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Caudalization by the amphibian organizer: *brachyury*, convergent extension and retinoic acid

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SUMMARY

Caudalization, which is proposed to be one of two functions of the amphibian organizer, initiates posterior pathways of neural development in the dorsalized ectoderm. In the absence of caudalization, dorsalized ectoderm only expresses the most anterior (archencephalic) differentiation. In the presence of caudalization, dorsalized ectoderm develops various levels of posterior neural tissues, depending on the extent of caudalization. A series of induction experiments have shown that caudalization is mediated by convergent extension: cell motility that is based on directed cell intercalation, and is essential for the morphogenesis of posterior axial tissues.

During amphibian development, convergent extension is first expressed all-over the mesoderm and, after mesoderm involution, it becomes localized to the posterior mid-dorsal mesoderm, which produces notochord. This expression pattern of specific down regulation of convergent extension is also followed by the expression of the *brachyury* homolog. Furthermore, mouse *brachyury* has been implicated in the regulation of tissue elongation on the one hand, and in the control of posterior differentiation on the other. These observations suggest that protein encoded by the *brachyury* homolog controls the expression of convergent extension in the mesoderm. The idea is fully corroborated by a genetic study of mouse *brachyury*, which demonstrates that the gene product produces elongation of the posterior embryonic axis. However, there exists evidence for the induction of posterior dorsal mesodermal tissues, if *brachyury* homolog protein is expressed in the ectoderm. In both cases the *brachyury* homolog contributes to caudalization.

A number of other genes appear to be involved in caudalization. The most important of these is *pintavallis*, which contains a fork-head DNA binding domain. It is first expressed in the marginal zone. After mesoderm involution, it is present not only in the presumptive notochord, but also in the floor plate. This is in contrast to the *brachyury* homolog, whose expression is restricted to mesoderm.

The morphogenetic effects of exogenous RA on anteroposterior specification during amphibian embryogenesis are reviewed. The agent inhibits archencephalic differentiation and enhances differentiation of deuterencephalic and trunk levels. Thus the effect of exogenous RA on morphogenesis of CNS is very similar to that of caudalization, which is proposed to occur through the normal action of the organizer.

According to a detailed analysis of the effect of lithium on morphogenesis induced by the *Cynops* organizer, lithium has a caudalizing effect closely comparable with that of RA. Furthermore, lithium induces convergent extension in the prechordal plate, which normally does not show cell motility. This raises the question of whether the caudalizing effect of RA may be also dependent on CE which RA may induce in the treated tissue.

A number of chick embryonic tissues are known to show polarizing activity for limb development. All these tissues are known to express, or expected to express CE. This strongly suggests that these tissues induce CE in limb mesenchyme, and CE functions as the polarizing activity. Recently RA was shown to have a positive effect in this respect. This supports our proposal that RA induces CE in treated tissues.

According to the expanded model of dorsalization-caudalization, anteroposterior specification in mesoderm and ectoderm is the combined effect of dorsalization and caudalization:while dorsalization occurs at all AP levels, caudalization is limited to posterior of the anteroposterior border-line. In agreement with this, genes involved in dorsalization become expressed at all anteroposterior levels, while the main caudalizing gene is expressed behind the anteroposterior border-line at the stage CNS is induced.

The study of the neural inducing effects of *noggin* protein on competent ectoderm indicates that it is able to cause ectodermal dorsalization. In the absence of mesoderm, *noggin* protein induces only neural tissues with the anterior markers. But in the presence of mesoderm, *noggin* protein can induce neural tissues with the posterior markers. These results are in full agreement with the model of dorsalization-caudalization.

Key words: amphibian organizer, *brachyury*, mesoderm, axis formation, retinoic acid, caudalization, *Xenopus*

INTRODUCTION

The central issue of this review is the regulation of the caudal shift of the positional value of cells, which is proposed to occur during the action of the organizer. The corresponding shift can be experimentally induced by RA and lithium. The discussion depends on a number of concepts and information related to the organizer, which are often ignored in the current literature. Some of these will be briefly explained to facilitate the understanding of the text.

The border-line of anteroposterior (AP) specification

During the 1940s-1950s the idea gradually became accepted that the regulation of AP specification is different in the anterior and posterior levels of the embryonic axis. The border-line is marked by the anterior end of the notochord. The mesoderm area anterior to the border-line induces, in the competent ectoderm, differentiation of archencephalon (telencephalon, diencephalon and optic rudiment), while the mesoderm area behind the border-line induces, in the competent ectoderm, differentiation of deuterencephalon (metencephalon and myelencephalon) and spinal cord (Lehmann, 1945; Yamada, 1950a; Nieuwkoop et al., 1952; Saxén and Toivonen, 1962; Dalcq, 1957). Opinion is divided as to whether mesencephalon belongs to the anterior or posterior zone. The weak expression of mesencephalic differentiation, which occurs after the separation of anterior and posterior parts, makes the decision difficult. It is proposed to call the border-line 'the AP border-line.'

This border-line, which has been defined purely from the inductive activities of parts of the organizer, now appears to coincide with the demarcation line of two groups of homeobox genes that may play a role in setting the positional values of cells along the AP axis: *Emx* genes and *Otx* genes, which characterize the anterior zone (Simeone et al., 1992a, 1992b, 1993) and *Hox* genes, which characterize the posterior zone (Gaunt et al., 1988; Graham et al., 1989; McGinnis and Krumlauf, 1992; Dekker et al., 1992, 1993). The situation here indicates that *Hox* genes do not function as the carrier of the positional value for all AP levels of the embryo, but only for the posterior zone. The superposition of the border-line of the inductive activity with the demarcation-line of two groups of homeobox genes may suggest that the regulation of AP specification and the expression of the positional value are based on a common principle.

Ap specification in the induction of CNS depends on the combined effects of ectodermal dorsalization and caudalization

Since the author's earlier research on the control of differentiation of trunk mesodermal tissues clearly indicated that the control is based on the shift of the cellular positional value along the dorso-ventral (DV) axis (Yamada, 1937, 1938, 1940, 1950b), it appeared best to use the positional value as the basic concept for studying the control in CNS induction. Obviously for this system one should look at the control of DV and AP positional values. Applying this concept to experimental research available at that time (reviews: Lehmann, 1945; Holtfreter and Hamburger, 1955; von Woellwarth, 1956; Hamburger, 1988; Slack and Tannahill, 1992) the following model appeared to be adequate for the induction of CNS by the amphibian organizer. Two basic events occur in the ectoderm responding to the organizer: ectodermal dorsalization (the increase of DV potential), which is expressed at all AP levels of the presumptive neural system, and caudalization (the shift of AP potential towards the posterior direction), which is limited to the region behind the AP border-line and functions in the reverse AP gradient. Archencephalic differentiation is expressed at the level where ectodermal dorsalization alone occurs. Deuterencephalic differentiation occurs where ectodermal dorsalization is associated with weak caudalization. Differentiation of trunk- or tail spinal cord occurs where moderate or strong caudalization is combined with ectodermal dorsalization (Yamada, 1950a) (Fig. 1). Thus the basic concept of the model is that the neuralized ectoderm, which by itself tends to show archencephalic differentiation is progressively shifted to the differentiation of more posterior levels by an event called caudalization. It appeared that caudalization may be mediated by convergent extension. This was the first model proposed for the action of the organizer, and the only model in which the regulation is strictly related to the positional value of cells.

Another model of AP specification of CNS which was essentially similar was proposed by Nieuwkoop et al. (1952). In this model the event corresponding to ectodermal dorsalization is designated as activation, and the event corresponding to caudalization as transformation.

The extended model of dorsalization-caudalization

Since the model of dorsalization-caudalization, proposed in 1950, was devoted to the regulation of neural induction, it did not address events occurring within the organizer. However, subsequent results of work on this area, including gene expression in the organizer (see the general discussion), strongly suggest that also in the mesoderm, dorsalization and caudalization constitute the main regulative events the combined effects of which control AP specification and developmental pathways. The mesoderm area that is dorsalized but not caudalized should develop as the prechordal plate, which induces archencephalic structures in competent ectoderm, while the mesoderm area that is dorsalized and caudalized should develop as notochord and somites and induce in competent ectoderm, deuterencephalon and spinal cord. The

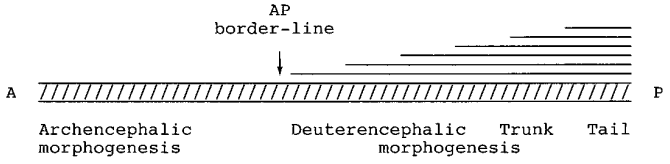

Fig. 1. Diagram of AP specification by the organizer according to the model of dorsalization-caudalization. In this diagram, the degree of dorsalization is assumed to be the same at all AP levels. Actually it may differ at various AP levels. What is proposed in this respect is that within certain limits the degree of dorsalization does not play any role in AP specification, although it may have a role in deciding dorsoventral parameters of archencephalic morphogenesis.

: dorsalization

: caudalization operating in gradient

gradient of caudalization with the apex at the posterior end should be responsible for different morphogenesis expressed at different levels of the posterior zone. This proposal will be called the extended model of dorsalization-caudalization, to distinguish it from the original model.

In connection with the extended model, it should be emphasized that mesodermal dorsalization and ectodermal dorsalization are closely related cell-biological events, because they can be inhibited or enhanced by the same agents in comparable ways. For instance, both types of dorsalization can be inhibited by lithium applied during gastrulation (for mesoderm see Lehmann, 1938; for ectoderm see Masui, 1958) and enhanced by sublethal cytolytic treatments (for mesoderm see Yamada, 1950b; Ogi, 1958; for ectoderm see Holtfreter, 1947; Karasaki, 1957) and concanavalin A (for mesoderm see Diaz et al., 1990; for ectoderm see Takata et al., 1981, 1984).

Convergent extension mediates caudalization

Convergent extension (CE) is one of the basic morphogenetic movements which operate in early development (Vogt, 1929; Keller et al., 1992), and is known for its role in the formation of the elongated posterior axis of vertebrate embryos by virtue of its directed cell intercalation (Keller and Tibetts, 1989; Keller et al., 1992). After mesoderm involution, CE is expressed behind the AP border-line, in the mid-dorsal part of the mesoderm, which forms notochord. From the late gastrula to neurula stages, CE is expressed also in the neural plate, behind the AP border-line (Vogt, 1929; Goerttler, 1925; Hama, 1978; Keller et al., 1992; Sharpe, 1992). In the neural plate, the highest level of CE is found along the floor plate area where the notochord forms a close contact with neural ectoderm. As emphasized by Vogt (1929), CE is also expressed before involution in mesoderm. In this case, it is found all over the marginal zone, at all dorsoventral levels. At the early gastrula stage, CE is strongly expressed at the most anterior level of the organizer before its involution. In *Xenopus* embryos, before mesoderm involution, the expression of convergence in the marginal zone is uniform at all dorsoventral levels, while that of extension is stronger at the dorsal level than at the ventral level (Keller and Danilchik, 1988).

In contrast to the classical work on AP specification of the organizer (Spemann, 1931), which indicated well defined AP specification depending on the early and late stage of the dorsal lip, some later experiments (Okada and Takaya, 1942a,b; Okada and Hama, 1943, 1944, 1945; Kato, 1958; Masui, 1960) showed a high apparent plasticity in AP specification by the organizer. When the presumptive prechordal plate is isolated before involution and immediately combined with competent ectoderm, it causes posterior induction. This is not how it normally functions. But if the isolated uninvoluted prechordal plate is kept in Holtfreter solution for 4 hours and then tested on competent ectoderm, it causes anterior induction, in conformity with its normal function. In subsequent extensive induction experiments combined with vital staining for visualizing morphogenetic movement of the organizer, Hama et al. (1985) demonstrated that the decisive factor for AP specification is whether or not the organizer is expressing CE at the time it is combined with competent ectoderm. The presumptive prechordal plate, like all other parts of the marginal zone, is expressing CE before involution. Association of the tissue at this stage with competent ectoderm favours the continued expression of CE and leads to posterior induction. However, the same tissue loses the expression of CE, if left in situ, or kept in Holtfreter solution for a few hours. The presumptive prechordal plate at this stage induces archencephalic differentiation if tested on competent ectoderm. However, the presumptive notochord expresses CE before and after involution, and induces posterior structures under both conditions. This means that the expression of CE determines the posterior specification by the organizer, irrespective of the presumptive fate of the organizer. This means that CE mediates caudalization.

There exists other evidence for the caudalizing role of CE. Nieuwkoop and Albers (1990) showed that when the presumptive archencephalon is converted into deuterencephalon and spinal cord by grafting it into the posterior zone of the neural plate where CE is occurring, the conversion is always associated with the expression of CE by the graft, and the extent of CE correlates with the extent of caudalization. Normally the presumptive archencephalon does not express CE, while the presumptive deuterencephalon expresses weak CE and the presumptive spinal cord strong CE.

TOPIC 1: ROLES OF *brachyury*, AND ITS HOMOLOGS IN CAUDALIZATION

It has been suspected throughout the long period of investigation of mouse *brachyury* (*T*) that it may be involved in the regulation of posterior specification. In early studies the loss of its function was reported to cause abnormal appearance of the primitive streak, alteration in cell motility and inhibition of notochord formation as well as the elongation of the posterior axis (Chesley, 1935; Gluecksohn-Schoenheimer, 1944; Grüneberg, 1958). In more recent work, the reduced number of mesoderm cells in *T/T* embryos was attributed to the alteration in cell migration, because no corresponding change in mesodermal proliferation was found (Yanagisawa et al., 1981). The possible function of the gene in AP specification was proposed (Yanagisawa, 1990). Successful cloning of the gene (Herrmann et al., 1990) allowed a series of studies of the localization of gene expression. According to Wilkinson et al. (1990) transcripts of the gene are detected in 8.5- to 9.5-days post coitum normal embryos at the gastrula stage at a high level, and in 10.5- to 12.5-day normal embryos at the neurula stage at a much lower level. Importantly, transcripts are not detected at later stages of normal development, in any normal adult tissues tested. In-situ hybridization studies of Wilkinson et al. (1990) indicate that *T* is expressed in normal embryos first in the primitive streak and in its direct vicinity. When the head process is formed, it is positive for the gene product. The anterior end of the positive head process is positioned underneath the AP border-line. At all more posterior levels, the gene product is present in axial mesoderm. An apparent increase in the gene product occurs during the early to middle streak stages. Herrmann (1991) describes the expression pattern of *T* in T^{Wis}/T^{Wis} embryos in relation to morphogenesis. In early stages of these embryos *T* expression is normal, but ceases prematurely during early organogenesis coincident with inhibition of the differentiation of mesodermal tissues. A decrease and final loss of *T* expression in primitive streak and head process are associated with the inhibition of axial elongation in head process and in the posterior axis, and followed by the sup-

pression of differentiation of notochord and somites, and the derangement of the neural tube. In a cell culture study the motility of mesoderm cells derived from the primitive streak and cultured on ECM is significantly reduced, if the cells originate from T/T embryos (Hashimoto et al., 1987). Beddington et al. (1992) using cell chimeras have shown that the reduced motility of T/T cells is a cell autonomous defect. According to an immunohistochemical study, T-protein is located in the nucleus, and at the tail-bud stage, the whole length of notochord is positive for T-protein (Kispert and Herrmann, 1994).

The expression of the *Xenopus* homolog of *brachyury*, *Xbra* (*XB*) during embryogenesis has been studied by Smith et al. (1991). RNAase protection analysis using an anti-sense probe prepared from the 3′ end of the cDNA shows that *Xbra* is strongly expressed from MBT to stage 12. The maximum level is attained around stage 11. The stage at which *Xbra* transcripts become undetectable varies according to blots. Some blots reveal the presence of transcripts still at stage 37. Around this stage the elongation of the tail bud stops. This means that the expression of *Xbra* is terminated when the last CE event of embryogenesis is finished. In situ hybridization studies of the early gastrula stage show that the whole marginal zone is positive for *Xbra* RNA. During involution of mesoderm through the blastopore, down regulation of transcripts occurs, so that the expression becomes limited to presumptive notochord. The similarity of the pattern of down regulation described here with that of CE during mesoderm involution as reviewed in the Introduction is impressive. Together with the above mentioned involvement of T in the elongation of the embryonic axis and the control of posterior differentiation, the coincidence of the specific pattern of down regulation strongly supports the hypothesis that the expression of CE is controlled by proteins of *brachyury* homologs, in vertebrate embryos. Decisive evidence in favour of this hypothesis has been produced by Stott et al. (1993) who show, by manipulating the copy number of the *T* gene in transgenic mice that the level of *T* product correlates with the extension of the AP axis, and conclude that *T* product is involved in axial elongation.

The following observations of experimental controls of the *Xbra* expression support the proposed hypothesis that *Xbra* protein controls the expression of CE: (1) In UV-ventralized *Xenopus* embryos, the expression of *Xbra* is suppressed (Ruizi i Altaba and Jessell, 1992), while the elongation of the AP axis, which depends on CE, becomes progressively inhibited as ventralization progresses (Scharf and Gerhart, 1983; Gerhart et al., 1989). (2) Amaya et al. (1993) used the expression of a dominant negative construct of the FGF receptor (XFD) to study the endogenous FGF signalling pathway in intact embryos, and found that the expression of *Xbra*, but not that of *goosecoid*, is inhibited by the expression of XFD. At the stage corresponding to the tail-bud stage of control embryos, XFD-embryos have a V-shaped dorsal side suggesting the lack of CE. The development of notochord is completely suppressed. Other trunk mesodermal tissues are also affected during development. However archencephalic differentiation occurs, suggesting the inducting activity of the prechordal palate. One question to be raised is whether in XFD-embryos the dorsalized mesoderm becomes extended to the tail level as it does in the normal embryos.

According to Niehrs et al. (unpublished observation in Niehrs et al., 1993), overexpression of *goosecoid* causes elongation of ventral marginal zone, interpreted as CE. Under this condition, the mesoderm produces notochord, muscle and neural tissues. Although the details of the experiment need to be known, the result appears to suggest that the dorsalization of the ventral mesoderm caused by microinjection of *goosecoid* mRNA leads to the expression of *Xbra*. This is in agreement with the observation that ventral microinjection of *goosecoid* mRNA gives rise to a secondary embryo, which often has the complete posterior axis (Blumberg et al., 1991; Cho et al., 1991; De Robertis et al., 1992). According to the extended model of dorsalization-caudalization, and the hypothesis proposed in this section, *Xbra* needs to be expressed under this condition to allow morphogenesis of the posterior zone.

In the ventrally located secondary embryo produced by overexpression of *goosecoid*, the archencephalic level is mostly lacking (Steinbeisser et al., 1993). This is in agreement with the observation that when the mid-ventral marginal zone of *Triturus* embryos, which forms the blood island in isolation, is dorsalized by ammonia or sodium thiocyanate, it differentiates notochord and somites, but no prechordal plate (Yamada, 1950b; Ogi, 1958). Since in the normal *Xenopus* embryo at the end of gastrulation, the length of the ventral midline of mesoderm is less than half that of the dorsal midline (Keller, 1976, Fig. 3), when mesoderm is dorsalized and *Xbra* is activated to the normal extent, the whole ventral mesoderm may be caudalized, and no prechordal plate will be formed, assuming that the form of the mesoderm mantle is controlled by mesoderm induction and not by dorsalization.

Smith and Howard (1992) showed that the activation of CE associated with mesoderm induction can be inhibited by the injection of *BMP-4* mRNA at the one cell-stage. *BMP-4* is normally expressed in the ventral level of mesoderm, and is involved in ventralization of mesoderm (Köster et al., 1991; Jones et al., 1992). Its over-expression should suppress mesodermal dorsalization, and hence inhibit CE expression. This observation together with the effect of mesodermal dorsalization show that the expression of *Xbra* depends on the dorsalized state of the mesoderm.

There is one type of induction experiment that suggests that the developmental regulation of CE is somehow related to the induction of posterior dorsal mesodermal tissues in ectoderm. Using competent ectoderm stained with Nile Blue sulphate as the lineage-marker, Suzuki et al. (1984) showed that when the uninvaginated presumptive prechordal plate is combined with competent ectoderm and cultured, it first induces notochord and somites in the ectoderm. The posterior neural structures, which regularly appear in this series are secondarily induced by notochord and somites, which in turn are derived from responding ectoderm. However, if different levels of the archenteric roof are used as the inducing tissues, no such mesoderm induction occurs. The neural structures are induced in these series directly by dorsal mesoderm derived from the archenteric roof in agreement with the classical concept of the organizer action. As already discussed in the Introduction, Hama et al. (1985) showed that the presumptive prechordal plate is expressing CE before invagination, and if it interacts with the competent ectoderm at this stage, its CE expression continues. However, after invagination the prechordal plate stops the expression of CE, and if it interacts with the competent ectoderm at this stage, no expression of CE occurs,

and archencephalic structures are induced in responding ectoderm. This appears to mean that the ectopic expression of *Xbra* may be responsible for the induction of notochord and somites in the competent ectoderm in the first series of Suzuki et al. (1984). This idea has been supported by the more recent experiment of Cunliffe and Smith (1992) who injected *Xbra* mRNA into the animal pole of the one cell stage *Xenopus* embryo. Subsequently the animal pole region was separated and cultured. It produced somites. Thus *Xbra* protein causes the expression of CE in the mesoderm, but induces posterior mesodermal tissues in the ectoderm.

TOPIC 2: OTHER GENES INVOLVED IN CAUDALIZATION

In addition to *Xbra*, other genes are known that may be involved in caudalization. A *Xenopus* gene containing the *fork head* DNA-binding domain called XFD-1, XFKH-1 or *pintallavis* has been shown to be expressed in the organizer region (Knöchel et al., 1992; Dirksen and Jamrich, 1992; Ruiz i Altaba and Jessell, 1992). Before the mesoderm involution it is expressed in the dorsal marginal zone. During gastrulation it is present at the dorsal lip and the surrounding area. At stage 12, the maximum over-all expression of the gene is attained. At stage 12.5 the expression is stronger in the presumptive notochord, and weaker in the presumptive prechordal plate. From stage 12 to 13, the expression becomes restricted to the dorsal midline. Cross sections of embryos demonstrate that the floor plate of the neural ectoderm, which is in the contact with the positive notochord, expresses the gene. In exogastrulation, the expression at the floor plate does not occur, if the ectoderm is not in the contact with the notochord, indicating that transmission of the activity from notochord to the floor plate occurs in the intact embryo. From stage 17-18, the mesodermal dorsal midline expression rapidly decreases from the anterior end toward the posterior, and a clear posterior to anterior gradient of expression becomes established. At around stage 28 (the tail-bud stage) the expression is restricted to the tail-bud including the floor plate. Both the inductive expression of the gene at the floor plate, and the progressive posterior restriction of the gene expression from the late neurula stage onwards correlate with the expression pattern of CE. This strongly suggests the possibility that *pintallavis* is involved in the expression of CE. It is significant that expression of ectodermal CE at the floor plate correlates with the expression pattern of *pintallavis* but not with the expression pattern of *Xbra*. Among genes involved in caudalization there may exist certain functional specialization. That overexpression of *pintallavis* can cause the inhibition of archencephalic differentiation strongly suggests the caudalizing role of the gene. The expression of the gene is dependent on the dorsalized state of mesoderm.

The possible involvement of a homeobox gene *Xnot* (von Dassow et al., 1993) in the regulation of caudalization is suggested by the progressive localization of its expression in the dorsal mesoderm behind the AP border-line around the time of involution. Its loss of expression from the anterior to posterior levels after the late neurula stage and final restriction to the tail-bud at the tail-bud stage again correspond to the similar posterior restriction of CE. The expression of *Xnot* at the floor plate can be interpreted as suggesting a relationship of the gene with CE. FGF induces the transcription of *Xnot*, and the dominant-negative mutant of FGF receptor inhibits the expression of *Xnot*.

Another homeobox gene, *Xhox-3*, is expressed in the posterior dorsal mesoderm of *Xenopus* gastrula and neurula embryos in the reverse AP gradient (Ruiz i Altaba and Melton, 1989a,b). Its possible involvement in caudalization has been discussed (Yamada, 1990). The injection of antibodies against the protein causes reduction of the AP axis and defects in posterior axial mesodermal tissues (Ruiz i Altaba et al., 1992). Thus Xhox-3 protein plays a regulatory role in caudalization.

That a homolog of *hedgehog* may play a caudalizing role during the action of the organizer is suggested by its regulation of the floor plate (Krauss et al., 1993; Echelard et al., 1993; Riddle et al., 1993; Roelink et al., 1994; Smith, 1994). This expanding area will be discussed in a separate review.

TOPIC 3: LITHIUM CAUSES CAUDALIZATION AND INDUCES CONVERGENT EXTENSION

It is rather surprising that a caudalizing effect very similar to that of RA can be produced by lithium, a fact that was first noted in 1958-1960. Although the information is contained in local Japanese publications, because of its present importance for understanding the regulation by RA, it will be briefly summarized here. It is highly desirable that corresponding studies should be conducted on *Xenopus* embryos.

Masui (1958) analyzed the effect of lithium on AP specification by the organizer in embryos of *Cynops* (*Triturus*) *pyrrhogaster*. In the control for the first series, competent stage 12 ectoderm is combined with the invaginated presumptive prechordal plate of the same stage, and grafted to the ventral part of a host embryo. In experimental series, one of combined tissues is treated with 0.06 M LiCl for 4 hours after separation. As controls, archencephalic structures including eyes and noses, and also occasionally deuterencephalic structures are induced. Lithium-treated presumptive prechordal plate combined with untreated ectoderm strongly inhibits archencephalic induction, increases deuterencephalic induction, and gives rise to the induction of spinal cord. The over-all neural frequency remains high. Deuterencephalic structures and spinal cord are always associated with myotome and notochord. When lithium-treated ectoderm is combined with untreated presumptive prechordal plate, again archencephalic structures are strongly reduced, deuterencephalic structures increase, and spinal cord appears. The frequency of total neural structures remains high. So too do the frequencies of myotome and notochord. The combination of untreated ectoderm with untreated presumptive trunk-tail mesoderm in the second control series gives rise to a high frequency of trunk-tail tissues. However, the combination of lithium-treated trunk-tail mesoderm with untreated ectoderm reduces the frequency of neural structures from 95% to 43%. A similar reduction occurs in notochord. Most of the neural structures formed are spinal cord accompanied by fins, notochord and myotomes. However, when lithium-treated ectoderm is combined with untreated trunk-tail mesoderm, results are closely similar to those of the control: frequent neural structures (100%), and well differentiated deuterencephalon and spinal cord. In a separate series,

Masui (1958) studied the effect of lithium on isolated presumptive forebrain of *Hynobius nebuloses* (a urodele species) by culturing isolated treated tissues. The treatment at the late gastrula stage inhibits the formation of archencephalon and eyes. The treatment at the early neurula stage affects the formation of eyes, but not of archencephalon, while treatment at the late neurula stage affects only the bilateral arrangement of eyes. Throughout those stages, the treatment increases the frequency of ears, and enhances the deuterencephalic mode of brain development. These results show that lithium shifts the system in the archencephalic pathway into the deuterencephalic pathway without the mediation of the organizer.

Gebhardt and Nieuwkoop (1964) studied the effect of lithium on the induction by anterior notochord of stage 15 *Amblystoma* neurula in 'sandwich' experiments. Untreated, this tissue induces a high percentage of archencephalic tissues in competent ectoderm, and a lower percentage of deuterencephalic tissues. Lithium treatment of 'sandwiches' causes a clear caudal shift of specification: high percentages of deuterencephalic tissues and spinal cords. The probable reason for the archencephalic induction by anterior notochord, which usually induces deuterencephalon and spinal cord, is the advanced stage of the donor. This is a phenomenon called posterior dominance by Slack and Tannahill (1992). The phenomenon can be explained in the following way. The cessation of expression of CE in intact embryos begins from the anterior notochord in the later part of the neurula stage. According to the model of dorsalization-caudalization, and the hypothesis of mediation of caudalization by CE, both discussed in the Introduction, the loss of CE in notochord should make the tissue an archencephalic inductor. From the viewpoint of the caudalizing effect of lithium, the experiments cited here are in full agreement with the earlier cited work of Masui (1958, 1960).

In summary, the experiments cited in the last paragraphs demonstrate that the lithium treatment of tissues involved in the induction of archencephalic differentiation leads to a posterior shift of specification. The separate treatment of the inducing dorsal mesoderm and responding ectoderm gives rise to the same shift. Such shift is not accompanied by the reduction of over-all neural induction. The shift corresponds to caudalization proposed in the model of dorsalization-caudalization for CNS induction (Introduction). Caudalization induced by lithium can also occur in isolated neural ectoderm. However, when differentiation of deuterencephalon and spinal cord is the main induced event in the control, lithium does not cause significant caudalization, but reduces the frequency of mid-dorsal structures: neural tissues and notochord. In this case, the target of lithium is restricted to the inducing activity of dorsal mesoderm, while the response of ectoderm is not affected. This effect of lithium may be the inhibition of mesodermal dorsalization which should be occurring at the time of the treatment.

Why does archencephalic differentiation alone becomes caudalized by lithium? Archencephalic differentiation is the only level of differentiation that occurs in the absence of CE in the organizer (Fig. 1). This suggests the possibility that lithium caudalizes the differentiation by inducing CE. This possibility is in agreement with the idea that CE mediates the differentiation of the posterior zone (Introduction). Masui (1960) reported that while the isolated, invaginated prechordal plate cultured with ectoderm forms a round mass and induces archencephalic differentiation, the same tissue treated with lithium and cultured with ectoderm shows marked stretching in all cases. Since under the latter condition the caudalization of differentiation occurs, it appears probable that the primary effect of lithium is the induction of CE, and the latter is responsible for caudalization of differentiation.

The observation of Masui that lithium causes caudalization and ventralization of gastrula cells appears to contradict the well known dorsalizing effect of the ion on *Xenopus* embryonic cells (Kao and Elinson, 1988, 1989; Kao et al., 1986; Ruiz i Altaba and Jessell, 1992). However, Yamaguchi and Shinagawa (1989) demonstrated that this is not a contradiction, but two different types of effects of the ion (dorsalizing or caudalizing-ventralizing effects) which occur at different stages of development. According to these authors, the dorsalizing effect of lithium is restricted to the early stages up to around MBT, and later the caudalizing-ventralizing effect prevails. When Lettice and Slack (1993) studied the effect of lithium on development of isolated ventral mesoderm, dorsalizing effect was found until stage 9 and disappeared at the stage 10. Since lithium is known to have cytolytic effect on early amphibian embryonic cells until around MBT (Grunz, 1968), it is possible that the dorsalizing effect of lithium is due to its subcytolytic stimulus similar to that of ammonia discussed in the Introduction.

TOPIC 4: MODIFICATION OF ANTEROPOSTERIOR SPECIFICATION BY EXOGENOUS RA, AND THE ROLE OF ENDOGENOUS RA

Durston et al. (1989) demonstrated that when stage 10-15 *Xenopus* embryos are treated with 30-minute pulses of 10^{-7} M-10^{-5} M RA and cultured up to stage 45 and studied in histological sections, defects in archencephalic structures including eyes and noses are evident. When the volume of neural tissues differentiated in archencephalon, deuterencephalon and spinal cord is measured separately at stage 45, a strong decrease in archencephalon, a moderate increase in deuterencephalon, and a weak increase in spinal cord are recorded. The total volume of neural tissue is not altered by the treatment, even at the highest dose of RA used. The shift of regional differentiation is also demonstrated in recombinates of stage 10 ectoderm and dorsal mesoderm, in which neural induction occurs during culture, and in isolated stage 11 ectoderm which has been neuralized before separation and differentiates archencephalic structures during culture. In both systems, RA treatment suppresses archencephalic differentiation. Furthermore stage 11 ectoderm-series shows that the respecificating effect of RA can occur without the mediation of mesoderm. The observed effect of RA is not associated with tissue damage or disturbance of gastrulation. It is further shown that at sensitive stages, the mean concentration of 1.5×10^{-7} M RA is present in *Xenopus* embryos, and ^{3}H-labelled RA is taken up by embryos. These findings are interpreted by the authors to mean that endogenous RA mediates caudalization (transformation) in intact embryos.

Sive et al. (1990) treated *Xenopus* embryos at late blastula and early gastrula stages with 1 μM RA for 5 to 320 minutes progressively, and described external morphology of embryos at stage 35. The most sensitive tissue was the eye, which was

reduced and lost after a treatment for 40 minutes. After this level of treatment, the outline of the head suggests reduction or loss of the archencephalic level of the brain. After 40-320 minutes treatment, the total size of the embryos is reduced and formation of tail suppressed. Whole embryos treated with RA and judged to have lost the brain, but retained the neural tube and somites were studied for transcripts of marker genes of various AP levels at stage 20-30, and compared with untreated control embryos. The treatment abolishes the expression of *XA-1* (Sive et al., 1989) normally expressed in cement glands and anterior neural tissue and *EN* (*engrailed*), which marks the boundary between mesencephalon and rhombencephalon (Hemmati-Brivanlou and Harland, 1989). However, *XIF-3* a marker of 'anterior neural tissue' is found to be increased, instead of lost as those authors anticipated. However, the following interpretation may explain this apparent anomaly. The in situ hybridization data of Sharpe et al. (1989) showed that in normal embryos *XIF-3* transcripts are mainly expressed at the deuterencephalic level, and probably also weakly in the anterior spinal cord, but are absent, or almost absent at the archencephalic level. The embryos judged by Sive et al. as headless, probably lost only archencephalic tissues but had retained the deuterencephalon. The conversion of archencephalon into deuterencephalon may have increased the amount of deuterencephalon. This may have elevated the level of *XIF-3*. The expression of *XIHbox-6* (Wright et al., 1990), the neural marker of the trunk-tail level, was found to be much greater in treated embryos, while the expression of *Xhox-36* (Condie and Harland, 1987), the neural marker of tail level, was reduced. In summary, this series shows that RA treatment respecifies positional values of the neural system in the process of induction, suppressing the differentiation of the archencephalic level as well as the tail level, and enhancing differentiation of deuterencephalic and trunk-spinal cord levels. Sive et al. (1990) further demonstrated that RA can directly alter the developmental pattern of the neural system in the absence of mesoderm. Dorsal ectoderm isolated at the middle gastrula stage is treated or not treated with RA, and cultured, and tested for markers of various AP levels. Control ectoderm shows a high expression level of archencephalic markers, and a very low expression level of deuterencephalic and trunk-tail markers. RA treatment suppresses the expression of the former group of markers, and enhances the expression of the latter group. Thus the pattern of alteration in AP specification found in whole embryos after RA treatment is repeated in isolated dorsalized ectoderm, suggesting that ectoderm has the full competence to react to RA, independently from dorsal mesoderm.

While the work of Sive et al. emphasized the ability of ectoderm to react to RA, Ruiz i Altaba and Jessell (1991a) emphasized the role of dorsal mesoderm in response to RA. In this work the effect of RA on AP specification of mesoderm induced by some peptide growth factors has been investigated. In one of the series, stage 8 ectoderm is treated with Activin A in the presence or absence of RA. In the control, 20% head and 60% tail are induced in host embryos. RA treatment in this system causes complete suppression of head induction, while the tail induction frequency remains high (65%). On the other hand, ectoderm treated with bFGF and implanted similarly shows 74% tail formation. This frequency of tail formation is not affected by RA-treatment. In a similar experiment, Cho et al. (1991) treated competent ectoderm with XTC-MIF, grafted it into host *Xenopus* gastrula and obtain induction of head (27%) and trunk-tail (61%). The addition of 10^{-5} M RA to this system inhibits head induction, with a slight increase in trunk-tail induction (64%).

Putting together the observations of Durston et al. (1989), Sive et al. (1989, 1990) and Ruiz i Altaba and Jessell (1991a,b), it appears likely that RA-treatment has a caudalizing effect on archencephalic induction, but a ventralizing effect on deuterencephalic and trunk-tail induction, as has been shown by Masui (1958) for lithium treatment (see Topic 4). If this is the case, RA-treatment of the whole embryos should considerably reduce the caudalizing effect of RA, because it is not possible to distinguish posterior structures produced by caudalization of archencephalic differentiation from the original posterior structures that are being diminished due to the ventralizing effect of RA. This may be one of reasons for the skeptic attitude of Ruiz i Altaba and Jessell (1991a) toward the caudalizing effect of RA.

The caudalizing effect of RA on neural induction by the organizer reviewed in this topic is very similar to that of lithium reviewed in the last topic. In view of the fact that both lithium (Hallcher and Sherman, 1980; Downes and Michell, 1985; Busa and Gimmlich, 1989) and RA (Ponzoni and Lanciotti, 1990) inhibit the polyphosphoinositide cycle, they may have the same mode of action. This appears to suggest that if lithium's caudalizing effect is mediated by CE as indicated in the last topic, the caudalizing effect of RA may be also dependent on CE induced in the responding tissue by RA. However, there exists no direct evidence for or against the induction of CE by RA treatment.

Chen et al. (1994) studied the distribution pattern of endogenous active retinoids in early *Xenopus* embryos. They show a 3-fold increase in concentration during stage 2 and stage 14, a clear posterior-anterior gradient of concentration at stage 14, and 5-fold increase in the concentration in the dorsal marginal zone during stage 10 to stage 12. Furthermore, retinoids are highly concentrated in the dorsal marginal zone compared to other parts of the embryo. The results do not support the view that all CE is induced by retinoid, but are in agreement with the view that CE expressed in presumptive notochord that becomes involved in caudalization is induced by retinoids.

TOPIC 5: THE CORRELATION BETWEEN THE POLARIZING ACTIVITY AND CONVERGENT EXTENSION (CELL INTERCALATION)

According to Hornbruch and Wolpert (1986) and Wagner et al. (1990), Hensen's node, notochord, and floor plate of chick embryos produce a high level of polarizing effects on limb development when grafted to the anterior margin of the limb bud. In contrast, other tissues of chick embryos similarly tested have no or only weak polarizing effect. It is known that Hensen's node and notochord of early chick embryos express CE (Spratt, 1955, 1957). In amphibian development, the floor plate region expresses the highest level of CE within the neural ectoderm (Goerttler, 1925; Keller et al., 1992; Hama, 1978; Sharpe, 1992). It is probable that the floor plate of chick embryos at early stages also expresses CE. The above facts would suggest that tissues that are expressing CE produce a

polarizing effect. Furthermore, we have discussed the possibility that retinoid-treatment may induce CE in responding tissues (Topic 4). Hence it is possible that the well documented polarizing effect of retinoid could be due to CE induced by retinoid.

Thus all these tests for the polarizing activity suggest the possibility that the polarizing activity is cell intercalation. In this regard, the observation of Muneoka and Murad (1987) that cell intercalation does occur during *Xenopus* limb duplication is highly interesting.

GENERAL DISCUSSION

Interaction between dorsalization and caudalization

Table 1 gives a summary of the sequence of events that are essential for the initial mode of dorsalization-caudalization. Although dorsalization and caudalization are two separate functions of the organizer, they are interdependent at the beginning and the terminal phase of the organizer action: (1) after mesoderm involution, the expression of *Xbra* and CE becomes dependent on the dorsalized state of the mesoderm; (2) the AP specification of the presumptive CNS is determined by the combinative effects of dorsalization and caudalization as indicated in Fig. 1. Also in the mesoderm AP specification of comparable nature may occur.

Table 1. Proposed sequence of events in dorsalization and caudalization during AP specification by the organizer

Main Events of Dorsalization	Main Events of Caudalization
Dorsalization of mesoderm anterior to the AP border-line at stage 9-10 (GD).	Expression of *Xbra* and CE over all areas of the marginal zone. CE is proposed to be controlled by *Xbra* protein (T-1).
Dorsalization of mesoderm behind the AP border-line around stage 11. The mesoderm becomes dorsalized at all AP levels probably within the gastrula stage. *goosecoid noggin*, and *XLIM-1* may be involved in mesodermal dorsalization (GD).	Restriction of the expression of *Xbra* and CE behind the AP border-line after mesoderm involution (IN,T-1).
	Expression of *Xbra* becomes dependent on mesodermal dorsalization (T-1).
The ectoderm is dorsalized (neuralized) by dorsalized mesoderm by the mediation of noggin protein (GD).	Mesoderm located behind the AP border-line is proposed to be caudalized by CE. The possible role of cell intercalation in this regulation (IN,GD).
In the ectoderm, dorsalization occurs at all AP levels of the presumptive CNS (GD).	Transmission of CE from notochord to neural ectoderm, behind the AP border-line (T-1,2).
	Pintallavis and *Xnot* may be involved in the expression of ectodermal CE (T-2).
	Morphogenesis of the posterior zone of neural ectoderm is caudalized by the ectodermal CE. The role of CE-dependent RA (T-5).

In the ectoderm, dorsalization and caudalization interact as indicated in Fig. 1 to give rise to the AP specification of CNS (IN).

Initials in parentheses indicate areas in the text where these subjects are discussed: IN, introduction; T, topics; GD, general discussion.

The expression of dorsalizing and caudalizing genes in the organizer

Among genes that are mainly expressed in the organizer region during gastrulation and neurulation, two groups can be distinguished. The group of dorsalizing genes contains *noggin* (Smith and Harland, 1992; Smith et al., 1993), *goosecoid* (Blumberg et al., 1991; Cho et al., 1991; De Robertis et al., 1992; Niehrs et al., 1993; Steinbeisser et al., 1993) and probably also *Xlim-1* (Taira et al., 1992). The other group contains genes proposed to function in caudalization (Topics 1, 2): *Xbra* (Smith et al., 1991), *pintallavis* (XFD-1 or XFKH-1) (Knöchel et al., 1992; Dirksen and Jamrich, 1992; Ruiz i Altaba and Jessell, 1992) and *Xnot* (von Dassow et al., 1993). The ectopic expression of the first group can give rise to more or less the complete embryonic axis, while the second group lacks this ability, but can caudalize the host anterior structures.

Both in *noggin* (Smith and Harland, 1992) and *goosecoid* (De Robertis et al., 1992) the expression at stage 9-10 is limited to the presumptive prechordal plate, and around stage 11, the expression is expanded to include the presumptive notochord. The *noggin* expression at stage 18 is restricted to the dorsal midline of the mesoderm, and starts from a level anterior to the AP border-line and continues uninterruptedly to the tail level. All levels of the presumptive notochord are positive for the gene transcript. But the most anterior part of the prechordal plate seems to have lost the *noggin* expression at this stage. This is probably related to the early loss of the inducing ability of the prechordal plate often reported (Suzuki et al., 1984). It appears from the above description that the dorsalizing genes are expressed at all AP levels of the organizer during gastrulation and early neurulation, when it induces ectodermal dorsalization.

As discussed, the expression of *Xbra* in front of the AP border-line disappears soon after the mesoderm invaginates, while the expression behind the AP border-line persists for some time (Topic 1). However, *pintallavis* is expressed weakly in front of the AP border-line soon after the mesoderm invagination (Topic 2). The disappearance of the *Xnot* expression in front of the AP border-line is intermediate in timing between that of *Xbra* and *pintallavis*. Thus all caudalizing genes loose the expression in front of the border-line first. Later a stepwise loss of the expression from the anterior to the posterior level is reported for *pintallavis* and *Xnot* (Topic 2). This point has not been studied for *Xbra*.

From the above discussion it is clear that during gastrulation and neurulation, the expression of two gene groups is occurring simultaneously at the dorsal midline of the mesoderm. The only difference between the expression of two groups is the location of the anterior limit. After mesoderm invagination, the expression of dorsalizing genes is extended more in the anterior direction than that of the caudalizing genes. Assuming that ectoderm receives the inductive effects of dorsalizing and caudalizing genes whenever they are expressed in the confronting regions of the mesoderm, the reported data for *Xbra* show that the anterior zone ectoderm receives only the effect of dorsalizing genes, while the posterior zone ectoderm receives the effects of both dorsalizing and caudalizing genes. The reported data for *pintallavis* tend to suggest that the anterior zone ectoderm receives a weak effect of caudalizing genes for a brief period after mesoderm involution.

Since both the original and extended models of dorsalization-caudalization depend on the assumption that dorsalization occurs at all AP levels and caudalization is limited to behind the AP border-line, the genetic data discussed in this section strongly support these two models.

The inducing effect of noggin on ectoderm

According to Lamb et al. (1993) *noggin* protein induces neural tissue in ectoderm in the absence of dorsal mesoderm, and the neural tissue thus induced contains anterior-brain markers, but not hindbrain or spinal cord markers. However, the last group of markers may appear when *noggin* induces ectoderm together with mesoderm. The result not only confirms the proposal made in this paper that dorsalizing genes in the organizer are responsible for ectodermal dorsalization, but supports, at the genetic level, one of basic assumptions made in the original model of dorsalization-caudalization (Yamada, 1950a): ectodermal dorsalization alone leads to the induction of archencephalic differentiation. The result is also in agreement with the assumption that dorsalization together with caudalization, which depends on dorsal mesoderm, induces posterior neural structures, as proposed by the model. Along the line of research of Lamb et al. it is also desirable to obtain information on whether the inducing ability of the posterior zone of the organizer disappears (together with that of the anterior zone) in the absence of *noggin* secretion by the organizer. To do this, simple inhibition of the expression of dorsalizing genes in the organizer cannot be used, because under these conditions, the expression of caudalizing genes in the organizer should be also inhibited (Topics 1,2). Probably the inhibition of *noggin* receptors in the responding ectoderm combined with the intact posterior organizer would be required.

The difference in the regulation of AP specification in mesoderm and ectoderm

As discussed in Topic 1, in the mesoderm, when dorsalizing genes are expressed, the expression of caudalizing genes are induced in the posterior levels of the dorsalized area. This guarantees the occurrence of normal AP specification. However, when the competent ectoderm is simply dorsalized by the organizer, it becomes neuralized and differentiates archencephalic structures. No posterior neural structures result in this situation. For the induction of posterior neural structures, the ectoderm has to receive both the dorsalizing and caudalizing signals from the organizer. Thus, in contrast to the mesoderm, the competent ectoderm does not have the ability to fulfill AP specification, when it is simply dorsalized. Hence the ectoderm is dependent on the organizer not only in dorsalization, but also in caudalization. This point is incorporated in the original model of dorsalization-caudalization.

The regulative role played by cell intercalation in caudalization

The suggestion made in this review that caudalization is mediated by CE implies that cell intercalation is the essential regulator of caudalization. The induction of cell intercalation in the dorsalized non-intercalating cell population should initiate the posterior pathway, and the gradient of cell intercalation should be able to give rise to a series of posterior pathways. Some details of experiments done by Herrmann (1991) can be interpreted to suggest that the posterior mesoderm is directly regulated in this way, while the posterior neural ectoderm is indirectly regulated. It is now pertinent to study the pattern of cell intercalation in various caudalizing systems from this view-point.

The dependence of development of the organizer on activin

The main tissues that differentiate in the organizer or in the ectoderm induced by the organizer can be produced in the activin-treated animal cap (Thomsen et al., 1990; Mitrani et al., 1990). Furthermore, dorsalizing genes like *goosecoid* (Blumberg et al., 1991; Cho et al., 1991); *Xlim-1* (Taira et al., 1992) as well as the caudalizing genes like *Xbra* (Smith et al., 1991); *XFKH-1* (Dirksen and Jamrich, 1992); *Xnot* (von Dassow et al., 1993); *Xhox 3* (Ruiz i Altaba et al., 1992) are inducible by activin. Thus, the function of the organizer is highly dependent on the mesoderm inducing ability of activin (Asashima et al., 1991; Dohrmann et al., 1993; Fukui et al., 1994).

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REFERENCES

Amaya, E., Stein, P. A., Musci, T. J. and Kirschner, M. W. (1993). FGF signalling in the early specification of mesoderm in *Xenopus*. *Development* **118**, 477-487.

Asashima, M., Nakano, H., Uchiyama, H., Sugino, H., Nakamura, T., Eto, Y., Ejima, D., Nishimatsu, S., Ueno, N. and Kinoshita, K. (1991). Presence of activin (erythroid differentiation factor) in unfertilized eggs and blastulae of *Xenopus laevis*. *Proc. Natl. Acad. Sci. USA* **88**, 6511-6514.

Beddington, R. S. P., Rashbass, P. and Wilson, V. (1992). Brachyury - a gene affecting mouse gastrulation and early organogenesis. *Development* **1992 Supplement**, 157-165.

Blumberg, B., Wright, C. V. E., De Robertis, E. M. and Cho, K. W. Y. (1991). Organizer-specific genes in *Xenopus laevis*. *Development* **103**, 193-209.

Busa, W. B. and Gimmlich, R. L. (1989). Lithium-induced teratogenesis in frog embryos prevented by a polyphsphoinositide cycle intermediate or a diacylglycerol analog. *Dev. Biol.* **132**, 315-324.

Chen, Y., Huang, L. and Solursh, M. (1994). A concentration gradient of retinoids in the early *Xenopus laevis* embryos. *Dev. Biol.* **161**, 70-76.

Chesley, P. (1935). Development of the short-tailed mutation in the house mouse. *Proc. Soc. Exp. Biol.* **29**, 437-438.

Cho, K. W. Y., Blumberg, B., Steinbeisser, H. and De Robertis, E. M. (1991). Molecular nature of Spemann's organizer: the role of the *Xenopus* homeobox gene *goosecoid. Cell* **67**, 1111-1120.

Condie, B. G. and Harland, R. M. (1987). Posterior expression of a homeobox gene in early *Xenopus* embryos. *Development* **101**, 93-105.

Cunliffe, V. and Smith, J. C. (1992). Ectopic mesoderm formation in *Xenopus* embryos caused by widespread expression of a *Brachyury* homologue. *Nature* **358**, 427-430.

Dalcq, A. M. (1957). Sur la terminologie de l'induction. *Acta anatomica* **30**, 242-253.

Dekker, E. J., Pannese, M., Houtzager, E., Timmermans, A., Boncinelli, E. and Durston, A. (1992). *Xenopus Hox-2* genes are expressed sequentially after the onset of gastrulation, and are differentially inducible by retinoic acid. *Development* **1992 Supplement**, 195-202.

Dekker, E. J., Pannese, M., Houtzager, E., Boncinelli, E. and Durston, A. (1993). Colinearity in the *Xenopus laevis Hox-2* complex. *Mech. Dev.* **40**, 3-12.

De Robertis, E. M., Blum, M., Niehrs, C. and Steinbeisser, H. (1992). *goosecoid* and the organizer. *Development* **1992 Supplement**, 167-171.

Diaz, M. R. M., Takahashi, T. C., Takeshima, K. and Takata, K. (1990). Concanavalin A acts as a factor in establishing the dorsoventral gradient in the ventral mesoderm of newt gastrula embryos. *Dev. Growth Differ.* **32**, 117-124.

Dirksen, M. L. and Jamrich, M. (1992). A novel, activin-inducible, blastopore lip-specific gene of *Xenopus laevis* contains a fork head DNA-binding domain. *Genes Dev.* **6**, 599-608.

Dohrmann, C. E., Hemmati-Brivanlou, S., Thomsen, G. H., Fields, A., Woolf, T. and Melton, D. A. (1993). Expression of activin mRNA during early development of *Xenopus laevis. Dev. Biol.* **157**, 474-483.

Downes, P. and Michell, R. (1985). Inositol phospholipid breakdown as a receptor controlled generator of second messengers. In *Molecular Mechanisms of Transmembrane Signalling* (ed. P. Cohen and M. D. Houslay), pp. 3-56. Amsterdam: Elsevier.

Durston, A. J., Timmermans, J. P. M., Hage, W. J., Hendriks, H. F. L., de Vries, N. J., Heideveld, M. and Nieuwkoop, P. D. (1989). Retinoic acid causes an anteroposterior transformation in the developing central nervous system. *Nature* **340**, 140-144.

Echelard, Y., Epstein, D. J., St-Jacques, B., Shen, L., Mohler, J., McMahon, J. A. and McMahon, A. P. (1993). Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. *Cell* **75**, 1417-1430.

Ellinger-Ziegelbauer, H. and Dreyer, C. (1991). A retinoic acid receptor expressed in the early development of *Xenopus laevis. Genes Dev.* **5**, 94-104.

Fukui, A., Nakamura, T., Uchiyama, H., Sugino, K., Sugino, H. and Asashima, M. (1994). Identification and characterization of *Xenopus* follistatin and activins. *Dev. Biol.* **159**, 131-139.

Gaunt, S. J., Sharpe, P. T. and Duboule, D. (1988). Spatially restricted domains of homeogene transcripts in mouse embryos: relation to a segmented body plan. *Development* **104 Supplement**, 169-180.

Gebhardt, D. O. E. and Nieuwkoop, P. D. (1964). The influence of lithium on the competence of the ectoderm in *Amblystoma mexicanum. J. Embryol. Exp. Morphol.* **12**, 317-331.

Gerhart, J., Danilchik, M., Doniach, T., Roberts, S., Rowning, B. and Stewart, R. (1989). Cortical rotation of the *Xenopus* egg: consequences for the anteroposterior pattern of embryonic dorsal development. *Development* **107 Supplement**, 37-51.

Gluecksohn-Schoenheimer, S. (1944). The development of normal and homozygous Brachyury (T/T) mouse embryos in the extra-embryonic coelome of the chick. *Proc. Natl. Acad. Sci. USA* **30**, 134-140.

Goerttler, K. (1925). Die Formbildung der Medullaranlage bei Urodelen im Rahmen der Verschiebungsvorgänage von Keimbezirken während der Gastrulation und als entwicklungsphysiologisches Problem. *Wilhelm Roux' Arch. Entw.Mech. Org.* **106**, 503-541.

Graham, A., Papalopulu, N. and Krumlauf, R. (1989). The murine and *Drosophila* homeobox complexes have common features of organization and expression. *Cell* **57**, 367-378.

Grüneberg, H. (1958). Genetical studies on the skeleton of the mouse. XXIII. The development of Brachyury and Anury. *J. Embryol. Exp. Morphol.* **6**, 424-443.

Grunz, H. (1968). Experimentelle Untersuchungen über die Kompetenzverhältnisse früher Entwicklungsstadien des Amphibien-Ektoderms. *Wilhelm Roux' Arch. Entw.Mech. Org.* **160**, 344-374.

Gurdon, J. B., Kao, K., Kato, K. and Hopwood, N. D. (1992). Muscle gene activation in *Xenopus* requires intercellular communication during gastrula as well as blastula stages. *Development* **1992 Supplement**, 137-142.

Hallcher, L. and Sherman, W. (1980). The effects of lithium and other agents on the activity of myo-inositol-phosphatase from bovine brain. *J. Biol. Chem.* **255**, 10896-10901.

Hama, T. (1978). Dynamics of the organizer. New findings on the regionality and morphogenetic movement of the organizer. In *Organizer-A Milestone of a Half-Century from Spemann* (ed. O. Nakamura and S. Toivonen), pp. 71-90. Amsterdam: Elsevier/North-Holland Biomedical Press.

Hama, T., Tsujimura, H., Kanéda, T., Takata, K. and Ohara, A. (1985). Inductive capacities for the dorsal mesoderm of the marginal zone and pharyngeal endoderm in the very early gastrula of the newt, and presumptive pharyngeal endoderm as an initiator of the organization centre. *Dev. Growth Diff.* **27**, 419-433.

Hamburger, V. (1988). *The Heritage of Experimental Embryology.* New York, Oxford: Oxford University Press.

Hashimoto, K., Fujimoto, H. and Nakatsuji, N. (1987). An ECM substratum allows mouse mesoderm cells isolated from the primitive streak to exhibit motility similar to that inside the embryo and reveals a deficiency in the T/T mutant cells. *Development* **110**, 325-330.

Hemmati-Brivanlou, A. and Harland, R. M. (1989). Expression of an engrailed-related protein is induced in the anterior ectoderm of early *Xenopus* embryos. *Development* **106**, 611-617.

Herrmann, B. G. (1991). Expression pattern of the Brachyury gene in whole mount T^{Wis}/T^{Wis} mutant embryos. *Development* **113**, 913-917.

Herrmann, B. G., Labeit, S., Poustka, A., King, T. R. and Lehrach, H. (1990). Cloning of the T gene required in mesoderm formation in the mouse. *Nature* **343**, 617-622.

Holtfreter, J. (1947). Neural induction in explants which have passed through a sublethal cytolysis. *J. Exp. Zool.* **106**, 197-222.

Holtfreter, J. and Hamburger, V. (1955). Embryogenesis: progressive differentiations, amphibians. In *Analysis of Development* (ed. H. Willier, P. A. Weiss, V. Hamburger), pp. 230-296. Philadelphia and London: Saunders.

Hornbruch, A. and Wolpert, L. (1986). Positional signalling by Hensen's node when grafted to the chick limb bud. *J. Embryol. Exp. Morphol.* **94**, 257-265.

Jones, C. M., Lyons, K. M., Lapan, P. M., Wright, C. V. E. and Hogan, B. M. L. (1992). DVR-4 (bone morphogenetic protein-4) as a posterior-ventralizing factor in *Xenopus* mesoderm induction. *Development* **115,** 639-648 .

Kao, K. R. and Elinson, R. P. (1988). The entire mesodermal mantle behaves as Spemann's organizer in dorso-anterior enhanced *Xenopus laevis* embryos. *Dev. Biol.* **127**, 64-77.

Kao, K. R. and Elinson, R. P. (1989). Dorsalization of mesoderm induction by lithium. *Dev. Biol.* **132**, 81-90.

Kao, K. R., Masui, Y. and Elinson, R. P. (1986). Lithium-induced respecification of pattern in *Xenopus laevis* embryos. *Nature* **322**, 371-373.

Karasaki, S. (1957). On the mechanism of the dorsalization in the ectoderm of *Triturus* gastrulae caused by precytolytic treatments. I. Cytological and morphogenetic effects of various agents. *Embryologia* **3**, 317-334.

Kato, K. (1958). Studies on the differentiation potencies of the blastoporal lip in *Triturus*-gastrula. I. Differentiation of mesodermal tissue in relation to the neural tissue. *Mem. College of Science, University of Kyoto* **B 25**, 1-10.

Keller, R. E. (1975). Vital dye mapping of the gastrula and neurula of *Xenopus laevis.* I. Prospective areas amd morphogenetic movement of the superficial layer. *Dev. Biol.* **42**, 222-241.

Keller, R. E. (1976). Vital dye mapping of the gastrula and neurula of *Xenopus laevis*. II. Prospective areas and morphogenetic movement of the deep layer. *Dev. Biol.* **51**, 118-137.

Keller, R. E. and Danilchik, M. (1988). Regional expression pattern and timing of convergent and extension during gastrulation of *Xenopus laevis. Development* **103**, 193-209.

Keller, R. E. and Tibbetts, P. (1989). Mediolateral cell intercalation in the dorsal axial mesoderm of *Xenopus laevis. Dev. Biol.* **131**, 539-549.

Keller, R. E., Shih, J. and Domingo, C. (1992). The patterning of protrusive activity during convergence and extension of the Xenopus organizer. *Development* **1992 Supplement**, 81-91.

Kimmel, C. B., Schilling, T. F. and Hatta, K. (1991). Patterning of body segment of the zebrafish embryo. *Current Topics Dev. Biol.* **25**, 77-110.

Kispert, A. and Herrmann, B. G. (1994). Immunohistochemical analysis of the brachyury protein in wild-type and mutant mouse embryos. *Dev. Biol.* **161**, 179-193.

Knöchel, W. (1992). Activin A induced expression of a fork head related gene in posterior chordamesoderm (notochord) of *Xenopus laevis* embryos. *Mech. Dev.* **38**, 157-165.

Köster, M., Plessow, S., Clement, J. H., Lorenz, A., Tiedemann, H. and Knöchel, W. (1991). Bone morphogenetic protein 4 (BMP-4) a member of the TGF-b family, in early embryos of *Xenopus laevis.* An analysis of mesoderm-inducing activity. *Mech. Dev.* **33**, 191-199.

Kraus, S., Concordet, J.-P. and Ingham, P. W. (1993). A functionally conserved homolog of the Drosophila segment polarity gene *hh* is expressed in tissues with polarizing activity in zebrafish embryos. *Cell* **75**, 1431-1444.

Lamb, T. M., Knecht, A. K., Smith, W. C., Stachel, S. E., Economides, A. N., Stahl, N., Yancopoulose, G. D. and Harland, R. M. (1993). Neural induction by the secreted polypeptide Noggin. *Science* **262**, 713-718.

Lehmann, F. E. (1938). Regionale Verschiedenheiten des Organisators von Triton, insbesondere in der vorderen und hinteren Kopfregion nachgewiesen durch phasenspezifische Erzeugung von Lithiumbedingten und operativ bewirkten Regionaldefekten. *Wilhelm Roux' Arch. EntwMech. Org.* **138**, 106-158.

Lehmann, F. E. (1945). Einführung in die physiologische Embryologie. Basel: Birkhäuser Verlag.

Lettice, L. A. and Slack, J. M. W. (1993). Properties of the dorsalizing signal in gastrulae of *Xenopus laevis. Development* **117**, 263-271.

Masui, Y. (1958). Effect of lithium upon the development of the head of amphibian embryo. *Japanese J. Exp. Morphol.* **13**, 33-53.

Masui, Y. (1960). Differentiation of the prechordal tissue under the influence of Lithium Chloride. *Mem. Konan University, Science* **Series no. 4**, 65-78.
McGinnis, W. and Krumlaut, R. (1992). Homeobox genes and axial patterning. *Cell* **68**, 283-302.
Mitrani, E., Ziv, T., Thomsen, G., Shimoni, Y., Melton, D. A. and Bril, A. (1990). Activin can induce the formation of axial structures and is expressed in the hypoblast of the chick. *Cell* **63**, 495-501.
Muneoka, K. and Murad, E. H. B. (1987). Intercalation and the cellular origin of supernumerary limbs in *Xenopus. Development* **99**, 521-526.
Niehrs, C., Keller, R., Cho, K. W. Y. and De Robertis, E. M. (1993). The homeobox gene *goosecoid* controls cell migration in *Xenopus* embryo. *Cell* **72**, 491-503.
Nieuwkoop, P. D. and Albers, B. (1990). The role of competence in the cranio-caudal segregation of the central nervous system. *Dev. Growth Differ.* **32**, 23-31.
Nieuwkoop, P. D., Bloemsma, F. F. S. N., Broterenbrood, E. C., Hoessels, E. L. M., Kremer, A., Meyer, G. and Verheyen, F. J. (1952). Activation and organization of the central nervous system in amphibians. Part I, II, III. *J. Exp. Zool.* **120**, 1-108.
Ogi, K. (1958). The effect of sodium thiocyanate on isolates of the presumptive ectoderm and medio-ventral marginal zone of *Triturus* gastrulae. *J. Embryol. exp. Morphol.* **6**, 412-417.
Okada, Y. K. and Hama, T. (1943). Examination of regional differences in the inductive activity of the organizer by means of transplantation into ectodermal vesicles. *Proc. Imp. Acad. (Tokyo)* **19**, 48-53.
Okada, Y. K. and Hama, T. (1944). On the different effects of the amphibian organizer following culture, transplantation and heat treatment. *Proc. Imp. Acad. (Tokyo)* **20**, 36-40.
Okada, Y. K. and Hama, T. (1945). Prospective fate and inductive capacity of the dorsal lip of the blastopore of *Triturus* gastrula. *Proc. Imp. Acad. (Tokyo)* **21**, 342-348.
Okada, Y. K. and Takaya, H. (1942a). Experimental investigation of regional differences in the inducing capacity of the organizer. *Proc. Imp. Acad. (Tokyo)* **18**, 505-513.
Okada, Y. K. and Takaya, H. (1942b). Further studies upon the regional differentiation of the inductive capacity of the organizer. *Proc. Imp. Acad. (Tokyo)* **18**, 514-519.
Ponzoni, M. and Lanciotti, M. (1990). Retinoic acid rapidly decreases phosphatidyl-inositol turn-over during neuroblastoma cell differentiation. *J. Neurochem.* **54**, 540-546.
Riddle, R. D., Johnson, R. L., Laufer, E. and Tabin, C. (1993). Sonic hedgehog mediates the polarizing activity of the ZPA. *Cell* **75**, 1401-1416.
Roelink, H., Augsburger, A., Heemskerk, J., Korzh, V., Norlin, S., Ruiz i Altaba, A., Tanabe, Y., Placzek, M., Edlund, T., Jessell, T. M. and Dodd, J. (1994). Floor plate and motor neuron induction by *vhh-1* a vertebrate homolog of hedgehog expressed by the notochord. *Cell* **76**, 761-775.
Ruiz i Altaba, A., Choi, T. and Melton, D. A. (1992). Expression of the Xhox-3 homeobox protein in *Xenopus* embryos. Blocking its early function suggests the requirement of Xhox-3 for normal posterior development of the neural axis. *Dev. Growth Differ.* **33**, 651-660.
Ruiz i Altaba, A. and Jessell, T. M. (1991a). Retinoic acid modifies mesodermal patterning in early *Xenopus* embryos. *Genes Dev.* **5**, 175-187.
Ruiz i Altaba, A. and Jessell, T. M. (1991b). Retinoic acid modifies the pattern of cell differentiation in the central nervous system of neurula stage *Xenopus* embryos. *Development* **112**, 945-958.
Ruiz i Altaba, A. and Jessell, T. M. (1992). *Pintallavis*, gene expressed in the organizer and mid-line cells of frog embryos: involvement in the development of the neural axis. *Development* **116**, 81-93.
Ruiz i Altaba, A. and Melton, D. A. (1989a). Involvement of the *Xenopus* homeobox gene Xhox-3 in pattern formation along the anterior-posterior axis *Cell* **57**, 317-329.
Ruiz i Altaba, A. and Melton, D. A. (1989b). Bimodal and graded expression of the *Xenopus* homeobox gene Xhox-3 during embryonic development. *Development* **106**, 173-183.
Saxén, L. and Toivonen, S. (1962). *Primary Embryonic Induction.* Engelwood Cliff, N.J.: Prentice-Hall Inc.
Scharf, S. R. and Gerhart, J. C. (1983). Axis determination of *Xenopus laevis*: A critical period before first cleavage, identified by the common effects of cold, pressure and ultraviolet irradiation. *Dev. Biol.* **99**, 75-87.
Sharpe, C. R. (1992). Retinoic acid and the late phase of neural induction. *Development* **1992 Supplement**, 203-207.
Sharpe, C. R., Pluck, A. and Gurdon, J. B. (1989). XIF3, a *Xenopus* peripherin gene, requires an inductive signal for enhanced expression in anterior neural tissue. *Development* **107**, 701-704.
Simeone, A., Acampra, D., Aricon, L., Andrews, P. W., Boncinelli, E. and Mavilio, F. (1990). Sequential activation of Hox 2 homeobox genes by retinoic acid in human embryonal carcinoma cells. *Nature* **346**, 763-766.
Simeone, A., Acampora, D., Gulisano, M., Stornaiuolo, A. and Boncinelli, E. (1992a). Nested expression domains of four homeobox genes in developing rostral brain. *Nature* **358**, 687-690.
Simeone, A., Gulisano, M., Acampora, D., Stornaiuolo, A., Rambaldi, M. and Boncinelli, E. (1992b). Two vertebrate homeobox genes related to the *Drosophila* empty spiracles gene are expressed in the embryonic cerebral cortex. *EMBO J.* **11**, 2541-2550.
Simeone, A., Acampora, D., Mallamaci, A., Stornaiuolo, A., D'Apice, M. R., Nigro, V. and Boncinelli, E. (1933). A vertebrate gene related to orthodenticle contains a homeodomain of the *bicoid* class demarcates anterior neuroectoderm in the gastrulating mouse embryo. *EMBO J.* **12**, 2735-2747.
Sive, H. L., Draper, B. W., Harland, R. M. and Weintraub, H. (1990). Identification of a retinoic acid-sensitive period during primary axis formation in *Xenopus laevis. Genes Dev.* **4**, 932-942.
Sive, H. L., Hattori, K. and Weintraub, H. (1989). Progressive determination during formation of the anteroposterior axis in *Xenopus laevis. Cell* **58**, 171-180.
Slack, J. M. W. and Tannahill, D. (1992). Mechanism of anteroposterior specification in vertebrates. Lessons from the amphibians. *Development* **114**, 285-302.
Smith, J. C. (1994). Hedgehog, the floor plate, and the zone of polarizing activity. *Cell* **76**, 193-196.
Smith, W. C. and Harland, R. M. (1992). Expression cloning of *noggin*, a new dorsalizing factor localized to the Spemann organizer in *Xenopus* embryos. *Cell* **70**, 829-840.
Smith, J. C. and Howard, J. E. (1992). Mesoderm-inducing factors and the control of gastrulation. *Development* **1992 Supplement**, 127-136.
Smith, J. C., Price, B. M. J., Green, J. B. A., Weigel, D. and Herrmann, B. G. (1991). Expression of a *Xenopus* homolog of Brachyury (T) is an immediate-early response to mesoderm induction. *Cell* **67**, 79-87.
Smith, W. C., Knecht, A. K., Wu, M. and Harland, R. M. (1993). Secreted *noggin* protein mimics the Spemann Organizer in dorsalizing *Xenopus* mesoderm. *Nature* **361**, 547-549.
Spemann, H. (1931). Über den Anteil von Implantat und Wirtskeimen an der Orientierung und Beschaffenheit der induzierten Embryonalanlage. *Wilhelm Roux' Arch. Entw.Mech. Org.* **123**, 389-517.
Spratt, N. T. (1955). Analysis of the organizer center in the early chick embryo. I. Localization of prospective notochord and somite cells. *J. Exp. Zool.* **128**, 121-163.
Spratt, N. T. (1957). Analysis of the organizer center in the early chick embryo. II. Studies of the mechanics of notochord elongation, and somite formation. *J. Exp. Zool.* **134**, 577-612.
Steinbeisser, H., De Robertis, E. M., Ku, M., Kesler, D. S. and Melton, D. A. (1993). *Xenopus* axis formation: induction of *goosecoid* by injected Xwnt-8 and activin mRNAs. *Development* **118**, 499-507.
Stott, D., Kispert, A. and Herrmann, B. G. (1993). Rescue of the tail defect of Brachyury mice. *Genes Dev.* **7**, 199-203.
Suzuki, A. S., Mifune, Y. and Kanéda, T. (1984). Germ layer interactions in pattern formation of amphibian mesoderm during primary embryonic induction. *Dev. Growth Differ.* **26**, 81-94.
Symes, K. and Smith, J. C. (1987). Gastrulation movement provides an early marker of mesoderm induction in *Xenopus laevis*. *Development* **101**, 339-349.
Taira, M., Jamrich, M., Good, P. J. and David, I. B. (1992). The LIM domain-containing homeobox gene *Xlim-1* is expressed specifically in the organizer region of *Xenopus* gastrula embryos. *Genes Dev.* **6**, 356-366.
Takata, K., Yamazaki-Yamamoto, K. and Ozawa, R. (1981). Use of lectin as probes for analyzing embryonic induction. *Roux' Arch. Dev. Biol.* **190**, 92-96.
Takata, K., Yamazaki-Yamamoto, K., Ishii, I. and Takahashi, N. (1984b). Glycoproteins responsive to the neural-inducing effect of Concanavalin A in *Cynops* presumptive ectoderm. *Cell Diff.* **14**, 25-31.
Thomsen, G. T., Woolf, T., Whitman, M., Sokol, S., Vaughan, J., Vale, W. and Melton, D. A. (1990). Activins are expressed early in *Xenopus* embryogenesis and can induce axial mesoderm and anterior structures. *Cell* **63**, 485-493.
Vogt, W. (1929). Gestaltungsanalyse am Amphibienkeimen mit örtlicher Vitalfärbung. II. Gastrulation und Mesodermbildung bei Urodelen und Anuren. *Wilhelm Roux' Arch. Entw.Mech. Org.* **120**, 384-706.
von Dassow, G., Schmidt, J. E. and Kimelman, D. (1993). Induction of the

Xenopus organizer: expression and regulation of *Xnot*, a novel FGF and activin-regulated homeobox gene. *Genes Dev.* **7**, 355-366.

von Woellwarth, C. (1956). Entwicklungsphysiologie der Wirbeltiere. *Fortschritte der Zoologie* **10**, 458-560.

Wagner, M., Thaller, C., Jessell, M. T. and Eichele, G. (1990). Polarizing activity and retinoid synthesis in the floor plate of the neural tube. *Nature* **345**, 819-822.

Wilkinson, D. G., Bhatt, S. S. and Herrmann, B. G. (1990). Expression pattern of the mouse T gene and its role in mesoderm formation. *Nature* **343**, 657-659.

Wright, C. V. E., Morita, E. A., Wilkin, D. J. and De Robertis, E. M. (1990). The *Xenopus* XIHbox-6 homeo protein, a marker of posterior neural induction, is expressed in proliferating neurons. *Development* **109**, 225-234.

Yamada, T. (1937). Der Determinationszustand des Rumpfmesoderms im Molchkeim nach der Gastrulation. *Wilhelm Roux' Arch. Entw.Mech. Org.* **137**, 151-270.

Yamada, T. (1939). Über bedeutungsfremde Selbstdifferenzierung der präsumptiven Rückemmuskulatur des Molchkeimes bei Isolation. *Okajimas Folia Anatomica Japonica* **18**, 565-568.

Yamada, T. (1940). Beeinflussung der Differenzierungsleistung des isoliertenMesoderms von Molchkeimen durch zugefügtes Chorda und neural Material. *Okajimas Folia Anatomica Japonica* **19**, 131-197.

Yamada, T. (1950a). Regional differentiation of the isolated ectoderm of the *Triturus* gastrula induced through a protein extract. *Embryologia* **1**, 1-20.

Yamada, T. (1950b). Dorsalization of the ventral marginal zone of the *Triturus* gastrula. I. Ammonia-treatment of the medioventral marginal zone. *Biol. Bull.* **98**, 98-121.

Yamada, T. (1990). Regulations in the induction of the organized neural system in amphibian embryos. *Development* **110**, 653-659.

Yamaguchi, Y. and Shinagawa, A. (1989). Marked alteration of midblastula transition in the effect of lithium on the larval body plan of *Xenopus laevis*. *Dev. Growth Diff.* **31**, 531-541.

Yanagisawa, K. O. (1990). Does the T gene determine the anteroposterior axis of a mouse embryo? *Japanese J. Genet.* **65**, 287-297.

Yanagisawa, K. O., Fujimoto, H. and Urushihara, H. (1981). Effects of the Brachyury (T) mutation on morphogenetic movement in the mouse embryo. *Dev. Biol.* **87**, 242-248.

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