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Notochord Patterning of the Endoderm

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Endodermally derived organs of the gastrointestinal and respiratory system form at distinct anterioposterior and dorsoventral locations along the vertebrate body axis. This stereotyped program of organ formation depends on the correct patterning of the endodermal epithelium so that organ differentiation and morphogenesis occur at appropriate positions along the gut tube. Whereas some initial patterning of the endoderm is known to occur early, during germ-layer formation and gastrulation, later signaling events, originating from a number of adjacent tissue layers, are essential for the development of endodermal organs. Previous studies have shown that signals arising from the notochord are important for patterning of the ectodermally derived floor plate of the neural tube and the mesodermally derived somites. This review will discuss recent evidence indicating that signals arising from the notochord also play a role in regulating endoderm development.

INTRODUCTION

The notochord is an axial structure of mesodermal origin. Its presence during embryonic development defines members of the Chordate phylum. Notochord precursors emerge during gastrulation from the organizer region of embryos, such as the blastopore lip of the amphibian embryo and Hensen's node of the mouse and chick. The notochord is one of the earliest embryonic structures to be formed and functions as a structural support for the entire organism, either transiently (as in higher vertebrates) or persistently (as in some lower vertebrates). The rigidity of the notochord maintains alignment of embryonic tissues during development and allows axis elongation (Spemann, 1938; Adams *et al.*, 1990). Many different studies have shown that in addition to its structural function, the notochord plays a critical role in patterning of ectodermal and mesodermal tissues, such as the neural tube and somitic derivatives. Recently, experimental evidence has been obtained showing that the notochord signals to the underlying endoderm. This complements a number of clinical and embryological observations made over the past 100 years that had previously implied a role for notochord in endodermal patterning. At present it is quite unclear how the notochord can achieve such a diverse range of inductive abilities. This diversity could result from differences in the inherent competence of the responding tissues or may perhaps be due to the localization of instructive signals within the notochord. In view of the importance of inductive interactions for embryonic development, the question of notochord-to-endoderm signaling certainly justifies further experimental investigation, particularly characterization of the tissue interactions at the molecular level. In the sections below, we will first review the evidence demonstrating that the notochord is a source of signaling molecules that influence the developmental fate of ectodermal and mesodermally derived tissues. We will then discuss the more recent studies which show that the notochord is also involved in patterning of endoderm.

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NOTOCHORD SIGNALING TO ECTODERM

Notochord Patterning of the Neural Tube

The developing neural tube exhibits a distinct dorsoventral (DV) polarity, characterized by differences in cell mor-

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phology and by the position of specific classes of neurons. In the early embryo, the notochord lies immediately beneath the floor plate, a specialized group of neuroepithelial cells in the ventral portion of the spinal chord. The role of the notochord in the induction of the floor plate has been studied intensively in a number of different organisms (for review see Jessell and Dodd, 1990–1991; Placzek *et al.,* 1991; Placzek, 1995; Dodd *et al.,* 1998). For example in *Xenopus laevis,* ultraviolet irradiation of fertilized eggs causes dose-dependent deficits in notochord development (Cooke, 1985). In these experiments, embryos which fail to form a notochord also show severe disruption of proper floor-plate formation in the neural tube (Youn and Malacinski, 1981; Clarke *et al.,* 1991). In chick embryos, when an ectopic notochord is grafted adjacent to the neural tube, cells in the lateral walls of the neural tube are ventralized and induced to exhibit the morphological and functional properties of the floor plate, including its associated motor neurons and bundles of efferent axons (van Straaten *et al.,* 1985, 1988; Smith and Shoenwolf, 1989; Placzek *et al.,* 1990; Yamada *et al.,* 1991). Dorsal neural tube markers such as *Pax-3, Pax-6,* and *dorsalin* are repressed in the vicinity of the grafted notochord (Goulding *et al.,* 1993; Basler *et al.,* 1993). Conversely, notochord extirpation in chick embryos results in the absence of the floor plate and of the adjacent motor neuron pools (van Straaten and Drukker, 1987; Placzek *et al.,* 1990; Hirano *et al.,* 1991; van Straaten and Hekking, 1991; Yamada *et al.,* 1991). As expected, this is accompanied by a ventral shift in the domain of expression of dorsal neural tube markers (Goulding *et al.,* 1993; Basler *et al.,* 1993). The induction of the floor plate by the notochord is thought to be mediated by the secreted protein Sonic Hedgehog (SHH), which is expressed in the notochord and can induce floor-plate markers both *in vivo* and *in vitro* (Echelard *et al.,* 1993; Fan and Tessier-Lavigne, 1994; Johnson *et al.,* 1994; Roelink *et al.,* 1994; Marti *et al.,* 1995; Munsterberg *et al.,* 1995; Ericson *et al.,* 1996).

When discussing the role of patterning by the notochord, it is impossible to ignore some recent studies that call into question the importance of notochord signaling for the development of the floor plate (LeDouarin *et al.,* 1998; Teillet *et al.,* 1998). Specifically, it is argued that the observed failure of floor-plate development, following the removal of the notochord, results from inadvertent removal of floor-plate cells, rather than the absence of inductive notochord signals. In addition, these investigators point to a number of zebrafish mutants, such as *flh, ntl, cyclops,* and *oep* mutants, which can develop either a notochord or a floor plate, apparently independent of each other. However, while these studies have raised some interesting questions about lineage relationships of axial tissues and certain aspects of notochord signaling, the ability of notochord to induce an ectopic floor plate in the lateral walls of the neural tube remains unquestioned and strongly implies an important role for notochord signals in neurectoderm patterning. Discussion of these arguments can be followed in the specialist literature (Vogel, 1998; Placzek *et al.,* 2000; LeDouarin and Halpern, 2000).

Notochord Patterning of Other Ectoderm Derivatives

In addition to neural tube patterning, the notochord appears to influence development of other ectodermal structures. It has been observed that the tip of the notochord contacts head ectoderm fated to become the anterior pituitary, thereby raising the possibility that the notochord might be involved in pituitary growth and development (Eyal-Giladi, 1958; Barteczko and Jacob, 1999). In support of this hypothesis, transplantation of anterior notochord into a lateral region of the head causes the stomodeal ectoderm to invaginate and form a pocket structure reminiscent of the early appearance of Rathke's pouch, the precursor of the anterior pituitary (Gleiberman *et al.,* 1999). Although notochord is not sufficient to induce complete formation of the anterior pituitary, these experiments clearly implicate the notochord in the early stages of development of an independent, ectodermally derived tissue.

NOTOCHORD SIGNALING TO MESODERM

Notochord Patterning of the Somites

Numerous studies show that the notochord is involved in patterning of the paraxial mesoderm. Classical embryological experiments demonstrated that, when the dorsal blastopore lip of the amphibian embryo is surgically removed at gastrulation, the resulting embryo fails to initiate notochord formation and subsequently develops irregular somites which fuse at the midline (Lehmann, 1926, 1928). Similarly, the zebrafish mutants *ntl* and *flh,* both of which lack a notochord, also exhibit fused somites which are characterized by disrupted somite chevron formation and by the lack of muscle pioneer cells (Halpern *et al.,* 1993, 1995). The somites of *ntl* mutant embryos correspondingly exhibit incorrect spatiotemporal expression of the muscle determination gene, *myoD* (Weinberg *et al.,* 1996). In the chick embryo, removal of the notochord results in the failure of sclerotome formation and a corresponding enlargement of the dermamyotome (Goulding *et al.,* 1994). At the molecular level, this is revealed by the absence of the sclerotome marker, *Pax1,* and expansion of the expression domains of the dermamyotome markers *Pax3* and *Pax-7.* In contrast, notochord grafts can induce more dorsal somitic cells to differentiate into axial cartilage (a sclerotome derivative) while repressing dermamyotome development. As expected, *Pax1* is upregulated in these tissues, while *Pax3* and *Pax-7* are repressed (Brand-Saberi *et al.,* 1993; Pourquié *et al.,* 1993; Goulding *et al.,* 1994). Like floor-plate formation, the determination of the sclerotome is thought to be mediated by SHH secreted from the notochord (Munster-

berg *et al.*, 1995; Bumcrot and McMahon, 1995; Lassar and Munsterberg, 1996).

Although the influence of the notochord on cartilage formation from sclerotome is firmly established, the precise role of the notochord on myogenic specification appears to be complex and is not completely understood (reviewed in Hall, 1977; Halpern, 1997). Nevertheless, both *in vitro* and *in vivo* studies provide strong evidence that the notochord does exert an important influence on muscle development and that, once again, this signaling may be mediated by SHH (Kenny-Mobbs and Thorogood, 1987; Bober *et al.*, 1994; Munsterberg *et al.*, 1995; Bumcrot and McMahon, 1995; Lassar and Munsterberg, 1996; Pownall *et al.*, 1996; Xue and Xue, 1996).

Notochord Patterning of Other Mesodermal Tissues

A number of independent studies provide evidence that notochord signaling is also important for development of the heart and the vasculature and for establishing the laterality of organs. Studies in zebrafish show a role for the notochord in regulation of early cardiac development (Goldstein and Fishman, 1998). More specifically, laser ablation of the anterior extremity of the notochord causes expansion of the expression domain of the homeobox gene *Nkx2-5*, a marker for the presumptive heart field. This suggests that the notochord might normally function to suppress cardiogenic fate in the underlying splanchnic mesoderm. Notochord signals have also been associated with formation of the dorsal aorta. The zebrafish mutants *ntl* and *flh*, both of which lack a notochord, also fail to form the dorsal aorta (Fouquet *et al.*, 1997; Sumoy *et al.*, 1997). When wild-type notochord cells are transplanted into *flh* mutants, some notochord development is restored and an aortic primordium forms. Finally, the notochord may be involved in assignment of left–right asymmetry. When the notochord is experimentally ablated or when it is absent in mutant embryos, asymmetric markers of lateral plate mesoderm are either randomized or expressed bilaterally. In *Xenopus* embryos, either surgical extirpation of the notochord or suppression of its development using UV irradiation leads to cardiac reversals and bilateral expression of the laterality marker *nodal* in the lateral plate mesoderm (Danos and Yost, 1995; Lohr *et al.*, 1997). Similar reversals are seen in notochord-deficient zebrafish mutants such as *ntl* and *flh* (Danos and Yost, 1996; Bisgrove *et al.*, 2000). Furthermore, in mice homozygous for the *no turning* mutation, both the notochord and the floor plate degenerate, and these embryos exhibit randomized cardiac looping and bilateral expression of the laterality markers *nodal* and *lefty* (Melloy *et al.*, 1998). Equivalent results are obtained when the node is surgically ablated in mouse embryos, resulting in the failure of notochord development and subsequent randomization of expression of *Pitx2*, a regulatory gene in the laterality pathway (Davidson *et al.*, 1999).

PATTERNING OF THE ENDODERM

Early Patterning of the Endoderm

The biological mechanisms responsible for patterning the endoderm are almost unexplored relative to those underlying ectoderm and mesoderm development. However, in order to generate organ primordia at appropriate locations along the gut tube, the endodermal epithelium must receive correct anterior–posterior (AP) and dorsoventral patterning signals. Coordination of these signals results in the formation of the respiratory system, the tympanic cavities, the thymus and thyroid gland, and the digestive system, including the esophagus, stomach, liver, pancreas, intestines, and colon.

What is the origin of regionalized signals during endoderm patterning? In frogs, the endoderm is derived from the vegetal hemisphere of the early cleavage-stage embryo. It is believed that expression of a number of different transcription factors, at the late blastula stage, results in the autonomous specification of endodermal cell fate (Hudson *et al.*, 1997; Rosa, 1989; Henry and Melton, 1998; Yasuo and Lemaire, 1999; Wessely and De Robertis, 2000). These transcription factors include *Sox17α*, *Mix.1*, *Mixer*, *GATA-4*, and *xBix-C*. In the short time between endodermal specification and gastrulation, the frog endoderm acquires a distinct molecular AP prepattern, which is dependent on TGF-β and FGF signaling (Henry *et al.*, 1996). This prepattern in the endoderm is clearly revealed in the expression patterns of endodermal marker genes such as *Xlhbox8*, *Hex*, *Cerberus*, and *xBix-C* (Henry *et al.*, 1996; Newman *et al.*, 1997; Bouwmeester *et al.*, 1996; Wessely and De Robertis, 2000). By the neurula stage, the frog embryo shows the first evidence of differential gene expression along the endodermal DV axis. This is illustrated by the expression patterns of genes such as *FrzA*, *SPARC*, and *Sox17α*, all of which are preferentially expressed in more dorsal regions of the endoderm (Xu *et al.*, 1998; Damjanovski *et al.*, 1994; Hudson *et al.*, 1997). In the mouse embryo, the endoderm acquires initial patterning information during gastrulation, as endodermal cells exit the primitive streak. This patterning is revealed by the regionalized expression of endodermal marker genes long before gut tube formation (Wells and Melton, 1999). For example, at the end of gastrulation, the anterior endoderm expresses *cerberus-like*, *Otx1*, and *Hesx1*, while the posterior endoderm expresses *IFABP* and *Cdx2* (Belo *et al.*, 1997; Thomas and Beddington, 1996; Biben *et al.*, 1998; Rhinn *et al.*, 1998; Wells and Melton, 2000). One factor likely to be important for early endoderm patterning in the mouse is the growth factor FGF4, which is expressed in the primitive streak and has the capacity to induce endoderm in a concentration-dependent manner (Wells and Melton, 2000).

Endoderm Patterning by Interactions with Mesodermal Tissues

Classical embryological methods have been used to investigate later patterning events involved in morphogenesis and differentiation of endodermal organs. These studies

show that reciprocal interactions between the endodermal epithelium and the adjacent mesodermal mesenchyme are critical for specifying regional identity within the gut tube. In response to endodermal signals, mesenchyme is initially recruited from the splanchnic mesoderm, after which it surrounds the gut tube and becomes visceral mesoderm (Kedinger *et al.*, 1986; Roberts *et al.*, 1995). Heterologous recombination experiments using visceral mesoderm and endoderm from different AP locations along the gut tube have been carried out with embryonic tissues from chicken, rat, and mouse (Fukamachi *et al.*, 1979; Fukamachi and Takayama, 1980; Yasugi, 1994). When chick foregut mesoderm is recombined with midgut endoderm, the endoderm becomes respecified to take on foregut endoderm morphology (Kedinger *et al.*, 1986). Similarly, intestinal mesenchyme can cause the respecification of stomach epithelium into intestinal epithelium (Yasugi and Mizuno, 1978, 1990; Ishizuya-Oka and Mizuno, 1984; Andrew and Rawdon, 1990). Experiments with mouse lung epithelium show an equivalent response. When lung bud epithelium is recombined with tracheal mesenchyme it fails to exhibit normal bronchial branching, but continues to grow, without branching, in a manner reminiscent of tracheal growth (Wessells, 1970). In the case of pancreatic bud development, the pancreatic mesenchyme which accumulates around the growing epithelial bud (endodermal tissue) is required for proliferation and branching of the epithelium. Interestingly, however, heterologous mesenchyme derived from other branching organs can substitute for pancreatic mesenchyme and promote pancreatic cell fate (Wessels, 1977; Ahlgren *et al.*, 1997). In summary therefore, the endoderm acquires at least some positional identity and morphogenic information through interactions with adjacent mesodermal tissues.

Hepatic Endoderm Specification

Specification of the developing liver represents an interesting example in which multiple inductive signals coordinate to influence endoderm development. Using a tissue explant system, Gualdi and colleagues showed that both dorsal and ventral foregut endoderm have the potential to differentiate along the hepatic pathway (Gualdi *et al.*, 1996). Furthermore, by culturing foregut endoderm with and without adjacent tissues, they demonstrated that signals from the cardiac mesoderm help to promote liver gene expression, while signals from the dorsal mesoderm and/or ectoderm, near the midline, repress liver gene expression. Although the authors do not identify the dorsal tissue responsible for the negative signals, it seems possible that the notochord is involved. This study also underlines the importance of inductive interactions from adjacent tissues in regulating development of the endodermal epithelium.

Proximity of Notochord and Endoderm

Several observations make it plausible to argue that the notochord is involved in endodermal patterning. In all species examined, the notochord is first formed in close association with the endoderm, and notochord precursors remain embedded in the dorsal endoderm as they coalesce into a rod-shaped structure. As development proceeds, the notochord resolves into an independent structure but continues to adhere to the underlying endoderm, even sharing a common basal lamina for a time (Jurand, 1974; Lamers *et al.*, 1987). Strictly, the notochord remains in contact with the endoderm from gastrulation until about E8 in mice (13-somite stage), stage 14 in chickens (22-somite stage), and stage 32 in frogs (26-somite stage) (Fig. 1). Subsequently, the notochord becomes separated from the endoderm by intervening endothelial tissue. This occurs during the fusion of the dorsal aortae at the midline ventral to the notochord (in mice and chickens) or during the *in situ* formation of a single dorsal aorta (in frogs and fish). The direct contact between the notochord and the endoderm is therefore sustained for much of early development, from gastrulation to well beyond the end of neurulation.

It is interesting to note that, although the spatial relationship between notochord and endoderm is effectively identical in different organisms, the relative size of the notochord varies dramatically (Fig. 1). In frog, the notochord is large and almost as wide as the neural tube, while the murine notochord is extremely narrow compared to adjacent structures. In both cases, however, the notochord is only a few cells in diameter. At present it is unclear whether these structural differences have any functional impact on the inductive signaling properties of the notochord.

Medical Examples Implicating the Notochord in Endodermal Development

There are a number of compelling observations in the medical literature illustrating a correlation between notochord defects and problems with development of endodermal tissues. For instance, human patients exhibiting developmental abnormalities in vertebral bone, apparently due to defects in notochord development, also show congenital gastrointestinal defects (Elliott *et al.*, 1970). This suggests that notochord signaling influences both sclerotome and endodermal patterning during human development. In another example, anomalous overgrowth of the notochord leads to foregut and hindgut abnormalities, such as duplications of the pharynx, esophageal and gastric cysts, rectovesical fistula, and rectal agenesis (Fallon, 1954). These observations imply that prolonged exposure to notochord signals is inhibitory to proper endoderm development. In a rat model for esophageal atresia and tracheoesophageal fistula, it is similarly proposed that sustained contact of the notochord with the foregut leads to abnormal foregut occlusion (Qi and Beasley, 1999). Overall, these observations are consistent with a role for the notochord in endoderm patterning, and moreover, they suggest that the timing of notochord signaling must be closely regulated for correct development of the gut tube.

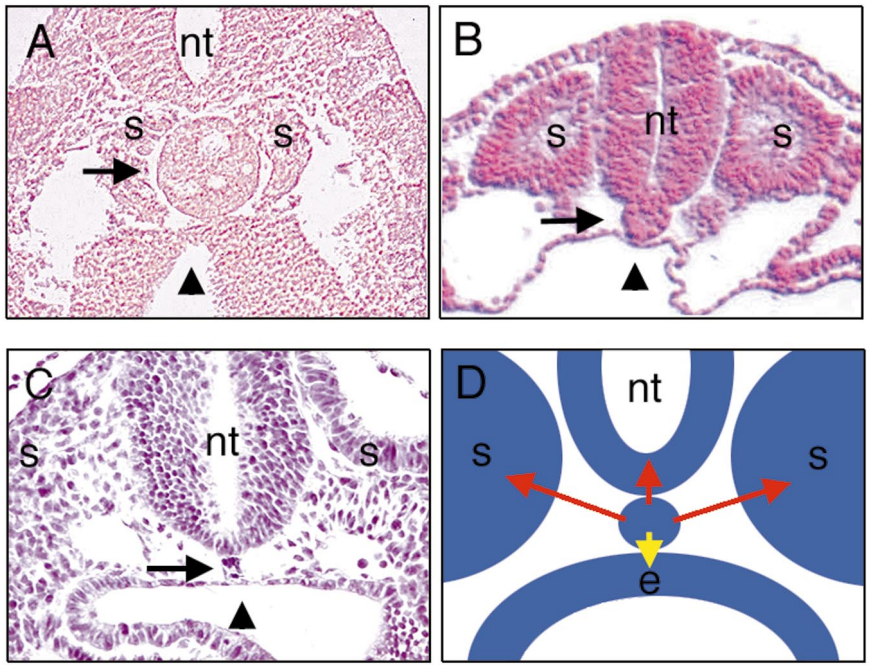

FIG. 1. Juxtaposition of the notochord and dorsal endoderm tissues in mouse, chick, and frog embryos. In all cases, the notochord (arrow) contacts the dorsal endoderm (arrowheads). The positions of the neural tube (nt) and somites (s) are indicated. (A) Transverse section through the foregut of a stage 34 frog embryo. (B) Transverse section through the hindgut of a HH stage 12 chick embryo. (C) Transverse section through the foregut of an E8.5 mouse embryo. (D) Diagram showing dorsolateral signaling from the notochord to the neural tube and somites (red) and ventral signaling to the endoderm (yellow). Note the marked difference in the size of the notochord relative to surrounding tissues in these three organisms.

NOTOCHORD SIGNALS ARE REQUIRED FOR PANCREAS DEVELOPMENT

Recent experiments using the chick embryo have provided strong evidence that the notochord plays a role in development of the pancreas. Removal of the notochord from chick embryos, at a stage when the notochord is in contact with the endoderm, eliminates subsequent expression of several markers of dorsal pancreas bud development, including both endocrine and exocrine cell markers, such as insulin, glucagon, and carboxypeptidase A (Kim *et al.*, 1997). The ventral pancreas, in contrast, does not normally contact the notochord and develops normally in these dissected embryos. Initiation of pancreatic budding still occurs in embryos lacking a notochord, but further branching and growth are arrested. Conversely, *in vitro* recombination experiments demonstrated that pancreatic markers could be induced in culture when notochord was combined with pancreatic endoderm. However, nonpancreatic endoderm grown in culture could not be induced to express pancreatic genes when recombined with notochord tissue. This suggests that notochord signals to pancreatic endoderm are permissive rather than instructive. These experiments also show that an anterior–posterior pattern preexists in the endoderm, prior to notochord signaling to the pancreatic primordia, since the notochord does not induce pancreas development along the entire length of the gut tube.

A specific molecular consequence of notochord signaling is repression of SHH expression in the endoderm (Kim *et al.*, 1997; Hebrok *et al.*, 1998). SHH is expressed in most portions of the gut tube except for those juxtaposed to the notochord (Hebrok *et al.*, 1998). In addition, the SHH receptor, Patched (Ptc), is expressed in all visceral mesoderm, except for pancreatic mesenchyme. When notochord tissue is grafted ventral to the gut tube, SHH expression is repressed in tissues in close proximity to the notochord. Conversely, removal of the notochord leads to expression of SHH in the pancreatic endoderm, to Ptc expression in the surrounding mesenchyme, and to the concomitant loss of pancreatic genes (Fig. 2). Further experiments demonstrated that this notochord-mediated repression of SHH is in fact necessary for activation of pancreatic gene expression, since addition of purified SHH protein inhibits induction of pancreatic markers and antibody inhibition of SHH activity is sufficient to activate pancreatic markers. Using *in vitro* culture of embryonic tissue, it was shown that activin-βB and FGF2 could effectively mimic the notochord signal by inhibiting SHH expression in endoderm and allowing pancreatic marker expression (Hebrock *et al.*, 1998). The same

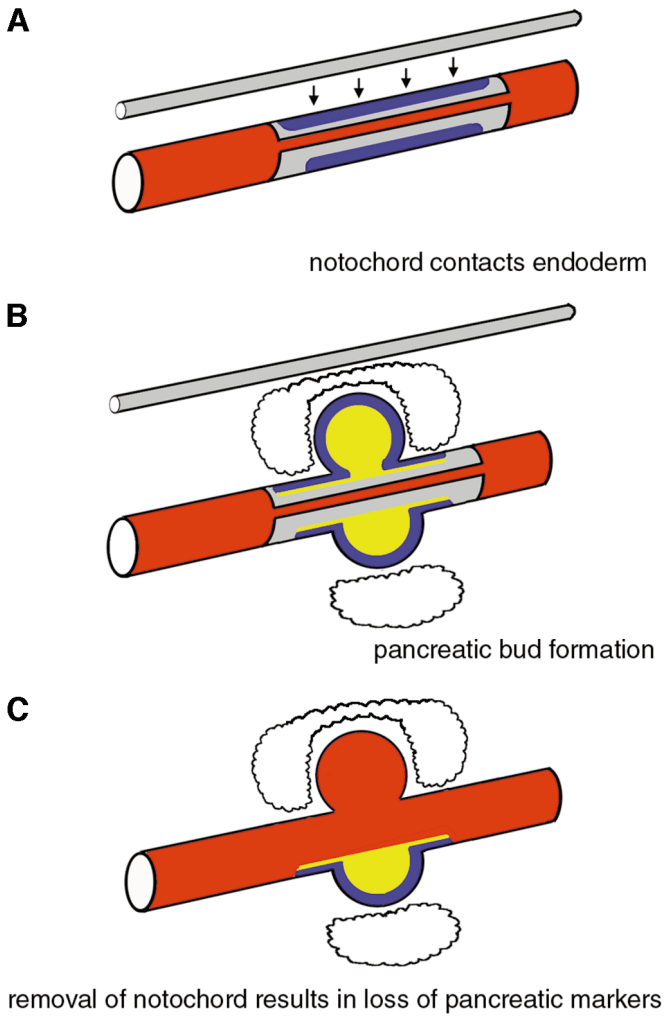

FIG. 2. Schematic diagram of notochord signaling to dorsal pancreatic endoderm. Red indicates SHH expression, blue indicates pdx1 expression, and yellow indicates pancreatic markers. (A) Signals from the notochord cause repression of SHH expression (red) in the pancreatic endoderm and expression of pancreatic genes, such as pdx-1 (blue). (B) The pancreatic buds develop on dorsal and ventral portions of the gut tube, surrounded by mesenchyme (indicated by squiggly lines). Both pancreatic buds express a range of pancreatic markers (yellow). (C) When the notochord is removed, the dorsal bud evaginates, but does not undergo further branching and morphogenesis. In addition, SHH expression is initiated in the dorsal pancreatic epithelium and pancreatic markers are repressed. The ventral bud develops unhindered.

results could be achieved using cyclopamine, a steroid alkaloid which blocks SHH signaling (Seung and Melton, 1998).

An interesting parallel has been observed between the regulation of neural and endodermal development by the notochord. In particular, the pancreatic endoderm and the neurectoderm express many of the same genes. These include *Pax4*, *Pax6*, *Isl-1*, *Nkx2-2*, *Nkx6-1*, *HNF3β*, *NeuroD*, *MNR2*, and *insulin* (Rudnick *et al.*, 1994; Turque *et al.*, 1994; Ahlgren *et al.*, 1997; Kim *et al.*, 1997; A. Grapin-Botton, personal communication). In addition, some other neural genes such as *HB9*, *Ngn3*, and *prox* are also expressed in the developing endoderm (Harrison *et al.*, 1994; Gradwhol, 2000; Olivier *et al.*, 1993). Expression of these genes in the neurectoderm is under the influence of notochord signals and it therefore seems likely that the notochord also regulates their expression in the endoderm.

NOTOCHORD SIGNALS ARE REQUIRED FOR HYPOCHORD FORMATION

Development of another endodermally derived tissue, the hypochord, is also regulated by the notochord. The hypochord is a transient rod-like structure in frog and fish embryos that develops along the embryonic axis immediately ventral to the notochord. In the *Xenopus* embryo, hypochord precursor cells are first detected in the dorsalmost endoderm at early neurula stages, shortly after the formation of the notochord. Lineage tracing experiments in the axolotl embryo confirm that the hypochord is an endodermal derivative (Lofberg and Collazo, 1997). The hypochord differentiates during tailbud stages and degenerates by apoptosis just prior to the swimming tadpole stage. The hypochord expresses high levels of vascular endothelial growth factor (VEGF) and is believed to play a role during formation of the dorsal aorta (Cleaver and Krieg, 1998).

Given the close juxtaposition of the notochord and the hypochord in the embryo, it is certainly plausible that the notochord might be involved in regulation of hypochord development. Using the *Xenopus* embryo, both notochord extirpations and transplantations have been carried out to address this question (Cleaver *et al.*, 2000). When the notochord is removed during early neurulation (stage 13–14), the hypochord fails to develop (Figs. 3A and 3B). However, if the notochord is removed later during neurulation (stage 17–18), hypochord development proceeds unhindered. These observations suggest that the notochord is necessary for the formation of the hypochord, but that this requirement is complete by the late neurula stages. It also appears that no maintenance signals from the notochord are required for hypochord development, after the initial signaling period. In notochord transplantation experiments, addition of a second notochord to the midline of the embryo results in enlarged hypochord tissue at the location of the graft (Fig. 3C). However, notochord transplantation ventrolateral to the somites does not induce the formation of an ectopic hypochord. By transplanting notochords next to the endoderm at different dorsolateral positions, it was demonstrated that competence to form hypochord is loosely restricted to the dorsalmost portion of the endoderm. As with the studies of pancreatic development, these results imply that a dorsoventral prepattern already exists in the amphibian endoderm by the early neurula stage.

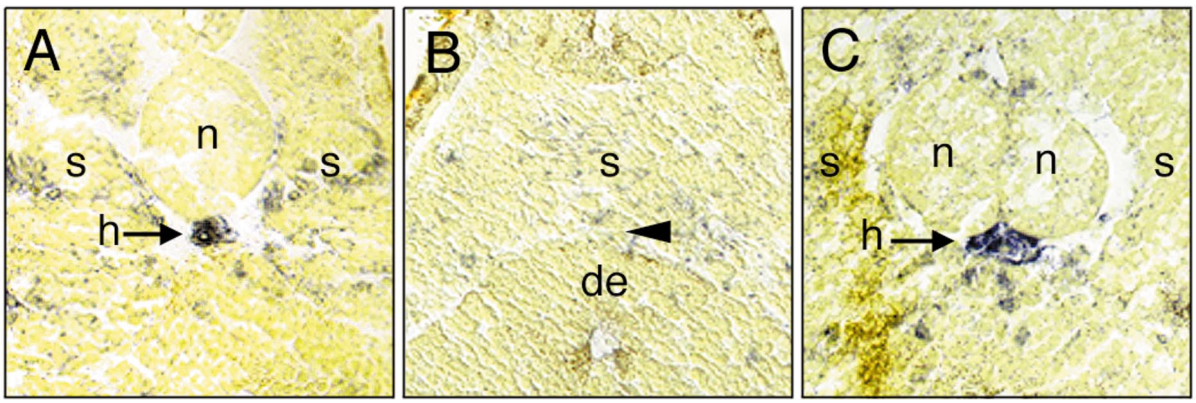

FIG. 3. Notochord signals influence development of the amphibian hypochord. VEGF expression, detected by *in situ* hybridization, is used as a marker for the hypochord. (A) Transverse section through the midgut region of a stage 32 frog embryo. The hypochord develops from the dorsal endoderm, immediately ventral to the notochord. The hypochord (h) is indicated. (B) Removal of the notochord at stage 14 results in the failure of hypochord formation. Somites fuse medially, separating the neural tube and the endoderm. Dorsal endoderm (de) and the normal position of the hypochord (arrowhead) are indicated. (C) Transplantation of an additional notochord results in the development of an enlarged hypochord. The endogenous notochord is on the left and the transplanted notochord on the right. Notochord (n) and somites (s) are indicated.

DOES THE NOTOCHORD PROVIDE AP INFORMATION TO THE ENDODERM?

While the role of the notochord in endodermal development is most clearly one of dorsoventral patterning, an interesting secondary question is raised by the studies of notochord signaling to the developing pancreas (Kim *et al.*, 1997). While it is easy to imagine how the notochord might transmit DV information to the underlying endodermal layer, as apparently occurs during hypochord and pancreas development, it is more difficult to explain how it could impart localized signals along the AP axis. Why does the notochord not induce pancreatic gene expression along the entire length of the dorsal gut tube? Does the notochord possess any localized signaling activity along the AP axis? The authors of the pancreas studies propose that notochord signals are permissive and affect only those cells that are competent to receive them, within a prepatterned endoderm (Kim *et al.*, 1997). It is intriguing to speculate, however, that the notochord itself may impart some degree of AP information.

At first glance the early notochord appears to be structurally homogeneous along the AP axis and there is currently no evidence to indicate that subdomains may exhibit different functions. However, a number of independent studies suggest that the notochord does indeed exhibit structural and gene expression differences along the AP axis. Classical studies in a number of species have described distinct segmental "flexures" and "dilations" in the notochord which correlate to the position of future, adjacent, intervertebral discs (Minot, 1907; Daws, 1930; Jurand, 1974). These observations prompted the proposal that cryptic segmentation exists within the notochord (Stern, 1990). Another feature which demonstrates heterogeneity within notochord tissue is the cranial flexure, a crook-shaped bending of the anterior notochord of mouse embryos, observed near Rathke's pouch (Jacobson *et al.*, 1979; Pikalow *et al.*, 1994). Indeed, it has long been suggested that the rostral part of the notochord has properties different from those of the more posterior notochord (Froriep, 1882). This conclusion is supported by experiments in frogs, which show that anterior notochord induces the expression of engrailed (en-2) in competent head ectoderm at a significantly higher frequency than posterior notochord (Hemmati-Brivanlou *et al.*, 1990). The most compelling evidence for notochord regionalization, however, is the heterogeneous expression of genes along the AP axis. For instance, the growth factor FGF4 is expressed transiently in the anterior notochord of stage 7 chick embryos (Shamim *et al.*, 1999). At various times during development, expression of a number of other genes is restricted to the posterior region of the notochord. These include follistatin, BMP-1/Tolloid, TGF-β5, a tolloid-related metalloprotease, BMP2, BMP7, and netrin (Graham and Lumsden, 1996; Marti, 2000; Kondaiah *et al.*, 2000; Liaubet *et al.*, 2000; Lyons *et al.*, 1995, and unpublished observations; Dale *et al.*, 1999). Furthermore, a number of Hox genes in the zebrafish exhibit a definite nested pattern, with sharp anterior boundaries of expression at different locations along the notochord AP axis (Prince *et al.*, 1998). At present however, none of the differential gene expression domains along the notochord AP axis have been correlated with clear differences in signaling properties.

POSSIBLE NATURE OF NOTOCHORD SIGNALS

The evidence that the notochord is an important source of patterning signals is undeniable, although the nature of

these signals is only beginning to be understood. During floor-plate induction and somite patterning, an excellent candidate molecule for the notochord signal is SHH. This is supported by a number of *in vitro* and *in vivo* experiments in which SHH is shown to directly affect floor-plate and somite development. For example, cells transfected with SHH can mimic the effect of the notochord and ventralize paraxial mesoderm or spinal cord (Johnson *et al.*, 1994; Fan *et al.*, 1995; Tanabe *et al.*, 1995). During pancreas development, when the notochord signals ventrally to the endoderm SHH, activin-βB, and FGF2 have been implicated (Hebrock *et al.*, 1998). What other potential signals in the notochord might affect development of the underlying endoderm? In addition to SHH, activin-βB, and FGF2, mentioned above, study of different organisms provides a long list of growth factors and secreted signaling molecules expressed in the notochord, including BMP7, BMP2, BMP3, follistatin, BMP1/tolloid, TGF-β3, TGF-β5, eFGF, FGF4, antivin (Xatv), nodal-related 2 (ndr2), Xnr4, noggin, chordin, and Hip (Echelard *et al.*, 1993; Dudley and Robertson, 1997; Dale *et al.*, 1999; Hemmati-Brivanlou *et al.*, 1994; Marti, 2000; Yamagishi *et al.*, 1999; Kondaiah *et al.*, 2000; Isaacs *et al.*, 1995; Shamim *et al.*, 1999; Cheng *et al.*, 2000; Rebagliati *et al.*, 1998; Joseph and Melton, 1997; Smith and Harland, 1992; Sasai *et al.*, 1994; Chuang and McMahon, 1999). Although the precise roles of these potent signaling molecules during embryonic patterning events are not completely understood, its seems likely that some at least will be important for the development of adjacent tissues, including endodermal derivatives.

PERSPECTIVES

Decades of experimental evidence have established a role for the notochord in patterning of ectodermal and mesodermal tissues. Perhaps belatedly, recent studies indicate that the notochord may also be the source of signals involved in endodermal patterning, especially those endodermal tissues that originate adjacent to its ventral surface. Several lines of evidence support this assertion. First, experiments in chick and frog embryos directly demonstrate a requirement for notochord signaling in formation of the pancreas and hypochord, respectively. Second, molecular studies have shown that the notochord is a source of numerous growth factors and signaling molecules, and so an influence on adjacent endodermal tissues would seem to be almost inevitable. This is particularly likely during early development when the notochord and the endoderm are in intimate association.

Historically, rather little attention has been directed toward understanding the early patterning and development of the endoderm, especially compared to the abundance of studies focused on development of mesoderm and neural tissues. This lack of progress can be ascribed, at least in part, to the absence of clear morphological markers in the relatively featureless endodermal tube, to the paucity of molecular markers of endodermal tissue, and also to the rather inaccessible location of the tissue within the embryo. Substantial progress, however, has been made in recent years, particularly in the identification of a broad range of molecular markers identifying different endodermal tissues. In addition, the development of tissue-specific gene ablation techniques, including the Cre–lox system, will permit the generation of mice in which notochord expression of important regulatory molecules has been specifically eliminated. This will facilitate precise dissection of the role that notochord-derived factors play in regulating development of adjacent tissues. With these tools in hand, advances toward understanding the molecular events underlying endodermal patterning will occur at a greatly accelerated pace.

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