RESEARCH ARTICLE

Neural Induction by the Secreted Polypeptide Noggin

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The Spemann organizer induces neural tissue from dorsal ectoderm and dorsalizes lateral and ventral mesoderm in *Xenopus*. The secreted factor noggin, which is expressed in the organizer, can mimic the dorsalizing signal of the organizer. Data are presented showing that noggin directly induces neural tissue, that it induces neural tissue in the absence of dorsal mesoderm, and that it acts at the appropriate stage to be an endogenous neural inducing signal. Noggin induces cement glands and anterior brain markers, but not hindbrain or spinal cord markers. Thus, noggin has the expression pattern and activity expected of an endogenous neural inducer.

Induction of the vertebrate nervous system is best understood in amphibians. Early experiments showed that transplants of Spemann's organizer (dorsal mesoderm) to the ventral side of a host gastrula result in twinned embryos. Although it was expected that the secondary embryo would be derived exclusively from the transplant, the organizer recruited the secondary nervous system from host tissues that would usually form skin (*1, 2*). The induction of this patterned nervous system has been investigated intensively, but little is known about the molecular nature of the factors responsible for the induction (*3*).

In contrast to neural induction, much progress has been made in understanding how mesoderm is induced. The mesoderm (which forms notochord, muscle, heart, mesenchyme, and blood) is induced in the equatorial region of the embryo (*4*). Candidates for the endogenous inducers include members of the fibroblast growth factor (FGF) family and activin (*5, 6*). Members of the wnt family (a family of cyteine-rich secreted proteins originally defined by the segment polarity gene *wingless* in *Drosophila* and the murine protooncogene *int-1*) and noggin modify the kind of mesoderm induced by activin and FGF, and may be important in the formation of the dorsal mesoderm (6). The use of dominant negative receptors for both FGF (*7*) and activin (*8*) in *Xenopus* embryos suggests that the signaling pathways activated by these molecules are essential for proper mesoderm formation.

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Until recently, there were no candidate molecules for the organizer signal that instructs lateral mesoderm to become muscle (*1, 9–11*). Noggin, a secreted protein lacking similarity to other known inducing factors, is expressed in the organizer, and noggin protein can dorsalize ventral mesoderm (*12*). Thus, noggin appeared to be a good candidate for this signal.

Early attempts to identify neural inducing factors were not productive because the newt and salamander ectoderm was poised to become neural, and therefore many heterologous chemicals (such as methylene blue) could elicit neuronal differentiation (*3*). Recently, neural induction has been studied in *Xenopus* embryos, which do not develop neural tissue so readily. Apart from isolated instances where a phorbol ester was used to induce neural tissue (*3*), no substances have been purified on the basis of their neural inducing activity. Activin, a mesoderm inducer, can promote formation of neural tissue in the blastula stage, but this is due to a secondary induction by the dorsal mesoderm that activin induces (*13–15*). However, in the gastrula, activin is ineffective at promoting the formation of neural tissue, since the gastrula ectoderm loses competence to form mesoderm in response to activin. In contrast, the endogenous neural inducer, dorsal mesoderm, can induce neural tissue until the end of gastrulation (*16*).

Studies in which the inducing effects of activin and dorsal mesoderm have been compared provide two criteria for the activities of an authentic neural inducer (*15*). First, the molecule should be able to induce neural tissue from animal cap ectoderm in the absence of dorsal mesoderm. If neural induction proceeds in the absence of dorsal mesoderm, it is considered a direct induction. Second, competent ectoderm should be responsive to the neural inducer at the gastrula stage, when dorsal mesoderm can still induce neural tissue (*15, 16*). In addition to displaying these activities, an endogenous neural inducer must be present at the right time and place to account for normal neural development. Finally, if a factor is required for neural induction, elimination of the activity should block normal neural development.

The *noggin* gene is expressed at the right time and place to be a neural inducer. The *noggin* cDNA was cloned because its RNA is able to rescue ventralized embryos (*17*). Ectopic *noggin* expression in the gastrula partially rescues ventralized embryos (*12*), an indication that noggin can mimic organizer signals. Zygotic *noggin* expression begins at the late blastula stage in the dorsal mesoderm and continues in the gastrula stage organizer (*17*). Later, *noggin* is expressed in the organizer derivatives, the head mesoderm, and notochord; the notochord directly underlies the neural plate and has been shown to be a potent neural inducer (*18, 19*). We now show that noggin activity satisfies the two criteria expected of an authentic neural inducer.

Direct neural induction by noggin. To determine whether noggin induces neural tissue directly, we added medium containing *Xenopus* noggin (*20*) to blastula animal caps and assayed the expression of neural and mesoderm specific transcripts. The markers used in a ribonuclease (RNase) protection assay (*21*) were NCAM (*22, 23*), which is a cell adhesion molecule expressed throughout the nervous system (*24*); an isoform of β-tubulin (*25–27*) expressed in the hind brain and spinal cord; a neurally expressed intermediate filament gene, XIF3 (*28*); and muscle actin (*29*). *Xenopus* noggin induces high levels of NCAM and XIF3 expression (Fig. 1B, lane 8), without inducing muscle actin (lane 13), while control medium fails to induce either muscle or neural tissues (lanes 7 and 12). In contrast, purified activin (*30*) induces muscle actin (lane 11) and all three neural markers (lane 6), demonstrating its ability to generate neural tissue indirectly. Noggin induces very little β-tubulin expression, while inducing high levels of NCAM, but activin induction has the converse effect.

Although noggin does not induce muscle in late blastula animal caps, noggin might induce other types of dorsal mesoderm. To address this, we asked whether noggin induces the expression of the early mesoderm markers goosecoid or brachyury (*Xbra*) (*31–33*). Goosecoid marks organizer tissue and subsequently head mesoderm, while *Xbra* appears to be expressed in all mesodermal precursors early, and subsequently is expressed in posterior mesoderm and notochord. Animal caps were treated at stage 9 (st9) and collected at stage 11 (st11), when expression of goosecoid and

Xbra in the whole embryo is high (*32, 33*). Noggin does not induce expression of these genes (Fig. 1C, lane 5), while the mesoderm inducer activin induces both goosecoid and *Xbra* expression (lane 4) (*32, 33*). Untreated animal caps show no expression of these mesodermal markers, and the amounts of RNA in the collected animal caps are comparable, as assessed by the ubiquitously expressed marker EF-1α (*34*). Thus, noggin induces neural tissue in the apparent absence of mesoderm as expected for a direct neural inducer.

Neural induction by purified noggin. To determine whether noggin protein is sufficient to induce neural tissue, COS cells were transfected with a human noggin expression plasmid, and noggin was purified to apparent homogeneity from the conditioned medium (Fig. 2) (*35*). This purified noggin is capable of neural induction (Fig. 3A, see below), therefore additional factors that may have been present in the crude medium are not required. Consistent with their 80 percent amino acid identity, both *Xenopus* and human noggin can induce neural tissue in *Xenopus*.

Purified noggin directly induces the expression of neural specific transcripts; however, it is possible that this is a transient induction. To address this, we treated animal caps with noggin and cultured to the late tailbud stage (st30) for antibody staining with the 6F11 antibody to NCAM, which marks the entire neural tube of a normal embryo (*36, 37*) (Fig. 4). Noggin-treated animal caps express this antigen, whereas untreated animal caps do not.

Neural induction at the gastrula stage. The organizer signal induces neural tissue from gastrula ectoderm. To assess the ability of noggin to induce neural tissue at different stages, we treated animal caps taken from blastula (st8), late blastula (st9), early gastrula (st10), and ventral animal caps from mid-gastrula (st10.5) embryos (Fig. 1A) with purified human noggin. Animal caps from similar stages were treated with activin medium (*30*) to contrast its effects with those of noggin (Fig. 3A). Noggin can induce neural tissue in animal caps taken from all of these stages without inducing either the notochord and somite marker, collagen type II (*38, 39*), or muscle actin (Fig. 3A). In this experiment responsiveness to noggin appeared to decline at the later stages, since there was a reduction of NCAM transcripts induced in animal caps. Upon repeating a similar experiment (*40*) twice, we found responsiveness to noggin at st8 and at st10.5 to be similar, indicating that there was not a loss of competence to noggin at the gastrula stage. Activin, however, promotes neural tissue formation only in conjunction with the induction of dorsal mesoderm, such as muscle and notochord. We have confirmed that the ability of activin to induce dorsal mesoderm, and consequently neural tissue (*41*), declines rapidly at the gastrula stage (Fig. 3A, lane 12) (*13, 15*). Thus, noggin induces neural tissue in animal caps at the time of normal neural induction, a time when mesoderm inducers are inactive.

Noggin can induce neural tissue in the absence of muscle; however, in some experiments noggin when added to gastrula (but not blastula) animal caps induced neural tissue and muscle. While the animal caps come from a region of the embryo that does not normally form mesoderm, there is evidence that gastrula animal caps receive a weak mesoderm-inducing signal. By itself, the signal that spreads into the gastrula animal cap is insufficient to induce mesoderm, but in the presence of either Xwnt-8 (*42*) or noggin, muscle can form. Since noggin can induce muscle from ventral mesoderm, it is not surprising that noggin

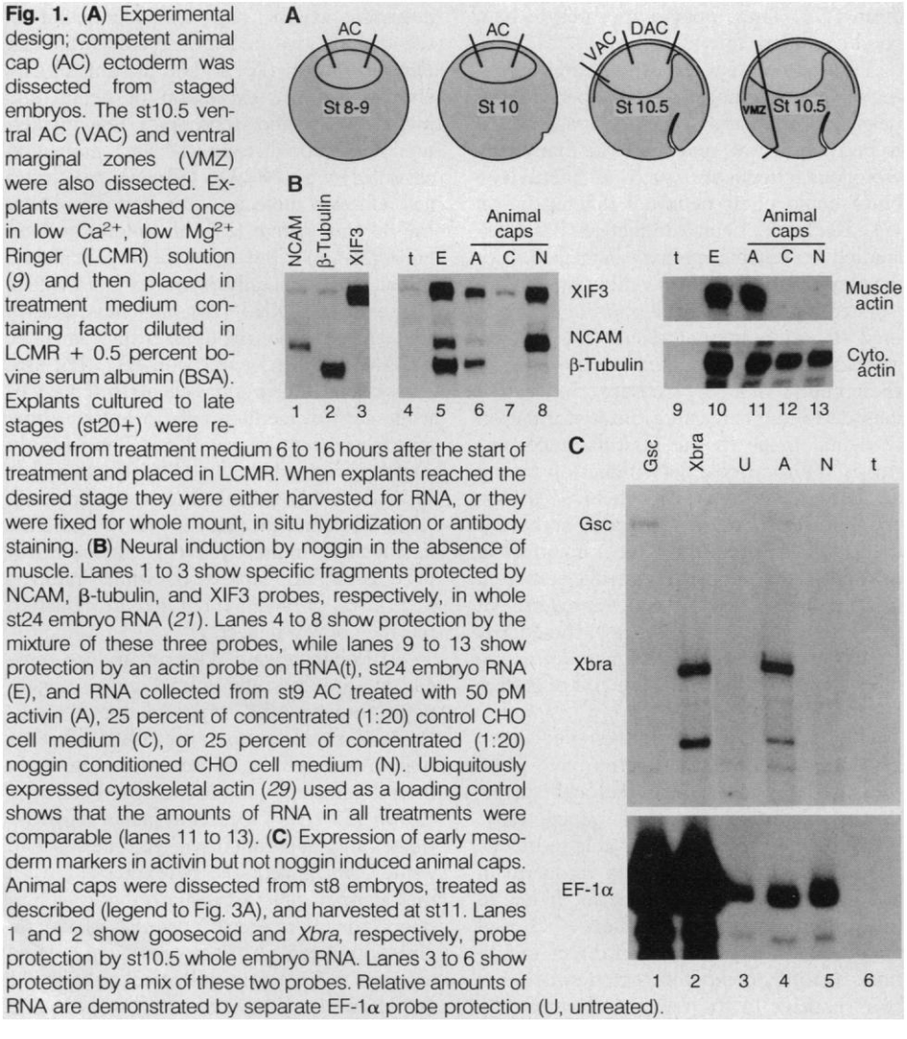

Fig. 1. (**A**) Experimental design; competent animal cap (AC) ectoderm was dissected from staged embryos. The st10.5 ventral AC (VAC) and ventral marginal zones (VMZ) were also dissected. Explants were washed once in low Ca^{2+}, low Mg^{2+} Ringer (LCMR) solution (*9*) and then placed in treatment medium containing factor diluted in LCMR + 0.5 percent bovine serum albumin (BSA). Explants cultured to late stages (st20+) were removed from treatment medium 6 to 16 hours after the start of treatment and placed in LCMR. When explants reached the desired stage they were either harvested for RNA, or they were fixed for whole mount, in situ hybridization or antibody staining. (**B**) Neural induction by noggin in the absence of muscle. Lanes 1 to 3 show specific fragments protected by NCAM, β-tubulin, and XIF3 probes, respectively, in whole st24 embryo RNA (*21*). Lanes 4 to 8 show protection by the mixture of these three probes, while lanes 9 to 13 show protection by an actin probe on tRNA(t), st24 embryo RNA (E), and RNA collected from st9 AC treated with 50 pM activin (A), 25 percent of concentrated (1:20) control CHO cell medium (C), or 25 percent of concentrated (1:20) noggin conditioned CHO cell medium (N). Ubiquitously expressed cytoskeletal actin (*29*) used as a loading control shows that the amounts of RNA in all treatments were comparable (lanes 11 to 13). (**C**) Expression of early mesoderm markers in activin but not noggin induced animal caps. Animal caps were dissected from st8 embryos, treated as described (legend to Fig. 3A), and harvested at st11. Lanes 1 and 2 show goosecoid and *Xbra*, respectively, probe protection by st10.5 whole embryo RNA. Lanes 3 to 6 show protection by a mix of these two probes. Relative amounts of RNA are demonstrated by separate EF-1α probe protection (U, untreated).

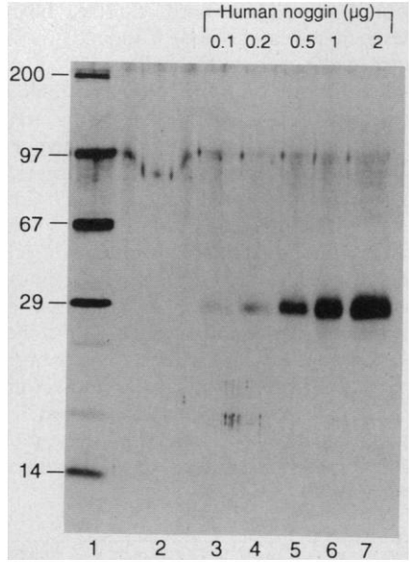

Fig. 2. Human noggin run on a 12 percent SDS-PAGE under reducing conditions. Proteins were visualized by silver staining. Lane 1 shows molecular size standards. Lanes 2 to 7 show 0, 0.1, 0.2, 0.5, 1, and 2 μg of purified human noggin.

added to ectoderm that has received a weak mesoderm-inducing signal also induces muscle. One interesting corollary of the induction of muscle is that the kinds of neural tissue seen in the explant are modified. Induction in explants that contain no muscle usually yields NCAM expression, but if muscle is present, expression of both NCAM and β-tubulin is seen. This phenomenon is demonstrated (i) in the secondary neural induction by activin in st9 animal caps (Fig. 1B) and (ii) in the comparison of neural tissue induced by noggin in ventral marginal zones and animal caps (see below, Fig. 5). In the ventral marginal zones and animal caps in which muscle is present, both NCAM and β-tubulin are expressed, whereas induced animal caps without muscle show only NCAM expression. This result suggests that the presence of mesoderm influences the type of neural tissue induced by noggin.

Neural induction after injection of DNA coding for *noggin*. To strengthen the arguments that noggin alone is the inducing activity and that noggin can induce neural tissue in gastrula animal caps, we have used an alternative experimental approach. *Noggin* expression was directed to gastrula stage animal caps by injecting the plasmid pCSKA-*noggin* into the animal pole of embryos at the one cell stage. In this plasmid, *noggin* is under the control of the cytoskeletal actin promoter, which turns on mRNA expression at the onset of gastrulation (*12*). The animal caps were dissected at the blastula stage and then matured to tailbud stages for molecular analysis. Animal caps injected with the *noggin* plasmid expressed NCAM without expressing either muscle or notochord markers (Fig. 3B). A control plasmid directing expression of *lacZ* induced no neural or mesodermal tissue as expected. This experiment shows that ectopic *noggin* expression is sufficient to induce neural tissue and refutes the possibility that a minor contaminant in the purified preparation was the active factor. Furthermore, *noggin* expressed in this manner is active at the gastrula stage, the time of neural induction in embryos.

Dose dependence. To determine how much noggin protein is required for neural inducing activity, we did a dose response experiment. In addition to determining the doses required for neural induction in animal caps, we examined the effect of noggin dose on the dorsalization of ventral marginal zones (VMZ) (*12*) in order to compare the doses required for these two types of inductions. Stage 9 animal caps or st10.5 VMZs were treated with purified human noggin, and NCAM and β-tubulin were used to assay neural induction, while muscle actin was used as a marker of dorsal mesoderm. Neural but not muscle induction by noggin occurs in animal caps only at a dose of 1 μg/ml (~10 nM). Since activin can induce muscle at picomolar doses, the noggin dose requirement for neural induction seems quite high. Ventral marginal zones are reproducibly induced to form muscle at doses of 50 ng/ml and above. This experiment shows that neural induction in animal caps requires a dose (1 μg/ml) that is 20 times higher than that required for dorsalization of VMZ (Fig. 5).

Several observations may account for the apparently high dose requirement. First, for maximal neural induction by dorsal mesoderm, the tissues must be left in contact through most of neurulation (*16*). Animal caps close up rapidly, inhibiting factor access (*43*) and consequently reducing the effective dose. The VMZs are much slower to close up, resulting in a longer exposure. This might account for both the large difference in dose required for the two kinds of induction and for the high absolute dose requirement for neural induction in animal caps. Second, it is likely that noggin is not the only neural inducer active in the embryo. The somites (*18, 19*) and the neural plate (*2, 44*) have neural inducing activity and noggin transcripts are not detected there. Thus, it is plausible that noggin is only one of several neural-inducing activities. Noggin induces neural tissue in ventral marginal zones at the same doses that dorsalize them to generate muscle, whereas other experiments show that induction of a similar amount of muscle at this stage by activin does not result in neural induction. Therefore, the mesoderm present may be producing an additional factor that reduces the noggin dose requirement for neural induction, yet by itself cannot induce neural tissue. Third, it may be that only a small fraction of the purified protein is active, and the experiment results in an overestimation of the amount of protein needed for neural induction. Finally, it is possible that the accessibility of exogenously added soluble noggin is lower than that of noggin protein secreted endogenously.

Patterning. Embryonic neural tissue initially forms as a tube with no obvious anterioposterior (A-P) pattern. Subsequently, brain structures, eyes, and the spinal cord form. Formation of A-P neural pattern requires the presence of dorsal mesoderm, whether it be adjacent to the responding ectoderm in a planar configuration (*23, 45–47*), or directly beneath it in a vertical interaction (*16, 19, 45, 48*). Both of these types of interactions occur normally, and both probably contribute to the resulting pattern (*49*). Noggin produced by the dorsal mesoderm could be responsible for inducing general neural tissue, or it may also be active in patterning. Initially we observed that noggin induces cement glands. In situ hybridization (*50*) confirms the expression of a cement gland specific transcript, *XAG-1* (*43*) in noggin treated, but not control treated animal caps (Fig. 4). Since cement

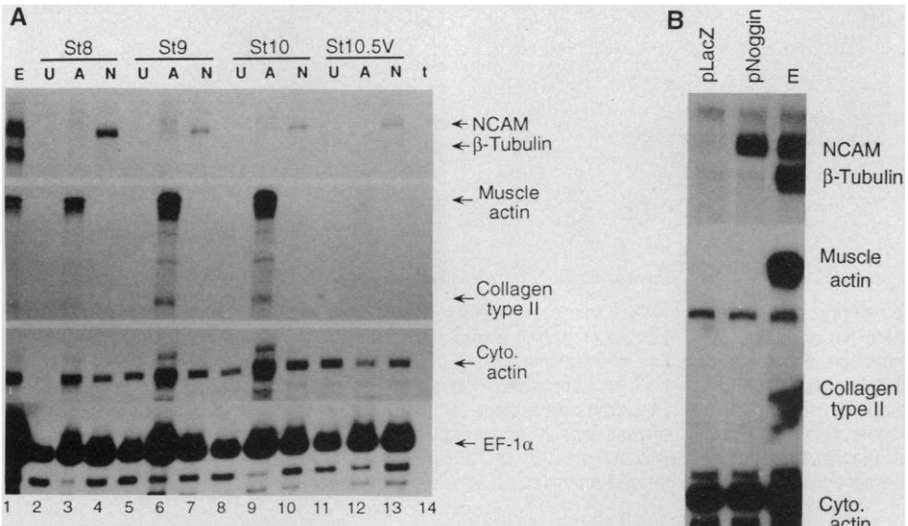

Fig. 3. (**A**) Staged treatments of animal caps with purified noggin and with activin; direct compared to indirect neural induction. Animal caps were dissected as shown in Fig. 1A and treated with LCMR plus 0.5 percent BSA (U), a 20 percent dilution of activin-conditioned medium (*30*) (A), or purified human noggin 1 μg/ml (N). RNA isolated from treated animal caps (lanes 2 to 13) along with st22 whole embryo RNA (lane 1) and tRNA (lane 14) was probed for NCAM, β-tubulin, muscle, cytoskeletal actins, collagen type II, and EF-1α. (**B**) Noggin expression directed to gastrula stages by plasmid injection induced neural tissue directly. One cell stage embryos were injected with 20 pg of pCSKA*lacZ* or pCSKA*noggin* (*12*) into the animal pole. Animal caps from injected embryos were dissected at st8 to st9 and cultured until st20 when they were harvested for analysis by RNase protection.

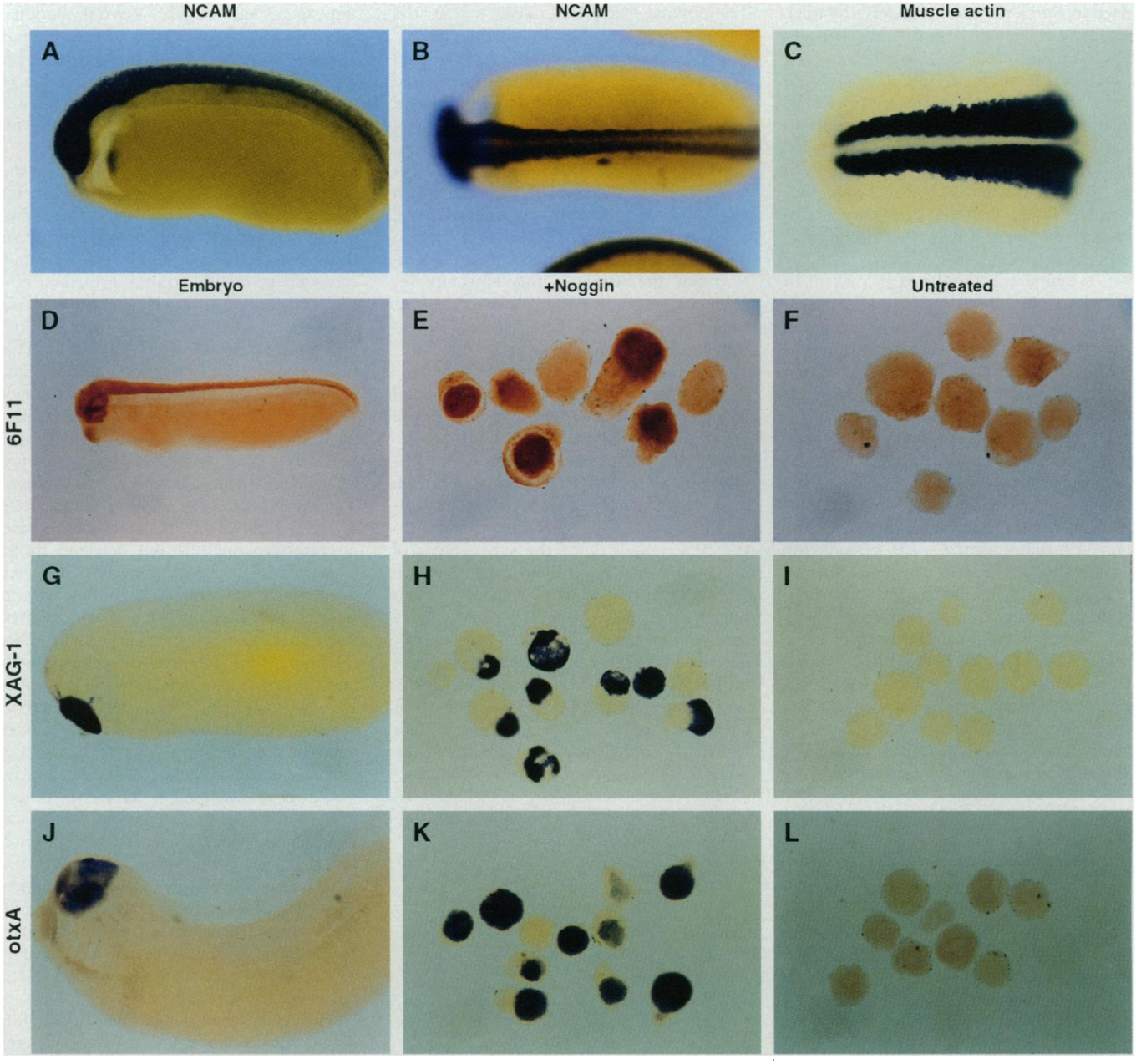

Fig. 4. In situ hybridization and antibody staining. Tailbud embryos stained for NCAM showing side and dorsal views (**A** and **B**); NCAM RNA was detected only in the neural tube and not the somites. For comparison, somites of a tailbud embryo stain with muscle actin, dorsal view (**C**). Neural specific 6F11 antibody staining (*62*) at st30 (**D** to **F**). Some cement gland pigment remained in these embryos after bleaching as seen in (D); however, this pigment is distinct from antibody staining. The inner mass of staining in the noggin-treated animal caps was due to the 6F11 antibody. Cement gland specific XAG-1 transcripts detected at st23 in whole embryos (**G**), human noggin treated (1 μg/ml) animal caps (17 of 30 explants were XAG positive) (**H**), untreated animal caps (1 of 31 explants were XAG positive) (**I**). Anterior brain *otxA* transcripts detected at st35 in whole embryos (**J**), human noggin treated (1 μg/ml) animal caps (14 of 20 explants were *otxA* positive) (**K**), untreated animal caps (0 of 16 explants were *otxA* positive) (**L**). No *en-2* (n = 28), *Krox20* (n = 25), or X1hBox6 (n = 10) expression was detected in noggin treated animal caps. Whole embryos have anterior to the left.

glands are induced organs of ectodermal origin found anterior to the neural plate, noggin may induce anterior neural structures also. To determine whether noggin induces patterned neural tissue, we used *otxA* from *Xenopus* (*51*) as a marker of anterior brain, *En-2* (*52*) as a marker of the mid brain–hind brain boundary, and *Krox-20* (*53*) as a marker of the third and fifth rhombomeres of the hind brain. Antibodies to X1Hbox6 mark posterior hind brain and spinal cord structures (*54*). Noggin induces *otxA* (Fig. 4); however, we have not detected *En-2*, *Krox20*, or X1Hbox6, suggesting that these more posterior markers are not induced by noggin. Noggin does not appear to induce expression of three antigens that are characteristic of various subclasses of differentiated neural cells. These include 2G9 (*18*), which stains most neural tissue, including peripheral neurons; Tor 25.44 (*55*), which stains sensory neurons; and Tor 23 (*55*), which stains a variety of neurons, including motor neurons. Furthermore, noggin treated animal caps cells failed to grow neuronal processes when plated on an appropriate growth matrix. Thus, noggin can induce neural

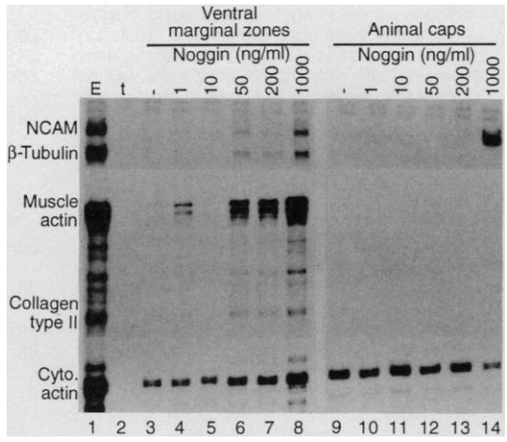

Fig. 5. Dose response of ventral marginal zones and animal caps to human noggin protein. st10.5 VMZs and st9 animal caps were dissected as shown in Fig. 1A, and treated with human noggin at 0, 1, 10, 50, 200, and 1000 μg/ml (lanes 3 to 8 and 10 to 15, respectively). RNA collected from treated explants and from control whole embryos, both aged to st26, was analyzed by RNase protection, with the probes NCAM, β-tubulin, actin, and collagen type II. In this experiment, muscle induction at the dose of 1 ng/ml was stronger than at 10 ng/ml, and there was a low level of muscle actin expression in the uninduced VMZs. In repeated experiments, muscle induction was observed only at the doses of 50 ng/ml and above.

tissue, but it fails to cause differentiation of mature neurons, a process that presumably requires additional factors.

To conclude, we have presented two kinds of evidence that noggin protein can induce neural tissue directly. First, neural tissue is induced in the absence of induced mesoderm. Second, neural tissue is induced in gastrula stage ectoderm that has lost competence to form mesoderm, but retains competence to be neuralized. Such ectoderm, when treated with activin, can no longer form neural tissue by an indirect induction. Since noggin is a secreted protein that is expressed in the Spemann organizer and its derivatives, noggin appears to be the only factor yet described that satisfies these criteria to be an endogenous neural inducer.

The type of neural tissue induced by noggin in the absence of mesoderm appears to be of an anterior nature because we detected *otxA*, but not *En-2*, *Krox20*, X1Hbox6, or β-tubulin expression. In the presence of mesoderm, however, the nature of neural tissue induced by noggin appears to be more caudal in that β-tubulin, a marker of hind brain and spinal cord, is expressed in addition to NCAM. These results may support the idea that neural pattern arises from an initial activation or neuralization that results in specification of forebrain, followed by transformation or caudalization to produce more posterior structures (*2*, *49*, *56*). Noggin's activity fits with a role in the initial neuralization.

Mechanism of noggin action. Dissociation of ectodermal cells for an extended period results in formation of neural tissue (*57*, *58*). This suggests that in normal ectoderm a signal may be distributed that prevents neuralization and promotes development of skin. Since inhibition of the activin receptor also promotes neuralization (*8*), this signal may be mediated in part by the activin receptor. That noggin also induces neural tissue by antagonizing the activin receptor seems unlikely because at the blastula stage activin synergizes with noggin to form dorsal mesoderm (*59*). Cloning of the noggin receptor should clarify the signal transduction pathway by which noggin mediates neural induction.

Noggin has been reported to contain a conserved spacing of seven cysteines characteristic of a motif found in Kunitz class protease inhibitors (*60*). The possibility that noggin acts as a protease inhibitor rather than interacting directly with a receptor is worth consideration, since dorsal-ventral pattern formation in *Drosophila* requires a protease cascade to initiate ventral-specific signaling (*61*).

We have shown that noggin has direct neural inducing activity. Noggin is made at the correct place and time to be an endogenous neural inducer. It can induce neural tissue at the gastrula stage, which is the time of endogenous neural induction. It is not yet clear that the physiological concentration of noggin is sufficient to be active in neural induction. To prove that noggin is a physiological neural inducer, it will be necessary to inhibit the noggin signal, or signaling pathway. However, noggin is found in the embryo and has the activities expected of an endogenous neural inducer. The noggin protein provides a valuable reagent to study the signal transduction pathway and early events in neural tissue formation.

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20. Conditioned medium was made from Chinese hamster ovary (CHO) cells after selecting for CHO cells stably transfected with *Xenopus* noggin. Dihydrofolate reductase-deficient (dhfr$^-$) CHO parental cells (J. Papkoff, Syntex Research) were transfected with a *Xenopus* noggin expression plasmid. Selection and amplification were carried out as described (*63*). The presence of noggin transcripts was tested by RNA Northern analysis. Clone B3 secreted noggin protein, since B3 conditioned medium was capable of dorsalizing ventral marginal zones. Furthermore, labeling B3 proteins with [^{35}S]methionine revealed noggin protein as a band of ~30 kD on reducing SDS-PAGE, and a band of 60 kD on nonreducing SDS-PAGE (indicating that it forms the expected dimer). These properties matched those of the noggin protein previously produced in *Xenopus* oocytes (*12*). B3-conditioned medium was collected in a mixture of one part of α-modified Eagle medium and nine parts of serum-free medium (CHO-S-SFMII) (Gibco-BRL). The cells conditioned the medium for 3 days. Control medium from parental cells (CHO dhfr$^-$) was collected in the same way. The medium was concentrated (Centriprep-10; Amicon) to 5 percent of its original volume.
21. RNase protection data were obtained as described (*64*), except that the preparation was digested at room temperature (22°C) with RNase T1 (Calbiochem 556785) alone at 10 U/ml. Animal caps (20 to 30) were harvested for each lane, and 80 percent of this material was used for neural markers and 10 percent for muscle actin and collagen type II. For goosecoid and brachyury, 20 caps were used. Exposures ranged from 12 hours to 5 days. In all cases, films were sensitized by pre-flashing. To confirm the absence of expression of mesoderm markers, phosphor imaging, a more sensitive detection method, was used.
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40. Animal caps at st10.5 were obtained by cutting animal caps at st8 or st9 and aging them to st10.5 in a 1:1 mixture of LCMR and Ca^{2+}- and Mg^{2+}-free medium (CMFM) (*65*). This medium kept the animal caps from closing up, without dissociation of cells, so that factors could be applied at later stages (st10.5). Activin treatment of these animal caps confirmed the loss of competence to induce mesoderm at st10.5, indicating that animal cap aging is parallel to whole embryo aging. Noggin treatment of these aged animal caps resulted in NCAM induction as strong as that of animal caps treated at st8, demonstrating that there was no loss of responsiveness to noggin in gastrula animal caps. Control medium did not induce NCAM.
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51. To isolate *Xenopus otx* clones, a tadpole head cDNA library (*36*) was screened with a mouse *otx* cDNA (S.-L. Ang and J. Rossant, Toronto) at low stringency. The clone used to make the probe, pXOT21.2, represents a class designated *otxA*. By in situ hybridization, transcripts are first detected before gastrulation throughout the marginal zone, but quickly become restricted to the superficial layer on the dorsal side. During neurulation a large anterior domain including both neural and nonneural tissues expresses the gene. After a decline in expression in the tailbud tadpole, the gene is again expressed specifically in the brain and eyes. At this stage there is no *otxA* expression in the mesoderm.
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