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Vertebrate Embryonic Induction: Mesodermal and Neural Patterning

Daniel S. Kessler and Douglas A. Melton

Within the fertilized egg lies the information necessary to generate a diversity of cell types in the precise pattern of tissues and organs that comprises the vertebrate body. Seminal embryological experiments established the importance of induction, or cell interactions, in the formation of embryonic tissues and provided a foundation for molecular studies. In recent years, secreted gene products capable of inducing or patterning embryonic tissues have been identified. Despite these advances, embryologists remain challenged by fundamental questions: What are the endogenous inducing molecules? How is the action of an inducer spatially and temporally restricted? How does a limited group of inducers give rise to a diversity of tissues? In this review, the focus is on the induction and patterning of mesodermal and neural tissues in the frog *Xenopus laevis*, with an emphasis on families of secreted molecules that appear to underlie inductive events throughout vertebrate embryogenesis.

A fundamental experiment in the history of embryology was the organizer transplant of Spemann and Mangold (*1*). In this impressive demonstration of induction in the newt, transplantation of a gastrula dorsal blastopore lip to a region fated to form ventral mesoderm resulted in formation of a second body axis. In the chick, fish, and mouse, transplants of the node, the anatomical equivalent of the amphibian blastopore lip, resulted in similar axial organization (*2–5*). This type of experiment became a much discussed example of induction (*6*) and has challenged biologists for decades to explain how one group of cells controls the fate of its neighbors.

Over the past century, numerous inductive events have been described in vertebrates including multiple interactions between the three germ layers (endoderm, mesoderm, and ectoderm) and within each germ layer. Reciprocal inductions occur throughout early development, and later multiple mesenchymal-epithelial inductions underlie organogenesis (*7*). In essence, virtually every vertebrate tissue and organ is formed by some type of induction. Mesoderm and neural induction have received considerable attention in recent years, and the molecules and principles used in these early events may be relevant to subsequent tissue and organ formation. With this in mind, we examine current advances in mesoderm and neural induction in vertebrates.

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Mesoderm Induction

The importance of endoderm in the induction of mesoderm in the frog, *Xenopus laevis*, was established by Nieuwkoop and colleagues. In isolation, explanted blastula animal and vegetal pole cells form only ectoderm and endoderm, respectively, but ectoderm can be induced to form mesodermal structures in recombinants containing both presumptive ectoderm and endoderm (*8–10*). In addition, although explants of the marginal zone (presumptive mesoderm; Fig. 1) from a 32-cell stage embryo fail to form mesoderm, blastula stage explants will form mesoderm, implicating a progressive interaction between endoderm and ectoderm to form mesoderm (*11*, *12*). These observations suggest that vegetal endoderm produces a mesoderm-inducing signal during cleavage stages.

In addition to inducing mesoderm, vegetal endoderm can confer a dorsal-ventral pattern on mesoderm. Dorsal vegetal cells induce dorsal mesoderm (notochord and muscle), whereas lateral and ventral vegetal cells induce ventrolateral mesoderm (mesenchyme, blood, and small amounts of muscle) (*13–15*). In addition, transplanted dorsal vegetal blastomeres can induce ectopic dorsal axial structures, an activity that has led to these cells being designated the endodermal organizer or Nieuwkoop center (*16–19*).

In *Xenopus*, the dorsal-ventral axis is established at fertilization with sperm entry stimulating a reorganization of egg contents by cortical rotation, leading to demarcation of future dorsal tissues opposite the site of sperm entry. Cortical rotation, a displacement of the surface (or cortex) of the egg relative to the inner cytoplasm, is thought to result in the formation of a "dorsal determinant" in the presumptive endoderm (Fig. 1) (*20*). Disruption of cortical rotation by ultraviolet (UV) irradiation results in the loss of dorsal axial structures (*19–22*). Both cortical rotation and subsequent axis formation can be rescued by manual tipping of the egg, which causes gravity-induced rearrangements (*23*, *24*). Gravity-driven rotation during the blastoderm stage is also responsible for axis formation in the chick (*25*).

Mesodermal patterning continues during gastrulation, as evidenced by the fact that as late as the gastrula stage explanted lateral marginal zone tissue forms ventral mesoderm rather than the intermediate mesodermal tissues (muscle and kidney) predicted from the fate map. Organizer tissue can induce ventral and lateral marginal zones to form intermediate mesoderm, suggesting that the gastrula stage organizer "dorsalizes" neighboring ventral mesoderm (Fig. 1) (*15*, *26*, *27*). Furthermore, examination of muscle formation indicates that local communication within a single tissue type is also required for differentiation. These studies showed that blastula or gastrula explants of presumptive muscle fail to form differentiated muscle if fewer than 100 cells are present. This community effect (*28*, *29*), distinct from other inductive interactions, appears to regulate the coordinate differentiation of specified mesodermal tissues and may be essential to the orderly patterning of the marginal zone (*30*, *31*).

These studies illuminate the cellular basis of mesoderm induction. Cortical rotation generates a dorsal determinant that resides in dorsal vegetal blastomeres, the endodermal organizer (Nieuwkoop center), and that subsequently induces formation of the mesodermal organizer (Spemann organizer) (Fig. 1). Although promising candidates for endogenous mesoderm inducers have been identified, it has not yet been possible to assign them to specific inducing functions in vivo.

Inducer action may be restricted by controlling inducer production and diffusion within the embryo (*32*). In addition, the competence (responsiveness) of target cells may be regulated and, for example, is thought to contribute to the restriction of mesoderm formation to the marginal zone, despite the capacity of animal pole cells to respond to mesoderm induction until the gastrula stage (*33*). The induction of diverse tissue types by a limited group of inducers is perplexing. Perhaps this can be accomplished by altering the response to an individual inducer, and a mechanistic understanding may be found in this issue: Can temporally or spatially restricted expression of distinct receptors, signal transducers, or nuclear factors result in dissimilar responses to a single inducer?

Mesoderm-Inducing Factors

The ease of culturing amphibian embryonic tissues has facilitated the identification of vertebrate gene products active in mesoderm induction and patterning. The principal mesoderm induction assay entails exposing blastula animal pole explants to candidate inducing factors, either by incubation in protein or injection of messenger RNA (mRNA). In this assay, based on Nieuwkoop's animal-vegetal recombinants (*8*), factors substitute for the vegetal signal, and induction is assessed by morphology, histology, and expression of tissue-specific markers. Although this protocol may identify factors that can induce or pattern mesoderm, the function of these factors, if any, in normal development must still be addressed. The embryonic expression and activity of various secreted and nuclear factors has been reviewed elsewhere (*34*, *35*), and here we summarize the evidence supporting a role for several secreted molecules in mesoderm induction.

Vg1. The studies of Nieuwkoop demonstrated the existence of a vegetally localized mesoderm-inducing factor. Vg1 is a maternally expressed member of the transforming growth factor–β (TGF-β) superfamily, localized to the vegetal pole of *Xenopus* oocytes and cleavage stage embryos (Fig. 2, A and B) (*36*–*38*). TGF-β–related molecules form disulfide-linked dimers that are subsequently cleaved, releasing the mature COOH-terminal peptide as a secreted bioactive dimer (*39*, *40*). Although the Vg1 precursor protein is abundantly expressed, the cleaved mature form has yet to be definitively detected, suggesting that processing of Vg1 is tightly regulated during development. Consistent with Vg1 activity being regulated at the level of posttranslational processing, microinjection of embryos with Vg1 mRNA results in little or no mature Vg1 protein and has no effect on the differentiation of animal pole explants or intact embryos (*37*, *41*, *42*).

Production of mature Vg1 can be directed by hybrid Vg1 molecules constructed by fusion of the NH_2-terminal pro-region and tetrabasic cleavage site of a bone morphogenetic protein (BMP) to the COOH-terminal mature region of Vg1. Microinjection of BMP-Vg1 mRNA directed synthesis and processing of this hybrid molecule, resulting in efficient production of mature Vg1 protein. Expression of processed Vg1 in animal pole explants strongly induced dorsal mesoderm, and neural tissue is obtained by secondary interactions between dorsal mesoderm and ectoderm (discussed below). However, blood, a ventral mesodermal tissue, is not induced, suggesting that additional factors are required during normal development. Injection of BMP-Vg1 mRNA into UV-ventralized embryos directed formation of a complete dorsal axis. The injected cells populate the endoderm of the rescued embryo, suggesting that Vg1 has activities similar to the endodermal organizer (*41*, *42*). In addition, treatment of animal pole explants with soluble, mature Vg1 induced formation of "embryoids" displaying axial organization and head structures (Fig. 2, C and D) (*43*).

Processed Vg1 is unique in the ability to induce dorsal mesoderm and organize a complete dorsal axis. These observations suggest that a transient or localized production of mature Vg1 may be sufficient for induction of dorsal mesoderm. It has been proposed that cortical rotation may stimulate Vg1 processing in dorsal vegetal cells, perhaps by localized translation or activation of posttranslational processing (*35*, *41*, *44*). Detection of endogenous, mature Vg1 and a description of its temporal and spatial regulation is now needed to substantiate the role of Vg1 in vivo.

A truncated, dominant inhibitory activin type II receptor fully inhibits the mesoderm-inducing activity of processed Vg1 in animal pole explants, suggesting that inhibition of endogenous mesoderm formation by this mutant receptor may be due to inhibition of Vg1 signaling (*43*, *45*, *46*).

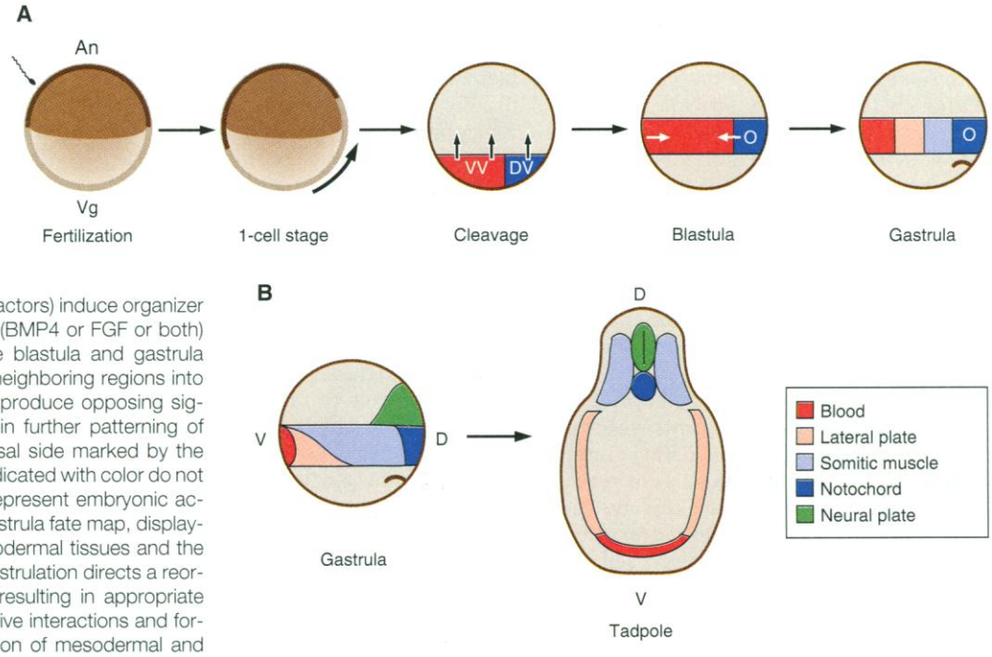

Fig. 1. Induction and patterning of the mesoderm in *Xenopus*. (**A**) The animal-vegetal (An-Vg) axis forms during oogenesis. After fertilization, cortical rotation (counterclockwise arrow) results in establishment of the dorsal-ventral axis. During cleavage, the dorsal-vegetal (DV; Nieuwkoop center) and ventral-vegetal (VV) regions of the prospective endoderm induce the overlying marginal zone cells to form mesoderm. Dorsal-vegetal signals (Vg1, activin, noggin, or Xwnt-11, or a combination of these factors) induce organizer mesoderm (O), and ventral-vegetal signals (BMP4 or FGF or both) induce ventral mesoderm. During the late blastula and gastrula stages, organizer signals (noggin) convert neighboring regions into lateral mesoderm; ventral mesoderm may produce opposing signals (BMP4 or Xwnt-8 or both), resulting in further patterning of mesoderm. An early gastrula with the dorsal side marked by the blastopore lip at the right is shown. Areas indicated with color do not correspond to anatomical structures but represent embryonic activities or regions of the fate map. (**B**) The gastrula fate map, displaying the dorsal-ventral (D-V) pattern of mesodermal tissues and the presumptive neural plate. The process of gastrulation directs a reorganization of the embryonic germ layers, resulting in appropriate positioning of tissues for subsequent inductive interactions and formation of the mature body. The organization of mesodermal and neural tissues is shown in a depiction of a tadpole transverse section.

Three observations, the potent activity of mature Vg1, the localization of Vg1 mRNA and protein to presumptive endoderm (the endogenous source of mesoderm-inducing signals), and the inhibition of its mesoderm-inducing activity by an inhibitor of endogenous mesoderm induction, establish Vg1 as a strong candidate for the natural inducer of dorsal mesoderm during *Xenopus* development.

Activin. Treatment of animal pole explants with soluble activin protein from a variety of sources induces dorsal mesodermal tissues (*47*–*49*). At high doses, "embryoids" are formed that display a rudimentary axial pattern and head structures (*48*, *49*). Despite the absence of maternal activin transcripts, an activin-like activity is detectable in oocytes and early embryos (*50*) and may be transported into oocytes from surrounding follicle cells (*51*, *52*). Injection of activin mRNA induces dorsal mesoderm in animal pole explants. Unlike expression of processed Vg1, activin mRNA injection organizes only a partial dorsal axis, lacking notochord and head structures (*49*).

Treatment of dispersed animal pole cells with activin, followed by reaggregation of the cells results in a homogeneous but highly dose-dependent response. Increasing activin concentration as little as 1.5-fold results in a dramatic alteration in molecular marker expression and tissue differentiation, causing, for example, a change from homogeneous muscle formation to notochord formation (*53*, *54*). On the basis of these observations, it has been proposed that an endogenous gradient of activin could direct mesodermal patterning (*54*). The initial response of dispersed cells may not be constrained by precise thresholds, and a mixed early response may be transformed into a precise pattern by cell interactions after reaggregation (*30*, *31*, *55*). Although these studies provide some insight into mesodermal patterning, the relation between normal development and results from dissociated cells must be considered with care, because dispersion alters the response of cells to inducers (*31*). In addition, the existence of an activin gradient during early *Xenopus* development is as yet unsupported, although other localized factors, perhaps Vg1, could function in this manner.

The axial organization obtained after incubation of animal pole explants with soluble activin relies on a pre-existing dorsal-ventral pattern within animal pole tissue. Prospective dorsal animal pole explants can form notochord, whereas ventral explants do not. This prepattern, lost upon cell dispersion, shows that the responding cells contain patterning information, and that pattern is unlikely to be established by inducer concentration alone (*56*). This prepattern, like dorsal-ventral patterning throughout the embryo, is a result of cortical rotation and can be abolished by UV irradiation (*57*, *58*).

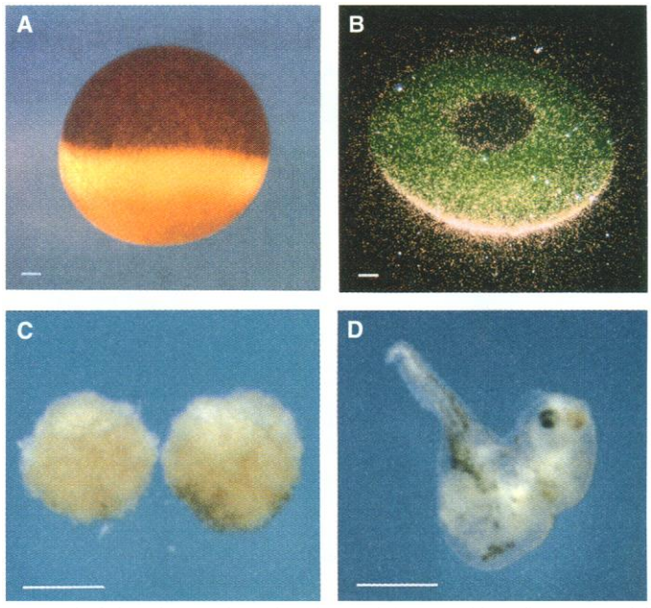

Fig. 2. Vg1 localization and activity. (**A**) The *Xenopus* oocyte has a single axis of polarity, evident from the pigmented animal pole and the unpigmented vegetal pole. (**B**) Vg1 mRNA, detected by in situ hybridization, is tightly localized to the vegetal pole of oocytes. The centrally located circle is the germinal vesicle, and the green coloration of the oocyte is due to Geimsa stain (*38*). (**C**) Control blastula animal pole explants, cultured to the late tadpole stage (stage 40), differentiate into a ball of ciliated epidermis. (**D**) Treatment of explants with soluble, mature Vg1 protein (*151*) induces formation of "embryoids" composed of dorsal mesodermal and neural tissues with rudimentary axial organization and head structures, including pigmented eye. Similar "embryoids" are observed after activin treatment (*48*, *49*). Scale bars, 100 μm. [Oocyte photograph courtesy of P. Klein]

Receptors for the TGF-β family are assigned to one of two classes (type I or type II), encode a cytoplasmic serine-threonine kinase, and function as heterodimeric complexes (*40*, *59*). In *Xenopus*, the activin type II receptor mRNA is maternally expressed and uniformly distributed (*60*). Expression of a truncated receptor that lacks the cytoplasmic kinase domain blocked activin activity in the animal pole assay. Microinjection of this dominant inhibitory receptor into the early embryo fully inhibited mesoderm formation, assessed with both histological and molecular criteria (*61*). This observation supports a role for activin in endogenous mesoderm induction. However, given the heterodimeric nature of the TGF-β receptor family, inhibition of signaling by overexpression of a truncated receptor may result from direct ligand binding or inactivation of an associated subunit within an existing or novel receptor complex. Therefore, in the absence of information regarding the structure of receptor complexes, it is difficult to draw any conclusions about the specificity of a truncated receptor for an individual TGF-β–related ligand. In fact, the truncated activin receptor does block signaling by additional members of the TGF-β superfamily (see Vg1 and BMP4).

A natural inhibitor of activin function is the activin-binding protein follistatin (*62*). *Xenopus* follistatin is maternally expressed and blocks activin-mediated induction of animal pole explants (*63*, *64*). However, unlike the truncated activin receptor, overexpression of follistatin in early embryos does not block mesoderm induction (*45*, *65*), nor does it inhibit mesoderm induction by Vg1 (*43*, *45*, *65*). These results raise questions about the role of activin in endogenous mesoderm induction.

Activin function has been examined in a number of vertebrate systems. In the chick, activin mRNA is expressed in the hypoblast (inducing tissue) during the period of axial mesoderm formation, and soluble activin induces axial organization in the epiblast (responding tissue) (*66*). Expression of a dominant inhibitory activin mutant in fish blocks mesoderm and axis formation, implicating maternal activin protein in these events (*67*). However, the interaction of this mutant activin protein with other inducing factors must be examined to determine the specificity of this inhibition. In the mouse, directed mutation of the gene encoding activin βB causes no serious developmental defects, but a second activin gene is up-regulated, suggesting that this redundancy compensates for the mutation (*68*). Preparation of double-mutant mice may elucidate the role of activin in mouse development. Furthermore, maternal protein may rescue this mutation, complicating the interpretation of these mouse studies (*69*).

Bone morphogenetic protein (*BMP*). The BMPs, capable of stimulating bone formation, are maternally expressed in *Xenopus* embryos (*70*–*72*). In mesoderm induction assays, BMP4 induces ventral mesodermal tissues, including blood and ventrolateral

molecular markers (*46, 70, 72–74*). In addition, BMP4 expression in animal pole explants modifies induction by activin, resulting in suppression of dorsal mesoderm formation. In the embryo, BMP4 inhibits development of dorsoanterior structures, consistent with a "ventralizing" effect on mesoderm (*72, 73*).

The recent isolation of vertebrate BMP2 and BMP4 receptors has allowed the examination of BMP function in the embryo. Expression of a dominant inhibitory mutant, similar in design to the truncated activin receptor, blocked mesoderm induction by BMP4 in animal pole explants (*74*) and BMP4-mediated ventralization in embryos (*75*). Embryonic overexpression did not block endogenous mesoderm induction but caused a "dorsalization" of mesoderm, resulting in formation of ectopic dorsal axial structures (*74, 75*). These studies indicate that BMP signaling is not essential for mesoderm induction but is involved in the dorsoventral organization of mesoderm and in the formation of ventral mesoderm in particular. Although the truncated BMP receptor does not block activin activity, the specificity of inhibition must be carefully considered in these overexpression experiments, as discussed above. The truncated activin receptor also inhibits mesoderm induction by BMP4 (*46*), suggesting that in blocking endogenous mesoderm formation, the truncated activin receptor inhibits several distinct TGF-β–related factors.

An additional TGF-β–related gene implicated in the induction of mesoderm is the mouse *nodal* gene. Embryos homozygous for a disruption of the *nodal* gene fail to form organized mesodermal tissues, and as a consequence, primitive streak formation and axial organization does not occur. This gene is expressed before the onset of gastrulation and is subsequently expressed in cells populating the node, consistent with a role in mesodermal and axial development (*76*).

Fibroblast growth factor (FGF). This growth factor was the first purified molecule demonstrated to induce mesoderm in animal pole explants (*77*). Transcripts and protein for several forms of *Xenopus* FGF and FGF receptors are maternally expressed (*78*). FGF protein induces ventrolateral mesoderm, including mesenchyme and small amounts of muscle, but fails to induce dorsal mesoderm and the definitive ventral tissue, blood.

Expression of a dominant inhibitory FGF receptor inhibits mesoderm induction by FGF in animal pole explants. In whole embryos this dominant inhibitory receptor causes defects in trunk and posterior development without affecting anterior development (*79*). Therefore, FGF signaling is required for formation of trunk and posterior mesoderm, including notochord and muscle, during normal development. The inhibition of notochord formation by the truncated FGF receptor is unexpected, given the inability of FGF to induce notochord efficiently in animal pole explants. However, activin signaling is dependent on functional FGF signaling (*80*). Therefore, FGF signaling is apparently necessary for the response of trunk and posterior cells to an activin-like induction, but formation of dorsoanterior mesodermal structures is not dependent on FGF signaling. It is not clear whether activin and FGF signaling pathways are stimulated within the same cell or at distinct periods within a common cell lineage, and further studies are required for an understanding of these interactions.

Wnt. The wnt family consists of genes related to the transforming int genes and the *Drosophila* segment polarity gene *wingless* (*81, 82*). The induction of ectopic dorsal axial structures after mouse *wnt-1* overexpression in *Xenopus* embryos stimulated the examination of *Xenopus* wnt (Xwnt) genes (*83–87*). Injection of Xwnt-1 or Xwnt-8 mRNA induced complete dorsal axis formation including head structures (*84, 85, 88*), but Xwnt-8 mRNA has little or no mesoderm-inducing activity in blastula animal pole explants, suggesting that ectopic axis formation results from modification of the dorsal-ventral patterning of existing mesoderm. Additional studies indicate that wnt genes can act as competence modifiers, altering the response of cells to induction and enhancing the formation of dorsal mesodermal structures (*89*).

However, the expression pattern of *Xwnt-8* is not consistent with a dorsal modifier role during development. In the gastrula, *Xwnt-8* transcripts are detected in ventral, not dorsal, mesoderm. Furthermore, overexpression of *Xwnt-8*, limited to later stages (zygotic expression), induces ventrolateral mesoderm. It has also been proposed that *Xwnt-8* can convert the fate of dorsal tissues to ventral (*90*). The paradoxical observation of both dorsalizing and ventralizing activity is not understood, but perhaps *Xwnt-8* dorsalizes by mimicking a maternal Xwnt molecule. Maternally expressed Xwnt genes have been described (*86, 87*), and one, *Xwnt-11*, is vegetally localized and induces partial dorsal axes lacking notochord and head structures (*86*).

Expression of the mouse *wnt-3A* gene is restricted to the primitive streak, the presumptive axial mesoderm (*91, 92*). *Wnt-3A* function is required for caudal mesodermal development as homozygous disruptions of this gene result in severe defects in posterior development, producing embryos lacking organized mesodermal structures posterior to the forelimb (*92*). A more complete understanding of the diverse developmental role of wnts would be facilitated by identification of wnt receptors and signaling components. The demonstrated function of the *Drosophila wingless* gene in vertebrate systems suggests a promising approach to this problem (*93*). Genetic analyses in *Drosophila* have identified a number of genes interacting with *wingless*, including *armadillo, dishevelled, porcupine, patched,* and *zeste-white3* (*94*), and studies of vertebrate homologs of these putative wnt signaling molecules are currently underway.

Noggin. The gene *noggin* encodes a secreted polypeptide identified in a screen for factors capable of rescuing a dorsal axis in UV-ventralized embryos. Expressed uniformly in *Xenopus* oocytes and cleavage embryos, noggin transcripts are localized to the dorsal blastopore lip (Spemann organizer) at the gastrula stage, and later in the notochord. Like processed Vg1 and Xwnt-8, injection of UV-ventralized embryos with noggin mRNA induces an endodermal organizer that directs formation of a complete dorsal axis with head structures (*95*). Noggin does not induce mesoderm in animal pole explants but can dorsalize gastrula ventral marginal zone explants, mimicking the effect of the gastrula organizer in recombinants of dorsal and ventral marginal zones (*96*). Thus, noggin can function as a dorsal modifier of mesodermal pattern, suggesting a role in organizer function. Experimental interference with endogenous noggin function is necessary to verify this interpretation.

Despite the obvious complexity of the described inducers, a rough correspondence can be made to activities observed in tissue recombination studies (Fig. 1). Even if future work indicates that particular factors do not play a role in endogenous mesoderm induction, a substantial overlap in activities will remain. This redundancy is twofold in that unrelated factors have similar activities and related factors have distinct activities. Of course, functional redundancy appears to be a fundamental aspect of vertebrate development and has been clearly demonstrated in mouse gene disruption studies. Important questions include the following: What is the order of action of endogenous inducers? How are these activities spatially regulated? How do multiple inducers result in the appropriate patterning of mesoderm into distinct tissues? An examination of downstream targets will provide some answers, and in fact, several nuclear factors have been described, including *goosecoid* and *brachyury* (*T*), that have mesoderm-inducing or -patterning activity (*97*). This diversity of activities presents developmental biologists with the challenging task of deriving a working model of mesoderm induction from a wealth of experimental data. Moreover, recent studies have demonstrated the induction of endodermal tissue by

mesoderm-inducing factors (98). This intriguing observation recalls the importance of endoderm in mesoderm induction and should stimulate the analysis of endoderm formation.

Neural Induction

Mesoderm induction and patterning are intimately connected with the complex cell movements of gastrulation. Axial mesodermal structures are formed from the dorsal marginal zone, which leads gastrulation movements and undergoes convergent extension to produce the dramatic elongation of dorsal mesoderm along the future anterior-posterior (A-P) axis (99). This reorganization of the germ layers ends with endoderm innermost, ectoderm at the surface, and mesoderm positioned in between (*100*). The transformation of tissue position generated by gastrulation permits the additional inductive events of later development, including aspects of neural induction.

The vertebrate central nervous system originates at gastrulation with an interaction between dorsal ectoderm and involuting dorsal mesoderm, first demonstrated in amphibia by Spemann and Mangold's (*1*) organizer transplant experiment (*6*, *101–103*). Two modes of signaling have been suggested to operate during neural induction. Involuting chordamesoderm (presumptive notochord) may vertically signal overlying ectoderm, or mesodermal organizer signals may travel horizontally within the plane of the ectoderm (Fig. 3). Evidence exists for both types of signals, but the identity and endogenous role of these signals remains to be established. In addition, the two modes of signaling do not necessarily reflect the existence of multiple inducers, because a single inducer may act in both a vertical and planar manner.

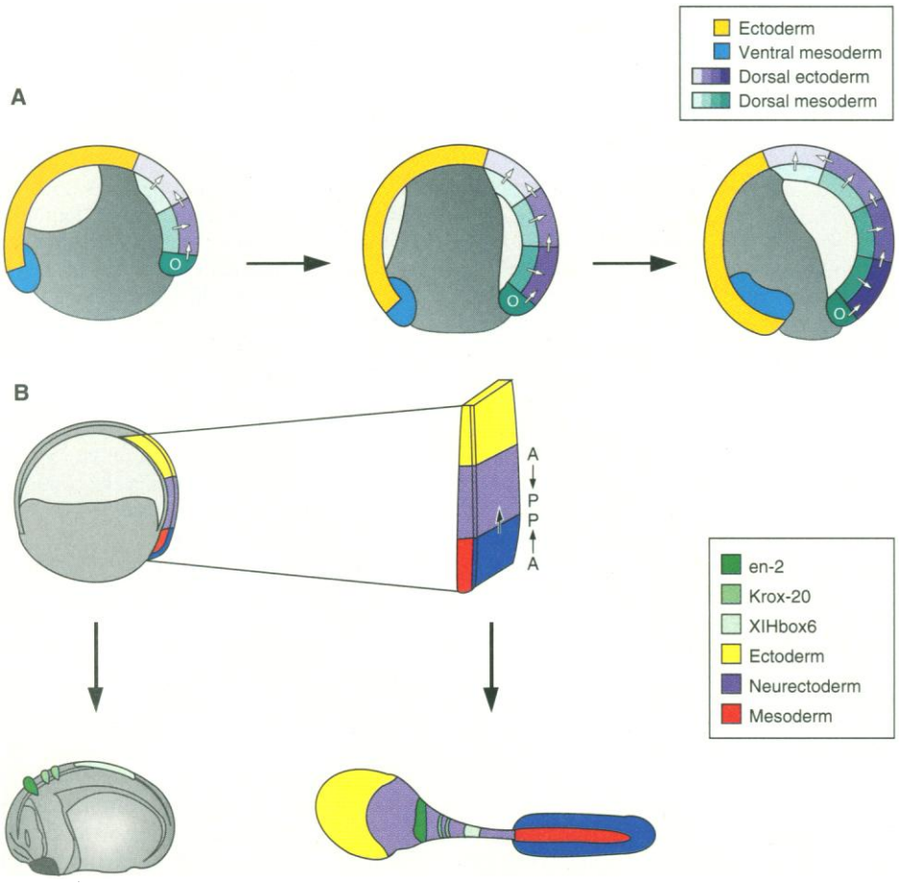

Fig. 3. Neural induction in *Xenopus*. (**A**) During gastrulation, dorsal mesoderm involutes and migrates underneath the ectodermal surface of the gastrula. As dorsoanterior mesoderm involutes, the adjacent ectoderm is initially induced to form anterior neural tissues (perhaps by noggin or follistatin or both). The mesoderm migrates toward the former animal pole, and newly contacted ectoderm is also induced to form anterior neural tissue. Ectoderm is progressively contacted by more posterior mesoderm resulting in posterior neural elements (darker shades of purple). As a result of this process, the anteroposterior pattern of induced neural tissue reflects the anteroposterior character of underlying dorsal mesoderm. Both vertical and planar signals emanating from dorsal mesoderm have been implicated in neural induction. Areas indicated with color do not fully correspond to anatomical structures, but represent embryonic activities or regions of the fate map. O, organizer mesoderm. (**B**) Planar induction of neural pattern demonstrated with Keller explants. Dorsal explants of the early gastrula, containing presumptive dorsal mesoderm, neurectoderm, and ectoderm, are cultured flat to prevent vertical signaling. Under appropriate conditions, the explants elongate similar to their movement within the embryo. In these explants, the region-specific neural markers *en-2*, *Krox-20*, and *XlHbox6* (*152*) are expressed in the same anteroposterior pattern observed in the intact embryo, providing evidence for planar signals in neural patterning [Adapted from (*121*)].

Mangold (*104*) demonstrated neural induction by vertical signals and, in addition, that the A-P pattern of induced neural tissue reflects the A-P character of the inducing mesoderm. In this study, dorsal mesoderm from differing A-P positions was isolated from the early neurula and transplanted to the blastocoel cavity of the early gastrula, resulting in formation of ectopic neural structures having an A-P identity consistent with the origin of the transplanted mesoderm. Similar results were obtained with recombinants of dorsal mesoderm and competent ectoderm (*4*, *105–113*). The diffusible nature of these signals is suggested by the ability of dorsal mesoderm to induce competent ectoderm despite the imposition of a permeable filter (*114*).

Nieuwkoop and colleagues performed a series of experiments that demonstrated the regional specificity of neural induction and provided evidence of planar signaling (*115*). The response of ectoderm distant from the site of mesodermal interaction was assessed by inserting folds of competent ectoderm into the presumptive neural plate of the early gastrula. Differentiation of the attached, or proximal, end of the ectodermal tissue matched that of the adjacent host tissue, and a posterior-to-anterior neural pattern formed along the proximal-distal axis of the inserted ectoderm. The A-P pattern present in the ectodermal tissue indicates that neural-inducing signals can function in a planar manner. These studies and others (*116*) suggest that an initial signal induces formation of anterior structures, and a second signal transforms this tissue to a posterior identity (Fig. 3A). Whereas the first signal is proposed to be present throughout the chordamesoderm, the posteriorizing signal may be expressed in a graded manner, with highest levels in the posterior mesoderm.

The role for vertical signals in neural induction was supported by early studies of amphibian exogastrulae (*4*, *106*). Under appropriate conditions involution of dorsal mesoderm, and subsequent vertical interaction with ectoderm, can be prevented, resulting in external chordamesoderm being connected to presumptive neuroectoderm by a narrow shaft of tissue. Mesodermal differentiation occurred in exogastrulae, but no neural structures were detected, suggesting that planar signals are not sufficient for

neural induction. However, more recent studies with molecular markers indicate that neural markers are expressed in ectoderm of exogastrulae (*108, 117, 118*). Additional studies were performed with explants of an intact sheet of presumptive chordamesoderm, neuroectoderm, and epidermis (Keller explants) to examine planar induction in a more controlled manner (*119*). Prepared from early gastrulae before any vertical interactions have occurred, these explants are cultured flat and undergo convergent extension in a planar fashion, allowing only planar signaling (Fig. 3B) (*120*). Neuronal cells, as well as general and region-specific neural markers, are detected in the elongated neuroectoderm of Keller explants, establishing the ability of planar signals to induce neural tissue in an appropriate A-P pattern (*110, 118, 121, 122*). However, the failure of these explants to undergo differentiation or morphogenesis indicates that planar signals are not sufficient for all aspects of neural induction (*110, 118*), and that underlying chordamesoderm is required for neural tube formation (*123, 124*).

The presumptive ectoderm is also prepatterned for neural induction (*125*). This prepattern can be visualized during cleavage stages with the epidermal marker, Epi1, which is expressed in presumptive non-neural ectoderm but not in future neural plate (*58, 126*). This bias is reflected in a differential response of dorsal and ventral ectoderm to neural induction. Recombinants of chordamesoderm and dorsal ectoderm result in strong expression of neural markers, whereas ventral ectoderm expresses low levels in response to the same signals (*125*). However, ventral ectoderm can produce the full spectrum of neural tissues, demonstrated most dramatically in the organizer transplant and also observed in planar recombinants of dorsal mesoderm and ventral ectoderm (*102, 127*).

The dissociation and culture of presumptive ectodermal cells has provided additional insight into neural development. In the absence of any treatment, intact animal pole explants differentiate into atypical epidermis, but if the explants are dissociated and cultured as isolated cells for several hours, both neural differentiation and expression of neural markers are observed in reaggregates (*128*). This result suggests that cell interactions inhibit neural development and dissociation relieves this repression. The relation of these observations to endogenous neural induction is unclear, but perhaps a neural-inducing signal antagonizes an endogenous inhibitor of neural fate.

At the completion of gastrulation, a neurala ectoderm is no longer responsive to induction by chordamesoderm. As early as the open neural plate stage, mediolateral patterning is detectable with molecular markers (*129*). This patterning is likely to rely on signals arising from midline chordamesoderm, with presumptive ventrolateral cell types of the neural tube close to the midline and presumptive dorsal cell types distant. The initial mediolateral pattern is converted into a dorsoventral pattern by neurulation, which transforms the open neural plate into the closed neural tube.

Secondary inductive interactions come into play during patterning and morphogenesis of the central nervous system. An especially well-described process is the regulation of dorsoventral patterning in the neural tube by underlying notochord (*103, 130*). The notochord underlies the midline cells of the neural plate, which form the ventral floor plate of the neural tube. Transplantation of notochord to the intermediate or dorsal neural tube results in ectopic floor plate formation, whereas removal of notochord prevents floor plate formation, as assessed by a chemoattractant assay and molecular markers (Fig. 4) (*123, 124, 131–134*). These results indicate that the notochord induces floor plate and ventrolateral motor neuron formation in adjacent neural tissue. Floor plate grafts or deletions result in similar alterations in patterning, suggesting that the notochord and floor plate share functional properties (*124, 133–136*). Further examination of interaction between notochord and neural tube indicated that floor plate induction was a contact-dependent process. In contrast, both notochord- and floor plate–conditioned media induce differentiation of ventrolateral motor neurons (*132–134*). In vivo, motor neurons differentiate before the attainment of motor neuron–inducing activity by the floor plate, suggesting that the notochord is responsible for motor neuron induction and that the floor plate may be involved in other aspects of ventral patterning. Consistent with this idea, the zebrafish cyclops mutant exhibits motor neuron differentiation despite the absence of the floor plate (*136*). Together these studies indicate that contact-dependent and diffusible signals, derived from the notochord, are responsible for floor plate and motor neuron differentiation.

Neural-Inducing Factors

Stimulated by Spemann and Mangold's organizer transplantation, the search for neural inducers has occupied embryologists for many years. Early studies in the newt identified various crude preparations that induced neural differentiation (*6*), but these preparations were difficult to work with and did not lend themselves to further purification. Molecular approaches have been used with success in *Xenopus* and the chick, and a number of promising candidates for endogenous secreted factors that induce or pattern neural tissue have been identified (*137*).

Activin inhibitors. The availability of two inhibitors of activin has allowed an exami-

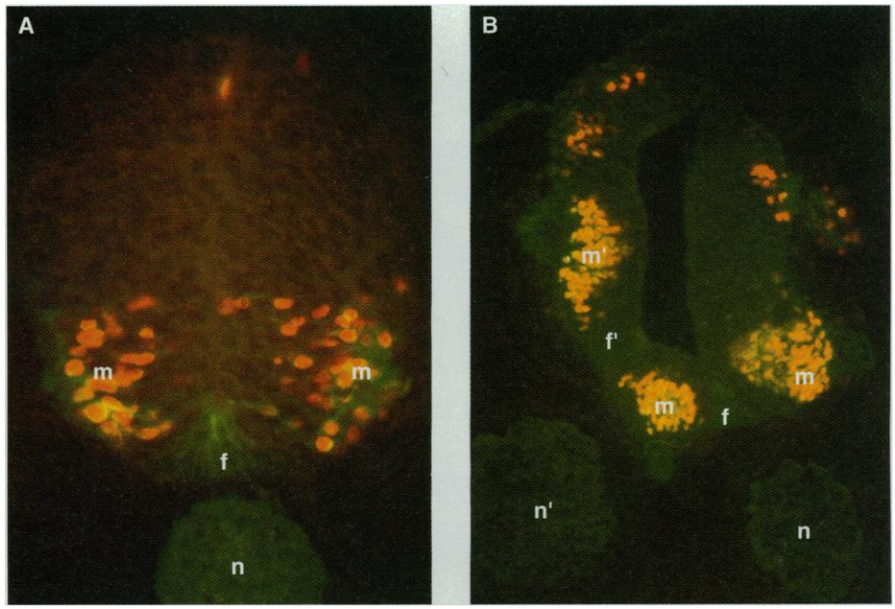

Fig. 4. Dorsoventral patterning of the chick neural tube. (**A**) Transverse section of a stage 17 chick spinal cord displaying immunofluorescence staining of notochord (n), floor plate (f), and ventrolateral motor neurons (m). (**B**) Transplantation of an additional notochord (n′) results in ectopic formation of floor plate (f′) and motor neurons (m′) at the lateral aspect of the neural tube, demonstrating the ability of notochord to induce ventral and ventrolateral patterning of the neural tube [for methods, see (*134*)]. [Photographs courtesy of T. M. Jessell]

nation of activin function in neural induction. Activin transcripts are uniformly distributed at the blastula and gastrula stages, restricted to dorsal tissues in the neurula, and expressed in the anterior notochord and head structures at later stages (*51*). A dominant inhibitory mutant of the activin type II receptor blocks the mesoderm-inducing activity of activin (*61*). Expression of the truncated receptor in animal pole explants resulted in expression of neural markers in the absence of detectable mesoderm, even without activin treatment. Induced tissue has an anterior neural pattern, expressing markers of the eye, forebrain, and midbrain, as well as general neural markers. Overexpression of the truncated receptor in embryos results in ectopic formation of neural structures, and in UV-ventralized embryos neural tissue is generated in the absence of axial structures (*138*). However, the target of inhibition by the truncated receptor is unknown, given the uncertain specificity of this mutant receptor for members of the TGF-β superfamily. The simplest interpretation of these results is that activin is an inhibitor of neural development and that neural induction involves disinhibition. This model is compatible with a number of other observations, including the stimulation of neural development by cell dissociation.

Support for this model of neural induction has come from studies of the secreted molecule follistatin, a natural antagonist of activin function. Follistatin was originally isolated as an inhibitor of pituitary follicle-stimulating hormone (FSH) secretion that acts by directly binding and inhibiting activin (*62*). *Xenopus* follistatin is expressed in the organizer at the gastrula stage and in the notochord and anterior nervous system at later stages (*64*). Expression of follistatin in animal pole explants results in expression of neural markers in the absence of detectable mesoderm. Like the truncated activin receptor, follistatin induces expression of general and anterior neural markers. Moreover, follistatin, unlike the truncated activin receptor, is a specific inhibitor of activin function that is localized to tissues having neural-inducing activity during normal development. Taken together, these results implicate the inhibition of activin signaling in neural induction.

Noggin. As discussed above, noggin is a secreted factor isolated on the basis of its ability to dorsalize mesoderm. Noggin transcripts are expressed in the organizer at the gastrula stage and throughout the notochord at later stages, both tissues implicated in neural induction (*95*). Addition of noggin protein to blastula ectoderm, competent for both mesoderm and neural induction, induces neural markers in the absence of detectable mesoderm, demonstrating a direct induction of neural tissue (*139*). Noggin treatment induces general and anterior neural markers, but explants fail to express markers of hindbrain and spinal cord. In relation to the two-signal model of neural induction, noggin may represent the initial anterior inducer. However, follistatin is also expressed in neural-inducing tissues and directly induces anterior neural tissues. In addition, noggin does not inhibit activin signaling and neither stimulates nor is stimulated by follistatin (*64*, *95*), suggesting that these factors may represent redundant or independent inducers of anterior neural structures. Future work will certainly address the interaction of noggin and follistatin in neural induction.

Notch. The *Drosophila Notch* gene encodes a large transmembrane protein implicated in the maintenance of an uncommitted state in many cell types, including precursors of neural and epidermal tissues (*140*). The ubiquitous expression of *Notch* and the pleiotropic effects of its mutations indicate a general role in cell fate decisions. The *Xenopus* homolog of *Notch* (*Xotch*) is maternally expressed and localized to dorsal ectoderm of the neurula, including both presumptive neural plate and non-neural ectoderm. A putative activated *Xotch* mutation, lacking most of the extracellular region, was overexpressed in embryos and resulted in a consistent hypertrophy of the neural tube and absence of anterior ectodermal structures. This effect is not due to a direct neural-inducing activity because expression in competent ectoderm did not produce substantial neural tissue. However, because mutant *Xotch* does cause an enhanced response of ectodermal explants to neural induction, the effect may be the result of an increased period of competence in ectodermal tissue and delay in their differentiation to epidermal cells. Overall, these results indicate that in the presumptive neural plate *Xotch* acts as a competence factor, regulating the proportion of uncommitted cells capable of responding to neural induction (*141*).

Wnt. Members of the wnt gene family are expressed in a variety of restricted patterns in the developing central nervous system (*82*). The importance of these factors in neural development is evident in homozygous disruptions of the mouse *wnt-1* locus, which is normally expressed in the dorsal regions of the mid- and hindbrain. Specific central nervous system defects were obtained, including loss of midbrain and cerebellar structures, whereas the remaining central nervous system and other embryonic structures were normal (*142*). Overexpression of *wnt-1* in the ventral neural tube results in clear perturbations of neural tube morphogenesis. However, these changes appear to be due to increased cell proliferation in ventral regions, whereas continued expression of ventral markers suggests that no change to dorsoventral pattern was obtained (*143*). Therefore, it appears that *wnt-1* may function by regulating the proliferation of specific regions of the developing neural tube. Additional mutagenesis and overexpression studies will elucidate the role of other wnt molecules.

Dorsalin. Dorsalin1 is a member of the TGF-β family isolated from chick spinal cord (*144*). First detected at the closure of the neural tube, dorsalin1 transcripts are restricted to the dorsal region of the spinal cord, including the roof plate and neural crest. Grafting of notochord to the dorsal neural tube extinguished dorsalin1 expression, consistent with the ectopic induction of ventral structures, including floor plate and motor neurons. Accordingly, removal of the notochord before floor plate induction resulted in an expansion of dorsalin1 expression ventrally. These studies suggest that the restriction of dorsalin1 expression results from inhibitory ventral signals. Furthermore, in neural plate explants, dorsalin1 stimulated the differentiation of certain neural crest-like cells (a dorsal tissue) and inhibited differentiation of motor neurons (a ventrolateral tissue).

These elegant experiments offer a simple model for dorsoventral patterning of the neural tube. Cellular differentiation along the dorsoventral axis is controlled by the local concentration of two diffusible signals, one produced in the dorsal neural tube (dorsalin1) and the second produced by ventrally located notochord. Initially, the entire neural tube is competent to express dorsalin1, but this expression is restricted to the dorsal third of the neural tube by diffusible notochord signals that also induce motor neuron differentiation. Expression of dorsalin1 protein stimulates neural crest differentiation in the dorsal neural tube, and diffusion of this factor ventrally overrides ventral signals, thus setting the upper boundary for motor neuron differentiation. Therefore, patterning of the vertebrate neural tube appears to involve opposing dorsal and ventral signals that specify cell fate along the dorsoventral axis. A vertebrate homolog of the *hedgehog* gene may be an excellent candidate for the notochord-derived ventral signal (*145–147*).

Hedgehog. The *Drosophila* segment polarity gene *hedgehog* is a secreted factor implicated in the control of cell patterning during segmentation and imaginal disc development (*148*). Vertebrate homologs of *hedgehog* have been isolated and are expressed in several tissues with demonstrated ability to regulate cell patterning, including the node, notochord, neural tube floor plate, and posterior limb bud (*145–147*, *149*). Missexpression of *hedgehog* by injec-

tion in the frog and zebrafish or in transgenic mice results in ectopic expression of floor plate markers and in formation of ectopic floor plate in the dorsal or intermediate neural tube (*145–147*). In addition, neural plate explants cultured on hedgehog-producing tissue culture cells express markers of floor plate and show differentiation of motor neurons. Conditioned medium of hedgehog-expressing cells fails to induce floor plate or motor neuron markers, suggesting that induction by hedgehog, in this assay, is a contact-dependent process (*147*). These results implicate notochord expression of hedgehog in the contact-dependent induction of floor plate. It is unclear whether motor neuron induction is a direct response to contact-dependent signaling or a response to a secondary signal from the induced floor plate. The involvement of a secondary diffusible signal is consistent with the ability of floor plate– or notochord-conditioned medium to induce motor neurons but not floor plate.

Similar to mesoderm induction, neural induction apparently involves both inducers of neural tissue (follistatin and noggin) and modifiers of neural patterning (Xotch, dorsalin1, and hedgehog). In addition, *Xash*, a *Xenopus* homolog of the *Drosophila achaete-scute* gene, and a mutant form of *brachyury* are nuclear factors implicated in the induction and patterning of neural tissue (*150*).

Conclusions

Advances of recent years have provided an abundance of information concerning the molecular basis of embryonic induction. With the identification of numerous inducing factors and a description of their embryonic expression and activities, the ground was set for recasting earlier models of embryogenesis in molecular terms. It is apparent from this brief review, however, that lurking behind the elegant embryological studies of the past 70 years is a vast complexity of multiple inducers with overlapping expression patterns and redundant functions. In vertebrate systems the balance of evidence suggests that the combinatorial action of inducers, having both redundant and antagonistic functions, underlies the regional specification of cell fate. The complexity of this problem is evident from the recurring involvement of members of large gene families, in particular the TGF-β superfamily. Studies in areas such as receptor binding affinities of related inducers, restriction of inducer diffusion by the extracellular matrix, and control of cell competence at the level of signal transduction will be the source for many answers.

Among the many remaining challenges for developmental biologists is the elucidation of mechanisms that integrate the actions of multiple inducers in generating a mature animal. The intense activity of those studying vertebrate development will undoubtedly generate further advances in the identification and characterization of embryonic inducers. It does not seem overly optimistic to believe that the combined approaches of current experimental systems will continue to illuminate the regulation of vertebrate development.

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