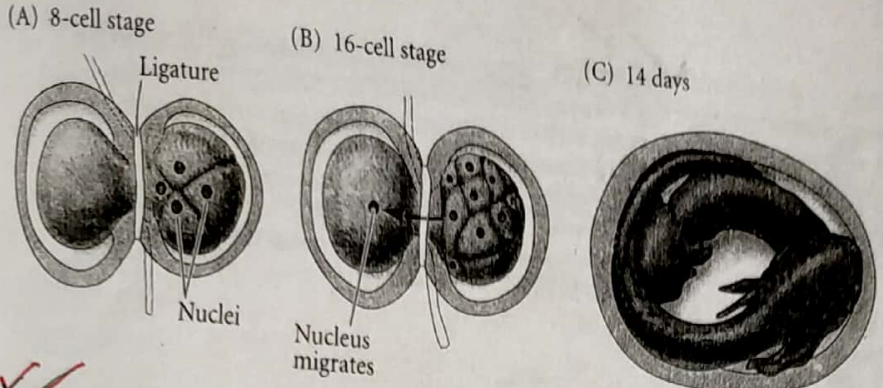



FIGURE 7.14 Spemann's demonstration of nuclear equivalence in newt cleavage. (A) When the fertilized egg of the newt *Triturus taeniatus* was constricted by a ligature, the nucleus was restricted to one half of the embryo. The cleavage on that side of the embryo reached the 8-cell stage, while the other side remained undivided. (B) At the 16-cell stage, a single nucleus entered the as-yet undivided half, and the ligature was further constricted to complete the separation of the two halves. (C) After 14 days, each side had developed into a normal embryo. (After Spemann 1938.)

## Hans Spemann: Inductive interactions in regulative development

Amphibian axis formation combines autonomous specification (*mosaic development*) and conditional specification (*regulative development*). The requirement for inductive interactions was demonstrated in Hans Spemann's laboratory at the University of Freiburg (see Hamburger 1988; De Robertis and Aréchaga 2001; Sander and Fässler 2001). Experiments by Spemann and his students framed the questions that experimental embryologists asked for most of the twentieth century and resulted in a Nobel Prize for Spemann in 1935. In recent times, the ongoing saga of discovery in identifying the molecules associated with these inductive processes has provided some of the most exciting moments in contemporary science.

The experiment that began this research program was performed in 1903, when Spemann demonstrated that early newt blastomeres have identical nuclei, each capable of producing an entire larva. His procedure was ingenious: Shortly after fertilizing a newt egg, Spemann used a baby's hair (taken from his infant daughter) to "lasso" the zygote in the plane of the first cleavage. He then partially constricted the egg, causing all the nuclear divisions to remain on one side of the constriction. Eventually—often as late as the 16-cell stage—a nucleus would escape across the constriction into the non-nucleated side. Cleavage then began on this side too, whereupon Spemann tightened the lasso until the two halves were completely separated. Twin larvae developed, one slightly more advanced than the other (Figure 7.14). Spemann concluded from this experiment that early amphibian nuclei were genetically identical and that each cell was capable of giving rise to an entire organism.

However, when Spemann performed a similar experiment with the constriction still longitudinal, but perpendicular to the plane of the first cleavage (i.e., separating the future dorsal and ventral regions rather than the right and left sides), he obtained a different result altogether. The nuclei continued to divide on both sides of the constriction, but only one side—the future dorsal side of the embryo—gave rise to a normal larva. The other side produced an unorganized tissue mass of ventral cells, which Spemann called the *Bauchstück*—the belly piece. This tissue mass was a ball of epidermal cells (ectoderm) containing blood and mesenchyme (mesoderm) and gut cells (endoderm), but it contained no dorsal structures such as nervous system, notochord, or somites.

Why should these two experiments give different results? One possibility was that when the egg was divided perpendicular to the first cleavage plane, some *cytoplasmic* substance was not equally distributed into the two halves. Fortunately, the salamander egg was a good place to test that hypothesis. As we saw earlier in this chapter, there are dramatic movements in the cytoplasm following the fertilization of amphibian eggs, and in some amphibians these movements expose a gray, crescent-shaped area of cytoplasm in the region directly opposite the point of sperm entry (see Figure 7.1D). The first cleavage plane normally splits this gray crescent equally between the two blastomeres. If these cells are then separated, two complete larvae develop (Figure 7.15A). However, should this cleavage plane be aberrant (either in the rare natural event or in an experiment), the gray crescent material passes into only one of the two blastomeres. Spemann's work revealed that when two blastomeres are separated such that only one of the two cells contains the crescent, only the blastomere containing the gray crescent develops normally (Figure 7.15B).

It appeared, then, that *something in the region of the gray crescent was essential for proper embryonic development*. But how did it function? What role did it play in normal development? The most important clue came from fate maps, which showed that the gray crescent region gives rise to those cells that form the dorsal lip of the blastopore. These dorsal lip cells are committed to invaginate into the blastula, initiating gastrulation and the formation of the head endomesoderm and notochord. Because all future amphibian development depends on the interaction of cells that

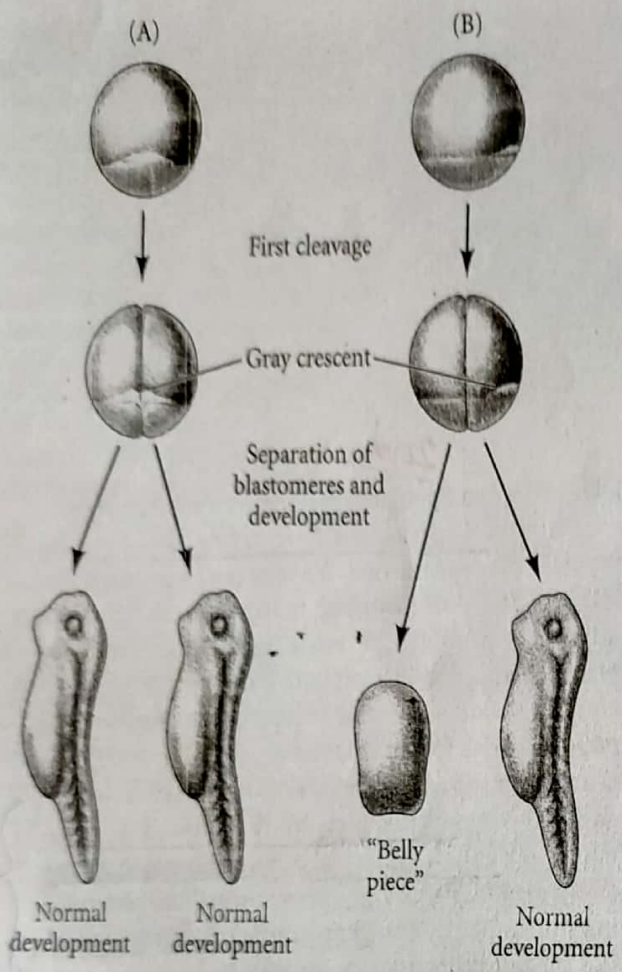



FIGURE 7.15 Asymmetry in the amphibian egg. (A) When the egg is divided along the plane of first cleavage into two blastomeres, each of which gets half of the gray crescent, each experimentally separated cell develops into a normal embryo. (B) When only one of the two blastomeres receives the entire gray crescent, it alone forms a normal embryo. The other blastomere produces a mass of unorganized tissue lacking dorsal structures. (After Spemann 1938.)

are rearranged during gastrulation, Spemann speculated that the importance of the gray crescent material lies in its ability to initiate gastrulation, and that crucial changes in cell potency occur during gastrulation. In 1918, he performed experiments that showed both statements to be true. He found that the cells of the *early* gastrula were uncommitted, but that the fates of *late* gastrula cells were determined.

Spemann's demonstration involved exchanging tissues between the gastrulae of two species of newts whose embryos were differently pigmented—the darkly pigmented *Triturus taeniatus* and the nonpigmented *T. cristatus*. When a region of prospective epidermal cells from an early gastrula of one species was transplanted into an area in an early gastrula of the other species and placed in a region where neural tissue normally formed, the transplanted cells gave rise to neural tissue. When prospective neural tissue from early gastrulae was transplanted to the region fated to become belly skin, the neural tissue became epidermal (Figure 7.16A; Table 7.1). Thus, cells of the *early* newt gastrula exhibit conditional (i.e., regulative, or dependent) development because their ultimate fate depends on their location in the embryo.

However, when the same interspecies transplantation experiments were performed on *late* gastrulae, Spemann obtained completely different results. Rather than differentiating in accordance with their new location, the transplanted cells exhibited *autonomous* (independent, or mosaic) development (see the introduction to Part II). Their prospective fate was *determined*, and the cells developed independently of their new embryonic location. Specifically, prospective neural cells now developed into brain tissue even when placed in the region of prospective epidermis (Figure 7.16B), and prospective epidermis formed skin even in the region of the prospective neural tube. Within the time separating early and late gastrulation, the potencies of these groups of cells had become restricted to their eventual paths of differentiation. Something was causing

**TABLE 7.1 Results of tissue transplantation during early- and late-gastrula stages in the newt**

| Donor region | Host region | Differentiation of donor tissue | Conclusion |
|---|---|---|---|
| EARLY GASTRULA | | | |
| Prospective neurons | Prospective epidermis | Epidermis | Conditional development |
| Prospective epidermis | Prospective neurons | Neurons | Conditional development |
| LATE GASTRULA | | | |
| Prospective neurons | Prospective epidermis | Neurons | Autonomous development (determined) |
| Prospective epidermis | Prospective neurons | Epidermis | Autonomous development (determined) |

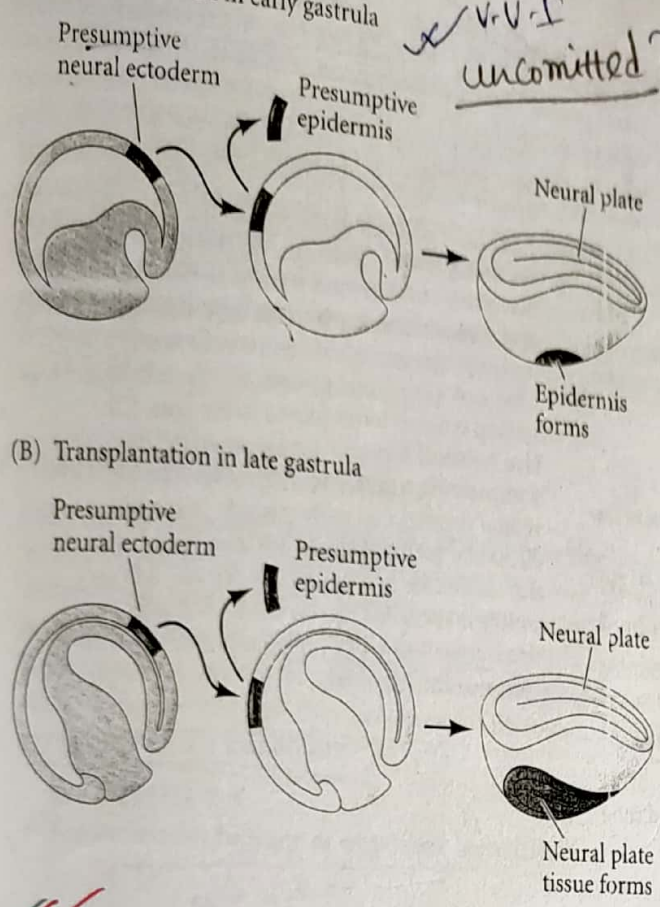



FIGURE 7.16 Determination of ectoderm during newt gastrulation. Presumptive neural ectoderm from one newt embryo is transplanted into a region in another embryo that normally becomes epidermis. (A) When the tissues are transferred between early gastrulae, the presumptive neural tissue develops into epidermis, and only one neural plate is seen. (B) When the same experiment is performed using late-gastrula tissues, the presumptive neural cells form neural tissue, thereby causing two neural plates to form on the host. (After Saxén and Toivonen 1962.)

them to become committed to epidermal and neural fates. What was happening?

## Hans Spemann and Hilde Mangold: Primary embryonic induction

The most spectacular transplantation experiments were published by Spemann and Hilde Mangold in 1924.* They showed that, of all the tissues in the early gastrula, only one has its fate autonomously determined. This self-determining tissue is the dorsal lip of the blastopore—the tissue derived from the gray crescent cytoplasm. When this tissue was transplanted into the presumptive belly skin region of another gastrula, it not only continued to be dorsal blastopore lip but also initiated gastrulation and embryogenesis in the surrounding tissue!

In these experiments, Spemann and Mangold once again used the differently pigmented embryos of *Triturus taeniatus* and *T. cristatus* so they could identify host and donor tissues on the basis of color. When the dorsal lip of an early *T. taeniatus* gastrula was removed and implanted into the region of an early *T. cristatus* gastrula fated to become ventral epidermis (belly skin), the dorsal lip tissue invaginated just as it would normally have done (showing self-determination) and disappeared beneath the vegetal cells (Figure 7.17A). The pigmented donor tissue then continued to self-differentiate into the chordamesoderm (notochord) and other mesodermal structures that normally form from the dorsal lip (Figure 7.17B). As the donor-derived mesodermal cells moved forward, host cells began to participate in the production of a new embryo, becoming organs that normally they never would have formed. In this secondary embryo, a somite could be seen containing both pigmented (donor) and unpigmented (host) tissue. Even more spectacularly, the dorsal lip cells were able to interact with the host tissues to form a complete neural plate from host ectoderm. Eventually, a secondary embryo formed, conjoined face to face with its host (Figure 7.17C). The results of these technically difficult experiments have been confirmed many times and in many amphibian species, including *Xenopus* (Figure 7.17D; Capuron 1968; Smith and Slack 1983; Recanzone and Harris 1985).

**See WEBSITE 7.3**
**Spemann, Mangold, and the organizer**

Spemann referred to the dorsal lip cells and their derivatives (notochord and head endomesoderm) as the **organizer** because (1) they induced the host's ventral tissues to change their fates to form a neural tube and dorsal mesodermal tissue (such as somites), and (2) they organized host and donor tissues into a secondary embryo with clear anterior-posterior and dorsal-ventral axes. He proposed that during normal development, these cells "organize" the dorsal ectoderm into a neural tube and transform the flanking mesoderm into the anterior-posterior body axis (Spemann 1938). It is now known (thanks largely to Spemann and his students) that the interaction of the chordamesoderm and ectoderm is not sufficient to organize the entire embryo. Rather, it initiates a series of sequential inductive events. Because there are numerous inductions during embryonic development, this key induction—in which the progeny of dorsal lip cells induce the dorsal axis and the neural tube—is traditionally called the **primary embryonic induction.**†

*Hilde Proescholdt Mangold died in a tragic accident in 1924, when her kitchen's gasoline heater exploded. She was 26 years old, and her paper was just being published. Hers is one of the very few doctoral theses in biology that have directly resulted in the awarding of a Nobel Prize. For more information about Hilde Mangold, her times, and the experiments that identified the organizer, see Ham-

†This classical term has been a source of confusion because the induction of the neural tube by the notochord is no longer considered the first inductive process in the embryo. We will soon discuss inductive events that precede this "primary" induction.

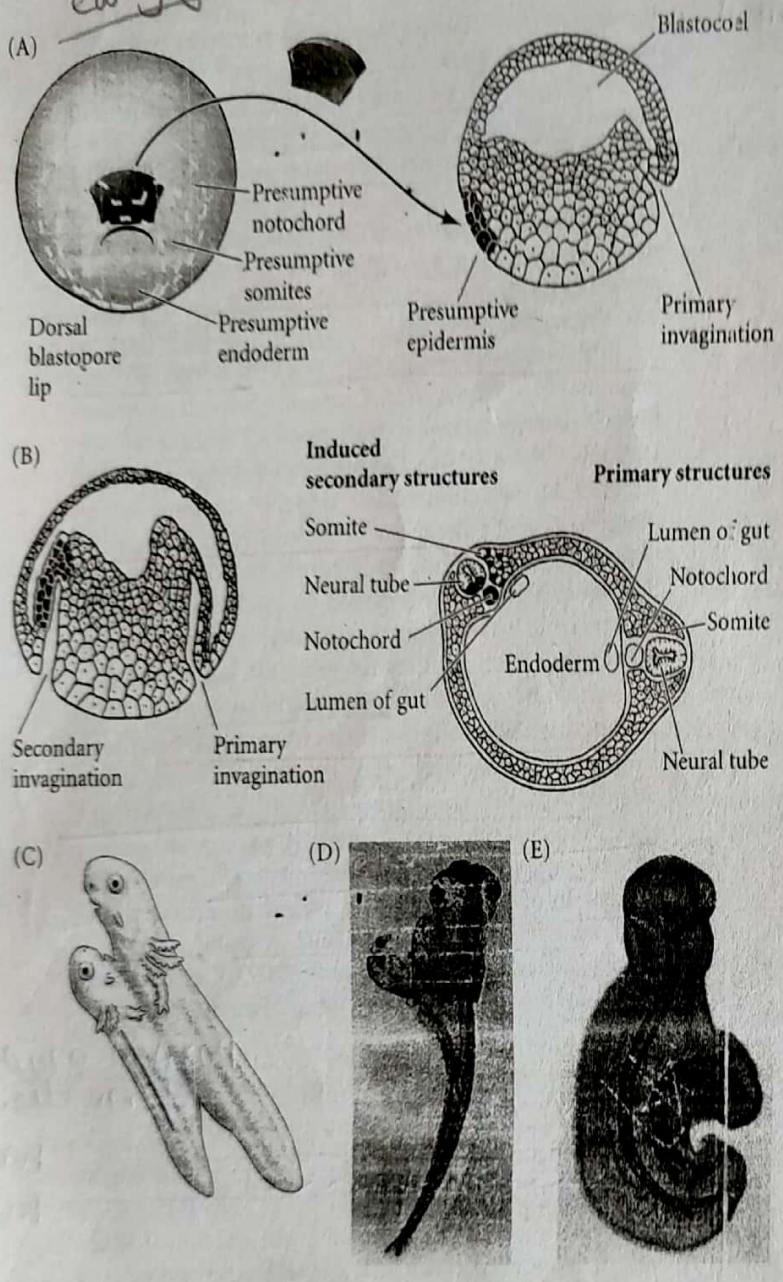



FIGURE 7.17 Organization of a secondary axis by dorsal blastopore lip tissue. (A–C) Spemann and Mangold's 1924 experiments visualized the process by using differently pigmented newt embryos. (A) Dorsal lip tissue from an early *T. taeniatus* gastrula is transplanted into a *T. cristatus* gastrula in the region that normally becomes ventral epidermis. (B) The donor tissue invaginates and forms a second archenteron, and then a second embryonic axis. Both donor and host tissues are seen in the new neural tube, notochord, and somites. (C) Eventually, a second embryo forms joined to the host. (D) Live twinned *Xenopus* larvae generated by transplanting a dorsal blastopore lip into the ventral region of an early-gastrula host embryo. (E) Similar twinned larvae are seen from below and stained for notochord; the original and secondary notochords can be seen. (A–C after Hamburger 1988; D,E photographs by A. Wills, courtesy of R. Harland.)

## Molecular Mechanisms of Amphibian Axis Formation

The experiments of Spemann and Mangold showed that the dorsal lip of the blastopore, along with the dorsal mesoderm and pharyngeal endoderm that form from it, constituted an "organizer" able to instruct the formation of embryonic axes. But the mechanisms by which the organizer itself was constructed and through which it operated remained a mystery. Indeed, it is said that Spemann and Mangold's landmark paper posed more questions than it answered. Among those questions were:

- How did the organizer get its properties? What caused the dorsal blastopore lip to differ from any other region of the embryo?
- What factors were being secreted from the organizer to cause the formation of the neural tube and to create the anterior-posterior, dorsal-ventral, and left-right axes?
- How did the different parts of the neural tube become established, with the most anterior becoming the sensory organs and forebrain, and the most posterior becoming spinal cord?

Spemann and Mangold's description of the organizer was the starting point of one of the first truly international scientific research programs: the search for the organizer molecules. Researchers from Britain, Germany, France, the United States, Belgium, Finland, Japan, and the former Soviet Union all tried to find these remarkable substances (see Gilbert and Saxén 1993). R. G. Harrison referred to the amphibian gastrula as the "new Yukon to which eager miners were now rushing to dig for gold around the blasto-

pore" (see Twitty 1966, p. 39). Unfortunately, their early picks and shovels proved too blunt to uncover the molecules involved. The proteins responsible for induction were present in concentrations too small for biochemical analyses, and the large quantity of yolk and lipids in the amphibian egg further interfered with protein purification (Grunz 1997). The analysis of organizer molecules had to wait until recombinant DNA technologies enabled investigators to make cDNA clones from blastopore lip mRNA and to see which of these clones encoded factors that could dorsalize the embryo. Today, however, we are able to take up each of the above four questions in turn.

## How does the organizer form?

Why are the dozen or so initial cells of the organizer positioned opposite the point of sperm entry, and what determines their fate so early? Recent evidence provides an unexpected answer: these cells are in the right place at the right time, at a point where two signals converge. The first signal tells the cells that they are dorsal. The second signal says that they are mesoderm.

See WEBSITE 7.4

### The molecular biology of organizer formation

**THE DORSAL SIGNAL: β-CATENIN** It turns out that one of the reasons the organizer cells are special is that these mesodermal cells reside above a special group of vegetal cells. One of the major clues in determining how the dorsal blastopore lip obtained its properties came from the experiments of Pieter Nieuwkoop (1969, 1973, 1977) and Osamu Nakamura. These studies showed that the organizer receives its properties from the ectoderm beneath it.

Nakamura and Takasaki (1970) showed that the mesoderm arises from the marginal (equatorial) cells at the border between the animal and vegetal poles. The Nakamura and Nieuwkoop laboratories then demonstrated that the properties of this newly formed mesoderm were induced by the vegetal (presumptive endoderm) cells underlying them. Nieuwkoop removed the equatorial cells (i.e., presumptive mesoderm) from a blastula and showed that neither the animal cap (presumptive ectoderm) nor the vegetal cap (presumptive endoderm) produced any mesodermal tissue. However, when the two caps were recombined, the animal cap cells were induced to form mesodermal structures such as notochord, muscles, kidney cells, and blood cells (Figure 7.18). The polarity of this induction (i.e., whether the animal cells formed dorsal mesoderm or ventral mesoderm) depended on whether the endodermal (vegetal) fragment was taken from the dorsal or the ventral side: ventral and lateral vegetal cells (those closer to the side of sperm entry) induced ventral (mesenchyme, blood) and intermediate (kidney) mesoderm, while the dorsalmost vegetal cells specified dorsal mesoderm components (somites, notochord)—including those having the properties of the organizer. These dorsalmost vegetal cells of the blastula, which are capable of inducing the organizer, have been called the **Nieuwkoop center** (Gerhart et al. 1989).

The Nieuwkoop center was demonstrated in the *Xenopus* embryo by transplantation and recombination experiments. First, Gimlich and Gerhart (Gimlich and Gerhart 1984; Gimlich 1985, 1986) performed an experiment analogous to the Spemann and Mangold studies, except that they used early *Xenopus* blastulae rather than newt gastrulae. When they transplanted the dorsalmost vegetal blas-

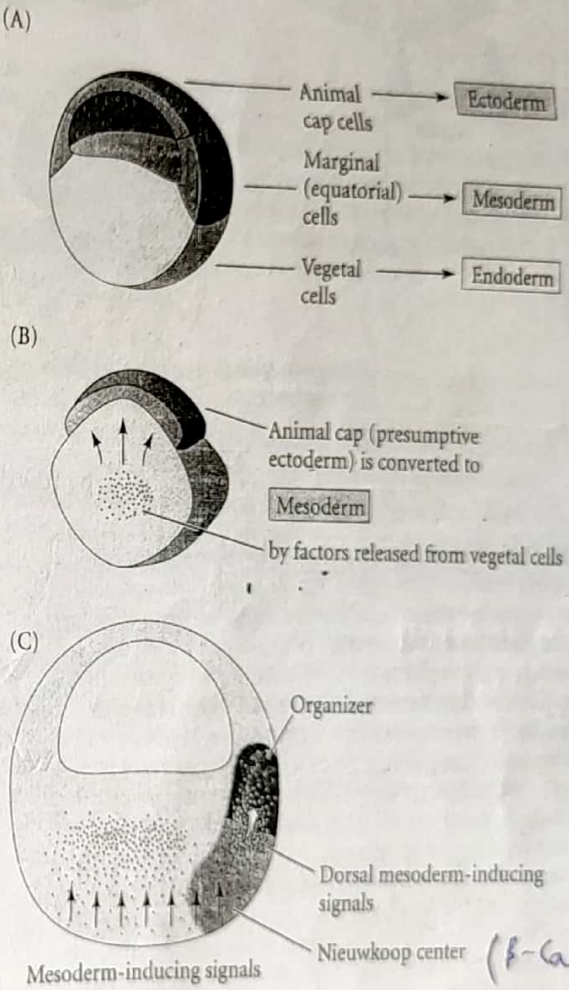
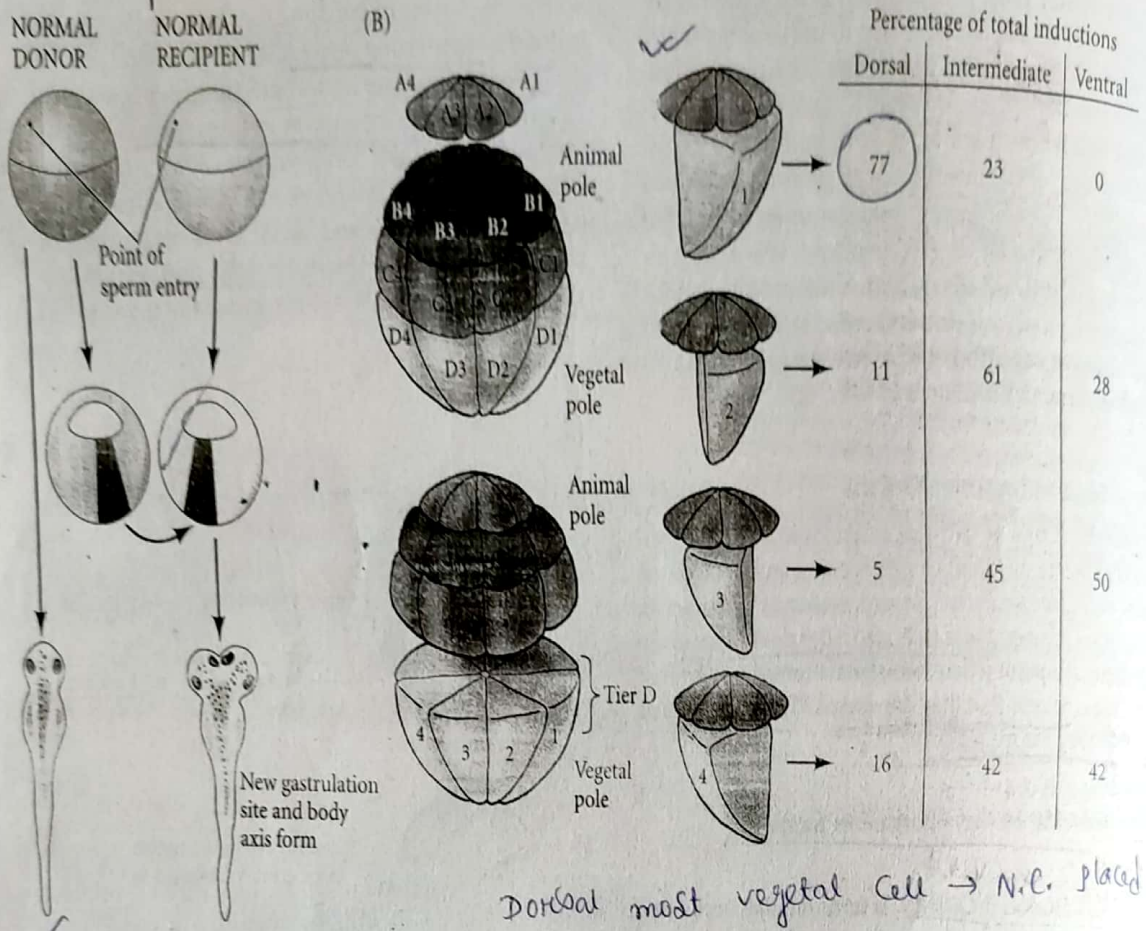



FIGURE 7.18 Summary of experiments by Nieuwkoop and by Nakamura and Takasaki, showing mesodermal induction by vegetal endoderm. (A) Isolated animal cap cells become a mass of ciliated ectoderm, isolated equatorial (marginal zone) cells become mesoderm, and isolated vegetal cells generate gutlike tissue. (B) If animal cap cells are combined with vegetal cap cells, many of the animal cells generate mesodermal tissue. (C) Simplified model for mesoderm induction in *Xenopus*. A ventral signal (probably a complex set of signals from activin-like TGF-β factors and FGFs) is released throughout the vegetal region of the embryo. This signal induces the marginal cells to become mesoderm. On the dorsal side (away from the point of sperm entry), a signal is released by the vegetal cells of the Nieuwkoop center. This dorsal signal induces the formation of the Spemann organizer in the overlying marginal zone cells. The possible identity of this signal will be discussed later in this chapter. (C after De Robertis et al. 1992.)

Transplantation (64 cell stage) Recombination (32 cell)




Dorsal most vegetal cell → N.C. placed

**FIGURE 7.19** Transplantation and recombination experiments on *Xenopus* embryos demonstrate that the vegetal cells underlying the prospective dorsal blastopore lip region are responsible for initiating gastrulation. (A) Formation of a new gastrulation site and body axis by the transplantation of the most dorsal vegetal cells of a 64-cell embryo into the ventralmost vegetal region of another embryo. (B) The regional specificity of mesoderm induction demonstrated by recombining blastomeres of 32-cell *Xenopus* embryos. Animal pole cells were labeled with fluorescent polymers so their descendants could be identified, then combined with individual vegetal blastomeres. The inductions resulting from these recombinations are summarized at the right. D1, the dorsalmost vegetal blastomere, was the most likely to induce the animal pole cells to form dorsal mesoderm. These dorsalmost vegetal cells constitute the Nieuwkoop center. (A after Gimlich and Gerhart 1984; B after Dale and Slack 1987.)

tomere from one blastula into the ventral vegetal side of another blastula, two embryonic axes formed (Figure 7.19A). Second, Dale and Slack (1987) recombined single vegetal blastomeres from a 32-cell *Xenopus* embryo with the uppermost animal tier of a fluorescently labeled embryo of the same stage. The dorsalmost vegetal cell, as expected, induced the animal pole cells to become dorsal mesoderm. The remaining vegetal cells usually induced the animal cells to produce either intermediate or ventral mesodermal tissues (Figure 7.19B). Holowacz and Elinson (1993) found that cortical cytoplasm from the dorsal vegetal cells of the 16-cell *Xenopus* embryo was able to induce the formation of secondary axes when injected into ventral vegetal cells. Thus, dorsal vegetal cells can induce animal cells to become dorsal mesodermal tissue.

So one important question became, What gives the dorsalmost vegetal cells their special properties? The major candidate for the factor that forms the Nieuwkoop center in these vegetal cells is **β-catenin**, a multifunctional protein that can act as an anchor for cell membrane cadherins (see Chapter 3) or as a nuclear transcription factor (induced by the Wnt pathway). As we saw in Chapter 5, β-catenin is responsible for specifying the micromeres of the sea urchin embryo. β-Catenin is a key player in the formation of the dorsal tissues, and experimental depletion of this molecule results in the lack of dorsal structures (Heasman et al. 1994a). Moreover, injection of exogenous β-catenin into the ventral side of an embryo produces a secondary axis (Funayama et al. 1995; Guger and Gumbiner 1995).

In *Xenopus* embryos, β-catenin is initially synthesized throughout the embryo from maternal mRNA (Yost et al. 1996; Larabell et al. 1997). It begins to accumulate in the dorsal region of the egg during the cytoplasmic movements of fertilization and continues to accumulate preferentially at the dorsal side throughout early cleavage. This accumulation is seen in the nuclei of the dorsal cells and appears to cover both the Nieuwkoop center and organizer regions (Figure 7.20; Schneider et al. 1996; Larabell et al. 1997).

If β-catenin is originally found throughout the embryo, how does it become localized specifically to the side oppo-

what happens when β-Catenin m-RNA injected into ventral vegetal Cells? ★

Narrate the summary of events hypothesized to bring about the induction of the organizer in the dorsal mesoderm. Fig → 7.22



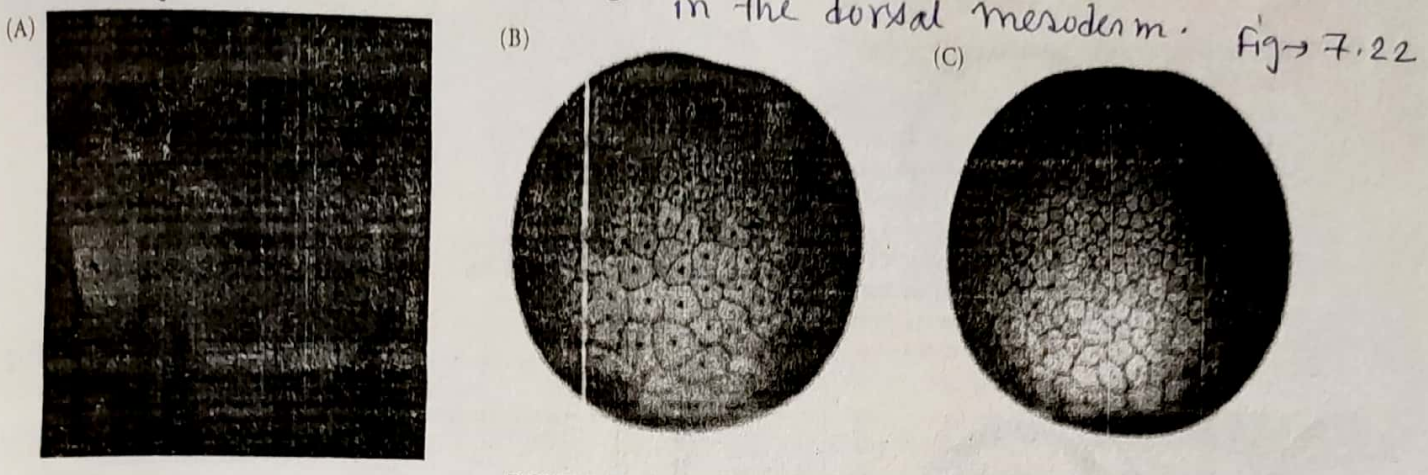



FIGURE 7.20 Role of Wnt pathway proteins in dorsal-ventral axis specification. (A–D) Differential translocation of β-catenin into *Xenopus* blastomere nuclei. (A) Early 2-cell stage, showing β-catenin (orange) predominantly at the dorsal surface. (B) Presumptive dorsal side of a blastula stained for β-catenin shows nuclear localization. (C) Such nuclear localization is not seen on the ventral side of the same embryo. (D) β-Catenin dorsal localization persists through the gastrula stage. (A,D courtesy of R. T. Moon; B,C from Schneider et al. 1996, courtesy of P. Hausen.)

V.V.I

site sperm entry? The answer appears to reside in the translocation of Wnt11 and the **Disheveled (Dsh)** protein from the vegetal pole to the dorsal side of the egg during fertilization. From research done on the Wnt pathway, we have learned that β-catenin is targeted for destruction by **glycogen synthase kinase 3 (GSK3**; see Chapter 3). Indeed, activated GSK3 destroys β-catenin and blocks axis formation when added to the egg, and if endogenous GSK3 is knocked out by a dominant negative form of GSK3 in the ventral cells of the early embryo, a second axis forms (see Figure 7.21F; He et al. 1995; Pierce and Kimelman 1995; Yost et al. 1996).

GSK3 can be inactivated by the **GSK3-binding protein (GBP)** and Disheveled. These two proteins release GSK3 from the degradation complex and prevent it from binding β-catenin and targeting it for destruction. During the first cell cycle, when the microtubules form parallel tracts in the vegetal portion of the egg, GBP travels along the microtubules by binding to kinesin, an ATPase motor protein that travels on microtubules. Kinesin always migrates toward the growing end of the microtubules, and in this case, that means moving to the point opposite sperm entry, i.e., the future dorsal side (Figure 7.21A–C). Disheveled, which is originally found in the vegetal pole cortex, grabs onto the GPB, and it too becomes translocated along the microtubular monorail (Miller et al. 1999; Weaver et al. 2003). The cortical rotation is probably important in orienting and straightening the microtubular array and in maintaining the direction of transport when the kinesin complexes occasionally jump the track (Weaver and Kimelman 2004). Once at the site opposite the point of sperm entry, GBP and Dsh are released from the microtubles. Here, on the future dorsal side of the embryo, they inactivate GSK3, allowing β-catenin to accumulate on the dorsal side while ventral β-catenin is degraded (Figure 7.21D,E).

But the mere translocation of these proteins to the dorsal side of the embryo does not seem to be sufficient for protecting β-catenin. It appears that a Wnt paracrine factor has to be secreted there to activate the β-catenin protection pathway; this is accomplished by **Wnt11** (see Figure 7.21). If Wnt11 synthesis is suppressed (by the injection of antisense Wnt11 oligonucleotides into the oocytes), the organizer fails to form (Tao et al. 2005). *Wnt11* mRNA is localized to the vegetal cortex during oogenesis and is thought to be translocated to the future dorsal portion of the embryo by the cortical rotation of the egg cytoplasm. Here it is translated into a protein that becomes concentrated in and secreted on the dorsal side of the embryo (Ku and Melton 1993; Schroeder et al. 1999; White and Heasman 2008).

Thus, during first cleavage, GBP, Dsh, and Wnt11 are brought into the future dorsal section of the embryo. Here, GBP and Dsh can *initiate* the inactivation of GSK3 and the consequent protection of β-catenin. The signal from Wnt11 *stabilizes* GBP and Dsh and organizes them to protect β-catenin. The β-catenin transcription factor can associate with other transcription factors to give them new properties. It is known that *Xenopus* β-catenin can combine with a ubiquitous transcription factor known as **Tcf3**, converting the Tcf3 repressor into an activator of transcription. Expression of a mutant form of Tcf3 (one that lacks the β-catenin binding domain) results in embryos without dorsal structures (Molenaar et al. 1996).

describe model by which the Dsh stabilizes β-Catenin. (7.21)

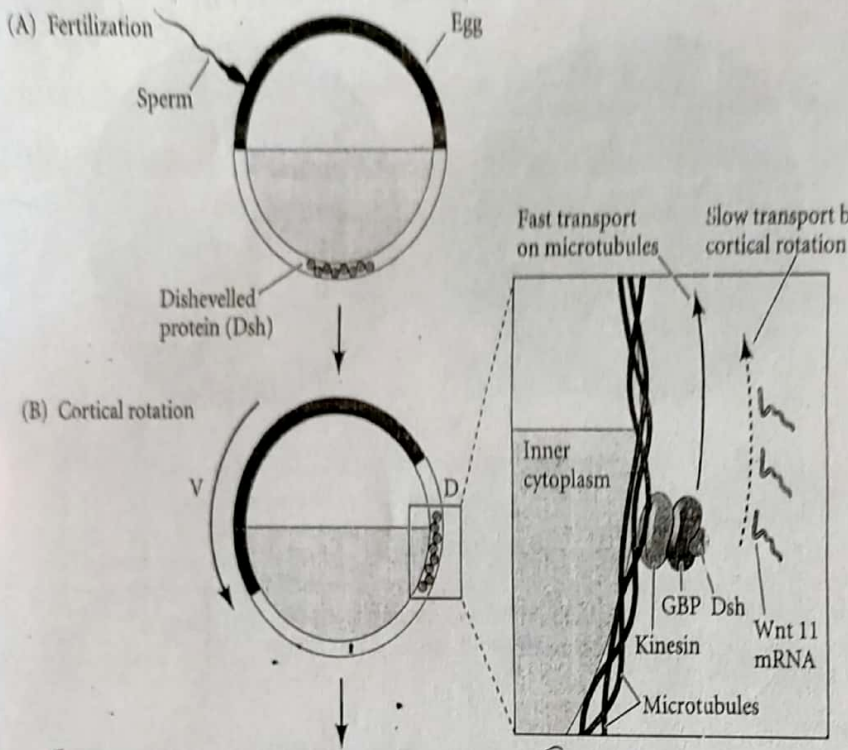




**FIGURE 7.21** Model of the mechanism by which the Disheveled protein stabilizes β-catenin in the dorsal portion of the amphibian egg. (A) Disheveled (Dsh) and GBP associate with kinesin at the vegetal pole of the unfertilized egg. Wnt11 is also in vesicles at the vegetal portion of the egg. (B) After fertilization, these vegetal vesicles are translocated dorsally along subcortical microtubule tracks. Cortical rotation adds a "slow" form of transportation to the fast-track microtubule ride. (C) Wnt11, Dsh, and GBP are then released from the microtubules and are distributed in the future dorsal third of the 1-cell embryo. (D) Dsh and GBP bind to and block the action of GSK3, thereby preventing the degradation of β-catenin on the dorsal side of the embryo. Wnt11 probably is needed to stabilize this reaction, keeping an active source of Dsh. (E) The nuclei of the blastomeres in the dorsal region of the embryo receive β-catenin, while the nuclei of those in the ventral region do not. (F) Formation of a second dorsal axis caused by the injection of both blastomeres of a 2-cell *Xenopus* embryo with dominant inactive *GSK3*. Dorsal fate is actively suppressed by wild-type *GSK3*. (A–E after Weaver and Kimelman 2004; F from Pierce and Kimelman 1995, courtesy of D. Kimelman.)

The β-catenin/Tcf3 complex appears to bind to the promoters of several genes whose activity is critical for axis formation. Two of these genes, *twin* and *siamois*, encode homeodomain transcription factors and are expressed in the organizer region immediately following the mid-blastula transition. If these genes are ectopically expressed in the ventral cells, a secondary axis emerges on the former ventral side of the embryo; and if cortical microtubular polymerization is prevented, *siamois* expression is eliminated (Lemaire et al. 1995; Brannon and Kimelman 1996). The Tcf3 protein is thought to inhibit *siamois* and *twin* transcription when it binds to those genes' promoters in the absence of β-catenin. However, when β-catenin binds to Tcf3, the repressor is converted into an activator, and *twin* and *siamois* are activated (Figure 7.22).

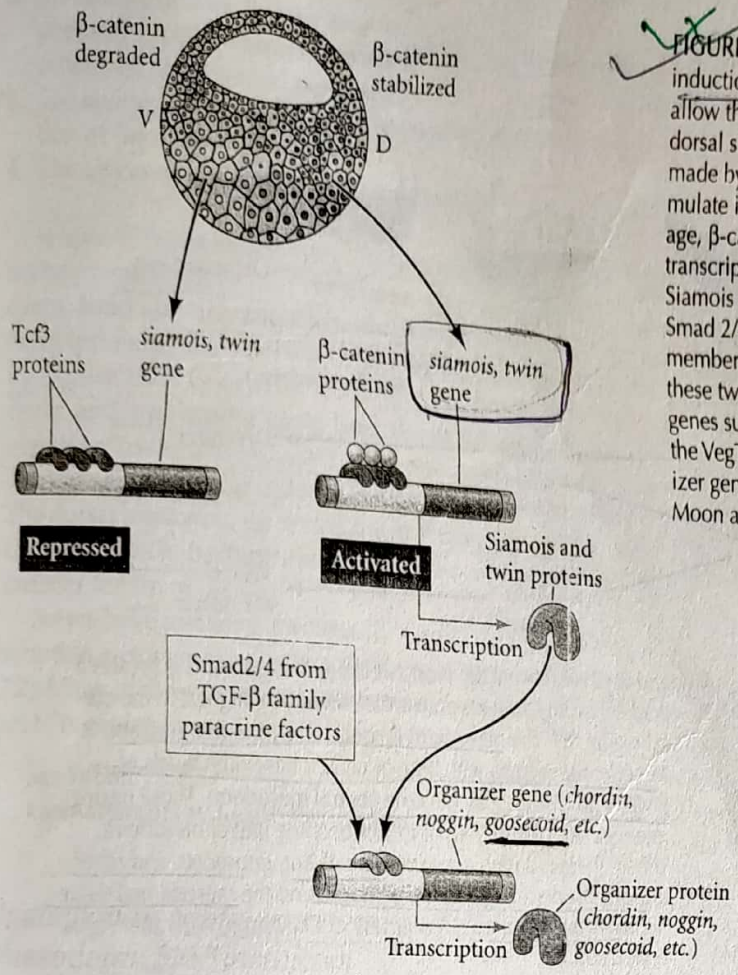



**FIGURE 7.22** Summary of events hypothesized to bring about induction of the organizer in the dorsal mesoderm. Microtubules allow the translocation of Disheveled and Wnt11 proteins to the dorsal side of the embryo. Dsh (from the vegetal cortex and newly made by Wnt11) binds GSK3, thereby allowing β-catenin to accumulate in the future dorsal portion of the embryo. During cleavage, β-catenin enters the nuclei and binds with Tcf3 to form a transcription factor that activates genes encoding proteins such as Siamois and Twin. Siamois and Twin interact in the organizer with Smad 2/4 transcription factors activated by vegetal TGF-β family members (Nodal-related proteins, Vg1, activin, etc.). Together, these two sets of transcription factors activate the "organizer" genes such as *chordin*, *noggin*, and *goosecoid*. The presence of the VegT transcription factor in the endoderm prevents the organizer genes from being expressed outside the organizer area. (After Moon and Kimelman 1998.)

Siamois and Twin bind to the enhancers of several genes involved in organizer function (Fan and Sokol 1997; Kessler 1997). These include genes encoding the transcription factors Goosecoid and Xlim1 (which appear to be critical in specifying the dorsal mesoderm) and the paracrine factor antagonists Noggin, Chordin, Frzb, and Cerberus (Laurent et al. 1997; Engleka and Kessler 2001). VegT appears to inhibit the expression of these genes in the vegetal cells (Brannon et al. 1997; Ishibashi et al. 2008). Thus one could expect that if the dorsal side of the embryo contained β-catenin, then β-catenin would allow this region to express Twin and Siamois proteins, and these proteins would initiate formation of the organizer.

**THE VEGETAL TGF-β-LIKE SIGNAL** Yet another transcription factor also appears to be critical in activating the genes that characterize the organizer cells. This other factor, Smad2/4, is induced in the dorsal mesoderm cells by TGF-β family paracrine factors secreted by the vegetal cells beneath them (Brannon and Kimelman 1996; Engleka and Kessler 2001). TGF-β proteins in the Nieuwkoop center induce the cells in the dorsal marginal zone above them to express Smad2/4 transcription factors, which then bind to the promoter of the organizer genes and cooperate with Twin and Siamois to activate them (see Figure 7.22; Germain et al. 2000).

Two maternal RNAs tethered to the vegetal cortex appear to be crucial for the ability of the vegetal cells to induce the cells above them to be mesodermal. One of these encodes Vg1, a member of the TGF-β superfamily (Figure 7.23A). Vg1 is critically important, since embryos whose Vg1 has been depleted lack organizer gene expression and also lack notochords (Birsoy et al. 2006). The other vegetally tethered mRNA encodes VegT, a transcription factor that instructs the endoderm to synthesize and secrete TGF-β family members activin, Derrière, and several Nodal proteins (Latinkic et al. 1997; Smith 2001). These proteins have overlapping functions. Each of them can activate the *Xbra* (*Brachyury*) gene encoding a transcription factor that instructs the cells to become mesoderm. Derrière can induce animal cap cells to become mesoderm over the long-range distances predicted by Nieuwkoop's experiments (White et al. 2002), and activin can induce different types of mesoderm at different concentrations. At moderate concentrations, activin activates the *Xbra* gene, while at higher concentrations it induces organizer genes to become expressed (Green and Smith 1990; Moriya and Asashima 1992; Piepenburg et al. 2004).

Thus the TGF-β-like signal from the endoderm is known to be critical for mesoderm induction; moreover, the *amount* of this signal may control the *type* of mesoderm induced. Since activin, Vg1, Derrier, and Nodal proteins all act

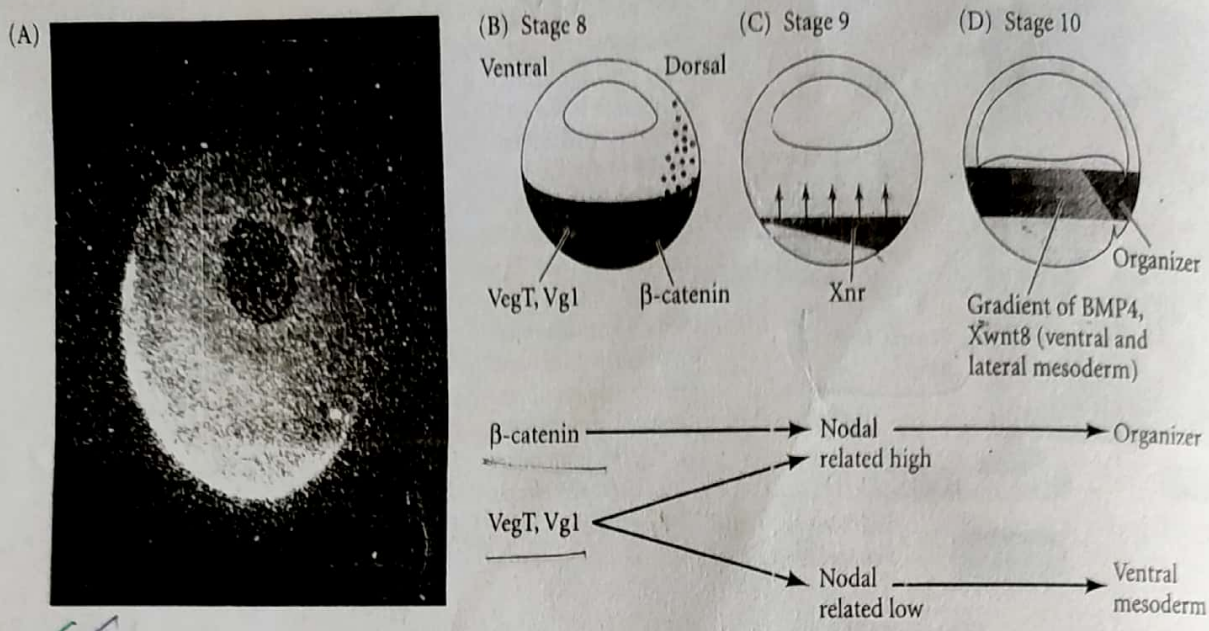



FIGURE 7.23 Vegetal induction of mesoderm. (A) The maternal RNA encoding Vg1 (bright white crescent) is tethered to the vegetal cortex of a *Xenopus* oocyte. The message (along with the maternal VegT message) will be translated at fertilization. Both proteins appear to be crucial for the ability of vegetal cells to induce cells above them to become mesodermal. (B–D) Model for mesoderm induction and organizer formation by the interaction of β-catenin and TGF-β proteins. (B) At late blastula stages, Vg1 and VegT are found in the vegetal hemisphere; β-catenin is located in the dorsal region. (C) β-Catenin acts synergistically with Vg1 and VegT to activate the Nodal-related (*Xnr*) genes. This creates a gradient of Xnr proteins across the endoderm, highest in the dorsal region. (D) The mesoderm is specified by the Xnr gradient. Mesodermal regions with little or no Xnr have high levels of BMP4 and Xwnt8; they become ventral mesoderm. Those having intermediate concentrations of Xnr become lateral mesoderm. Where there is a high concentration of Xnr, *goosecoid* and other dorsal mesodermal genes are activated and the mesodermal tissue becomes the organizer. (A courtesy of D. Melton; B–D after Agius et al. 2000.)

through the same pathway (activating the Smad2/4 transcription factor; see Figure 7.22), the amount of each of these is expected to be additive (Agius et al. 2000). Indeed, this appears to be the case.

During the late blastula stage, several Nodal-related proteins (including Xnr1–6) are expressed in a gradient throughout the endoderm with a low concentration ventrally and a high concentration dorsally (Onuma et al. 2002; Rex et al. 2002; Wright et al. 2005). This gradient is formed by the activation of *Xenopus* Nodal-related gene expression by the synergistic action of VegT with β-catenin. Agius and his colleagues presented a model, shown in Figure 7.23B–D, in which the dorsally located β-catenin and the vegetally located Vg1 signals interact to create a gradient of the Nodal-related proteins across the endoderm. Those regions with little Nodal-related protein induce the cells above them to become ventral mesoderm; regions above vegetal cells with some Nodal-related protein become lateral mesoderm; and regions above vegetal cells containing large amounts of these proteins (plus Vg1) become the organizer. Activin, Vg1, and Derrière gradients may behave in the same way. Thus, the initial specification of the mesoderm along the dorsal-ventral axis appears to be accomplished by Nodal-like TGF-β paracrine factors. The region with the highest concentration of these factors may provide the vegetal signal for dorsal mesoderm (organizer) specification, particularly when combined with dorsal β-catenin signal.

In summary, then, the formation of the dorsal mesoderm and the organizer originates through the activation of critical transcription factors by intersecting pathways. The first pathway is the Wnt/β-catenin pathway that activates genes encoding the Siamois and Twin transcription factors. The second pathway is the maternal VegT pathway that activates the expression of Vg1 and other Nodal-related paracrine factors, which in turn activate the Smad2/4 transcription factor in the mesodermal cells above them. The high levels of Smad2/4 and Siamois/Twin transcription factor proteins work within the dorsal mesoderm cells and activate the genes that give these cells their "organizer" properties (review Figures 7.20–7.23).

## Functions of the organizer

While the Nieuwkoop center cells remain endodermal, the cells of the organizer become the dorsal mesoderm and migrate underneath the dorsal ectoderm. There, the dorsal mesoderm induces the central nervous system to form. The properties of the organizer tissue can be divided into four major functions:

1. The ability to self-differentiate dorsal mesoderm (prechordal plate, chordamesoderm, etc.)

2. The ability to dorsalize the surrounding mesoderm into paraxial (somite-forming) mesoderm when it would otherwise form ventral mesoderm
3. The ability to dorsalize the ectoderm and induce formation of the neural tube
4. The ability to initiate the movements of gastrulation

It is now thought that the cells of the organizer ultimately contribute to four cell types: pharyngeal endoderm, head mesoderm (prechordal plate), dorsal mesolip (Keller 1976; Gont et al. 1993). The pharyngeal endoderm and prechordal plate lead the migration of the organizer tissue and induce the forebrain and midbrain. The dorsal mesoderm induces the hindbrain and trunk. The dorsal blastopore lip remaining at the end of gastrulation eventually becomes the chordaneural hinge that induces the tip of the tail.

As we have just seen, the Smad2/4 and β-catenin transcription factors cooperate to activate several genes (Table 7.2). Many of these genes encode secreted proteins that will act to organize the embryo.

**TABLE 7.2 Proteins expressed solely or almost exclusively in the organizer (partial list)**

| Nuclear proteins | Secreted proteins |
|---|---|
| Twin | Chordin |
| Siamois | Dickkopf |
| Xlim1 | ADMP |
| Xnot | Frzb |
| Otx2 | Noggin |
| XFD1 | Follistatin |
| XANF1 | Sonic hedgehog |
| Goosecoid | Cerberus |
| HNF3β | Nodal-related proteins (several) |

See WEBSITE 7.5
Early attempts to locate the organizer molecules

## Induction of neural ectoderm and dorsal mesoderm: BMP inhibitors

Evidence from experimental embryology showed that one of the most critical properties of the organizer was its production of soluble factors. The evidence for such diffusible signals from the organizer came from several sources. First, Hans Holtfreter (1933) showed that if the notochord fails to migrate beneath the ectoderm, the ectoderm will not become neural tissue (and will become epidermis). More definitive evidence for the importance of soluble factors came later from the transfilter studies of Finnish investigators (Saxén 1961; Toivonen et al. 1975; Toivonen and Wartiovaara 1976). Here, newt dorsal lip tissue was placed on one side of a filter fine enough so that no processes could fit through the pores, and competent gastrula ectoderm was placed on the other side. After several hours, neural structures were observed in the ectodermal tissue (Figure 7.24). The identities of the factors diffusing from the organizer, however, took another quarter of a century to find.

It turned out that scientists were looking for the wrong mechanism. They were searching for a molecule secreted by the organizer and received by the ectoderm that then converted the ectoderm into neural tissue. However, molecular studies led to a remarkable and non-obvious conclusion: *it is the epidermis that is induced to form, not the neural tissue.* The ectoderm is induced to become epidermal tissue by binding **bone morphogenetic proteins** (BMPs), while the nervous system forms from that region of the ectoderm that is *protected* from epidermal induction by BMP-inhibiting molecules (Hemmati-Brivanlou and Melton 1994, 1997). In other words, (1) the "default fate" of the ectoderm is to become neural tissue; (2) certain parts of the embryo induce the ectoderm to become epidermal tissue by secreting BMPs; and (3) the organizer tissue acts by secreting molecules that block BMPs, thereby allowing the ectoderm "protected" by these BMP inhibitors to become neural tissue.

See WEBSITE 7.6 Competence and bias

Three of the major BMP inhibitors secreted by the organizer are Noggin, chordin, and follistatin. The *noggin*, *chordin*, and *follistatin* genes are some of the most critical genes activated by Smad2/4 and Siamois/Twin (Carnac et al. 1996; Fan and Sokol 1997; Kessler 1997).

Narrate the function of $BMP_4$ ?

FIGURE 7.24 Neural structures induced in presumptive ectoderm by newt dorsal lip tissue, separated from the ectoderm by a nucleopore filter with an average pore diameter of 0.05 mm. Anterior neural tissues are evident, including some induced eyes. (From Toivonen 1979, courtesy of L. Saxén.)

State the function of chordin, noggin & follistatin.

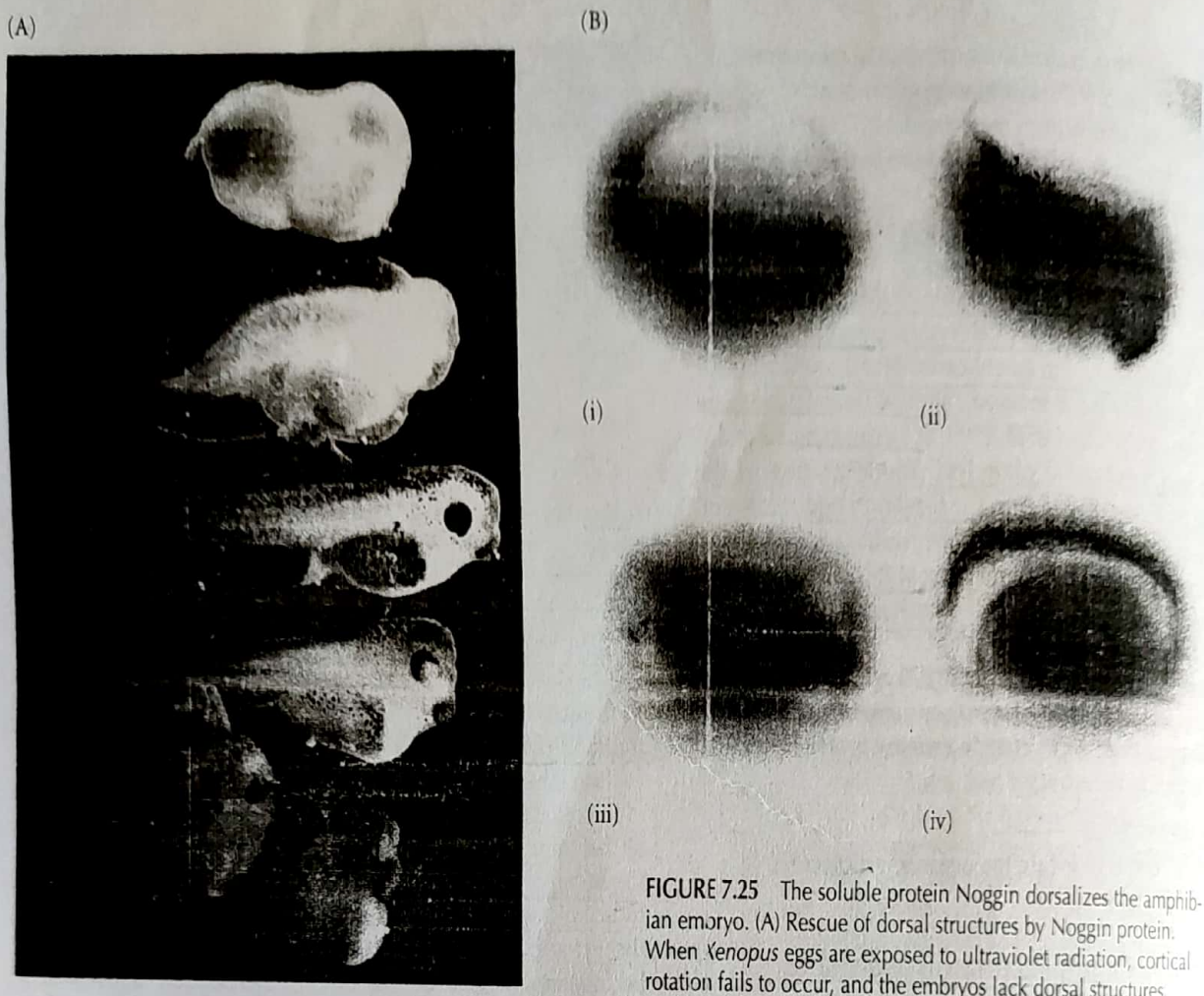



**FIGURE 7.25** The soluble protein Noggin dorsalizes the amphibian embryo. (A) Rescue of dorsal structures by Noggin protein. When *Xenopus* eggs are exposed to ultraviolet radiation, cortical rotation fails to occur, and the embryos lack dorsal structures (top). If such an embryo is injected with *noggin* mRNA, it develops dorsal structures in a dosage-related fashion (top to bottom). If too much *noggin* message is injected, the embryo produces dorsal anterior tissue at the expense of ventral and posterior tissue, becoming little more than a head (bottom). (B) Localization of *noggin* mRNA in the organizer tissue, shown by in situ hybridization. At gastrulation (i), *noggin* mRNA (dark areas) accumulates in the dorsal marginal zone. When cells involute (ii), *noggin* mRNA is seen in the dorsal blastopore lip. During convergent extension (iii), *noggin* is expressed in the precursors of the notochord, prechordal plate, and pharyngeal endoderm, which (iv) extend beneath the ectoderm in the center of the embryo. (Courtesy of R. M. Harland.)

Function

**NOGGIN** In 1992, Smith and Harland constructed a cDNA plasmid library from dorsalized (lithium chloride-treated) gastrulae. Messenger RNAs synthesized from sets of these plasmids were injected into ventralized embryos (having no neural tube) produced by irradiating early embryos with ultraviolet light. Those plasmid sets whose mRNAs rescued dorsal structures in these embryos were split into smaller sets, and so on, until single-plasmid clones were isolated whose mRNAs were able to restore the dorsal tissue in such embryos. One of these clones contained the gene for the protein **Noggin** (Figure 7.25A). Injection of *noggin* mRNA into 1-cell, UV-irradiated embryos completely rescued dorsal development and allowed the formation of a complete embryo.

Noggin is a secreted protein that is able to accomplish two of the major functions of the organizer: it induces dorsal ectoderm to form neural tissue, and it dorsalizes mesoderm cells that would otherwise contribute to the ventral mesoderm (Smith et al. 1993). Smith and Harland showed that newly transcribed *noggin* mRNA is first localized in the dorsal blastopore lip region and then becomes expressed in the notochord (Figure 7.25B). Noggin binds to BMP4 and BMP2 and inhibits their binding to receptors (Zimmerman et al. 1996).

**CHORDIN** The second organizer protein found was **chordin**. It was isolated from clones of cDNA whose mRNAs were present in dorsalized, but not in ventralized, embryos (Sasai et al. 1994). These clones were tested by injecting them into ventral blastomeres and seeing whether they induced secondary axes. One of the clones capable of inducing a secondary neural tube contained the *chordin* gene. *Chordin* mRNA was found to be localized in the dorsal blastopore lip and later in the notochord (Figure 7.26). Of all organizer genes observed, *chordin* is the one most acutely activated by β-catenin (Wesseley et al. 2004). Morpholino antisense oligomers directed against the *chordin* message blocked the ability of an organizer graft to induce a secondary central nervous system (Oelgeschläger et al.

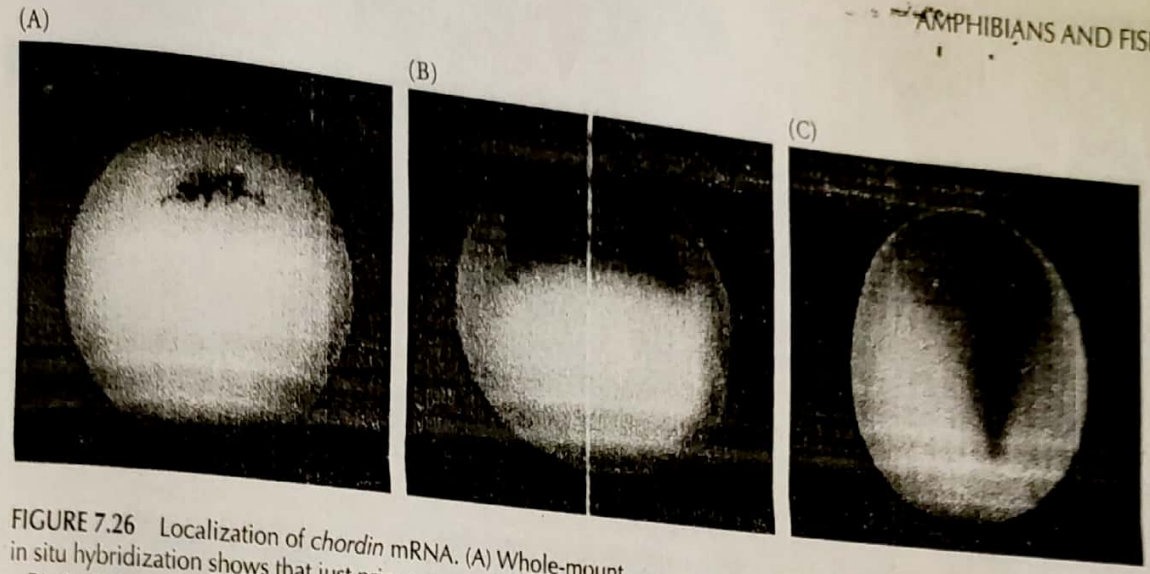



FIGURE 7.26 Localization of *chordin* mRNA. (A) Whole-mount in situ hybridization shows that just prior to gastrulation, *chordin* mRNA (dark area) is expressed in the region that will become the dorsal blastopore lip. (B) As gastrulation begins, *chordin* is expressed at the dorsal blastopore lip. (C) In later stages of gastrulation, the *chordin* message is seen in the organizer tissues. (From Sasai et al. 1994, courtesy of E. De Robertis.)

2003). Like Noggin, chordin binds directly to BMP4 and BMP2 and prevents their complexing with their receptors (Piccolo et al. 1996).

**FOLLISTATIN** The mRNA for a third organizer-secreted protein, **follistatin**, is also transcribed in the dorsal blastopore lip and notochord. Follistatin was found in the organizer through an unexpected result of an experiment that was looking for something else. Ali Hemmati-Brivanlou and Douglas Melton (1992, 1994) wanted to see whether the protein activin was required for mesoderm induction. In searching for the mesoderm inducer, they found that follistatin, an inhibitor of both activin and BMPs, caused ectoderm to become neural tissue. They then proposed that under normal conditions, ectoderm becomes neural unless induced to become epidermal by the BMPs. This model was supported by, and explained, certain cell dissociation experiments that had also produced odd results. Three 1989 studies—by Grunz and Tacke, Sato and Sargent, and Godsave and Slack—had shown that when whole embryos or their animal caps were dissociated, they formed neural tissue. This result would be explainable if the "default state" of the ectoderm was not epidermal, but neural, and tissue had to be induced to have an epidermal phenotype. Thus we conclude that *the organizer blocks this epidermalizing induction by inactivating BMPs.*

See WEBSITE 7.7 Specification of the endoderm

## Epidermal inducers: The BMPs

In *Xenopus*, the epidermal inducers are the bone morphogenetic protein BMP4 and its close relatives BMP2, BMP7, and ADMP (anti-dorsalizing morphogenetic protein, a BMP-like paracrine factor). There is an antagonistic relationship between these BMPs and the organizer. If the mRNA for BMP4 is injected into *Xenopus* eggs, all the mesoderm in the embryo becomes ventrolateral mesoderm. Involution is delayed and, when it does occur, has a ventral rather than a dorsal character (Dale et al. 1992; Jones et al. 1992). Conversely, overexpression of a dominant negative BMP4 receptor results in the formation of twinned axes (Graff et al. 1994; Suzuki et al. 1994). In 1995, Wilson and Hemmati-Brivanlou demonstrated that BMP4 induces ectodermal cells to become epidermal. By 1996, several laboratories had demonstrated that Noggin, chordin, and follistatin are all secreted by the organizer, and that each of them prevents BMP from binding to and inducing the ectoderm and mesoderm cells near the organizer (Piccolo et al. 1996; Zimmerman et al. 1996; Iemura et al. 1998).

BMP4 is expressed initially throughout the ectodermal and mesodermal regions of the late blastula. However, during gastrulation, *bmp4* transcripts become restricted to the ventrolateral marginal zone. This is because the Goosecoid protein (and some other transcription factors) are induced by the Siamois/Twin and Smad2/4 interactions in the dorsal (organizer) mesoderm starting at the beginning of gastrulation (Blitz and Cho 1995; Yao and Kessler 2001). These transcription factors repress *bmp4* and *wnt8* transcription (Hemmati-Brivanlou and Thomsen 1995; Northrop et al. 1995; Steinbeisser et al. 1995; Glavic et al. 2001). In the ectoderm, BMPs repress the genes (such as *neurogenin*) involved in forming neural tissue, while activating other genes involved in epidermal specification (Lee et al. 1995). In the mesoderm, it appears that graded levels of BMP4 activate different sets of mesodermal genes, thereby specifying the dorsal, intermediate, and lateral mesodermal tissues (Figure 7.27; Gawantka et al. 1995; Hemmati-Brivanlou and Thomsen 1995; Dosch et al. 1997).

In 2005, two important sets of experiments confirmed the default model and the importance of blocking BMPs to specify the nervous system. First, Khokha and colleagues

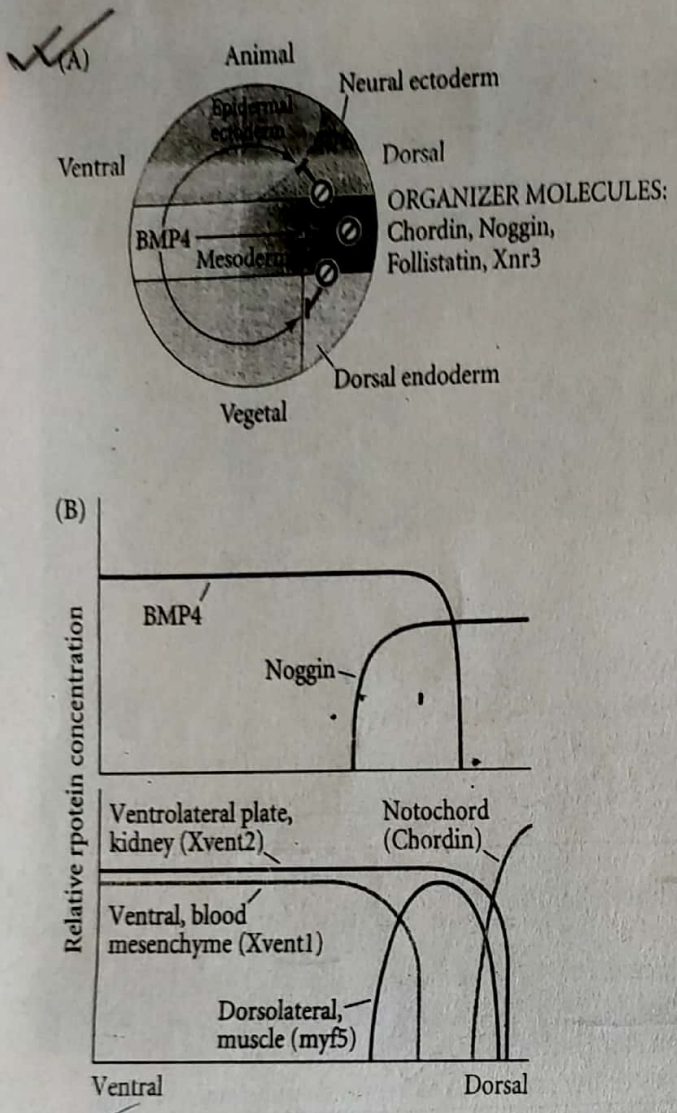



**FIGURE 7.27** Model for the action of the organizer. (A) BMP4 (along with certain other molecules) is a powerful ventralizing factor. Organizer proteins such as chordin, Noggin, and follistatin block the action of BMP4; their inhibitory effects can be seen in all three germ layers. (B) BMP4 may elicit the expression of different genes in a concentration-dependent fashion. Thus, in the regions of *noggin* and *chordin* expression, BMP4 is totally prevented from binding, and these tissues become notochord (organizer) tissue. Slightly farther away from the organizer, the *myf5* gene is activated, producing a marker for the dorsolateral muscles. As more and more BMP4 molecules are allowed to bind to the cells, the *Xvent2* (ventrolateral) and *Xvent1* (ventral) genes become expressed. (After Dosch et al. 1997; De Robertis et al. 2000.)

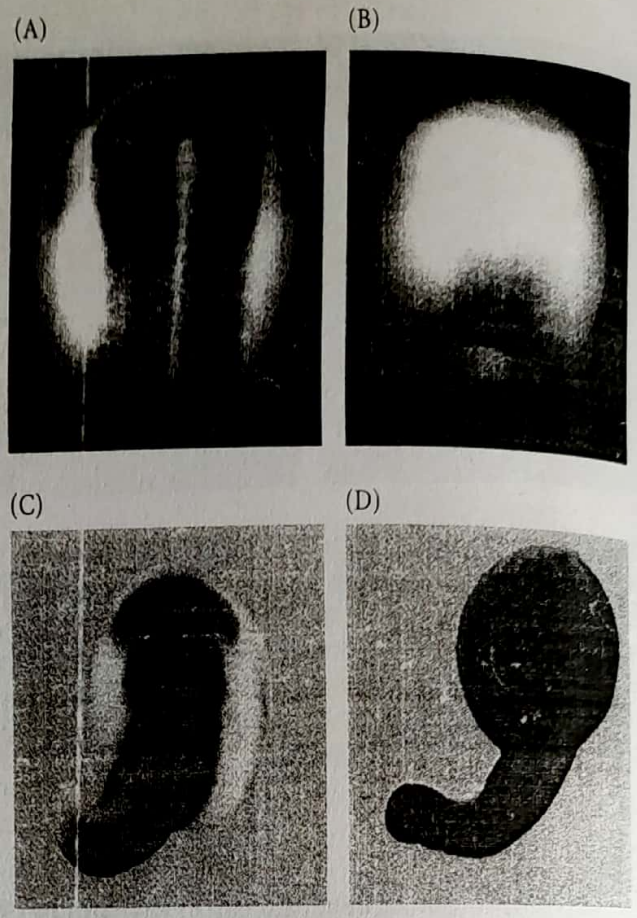



**FIGURE 7.28** Control of neural specification by the levels of BMPs. (A,B) Lack of dorsal structures in *Xenopus* embryos whose BMP-inhibitor genes (*chordin*, *noggin*, and *follistatin*) were eliminated by antisense morpholino oligonucleotides. (A) Control embryo with neural folds stained for the expression of the neural gene *Sox2*. (B) Lack of neural tube and *Sox2* expression in an embryo treated with the morpholinos against all three BMP inhibitors. (C,D) Expanded neural development. (C) The neural tube, visualized by *Sox2* staining, is greatly enlarged in embryos treated with antisense morpholinos that destroy BMPs 2, 4, and 7. (D) Complete transformation of the entire ectoderm into neural ectoderm (and loss of the dorsal-ventral axis) by inactivation of ADMF as well as BMPs 2, 4, and 7. (A,B from Khokha et al. 2005, courtesy of R. Harland; C,D from Reversade and De Robertis 2005.)

(2005) used antisense morpholinos to eliminate the three BMP antagonists (i.e., Noggin, chordin, and follistatin) in *Xenopus*. The resulting embryos had catastrophic failure of dorsal development and lacked neural plates and dorsal mesoderm (Figure 7.28A,B). Second, Reversade and colleagues blocked BMP activity with antisense morpholinos (Reversade et al. 2005; Reversade and De Robertis 2006). When they simultaneously blocked the formation of BMPs 2, 4, and 7, the neural tube became greatly expanded, taking over a much larger region of the ectoderm (Figure 7.28C). When they did a quadruple inactivation of the three BMPs *and* ADMP, the entire ectoderm became neural—no dorsoventral polarity was apparent (Figure 7.28D). Thus the epidermis is instructed by BMP signaling, and the organizer works by blocking that BMP signal from reaching the ectoderm above it.

SPECULATIONS

## BMP4 and Geoffroy's Lobster

The hypothesis that the organizer secretes proteins that block BMPs received further credence from an unexpected source—the emerging field of evolutionary developmental biology (see Chapter 19). Researchers have discovered that the same chordin-BMP4 interaction that instructs the formation of the neural tube in vertebrates also forms neural tissue in fruit flies (Holley et al. 1995; Schmidt et al. 1995; De Robertis and Sasai 1996). The dorsal neural tube of the vertebrate and the ventral neural cord of the fly appear to be generated by the same set of instructions.

The *Drosophila* homologue of the *bmp4* gene is *decapentaplegic* (*dpp*). As discussed in Chapter 6, Dpp protein is responsible for patterning the fly's dorsal-ventral axis; it is present in the dorsal portion of the fly embryo and diffuses ventrally. Dpp is opposed by a protein called Short-gastrulation (Sog), which is the *Drosophila* homologue of chordin. These insect homologues not only appear to be similar to their vertebrate counterparts, they can actually substitute for each other. When *sog* mRNA is injected into ventral regions of *Xenopus* embryos, it induces the amphibian notochord and neural tube. Injecting *chordin* mRNA into *Drosophila* embryos produces ventral nervous tissue.

Although chordin dorsalizes the *Xenopus* embryo, it ventralizes *Drosophila*. In *Drosophila*, Dpp is made dorsally; in *Xenopus*, BMP4 is made ventrally. In both cases, Sog/chordin helps specify neural tissue by blocking the effects of Dpp/BMP4. In *Drosophila*, Sog interacts with Tolloid and several other proteins to create a gradient of Sog proteins. In *Xenopus*, the homologues of the same proteins act to create a gradient of chordin (see Figure 19.4; Hawley et al. 1995; Holley et al. 1995; De Robertis et al. 2000).

In 1822, the French anatomist Étienne Geoffroy Saint-Hilaire provoked one of the most heated and critical confrontations in biology when he proposed that the lobster was but a vertebrate upside down. He claimed that the ventral side of the lobster (with its nerve cord) was homologous to the dorsal side of the vertebrate (Appel 1987). It seems that he was correct on the molecular level, if not on the anatomical level. The instructions for producing a nervous system in fact may have evolved only once, and the myriad animal lineages may all have used this same set of instructions—just in different places. The BMP4 (Dpp)/chordin (Sog) interaction is an example of "homologous processes," suggesting a unity of developmental principles among all animals (Gilbert and Bolker 2001).

## The Regional Specificity of Neural Induction

One of the most important phenomena in neural induction is the regional specificity of the neural structures that are produced. Forebrain, hindbrain, and spinocaudal regions of the neural tube must be properly organized in an anterior-to-posterior direction. The organizer tissue not only induces the neural tube, it also specifies the regions of the neural tube. This region-specific induction was demonstrated by Hilde Mangold's husband, Otto Mangold, in 1933. He transplanted four successive regions of the archenteron roof of late-gastrula newt embryos into the blastocoels of early-gastrula embryos. The most anterior portion of the archenteron roof (containing head mesoderm) induced balancers and portions of the oral apparatus; the next most anterior section induced the formation of various head structures, including nose, eyes, balancers, and otic vesicles; the third section (including the notochord) induced the hindbrain structures; and the most posterior section induced the formation of dorsal trunk and tail mesoderm* (Figure 7.29A–D).

In further experiments, Mangold demonstrated that when dorsal blastopore lips from early salamander gastrulae were transplanted into other early salamander gastrulae, they formed secondary heads. When dorsal lips from later gastrulas were transplanted into early salamander gastrulae, however, they induced the formation of secondary tails (Figure 7.29E,F; Mangold 1933). These results show that the first cells of the organizer to enter the embryo induce the formation of brains and heads, while those cells

*The induction of dorsal mesoderm—rather than the dorsal ectoderm of the nervous system—by the posterior end of the notochord was confirmed by Bijtel (1931) and Spofford (1945), who showed that the posterior fifth of the neural plate gives rise to tail somites and the posterior portions of the pronephric kidney duct.

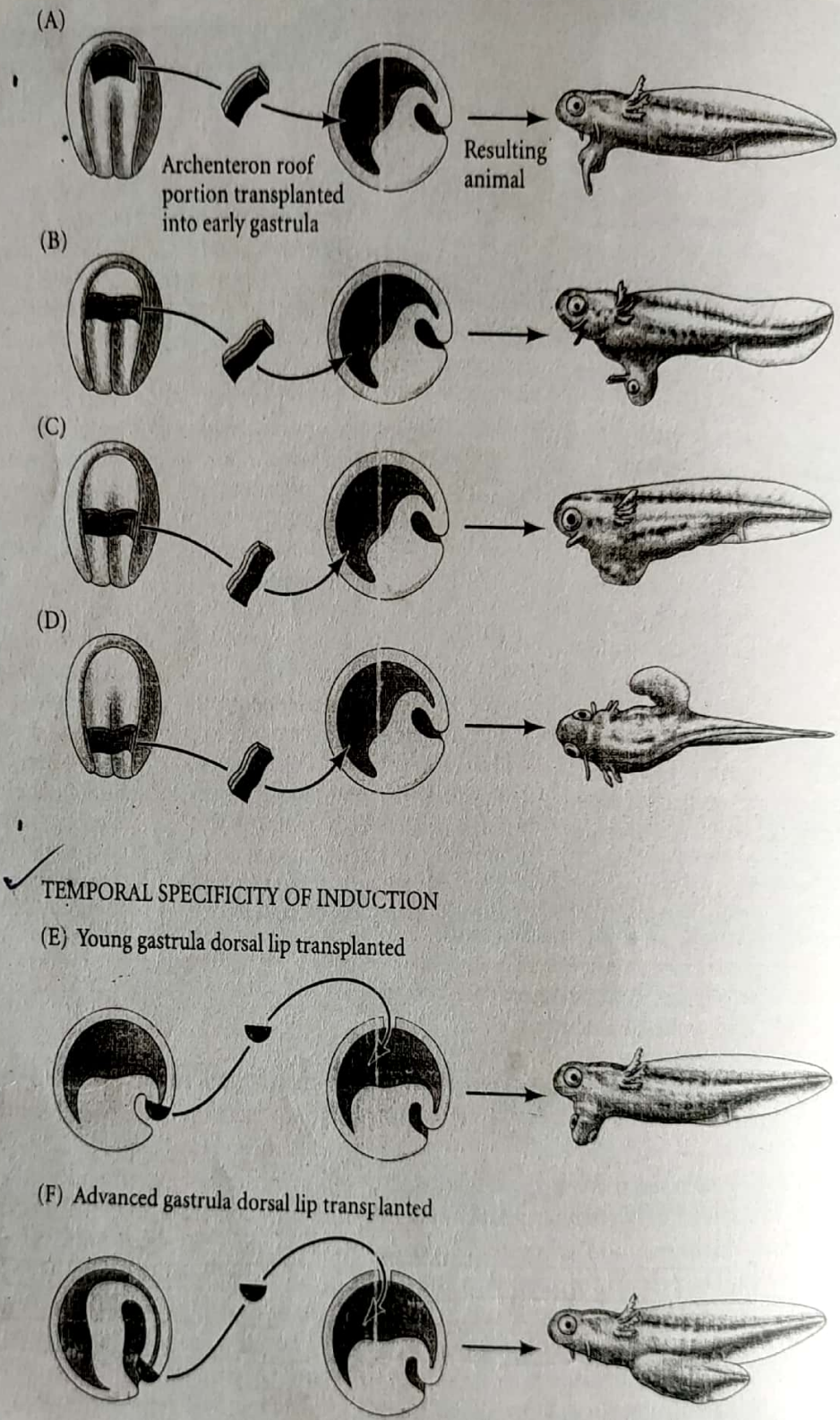



**FIGURE 7.29** Regional and temporal specificity of induction. (A–D) Regional specificity of structural induction can be demonstrated by implanting different regions (color) of the archenteron roof into early *Triturus* gastrulae. The resulting embryos develop secondary dorsal structures. (A) Head with balancers. (B) Head with balancers, eyes, and forebrain. (C) Posterior part of head, diencephalon, and otic vesicles. (D) Trunk-tail segment. (E,F) Temporal specificity of inducing ability. (E) Young dorsal lips (which will form the anterior portion of the organizer) induce anterior dorsal structures when transplanted into early newt gastrulae. (F) Older dorsal lips transplanted into early newt gastrulae produce more posterior dorsal structures. (A–D after Mangold 1933; E,F after Saxén and Toivonen 1962.)

that form the dorsal lip of later-stage embryos induce the cells above them to become spinal cords and tails.

The question then became, What are the molecules being secreted by the organizer in a regional fashion such that the first cells involuting through the blastopore lip (the endomesoderm) induce head structures, while the next portion of involuting mesoderm (notochord) produces trunk and tail structures? Figure 7.30 shows a possible model for these inductions, the elements of which we will now describe in detail.

## The head inducer: Wnt inhibitors

The most anterior regions of the head and brain are underlain not by notochord but by pharyngeal endoderm and head (prechordal) mesoderm (see Figures 7.6C,D and

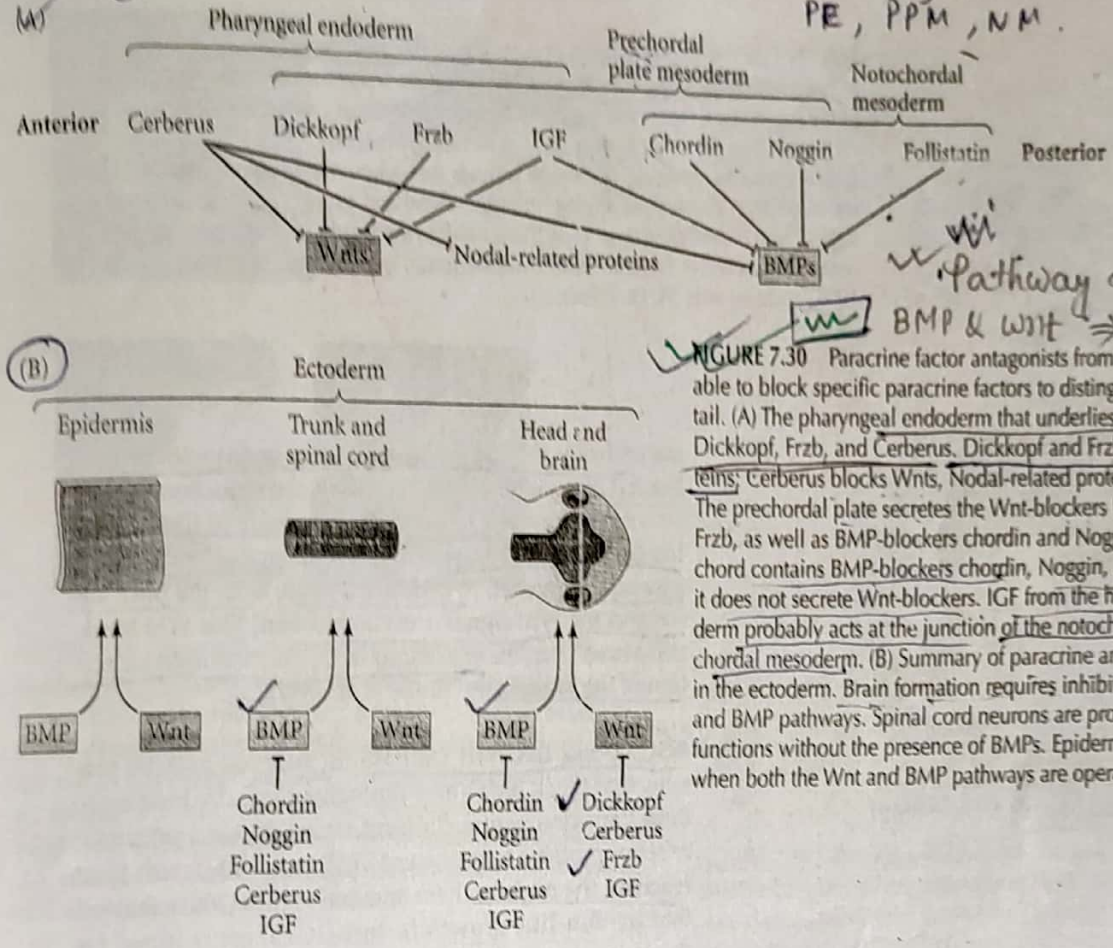



FIGURE 7.30 Paracrine factor antagonists from the organizer are able to block specific paracrine factors to distinguish head from tail. (A) The pharyngeal endoderm that underlies the head secretes Dickkopf, Frzb, and Cerberus. Dickkopf and Frzb block Wnt proteins; Cerberus blocks Wnts, Nodal-related proteins, and BMPs. The prechordal plate secretes the Wnt-blockers Dickkopf and Frzb, as well as BMP-blockers chordin and Noggin. The notochord contains BMP-blockers chordin, Noggin, and follistatin, but it does not secrete Wnt-blockers. IGF from the head endomesoderm probably acts at the junction of the notochord and prechordal mesoderm. (B) Summary of paracrine antagonist function in the ectoderm. Brain formation requires inhibiting both the Wnt and BMP pathways. Spinal cord neurons are produced when Wnt functions without the presence of BMPs. Epidermis is formed when both the Wnt and BMP pathways are operating.

7.30A). This endomesodermal tissue constitutes the leading edge of the dorsal blastopore lip. Recent studies have shown that these cells not only induce the most anterior head structures, but that they do it by blocking the Wnt pathway as well as by blocking BMP4.

**CERBERUS** In 1996, Bouwmeester and colleagues showed that the induction of the most anterior head structures could be accomplished by a secreted protein called Cerberus.* Unlike the other proteins secreted by the organizer, Cerberus promotes the formation of the cement gland (the most anterior region of tadpole ectoderm), eyes, and olfactory (nasal) placodes. When *cerberus* mRNA was injected into a vegetal ventral *Xenopus* blastomere at the 32-cell stage, ectopic head structures were formed (Figure 7.31). These head structures arose from the injected cell as well as from neighboring cells.

The *cerberus* gene is expressed in the pharyngeal endomesoderm cells that arise from the deep cells of the early dorsal lip. Cerberus protein can bind BMPs, Nodal-related proteins, and Xwnt8 (see Figure 7.30; Piccolo et al. 1999). When Cerberus synthesis is blocked, the levels of BMP, Nodal-related proteins, and Wnts all rise in the anterior of the embryo, and the ability of the anterior endomesoderm to induce a head is severely diminished (Silva et al. 2003).

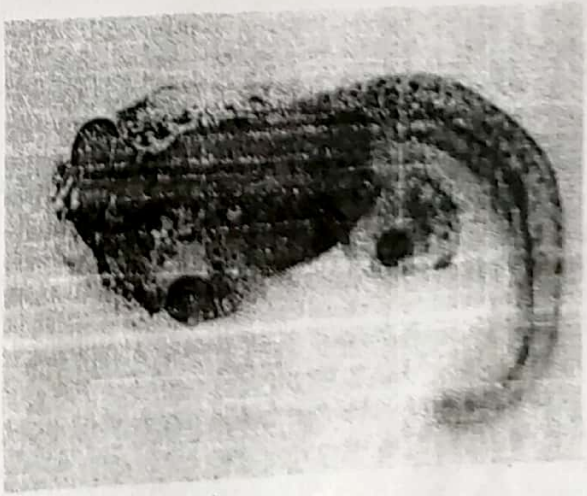


FIGURE 7.31 *Cerberus* mRNA injected into a single D4 (ventral vegetal) blastomere of a 32-cell *Xenopus* embryo induces head structures as well as a duplicated heart and liver. The secondary eye (a single cyclopic eye) and olfactory placode can be readily seen. (From Bouwmeester et al. 1996, courtesy of E. M. De Robertis.)

*Cerberus is named after the three-headed dog that guarded the entrance to Hades in Greek mythology.

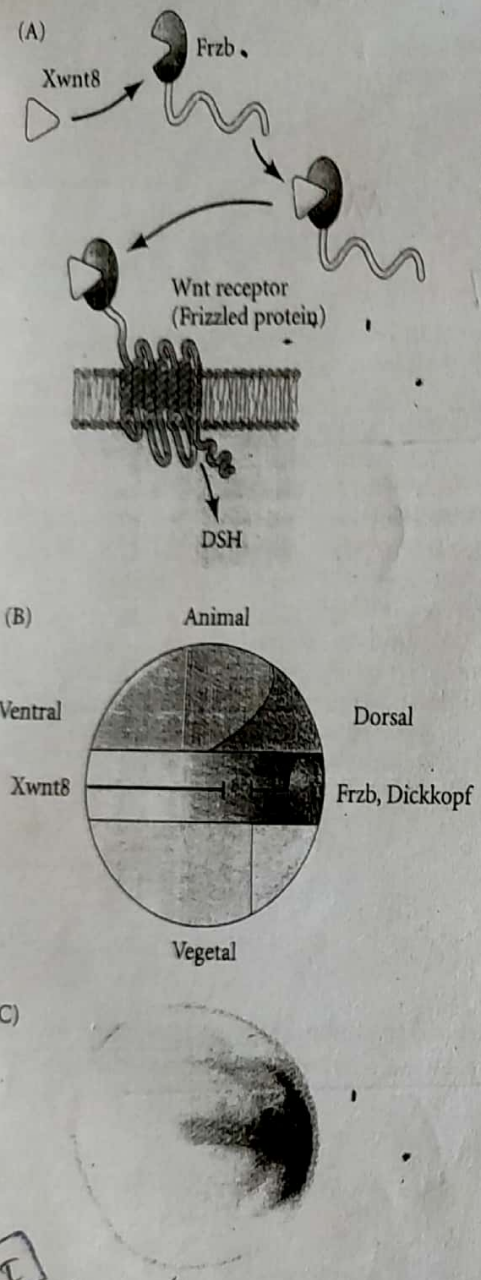



FIGURE 7.32 Xwnt8 is capable of ventralizing the mesoderm and preventing anterior head formation in the ectoderm. (A) Frzb protein is secreted by the anterior region of the organizer. It must bind to Xwnt8 before that inducer can bind to its receptor. Frzb resembles the Wnt-binding domain of the Wnt receptor (Frizzled protein), but Frzb is a soluble molecule. (B) Xwnt8 is made throughout the marginal zone. (C) Double in situ hybridization localizing Frzb (dark stain) and chordin (reddish stain) messages. The *frzb* mRNA is seen to be transcribed in the head endomesoderm of the organizer, but not in the notochord (where *chordin* is expressed). (From Leyns et al. 1997, courtesy of E. M. De Robertis.)

**FRZB AND DICKKOPF** Shortly after the attributes of Cerberus were demonstrated, two other proteins, Frzb and Dickkopf, were discovered to be synthesized in the involuting endomesoderm. **Frzb** (pronounced "frisbee") is a small, soluble form of Frizzled (the Wnt receptor), and it is capable of binding Wnt proteins in solution (Figure 7.32; Leyns et al. 1997; Wang et al. 1997). Frzb is synthesized predominantly in the endomesoderm cells beneath the brain (see Figure 7.32B,C). If embryos are made to synthesize excess Frzb, Wnt signaling fails to occur throughout the embryo; such embryos lack ventral posterior structures and become "all head." The **Dickkopf** protein (German, "thick head," "stubborn") also appears to interact directly with the Wnt receptors, preventing Wnt signaling (Mao et al. 2001, 2002). Injection of antibodies against Dickkopf causes the resulting embryos to have small, deformed heads with no forebrain (Glinka et al. 1998). Therefore, the induction of trunk structures may be caused by the blockade of BMP signaling from the notochord, while Wnt signals are allowed to proceed. However, to produce a head, both the BMP signal and the Wnt signal must be blocked. This Wnt blockade comes from the endomesoderm, the most anterior portion of the organizer (Glinka et al. 1997).

**INSULIN-LIKE GROWTH FACTORS** In addition to those proteins that block BMPs and Wnts by physically binding to these paracrine factors, the head region contains yet another set of proteins that prevent BMP and Wnt signals from reaching the nucleus. Pera and colleagues (2001) showed that **insulin-like growth factors** (**IGFs**) are required for the formation of the anterior neural tube, including the brain and sensory placodes. IGFs accumulate in the dorsal midline and are especially prominent in the anterior neural tube (Figure 7.33A). When injected into ventral mesodermal blastomeres, mRNA from IGFs causes the formation of ectopic heads, while blocking the IGF receptors results in the lack of head formation (Figure 7.33B,C).

Insulin-like growth factors appear to work by initiating a receptor tyrosine kinase (RTK) signal transduction cascade (see Chapter 3) that interferes with the signal transduction pathways of both BMPs and Wnts (Richard-Parpaillon et al. 2002; Pera et al. 2003).

## *Trunk patterning: Wnt signals and retinoic acid*

Toivonen and Saxén provided evidence for a gradient of a posteriorizing factor that would act to specify the trunk and tail tissues of the amphibian embryo* (Toivonen and

*The tail inducer was initially thought to be part of the trunk inducer, since transplantation of the late dorsal blastopore lip into the blastocoel often produced larvae with extra tails. However, it appears that tails are normally formed by interactions between the neural plate and the posterior mesoderm during the neurula stage (and thus are generated outside the organizer). Here, Wnt, BMPs, and Nodal signaling all seem to be required (Tucker and Slack 1995; Niehrs 2004). Interestingly, all three of these signaling pathways have to be inactivated if the head is to form.

* Name the secrete of Pharyngeal endoderm which block Wnts
⇒ Frz B, Dickkopf



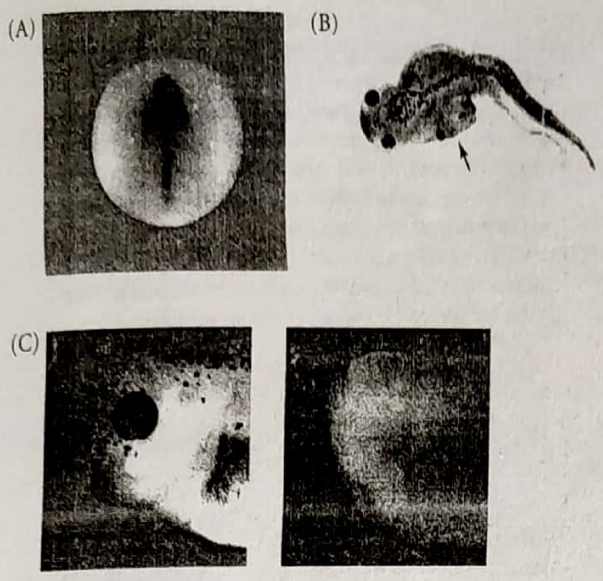



FIGURE 7.33 Insulin-like growth factors enhance anterior neural development. (A) Expression pattern of *Igf3*, showing protein accumulation in the anterior neural tube. (B) An ectopic headlike structure (complete with eyes and cement gland) formed when *Igf2* mRNA was injected into ventral marginal zone blastomeres. (C) Anterior of 3-day control tadpole (left) compared with a tadpole whose 4-cell embryonic blastomeres were injected with an IGF inhibitor. The cement gland and eyes are absent. (From Pera et al. 2001, courtesy of E. M. De Robertis.)

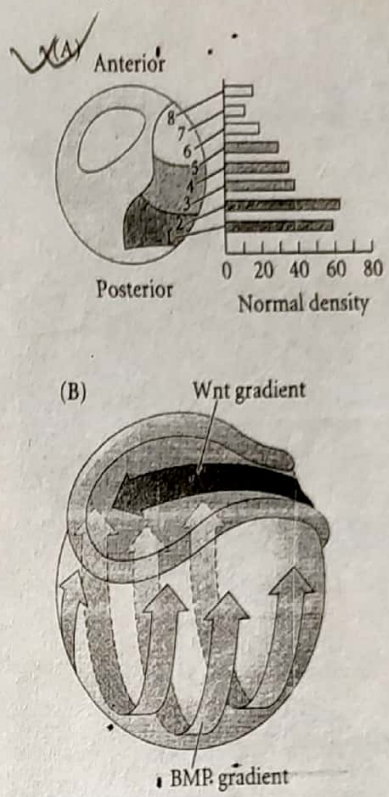



FIGURE 7.34 Wnt signaling pathway and posteriorization of the neural tube. (A) Gradient of β-catenin in the presumptive neural plate during gastrulation. Gastrulating embryos were stained for β-catenin and the density of the stain compared between regions of the ectodermal cells. (B) Double-gradient model whereby a gradient of BMP expression specifies the frog dorsal-ventral axis while a gradient of Wnt proteins specifies the anterior-posterior axis. (After Kiecker and Niehrs 2001; Niehrs 2004.)

Saxén 1955, 1968; reviewed in Saxén 2001). This factor's activity would be highest in the posterior of the embryo and weakened anteriorly. Recent studies have extended this model and have proposed candidates for posteriorizing molecules. The primary protein involved in posteriorizing the neural tube is thought to be a member of the Wnt family of paracrine factors, most likely Xwnt8 (Domingos et al. 2001; Kiecker and Niehrs 2001).

It appears that a gradient of Wnt proteins is necessary for specifying the posterior region of the neural plate (the trunk and tail; Hoppler et al. 1996; Niehrs 2004). In *Xenopus*, an endogenous gradient of Wnt signaling and β-catenin is highest in the posterior and absent in the anterior (Figure 7.34A). Moreover, if Xwnt8 is added to developing embryos, spinal cord-like neurons are seen more anteriorly in the embryo, and the most anterior markers of the forebrain are absent. Conversely, suppressing Wnt signaling (by adding Frzb or Dickkopf to the developing embryo) leads to the expression of the anteriormost markers in more posterior neural cells. Therefore, there appear to be two major gradients in the amphibian gastrula—a BMP gradient that specifies the dorsal-ventral axis and a Wnt gradient specifying the anterior-posterior axis (Figure 7.34B). It must be remembered, too, that both of these axes are established by the initial axes of Nodal-like TGF-β factors and β-catenin across the vegetal cells. The basic model of neural induction, then, looks like the diagram in Figure 7.35.

While the Wnt proteins probably play a major role in specifying the anterior-posterior axis, they are probably not the only agents involved. Fibroblast growth factors appear to be critical in allowing the cells to respond to the Wnt signal (Holowacz and Sokol 1999; Domingos et al. 2001). Retinoic acid also is seen to have a gradient highest at the posterior end of the neural plate, and RA can also posteriorize the neural tube in a concentration-dependent manner (Cho and De Robertis 1990; Sive and Cheng 1991; Chen et al. 1994). RA signaling appears to be especially important in patterning the hindbrain and appear to interact with Fgf signals to activate the posterior Hox genes (Kolm et al. 1997; Dupé and Lumsden 2001; Shiotsugu et al. 2004).

**See WEBSITE 7.8 Regional specification**

**See VADE MECUM The primary organizer and double gradient hypothesis**

* wnt is responsible for anterior & posterior axis.
* Ratinoic acid " " formation of hindbrain.