that the ectoderm of a Keller sandwich/recombinant undergoes a significant amount of differentiation including the formation of structures remarkably similar to a forehead of normal embryos. Histological sections through these structures reveal the presence of anterior neural structures such as eyes which are never found in sections through a Keller sandwich or a recombinant (data not shown). It appears therefore that involuting, dorsal mesoderm can have profound qualitative effects on the formation of neural tissue in the ectoderm of a Keller sandwich.

## Discussion

### *Contact between ectoderm and dorsal mesoderm at the IMZ–NIMZ boundary*

The results obtained with exogastrulae and Keller sandwiches show that ectoderm and mesoderm need not be in contact as apposed cell layers in order to form almost as much neural tissue as a normal embryo. These results indicate that the contact between dorsal mesoderm and ectoderm before gastrulation, as diagrammed in Fig. 1B, is sufficient for the inducer to pass laterally within a plane of tissue (Jacobson & Sater, 1988; Keller & Danilchik, 1988). It is not known whether the inducer passes between the dorsal mesoderm and ectoderm by this route before or during gastrulation (Savage & Phillips, 1989). In other experiments to determine whether the inducer passes before gastrulation, we have dissected a thin strip of ectoderm that lies just above dorsal mesoderm from blastula embryos (the AC above the NIMZ in Fig. 3B) and placed it in culture for 24 h. About 75% of these individual explants express both markers for neural and a muscle tissue at high levels. About 25% of the individual explants, however, express the neural transcripts at levels comparable to normal embryos but do not express the muscle transcripts. The interpretation of the latter result is that before gastrulation, a portion of animal cap tissue has already gained the ability to form large amounts of neural tissue and, if dissected appropriately, it can be isolated from presumptive dorsal mesoderm. It is difficult to rule out in these experiments, however, that the isolated tissue completely lacks inducing mesodermal tissue. Therefore, while there are suggestions that neural induction may occur to some extent before gastrulation, the actual timing of induction is very difficult to pinpoint. A second point is that even if ectoderm has already been neuralized before gastrulation, as suggested by these experiments, it is not known if this occurs by induction. None of the experiments reported here show formally that the neural tissue formed in exogastrulae or Keller sandwiches results from a direct interaction between the organizer and ectoderm.

The idea of an interaction between the organizer and ectoderm before gastrulation in the context of neural induction is certainly not new (for example see Ham-

burger, 1988). Experiments using a homeobox-containing neural marker indicated that the dorsal ectoderm is predetermined in its ability to respond to neural induction (Sharpe *et al.* 1987). A series of experiments by Phillips and colleagues have shown that the expression of an epidermal marker Epi 1 is different in dorsal *versus* ventral ectoderm and that the expression pattern in dorsal ectoderm is set up by an interaction with the organizer before gastrulation (Akers *et al.* 1986; London *et al.* 1988; Savage & Phillips, 1989). The results reported here extend these observations by showing that contact between dorsal mesoderm and ectoderm before gastrulation not only appears to predetermine ectoderm to undergo neural development but also might quantitatively explain the formation of a large proportion of the neural tissue in the embryo.

### *Contact between ectoderm and dorsal mesoderm by apposition*

The results obtained with recombinants between ectoderm and involuting, anterior–dorsal mesoderm indicate that neural induction occurs extremely poorly when these two tissues come into contact by apposition experimentally. The ectoderm in these recombinants included ectoderm on the dorsal side up to the AC–NIMZ border and so the poor induction obtained in these experiments does not reflect the absence of predetermination (Sharpe *et al.* 1987). In addition, the poor induction obtained in these recombinant experiments is in sharp contrast to the results obtained with mesodermal induction where apposition of vegetal and animal cells efficiently induces mesodermal tissue (Nieuwkoop, 1973; Gimlich & Gerhart, 1984; Gurdon *et al.* 1985). The negative results obtained with the recombinants on their own, however, are not easy to interpret. It could be that the anterior dorsal mesoderm is an extremely poor neural inducer or the contact between it and ectoderm is sufficiently complex that induction fails when apposition is arranged experimentally.

The results obtained when the anterior–dorsal mesoderm is recombined with the ectoderm of Keller sandwich provide an important control and indicate that the involuting mesoderm is a potent neural inducer of ectoderm when placed **in the right context**. The involuting dorsal mesoderm is apparently capable of inducing more neural tissue but only in ectoderm that has remained in contact with presumptive mesoderm at the boundary between the IMZ and NIMZ. Moreover, the involuting, anterior–dorsal mesoderm can have a qualitative effect when placed in apposition to the ectoderm of a Keller sandwich where it induces the formation of anterior structures such as eyes. This result argues strongly that apposition between ectoderm and involuting mesoderm plays an important role in regionalizing neural tissue (Holtfreter & Hamburger, 1955).

In summary, we are proposing that the contact between ectoderm and anterior–dorsal mesoderm during gastrulation makes only a minor contribution quantitatively to the induction of neural tissue in the embryo. Neural tissue can form efficiently when the



only contact between dorsal mesoderm and ectoderm is at the IMZ–NIMZ boundary. Nonetheless, qualitative aspects of neural induction appear to depend critically on ectoderm and dorsal mesoderm coming in apposition during gastrulation. For example, apposition may allow contacts that are important in increasing the region of neuralized ectoderm or are critical for regionalizing the neural ectoderm. It appears therefore that the initiation of neurogenesis depends on several inductive signals working in tandem and that some of these inductive events may occur even before the beginning of gastrulation (Sharpe *et al.* 1987; Savage & Phillips, 1989).

The authors would like to thank Drs Greg Lemke and R. Jennifer Detrick for critical comments on the manuscript, Doug Melton who collaborated in the early stages of this work and Lawrence Charnas for providing the NF-3 cDNA. We acknowledge support from the National Institute of Health. C.K. is a research fellow of the Alfred P. Sloan and the McKnight foundation.

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(Accepted 19 April 1989)