Int. J. Dev. Biol. 45: 165-175 (2001)

Formation and maintenance of the organizer among the vertebrates

KATHERINE JOUBIN[1] and CLAUDIO D. STERN*

Department of Genetics and Development and Center for Neurobiology and Behavior, Columbia University, New York, U.S.A.

ABSTRACT The organizer is established at the blastula stage of development, under the influence of a special region of cells known as the Nieuwkoop center in amphibians, where Vg1/activin-like signals overlap with activity of the Wnt-pathway. Despite differences in their mode of early development, a similar region can be identified in other vertebrates. It has widely been assumed that once the organizer property is assigned to cells at this early stage, it is fixed so that by the gastrula stage, no new cells acquire organizer properties. However, when the organizer is ablated, it can regenerate for a limited period during gastrulation, a process regulated by both positive and negative signals emanating from various domains in the embryo. Here we compare the mechanisms that initially establish the organiser in the blastula with those that maintain it during gastrulation in different vertebrate classes, and argue that similar molecular mechanisms may be involved in the two processes. We also suggest that these mechanisms are required to ensure the appropriate location of the organizer property in the gastrula, where cells are continuously moving.

KEY WORDS: *Regeneration, regulation, cell movements, gastrulation, Hensen´s node, Nieuwkoop center, posterior marginal zone, primitive streak*

"The phenomena of regeneration make it clear that no discussion of the developmental factors at work in the blastoderm can be complete if it is confined to a consideration of the primitive streak. The epiblast must be considered as a whole". (Waddington, 1932)

Introduction

The activity of the "organizer", first described by Spemann and Mangold in 1924, is one of the most remarkable demonstrations of the importance of cell-cell communication in generating tissue diversity during embryonic development. The ability of a small group of cells from the dorsal lip of the blastopore to determine the developmental dynamics of the entire embryo made it clear that inductive interactions between cell types generate cell fate diversity during embryogenesis, and that they are indeed responsible for the formation of the nervous system. Following Spemann and Mangold's discovery (Spemann and Mangold, 1924), organizers were identified in many different vertebrates, demonstrating that despite very different reproductive strategies and geometries of the egg, development proceeds through very similar mechanisms in all vertebrate classes. It was further shown that the organizers from different species are interchangeable, and, in the quest for the identification of the "organizing" substance, it was found that tissue extracts from many different animals contain potent organizing activity (Holtfreter, 1934). Subsequent demonstrations that the responses induced by the organizer are themselves not specific, but dependent on the developmental context of the responding tissue, further highlighted the conservation of developmental mechanisms.

Despite the generally accepted significance of the organizer, the surprising discovery that experimental ablation of the organizer results in subsequently normal development (Waddington, 1932; Oppenheimer, 1934; 1936a; Abercrombie and Bellairs, 1954; Butros, 1967; Gallera and Nicolet, 1974; see also Yuan *et al.*, 1995a; 1995b; Psychoyos and Stern, 1996; Yuan and Schoenwolf, 1998) led some embryologists to conclude that the organizer is not essential for proper development (Waddington, 1932). These studies confirmed that the early embryo is remarkably plastic and demonstrated that not only does the organizer control the developmental fate of its surrounding tissues, but that it is, in turn, under reciprocal control by similar interactions. Recent reinvestigations of these results in the chick embryo now suggest that molecular mechanisms similar to those that initially set up the organizer are also responsible for the regeneration and the maintenance of the organizer during the cell movements of gastrulation (Joubin and Stern, 1999). In addition, an antagonism between the node and the periphery of the embryo

Abbreviations used in this paper: ADMP, anti-dorsalizing morphogenetic protein; BMP, bone morphogenetic protein.

***Present address and correspondence to:** Dr. Claudio D. Stern. Department of Anatomy and Developmental Biology. University College London, Gower Street, London, WC1E 6BT, England. e-mail: c.stern@ucl.ac.uk

[1]Present address: Dr. Katherine Joubin. Skirball Institute for Biomolecular Medicine. NYU School of Medicine, 540 First Avenue, New York, NY 10016, USA.

0214-6282/2001/$25.00

Printed in Spain
www.ijdb.ehu.es

imposes spatial restrictions on the organizer, thereby confining it to just the center of the embryo. The phenomenon of organizer regeneration has been demonstrated, at least partially, in other vertebrates, suggesting that it is not unique to the chick embryo, but rather, that it may be a general consequence of early developmental events.

Here, we will review some of the early literature following Spemann and Mangold's publication, as it pertains to early embryonic regulation in different vertebrate classes. We will begin with a brief introduction to the history of the organizer and its induction by the Nieuwkoop center. Then a description of the phenomenon of organizer regeneration will be provided and compared to the initial establishment of polarity in the early embryo. This historical overview will provide the framework to present some recent insights regarding the significance of early embryonic plasticity and regulation in different vertebrates.

The Spemann-Mangold Organizer

The observation that grafts of the dorsal lip of the amphibian blastopore can result in the formation of a second body axis upon transplantation was first demonstrated (Lewis, 1907; Spemann, 1918) more than 15 years before Spemann and Mangold's publication in 1924, but because it was impossible to distinguish between host and donor contributions, the phenomenon was attributed solely to the graft's capacity for self-differentiation. The idea that a tissue could "induce" (cause a change of fate in a neighboring tissue), had entered Spemann's mind because of his earlier work on the lens (Spemann 1901; 1905) and led him to repeat the dorsal lip grafts to determine whether an inductive event might be responsible for the formation of the secondary axis. Using differently pigmented amphibian embryos to distinguish between host and donor tissues, it became clear that the dorsal lip of the blastopore has the ability not only to initiate gastrulation movements and differentiate into axial mesodermal derivatives upon transplantation, but also to induce a nervous system from the host's ventral epidermal tissue (Spemann and Mangold, 1924). Spemann and Mangold called the dorsal lip an *organizer* - "a region of the embryo that has preceded the other parts in determination and thereupon emanates differentiation effects of a certain quantity in certain directions.".

These findings highlighted the role of cell-cell interactions during embryogenesis and instigated the search for organizers in other animals (Fig. 1). Waddington found that grafts containing the anterior half of the primitive streak of the chick embryo also induce neural tissue in host non-neural and extra-embryonic ectoderm and demonstrated the existence of an organizer in amniotes (Waddington, 1930; 1932; 1934). His experiments grafting duck into chick (Waddington and Schmidt, 1933) confirmed that the effects are due to induction and demonstrated that organizer activity is most potent in Hensen's node at the anterior tip of the primitive streak, although also present in lesser degrees throughout the anterior third of the primitive streak. Similarly, Oppenheimer, using vital dyes, demonstrated that the dorsal shield of the perch is the teleost equivalent of the Spemann-Mangold organizer and can induce the formation of a secondary embryo upon transplantation into the embryonic or extra-embryonic region of host embryos (Oppenheimer, 1934a; 1934b; 1936a). Until recently (Beddington, 1994), the only demonstrations that the mammalian organizer also resides in Hensen's node at the tip of the primitive streak were those of Waddington (1934, 1936, 1937) who transplanted organizers between rabbit and chick and vice-versa, and Blum *et al.* (1992) who transplanted the tip of the mouse primitive streak into *Xenopus*. Due to the technical difficulty in manipulating the mouse, a convincing demonstration that the mouse node can induce a secondary axis when grafted into posterolateral locations of a host mouse embryo was provided only recently (Beddington, 1994).

In most of these initial experiments, inter-species grafts were carried out primarily to distinguish between host and donor contributions. However, in so doing, these experiments also demonstrated the lack of specificity among organizers from different species. Cross-species organizer grafts between chick, rabbit, fish, amphibians and mouse demonstrated that, in every combination, neural tissue is induced in host tissue (Waddington, 1934; 1936; 1937; Oppenheimer, 1936b; Dodd and Kintner, 1991; Blum *et al.*, 1992; Hatta and Takahashi, 1996; Zhu *et al.*, 1999).

In characterizing the response induced by the organizer, it was noticed that the capacity for self-differentiation and the regional character of the nervous system induced by the organizer change depending on the age of the graft and host (Holtfreter, 1936; Oppenheimer, 1934a; 1936a; Gallera and Ivanov, 1964; Vakaet, 1965; Dias and Schoenwolf, 1990; Storey *et al.*, 1992). Such observations led to the distinction between *evocation*, defined as "the determination that an embryonic axis shall be developed" and *individuation* which was "the determination of the character of that axis" (Needham *et al.*, 1934). In general, grafts of gastrula stage organizers induce complete neural axes in gastrula stage hosts. As the age of the graft and host is increased, there is a gradual decrease in the frequency of induction and in the ability to induce anterior structures, and instead, much of the nervous system in the ectopic axes is differentiated graft tissue itself (Holtfreter, 1936; Oppenheimer, 1934a; 1936a; Gallera and Ivanov, 1964; Vakaet, 1965; Dias and Schoenwolf, 1990; Storey *et al.*, 1992). These results suggest that during normal development, the activity of the organizer is most potent during gastrulation.

Formation of the organizer - the Nieuwkoop center

The formation of the organizer in amphibians was first elucidated by Nieuwkoop in his studies on mesoderm formation, which first demonstrated that the mesoderm is induced in ectodermal tissue under the influence of signals emanating from the yolky endoderm in the vegetal pole (Nieuwkoop, 1969a). The initial pattern of the mesoderm induced is completely dependent on the inherent pattern of the vegetal pole, so that the organizer (the most dorsal mesoderm) is induced by the most dorsal part of the vegetal tissue (Nieuwkoop, 1969b). The pattern of the vegetal tissue is itself established by rotation of the egg cortex following sperm entry (Vincent and Gerhart, 1987). The fate of the dorsal vegetal cells is non-axial endoderm (perhaps mainly gut contents), and the Nieuwkoop center (dorsal vegetal tissue) is now defined by its ability to induce organizer tissue without making a cellular contribution to the organizer or to its axial derivatives (see Bachvarova *et al.*, 1998).

In fish, it was recently shown that mesoderm and organizer induction may occur by a very similar mechanism to amphibians. Like the vegetal endoderm in amphibians, the yolk syncytium of the egg that underlies the developing blastoderm induces mesoderm, and the type of mesoderm induced is influenced by the polarity of the yolk (Mizuno *et al.*, 1996). Polarity in the yolk itself may be determined initially by the location of the egg nucleus and the increased amount of cytoplasmic streaming present around it (Roosen-Runge, 1938).

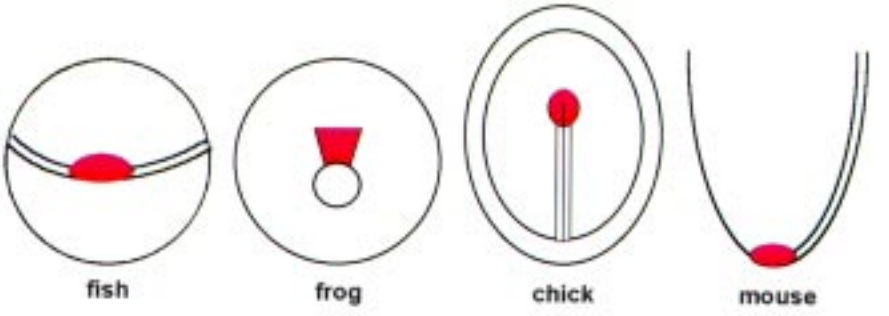

Fig. 1. Position of the organizer in four different vertebrate classes. *In the fish (here viewed from the dorsal side of the equator), the organizer (red) is centered on the embryonic shield; in the frog (viewed obliquely from the blastopore – circle), the organizer extends from the dorsal lip of the blastopore; in the chick (viewed here from the epiblast side – inner circle, area pellucida; outer circle, area opaca; posterior to the bottom), it is situated at the tip of the primitive streak; in the 7.5 d.p.c. mouse, the organizer (node) lies at the distal tip of the cylinder (the primitive streak is shown to the right, posterior top right and anterior top left).*

The identification of a comparable region in amniotes has been a subject of debate. In the chick, emphasis was initially put on the hypoblast, which like the Nieuwkoop center is an endodermal tissue present before gastrulation and which does not itself contribute to axial tissues. The hypoblast forms as a delicate layer underlying the embryonic epiblast before gastrulation, and spreads in a posterior to anterior direction. When Waddington (1932; 1933b) and more recently Azar and Eyal-Giladi (1981) rotated the hypoblast, they found that the orientation of the primitive streak followed that of the rotated hypoblast. However, it was difficult to distinguish between an inductive event and mechanical interference with normal movements (see Waddington, 1932). Moreover, other studies were unable to confirm the claim (Waddington, 1933) that hypoblast rotation can sometimes initiate formation of a new primitive streak (Khaner, 1995), and showed that removal of the hypoblast does not prevent streak formation (Stern, 1990; Khaner, 1995). Most recently, it was shown that hypoblast rotation does indeed alter the orientation of the axis, not by a process of induction but rather by redirecting cell movements in the overlying epiblast (Foley *et al*., 2000).

A second region that has received attention as a putative avian homologue of the Nieuwkoop center is Koller's sickle (also known as Rauber's sickle; Callebaut *et al*., 1998a; 1998b). Although Koller's sickle does have some primitive streak inducing ability (Izpisua-Belmonte *et al*., 1993; Callebaut *et al*., 1998a; 1998b; Khaner, 1998), if the cells of the sickle are labeled, the labeled cells are found to contribute to the node and to definitive endoderm derived from it (Izpisua-Belemonte *et al*., 1993; Bachvarova *et al*., 1998).

The third candidate region is the posterior marginal zone. This can induce a primitive streak if grafted into a young enough host embryo (Khaner *et al.,* 1985), and gives rise only to extra-embryonic tissue without making a cellular contribution to the induced streak (Bachvarova *et al*., 1998). The posterior marginal zone seems therefore to be the only region that meets the criteria required for an avian equivalent of the Nieuwkoop center - it acts before gastrulation to induce an organizer but it does not itself contribute to the axial structures.

At least two populations of cells contibute to the organizer in the chick embryo (Fig. 2), and it seems likely from their initial location (stages X-XI) that they might both be induced by signals from the posterior marginal zone. One of these populations is located in the middle layer, associated with the inner face of Koller's sickle (Izpisua-Belmonte *et al*., 1993; Bachvarova *et al*., 1998; Streit *et al*., 2000) – these give rise mostly to embryonic endoderm. The second population resides in the epiblast just above the anterior face of Koller's sickle at stage X (Hatada and Stern, 1994; Streit *et al*., 2000). Between stages XI and XIII, this second population moves anteriorly within the epiblast until it reaches the center of the embryo, where it remains. The precursors in the middle layer migrate later, with the tip of the forming primitive streak, until the two populations meet in the center of the embryo at the mid-

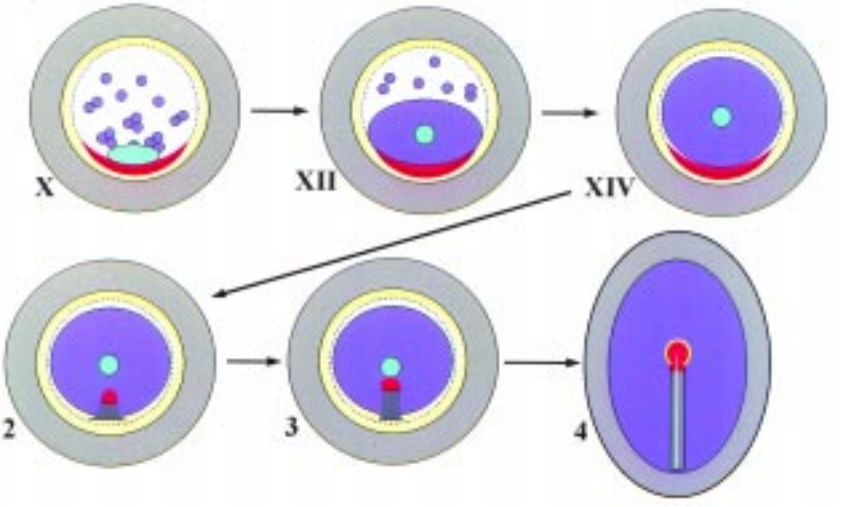

Fig. 2. Formation of the organizer in the chick. *The diagrams represent embryos at progressively older stages, showing the position of the precursors of the organizer and other major features. At stage X (time of egg laying), the embryo consists of an inner region (white, area pellucida) composed of a single cell thick epithelium: the epiblast. Its ventral surface is dotted with islands of primary hypoblast cells (deeper blue). Surrounding the area pellucida is the marginal zone (light yellow), which separates it from the peripheral area opaca (grey). One group of cells that contributes to the organizer is initially found at the posterior edge of the area pellucida (red), associated with the inner face of Koller's sickle. A second group of organizer precursors (light blue) are located in the epiblast immediately overlying the first group at this stage. As the embryo develops, the hypoblast islands coalesce into a layer (deep blue at stages XII-2) in a posterior-to-anterior direction, and the second group of precursors (light blue) follows this movement until it reaches the center of the blastoderm at about stage XIII. The first group (red) remains posteriorly until the primitive streak starts to form at stage 2, and then moves anteriorly together with the elongating streak. The two groups meet each other at about stage 3, when a fully functional organizer develops (red node with deeper yellow outline at stage 4). By stage 4 the lower layer is composed of several different cell types including the definitive endoderm which is derived from the streak, but these have been omitted for clarity.*

primitive streak stage; it is only after these two populations meet that a fully functional organizer develops (Streit *et al.*, 2000).

Timing of mesoderm induction and Nieuwkoop center activity

The timing of Nieuwkoop center activity has not been extensively studied, but in amphibians, already at the 4-cell stage, the dorsal and ventral blastomeres are specified in their dorsal/ventral character (Cooke and Webber, 1985a). At the 64 cell stage, it was shown that 1-3 dorsal vegetal cells can induce an organizer and thus rescue a UV ventralized host embryo (Gimlich and Gerhart, 1984), demonstrating that by this stage and perhaps even earlier, there is Nieuwkoop center activity. In terms of when Nieuwkoop center activity ends, the ability to induce mesoderm (and possibly organizer) in explants of amphibian embryos was found to extend into, though weaken during, gastrulation (Boterenbrood and Nieuwkoop, 1973; Jones and Woodland, 1987). Additionally, small grafts of the ventral marginal zone or animal cap ectoderm can acquire organizer properties when transplanted into the organizer region, up until the end of gastrulation (Domingo and Keller, 1995). This finding suggests the existence of organizer-inducing signals in the embryo throughout gastrulation.

In the chick it was found that the posterior marginal zone can induce a streak only from pre-primitive streak stages X to XII (Khaner *et al.*, 1985; Bachvarova *et al.*, 1998). Afterwards, other tissues, such as the posterior marginal zone with Koller´s sickle (Izpisua-Belmonte *et al.*, 1993; Bachvarova *et al.*, 1998; Khaner *et al.*, 1998) can induce a streak until stage XIII. At gastrula stages, it was shown that grafts of portions of the primitive streak can induce another primitive streak until the end of gastrulation (Waddington and Schmidt, 1933; Gallera and Nicolet, 1969; Vakaet, 1964), demonstrating that mesoderm induction can occur during gastrulation. Additionally, grafting pieces of ectoderm into the primitive streak of host embryos results in the conversion of the graft into mesoderm suggesting that mesoderm inducing signals are also present in the embryo during gastrulation (Waddington and Taylor, 1937).

In the fish, the timing of mesoderm induction has not been systematically studied. The only studies addressing this were done by Oppenheimer (1934b; 1936c) (and only in retrospect to the studies of Mizuno *et al.*, 1996). Oppenheimer separated blastoderms from the yolk at different stages and found that only when blastoderms were isolated after the 32-cell stage could they initiate gastrulation and form embryonic structures. She also mentions that adding material from the yolk to a blastoderm removed from the yolk before the 32-cell stage results in some recovery of structures. In these studies, she also noticed that embryos developing from blastoderms isolated after the 32-cell stage form normal head structures, but fail to form tails, demonstrating that continuous interaction with the yolk is necessary for the completion of gastrulation. Additional studies in which blastoderms were rotated 180° on the yolk at the blastula and gastrula stages demonstrated that the polarity of the embryo is already set by this time; however, the polarizing ability of the yolk itself continues into gastrulation and is able to pattern blastoderm grafts (Devillers, 1951; Long, 1983).

Additionally, experiments in which the organizer was ablated and subsequently regenerated suggest that new organizer formation can occur (albeit under experimental conditions) throughout gastrulation. This will be reviewed in greater depth in the next section.

Regeneration of the organizer

In the same study that initially described organizer activity in the chick embryo, Waddington made the additional observation that ablation of Hensen's node and other parts of the primitive streak can be followed by completely normal development (Waddington, 1932). Following node ablations, there is a doubling of the mitotic index as well as extensive migration of lateral cells towards the anterior portion of the remaining streak, as shown by labeling with carbon particles (Grabowski, 1956). Coincidently, node regeneration can only occur during gastrulation when lateral cells are normally undergoing movements towards the streak. As soon as the head process starts to form at the end of gastrulation, transverse movements no longer occur and regeneration becomes very limited; even when the hole closes at these stages, no notochord forms. In experiments on node ablations during gastrulation, even in cases in which the hole from the ablation did not heal, it appeared that normal development proceeds on either side of the wound (Waddington, 1932). Similarly, removal of the node and its replacement with a piece of posterior primitive streak results in the appearance of two regions with node properties on either side of the graft, and ultimately the formation of embryos with anterior axial duplications. The graft itself does not contribute to the axis, suggesting instead that "the operation releases notochordal potency in material which is not presumptive notochord at either side of the graft" (Abercrombie and Bellairs, 1954; see also Yuan *et al.*, 1995a; 1995b; Psychoyos and Stern, 1996; Yuan and Schoenwolf, 1998). Experiments involving rotations of different parts of the primitive streak including the node also result in complete regulation (although sometimes a doubling of the axis). A reversed axis rarely forms, demonstrating that the polarity of the primitive streak is subject to control by neighboring tissue and that organizer activity itself can be downregulated (Abercrombie, 1950). This remarkable regulative ability of the embryo led Waddington to conclude that "there are no grounds for supposing that the unaided primitive streak, in normal development, induces the medullary plate from non-presumptive ectoderm, since under normal conditions it is aided by the material movements, which have been initiated at an earlier stage ... Similarly it is clear that the transverse material movements themselves do not in normal development give rise to the axial region of the embryo, since they are in fact added by the primitive streak, which has been built up by the longitudinal movements of a still earlier stage" (Waddington, 1932).

Together, these studies suggest that regeneration occurs through the acquisition of organizer properties by cells which normally do not possess them, raising the questions of how these properties are acquired, and what prevents these regions from becoming organizer during normal development. Recently, the phenomenon of organizer regeneration in the chick has been reinvestigated (Yuan *et al.*, 1995a; 1995b; Psychoyos and Stern, 1996; Yuan and Schoenwolf, 1998; Joubin and Stern, 1999). These studies demonstrate that there is a gradual acquisition of organizer properties by regions surrounding the site of ablation as shown by neural inducing ability and gene expression. Furthermore, organizer properties first reappear close to the stump of the primitive streak, suggesting that this region may be responsible for inducing new organizer tissue during regeneration. Indeed, the middle of the primitive streak can induce organizer gene expression when grafted to an ectopic site (Joubin and Stern, 1999), consistent with previous observations that the middle of the primitive streak can

induce a new streak in host embryos during gastrulation (Waddington, 1932; Gallera and Nicolet, 1969; Vakaet, 1964). This could explain why removal of the anterior third of the primitive streak results in complete regeneration, but removal of the entire streak results in no further development, although healing still occurs (Waddington, 1932; Spratt, 1960). After gastrulation, the inducing ability of the middle of the primitive streak is lost (Gallera and Nicolet, 1969), consistent with the observations that full regeneration of the node can occur only during gastrulation. It therefore appears that the middle of the primitive streak can induce organizer properties, but what prevents the induction of ectopic organizer tissue during normal development? Grafts of the middle of the primitive streak induce organizer properties in host tissue much more frequently in the absence of Hensen's node, suggesting the existence of an inhibitory effect emanating from the node itself that prevents adjacent cells from becoming organizer during normal development. In the absence of Hensen's node, neighboring cells would be released from the inhibition and therefore become organizer (Joubin and Stern, 1999).

Can regeneration occur in other vertebrates? Similar experiments have been done in amphibians and fish but have given inconsistent results. Bautzmann (1933) was the first to describe organizer regeneration in amphibians, and the observation was followed up by extensive studies performed by Cooke in the 1970s. If the entire dorsal lip and presumptive foregut endoderm is ablated, the wound closes in just 30 minutes and is followed by normal gastrulation movements and subsequently normal development (Cooke, 1975). In a subsequent study, however, it was reported that regeneration does not occur. In this study, a more rigorous analysis of "regulated" embryos involving serial sections demonstrated that embryos with a superficially normal external appearance often revealed anterior deficits and a lack of complete regeneration (Cooke, 1985). Therefore, at this point, we can only conclude that organizer ablation in *Xenopus* results in partial regeneration. Additional evidence for regulatory tendencies in the amphibian gastrula came from experiments in which an organizer was grafted onto the ventral side of hosts whose own organizer had been ablated, either at the time of the organizer graft or hours later (Cooke, 1973). In normal hosts, usually the secondary axis induced by an organizer graft is complete though much smaller than the host's axis. Similarly, in hosts in which an organizer was grafted at the same time as the ablation, the induced axis is also smaller. However, if an organizer is grafted a few hours before the ablation of the host's organizer, the size of the second axis is significantly larger, suggesting that the amount of territory "captured" by an organizer can be inhibited by the presence of another organizer (Cooke, 1973).

Similarly, in the fish, different studies have yielded often variable results and conclusions regarding organizer regeneration. Some investigators have found regeneration (Sumner, 1904; Oppenheimer, 1934; 1936a; Luther, 1937), whereas others have not (Brummet, 1968a; 1968b; Shih and Fraser, 1996). It appears that one reason for this discrepancy may lie in the stage of the embryos used in the studies - ablation of young shields resulting in at least partial regeneration, whereas a marked decrease in regulation is found at older stages. This explanation is consistent with a series of rigorous experiments designed to reveal the stage at which axial cell fates become localized in the shield (Hoadley, 1928). Portions of tissue were removed from the shield at different stages. Until the middle shield stages, tissue could be removed from anywhere along the shield without any resulting deficiency. However, at the late shield stage, removal of cells results in a higher mortality rate as well as deficits appropriate to the location of the removed cells, suggesting that plasticity is lost at this time. A more recent study confirms the idea that the lack of regulation may be correlated with the age of the embryo. Shih and Fraser (1996) found that ablation of the shield is never followed by regeneration of the notochord. In the same study, it was found that transplants of the shield to the ventral side of host embryos result in the formation of a secondary body axis, derived solely from the graft. This is similar to the results of ablation and transplantation of the node from post-gastrula stage hosts in the chick (Grabowski, 1956). Therefore, it seems possible that the lack of regeneration observed in many of these studies could be due to the embryonic stages used in the experiments.

Only one study on organizer ablation in the mouse has been published to date (Davidson *et al.*, 1999). In this report, the node was ablated during late gastrulation and resulted in the absence of notochord regeneration. It seems likely that a similar manipulation carried out at an earlier stage will lead to more extensive regulation.

Therefore, although organizer regeneration can occur in the chick, still inconclusive data from other vertebrates are consistent with the idea that at least partial regeneration can occur. Additional examples of regulatory tendencies are demonstrated by experiments in the early embryo. In early chick and fish embryos (in contrast to the more "mosaic" frog egg) we are confronted by the problem on how only one embryonic axis forms during normal development from a seemingly totipotent blastoderm. As we will see, it appears that mechanisms similar to those that prevent the formation of ectopic organizers in the gastrula could also serve to prevent the formation of more than one axis during the initial polarization of the embryonic blastoderm.

Regulation in the early embryo

When the chick egg is laid, the embryo is a non-polarized one-cell thick blastoderm lying on top of a yolk mass. Soon after, Koller's sickle appears as a thickening which marks the future posterior edge of the embryo, where the primitive streak will start to form. At these pre-streak stages, although the fate map reveals a spatial distribution of tissue precursors (Hatada and Stern, 1994), all parts of the blastoderm have the potential to initiate an embryonic axis. Lutz (1948) first demonstrated that if unincubated duck blastoderms are cut into either four parallel bands or into quadrants, embryos can develop in each piece, even though only one does so in the intact blastoderm. This observation was followed up by a series of studies by Spratt in the 1960s looking at the regulative potential of different regions of the pre-streak chick embryo (Spratt and Haas, 1960; 1961). Spratt's studies, which also involved cutting blastoderms into many different pieces and culturing the individual pieces, revealed that although during normal development, only one primitive streak forms at the posterior side of the embryo, streaks can potentially form from anywhere along the circumference of the marginal zone, although at different frequencies. The frequency of streak formation is highest posteriorly, gradually declining to a lower frequency anteriorly. At early streak stages, blastoderm fragments can no longer develop a streak. These results already raise the intriguing problem of why only one primitive streak (and organizer) forms during normal development. Spratt hypothesized that the force of the growing and radially

moving cells of the lower layer could act mechanically to prevent streak formation from anywhere except one point. However, a simple mechanical explanation does not account for the differences in streak forming potential of cultured explants. These results point to the existence of mechanisms in the unincubated embryo, in addition to those that induce the streak, which serve to prevent the formation of ectopic streaks during normal development and thereby integrate the entire blastoderm into forming just one organizer and one embryo. Based on the frequency of streak formation in cultured blastoderm isolates, one could imagine that streak formation could be controlled simply through the action of a gradient of a positive streak inducing signal from the posterior end and/or an inhibitor emanating from the anterior margin.

Studies in which the posterior marginal zone was ablated and/or grafted into ectopic positions (Khaner and Eyal-Giladi, 1986; 1989) demonstrated that in addition to a gradient of streak forming potential which determines the position of the streak, there also exists an inhibitory effect exerted by the forming streak which prevents the formation of ectopic streaks in the near vicinity. If the posterior marginal zone is cut out, a streak still forms in the original position. If, however, the hole is plugged with a bead, then two streaks form, one on each side of the implant (similar to what occurs after ablation of Hensen's node if the hole is prevented from healing; Waddington, 1932). Additionally, if a posterior marginal zone is grafted at 90° from its initial position, it is able to induce an ectopic primitive streak at its new site, while another streak continues to form at the original posterior pole. As grafts are placed closer to the host's posterior margin, there is increasing competition between the two sites and only one streak forms. Temporal constraints also govern this response: as mentioned earlier, the ability of the posterior marginal zone to initiate a streak ends at stage XII. Its streak inhibiting activity appears to end earlier, at stage XI (Eyal-Giladi and Khaner, 1989; Khaner and Eyal-Giladi, 1986; 1989). These results suggest the existence of a regulatory mechanism, which would serve to ensure the formation of just one streak, perhaps similar to the mechanisms that regulate the organizer during gastrulation.

In fish, the first morphological signs of bilateral symmetry appear at the beginning of gastrulation with a thickening of the dorsal shield. As in the chick, it appears that until this stage, there is a high degree of multipotency among blastomeres. Indeed, in lineage tracing experiments, labeled blastomeres disperse randomly up until gastrulation demonstrating that at these early stages, in the fish, unlike the chick, there is no correlation between position and fate (Kimmel and Law, 1985). Regulation in the early fish embryo has also been documented. Removal of one cell at the 2-cell stage, any two cells at the 4-cell stage, and any fifth of the embryo at the blastoderm stage results in normal (though small) embryos (Morgan, 1895; Lewis, 1912a; Hoadley, 1928). Similar to Spratt's studies, Luther (1936) divided blastula stage blastoderms of the trout into quarters and found that each piece can regulate and form embryos, though the topography of many of the differentiated structures is abnormal. Additional experiments in fish at later stages demonstrated equally dramatic regulatory properties at both blastula and early gastrula stages (Luther, 1937; Devilliers, 1951). If either the dorsal or ventral half is removed, the embryo can regenerate the missing half. Removal of a dorsal half and replacement with a grafted ventral half (to make double-ventral embryos) results in the formation of a single axis arising from the position of the original dorsal half, suggesting that a polarizing activity emanates from the yolk. However, 180° rotation of the blastoderm with respect to the yolk does not repolarize the embryo, demonstrating that the formation of an axis in combined ventral halves is not simply due to an induction of dorsal tissue by the underlying yolk but also the release from an inhibitory influence normally exerted by the dorsal tissue. Combination of two dorsal halves at either blastula or early gastrula stages, however, results in two axes (Luther, 1937; Devilliers, 1951). More recently, it was shown that transplants of cells taken from blastula and early gastrula stages always result in the graft adopting fates appropriate to the new environment. In contrast, cells taken from later gastrula stages and transplanted into new environments remained committed to their initial fates (Ho and Kimmel, 1993). As with the chick experiments previously described, these results highlight the plasticity of the early fish embryo, and suggest that antagonistic influences between different parts of the embryo may ensure that only one embryo forms.

The regulative capacity of early chick and fish embryos is in marked contrast to the largely "mosaic" development of the amphibian embryo. In *Xenopus*, separation of the left and right blastomeres at the two cell stage results in small embryos that are normal except for having, surprisingly, nearly twice as much notochord tissue as normal embryos (Cooke and Webber, 1985b). However, at the 4-cell stage, separation of dorsal from ventral blastomeres results in embryos completely deficient for ventral or dorsal structures, respectively. Regulation is never observed (Cooke and Webber, 1985a). In fact, the complementation in the structures present in embryos derived from dorsal or ventral halves suggested that "further communication between their descendent endomesodermal cell populations would have been minimal and unnecessary during normal embryogenesis" (Cooke and Webber, 1985a).

Parallels of organizer regulation with organizer induction

The plasticity of early fish and chick embryos opens the question of how the blastoderm is integrated into forming just one embryo. Do similar mechanisms prevent both the formation of ectopic organizers during gastrulation and ectopic streaks during the initial polarization of the blastoderm?

The activity of the Nieuwkoop center is mediated at least in part by cooperativity between the TGF-β (specifically, Vg1/activin/Nodal) and Wnt signaling pathways. These two pathways synergize in the induction of organizer-specific genes (Watabe *et al.*, 1995; Cui *et al.*, 1996; Crease *et al.*, 1998; Zorn *et al.*, 1999; Nishita *et al.*, 2000). In *Xenopus*, factors with mesoderm inducing activity include maternally deposited Vg1, as well as the Nodals, Activin and Derriere, which are activated zygotically by the maternal transcription factor VegT (Dale *et al.*, 1989; Kessler and Melton, 1995; Zhang *et al.*, 1998; Clements *et al.*, 1999; Kofron *et al.*, 1999; Sun *et al.*, 1999; Agius *et al.*, 2000). Activation of the Wnt signaling pathway (by an unknown mechanism) on the dorsal side of the embryo results in nuclear localization of β-catenin only on the dorsal side of the embryo, so that these pathways intersect in dorsal vegetal cells. These two pathways (Vg1/Nodal and Wnt8C/nuclear β-catenin) are also active in the posterior marginal zone of the chick (Hume and Dodd, 1993; Seleiro *et al.*, 1996; Shah *et al.*, 1997; Roesser *et al.*, 1999). In the zebrafish, *squint* (a Nodal family

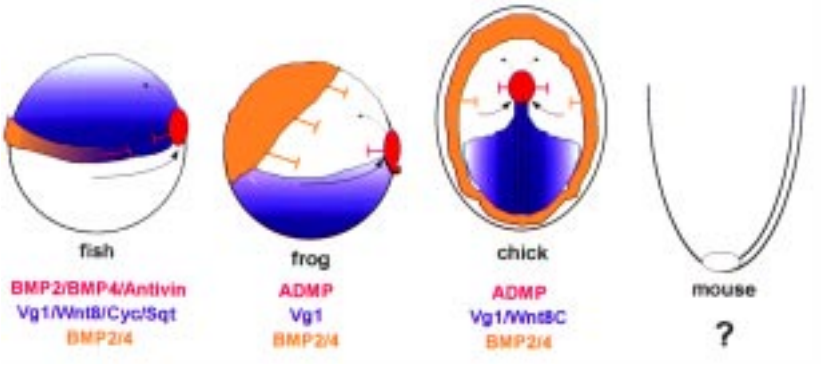

Fig. 3. Signals that contribute to pattern the embryo and position the organizer in different vertebrate classes. *"Positive" signals are shown in shades of blue, "negative" (inhibitory) signals in red/orange. In the frog , the TGFβ-related factor Vg1 is expressed vegetally, while both the organizer (right, red) and the ventral side of the animal cap (left, orange) express the inhibitors ADMP and BMP2/4, respectively. In the fish the shield expresses the Nodal-related proteins (Sqt and Cyc), Antivin as well as BMPs, and the latter are also expressed ventrally, while Vg1 and Wnt8 are fairly ubiquitous. In the chick, the inducers Vg1 and Wnt8C partially overlap in the primitive streak, while the inhibitors BMP2/4 are expressed peripherally at the edge of the area pellucida and ADMP in the node itself. Few comparable data are available in the mouse, as neither ADMP nor Vg1 have yet been clone*d.

member), *Vg1, activin* and *Wnt8* are all expressed maternally (Helde and Grunwald, 1993; Kelly *et al*., 1995; Dohrmann *et al*., 1996). After the onset of zygotic transcription, Squint along with the nuclear localization of β-catenin become localized to the dorsal yolk syncytial layer (Schneider *et al*., 1996; Erter *et al*., 1998). While a similar region has not yet been identified in the mouse, *nodal* and *Wnt3A* are expressed in the early embryo, and mouse mutants for *nodal* and β-*catenin* are deficient for most mesoderm (Conlon *et al*., 1994; Haegel *et al*., 1995).

The expression of components of both pathways that underlie the activity of the Nieuwkoop center/posterior marginal zone continues into gastrulation, and is consistent with the same pathways playing a role in regulating the position of the organizer during chick gastrulation (Joubin and Stern, 1999) (Fig. 3). *Vg1* and *Wnt8c* are both expressed in the middle of the primitive streak of the chick embryo – the same region that acts as a "node inducing center" upon transplantation (see above). Indeed, grafts of either the middle of the primitive streak or of cells secreting Vg1 and Wnt protein can induce an ectopic organizer. Therefore, the same pathways that mediate Nieuwkoop center activity may account for the continuous allocation of cells to the organizer territory. However, it is important to emphasize that the cells expressing these factors during gastrulation (the middle of the primitive streak) are not direct descendants of those that express them before the primitive streak stage (the posterior marginal zone) (Bachvarova *et al*., 1998; Joubin and Stern, 1999).

Grafts of the middle of the primitive streak or a source of Vg1/Wnt into different positions in a host embryo revealed that organizer-forming potential declines towards the periphery of the embryo and in the vicinity of Hensen's node itself (Joubin and Stern, 1999), suggesting the existence of inhibitors released from these regions which would impose spatial constraints to the response to organizer inducing signals. Molecules that may mediate these inhibitory activities include members of the BMP gene family: *BMP4* and *BMP7*, which are expressed at the edges of the area pellucida, and *ADMP,* which is expressed in the node itself. This working model accounts for the regeneration of a new organizer after removal of the node: after ablation, cells lateral to the node are released from the inhibitory effect of ADMP and are induced to become organizer by Vg1 and Wnt activity. It also raises a paradox of why during normal development cells outside the organizer region are affected by ADMP, whereas the organizer cells themselves which produce ADMP are immune. This is a problem raised with many genetic cascades involving feedback inhibitors expressed at sites of highest signaling activity. Interestingly, *ADMP* is one of the last organizer genes to be expressed by cells receiving organizer inducing signals during regeneration, and its expression peaks during the definitive streak stage when there is maximum organizer activity. It is therefore possible that ADMP production is a property of cells already induced to be organizer, and therefore not as vulnerable to its effects.

These ideas are consistent with mathematical models of organizer function in moving fields of cells, which suggest that "autocatalysis" (self-perpetuating induction of organizer) is sufficiently high in the organizer region to overcome the effects of an inhibitor (Meinhardt, 1993; 1995). This interplay of inducers and inhibitors, which will likely be elaborated in the future to include many more components, provides a mechanism for the somewhat paradoxical finding that both organizer-inducing and -inhibiting signals are present during gastrulation.

Could the same molecular mechanisms that regulate the organizer during gastrulation also ensure the formation of only one primitive streak at earlier stages of development? Although both *Vg1* and *Wnt8c* are expressed in the posterior marginal zone, *ADMP* is not expressed in the pre-streak embryo, and *BMP4* is expressed only very weakly throughout the epiblast (Streit *et al*., 1998). Therefore for the moment it appears that the inducing signals are common to both situations, but different inhibitory molecules may be involved in each.

In zebrafish, it appears that similar pathways may be involved in regulating mesoderm formation during gastrulation. The Nodal family members Cyclops (*Cyc*) and Squint (*Sqt*), have been shown both embryologically and genetically to be essential for the formation of the germ layers during gastrulation (Feldman *et al*., 1998; Rebagliatti *et al*., 1998). *Sqt* and *cyc* are both expressed along the blastoderm margin and later in the shield, and double mutants for *cyc* and *sqt* display gastrulation defects and lack mesodermal and endodermal derivatives (Erter *et al*., 1998; Feldman *et al*., 1998; Regagliatti *et al*., 1998; Sampath *et al*., 1998). The activity of Nodal is regulated through a complex interplay of secreted inhibitors, competence factors, and intracellular processing proteins which impose temporal and spatial constraints on the duration and intensity of the signals. In particular, the TGFβ family member Antivin (also known as Lefty1) appears to restrict Nodal signaling during gastrulation possibly by competing with Nodal for binding to its receptor (Bisgrove *et al*., 1999; Meno *et al*., 1999; Thisse and Thisse, 1999). Interestingly, Antivin is also induced by Cyc and Sqt and thereby acts as a feedback inhibitor, similar to ADMP in the chick. Additionally, BMP2 and BMP4 also are required for dorsoventral patterning of the mesoderm during gastrulation (Kishimoto *et al*., 1997). They are expressed in ventrolateral parts of the

blastoderm margin, and have an additional, somewhat surprising, expression in the dorsal shield itself. ADMP has not been cloned yet in the fish, but it is tempting to speculate that this expression of BMPs in the shield may be performing a similar role to that of ADMP. Is there a region in the fish embryo which would act as a source of organizer inducing signals during gastrulation, comparable to the middle of the primitive streak in the chick? One candidate is the dorsolateral region of the blastoderm margin, which expresses *cyc, sqt, wnt8* (Kelly *et al.*, 1995), and *vg1* (which is ubiquitous in zebrafish; Helde and Grunwald, 1993; Dohrmann *et al.*, 1996) during gastrulation. Based on the results of Oppenheimer's experiments in which she separated blastoderms from the yolk (1934b; 1936a), one might expect that the yolk could also be a source of signals during gastrulation. However, the distribution of these gene products in the yolk during gastrulation has not been documented.

The Nodal-related proteins Cyc and Sqt are also involved in the initial induction of mesoderm, as demonstrated by their mutant phenotypes. *Sqt* is initially expressed in the yolk syncytial layer, and also in the dorsal sector of the blastoderm along with *cyc* (Erter *et al.*, 1998; Feldman *et al.*, 1998; Rebagliatti *et al.*, 1998; Sampath *et al.*, 1998). Injection of *sqt* mRNA specifically into the YSL results in ectopic organizer gene expression and, as expected for a Nieuwkoop center molecule, secondary axes are induced non-cell autonomously by *sqt* overexpressing cells (Erter *et al.*, 1998). Additionally, maternal Activin, Vg1, and Wnt8 are also implicated in mesoderm induction in the fish, and may be part of this regulatory pathway (Helde and Grunwald, 1993; Wittrobt and Rosa, 1994; Kelly *et al.*, 1995; Dohrmann *et al.*, 1996). Surprisingly, BMPs do not appear to have a role in the initial induction and patterning of the mesoderm (Kishimoto *et al.*, 1997), but Antivin has been shown also to inhibit mesoderm inducing signals at these early stages (Thisse and Thisse, 1999).

Genetic evidence from the mouse also implicates the TGFβ pathway in regulating axis development. Interactions between Nodal and Antivin/Lefty family members in germ-layer formation during gastrulation have been demonstrated (Meno *et al.*, 1999). Misexpression of chick Wnt8c can cause axis duplication (Popperl *et al.*, 1997), while mice carrying severe alleles of the *fused* mutation (a locus that encodes Axin, an antagonistic component of the Wnt pathway) develop multiple primitive streaks (Zeng *et al.*, 1997), suggesting that the Wnt pathway plays a role in induction of the primitive streak.

In *Xenopus*, the various TGF-βs (Nodals, Activins, Vg1, BMPs, ADMP) have been implicated both in the initial induction of mesoderm and in its dorsoventral subdivision during gastrulation (Dale *et al.*, 1992; Jones *et al.*, 1992; 1995; 1996; Hemmati-Brivanlou and Melton, 1993; Kessler and Melton, 1995; Moos *et al.*, 1995). Additionally, a family of secreted Wnt inhibitors, called the Fzb's, has been characterized (Leyns *et al.*, 1997; Salic *et al.*, 1997; Wang *et al.*, 1997a; 1997b). Interestingly, Fzb1 is expressed in the organizer and may act as a feedback inhibitor of organizer induction, similar to ADMP. However, the only evidence supporting a requirement for organizer maintenance during gastrulation comes from results in which mutant Vg1 ligands were used to block Vg1 signaling in early *Xenopus* embryos (Joseph and Melton, 1998). In this study, it was found that while dorsal gene expression was intact at the beginning of gastrulation, ultimately there resulted a deficit in dorsal mesoderm derivatives. This suggests that although the initial induction of the organizer can occur in the absence of Vg1, there does appear to be a requirement for continuous Vg1 activity throughout gastrulation as well. This effect of mutant Vg1 ligands is similar to the results of overexpressing BMP4 - in this case, the initial induction of organizer is genes is normal, but their expression is not maintained and quickly downregulated (Jones *et al.*, 1996).

Reasons for regulation

After the initial induction of organizer cells, the cell movements of gastrulation reorganize the blastoderm into the three germ layers. In the chick, gastrulation is marked by the formation and elongation of the primitive streak, the amniote equivalent of the amphibian blastopore. As the streak takes shape, some of the induced organizer cells move with it and end up resident in Hensen's node at its anterior tip. The cells of the posterior marginal zone are excluded from these movements and remain in the posterior extra-embryonic area (Bachvarova *et al.*, 1998). In addition to these movements, cells in the epiblast layer are continuously moving into and out of the primitive streak, including the node, so that the organizer region consists of a continuously changing population of cells, which acquire and lose organizer properties according to their position at a particular time (Joubin and Stern, 1999). This observation raises the question of how organizer activity is maintained during the time that it is required. We believe that the same molecular pathway that mediates node regeneration is a reflection of cell interactions that function during normal development to position the organizer at the tip of the primitive streak during gastrulation. We propose that regeneration is a consequence of the normal maintenance mechanism, made evident by experimental manipulation. Such a mechanism may also ensure that there is a constant pool of non-organizer cells (cells which have not yet entered the node) which can respond to organizer derived signals and become, for example, neural tissue. Moreover, it may also allow for the migration of cells out of the node after receiving their fate instructions, without undermining the organizer itself. According to Meinhardt's model (1995), it is the presence of an inhibitor that "poisons" the organizer region and causes cells to leave the position of the organizer, thereby acting as a key player in coordinating cell movements.

Spratt concluded that the plasticity of the early embryo serves the function of compensating for cellular "excess" in the pre-streak embryo (Spratt and Haas, 1960). Indeed, the unincubated blastoderm consists of about 60,000 cells, of which only about 500 will participate in forming the embryo. This is in sharp contrast with more mosaic animals such as *Xenopus* with much fewer cells in the blastula, most of which appear to be committed to specific fates according to their positions and lineage history. Although the initial establishment of polarity in the chick has been difficult to study since it starts before the egg is laid, it seems unlikely that maternal localization of determinants is involved (Eyal-Giladi, 1997), and sperm entry is even more unlikely to play a role because bird embryos are highly polyspermic. Polarity is probably initiated stochastically, under the influence of gravity (Kochav and Eyal-Giladi, 1971), therefore making it necessary that cells in all regions in the embryo be able to contribute to the body axis. The stabilization of the initial asymmetry may result from amplification of an initially subtle bias across the embryonic field, through a combination of mechanical and chemical inhibition of neighboring regions. These regulatory tenden-

cies may therefore also function to control the delicate balance between cell specification and multipotency.

Acknowledgements

Our research on this topic is currently funded by grants from the National Institutes of Health, USA (R01-GM53456, R01-GM56656 and R01-MH60156) and the Medical Research Council (U.K.).

References

ABERCROMBIE, M. (1950). The effects of antero-posterior reversal of lengths of the primitive streak in the chick. *Phil. Trans. Roy. Soc. B.* 234: 317-338.

ABERCROMBIE, M. and BELLAIRS, R. (1954). The effects in chick blastoderms of replacing the primitive node by a graft of posterior primitive streak. *J. Embryol. Exp. Morph.* 2: 55-72.

AGIUS, E., OELGESCHLAGER, M., WESSELY, O., KEMP, C. and DE ROBERTIS, E.M. (2000). Endodermal Nodal-related signals and mesoderm induction in *Xenopus. Development* 127: 1173-1183.

AZAR, Y. and EYAL-GILADI, H. (1981). Interaction of epiblast and hypoblast in the formation of the primitive streak and the embryonic axis in chick, as revealed by hypoblast rotation experiments. *J. Embryol. Exp. Morph.* 52: 79-88,

BACHVAROVA, R.F., SKROMNE, I. and STERN, C.D. (1998). Induction of primitive streak and Hensen's node by the posterior marginal zone in the early chick embryo. *Development* 125: 3521-3534.

BAUTZMANN, H. (1933). Ueber determinationsgrad und wirkungs-beziehungen der Randzonenteilanlagen (Chorda, Ursegmente, Seitenplatten und Kopfdarmanlage) bei Urodelen und Anuren. *W. Roux' Archiv. EntwMech. Org.* 128: 665-765.

BEDDINGTON, R.S. (1994). Induction of a second neural axis by the mouse node. *Development* 120: 613-620.

BISGROVE, B.W., ESSNER, J.J. and YOST, H.J. (1999). Regulation of midline development by antagonism of *lefty* and *nodal* signaling. *Development* 126: 3253-3262.

BLUM, M., GAUNT, S.J., CHO, K.W.Y., STEINBEISSER, H., BLUMBERG, B., BITTNER, D. and DE ROBERTIS, E.M. (1992). Gastrulation in the mouse: the role of the homeobox gene *goosecoid. Cell* 69: 1097-1106.

BOTERENBROOD, E.C. and NIEUWKOOP, P.D. (1973). The formation of the mesoderm in urodelean amphibians. V. Its regional induction by the endoderm. *W. Roux' Archiv. EntwMech. Org.* 173: 319-332.

BRUMMET, A.R. (1968a). Deletion-transplantation experiments on embryos of Fundulus heteroclitus I. The posterior embryonic shield. *J. Exp. Zool.* 169: 315-334.

BRUMMET, A.R. (1968b). Deletion-transplantation experiments on embryos of Fundulus heteroclitus II. The anterior embryonic shield. *J. Exp. Zool.* 172: 443-464.

BUTROS, J. (1967) Limited axial structures in nodeless chick blastoderms. *J. Embryol. Exp. Morph.* 17: 119-130.

CALLEBAUT, M., VAN NUETEN, E., HARRISON, F., VAN NASSAUW, L. and SCHREVENS, A. (1998a). Induction of (pre) gastrulation and/or (pre) neurulation by subgerminal ooplasm and rauber's sicle in cultured anti-sicle regions of avian unincubated blastoderms. *Eur. J. Morph.* 36: 1-10.

CALLEBAUT, M., VAN NEUTEN, E., VAN NASSAUW, L., BORTIER, H. and HARRISSON, F. (1998b). Only the endophyll-Rauber's sickle complex and not cells derived from the caudal marginal zone induce a primitive streak in the upper layer of avian blastoderms. *Reprod. Nutr. Dev.* 38: 449-463.

CLEMENTS, D., FRIDAY, R.V. and WOODLAND, H.R. (1999). Mode of action of VegT in mesoderm and endoderm formation. *Development* 126: 4903-4911.

CONLON, F.L., LYONS, K.M., TAKAESU, N., BARTH, K.S., KISPERT, A.K., HERRMANN, B. and ROBERTSON, E.J. (1994). A primary requirement of *nodal* in the formation and maintenance of the primitive streak in mouse. *Development* 120: 1919-1928.

COOKE, J. (1973). Properties of the primary organization field in the embryo of *Xenopus laevis* V. Regulation after removal of the head organizer, in normal early gastrulae and in those already possessing a second implanted organizer. *J. Embryol. Exp. Morph.* 30: 283-300.

COOKE, J. (1975). Local autonomy of gastrulation movements after dorsal lip removal in two anuran amphibians. *J. Embryol. Exp. Morph.* 33: 147-157.

COOKE, J. (1985). Dynamics of the control of body pattern in the development of *Xenopus laevis* III. Timing and pattern after u.v. irradiation of the egg and after excision of presumptive head endo-mesoderm. *J. Embryol. Exp. Morph.* 88: 135-149.

COOKE, J. and WEBBER, J.A. (1985a). Dynamics of the control of body pattern in the development of *Xenopus laevis* I. Timing and pattern in the development of dorsoanterior and posterior blastomere pairs, isolated at the 4- cell stage. *J. Embryol. Exp. Morph.* 88: 85-112.

COOKE, J. and WEBBER, J.A. (1985b). Dynamics of the control of body pattern in the development of *Xenopus laevis* II. Timing and pattern in the development of single blastomeres (presumptive lateral halves) isolated at the 2-cell stage. *J. Embryol. Exp. Morph.* 88: 113-133.

CREASE, D.J., DYSON, S. and GURDON, J.B. (1998). Cooperation between the activin and Wnt pathways in the spatial control of organizer gene expression. *Proc. Natl. Acad. Sci. U.S.A.* 95: 4398-4403.

CUI, Y., TIAN, Q. and CHRISTIAN, J.L. (1996). Synergistic effects of Vg1 and Wnt signals in the specification of dorsal mesoderm and endoderm. *Dev. Biol.* 180: 22-34.

DALE, L., HOWES, G., PRICE, B.M. and SMITH, J.C. (1992). Bone morphogenetic protein 4: A ventralizing factor in early Xenopus development. *Development* 115: 573-585.

DALE, L., MATTHEWS, G., TABE, L. and COLMAN, A. (1989). Developmental expression of the protein product of Vg1, a localized maternal mRNA in the frog *Xenopus laevis. EMBO J.* 8: 1057-1065.

DAVIDSON, B.P., KINDER, S.J., STEINER, K., SCHOENWOLF, G.C. and TAM, P.P.L. (1999). Impact of node ablation on the morphogenesis of the body axis and the lateral asymmetry of the mouse embryo during early organogenesis. *Development* 211: 11-26.

DEVILLERS, C. (1951). Symetrisation et régulation du germ chez la truite. *C. R. Assoc. Anat.* 38: 418-424.

DIAS, M.S. and SCHOENWOLF, G.C. (1990). Formation of ectopic neuroepithelium in chick blastoderms: age-related capacities for induction and self-differentiation following transplantation of quail Hensen's nodes. *Anat. Rec.* 228: 437-448.

DOHRMANN, C.E., KESSLER, D.S. and MELTON, D.A. (1996). Induction of axial mesoderm by zDVR-1, the zebrafish orthologue of Xenopus Vg1. *Dev. Biol.* 175: 108-117.

DOMINGO, C. and KELLER, R. (1995). Induction of notochord cell intercalation behavior and differentiation by progressive signals in the gastrula of *Xenopus laevis. Development* 121: 3311-3321.

ERTER, C.E., SOLNICA-KREZEL, L. and WRIGHT, C.V.E. (1998). Zebrafish nodal-related 2 encodes an early mesendodermal inducer signaling from the extraembryonic yolk syncytial layer. *Dev. Biol.* 204: 361-372.

EYAL-GILADI, H. (1997). Establishment of the axis in chordates: pure speculations. *Development* 124: 2285-2296.

EYAL-GILADI, H. and KHANER, O. (1989). The chick's marginal zone and primitive streak formation II. Quantification of the marginal zone's potencies - temporal and spatial aspects. *Dev. Biol.* 134: 215-221.

FELDMAN, B., GATES, M.A., EGAN, E.S., DOUGAN, S.T., RENNEBECK, G., SIROTKIN, H.I., SCHIER, A.F. and TALBOT, W.S. (1998). Zebrafish organizer development and germ-layer formation require nodal-related signals. *Nature* 395: 181-185.

FOLEY, A.C., SKROMNE, I. and STERN, C.D. (2000). Reconciling different models of forebrain induction and patterning: a dual role for the hypoblast. *Development* 127: 3839-3854.

GALLERA, J. and IVANOV, I. (1964). La competence neurogène du feuillet externe du blastoderme de poulet en fonction du facteur "temps*". J. Embryol. Exp. Morph.* 12: 693-711.

GALLERA, J. and NICOLET, G. (1969). Le pouvoir inducteur de l'endoblaste presomptif contenu dans la ligne primitive jeune de Poulet. *J. Embryol. Exp. Morph.* 21: 105-118.

GALLERA, J. and NICOLET, G. (1974). Regulation in nodeless chick blastoderms. *Experientia* 30: 183-185.

GIMLICH, R.L. and GERHART, J.C. (1984). Early cellular interactions promote embryonic axis formation in *Xenopus laevis. Dev. Biol.* 104: 117-130.

GRABOWSKI, C.T. (1956). The effects of the excision of Hensen's node on the early development of the chick embryo. *J. Exp. Zool.* 133: 301-344.

HAEGEL, H., LARUE, L., OHSUGI, M., FEDOROV, L., HERRENKNECHT, K. and KEMLER, R. (1995). Lack of β-catenin affects mouse development at gastrulation. *Development* 121: 3529-3537.

HATADA, Y. and STERN, C.D. (1994) A fate map of the epiblast of the early chick embryo. *Development* 120: 2879-2890.

HATTA, K. and TAKAHASHI, Y. (1996). Secondary axis induction by heterospecific organizers in zebrafish. *Dev. Dyn.* 205: 183-195.

HELDE, K.A. and GRUNWALD, D.J. (1993). The DVR-1 (Vg1) transcript of zebrafish is maternally supplied and distributed throughout the embryo. *Dev. Biol.* 159: 418-426.

HEMMATI-BRIVANLOU, A. and MELTON, D.A. (1992). A truncated activin receptor inhibits mesoderm induction and formation of axial structures in Xenopus embryos. *Nature* 359: 609-614.

HO, R.K. and KIMMEL, C.B. (1993). Commitment of cell fate in the early zebrafish embryo. *Science* 261: 109-111.

HOADLEY, L. (1928). On the localization of developmental potencies in the embryo of Fundulus heteroclitus. *J. Exp. Zool.* 52: 7-44.

HOLFRETER, J. (1934). Der Einfluss thermischer, mechanischer und chemischer Eingriffe auf die Induktionfaehigkeit von *Triton*-Keimteil. *W. Roux' Arch. EntwMech. Org.* 132: 307-383.

HOLTFRETER, J. (1936). Regionale Induktionen in xenoplastisch zusammengesetzten Explantaten. *W. Roux' Arch. EntwMech. Org.* 134: 466-561.

HUME, C.R. and DODD, J. (1993). Cwnt-8C: a novel Wnt gene with a potential role in primitive streak formation and hindbrain organization. *Development* 119: 1147-1160.

IZPISUA-BELMONTE, J.C., DE ROBERTIS, E.M., STOREY, K. and STERN, C.D. (1993). The homeobox gene goosecoid and the origin of organizer cells in the early chick blastoderm. *Cell* 74: 645-659.

JONES, C.M., DALE, L., HOGAN, B.L., WRIGHT, C.V. and SMITH, J.C. (1996). Bone morphogenetic protein-4 (BMP-4) acts during gastrula stages to cause ventralization of Xenopus embryos. *Development* 122: 1545-1554.

JONES, C.M., KUEHN, M.R., HOGAN, B.L.M., SMITH, J.C. and WRIGHT, C.V.E. (1995). Nodal-related signals induce axial mesoderm and dorsalize mesoderm during gastrulation. *Development* 121: 3651-3662.

JONES, C.M., LYONS, K.M., LAPAN, P.M., WRIGHT, C.V. and HOGAN, B.L. (1992). DVR-4 (bone morphogenetic protein-4) as a posterior-ventralizing factor in Xenopus mesoderm induction. *Development* 115: 639-647.

JONES, E.A. and WOODLAND, H.R. (1987). The development of animal cap cells in *Xenopus*: a measure of the start of animal cap competence to form mesoderm. *Development* 101: 557-563.

JOSEPH, E.M. and MELTON, D.A. (1998). Mutant Vg1 ligands disrupt endoderm and mesoderm formation in Xenopus embryos. *Development* 125: 2677-2685.

JOUBIN, K. and STERN, C.D. (1999). Molecular interactions continuously define the organizer during the cell movements of gastrulation. *Cell* 98: 559-571.

KELLY, G.M., GREENSTEIN, P., EREZYILMAZ, D.F. and MOON, R.T. (1995). Zebrafish *wnt8* and *wnt8b* share a common activity but are involved in distinct developmental pathways. *Development* 121: 1787-1799.

KESSLER, D.S. and MELTON, D.A. (1995). Induction of dorsal mesoderm by soluble, mature Vg1 protein. *Development* 121: 2155-2164.

KHANER, O. (1995). The rotated hypoblast of the chicken embryo does not initiate an ectopic axis in the epiblast. *Proc. Natl. Acad. Sci. U.S.A.* 92: 10733-10737.

KHANER, O. (1998). The ability to initiate an axis in the avian blastula is concentrated mainly at a posterior site. *Dev. Biol.* 194: 257-266.

KHANER, O. and EYAL-GILADI, H. (1986). The embryo-forming potency of the posterior marginal zone in stages X through XII of the chick. *Dev. Biol.* 115: 275-281.

KHANER, O. and EYAL-GILADI, H. (1989). The chick's marginal zone and primitive streak formation I. Coordinative effect of induction and inhibition. *Dev. Biol.* 134: 206-214.

KHANER, O., MITRAI, E. and EYAL-GILADI, H. (1985). Developmental potencies of area opaca and marginal zone areas of early chick blastoderms. *J. Embryol. Exp. Morph.* 89: 235-241.

KIMMEL, C.B. and LAW, R.D. (1985). Cell lineage of zebrafish blastomeres III. Clonal analysis of the blastula and gastrula stages. *Dev. Biol.* 108: 94-101.

KINTNER, C.R. and DODD, J. (1991). Hensen's node induces neural tissue in Xenopus ectoderm. Implications for the action of the organizer in neural induction. *Development* 113: 1495-1505.

KISHIMOTO, Y., LEE, K., ZON, L., HAMMERSCHMIDT, M. and SCHULTE-MERKER, S. (1997). The molecular nature of zebrafish *swirl*: BMP2 function is essential during early dorsoventral patterning. *Development* 124: 4457-4466.

KOCHAV, S. and EYAL-GILADI, H. (1971). Bilateral symmetry in chick embryo determination by gravity. *Science* 171: 1027-1029.

KOFRON, M., DEMEL, T., XANTHOS, J., LOHR, J., SUN, B., SIVE, H., OSADA, S., WRIGHT, C., WYLIE, C. and HEASMAN, J. (1999). Mesoderm induction in *Xenopus* is a zygotic even regulated by maternal VegT via TGFβ growth factors. *Development* 126: 5759-5770.

LEWIS, W.H. (1907). Transplantation of the lips of the blastopore in *Rana palustris. Am. J. Anat.* 7: 137-143.

LEWIS, W.H. (1912a). Experiments on localization in the eggs of a teleost fish (Fundulus heteroclitus). *Anat. Rec.* 6: 1-6.

LEWIS, W.H. (1912b). Experiments on localization and regeneration in the embryonic shield and germ ring of a teleost fish (Fundulus heteroclitus). *Anat. Rec.* 6: 325-333.

LEYNS, L., BOUWMEESTER, T., KIM, S.H., PICCOLO, S. and DE ROBERTIS, E.M. (1997) Frzb-1 is a secreted antagonist of Wnt signaling expressed in the Spemann organizer. *Cell* 88: 747-756.

LONG, W.L. (1983). The role of the yolk syncytial layer in determination of the plane of bilateral symmetry in the rainbow trout, *Salmo gairdneri. J. Exp. Zool.* 229: 91-97.

LUTHER, W. (1936). Potenzprüfungen an isolierten Teilstuecken der Forellenkeimscheibe. *W. Roux' Arch. EntwMech. Org.* 135: 359-383.

LUTHER, W. (1937). Transplantations- und Defektversuche am Organisationszentrum der Forellenkeimscheibe. *W. Roux' Arch. EntwMech. Org.* 137: 404-424.

LUTZ, H. (1948). Sur l'obtention experimentale de la polyembryonie chez le Canard. *C. R. Soc. Biol. Strasbourg* 142: 384-385

MEINHARDT, H. (1993). A model for pattern formation of hypostome, tentacles, and foot in hydra: how to form structures close to each other, how to form them at a distance. *Dev. Biol.* 157: 321-333.

MEINHARDT, H. (1995). The algorithmic beauty of sea shells. Berlin: Springer.

MENO, C., GRITSMAN, K., OHISHI, S., OHFUJI, Y., HECKSCHER, E., MOCHIDA, K., SHIMONO, A., KONDOH, H., TALBOT, W.S., ROBERTSON, E.J., SCHIER, A.F. and HAMADA, H. (1999). Mouse Lefty2 and zebrafish Antivin are feedback inhibitors of Nodal signaling during vertebrate gastrulation. *Molec. Cell* 4: 287-298.

MIZUNO, T., YAMAHA, E., WAKAHARA, M., KOROIWA, A. and TAKEDA, H. (1996). Mesoderm induction in zebrafish. *Nature* 383: 131-132.

MOOS, M. JR., WANG, S. and KRINKS, M. (1995) Anti-dorsalizing morphogenetic protein is a novel TGF-β homolog expressed in the Spemann organizer. *Development* 121: 4293-4301.

MORGAN, T.H. (1895). The formation of the fish embryo. *J. Morph.* 10: 419-465.

NEEDHAM, J., WADDINGTON, C.H. and NEEDHAM, D.M. (1934). Experiments on the amphibian organizer. *Proc. Roy. Soc. B.* 114: 393-342.

NIEUWKOOP, P.D. (1969a). The formation of the mesoderm in urodelean amphibians. I. Induction by the endoderm. *W. Roux' Archiv. EntwMech. Org.* 162: 341-373.

NIEUWKOOP, P.D. (1969b). The formation of the mesoderm in urodelean amphibians. II. The origin of the dorso-ventral polarity of the mesoderm. *W. Roux' Archiv. EntwMech. Org.* 163: 298-315.

NISHITA, M., HASHIMOTO, M.K., OGATA, S., LAURENT, M.N., UENO, N., SHIBUYA, H. and CHO, K.W. (2000). Interaction between Wnt and TGF-beta signalling pathways during formation of Spemann's organizer. *Nature* 403: 781-785.

OPPENHEIMER, J.M. (1934a). Experimental studies on the developing perch (Perca flavescens Mitchill). *Proc. Soc. Exp. Biol. Med.* 31: 1123-1124.

OPPENHEIMER, J.M. (1934b). Experiments on early developing stages of Fundulus. *Proc. Natl. Acad. Sci. U.S.A.* 20: 536-538.

OPPENHEIMER, J.M. (1936a). Transplantation experiments on developing teleosts (Fundulus and Perca). *J. Exp. Zool.* 172: 409-437.

OPPENHEIMER, J.M. (1936b). Structures developed in amphibians by implantation of living fish organizer. *Proc. Soc. Exp. Biol. Med.* 34: 461-463.

OPPENHEIMER, J.M. (1936c). The development of isolated blastoderms of Fundulus heteroclitus. *J. Exp. Zool.* 72: 247-269.

POPPERL, H., SCHMIDT, C., WILSON, V., HUME, C.R., DODD, J., KRUMLAUF, R. and BEDDINGTON, R.S. (1997). Misexpression of Cwnt8C in the mouse induces

an ectopic embryonic axis and causes a truncation of the anterior neuroectoderm. *Development* 124: 2997-3005.

PSYCHOYOS, D. and STERN, C.D. (1996) Restoration of the organizer after radical ablation of Hensen's node and the anterior primitive streak in the chick embryo. *Development* 122: 3263-3273.

REBAGLIATI, M.R., TOYAMA, R., HAFFTER, P. and DAWID, I.B. (1998). *Cyclops* encodes a nodal-related factor involved in midline signaling. *Proc. Natl. Acad. Sci. U.S.A.* 95: 9932-9937.

ROESSER, T., STEIN, S. and KESSEL, M. (1999). Nuclear β-catenin and the development of bilateral symmetry in normal and LiCl-exposed chick embryos. *Development* 126: 2955-2965.

ROOSEN-RUNGE, E.C. (1938). On the early development - bipolar differentiation and cleavage - of the zebrafish, Brachydanio rerio. *Biol. Bull.* 75: 119-133.

SALIC, A.N., KROLL, K.L., EVANS, L.M. and KIRSCHNER, M.W. (1997). Sizzled: a secreted Xwnt8 antagonist expressed in the ventral marginal zone of Xenopus embryos. *Development* 124: 4739-4748.

SAMPATH, K., RUBINSTEIN, A.L., CHENG, A.M.S., LIANG, J.O., FEKANY, K., SOLNICA-KREZEL, L., KORZH, V., HALPERN, M.E. and WRIGHT, C.V.E. (1998). Induction of the zebrafish ventral brain and floorplate requires cyclops/nodal signalling. *Nature* 395: 185-189.

SCHNEIDER, S., STEINBEISSER, H., WARGA, R.M. and HAUSEN, P. (1996). β-catenin translocation into nuclei demarcates the dorsalizing centers in frog and fish embryos. *Mech. Dev.* 57: 191-198.

SELEIRO, E.A., CONNOLLY, D.J. and COOKE, J. (1996). Early developmental expression and experimental axis determination by the chicken Vg1 gene. *Curr. Biol.* 6: 1476-1486.

SHAH, S.B., SKROMNE, I., HUME, C.R., KESSLER, D.S., LEE, K.J., STERN, C.D. and DODD, J. (1997). Misexpression of chick Vg1 in the marginal zone induces primitive streak formation. *Development* 124: 5127-5138.

SHIH, J. and FRASER, S.E. (1996). Characterizing the zebrafish organizer: microsurgical analysis at the early-shield stage. *Development* 122: 1313-1322.

SPEMANN, H. (1901). Ueber correlationen in der Entwicklung des Auges. *Verhandl. Anat. Gesellsch.* 15: 61-79.

SPEMANN, H. (1905). Ueber Linsenbildung nach experimenteller Entfernung der primären Linsenbildungszellen. *Zool. Anz.* 3: 419-432.

SPEMANN, H. (1918). Ueber die Determination der ersten Organanlagen des Amphibienembryo. *W. Roux' Arch. EntwMech. Org.* 43: 448-555.

SPEMANN, H. and MANGOLD, H. (1924). Ueber Induktion von Embryoalanlagen dürch Implantation artfremder Organisatoren. *W. Roux' Arch. EntwMech. Org.* 100: 599-638.

SPRATT, N.T. and HAAS, H. (1960). Integrative mechanisms in development of the early chick blastoderm. I. Regulative potentiality of separated parts. *J. Exp. Zool.* 145: 97-137.

SPRATT, N.T. and HAAS, H. (1961a). Integrative mechanisms in development of the early chick blastoderm. II. Role of morphogenetic movements and regenerative growth in synthetic and topographically disarranged blastoderms. *J. Exp. Zool.* 147: 57-93.

SPRATT, N.T. and HAAS, H. (1961b). Integrative mechanisms in development of the early chick blastoderm. III. Role of cell population size and growth potentiality in synthetic systems larger than normal. *J. Exp. Zool.* 47: 271-294.

STERN, C.D. (1990). The marginal zone and its contribution to the hypoblast and primitive streak of the chick embryo. *Development* 109: 667-682.

STOREY, K.G., CROSSLEY, J.M., DE ROBERTIS, E.M., NORRIS, W.E. and STERN, C.D. (1992). Neural induction and regionalization in the chick embryo. *Development* 114: 729-741.

STREIT, A., BERLINER, A.J., PAPANAYOTOU, C., SIRULNIK, A. and STERN, C.D. (2000). Initiation of neural induction by FGF signalling before gastrulation. *Nature* 406: 74-78.

STREIT, A., LEE, K.J., WOO, I., ROBERTS, C., JESSELL, T.M. and STERN, C.D. (1998). Chordin regulates primitive streak development and the stability of induced neural cells, but is not sufficient for neural induction in the chick embryo. *Development* 125: 507-519.

SUMNER, F.B. (1904). A study of early fish development. Experimental and morphological. *W. Roux' Arch. EntwMech. Org.* 17: 92-149.

SUN, B.I., BUSH, S.M., COLLINS-RACIE, L.A., LA VALLIE, E.R., DIBLASIO-SMITH, E.A., WOLFMAN, N.M., McCOY, J.M. and SIVE, H.L. (1999). *Derriere:* a TGF-beta family member required for posterior development in *Xenopus. Development* 126: 1467-1482.

THISSE, C. and THISSE, B. (1999). Antivin, a novel and divergent member of the TGFβ superfamily, negatively regulates mesoderm induction. *Development* 126: 229-240.

VAKAET, L. (1964). Diversité fonctionelle de la ligne primitive du blastoderme de poulet. *C. R. Soc. Biol.* 158: 1964-1966.

VAKAET, L. (1965). Resultats de la greffe de noeud de Hensen d'age différent sur le blastoderme de poulet. *C.R. Soc. Biol.* 159: 232-233.

VINCENT, J. and GERHART, J.C. (1987). Subcortical rotation in *Xenopus* eggs: an early step in embryonic axis specification. *Dev. Biol.* 123: 526-539.

WADDINGTON, C.H. (1930). Developmental mechanics of chick and duck embryos. *Nature* 125: 924-925.

WADDINGTON, C.H. (1932) Experiments on the development of chick and duck embryos, cultivated in vitro. *Phil. Trans. Roy. Soc. B.* 221: 179-230.

WADDINGTON, C.H. (1933a). Induction by the primitive streak and its derivatives in the chick. *J. Exp. Biol.* 10: 38-46.

WADDINGTON, C.H. (1933b). Induction by the endoderm in birds. *W. Roux' Arch. EntwMech. Org.* 128: 502-521.

WADDINGTON, C.H. (1934). Experiments on embryonic induction. *J. Exp. Biol.* 11: 211-227.

WADDINGTON, C.H. (1936). Organizers in mammalian development. *Nature* 138: 125.

WADDINGTON, C.H. (1937). Experiments on determination in the rabbit embryo. *Arch. Biol.* 48: 273-290.

WADDINGTON, C.H. and SCHMIDT, G.A. (1933). Induction by heteroplastic grafts of the primitive streak in birds. *W. Roux' Arch. EntwMech. Org.* 128: 522-563.

WADDINGTON, C.H. and TAYLOR, J. (1937). Conversion of presumptive ectoderm to mesoderm in the chick. *J. Exp. Biol.* 14: 335-339.

WANG, S., KRINKS, M., LIN, K., LUYTEN, F.P. and MOOS, M. JR. (1997a). Frzb, a secreted protein expressed in the Spemann organizer, binds and inhibits Wnt-8. *Cell* 88: 757-766.

WANG, S., KRINKS, M. and MOOS, M. JR. (1997b). Frzb-1, an antagonist of Wnt-1 and Wnt-8, does not block signaling by Wnts -3A, -5A, or -11. *Biochem. Biophys. Res. Commun.* 236: 502-504.

WATABE, T., KIM, S., CANDIA, A., ROTHBACHER, U., HASHIMOTO, C., INOUE, K. and CHO, K.W. (1995). Molecular mechanisms of Spemann organizer formation: conserved growth factor synergy between Xenopus and mouse. *Genes Dev.* 9: 3038-3050.

WITTBRODT, J. and ROSA, F.M. (1994). Disruption of mesoderm and axis formation in fish by ectopic expression of activin variants: the role of maternal activin. *Genes Dev.* 8: 1448-1462.

YUAN, S. and SCHOENWOLF, G.C. (1998). De novo induction of the organizer and formation of the primitive streak in an experimental model of notochord reconstitution in avian embryos. *Development* 125: 201-213.

YUAN, S.G., DARNELL, D.K. and SCHOENWOLF, G.C. (1995a) Mesodermal patterning during avian gastrulation and neurulation: experimental induction of notochord from non-notochordal precursor cells. *Dev. Genet.* 17: 38-54.

YUAN, S.G., DARNELL, D.K. and SCHOENWOLF, G.C. (1995b) Identification of inducing, responding and suppressing regions in an experimental model of notochord formation in avian embryos. *Dev. Biol.* 172: 567-584.

ZENG, L., FAGOTTO, F., ZHANG, T., HSU, W., VASICEK, T.J., PERRY, W.L., LEE, J.J., TILGHMAN, S.M., GUMBINER, B.M. and CONSTANTINI, F. (1997). The mouse Fused locus encodes Axin, an inhibitor of the Wnt signaling pathway that regulates embryonic axis formation. *Cell* 94: 515-524.

ZHANG, J., HOUSTON, D.W., KING, M.L., PAYNE, C., WYLIE, C. and HEASMAN, J. (1998). The role of maternal VegT in establishing the primary germ layers in *Xenopus* embryos. *Cell* 94: 515-524.

ZHU, L., BELO, J.A., DE ROBERTIS, E.M. and STERN, C.D. (1999). Goosecoid regulates the neural inducing strength of the mouse node. *Dev. Biol.* 216: 276-281.

ZORN, A.M., BUTLER, K. and GURDON, J.B. (1999). Anterior endomesoderm specification in Xenopus by Wnt/β-catenin and TGF-β signalling pathways. *Dev. Biol.* 209: 282-297.