J. Anat. (2001) **199**, pp. 35–52, with 4 figures Printed in the United Kingdom

Evolution of vertebrate forebrain development: how many different mechanisms?

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(*Accepted 6 April 2001*)

ABSTRACT

Over the past 50 years and more, many models have been proposed to explain how the nervous system is initially induced and how it becomes subdivided into gross regions such as forebrain, midbrain, hindbrain and spinal cord. Among these models is the 2-signal model of Nieuwkoop & Nigtevecht (1954), who suggested that an initial signal ('activation') from the organiser both neuralises and specifies the forebrain, while later signals ('transformation') from the same region progressively caudalise portions of this initial territory. An opposing idea emerged from the work of Otto Mangold (1933) and other members of the Spemann laboratory: 2 or more distinct organisers, emitting different signals, were proposed to be responsible for inducing the head, trunk and tail regions. Since then, evidence has accumulated that supports one or the other model, but it has been very difficult to distinguish between them. Recently, a considerable body of work from mouse embryos has been interpreted as favouring the latter model, and as suggesting that a 'head organiser', required for the induction of the forebrain, is spatially separate from the classic organiser (Hensen's node). An extraembryonic tissue, the 'anterior visceral endoderm' (AVE), was proposed to be the source of forebrain-inducing signals. It is difficult to find tissues that are directly equivalent embryologically or functionally to the AVE in other vertebrates, which led some (e.g. Kessel, 1998) to propose that mammals have evolved a new way of patterning the head. We will present evidence from the chick embryo showing that the hypoblast is embryologically and functionally equivalent to the mouse AVE. Like the latter, the hypoblast also plays a role in head development. However, it does not act like a true organiser. It induces pre-neural and pre-forebrain markers, but only transiently. Further development of neural and forebrain phenotypes requires additional signals not provided by the hypoblast. In addition, the hypoblast plays a role in directing cell movements in the adjacent epiblast. These movements distance the future forebrain territory from the developing organiser (Hensen's node), and we suggest that this is a mechanism to protect the forebrain from caudalising signals from the node. These mechanisms are consistent with all the findings obtained from the mouse to date. We conclude that the mechanisms responsible for setting up the forebrain and more caudal regions of the nervous system are probably similar among different classes of higher vertebrates. Moreover, while reconciling the two main models, our findings provide stronger support for Nieuwkoop's ideas than for the concept of multiple organisers, each inducing a distinct region of the CNS.

Key words: Neural induction; regionalisation; forebrain; head organiser; Hensen's node; hypoblast; anterior visceral endoderm.

INTRODUCTION

One of the most striking changes in body plan to arise during the evolution of chordates is the gradual elaboration of an increasingly complex forebrain. Gans & Northcutt (1983) viewed this as an important evolutionary innovation and coined the concept of the 'New Head', to account for the appearance of both an elaborate forebrain and of neural crest and placodal tissues. For developmental biologists, a long-standing challenge has been the search for the cellular and molecular mechanisms that establish this increasingly complex forebrain. One recent view (Knoetgen et al. 1999*a*, *b*; de Souza & Niehrs, 2000)

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holds that mammals have invented a new mechanism for specifying the forebrain, where signals emanate from a tissue unique to the mammalian class, the anterior visceral endoderm (AVE). Here we examine this issue by reviewing some of the earliest steps in neural patterning, starting with an historical overview of different models that have been proposed to explain this process, and end by proposing a new model for forebrain development that fits the currently available data.

MODELS FOR ROSTROCAUDAL PATTERNING OF THE VERTEBRATE NERVOUS SYSTEM

Over the years a number of different theories have been put forward to explain how the vertebrate nervous system becomes subdivided into major regions (forebrain, midbrain, hindbrain, etc.). These models fall roughly into 2 groups depending on whether they emphasise qualitative or quantitative mechanisms (Saxén & Toivonen, 1962). *Qualitative models* propose that regional identities in the nervous system result primarily from regional differences in the signals emanating from the underlying mesoderm. The *quantitative models* postulate that patterning occurs primarily through the interaction of a small number (2 or 3) of graded 'morphogenetic signals' that specify regional identity in a combinatorial way. 'Graded signals' may be viewed either as literal *molecular gradients*, inducing different identities depending on local concentrations of morphogens (Wolpert, 1969; Crick, 1970), or as *temporal gradients* whereby identity depends on how long cells are exposed to a given signal.

Qualitative models

Spemann's notion of separate organisers. In 1924, ground-breaking work by Spemann and Mangold showed that when the dorsal lip of the blastopore of an amphibian embryo is grafted to the ventral side of a host embryo, a second neural axis forms (Spemann & Mangold, 1924). Importantly, the grafted dorsal lip contributes primarily to notochord and somites of the new axis, while the ectopic nervous system is derived almost exclusively from host cells. This demonstrated that signals from the dorsal lip could induce a change of fate in the host ectoderm, from prospective ventral epidermis to nervous system. Moreover, the induced nervous system is coherently organised, with clear rostrocaudal and dorsoventral pattern. Because of its unique ability to induce such a patterned array of structures from tissue not fated to do so, the dorsal lip of the blastopore became known as 'the organiser' (Spemann & Mangold, 1924). Similar organising centres have been found in most vertebrate classes, whose signals can induce nervous system even in heterospecific combinations (e.g. Waddington, 1930, 1932, 1933*a*, 1937, 1940; Kintner & Dodd, 1991; Blum et al. 1992; Beddington, 1994; Hatta & Takahashi, 1996; Zhu et al. 1999; Knoetgen et al. 2000).

Subsequent work has shown that the axial mesoderm that migrates out of the dorsal lip in amphibians, and which comes to underlie the presumptive neural plate, can also induce neural tissue (e.g. Mangold, 1929; Holtfreter, 1933*a*, *b*, 1936; Doniach 1992, 1993; Ruiz i Altaba, 1993; Lemaire & Kessel, 1997), as does the head process mesoderm of avian embryos (Izpisúa-Belmonte et al. 1993; Rowan et al. 1999). These findings have led to the general assumption that neural induction occurs largely via vertical signalling between the mesoderm and the overlying ectoderm.

Work by Spemann and his colleagues compared the inducing ability of the dorsal lip from early gastrulae to that of the dorsal lip from late gastrulae. They found that the young dorsal lip induces secondary axes possessing a full range of regional identities, whereas grafts of older dorsal lip can only induce posterior structures. This led them to suggest that the mesoderm might be divided into different regions, each with a unique ability to induce a specific part of the nervous system. In this model, the first mesoderm to involute during gastrulation (the prechordal mesoderm) would act as a head inducer. Trunk and tail structures would be induced by the later involuting, more posterior mesoderm (Spemann, 1931, 1938; Mangold, 1933; Holtfreter, 1936, 1938). Similar studies in avian embryos concur with these findings. Grafts of young Hensen's node induce a full rostrocaudal range of markers in the epiblast of host embryos, but the ability of the node to induce anterior parts of the nervous system is lost from the node as soon as the prechordal mesoderm begins to migrate out (Dias & Schoenwolf, 1990; Storey et al. 1992).

Later work, chiefly by Toivonen (1938, 1940) led to the more extreme view that there may be separate head and trunk inducers. He studied the effects of many different heterologous inducers and noticed that most of these induced either trunk or tail but never both. These data led the author to suggest that different regions of the nervous system are induced by chemically different substances. This raises the inevitable question: how many distinct inducers are required to generate the entire nervous system?

The 'head organiser' hypothesis. Recent evidence, particularly from the mouse, has renewed interest in

the idea of 2 separate organisers for the head and for the rest of the axis. Mice possessing a homozygous deletion of the homeodomain-containing-gene *Lim1* possess normal trunks and tails but lack head structures (Shawlot & Behringer, 1995). It was later found that injection of *Xenopus* blastomeres with RNA encoding a constitutively active form of *Xlim-1* can induce head structures in animal caps (Taira et al. 1997). These findings led to the view that tissues expressing *Lim-1* might correspond to the head organiser in vertebrates (Shawlot & Behringer, 1995; Taira et al. 1997). In keeping with the earlier work of Spemann, the authors suggested that this head organiser might reside in the prechordal mesoderm. However, the subsequent revelation that *Lim-1* is also expressed in the visceral endoderm (VE) of mice, and in the dorsal endoderm of *Xenopus* (Taira et al. 1997), raised the possibility that head organiser activity might reside in these tissues instead, or as well (Shawlot et al. 1999).

Many recent data in mice appear to support the notion that signals from outside the node are required for induction of the anterior nervous system and that a head organiser might reside in the AVE (Thomas & Beddington, 1996; Bouwmeester & Leyns, 1997; Beddington & Robertson, 1998, 1999) and are reviewed briefly below.

First, grafting experiments of either late streak stage mouse nodes (Beddington, 1994) or of the 'early gastrula organiser' (EGO) (Tam & Steiner, 1999) suggested that the mouse node may lack the ability to induce anterior neural structures, raising the interesting possibility that forebrain induction is carried out by signals from structures other than the node. This notion is supported by the finding that mice with a homozygous deletion of the transcription factor *HNF3β*, which lack a morphological node (Ang et al. 1993; Weinstein et al. 1994; Klingensmith et al. 1999), nevertheless have an essentially complete nervous system (Klingensmith et al. 1999).

It was noticed that the transcription factor *Hex* is expressed in a portion of the mouse visceral endoderm prior to primitive streak formation, and that this region moves anteriorly during gastrulation together with the prospective forebrain territory in the epiblast (Thomas & Beddington, 1996; Thomas et al. 1998). This suggested the possibility that the AVE may provide the initial inducing signals for the forebrain. Consistent with this, physical ablation of the mouse AVE leads to the formation of embryos lacking head structures (Thomas & Beddington, 1996). Finally, it was found that the rabbit hypoblast (which is likely to be the equivalent of the mouse AVE), induces expression of early neural markers when grafted to a region of a chick host that is competent to respond to neural inducing signals (Knoetgen et al. 1999*a*, *b*). Collectively, these data suggest that mammalian extra-embryonic endoderm might indeed act as a distinct head inducer. It was the finding that chick hypoblast did not induce expression of these markers in similar assays that led to the speculation that head induction may occur through different mechanisms in mammals as opposed to other vertebrates (Knoetgen et al. 1999*a*, *b*)

Further molecular evidence also supports the idea that a head inducer may reside in the AVE. Several of the genes that have been implicated in head formation (*HNF3β*: Ang & Rossant, 1994; Weinstein et al. 1994; *Lim1*: Shawlot & Behringer, 1995; Shawlot et al. 1999; *Otx-2*: Acampora et al. 1995; Matsuo et al. 1995; Ang et al. 1996; *nodal*: Conlon et al. 1994) are expressed in the AVE, as well as in embryonic structures. Chimaeric embryos in which extra-embryonic structures are derived almost exclusively from wild-type cells while embryonic structures are mutant or vice versa have provided a useful tool to identify the primary sites of action of these genes, and several mutants known to have defects in head development have now been analysed using this technique. For example, chimaera studies in combination with explant cultures have revealed that *Lim1* is required in both the anterior axial mesoderm and in the VE for proper head development (Shawlot et al. 1999). Similar experiments have shown that normal patterning of the forebrain requires *Otx-2* expression in both the VE and epiblast (Ang et al. 1994, 1996; Acampora et al. 1995, 1998; Matsuo et al. 1995; Rhinn et al. 1998; Suda et al. 1999).

Mice homozygous for the *413.d* insertional mutation (Conlon et al. 1991; Iannaccone et al. 1992), which disrupts *nodal* gene function, have many serious developmental defects due to a general failure of gastrulation (Conlon et al. 1994). Chimaeric mice that have a mutant VE, but wild-type embryonic tissues, show significant rescue of the gastrulation defects but still have extremely abnormal heads. The converse chimaeras, with wild type VE but embryonic structures made up largely of mutant cells, show essentially normal rostrocaudal patterning and no head defects (Varlet et al. 1997). These results suggest that although the embryonic expression of *nodal* can partially compensate for a loss of function in the VE, its function is primarily required in the VE.

Taken together, these results suggest that the visceral endoderm plays an important role in head development. We will return to discuss this idea later.

Quantitative models

The 2-signal model. In the 1930s Dalcq & Pasteels (1937) performed a series of experiments in which they restrained amphibian eggs between plates such that the position of their animal and vegetal poles were reversed. In this manner, they were able to alter the position at which the blastopore lip formed and sometimes observed the formation of a second blastopore. Based on these observations, they determined that lip formation, and thus the site of gastrulation movements, could occur at any point on the egg surface where yolky mass from the vegetal half of the egg came into contact with the outer cortex. They also noted that the dorsal side of the embryo possesses a greater potential for lip formation. Based on these results, they proposed a model for axis specification postulating the existence of 2 morphogenetic gradients, a vitelline (V) gradient with a high point in the vegetal portion of the egg, and a C gradient, with a high point in the grey crescent on the dorsal side of the embryo. They assigned different regions of the embryo a 'morphogenetic potential' based on the equation $C \times V$. Regions with the same value of $C \times V$ were considered to have equal morphogenetic potential. On the other hand, they realised that regions with equal morphogenetic potential could see different values of C and V. They therefore proposed a second field based on the ratio (C/V) such that those points with the highest C/V ratio would develop the most dorsal/anterior character, whereas regions with lower C/V ratios would develop into progressively more lateral and posterior structures. This model therefore provides a mechanism whereby a limited number of graded signals can generate a complete embryonic pattern. Although this model was proposed to explain how the whole embryo becomes patterned, it could possibly be extended to explain patterning of the neural tube.

The double-potency model. A hypothesis with some similarity to the 2-signal model was proposed by Yamada (1940, 1950). In its original formulation, the double-potency model simply proposed that two opposing 'potentials' (which he termed the cephalocaudal potential, *Pcc* and the dorsoventral potential, *Pdv*) could work in dynamic tension to generate positional information. However, subsequent work by Takaya and others made it clear that the ability of the archenteron roof to induce neural tissue with regional character depends in part on morphogenetic movements (Okada & Takaya, 1942; Okada, 1942). By the 1950s Yamada (1950) had modified his model to suggest that only the mediator of dorsoventral potential (*Mdv*) is a biochemical entity, whereas the mediator of cephalocaudal potential (*Mcc*), must primarily be connected to morphogenetic movements. According to this model, sources of inductive activity would be stationary, while morphogenetic movements would direct cells towards or away from these signals, thus indirectly regulating the timing of inductive interactions. According to Yamada, the identity of cells along the rostrocaudal axis would depend on when cells came into contact with inductive signals and on how long that contact is maintained.

The activation-transformation model of Nieuwkoop. Perhaps the best known of all the models put forward to explain neural patterning is the activation/transformation model of Nieuwkoop and coworkers (Nieuwkoop et al. 1952; Nieuwkoop & Nigtevecht, 1954). In an elegant series of experiments, folds of ectoderm were inserted into the neural plate of host embryos. The most distal part of the fold remained undifferentiated, intermediate regions differentiated into mesodermal and ectodermal tissues and finally, closest to the host axis, a region of patterned neural tissue formed. Within this neural tissue, the more distal part was always rostral whereas the more proximal part was always caudal. More importantly, the level of the graft in the host embryo always determined the regional character of the most caudal neural tissue in the fold. This suggested a 2-step model for neural patterning, in which neural tissue is first 'activated' to a general anterior neural character, some of which later becomes progressively 'transformed' by factors that give it a more posterior character.

This model challenged contemporary views of neural induction and patterning in several ways. First, it suggested that the induction of anterior neural character was a necessary early step in the formation of the nervous system. Second, it suggested that neural induction could spread through the plane of the ectoderm, quite a departure from the classic view of induction as signals passing from one cell layer to another.

HOW WELL DO RECENT EXPERIMENTAL RESULTS AGREE WITH THESE MODELS?

Many results have been gathered in vertebrate systems regarding the establishment of the rostrocaudal axis. Some of these data appear to support quantitative models such as Nieuwkoop's activation/transformation model. Other data seem to support the notion of qualitatively different inducers for different regions of the nervous system, as proposed by Spemann and

colleagues. Some results have been interpreted as supporting one or the other model but could equally well support either. We will now review some of these data in the context of the models described above.

Transient induction of anterior character

The activation/transformation model of Nieuwkoop predicts that the first step in neural induction should be an early, transient induction of anterior neural character. However, there are conflicting data about whether such a transient anterior state exists during development. Eyal-Giladi (1954) described what she interpreted as an early transient induction of anterior neural character, using experiments similar to those carried out by Nieuwkoop. She isolated folds of presumptive neural plate and grafted them back to the archenteron of the same embryo from which they had been excised. Epiblast from early gastrula stage embryos developed into a more limited set of neural structures than its normal fate: even when the excised tissue comes from a region fated to contribute extensively to the rostrocaudal axis, the grafted fold gave rise only to forebrain. She interpreted this result as supporting Nieuwkoop's model. However, the data could also be consistent with a qualitative model such as Spemann's. In these explants, neural character developed only in early gastrula stage epiblast, in excised tissue that had been exposed to signals from the involuting mesoderm. Furthermore, since the first mesoderm that involutes in amphibian embryos is the presumptive prechordal mesoderm, which has been described as a potent inducer of forebrain character in amphibians (Dalcq & Pasteels, 1937; Damas, 1947; Eyal-Giladi, 1954; Nieuwkoop & Nigtevecht, 1954; Blitz & Cho, 1995), it is possible that the early gastrula fold developed this anterior character because it had been exposed to head-inducing signals.

Factors that might be involved in specifying rostral and caudal identity

The discovery of molecular factors capable of inducing rostral neural character in the absence of caudal ones has been taken to support an activation/transformation-like model for neural patterning. Putative 'activating' factors include several BMP antagonists, all of which have also been implicated as neural inducers in *Xenopus*. Chordin, noggin, follistatin and cerberus all induce forebrain but not more posterior character in amphibian animal caps (chordin: Sasai et al. 1995; noggin: Lamb et al. 1993; follistatin, Hemmati-Brivanlou et al. 1994; cerberus, Bouwmeester et al. 1996; Piccolo et al. 1999). The Wnt-antagonist Dickkopf (Glinka et al. 1998) induces head structures when coexpressed with molecules that disrupt the BMP signalling pathway. On the other hand, these molecules might also be seen to correspond to the head inducers predicted by the qualitative models. Furthermore, in chick, there is no known graft that induces anterior neural regions in the absence of more posterior structures.

Several putative caudalising/transforming factors have also been identified. FGF (Cox & Hemmati-Brivanlou, 1995; Pownall et al. 1996; Kolm et al. 1997; Xu et al. 1997; Holowacz & Sokol, 1999) or an activated form of its receptor (Amaya et al. 1991) caudalise the neural plate in *Xenopus*. FGF can also elicit a range of rostrocaudal neural markers in dissociated cells from *Xenopus* animal caps (Kengaku & Okamoto, 1995).

Two other factors, Wnt3a (McGrew et al. 1997) and retinoic acid (Durston et al. 1989; Boncinelli et al. 1991; Papalopulu et al. 1991; Marshall et al. 1992; Conlon, 1995; Hill et al. 1995; Simeone et al. 1995; Avantaggiato et al. 1996; Blumberg et al. 1997; Kolm et al. 1997) have also been proposed to act as caudalising factors in the embryo. The observation that most of the factors studied to date are either specific 'inducers' of anterior character or posteriorising factors that do not induce neural tissue on their own seems to support the activation/transformation model of Nieuwkoop.

On the other hand, the finding that organiser grafts from late gastrula stage embryos induce caudal neural tissue without inducing forebrain structures (chick: Dias & Schoenwolf, 1990; Storey et al. 1992; amphibians: Mangold, 1929, 1933; Spemann, 1938) has been taken as strong support for Spemann's notion of separate organisers. The observation of caudal induction in the absence of rostral structures is particularly difficult to reconcile with Nieuwkoop's model.

Planar induction

Another prediction of Nieuwkoop's model is that neural induction should be able to spread through the plane of the induced neural tissue. Early work in amphibians suggested that this is not likely to be the case. Holtfreter observed that hypertonic solutions cause amphibian embryos to undergo aberrant gastrulation movements so that the mesoderm fails to involute, but rather forms a separate pocket of tissue outside the ectoderm ('exogastrula'). The observation that these exogastrulae fail to form morphologically distinct neural tissue (Holtfreter, 1933*a*) was taken as

strong evidence that neural induction requires vertical signalling between the ectoderm and the underlying mesoderm. More recent work however, contradicts these findings. *Xenopus* exogastrulae do express the pan-neural marker N-CAM (Kintner & Melton, 1987) and thus, form neural tissue in the apparent absence of vertical signalling from the mesoderm. Of course, with exogastrulated embryos, it is impossible to rule out completely the possibility that some transient vertical contact between the ectoderm and underlying mesoderm has occurred. To overcome this problem, Keller and his group developed a new approach to this problem in which they sandwiched together two pieces of ectoderm that had been isolated from early gastrula stage embryos (before mesoderm has started to involute; Keller & Danilchik, 1988). It was observed that, as long as presumptive mesoderm from the organiser is present in these explants, the ectodermal component of the sandwich undergo normal gastrulation movements and develop into neural tissue; this proved to be the case even though this ectoderm had never come into vertical contact with the mesodermal cells of the explant (Dixon & Kintner, 1989; Keller & Jansa, 1992; Sater et al. 1993). Subsequently, it was shown that the neural tissue in these explants expresses a range of regional neural markers, in the correct positions relative to one another (Doniach, 1992; Doniach et al. 1992). These data indicate that neither the induction nor the patterning of the nervous system requires vertical signals from the mesoderm. However, given the observation that the ectodermal component of these 'Keller explants' undergoes considerable morphogenetic movements (Keller & Jansa, 1992; Keller et al. 1992), these experiments cannot rule out the possibility that patterning requires cell movement (Yamada, 1940, 1950; Yamada, 1994).

A recent result in zebrafish also supports the notion that planar signals can induce and pattern the nervous system. *Squint* and *cyclops* (Erter et al. 1998; Feldman et al. 1998; Rebagliati et al. 1998*a*, *b*; Sampath et al. 1998) are 2 genes that encode different homologs of *nodal*. Double mutants lack mesoderm but nevertheless possess a fully patterned neural tissues (Feldman et al. 2000).

Rostrocaudal polarity of the axial mesoderm and models for neural patterning

Qualitative models, such as Spemann's, predict that the dorsal mesoderm, which induces the nervous system, should be divided into different regions along the rostrocaudal axis. There are molecular and functional differences between different regions of the axial mesoderm as soon as (or shortly after) it emerges from the organiser (Wilkinson et al. 1990; Izpisúa-Belmonte et al. 1993; Kispert et al. 1995*a*, *b*; Stein & Kessel, 1995; Filosa et al. 1997; McMahon et al. 1998; Dale et al. 1999; Vesque et al. 2000). In *Xenopus* both ter Horst (1948) and Sala (1955) demonstrated that different parts of the archenteron roof induce different regions of the nervous system when combined with regions of competent ectoderm. In mouse, it has been demonstrated that the anterior, but not the posterior notochord can induce expression of the mid/hindbrain marker *engrailed* (Hemmati-Brivanlou et al. 1990) and in chick, different regions of the head process mesoderm induce neural tissue with different regional character (Rowan et al. 1999). Superficially, these results appear to support a qualitative model for neural induction. However, Sala (1955) pointed out that differences in inducing ability of different regions of the amphibian archenteron roof might correspond to locally different concentrations of graded molecular signals, rather than the presence of specific inducing factors. Also, ter Horst (1948) observed that in amphibians, the inducing ability of individual regions did not quite correspond to the normal fate of the ectoderm overlying it. In fact, the archenteron roof tends to induce neural tissue of somewhat broader regional character than would be expected from its position in the embryo. In the chick embryo grafts of the anterior-most portion of the head process induce neural tissue possessing a more posterior character than would be expected based on its normal position in the embryo (Rowan et al. 1999). Thus, while it seems unlikely that there is a one-to-one correspondence between inducer and induced regional character, these data might still support either a qualitative model, like the 2-gradient hypothesis of Toivonen and Saxén or a quantitative model such as Nieuwkoop's.

The head inducer hypothesis, revisited

Both of the major premises of the head inducer model, that induction of the forebrain in mice requires signals from outside the node and that these head inducing signals reside in the AVE, have been called into question by recent embyrological data. First, the avian equivalent of the AVE, the hypoblast, does not induce neural tissue, although it does induce transient expression of early neural markers (Foley et al. 2000). Second, the AVE, like the chick hypoblast, fails to induce neural tissue when grafted to a lateral region of a mouse egg cylinder (Tam, 1999), and has only been

shown to pattern the anterior nervous system in conjunction with signals from both the EGO and the anterior epiblast (Tam, 1999).

It is also important to consider that the mouse EGO and node transplantation experiments mentioned previously (Beddington, 1994; Tam et al. 1997; Tam & Steiner, 1999) probably do not reveal the full inducing ability of the node. Nodes from 0B stage embryos are of a stage equivalent to the old dorsal lips of amphibians (Spemann, 1931, 1938; Waddington, 1960) and old nodes of chick (Dias & Schoenwolf, 1990; Storey et al. 1992) which have lost their ability to induce forebrain. Second, the EGO may be too young to induce forebrain, although fate maps of the mouse epiblast do not reveal the stage at which precursors of the prechordal mesoderm become contained within the tip of the primitive streak (Lawson et al. 1991; Tam et al. 1997). In the chick, at least some precursors of these tissues are located in the epiblast anterior to the primitive streak until the streak reaches its full length (Hatada & Stern, 1994). It is therefore possible that the failure of EGO grafts to induce anterior neural tissue is due to the absence of prechordal mesendoderm precursors in the graft. Indeed, it has recently been shown that definitive streak stage mouse nodes do induce forebrain markers when grafted to chick hosts (Knoetgen et al. 2000).

So, while it is undeniable that the AVE plays an important role in axial patterning of the mouse embryo, it is still not clear in what capacity it functions. Indeed, it appears that the issue of whether mammalian embryos require separate 'organisers' for the head and trunk, is still open to debate. Despite some evidence from different systems supporting either the head/trunk-tail or the activation/transformation hypotheses, none of the existing models is entirely satisfactory to explain forebrain development. We therefore propose a modification of the Nieuwkoop model that can accommodate the available data from various animal systems. The salient features of this model are discussed below.

A MODIFIED NIEUWKOOP MODEL FOR EARLY PATTERNING OF THE CNS

We propose that in all vertebrates the gross subdivisions of the CNS are established by similar mechanisms, which can be subdivided into 3 main steps. We will describe these with reference to avian and mammalian embryos before discussing how these proposals can be extended to explain patterning of the CNS in other vertebrates. First, signals from the hypoblast/AVE induce an unstable pre-forebrain state in the epiblast. At the same time, signals from the hypoblast also initiate cell movements that direct the future forebrain away from the posteriorising influence of the node. In a second step, signals from the node provide signals required for cells that had received the earlier ones to differentiate in a neural direction. In addition, the node also emits posteriorising signals that act on adjacent cells of the forming neural plate. Finally, the prechordal mesendoderm emits neural stablising signals, which allow neuralisation of the forebrain regions and also signals that actively protect these regions from posteriorisation by the node.

The hypoblast induces an early, 'pre-forebrain' but unstable state

The chick hypoblast, which has recently been identified as the avian equivalent of the mouse AVE (Foley et al. 2000), induces expression of the early neural markers *Sox3* and *Otx-2* in competent epiblast, but only transiently. Over a longer period no definitive neural or forebrain markers are expressed and both *Sox3* and *Otx-2* expression are lost.

In both the chick and mouse, *Otx-2* is expressed in three distinct but overlapping phases (Bally-Cuif et al. 1995; Simeone, 1998). The first (in the hypoblast/AVE and the overlying epiblast) and second (in the node) phases are transient. In the final phase, expression is only seen in the anterior neurectoderm. Since the hypoblast induces *Otx-2* expression in the absence of a neural plate like morphology, it is likely that this expression corresponds to the early pre-neural phase of its expression. Two other pre-neural markers, *Sox3* and *ERNI* (Streit et al. 2000), are also transiently expressed in a broad region that includes the entire prospective neural plate. Grafts of Hensen's node into the area opaca induce both *Sox3* and *ERNI* in a few hours but if the node is removed some 5 h after grafting, expression declines and is not followed by the later neural marker *Sox2* (Streit et al. 1998, 2000). These observations suggest that early neural inducing signals result in transient, unstable induction of *Sox3*, but that continued signalling from the organiser is required both to maintain its expression and to initiate expression of later, definitive neural markers. In this context, the finding that grafts of chick hypoblast to a region of competent epiblast induce transient expression of *Sox3* and *Otx-2* (Foley et al. 2000) suggests that the hypoblast may be a source of early inducing signals (that are also present in the

node) but that it lacks the ability to maintain expression of these markers or initiate the induction of definitive neural tissue. The same conclusion was reached based on experiments in mice, where it was shown that interactions between the mesendoderm and the ectoderm are required to maintain *Otx2* expression and forebrain development (Ang et al. 1994; Shawlot et al. 1999).

Also consistent with this interpretation is the recent finding that the rabbit hypoblast (a tissue similar to both the mouse VE and the chick hypoblast) can induce expression of the early pre-neural marker *Sox-3* and the anterior marker *GANF* (Knoetgen et al. 1999). However, like the chick hypoblast, the rabbit hypoblast is unable to induce a recognisable forebrain and moreover, the operated embryos were only allowed to develop for 6–10 h in these experiments, which leaves open the possibility that induction by the rabbit hypoblast is as transient as that by the chick hypoblast.

The hypoblast directs cell movements to protect against caudalisation by the organiser

Waddington (1930) found that rotation of the chick hypoblast by 90° from its original position results in reorientation of the axis. He recognised that this could indicate either that the hypoblast has primitive-streak-inducing ability, or that it plays a role in directing cell movements, and acknowledged the difficulties in distinguishing between these 2 possibilities. To address this question, he rotated the hypoblast by 180° (Waddington, 1933*b*). This occasionally resulted in the transient formation of a second streak, or (in one case) in the formation of a second axis, the head of which was fused with that of the host axis. Subsequent researchers who repeated these experiments also determined that rotation of the hypoblast could affect primitive streak formation but neglected the possibility that it might direct cell movements, concluding that the hypoblast is a direct inducer of the streak (Azar & Eyal-Giladi, 1979, 1981; Mitrani & Eyal-Giladi, 1981; Mitrani et al. 1983; Callebaut & Van Nueten, 1996; Callebaut et al. 1998). This conclusion was strengthened by the finding that the hypoblast expresses a number of genes associated with axis induction, such as *activin* (Mitrani et al. 1990), *Otx-2* (Bally-Cuif et al. 1995), *HNF3β* (Ruiz i Altaba et al. 1995) and *goosecoid* (Hume & Dodd, 1993).

We have recently described results that give direct support to Waddington's initial conclusions, by showing that the hypoblast does indeed direct cell movements in the epiblast and that its effect on the orientation of the embryonic axis is not accompanied by changes of fate in the adjacent epiblast. Rather, rotation of the hypoblast distorts the fate map (Foley et al. 2000).

Fate maps of the pre-streak stage chick embryo (Hatada & Stern, 1994) place the prospective forebrain territory at the posterior midline at stage X, adjacent to Koller's sickle (which contains precursors of the organiser; Izpisúa-Belmonte et al. 1993; Streit et al. 2000). During stages XI–XIII, the prospective forebrain territory moves anteriorly so that by the time the primitive streak appears, it lies in front of the tip of the streak. As the streak elongates to its full length, the node ends adjacent to prospective hindbrain; most, if not all of the forebrain territory lies well forward of Hensen's node (Spratt, 1952; Rosenquist, 1966; Schoenwolf & Sheard, 1990; Bortier & Vakaet, 1992). The embryos of all of the major vertebrate model systems have been extensively fate mapped at pregastrula and gastrula stages and the resulting maps seem to support the general conclusion that the forebrain territory first arises in the posterior part of the epiblast and then moves forward to its final position during gastrulation (see Vogt, 1929, for urodeles, Woo & Fraser, 1995 and Woo et al. 1995 for zebrafish, Hatada & Stern, 1994, for the chick).

If neural induction is initiated by signals from the node, how is the forebrain ever induced? One possibility is that the initial signals emanate not from the node itself, but from some of its precursors at the posterior end of the embryo, before primitive streak formation. A recent finding strongly supports this possibility: middle layer cells associated with Koller's sickle can induce transient expression of the early preneural markers *ERNI* and *Sox3* (Streit et al. 2000) in competent epiblast of the area opaca. The data of Foley et al. (2000) suggest that the hypoblast could emit similar signals, which can initiate the process of neural induction, but are not sufficient to complete it.

The organiser appears to be a source of strong caudalising signals. The spreading hypoblast may act to direct cell movements in the epiblast to ensure that cells that received early inducing signals (the prospective forebrain territory) are kept separate from the developing node and thus protected from its caudalising activity (Kimura et al. 2000). The ability of the prechordal mesoderm to protect the forebrain against caudalising signals from the organiser was also discussed above. However, the activities of the hypoblast and of the prechordal mesoderm seem quite different. The prechordal mesoderm can alter the fate of hindbrain cells to forebrain at stages 3^{+}-4 (Foley et al. 1997), while the hypoblast cannot (Foley et al.

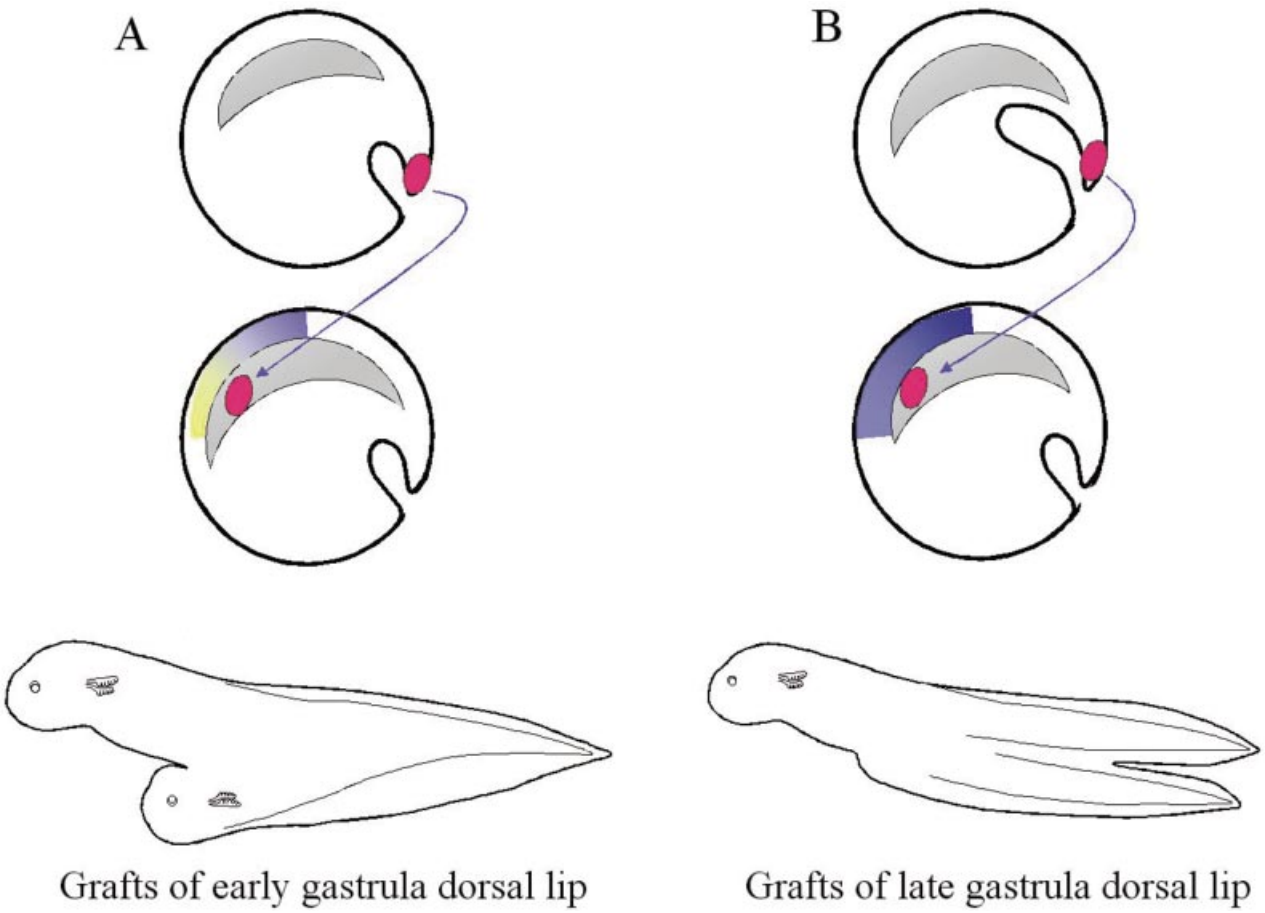

Fig. 1. Head and tail organisers in the amphibian embryo (based on experiments by Spemann, 1931, 1938; Mangold, 1933). (*A*) When a young organiser (red circle) is grafted to the ventral side of a host embryo, a complete secondary axis forms, which includes the full rostrocaudal range of neural structures. (*B*) When an organiser from a late gastrula stage donor embyro (red circle) is grafted, only caudal structures develop.

2000). Therefore the hypoblast may protect prospective forebrain cells against caudalising signals indirectly, directing its movement away from the caudalising influence of the organiser, whereas the prechordal mesoderm protects them directly, by antagonising these signals. In addition, it is possible that the prechordal mesoderm (perhaps together with anterior head-process; see Rowan et al. 1999) also acts to reinforce the initial induction, since the early events are insufficient to lead to the formation of definitive forebrain structures.

How consistent is this model with data from mouse?

Although the role of the mouse VE in directing cell movements in other cell layers has not been demonstrated directly, the phenotypes of several mouse mutants are consistent with the idea that the VE may have such a role (Kimura et al. 2000). One characteristic shared by all of the 'headless' mutants is an unusual constriction between the embryonic and extra-embryonic regions of the egg cylinder at E6.5–7.5. This phenotype has generally been thought to result from aberrant cell movements during gastrulation and is also rescued in chimeric mice with a wild-type VE (*Lim-1*, Shawlot & Behringer, 1995; Shawlot et al. 1999; *HNF3β*, Dufort et al. 1998; *Otx-2*, Rhinn et al. 1998; *nodal*, Varlet et al. 1997). Analysis of individual mouse mutants also supports this idea. In mice with a homozygous deletion of *Otx-2*, genes that are normally restricted anteriorly (such as *Hesx1/Rpx*, *Lim-1* and *cerberus*), remain abnormally located at the distal tip of the egg cylinder (Acampora et al. 1998; Rhinn et al. 1998). This defect can be rescued in transgenic mice with VE-restricted synthesis of Otx-1 (Acampora et al. 1998). Although these studies did not include analysis of cell movements, the results could be explained by proposing that the Otx-1-expressing VE rescues normal cell movements in the epiblast. Furthermore, these findings are consistent with the idea that one role of the

VE is to facilitate, or even direct, cell movements in the adjacent epiblast.

Other results support the idea that elongation of the streak positions caudalising signals at the distal tip. The *Cripto* mutant is characterised both by a failure of forebrain marker expression to move to the anterior part of the cylinder and by a failure of the primitive streak to elongate; despite the double-defect, forebrain markers nevertheless develop (Ding et al. 1998). This phenotype could be interpreted by proposing that the forebrain can develop in an abnormal, distal location because failure of the streak to elongate keeps the node and its caudalising signals at a proximal location, and these signals therefore fail to act on the prospective forebrain, which is stuck at the distal tip. Even though the physical distance between these regions is small in the mouse, it is conceivable that the patterning molecules act over a distance of very few cell diameters.

Finally, several results show that elongation of the axial mesoderm (head process and prechordal mesendoderm) is important for proper forebrain development, perhaps consistent with a maintenance/protection role for these tissues. Interestingly, the VE (like the chick hypoblast, whose rotation causes bending of the streak) may also play a role in regulating cell movements that facilitate the elongation of these structures. The partial rescue of a forebrain in *Lim-1* chimaeric mice may be due in part to the rescue of normal gastrulation movements, allowing for the formation of head process/prechordal mesendoderm (Perea-Gómez et al. 1999; Shawlot et al. 1999; see also Morriss-Kay & Tuckett, 1987; Dale et al. 1999; Rowan et al. 1999). Also, expression of either Otx-2 or Otx-1 in the VE of *Otx-2* mutant mice rescues the formation of anterior axial mesoderm (Acampora et al. 1998; Rhinn et al. 1998). Moreover, VE-restricted expression of Nodal in chimaeric mice that lack Nodal function in embryonic tissues rescues the severe morphological defects observed in homozygous mutant embryos, and one of the more striking features of these chimaeras is the proper elongation of the axial mesoderm (Varlet et al. 1997). Only one experimental finding is more difficult to explain with this model: the fact that relatively late ablation of the AVE causes a loss of expression of anterior epiblast markers (Thomas & Beddington, 1996). This finding can be accommodated by suggesting that, at least in the mouse, the AVE provides protective signals until the prechordal mesendoderm develops in the appropriate position.

Thus the major elements of these models are consistent and, in fact, have been proposed separately in the mouse (see for example Kimura et al. 2000), but a critical comparison of how these individual ideas relate to each other or to different classical models of forebrain development has not yet been undertaken. All of these findings can be accounted for by a modification of the Nieuwkoop model, in which early morphogenetic movements directed by the VE contribute to protect the prospective forebrain against caudalising signals from the organiser, and the prospective forebrain is maintained and further protected by signals first from the AVE and later from the head process/prechordal mesendoderm.

Extension to teleost embryos

In the zebrafish, there is no obvious equivalent of the hypoblast/VE; however, some data suggest that the present model may also apply to this species. In the fish, induction and patterning of the nervous system does not appear to require signals from the axial mesoderm but rather requires signals from the nonaxial mesoderm of the germ ring. Similar to the posteriorising function that was proposed above for the node, signals from the germ ring/embryonic shield can posteriorise prospective forebrain (Woo & Fraser, 1997). Furthermore, fate maps reveal that at the start of gastrulation the presumptive ventral forebrain is located posteriorly, in the epiblast adjacent to the embryonic shield. Subsequent movements carry these prospective forebrain cells to the centre of the blastoderm, far from the posteriorising influence of the germ ring/embryonic shield. Furthermore, epiboly movements during gastrulation also move the embryonic shield, and its posteriorising influence, away from the presumptive forebrain (Woo & Fraser, 1995, 1997). In future, it may be interesting to investigate whether the yolk syncytial layer (YSL), which expresses *Hex* like the mouse VE and chick hypoblast (Ho et al. 1999), can also direct cell movements in the adjacent ectoderm.

Amphibian embryos

As in teleosts, amphibian embryos do not have a region that is obviously homologous to the AVE/hypoblast. The yolky vegetal pole is generally considered to be endodermal, but its ultimate fate is mostly as gut contents, rather than gut lining, most of the latter being derived from the dorsal side of the embryo during gastrulation (Keller, 1975, 1976).

Nonetheless, several groups have explored the possibility that the anteriormost endoderm, which co-expresses *cerberus* and *Hex*, might act as a head organiser (Glinka et al. 1998; Osada & Wright, 1999; Schneider & Mercola, 1999). Injection of nodal induces the expression of these anterior endoderm markers and initiates gastrulation movements (Osada & Wright, 1999). This region also expresses the head-inducing factor *Dickkopf* (Glinka et al. 1998). However, Schneider and Mercola have shown through ablation experiments that this anterior endoderm does not induce the forebrain (Schneider & Mercola, 1999).

It is also interesting to note that one of the first proposals that morphogenetic movements play an important role in prosencephalic specification, and that this state requires reinforcing signals from prechordal tissue, was based on experiments in *Triturus* (Yamada, 1950).

A few more, albeit scattered, observations also seem to support the general hypothesis that cell movements are important for forebrain development. Although the anterior part of the presumptive archenteron roof has been shown to be a potent head inducer when inserted into the blastocoele of a gastrula stage embryo using Mangold's *Einsteckung* method (Spemann, 1931, 1938; Mangold, 1933), in *Cynops* at least, this same presumptive anterior mesoderm fails to induce forebrain in sandwich explants (Hama et al. 1985), which tends to limit morphogenetic movements.

Also, when cell movements are disrupted midway through gastrulation by agents such as Trypan blue or suramin, the embryos that develop lack a head but have normal trunk and tail development, suggesting that cell movements are particularly critical for head development (Gerhart et al. 1989).

MOLECULAR NATURE OF HYPOBLAST-DERIVED SIGNALS

Although we do not yet know the molecular nature of the signals emitted by the hypoblast that are responsible for either the transient induction of *Sox3*/*Otx2* or for its effects on cell movements, several recent results point to some likely candidates. FGFs, and specifically FGF8, is a good candidate to mediate the transient induction of early neural markers: the hypoblast (as well as prospective organiser cells at the posterior edge of the pre-streak embryo) expresses *FGF8*. Misexpression of FGF8 can also transiently induce *Sox3* and *ERNI*, while FGF antagonists abolish induction of these markers both by the organiser and by posterior precursor cells (Streit et al. 2000). It is conceivable that FGFs also contribute to the effects on cell movements, particularly because FGFs have been implicated in directing cell movements in both vertebrate and invertebrate systems (see in particular, Deng et al. 1994; Kroll & Amaya, 1996; Ciruna et al. 1997; Sun et al. 1999; reviewed by Montell, 1999).

In addition to FGFs, other likely candidates include components of the Wnt pathway or its antagonists. In zebrafish, a requirement for one member of this family, *Silberblick* (*Wnt11*), acting through a β-catenin-independent pathway, has been demonstrated to be essential for the cell movements of convergence and extension that drive major cell rearrangements during gastrulation (Heisenberg et al. 2000). *Silberblick* mutants are defective both in convergence/extension of the mesoderm and in forebrain patterning (Heisenberg et al. 2000). Likewise in *Xenopus*, a β-catenin-independent Wnt pathway has recently been shown to be important in regulating cell polarity and cell protrusions (Tada & Smith, 2000, Wallingford et al. 2000). The hypoblast expresses several secreted Wnt antagonists, including *Dkk1* and *crescent* (Foley et al. 2000), and it is therefore possible that these antagonists contribute to its effects on extension of the primitive streak and/or the forward migration of epiblast territories.

Finally, the Nodal pathway may also be involved in the regulation of cell movements. Nodal is expressed transiently in the mouse VE (Conlon et al. 1994; Varlet et al. 1997). Both Nodal and Cripto, a modulator of Nodal signaling, are required for both extension of the primitive streak and normal forebrain development (see above and Ding et al. 1998; Schier & Shen, 2000). Although the chick hypoblast does not appear to express the only *nodal* gene identified to date, it produces a Nodal antagonist (*cerberus*/*caronte*; Foley et al. 2000) and may also produce as yet unidentified members of the Nodal family.

MIGHT THIS MODEL SHED LIGHT ON THE CONCEPT OF THE NEW HEAD?

It has been proposed (Gans & Northcutt, 1983) that the evolutionary elaboration of the forebrain is related to the innovations of neural crest and placodes (the 'new head' hypothesis). Some elements of the model presented above might support this notion and

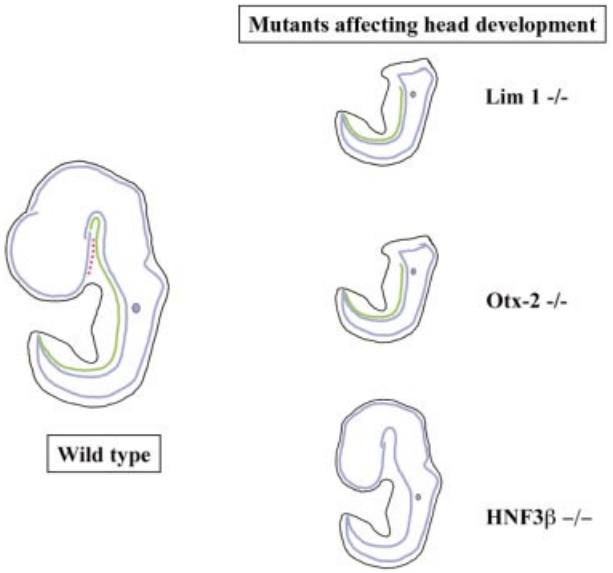

Fig. 2. Mouse mutations that affect forebrain development. *Lim-1* mutants lack a forebrain (neural tube shown blue in all embryos) and also lack prechordal mesoderm (red area in wild type) and have defects in extraembryonic tissues (not shown). *Otx-2* mutants have a similar phenotype. By contrast, *HNF3β* mutants, which lack tissues derived from the node such as the notochord (light green in all embryos) and prechordal mesoderm, express a full range of anterior/posterior neural markers, although they show many dorsal/ventral patterning defects (not shown).

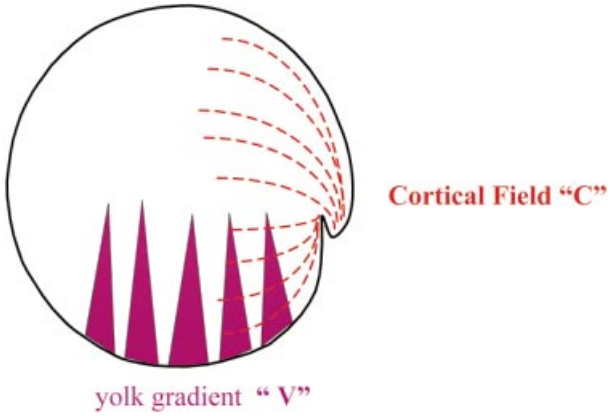

Fig. 3. The 2-signal model of Dalcq and Pasteels (1937). Two morphogenetic fields, the vitelline or V gradient (purple arrows) from the yolk and the cortical or C gradient (red dashed lines) interact to pattern the embryonic axis. Note that in this diagram, the 2 gradients are shown projected on to a gastrula stage embryo, with the dorsal lip to the right, but the original proposal concerned embryos at much earlier stages of development.

provide a mechanism for how the forebrain became more elaborate during evolution.

The notochord of lower chordates extends into the most anterior regions of the axis; therefore, lower chordates appear to lack prechordal mesoderm. This is likely to explain the lack of bilaterally symmetric eyes in these species (Li et al. 1997). However, it is also interesting that the appearance of the prechordal mesoderm during evolution correlates with the appearance of an elaborate forebrain, consistent with the idea that this mesendodermal population provides protective signals required for forebrain development.

Some recent results (Foley et al. 2000; Streit et al. 2000) suggest that specification of the vertebrate forebrain begins very early, before gastrulation, when its prospective location is adjacent to cells fated to become the organiser. Cell movements just prior to gastrulation then invert the nervous system, moving the forebrain away from the organiser. Fate maps suggest that this inversion is a general feature of many, if not all, classes of vertebrates. Interestingly, the fate map of the 110-cell ascidian embryo does not suggest a similar inversion: cells fated to give rise to the anterior nervous system are already located anteriorly (Hirano & Nishida, 1997).

CONCLUSIONS

We favour a model (Foley et al. 2000; Kimura et al. 2000) in which early inducing signals, starting before the onset of gastrulation, generate a region expressing early pre-neural and anterior neural markers (including *Sox3* and *Otx2*), but these signals are not sufficient to give rise to the definitive rostral CNS. Later in development, the organiser and/or its derivatives produce stabilising signals that complete the process. The organiser also emits strong posteriorising signals that can transform cells that have received neural inducing signals into more caudal regions of the CNS. The rostral CNS can only develop if it is protected from these caudalising signals. This occurs in 2 stages: shortly before the start of gastrulation, the prospective forebrain territory moves anteriorly under the control of the spreading hypoblast, and this movement protects the territory from the organiser by maintaining its distance from it. Later, the prechordal mesendoderm (perhaps with the anterior head process) provides signals that actively protect the forebrain against caudalisation. This model is closer to Nieuwkoop's activation/transformation hypothesis (Nieuwkoop et al. 1952; Nieuwkoop & Nigtevecht, 1954) than to the idea of separate organisers for different regions of the CNS, and accommodates data from fish, chick and mouse. It is therefore proposed that, unlike a previous suggestion that mammals have evolved a new way of patterning the rostral CNS (Knoetgen et al. 1999*a*, *b*), the mechanisms that establish this region are conserved among all vertebrate classes.

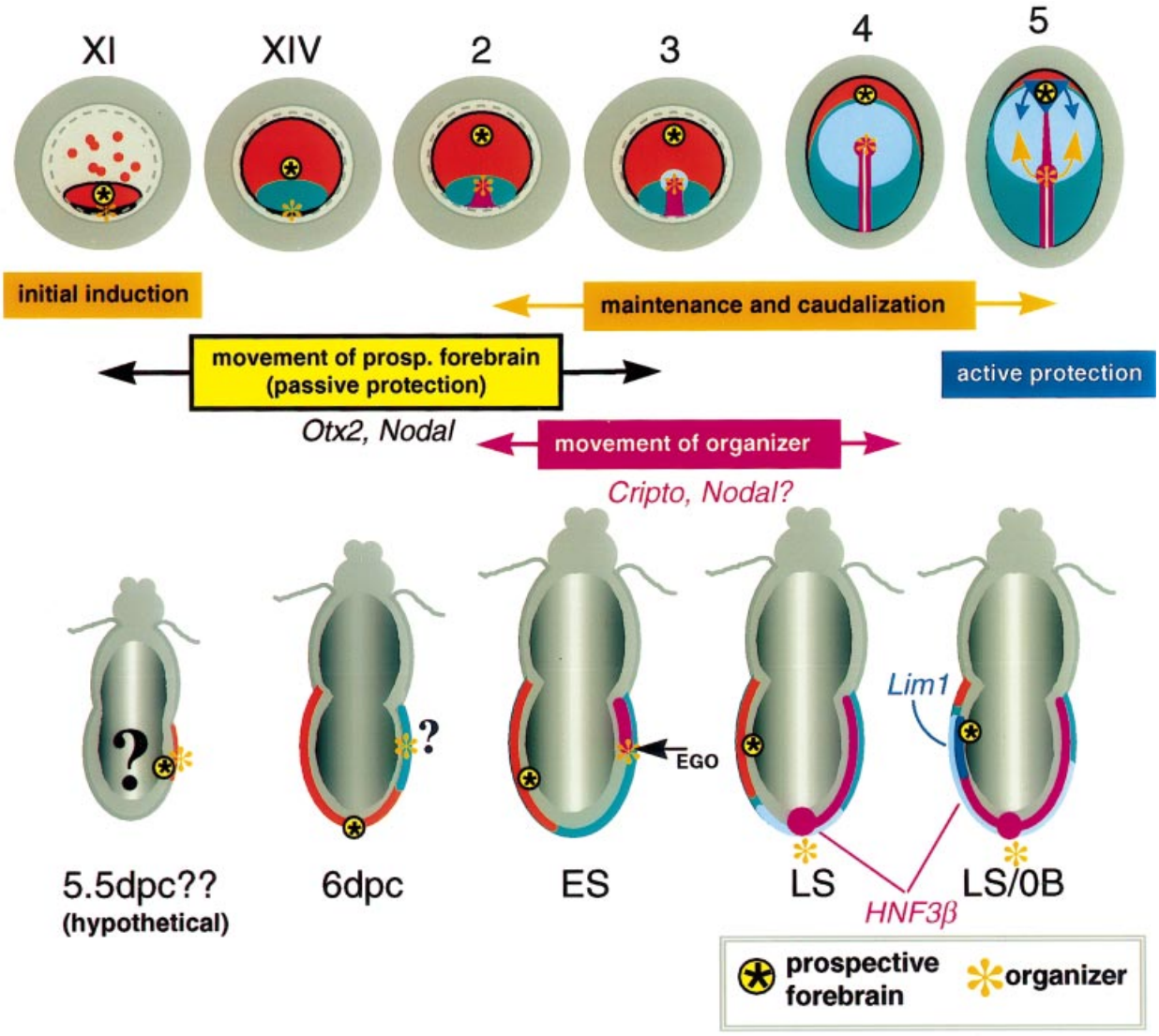

Fig. 4. A model for induction and patterning of the forebrain in chick and mouse. A series of steps lead to the formation of the forebrain. The upper row of diagrams illustrates events at different stages in the chick embryo; the lowest row extrapolates these to equivalent stages in the mouse (the question mark has been included to signify that the positions of the organiser and forebrain precursors have not been established for stages earlier than about E5.5 and there is therefore no equivalent to the first diagram in the chick). Between the 2 rows of diagrams, the text boxes indicate the major events proposed by the model, and the mouse genes whose mutation appears to interfere with these events. An initial induction occurs early in development when the prospective forebrain territory (yellow/black star) lies close to precursors of the organiser (orange star), but this induction is not sufficient to specify a forebrain. Soon afterwards, the prospective forebrain territory moves anteriorly, while the organiser stays posterior. As the primitive streak appears, the organiser moves forward with the tip of the streak. By early head-process sage, the prechordal mesendoderm (blue) that has emerged from the node protects the forebrain territory against caudalising signals from the node and also reinforces the initial induction (blue arrows). In more caudal region of the CNS, the reinforcement is due to signals from the node itself which also caudalise (orange arrows). Colour code: red, hypoblast/AVE; green, endoblast/non-anterior VE; light blue, gut endoderm; purple, primitive streak/head process; dark blue, prechordal mesendoderm; yellow/black star, center of future forebrain: orange star, organiser/node. Mouse stages according to Downs & Davies (1993): ES, early streak; LS, late streak; 0B, no allantoic bud. Chick embryos are viewed from the ventral side, mouse embryos in midsagittal section with caudal to the right and rostral to the left.

REFERENCES

ACAMPORA D, MAZAN S, LALLEMAND Y, AVANTAGGIATO V, MAURY M, SIMEONE A et al. (1995) Forebrain and midbrain regions are deleted in Otx2-/- mutants due to a defective anterior neuroectoderm specification during gastrulation. *Development* **121**, 3279–3290.

ACAMPORA D, AVANTAGGIATO V, TUORTO F, BRIATA P, CORTE G, SIMEONE A (1998) Visceral endoderm-restricted translation of Otx1 mediates recovery of Otx2 requirements for specification of anterior neural plate and normal gastrulation. *Development* **125**, 5091–5104.

AMAYA E, MUSCI TJ, KIRSCHNER MW (1991) Expression of a dominant negative mutant of the FGF receptor disrupts mesoderm formation in Xenopus embryos. *Cell* **66**, 257–270.

ANG S-L, ROSSANT J (1994) HNF-3 beta is essential for node and notochord formation in mouse development. *Cell* **78**, 561–574.

ANG S-L, WIERDA A, WONG D, STEVENS KA, CASCIO S, ROSSANT J et al. (1993) The formation and maintenance of the

definitive endoderm lineage in the mouse: involvement of HNF3/forkhead proteins. *Development* **119**, 1301–1315.

ANG S-L, CONLON RA, JIN O, ROSSANT J (1994) Positive and negative signals from mesoderm regulate the expression of mouse Otx2 in ectoderm explants. *Development* **120**, 2979–2989.

ANG S-L, JIN O, RHINN M, DAIGLE N, STEVENSON L, ROSSANT J (1996) A targeted mouse Otx2 mutation leads to severe defects in gastrulation and formation of axial mesoderm and to deletion of rostral brain. *Development* **122**, 243–252.

AVANTAGGIATO V, ACAMPORA D, TUORTO F, SIMEONE A (1996) Retinoic acid induces stage-specific repatterning of the rostral central nervous system. *Developmental Biology* **175**, 347–357.

AZAR Y, EYAL-GILADI H (1979) Marginal zone cells – the primitive streak-inducing component of the primary hypoblast in the chick. *Journal of Embryology and Experimental Morphology* **52**, 79–88.

AZAR Y, 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. *Journal of Embryology and Experimental Morphology* **61**, 133–144.

BALLY-CUIF L, GULISANO M, BROCCOLI V, BONCINELLI E (1995) c-otx2 is expressed in two different phases of gastrulation and is sensitive to retinoic acid treatment in chick embryo. *Mechanisms of Development* **49**, 49–63.

BEDDINGTON RS (1994) Induction of a second neural axis by the mouse node. *Development* **120**, 613–620.

BEDDINGTON RS, ROBERTSON EJ (1998) Anterior patterning in mouse. *Trends in Genetics* **14**, 277–284.

BEDDINGTON RS, ROBERTSON EJ (1999) Axis development and early asymmetry in mammals. *Cell* **96**, 195–209.

BLITZ IL, CHO KW (1995) Anterior neurectoderm is progressively induced during gastrulation: the role of the Xenopus homeobox gene orthodenticle. *Development* **121**, 993–1004.

BLUM M, GAUNT SJ, CHO KW, STEINBEISSER H, BLUMBERG B, BITTNER D et al. (1992) Gastrulation in the mouse: the role of the homeobox gene goosecoid. *Cell* **69**, 1097–1106.

BLUMBERG B, BOLADO J JR, MORENO TA, KINTNER CR, EVANS RM, PAPALOPULU N (1997) An essential role for retinoid signaling in anteroposterior neural patterning. *Development* **124**, 373–379.

BONCINELLI E, SIMEONE A, ACAMPORA D, MAVILIO F (1991) HOX gene activation by retinoic acid. *Trends in Genetics* **7**, 329–334.

BORTIER H, VAKAET LC (1992) Fate mapping the neural plate and the intraembryonic mesoblast in the upper layer of the chicken blastoderm with xenografting and time-lapse videography. *Development* (suppl.), 93–97.

BOUWMEESTER T, KIM S-H, SASAI Y, LU B, DE ROBERTIS EM (1996) Cerberus is a head-inducing secreted factor expressed in the anterior endoderm of Spemann's Organizer. *Nature* **382**, 595–601.

BOUWMEESTER T, LEYNS L (1997) Vertebrate head induction by anterior primitive endoderm. *Bioessays* **19**, 855–863.

CALLEBAUT M, VAN NEUTEN E (1996) Ectopic avian endophyll induces a neural plate or a miniature embryo in the caudal marginal zone and siamese twins in the area centralis. *Biol Jb Dodonaea* **64**, 39–63.

CALLEBAUT M, VAN NUETEN E, VAN NASSAUW L, BORTIER H, HARRISSON F (1998) 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. *Reproduction, Nutrition, Development* **38**, 449–463.

CIRUNA BG, SCHWARTZ L, HARPAL K, YAMAGUCHI TP, ROSSANT J (1997) Chimeric analysis of fibroblast growth factor receptor-1 (Fgfr1) function: a role for FGFR1 in morphogenetic movement through the primitive streak. *Development* **124**, 2829–2841.

CONLON FL, BARTH KS, ROBERTSON EJ (1991) A novel retrovirally induced embryonic lethal mutation in the mouse: assessment of the developmental fate of embryonic stem cells homozygous for the 413.d proviral integration. *Development* **111**, 969–981.

CONLON FL, LYONS KM, TAKAESU N, BARTH KS, KISPERT A, HERRMANN BG et al. (1994) A primary requirement for nodal in the formation and maintenance of the primitive streak in the mouse. *Development* **120**, 1919–1928.

CONLON RA (1995) Retinoic acid and pattern formation in vertebrates. *Trends in Genetics* **11**, 314–319.

COX WG, HEMMATI-BRIVANLOU A (1995) Caudalization of neural fate by tissue recombination and bFGF. *Development* **121**, 4349–4358.

CRICK F (1970) Diffusion in embryogenesis. *Nature* **225**, 420–422.

DALCQ A, PASTEELS J (1937) Une conception nouvelle des bases physiologiques de la morphogénèse. *Archives de Biologie de Liège* **48**, 669–710.

DALE K, SATTAR N, HEEMSKERK J, CLARKE JD, PLACZEK M, DODD J (1999) Differential patterning of ventral midline cells by axial mesoderm is regulated by BMP7 and chordin. *Development* **126**, 397–408.

DAMAS H (1947) Effet de la suspension précoce du flux inducteur sur la détermination du neurectoblaste médullaire. *Archives de Biologie Liège* **58**, 15–57.

DE SOUZA FS, NIEHRS C (2000) Anterior endoderm and head induction in early vertebrate embryos. *Cell and Tissue Research* **300**, 207–217.

DENG CX, WYNSHAW-BORIS A, SHEN MM, DAUGHERTY C, ORNITZ DM, LEDER P (1994) Murine FGFR-1 is required for early postimplantation growth and axial organization. *Genes and Development* **8**, 3045–3057.

DIAS MS, SCHOENWOLF GC (1990) Formation of ectopic neurepithelium in chick blastoderms: age-related capacities for induction and self-differentiation following transplantation of quail Hensen's nodes. *Anatomical Record* **228**, 437–448.

DING J, YANG L, YAN YT, CHEN A, DESAI N, WYNSHAW-BORIS A et al. (1998) Cripto is required for correct orientation of the anterior-posterior axis in the mouse embryo. *Nature* **395**, 702–707.

DIXON JE, KINTNER CR (1989) Cellular contacts required for neural induction in Xenopus embryos: evidence for two signals. *Development* **106**, 749–757.

DONIACH T (1992) Induction of anteroposterior neural pattern in Xenopus by planar signals. *Development* (suppl.), 183–193.

DONIACH T (1993) Planar and vertical induction of anteroposterior pattern during the development of the amphibian central nervous system. *Journal of Neurobiology* **24**, 1256–1275.

DONIACH T, PHILLIPS CR, GERHART JC (1992) Planar induction of anteroposterior pattern in the developing central nervous system of Xenopus laevis. *Science* **257**, 542–545.

DUFORT D, SCHWARTZ L, HARPAL K, ROSSANT J (1998) The transcription factor HNF3 beta is required in viscera l endoderm for normal primitive streak morphogenesis. *Development* **125**, 3015–3025.

DURSTON AJ, TIMMERMANS JP, HAGE WJ, HENDRIKS HF, DE VRIES NJ, HEIDEVELD M et al. (1989) Retinoic acid causes an anteroposterior transformation in the developing central nervous system. *Nature* **340**, 140–144.

ERTER CE, SOLNICA-KREZEL L, WRIGHT CV (1998) Zebrafish nodal-related 2 encodes an early mesendodermal inducer signaling from the extraembryonic yolk syncytial layer. *Developmental Biology* **204**, 361–372.

EYAL-GILADI H (1954) Dynamic aspects of neural induction in Amphibia. *Archives de Biologie* **65**, 179–259.

FELDMAN B, GATES MA, EGAN ES, DOUGAN ST, RENNEBECK G, SIROTKIN HI et al. (1998) Zebrafish organizer development and germ-layer formation require nodal- related signals. *Nature* **395**, 181–185.

FELDMAN B, DOUGAN ST, SCHIER AF, TALBOT WS (2000) Nodal-related signals establish mesendodermal fate and trunk neural identity in zebrafish. *Current Biology* **10**, 531–534.

FILOSA S, RIVERA-PÉREZ JA, GÓMEZ AP, GANSMÜLLER A, SASAKI H, BEHRINGER RR et al. (1997) Goosecoid and HNF-3β genetically interact to regulate neural tube patterning during mouse embryogenesis. *Development* **124**, 2843–2854.

FOLEY AC, STOREY KG, STERN CD (1997) The prechordal region lacks neural inducing ability, but can confer anterior character to more posterior neuroepithelium. *Development* **124**, 2983–2996.

FOLEY AC, SKROMNE IS, STERN CD (2000) Reconciling different models of forebrain induction and patterning: a dual role for the hypoblast. *Development* **127**, 3839–3854.

GANS C, NORTHCUTT RG (1983) Neural crest and the origin of vertebrates: A new head. *Science* **220**, 268–274.

GERHART J, DANILCHIK M, DONIACH T, ROBERTS S, ROWNING B, STEWART T (1989) Cortical rotation of the Xenopus egg: consequences for the anteroposterior pattern of embryonic dorsal development. *Development* **107**, 37–51.

GLINKA A, WU W, DELIUS H, MONAGHAN AP, BLUMENSTOCK C, NIEHRS C (1998) Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature* **391**, 357–362.

HAMA T, TSUJIMURA H, KANÉDA T, TAKATA K, OHARA A (1985) Inductive capacities for the dorsal mesoderm of the dorsal marginal zone and pharyngeal endoderm and presumptive pharyngeal endoderm as an intiator for the organizer center. *Development, Growth and Differentiation* **27**, 419–433.

HATADA Y, STERN CD (1994) A fate map of the epiblast of the early chick embryo. *Development* **120**, 2879–2889.

HATTA K, TAKAHASHI Y (1996) Secondary axis induction by heterospecific organizers in zebrafish. *Developmental Dynamics* **205**, 183–195.

HEISENBERG CP, TADA M, SAUDE L, CONCHA M, RAUCH J, GEISLER R et al. (2000) Silberblick/Wnt11 activity in paraxial tissue mediates convergent extension movements during zebrafish gastrulation. *Nature* **405**, 76–81.

HEMMATI-BRIVANLOU A, STEWART RM, HARLAND RM (1990) Region-specific neural induction of an engrailed protein by anterior notochord in Xenopus. *Science* **250**, 800–802.

HEMMATI-BRIVANLOU A, KELLY G, MELTON DA (1994) Follistatin, an antagonist of activin, is expressed in the Spemann organizer and displays direct neuralizing activity. *Cell* **77**, 283–295.

HILL J, CLARKE JD, VARGESSON N, JOWETT T, HOLDER N (1995) Exogenous retinoic acid causes specific alterations in the development of the midbrain and hindbrain of the zebrafish embryo including positional respecification of the Mauthner neuron. *Mechanisms of Development* **50**, 3–16.

HIRANO T, NISHIDA H (1997) Developmental fates of larval tissues after metamorphosis in ascidian Halocynthia roretzi. I. Origin of mesodermal tissues of the juvenile. *Developmental Biology* **192**, 199–210.

HO CY, HOUART C, WILSON SW, STAINIER DY (1999) A role for the extraembryonic yolk syncytial layer in patterning the zebrafish embryo suggested by properties of the hex gene. *Current Biology* **9**, 1131–1134.

HOLOWACZ T, SOKOL S (1999) FGF is required for posterior neural patterning but not for neural induction. *Developmental Biology* **205**, 296–308.

HOLTFRETER J (1933*a*) Die totale Exogastrulation, eine Selbstablösung des Ektoderms von Entomesoderm. *Wilhelm Roux' Archiv für Entwickslungsmechanik des Organismen* **129**, 669–793.

HOLTFRETER J (1933*b*) Eigenschaften und Verbreitung induzierender Stoffe. *Naturwissenschaften* **21**, 766–770.

HOLTFRETER J (1936) Regionale Induktionen in xenoplastisch zusammengesetzten Explantaten. *Wilhelm Roux' Archiv für Entwickslungsmechanik des Organismen* **134**, 466–550.

HOLTFRETER J (1938) Differenzierungspotenzen isolierter Teile der Urodelengastrula. *Wilhelm Roux' Archiv für Entwickslungsmechanik des Organismen* **138**, 522–656.

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

IANNACCONE PM, ZHOU X, KHOKHA M, BOUCHER D, KUEHN MR (1992) Insertional mutation of a gene involved in growth regulation of the early mouse embryo. *Developmental Dynamics* **194**, 198–208.

IZPISÚA-BELMONTE JC, DE ROBERTIS EM, STOREY KG, STERN CD (1993) The homeobox gene goosecoid and the origin of organizer cells in the early chick blastoderm. *Cell* **74**, 645–659.

KELLER RE (1975) Vital dye mapping of the gastrula and neurula of Xenopus laevis. I. Prospective areas and morphogenetic movements of the superficial layer. *Developmental Biology* **42**, 222–241.

KELLER RE (1976) Vital dye mapping of the gastrula and neurula of Xenopus laevis. II. Prospective areas and morphogenetic movements of the deep layer. *Developmental Biology* **51**, 118–37.

KELLER RE, DANILCHIK M (1988) Regional expression, pattern and timing of convergence and extension during gastrulation of Xenopus laevis. *Development* **103**, 193–209.

KELLER RE, JANSA S (1992) Xenopus gastrulation without a blastocoel roof. *Developmental Dynamics* **195**, 162–176.

KELLER RE, SHIH H, SATER AK, MORENO C (1992) Planar induction of convergence and extension of the neural plate by the organizer of xenopus. *Developmental Dynamics* **193**, 218–234.

KENGAKU M, OKAMOTO H (1995) bFGF as a possible morphogen for the anteroposterior axis of the central nervous system in Xenopus. *Development* **121**, 3121–3130.

KIMURA C, YOSHINAGA K, TIAN E, SUZUKI M, AIZAWA S, MATSUO I (2000) Visceral endoderm mediates forebrain development by suppressing posteriorizing signals. *Developmental Biology* **225**, 304–321.

KINTNER CR, MELTON DA (1987) Expression of Xenopus N-CAM RNA is an early response to neural induction. *Development* **99**, 311–325.

KINTNER CR, 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.

KISPERT A, KOSCHORZ B, HERRMANN BG (1995*a*) The T protein encoded by Brachyury is a tissue-specific transcription factor. *EMBO Journal* **14**, 4763–4772.

KISPERT A, ORTNER H, COOKE J, HERRMANN BG (1995*b*) The chick Brachyury gene: developmental expression pattern and response to axial induction by localized activin. *Developmental Biology* **168**, 406–415.

KLINGENSMITH J, ANG S-L, BACHILLER D, ROSSANT J (1999) Neural induction and patterning in the mouse in the absence of the node and its derivatives. *Developmental Biology* **216**, 535–549.

KNOETGEN H, TEICHMANN U, KESSEL M (1999*a*) Head-organizing activities of endodermal tissues in vertebrates. *Cell and Molecular Biology* **45**, 481–492.

KNOETGEN H, VIEBAHN C, KESSEL M (1999*b*) Head induction in the chick by primitive endoderm of mammalian, but not avian origin. *Development* **126**, 815–825.

KNOETGEN H, TEICHMANN U, WITTLER L, VIEBAHN C, KESSEL M (2000) Anterior neural induction by nodes from rabbits and mice. *Developmental Biology* **225**, 370–380.

KOLM PJ, APEKIN V, SIVE H (1997) Xenopus hindbrain patterning requires retinoid signaling. *Developmental Biology* **192**, 1–16.

KROLL KL, AMAYA E (1996) Transgenic Xenopus embryos from sperm nuclear transplantations reveal FGF signaling requirements during gastrulation. *Development* **122**, 3173–3183.

LAMB TM, KNECHT AK, SMITH WC, STACHEL SE, ECONOMIDES AN, STAHL N et al. (1993) Neural induction by the secreted polypeptide noggin. *Science* **262**, 713–718.

LAWSON KA, MENESES JJ, PEDERSEN RA (1991) Clonal analysis of epiblast fate during germ layer formation in the mouse embryo. *Development* **113**, 891–911.

LEMAIRE L, KESSEL M (1997) Gastrulation and homeobox genes in chick embryos. *Mechanisms of Development* **67**, 3–16.

LI H, TIERNEY C, WEN L, WU JY, RAO Y (1997) A single morphogenetic field gives rise to two retina primordia under the influence of the prechordal plate. *Development* **124**, 603–615.

MANGOLD O (1929) Experimente zur Analyse der Determination und Induktion der Medullarplatte. *Wilhelm Roux' Archiv für Entwicklungsmechanik des Organismen* **117**, 586–696.

MANGOLD O (1933) Über die Induktionsfähighkeit der verschiedenen Bezirke der Neurula von Urodelen. *Naturwissenshaften* **21**, 761–766.

MARSHALL H, NONCHEV S, SHAM MH, MUCHAMORE I, LUMSDEN A, KRUMLAUF R (1992) Retinoic acid alters hindbrain hox code and induces transformation of rhombomeres 2/3 into a 4/5 identity. *Nature* **360**, 737–741.

MATSUO I, KURATANI S, KIMURA C, TAKEDA H, AIZAWA S (1995) Mouse Otx2 functions in the formation and patterning of rostral head. *Genes and Development* **9**, 2646–2658.

McGREW LL, HOPPLER S, MOON RT (1997) Wnt and FGF pathways cooperatively pattern anteroposterior neural ectoderm in Xenopus. *Mechanisms of Development* **69**, 105–114.

McMAHON JA, TAKADA S, ZIMMERMAN LB, FAN CM, HARLAND RM, McMAHON AP (1998) Noggin-mediated antagonism of BMP signaling is required for growth and patterning of the neural tube and somite. *Genes and Development* **12**, 1438–1452.

MITRANI E, EYAL-GILADI H (1981) Hypoblastic cells can form a disk inducing an embryonic axis in chick epiblast. *Nature* **289**, 800–802.

MITRANI E, SHIMONI Y, EYAL-GILADI H (1983) Nature of the hypoblastic influence on the chick embryo epiblast. *Journal of Embryology and Experimental Morphology* **75**, 21–30.

MITRANI E, ZIV T, THOMSEN G, SHIMONI Y, MELTON DA, BRIL A (1990) Activin can induce the formation of axial structures and is expressed in the hypoblast of the chick. *Cell* **63**, 495–501.

MONTELL D (1999) The genetics of cell migration in Drosophila melanogaster and Caenorhabditis elegans. *Development* **126**, 3035–3046.

MORRISS-KAY G, TUCKETT F (1987) Fluidity of the neural epithelium during forebrain formation in rat embryos. *Journal of Cell Science* (Suppl. 8), 433–449.

NIEUWKOOP PD, BOTTERENBROOD EC, KREMER A, BLOESMA FFSN, HOESSELS ELMJ, MEYER G et al. (1952) Activation and organization of the central nervous system in Amphibians. *Journal of Experimental Zoology* **120**, 1–108.

NIEUWKOOP PD, NIGTEVECHT GV (1954) Neural activation and transformation in explants of competent ectoderm under the influence of fragments of anterior notochord in urodeles. *Journal of Embryology and Experimental Morphology* **2**, 175–193.

OKADA YK (1942) Further studies upon the regional differentiation of the inductive capacity of the organizer. *Proceedings of the Imperial Academy of Japan* **18**, 514–519.

OKADA YK, TAKAYA H (1942) Experimental investigation of regional differences in the inductive capacity of the organizer. *Proceedings of the Imperial Academy of Japan* **18**, 505–513.

OSADA SI, WRIGHT CV (1999) Xenopus nodal-related signaling is essential for mesendodermal patterning during early embryogenesis. *Development* **126**, 3229–3240.

PAPALOPULU N, CLARKE JD, BRADLEY L, WILKINSON D, KRUMLAUF R, HOLDER N (1991) Retinoic acid causes abnormal development and segmental patterning of the anterior hindbrain in Xenopus embryos. *Development* **113**, 1145–1158.

PEREA-GÓMEZ A, SHAWLOT W, SASAKI H, BEHRINGER RR, ANG S-L (1999) HNF3beta and Lim1 interact in the visceral endoderm to regulate primitive streak formation and anterior-posterior polarity in the mouse embryo. *Development* **126**, 4499–4511.

PICCOLO S, AGIUS E, LEYNS L, BHATTACHARYYA S, GRUNZ H, BOUWMEESTER T et al. (1999) The head inducer Cerberus is a multifunctional antagonist of Nodal, BMP and Wnt signals. *Nature* **397**, 707–710.

POWNALL ME, TUCKER AS, SLACK JMW, ISAACS HV (1996) eFGF, Xcad3 and Hox genes form a molecular pathway that establishes the anteroposterior axis in Xenopus. *Development* **122**, 3881–3892.

REBAGLIATI MR, TOYAMA R, FRICKE C, HAFFTER P, DAWID IB (1998*a*) Zebrafish nodal-related genes are implicated in axial patterning and establishing left-right asymmetry. *Developmental Biology* **199**, 261–272.

REBAGLIATI MR, TOYAMA R, HAFFTER P, DAWID IB (1998*b*) Cyclops encodes a nodal-related factor involved in midline signaling. *Proceedings of the National Academy of Sciences of the USA* **95**, 9932–9937.

RHINN M, DIERICH A, SHAWLOT W, BEHRINGER RR, LE MEUR M, ANG S-L (1998) Sequential roles for Otx2 in visceral endoderm and neuroectoderm for forebrain and midbrain induction and specification. *Development* **125**, 845–856.

ROSENQUIST GC (1966) A radioautographic study of labelled grafts in the chick blastoderm. Development from primitive-streak stages to stage 12. *Carnegie Institute of Washington: Contributions to Embryology* **38**, 71–110.

ROWAN AM, STERN CD, STOREY KG (1999) Axial mesendoderm refines rostrocaudal pattern in the chick nervous system. *Development* **126**, 2921–2934.

RUIZ I, ALTABA A (1993) Induction and axial patterning of the neural plate: planar and vertical signals. *Journal of Neurobiology* **24**, 1276–1204.

RUIZ I, ALTABA A, PLACZEK M, BALDASSARE M, DODD J, JESSELL TM (1995) Early stages of notochord and floor plate development in the chick embryo defined by normal and induced expression of HNF-3 beta. *Developmental Biology* **170**, 299–313.

SALA M (1955) Distribution of activating and transforming influences in the archenteron roof during the induction of the nervous system in amphibians, I. Distribution in craniocaudal direction. *Proceedings of the Koninklije Nederlandse Akademie Wetenschappen Serie C* **58**, 635–647.

SAMPATH K, RUBINSTEIN AL, CHENG AM, LIANG JO, FEKANY K, SOLNICA-KREZEL L et al. (1998) Induction of the zebrafish ventral brain and floorplate requires cyclops/nodal signalling. *Nature* **395**, 185–189.

SASAI Y, LU B, STEINBEISSER H, DE ROBERTIS EM (1995) Regulation of neural induction by the Chd and Bmp-4 antagonistic patterning signals in Xenopus. *Nature* **377**, 757

SATER A, STEINHARDT RA, KELLER RE (1993) Induction of neuronal differentiation by planar signals in Xenopus embryos. *Developmental Dynamics* **197**, 268–280.

SAXÉN L, TOIVONEN S (1962). Primary embryonic induction. Academic Press, London

SCHIER AF, SHEN MM (2000) Nodal signalling in vertebrate development. *Nature* **403**, 385–389.

SCHNEIDER VA, MERCOLA M (1999) Spatially distinct head and heart inducers within the Xenopus organizer region. *Current Biology* **12**, 800–809.

SCHOENWOLF GC, SHEARD P (1990) Fate mapping the avian epiblast with focal injections of a fluorescent- histochemical marker: ectodermal derivatives. *Journal of Experimental Zoology* **255**, 323–339.

SHAWLOT W, BEHRINGER RR (1995) Requirement for Lim1 in head-organizer function. *Nature* **374**, 425–430.

SHAWLOT W, WAKAMIYA M, KWAN KM, KANIA A, JESSELL TM, BEHRINGER RR (1999) Lim1 is required in both primitive streak-derived tissues and visceral endoderm for head formation in the mouse. *Development* **126**, 4925–4932.

SIMEONE A, AVANTAGGIATO V, MORONI MC, MAVILIO F, ARRA C, COTELLI F et al. (1995) Retinoic acid induces stage-specific antero-posterior transformation of rostral central nervous system. *Mechanisms of Development* **51**, 83–98.

SPEMANN H (1931) Über den Anteil von Implantat und Wirtskeim an der Orientierung und Beschaffenheit der induzierten Embryonalanlage. *Wilhelm Roux' Archiv für Entwickslungsmechanik des Organismen* **123**, 389–517.

SPEMANN H (1938) *Embryonic Development and Induction* Yale: Yale University Press.

SPEMANN H, MANGOLD H (1924) Über Induktion von Embryonalanlangen durch Implantation artfremder Organisatoren. *Wilhelm Roux' Archiv für Entwickslungsmechanik des Organismen* **100**, 599–638.

SPRATT NT JR (1952) Localization of the prospective neural plate in the early chick blastoderm. *Journal of Experimental Zoology* **120**, 109–130.

STEIN S, KESSEL M (1995) A homeobox gene involved in node, notochord and neural plate formation of chick embryos. *Mechanisms of Development* **49**, 37–48.

STOREY KG, CROSSLEY JM, DE ROBERTIS EM, NORRIS WE, STERN CD (1992) Neural induction and regionalisation in the chick embryo. *Development* **114**, 729–741.

STREIT A, LEE KJ, WOO I, ROBERTS C, JESSELL TM, STERN CD (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**, 507–19.

STREIT A, BERLINER AJ, PAPANAYOTOU C, SIRULNIK A, STERN CD (2000) Initiation of neural induction by FGF signalling before gastrulation. *Nature* **406**, 74–78.

SUDA Y, NAKABAYASHI J, MATSUO I, AIZAWA S (1999) Functional equivalency between Otx2 and Otx1 in development of the rostral head. *Development* **126**, 743–757.

SUN X, MEYERS EN, LEWANDOSKI M, MARTIN GR (1999) Targeted disruption of Fgf8 causes failure of cell migration in the gastrulating mouse embryo. *Genes and Development* **13**, 1834–1846.

TADA M, SMITH JC (2000) Xwnt11 is a target of Xenopus Brachyury: regulation of gastrulation movements via Dishevelled, but not through the canonical Wnt pathway. *Development* **127**, 2227–2238.

TAIRA M, SAINT-JEANNET JP, DAWID IB (1997) Role of the Xlim-1 and Xbra genes in anteroposterior patterning of neural tissue by the head and trunk organizer. *Proceedings of the National Academy of Sciences of the USA* **94**, 895–900.

TAM PP, STEINER KA, ZHOU SX, QUINLAN GA (1997) Lineage and functinal analyses of the mouse organizer. *Cold Spring Harbor Symposiusm on Quantative Biology* **62**, 135–144.

TAM PP, STEINER KA (1999) Anterior Patterning by synergistic activity of the early gastrula organizer and the anterior germ layer tissues of the mouse embryo. *Development* **126**, 5171–5179.

TER HORST J (1948) Differenzierungs- und Induktionsleistungen verscheidener Abschnitte der Medullarplatte und des Urdamdachs von Triton im Kombinat. *Wilhelm Roux' Archiv für Entwickslungsmechanik des Organismen* **143**, 275–303.

THOMAS PQ, BEDDINGTON RS (1996) Anterior primitive endoderm may be responsible for patterning the anterior neural plate in the mouse embryo. *Current Biology* **6**, 1487–1496.

THOMAS PQ, BROWN A, BEDDINGTON RS (1998) Hex: a homeobox gene revealing peri-implantation asymmetry in the mouse embryo and an early transient marker of endothelial cell precursors. *Development* **125**, 85–94.

TOIVONEN S (1938) Spezifische Induktionsleistungen von abnormen Induktoren im Implantatversuch. *Annales Societatis Zoologicae Botanicae Fennicae Vanamo* **6**, 1–12.

TOIVONEN S (1940) Über die Leistungsspezifität der abnormen Induktoren im Implantatversuch bei Triton. *Annales Academiae Scientiarum Fennicae Ser A IV* **55**, 1–150.

VARLET I, COLLIGNON J, ROBERTSON EJ (1997) Nodal expression in the primitive endoderm is required for specification of the anterior axis during mouse gastrulation. *Development* **124**, 1033–1044.

VESQUE C, ELLIS S, LEE A, SZABO M, THOMAS PQ, BEDDINGTON RS et al. (2000) Development of chick axial mesoderm: specification of prechordal mesoderm by anterior endoderm-derived TGF-β family signalling. *Development* **127**, 2795–2809.

VOGT W (1929) Gestaltungsanalyse am Amphibienkeim mit örtlicher Vitalfärbung. II. Gastrulation und Mesodermbildung bei Urodelen und Anuren. *Wilhelm Roux' Archiv für Entwickslungsmechanik des Organismen* **120**, 384–706.

WADDINGTON CH (1930) Developmental mechanics of chick and duck embryos. *Nature* **125**, 924–925.

WADDINGTON CH (1932) Experiments on the development of chick and duck embryos cultivated in vitro. *Philosophical Transactions of the Royal Society of London, Series B* **221**, 179–230.

WADDINGTON CH (1933*a*) Induction by the primitive streak and its derivatives in the chick. *Journal of Experimental Biology* **10**, 38–48.

WADDINGTON CH (1933*b*) Induction by the endoderm in birds. *Wilhelm Roux' Archiv für Entwickslungsmechanik des Organismen* **128**, 502–521.

WADDINGTON CH (1937) Experiments on determination in the rabbit embryo. *Archives de Biologie* **48**, 273–290.

WADDINGTON CH (1960) *Principles of Embryology*. New York: Macmillan.

WALLINGFORD JB, ROWNING BA, VOGELI KM, ROTHBACHER U, FRASER SE, HARLAND RM (2000) Dishevelled controls cell polarity during Xenopus gastrulation. *Nature* **405**, 81–85.

WEINSTEIN DC, RUIZ I, ALTABA A, CHEN WS, HOODLESS P, PREZIOSO VR, JESSELL TM et al. (1994) The winged-helix transcription factor HNF-3 beta is required for notochord development in the mouse embryo. *Cell* **78**, 575–588.

WILKINSON DG, BHATT S, HERRMANN BG (1990) Expression pattern of the mouse T gene and its role in mesoderm formation. *Nature* **343**, 657–659.

WOLPERT L (1969) Positional information and the spatial pattern of cellular differentiation. *Journal of Theoretical Biology* **25**, 1–47.

WOO K, FRASER SE (1995) Order and coherence in the fate map of the zebrafish nervous system. *Development* **121**, 2595–2609.

WOO K, FRASER SE (1997) Specification of the zebrafish nervous system by nonaxial signals. *Science* **277**, 254–257.

WOO K, SHIH J, FRASER SE (1995) Fate maps of the zebrafish embryo. *Current Opinion in Genetics and Development* **5**, 439–43.

XU RH, KIM J, TAIRA M, SREDNI D, KUNG H (1997) Studies on the role of fibroblast growth factor signaling in neurogenesis using conjugated/aged animal caps and dorsal ectoderm- grafted embryos. *Journal of Neuroscience* **17**, 6892–6898.

YAMADA T (1940) Beeinflussung der Differenzierungsleistung des

isolierten Mesoderms von Molchkeimen durch zugefügtes Corda- und Neuralmaterial. *Okajimas Folia Anatomica Japonica* **19**, 131–197.

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

YAMADA T (1994) Caudalization by the amphibian organizer: brachyury, convergent extension and retinoic acid. *Development* **120**, 3051–3062.

ZHU L, BELO JA, DE ROBERTIS EM, STERN CD (1999) Goosecoid regulates the neural inducing strength of the mouse node. *Developmental Biology* **216**, 276–281.