COMMENT

Unexpected requirements for neural induction in the avian embryo

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Spemann's organizer experiment in 1924 suggested that neural tissue is induced from prospective epidermal tissue by a secreted factor[1]. Since then, neural induction has been a focus of biological interest; however attempts to isolate a neural inducing factor remained unsuccessful for decades. Therefore, it came as a major relief when the molecular basis of neural induction in amphibia was unravelled by several major breakthroughs (reviewed in Ref. 2). Although research on neural induction has been dominated by work on amphibia, it is clear that the experimental advantages of other animals need to be exploited. In several recent papers, neural induction in the chick embryo has been analyzed at the molecular level[3–5]. Some of the striking results and interpretations challenge models derived purely from work on amphibia.

The amphibian and the avian organizer

In amphibia, the formation of the organizer, the dorsal blastopore lip, indicates the presence of a dorsal–ventral axis and the beginning of gastrulation (Fig. 1a). Cells of the blastopore lip invaginate and become dorsal midline structures, first the prechordal mesendoderm and then notochord. Before, and soon after, internalization these cells can induce neural tissue from otherwise epidermally fated cells. Clearly, the appearance of the organizer precedes the anlage of the neural plate[6].

In birds, reptiles and mammals, the first recognizable embryonic axis is the primitive streak, which demarcates the later anterior–posterior axis. The fate of mesodermal cells along the streak resembles the alignment of mesodermal fates in the amphibian marginal zone. Prospective dorsal mesoderm is located in the tip, and ventral mesoderm more towards the base (reviewed in Ref. 7). The avian primitive streak originates in the posterior embryonic region from a primordium near Köller's sickle (Fig. 1b). Cells from the posterior embryonic region can be traced into the tip of the streak by expression of the homeobox gene *goosecoid*[8]. These cells are the first indication of the organizer in the chick embryo. The fully developed avian organizer is Hensen's node, the tip of the maximally elongated primitive streak. Cells from Hensen's node ingress to become anterior mesendoderm, which before and after internalization are also identified by the molecular marker *goosecoid* and maintain some organizer functions[7–10].

Thus, while the amphibian early organizer is equivalent to the blastopore lip, the avian organizer is first recognizable in or near Köller's sickle in the posterior embryo, and only 18 hours later it is fully established in the node located 1.8 mm away. It is of note that the early avian, as well as the amphibian, organizer precedes the anlage of the neural plate, whereas the node cells are already surrounded by a neural plate.

Neural induction by BMP-antagonism in amphibia

In the amphibian *Xenopus laevis*, genes for the three secreted proteins, Follistatin, Noggin and Chordin are expressed in the dorsal mesoderm, that is, the organizer, the prechordal mesendoderm and the notochord[11–13]. It turned out that each factor alone can induce neural ectoderm from prospective surface ectoderm, thus qualifying as the long sought neural inducing factor. In addition, the factors possess a more general dorsalizing ability and inhibit epidermis formation. Ventralizing factors, which inhibit neuralization, were identified among the bone morphogenetic proteins, in particular BMP4 (Ref. 14). At the beginning of gastrulation, the *BMP4* gene is active in the complete ectoderm of the animal
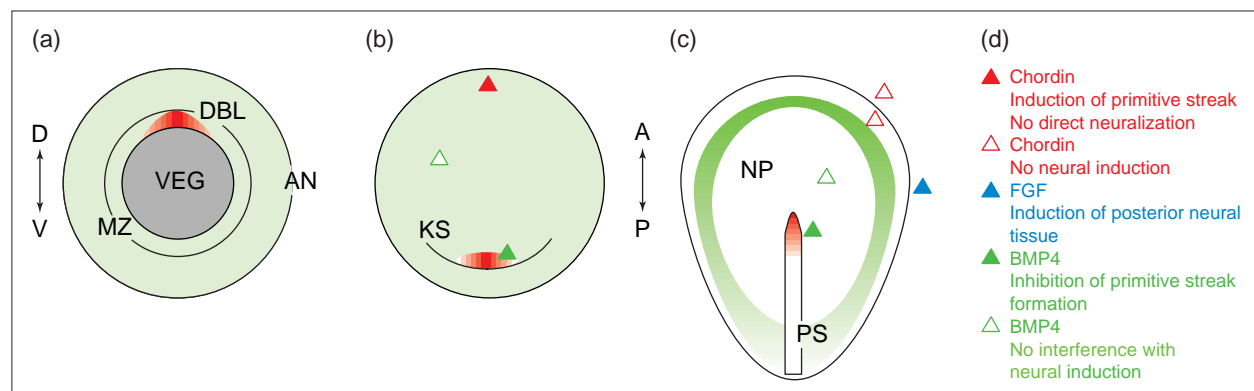

FIGURE 1. Chordin (red) and BMP4 (green) expression in early frog and chick embryos. (Data taken from Refs 4, 5, 14 and 15.) (a) Vegetal view onto the forming blastopore of an early frog gastrula. Vegetal cells (VEG), animal cells (AN), the dorsal blastopore lip (DBL) and the marginal zone (MZ) are indicated, the double arrow points in the dorsal (D) and ventral (V) directions. (b) A pre-streak and (c) a mid-streak chick embryo showing the epiblast and the forming streak, i.e. the blastopore. Köller's sickle (KS), the neural plate (NP) and the primitive streak (PS) are indicated, the double arrow points in the anterior (A) and posterior (P) direction. Note the BMP4 RNA expression adjacent to the Chordin domain in the early embryo (b), but far away from the Chordin positive tip of the primitive streak later in development (c). (d) Triangles indicate the experimental positioning of ectopic sources of factors.

half and the prospective mesoderm in the equatorial marginal zone, but not in the organizer region (Fig. 1a). The molecular mechanism underlying neural induction became clear when binary protein complexes between BMP4 and Chordin, BMP4 and Noggin, and Follistatin and BMP7 were found[2]. These observations suggest that the function of a neural inducing factor is to remove free BMP proteins from the area around the organizer and above the dorsal mesoderm, resulting in the differentiation of a neural plate. The evolving model focusing on BMP-antagonism implies that neuroectoderm is a default state, whereas epidermal fate is induced by BMPs (Ref. 2). This contrasts with initial presumptions based on Spemann's experiments. However, it remains to be seen to what extent neural induction requires an instructive component.

Chordin and BMP4 in the avian embryo

A recent paper from C. Stern's laboratory describes BMP4 and Chordin in the avian embryo[5]. A comparison of the expression patterns in the chick with homologous genes in the frog is not straightforward, given the complex developmental differences. The chick BMP4 gene is widely expressed in the ectoderm of pre-streak embryos, then transcription becomes restricted to the area opaca, before it surrounds the neural plate forming around the node (Fig. 1b, c). The chick *Chordin* gene is first expressed in a relatively strong domain just anterior to Köller's sickle of pre-streak embryos, then in the tip of the primitive streak, the node, and later on in the notochord. Thus, the early expression domain of Chordin is adjacent to BMP4-expressing ectoderm in the chick, as is the case near the dorsal blastopore lip of the frog (compare Fig. 1a with Fig. 1b). However, the fully developed avian organizer is significantly distant from BMP4-expressing, prospective epidermal ectoderm, making the formation of BMP4–Chordin protein complexes unlikely (Fig. 1c).

A. Streit *et al.* have analyzed the inductive capacities and the interplay between Chordin and BMP4 by localized ectopic expression from transfected cells (Fig. 1b–d). They obtained what appear, at first sight, to be surprising negative results: (1) Chordin cannot induce neural ectoderm in the competent periphery of the anterior embryo, either before or during streak formation; and (2) BMP4 does not interfere with neural development, when positioned beneath the neural plate anlage. It seems that Chordin and BMP4 affect gastrulation rather than neural induction after ectopic application. Streit *et al.* demonstrate that Chordin can induce ectopic primitive streaks in the anterior epiblast of pre- and mid-streak embryos. The induced streaks are complete and go on to generate their own neural plate. It is of note that transplants containing the early Chordin expression domain appear to induce a primitive streak, rather than neural ectoderm[8,9]. When BMP4 protein is applied near Köller's sickle or near the node, it inhibits the formation of a primitive streak and consequently any further development. Thus, BMP4 indeed antagonizes Chordin in the chick embryo. Under natural conditions the only time and place for a physical interaction between Chordin and BMP4 is before streak formation near Köller's sickle, at least this is what the RNA expression data suggest. In chick embryogenesis, this time and location mark the onset of gastrulation, neural induction has not yet occurred, and the ingression of prechordal mesendoderm is some 18 hours away.

This is in contrast to the frog embryo, where the formation of the dorsal lip (Fig. 1a) is followed directly by ingression of mesendoderm and formation of the neural plate. The developmental differences between chick and frog embryos are reflected in the inductive capacity of Chordin, namely to elicit gastrulation or neural induction, respectively. The outcome of Chordin activity is, however, not so dissimilar. Chordin can induce secondary embryos in chicks as well as in frogs, even under completely different experimental contexts, that is, the application of factor or the injection of RNA (Refs 5, 15). Observations from the Stern laboratory therefore clearly recognize the conservation of Chordin–BMP4 antagonism as a major principle in dorsal–ventral patterning of avian embryos.

But what about neural induction? Why is Chordin unable to induce neural fates in the periphery of the chick embryo? Currently, the only conclusion that can be made is that other factors, possibly emitted by the node, are involved. Streit *et al.* provide preliminary evidence to show that such a factor could act upstream of Chordin. Neither Noggin nor Follistatin seems to be the putative factor, although combinatorial experiments have not been reported. At least for the chicken Noggin gene no apparent role in neuralization can be detected[16]. An insight into neuralization has been gained, however, in amniotes because a factor capable of inducing neural fate from uncommitted ectoderm has been identified.

Neural induction by FGF in chick embryos

The laboratories of Alvarez and Storey have demonstrated neural induction in avian embryos by fibroblast growth factor (FGF), in particular FGF4 (Refs 3, 4). Storey *et al.* describe the activation of *FGF8*, *SAX1* and *CASH4*, all genes normally found active in the primitive streak and the posterior neural plate, which supports the notion of direct signalling within the prospective neural epithelium[4]. Alternatively, FGF might act indirectly through induced mesoderm, and the available experimental data need further analysis (e.g. in order to eliminate a discrepancy with respect to the brachyury marker). The fast activation of brachyury by factors such as FGF2, FGF4, FGF8b and FGF9 suggests that the posterior neural genes can also be induced by mesodermal tissue[4].

Are signals required from the primary embryo in addition to the ectopic FGF signal? Both laboratories demonstrate that FGF does not redirect cells from the primary neural plate to the periphery, but induces neural fate *de novo*[3,4]. The induced neuroepithelium either becomes fused, or remains independent of the primary embryo, apparently depending on the stage of the host and the site of FGF bead implantation. Neural induction by FGF is restricted to early posterior neuroectoderm, and neither anterior nor late neural markers are switched on. Storey *et al.* conclude that FGF signalling only elicits the initial steps of a neural programme, including the maintenance of neural competence[4]. These observations in the avian embryo are reminiscent of the situation in *Xenopus laevis*, where some evidence for FGF-induced neural fate is found, depending on experimental conditions and markers[17,18]. Thus, while some details of FGF involvement

in neural competence, induction and patterning are still unclear, there remains the major finding that FGF signalling is sufficient for the initiation events underlying the formation of the posterior neural plate.

Conclusion

The analysis of Chordin and BMP4 in the chick embryo shows that the basic mechanism for dorsal–ventral patterning is conserved from the fruit fruitfly to the frog, and also to the chick. However, it discourages the idea derived from analysis of amphibia, that dorsalization of ectoderm is equivalent to neuralization. Up to now it has only been possible to describe a role for Chordin in the early phase of organizer formation, but not for its later function in the node. We now understand more about the first definition of 'dorsal' in the chick, we have identified a factor that is sufficient for determining the posterior region, but we still do not understand the onset of neuralization. This is a very complex issue, but with two new factors having been identified we are a few steps nearer to understanding the great secret of neural induction in amniotes.

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A new mechanism broadening the role of prion proteins in neurodegeneration

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Over the past 15 years our understanding of the underlying mechanisms in the transmission and pathogenesis of the neurodegenerative diseases classed as 'transmissible spongiform encephalopathies' or 'prion diseases' has fundamentally changed our concept of an infectious agent[1]. Research from many disciplines indicates that the infectious agent causing prion diseases is, at least in part, an abnormally folded isoform (PrP^{Sc}) of a normal cellular protein called PrP^C (Ref. 1). The protein isoforms are known collectively as PrP. Prion diseases in humans present as sporadic, dominantly inherited, or transmissible neurological disorders, while the known animal prion diseases are all transmissible (Table 1).

Prion proteins and disease transmission

The PrP-encoding gene (*PRNP*, human; *Prn-p*, mouse) plays a key role in the development and transmission of prion diseases in both humans and animals[1]. The transmissible and most of the inherited forms of these diseases are characterized by the accumulation and deposition of an altered conformational form of the prion protein designated PrP^{Sc} (Ref. 2). Inherited forms of the disease are characterized by mutations in the PrP-encoding gene leading to amino acid substitutions and insertions in the prion protein (Fig. 1 and Table 1). The ability to transmit human prion diseases to experimental animals has been, until recently, a prerequisite for diagnosis. However, rates of transmission of prion disease have been variable (Table 1), ranging from almost 100% transmission for iatrogenic* Creutzfeldt–Jakob disease (CJD) cases, to 38% for Gerstmann–Sträussler–Scheinker syndrome GSS(P102L)‡ and to non-transmission of some inherited forms, such as GSS(A117V)[2–4].

The role of PrP^{Sc} in the transmission of the disease is well established but its role in neurodegeneration is less well defined. The use of transgenic mice expressing chimeric and mutant PrP-encoding genes and the development of transgenic PrP knockout mice have convincingly shown the importance of the prion protein in these diseases. The expression of the normal form of the prion protein PrP^C is required for the replication of the infectious prion[5], modulates the incubation time of the disease[6,7], and controls the development

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* Exposure to infectious agent through an accident.
‡ GSS(P102L) refers to a mutation in the human *PRNP* coding sequence resulting in a proline to leucine substitution at position 102 that is associated with Gerstmann–Sträussler–Scheinker syndrome. A similar nomenclature is used for other mutations in the prion protein gene.