Induction and initial patterning of the nervous system – the chick embryo enters the scene

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Until recently, almost everything known about the molecular controls of early neural development came from studies in amphibians. It is now possible to misexpress factors in chick embryos at relatively late stages in development, allowing careful dissection of the timing of cell interactions. This is starting to contribute significantly to our understanding of neural induction and early patterning.

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Abbreviations

ANR	anterior neural ridge
AVE	anterior visceral endoderm (mouse)
BMP	bone morphogenetic protein
Dkk-1	Dickkopf-1
FGF	fibroblast growth factor

Introduction

The earliest, and some of the most important events during development of vertebrate embryos include a choice between neural and epidermal fates by cells of the ectoderm, and the initial subdivision of the early nervous system into broad domains — forebrain, midbrain, hindbrain and spinal cord — along the head-tail axis. Three quarters of a century ago, Spemann and Mangold discovered that a special region of the gastrula-stage amphibian embryo, the dorsal lip of the blastopore, has the unique ability to induce neighbouring cells to acquire a neural fate (for recent reviews, see [1,2,3••,4••]). They noticed that the induced nervous system is coherently organised along the head–tail and dorso-ventral axes, which led them to coin the term 'organiser' for this group of signalling cells. Ever since, researchers have striven intensively to elucidate the molecular nature both of the neural inducing and of the head–tail patterning signals. Until recently, very little progress had been made in these areas.

The first molecular insights on the mechanisms of neural induction all came from experiments in *Xenopus* embryos. First, embryos in which 'activin' signalling had been inhibited [5] failed to form mesoderm but developed an ectopic nervous system. Other groups [6,7] had discovered that dissociation of the *Xenopus* ectoderm alone is sufficient to induce cells to differentiate into neurons, suggesting that dissociation releases them from the action of a neural antagonist; together, these findings led to the widely known 'default model' [8]. Indeed, it was discovered that the antagonists are members of the bone morphogenetic protein (BMP) family and, in *Xenopus*, that all known neural-inducing treatments seem to act through BMP inhibition (for review see [3••]). However, in the past few years, several observations, now mostly made in amniotes, soon started to reveal additional complexity, which is reviewed briefly here.

The search for a mechanism responsible for the initial subdivision of the nervous system into broad domains was also fruitless for many decades. Many models were proposed, among which two — the 'head–trunk–tail organisers' model based on Otto Mangold's 1933 experiments and the 1954 'activation–transformation' model of Pieter Nieuwkoop — recruited the greatest number of followers (reviewed in [9]). However, the experimental evidence tended to favour both models equally, which led to several hybrid versions in some of the more recent literature. Renewed interest in the earlier model came mostly from experiments in mouse, where Rosa Beddington's group first drew attention to a tissue outside of the organiser region, the anterior visceral endoderm (AVE). It was discovered that, in the absence (either through physical ablation or in mutants where this tissue is defective) of the AVE, the forebrain is either lacking or severely defective (reviewed by [9]). Many quickly jumped to the conclusion that the AVE is the 'head organiser' proposed by Mangold, whereas Hensen's node — the amniote equivalent of the dorsal lip of the blastopore — acts as a 'trunk–tail organiser'. However, more recent work in both chick and mouse has started to reveal that Nieuwkoop's model is the more accurate. Although the molecular players are not yet identified, at least some knowledge is beginning to accrue concerning the roles of different cellular sources of the signals that should soon lead to understanding how this initial regional subdivision is established.

Neural-inducing signals

Despite the apparently overwhelming evidence supporting the 'default model' for neural induction, several observations in the literature suggested that other pathways may participate in the selection of either neural or epidermal cell fates in addition to BMPs. One was fibroblast growth factor (FGF). In *Xenopus*, two groups had found that the presence of a dominant-negative version of the FGF receptor-1 could block the neural-inducing activity of the two BMP antagonists Noggin [10] and Chordin [11]. However, these findings were generally dismissed because the same dominant-negative FGF receptor by itself does not block neural development (see [3••]). More recently, it was shown that a dominant-negative version of a different FGF receptor, FGF4 [12] does block neural development.

It is surprising that even then the community was reluctant to accept that FGF might play a role in neural induction. The simplicity of the 'default model' has endowed it with considerable appeal.

However, findings in amniotes also cast some doubt on the 'default model' because BMP antagonists do not induce neural tissue, BMPs do not suppress neural fates, and mouse mutants lacking some of these components have surprisingly normal nervous systems (reviewed in [4••,13]). Now, two groups also found that in the chick, FGF signalling is involved in, and required for, the acquisition of neural fates both in cultured explants [14•] and in whole embryos [15••]. However, FGF is not sufficient, even in combination with BMP antagonists, to direct ectodermal cells towards a stable neural fate. Interestingly, similar findings have now been made in ascidian embryos [16,17].

The finding that FGF signalling plays a role in neural induction in the chick, and the identification of *ERNI* [15••], a molecular marker for a very early response to this, first revealed that this signalling pathway acts at a very early stage, even before the formation of the organiser [14•,15••]. Indeed, it was proposed that the requirement for FGF could begin as early as stage VIII (equivalent to a very early blastula) [14•].

Other evidence implicates another group of factors, the Wnts, in neural induction both in amphibians and in amniotes. However, there are also some contradictions: in *Xenopus*, activation of the Wnt pathway appears to facilitate neural induction [18,19], while in the chick it inhibits neural fates [20••]. The apparent discrepancy was interpreted [4••] as being as a result of different Wnt signalling roles at different stages of development: early in development, the Wnt pathway dorsalises the whole embryo, a consequence of which includes the positioning of the future neural plate dorsally [18]; later on, Wnt signals appear to block neural differentiation [18,20••]. Indirectly this is also a consequence of the different ways in which candidate factors can be tested in *Xenopus* and in the chick. In *Xenopus*, many experiments are conducted by injection of capped mRNAs at early cleavage stages; in chick, most experiments involve the introduction of factors locally at later stages — by *in situ* electroporation or by grafting secreting cells — followed by assays of the consequences in time-course. With new technologies such as electroporation, the chick now lends itself better to analysis of later developmental events, and particularly to resolving different roles for signalling pathways such as FGFs, BMPs and Wnts, which have multiple functions.

Taken together, these new findings have revealed that, at least in amniotes, neural induction involves a cascade of molecular events, beginning at a very early stage (before gastrulation) and ending at the full primitive streak stage [21,22]. A critical question remaining to be addressed is whether different vertebrate classes use different mechanisms to specify the neural territory, or whether the differences are a consequence of the methods used to study them. I favour the latter interpretation. As we begin to disentangle the precise sequence of biological events in time and space, which has now become easier in the chick than in *Xenopus*, the common features will no doubt emerge.

How many organisers?

If neural induction begins before gastrulation, where do the earliest signals come from? One possible source in the chick is the hypoblast, a tissue with extraembryonic fate that comes to cover the surface of the embryo just before primitive streak formation. Consistent with this, the hypoblast expresses both FGF8 [15••] and antagonists of the Wnt pathway such as *crescent* and *Dickkopf-1* (*Dkk-1*) [23••]. Indeed, grafts of the hypoblast induce ectopic expression of the earliest *pre*-neural markers, *ERNI* and *Sox3* as well as *Otx2* [23••]. In addition, the hypoblast also controls the expression of another epiblast marker, *Not1/GNOT1* [24•]. However, these grafts do not induce stable neural markers, and expression of the early markers gradually decays. These findings are consistent with the notion, derived from experiments on mouse embryos, that the AVE (equivalent to the hypoblast) is required for the induction of the anterior nervous system (reviewed in [9]). It was initially thought that the AVE could correspond to Mangold's 'head organiser' but it has now been shown that the AVE can only induce neural fates when combined both with the node and with responsive ectoderm [25]. In one of the studies discussed earlier [14•], central epiblast explants isolated from stage VIII embryos were shown spontaneously to develop expression of neural markers when explanted into culture, and this expression was blocked by the FGF antagonist, SU5402. The authors suggested that FGF signalling is constitutive in the epiblast at these stages; however, at this time the epiblast contains precursors of the hypoblast that have not yet ingressed, and it is therefore possible that these are the source of the signals. Together, these findings led to the proposal that neural induction may be initiated by signals (such as FGF and perhaps Wnt antagonists) from the hypoblast, which are later stabilised by other signals emanating from the organiser (Hensen's node) and its derivatives [3••,4••,9,23••]. There is, therefore, as yet no evidence that the chick hypoblast or the mouse AVE are true 'organisers', because neither tissue can induce nervous system, and neither is sufficient to pattern a nervous system induced by grafts of the node [23••].

Early patterning: cells and signals

Mangold's experiments led to the proposal that each of the major regions of the neural tube is specified by signals emanating from a different set of organiser cells. The model proposes at least two separate organisers (one for the head, one for trunk/tail) must exist, but a logical extension is that there may be as many organisers as there are regions of the nervous system. This model is intrinsically unappealing because it does not explain how the embryo

increases in complexity as it develops, and it is therefore surprising that it has recruited many followers. Other models, such as Nieuwkoop's 'activation–transformation model' are more attractive because they account for the generation of more than two regions with a smaller number of signals. Nieuwkoop's proposal was that initial induction (activation) produces nervous system of forebrain character, and that later signals 'transform' (i.e. caudalise) some of it.

As mentioned above, the AVE of the mouse embryo seemed initially to fulfil the criteria required of a 'head organiser', but now it appears that the signals it emits are not sufficient to induce forebrain or even nervous system [25]. Indeed, the mammalian node was thought to be incapable of inducing head structures, unlike its avian counterpart. However, it now appears that the rabbit and mouse node can induce expression of forebrain markers when grafted into host embryos [26], as can the chick node. If the AVE/hypoblast is not the 'head organiser', what is its role in head formation? The recent work of three groups has now led to the idea that, in addition to the transient induction of neural markers (which includes the anterior marker Otx2 [23••]), its principal role is to direct cell movements in the adjacent epiblast, to keep portions of the prospective neural plate away from the caudalising (posteriorising) influence of the node [23••,27••,28•,29]. This notion is far closer to Nieuwkoop's 'activation–transformation model' than to the idea of multiple organisers.

Thus, the hypoblast/AVE is not a 'head organiser' but may be responsible for supplying initial, 'pre-neural' inducing signals and initiating the expression of an early anterior marker, Otx2, in the epiblast. However, this early state needs to be maintained and reinforced by signals from other regions. The organiser and/or some of its descendants might supply these signals. It appears that the node not only maintains the neural state but also 'transforms' (caudalises), as proposed by Nieuwkoop. Recently, it was shown that FGF signalling in the vicinity of the chick node can maintain the cells of the caudal neural plate in an immature state, in which they may be able to respond to caudalising influences [30••,31••] (see also [32]). In fact, some cells in this region are so immature that they can still produce progeny with both neural and epidermal fate [33]. Therefore, FGF plays multiple roles in neural induction and patterning; at very early stages it induces (transiently) the expression of early neural and anterior markers but later in development it plays a role in caudalisation.

In addition to FGF, Wnts also seem to be important, at least for caudalising the most cranial regions of the axis [34,35]. It now appears that a unique population of ectoderm cells at the anteriormost tip of the neural plate, the anterior neural ridge (ANR), also acts to protect future forebrain cells against posteriorising signals [36]. To date, this population of cells and their activity have only been described in teleosts. If equivalent cells also exist in other vertebrates, the question remains as to where they reside. This protection is probably collaborative, between the ANR and the prechordal mesendoderm, which has similar properties [37].

In *Xenopus*, a 'head-inducing' molecule, Dkk-1, has been described, which acts as a multifunctional antagonist of both BMP and Wnt signals [38]. Now, Kazanskaya *et al*. [34] show that Dkk-1 is required for the formation of the prechordal plate, a region previously implicated in 'head induction' in many different species. Moreover, mouse mutants lacking functional Dkk-1 lack the most cranial portions of the nervous system [39]. Paradoxically in the chick embryo, the prechordal mesendoderm expresses BMP7 and this expression is regulated by BMPs themselves, emanating from the underlying definitive endoderm [40]. This finding could explain reports that definitive endoderm itself is involved in head formation [41].

Finally, it is important to mention that several lines of evidence suggest a role for some degree of 'pre-patterning' of the epiblast in this process. Recently, it was shown that combinations of transcription factors are probably responsible for this, establishing domains of 'competence' to respond to the appropriate patterning signals [42,43].

Conclusions

The ease with which genes can be misexpressed by RNA injection in *Xenopus* has greatly facilitated the elucidation of many signalling pathways and of the interactions between them. However, like genetics, this approach makes it particularly difficult to disentangle the individual biological functions of factors that play multiple roles at closely spaced periods of development, such as the BMPs, FGFs and Wnts. To do so, a system that lends itself to misexpression of factors locally at any time during development is advantageous. Recent advances in the chick embryo now make it an excellent system in which to achieve this. Its large size, relative transparency and flat morphology also facilitates examination of cellular events such as morphogenetic movements. These advances have started to bring the chick embryo back into a prominent position as a major experimental system. In the area of neural induction and early patterning, it has now started to reveal the importance of cell movements and the very complex cell interactions that initiate the development of the central nervous system.

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