MEDAL REVIEW

The Spemann organizer and embryonic head induction

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Introduction
My first thought was that someone had played a practical joke on me when I saw the fax and I asked the EMBO office for confirmation. During my postdoc days in Eddy De Robertis’s lab, we would send fake letters to colleagues with the letterhead of Cell or Nature containing rave reviews or flattering invitations and, for the brief moment when vanity dominated reason, we had a good laugh. I was at EMBL in 1986 when the EMBO gold medal was born and for the first time awarded to John Tooze, then head of EMBO, and hence followed its annual dedication with interest. It is noteworthy that the medal is now awarded for the second time (Smith, 1993) for work carried out in the Xenopus embryo, despite the fact that it has been said that more sophisticated model systems will eventually make the frog obsolete.

When conceiving this biographical account, I realize that many of my experiences have been of a dialectic nature, in the sense of a Hegelian ‘thesis–antithesis–synthesis’: from the development of my research interest and the approach towards science of my teachers, to the lessons that the object of my study—the embryo—taught me. I will try to point these out in the following passages. With my father being a theoretical physicist, I was impregnated with a sense for the ‘hard’ natural sciences, in particular organic chemistry, when I began my studies at the Free University of Berlin (in the former West). While the complexity of the living fascinated me increasingly, pure biology was too descriptive for my taste and I settled on studying biochemistry, which still qualified as a quantitative science. During those early student days, I became familiar with the work of Heinz Thiedemann at the Free University, whose life-work was the characterization and purification of inducing factors from amphibian and chicken embryos, capable of inducing neural and mesodermal tissue (‘vegetalizing factor’) (for a review see Thiedemann et al., 1995). The synthesis he represented was that of the chemist and the embryologist. The phenomena were spectacular, e.g. induction of different tissues in a concentration-dependent fashion. His work and approach probably had an important influence on my thinking.

It was during a course at the Max-Planck Institute in Munich that I approached Wieland Huttner, whose seminar on tyrosine sulfation I had recently attended. Still an undergraduate, I congratulated him for his nice work (as if I was in a position to pass judgements on principal investigators!). After he recovered from the surprise, he invited me to visit his lab and I ended up carrying out my graduate work with him after his move to the EMBL Cell Biology Program. Purification and characterization of tyrosylprotein sulfotransferase—a Golgi transmembrane enzyme catalysing the post-transcriptional sulfation of secreted proteins—gave me the hard-core biochemical training I wanted, and many hours in the cold room, which somewhat dampened my romantic inclination for biochemistry. Needless to say, the EMBL was a fantastic place to become exposed to both cell and developmental biology, and, like many colleagues, I became spoiled for life after this experience with regard to scientific environments.

After my thesis, I decided finally to make the move to developmental biology and to study Xenopus, because it was amenable to direct experimental manipulation and biochemical analysis. Before joining the laboratory of Eddy De Robertis, I participated in the Woods Hole embryology course organized by Eric Davidson, which was an eye opener to the amazing variety of embryogenesis of different organisms, including sea urchins, chicken, frogs, worms, flies, snails and ascidians. I could not have been primed better, and my first project in Eddy’s lab—experimental cell fate studies using a novel lineage tracer—was very antithetic to enzyme purification (Niehrs and De Robertis, 1991). The other surprise was the difference in approach towards research in Eddy’s lab. Where Wieland Huttner instructed me to pay attention to the minutest detail, Eddy De Robertis taught me to concentrate on the big picture, and so I learned the best of both worlds.

Dialectic of embryonic induction
Soon I was to work on the Spemann organizer, the Holy Grail of vertebrate developmental biology. It is a piece of tissue corresponding to the upper dorsal blastopore lip of the amphibian embryo, consisting of presumptive mesoderm and endoderm. The properties of the organizer were identified in 1926 by Hans Spemann and Hilde Mangold when they showed that the transplanted dorsal lip is able to induce a twinned embryo in a receiving host, which earned Spemann the Nobel Prize in 1935. The dorsal lip cells only form a small part of the Siamese twin formed; the majority of the cells are recruited and organized under its inductive influence, hence the name organizer. As it turned out, the so-called primary embryonic induction by the Spemann organizer was only one of the earliest inductive events of a whole cascade of inductions by which embryogenesis proceeds, and which occur in a dialectic fashion: two different cell layers contact each other, they communicate at their boundary and, as a result, a new cell type is formed between them. This cell type now has two new boundaries where further inductions can take place, and so on.
Generations of scholars have since devoted their careers to characterizing the organizer and to identifying the inducing molecules, but little progress had been made molecularly. In fact, the entire concept of the organizer was at times considered something of an artefact when it was realized that agents as unspecific as methylene blue could bring about similar inductions. During my postdoc days, history was made in Eddy’s lab when Ken Cho and Bruce Blumberg cloned the first organizer-specific gene, encoding the homeodomain protein Goosecoid (gsc; Blumberg et al., 1991). It was Herbert Steinbeisser who for the first time in history saw the dorsal lip light up in an in situ hybridization. However, gsc was not only a great marker but turned out to be capable of reproducing embryonic twinning when overexpressed in Xenopus embryos (Cho et al., 1991). We knew that we would hardly be witnessing such pioneering discoveries again during our careers. With orthologous gsc probes, the organizer equivalent was subsequently stained in other vertebrates, e.g. mouse, chicken and zebrafish, and I was fortunate to be involved in characterizing the effects of gsc in Xenopus (Niehrs et al., 1993, 1994). Today, genes specifically expressed in the organizer go by the dozen.

**Antagonizing the organizer: anti-BMPs**

Following the discovery of gsc, the field exploded with the discovery of the secreted factors Noggin, Chordin and Follistatin, which are released from the organizer and are able to induce secondary embryonic axes when overexpressed in Xenopus embryos, thus mimicking organizer activity. The view of organizer function changed dramatically when it was realized that these inducers act in a permissive fashion: they all antagonize signalling by bone morphogenetic proteins (BMPs). BMPs are members of the transforming growth factor-β (TGF-β) superfamily. The antagonists inhibit BMPs by sequestering BMP proteins into inactive complexes (reviewed in Harland and Gerhart, 1997). Thus, a central molecular mechanism of induction by the amphibian organizer resides in the inhibition of BMP signalling.

The role of BMPs and their antagonists turned out to be evolutionarily conserved between arthropods and vertebrates during dorsoventral patterning (reviewed in De Robertis and Sasai, 1996). Since the fly Bmp (dpp) functions as a morphogen to regulate the development of the blastoderm and appendages in a dose-dependent fashion, this raised the possibility that, like dpp in Drosophila (reviewed in Neumann and Cohen, 1997), Bmps act as a morphogen in dorsoventral patterning of vertebrate mesoderm (Ferguson, 1996; Hogan, 1996; Holley et al., 1996; Piccolo et al., 1996; Zimmerman et al., 1996).

We investigated the possibility that Bmp4 (Köster et al., 1991; Dale et al., 1992; Jones et al., 1992) functions dose dependently in dorsoventral patterning of Xenopus mesoderm, and found that it ventralizes dorsal (i.e. organizer) mesoderm in a dose-dependent manner, from notochord, to muscle, to pronephros, to blood (Doshch et al., 1997). Inversely, a dominant-negative BMP receptor dorsalizes ventral mesoderm dose dependently from blood, to pronephros, to muscle, to notochord. The results indicated that different BMP doses are both necessary and sufficient for patterning of at least three domains in the early gastrula, as well as for terminal differentiation into four mesodermal tadpole tissues.

A gradient of BMP signalling in the embryo may be established by two mechanisms: diffusion of BMP protein and diffusion of its antagonists. In an experimental design analogous to that used to show direct and long-range action of the DPP morphogen in Drosophila (Lecuit et al., 1996; Nellen et al., 1996), we found that, unlike in Xenopus animal caps (Jones et al., 1996), in mesoderm BMP4 is able to elicit responses over a distance of up to 10 cell diameters beyond expressing cells. This is unlike its intracellular transducer Smad1, whose action is strictly cell autonomous (Doshch et al., 1997). The other mechanism contributing to the generation of the BMP activity gradient is diffusion of the antagonists Noggin, Chordin and Follistatin (reviewed in Dale and Wardle, 1999). Xenopus noggin has dose-dependent effects on mesodermal patterning (ReemKalma et al., 1995; Doshch et al., 1997; Jones and Smith, 1998), as would be expected if the ratio between BMP and BMP antagonists determines cell fates. Consistent with this scenario, the BMP-inhibiting effect of Noggin spreads far beyond expressing cells, indicating that the protein is highly diffusible and hence capable of generating a BMP activity gradient (Doshch et al., 1997; Jones and Smith, 1998). These results argued for a model where positional information in the gastrula marginal zone is provided by graded BMP activity that is high ventrally and low dorsally. Graded BMP activity is the result of superimposition of antagonizing BMP and BMP antagonists, e.g. Noggin, Chordin and Follistatin. Put differently, what appeared macroscopically to be induction, is microscopically modification of a ventral morphogen read-out. This model has been supported independently by genetic evidence in zebrafish (reviewed in Holley and Ferguson, 1997).

An important question is how quantitative differences in morphogen signalling are converted into qualitative discrete cellular responses (McDowell and Gurdon, 1999). Our work focused on transcriptional targets of BMP signalling with the discovery of the Xvent genes (Gawantka et al., 1995). In Xenopus, candidate transcriptional targets that mediate the effects of BMPs are msx1 in ectoderm (Suzuki et al., 1997), and the Xvent-1 and Xvent-2 homeobox genes in ecto- and mesoderm (Gawantka et al., 1995; Onichtchouk et al., 1996). The fact that Xvents were reported independently five times in 1996 (called PV.1, Xbr1, Vox and Xom; Ladher et al., 1996; Papalopulu and Kintner, 1996; Schmidt et al., 1996; Tidman-Ault et al., 1996) highlights how mature the field of organizer regulation had become by then. Xvent-1 and -2 are expressed in a nested fashion in the gastrula marginal zone, which is regulated by the BMP morphogen (Doshch et al., 1997). They function as transcriptional repressors and appear to play a role in regulating target gene expression in cells responding to the BMP activity gradient (Friedle et al., 1998; Onichtchouk et al., 1998, 1999; Rastegar et al., 1999; Trindade et al., 1999; Melby et al., 1999, 2000; Schuler-Metz et al., 2000).

BMP signalling is subject to delicate regulation at multiple levels, both extracellularly and intracellularly (reviewed in Cho and Blitz, 1998). As a novel regulator, we identified the transmembrane protein BAMBI, which
plays a role in attenuating BMP signalling. \textit{BAMBI} encodes a TGF-\(\beta\) pseudoreceptor, which interferes with TGF signalling by intercalating in the TGF complex, as we found out in a collaboration with Ye-Guang Chen and Joan Massagué (Onichtchouk et al., 1999).

**Antagonizing the head organizer: anti-Wnts**

Spemann, and subsequently Otto Mangold, had shown that the organizer is not a homogenous tissue but a dynamic structure; while cells involute in the dorsal blastopore lip during gastrulation, they acquire different fates and inducing properties. Early involving endomesoderm induces head structures while later involuting mesoderm induces trunks (reviewed in Gilbert and Saxen, 1993; Nieuwkoop, 1997). Inductions elicited by overexpressed BMP antagonists are predominantly of trunk nature, which left open the question of the nature of the head inducer. Moon and colleagues had already shown that Wnts can act as potent antagonists of head formation in \textit{Xenopus} when expressed after midblastula transition, by injecting plasmid DNA instead of mRNA (Christian and Moon, 1993). This was a surprising finding, since at the time Wnts were thought to act in setting up the Nieuwkoop centre, a signalling centre inducing the Spemann organizer at the blastula stage. So unorthodox was this posteriorizing property of Wnts that not only did Randy Moon’s study face strong initial resistance, but Olivier Destree, who had reported the same finding 3 years earlier at a meeting, could never publish it (O. Destree, personal communication).

We observed that co-expression of Wnt and BMP antagonists is sufficient to induce ectopic heads in \textit{Xenopus} and, furthermore, that the first head inducer discovered, \textit{cerberus} (Bouwmeester et al., 1996), is a potent Wnt inhibitor (Glinka et al., 1997). This suggested a two-inhibitor model for regional specific induction with anti-BMPs alone inducing trunk and anti-BMP + anti-Wnts inducing heads (Figure 1). It also made sense in light of the expression of the Wnt inhibitors \textit{cerberus} and \textit{fzrb} (Leyns et al., 1997; Wang et al., 1997) in anterior endomesoderm, i.e. the region that harbours head organizer activity, and this predicted that a distinguishing feature of head inducers is their ability to inhibit Wnt signalling. The prediction was confirmed when Andrei Glinka subsequently cloned another Wnt inhibitor of the head organizer in the lab, which we named \textit{dickkopf} (German for thick-headed, stubborn), since its expression leads to big-headed embryos (Glinka et al., 1998). The term Dickkopffrosch existed already, as I learned later, having been coined for a big-headed species described in the 19th century, as depicted by E. Haeckel (Figure 2). \textit{dickkopf1} (dkk1) is a member of a novel gene family encoding secreted proteins, and its co-expression with BMP inhibitors induces entire secondary heads (Figure 3A). Such double heads also occur in humans, and an early specimen from the famous collection of Meckel (Halle, Germany) is shown in Figure 4. Luckily, we obtained an inhibitory antibody against Dkk1, as loss-of-function is the weak point of the \textit{Xenopus} system. Microinjection of this antibody induced small-headed tadpoles, indicating that the protein was indeed required for head induction (Figure 3B).

![Fig. 1. Two-inhibitor model for organizer regionalization. Trunk induction requires inhibition of BMP signalling while head induction requires dual inhibition of Wnt and BMP signalling. The organizer produces factors that inhibit both types of signals (anti-wnt, Dkk1, Cerberus and Frzb; anti-BMP: Cerberus, Noggin, Chordin and Follistatin). Regional specificity of induction results from differential expression of Wnt and BMP inhibitors in endomesoderm and chordamesoderm. Note that Cerberus also inhibits Nodal and Activin signalling, which may be important to maintain anterior endomesodermal fate (adapted from Glinka et al., 1997).](image1)

![Fig. 2. Dickkopffrosch. Drawing of a specimen of \textit{Brevicpes mossambicus}, indigenous to east Africa. From Haeckel (1998).](image2)

Our two-inhibitor model for regional specific induction obviously was very simplified (Glinka et al., 1997). Indeed, further work showed that the head inducer \textit{cerberus} is in fact an antagonist of BMPs, Wnts and Nodals (Piccolo et al., 1999). Nodals, like BMPs, are members of the TGF growth factor superfamily and are related to activin. They play important roles in mesoderm and endoderm induction and can act in a graded fashion to induce distinct cell fates at different concentrations (De Robertis et al., 2000; Schier and Shen, 2000). The role of activins in mesoderm induction was the topic of the pioneering work of J. Smith and is reviewed in his EMBO medal review (Smith, 1993).

Attenuation of Nodal signalling by the inhibitors \textit{cerberus}, \textit{activin} and \textit{lefty} (Meno et al., 1999; Piccolo et al., 1999; Thissen and Thissen, 1999) may be essential in the formation of anterior endomesoderm. Furthermore, signalling by FGF8 may finally constitute an instructive signal required for anterior neural induction in addition to inhibition of Wnt and BMP growth factors (Alvarez et al., 1998; Streit et al., 2000; Wilson et al., 2000). Yet the
predictions of the two-inhibitor model prove valid and help to focus research (Niehrs, 1999). For example, it fits nicely with the observations that the zebrafish bozozok (boz) gene, encoding a homeodomain protein, promotes BMP and Wnt antagonism in the organizer, and that boz mutation leads to microcephaly (Fekany-Lee et al., 2000). Furthermore, it explains the microcephaly observed in zebrafish mutant for the Wnt pathway inhibitor tcfβ (Kim et al., 2000). Finally, it is in line with the observation of anterior defects in mice double mutant for the BMP antagonists noggin and chordin (Bachiller et al., 2000), and of posterior defects seen in mice mutant for Wnt3a (Takada et al., 1994).

**Biology à grande échelle**

An important factor for progress made in our lab was the venture into a new type of large-scale screen, based on simply screening randomly picked cDNAs for their expression patterns at moderate resolution by whole-mount *in situ* hybridization in *Xenopus* embryos and expressed sequence tag (EST) sequencing of the differentially expressed genes (Gawantka et al., 1998). This screen was set in the middle of two opposite experimental paradigms: in the terms of Hans Lehrach, learning a lot about one gene versus learning little about lots of genes. In the beginning, we earned ridicule because such brute-force screening was thought unlikely to yield interesting genes or concepts, and this kind of work would probably never have gained funding in the USA. Yet, the *in situ* screen paid off quickly. By screening 1800 cDNAs, not only did we find useful novel marker genes, but also identified interesting developmental control genes, including *Xvent* and the TGF-β pseudoreceptor *BAMBI* discussed above; the *Xblimp1* zinc finger gene, a regulator of anterior endomesoderm and essential for head induction (de Souza et al., 1999); and the basic helix–loop–helix gene *ESR4*, involved in Delta–Notch signalling (Jen et al., 1999).

**Fig. 3.** Head induction by *dickkopf1*. (A) A tadpole-stage embryo that has been injected with mRNA encoding a dominant-negative BMP receptor and *dkk1* forms a complete secondary head. (B) A tadpole embryo microinjected with inhibitory anti-Dkk1 antibodies lacks anterior head structures. Reproduced from de Souza and Niehrs (2000) with permission.

**Fig. 4.** Human diccephalus. An 18th century skeleton of a young child, displaying axial and head duplication, from the anatomical collection of Johann Friedrich Meckel (Halle, Germany). Reproduced from Schumacher (1996) with permission.

Refuting the notion that this type of screen does not teach any new concepts, the screen led us to the discovery of synexpression groups. Fifteen per cent of the 270 differentially expressed genes identified could be grouped into four gene sets which each share a very distinctive, complex expression pattern. An example is the BMP4 group (Figure 5), members of which are expressed like this growth factor dorsally in the eye, the heart, tailbud and lateral plate mesoderm of tailbud-stage *Xenopus* embryos. This group consists of seven members which all encode components of the BMP signalling pathway as studied in early dorsoventral patterning of mesoderm, including ligands, receptor and downstream components of the pathway. Such sets of coordinately expressed genes that act in the same process are referred to as ‘synexpression groups’ (Niehrs, 1997; Gawantka et al., 1998). The expression pattern of these genes is similar to that of growth factor since they themselves are transcriptional targets of the cascade. This group illustrates three other points: (i) the proteins encoded by genes of synexpression groups are not homologous (e.g. receptor, ligand and transcription factor), and hence co-expression is not the consequence of gene duplication; (ii) while the co-regulation is relatively tight, there are minor differences in the pattern, e.g. unlike the other members of the group, BMP4 itself is expressed in the otic vesicle; and (iii) not all components of a pathway are coordinately expressed. For example, *Smad1* and *Smad4*, which also participate in BMP4 signalling, are ubiquitously expressed during early embryogenesis (Lagna et al., 1996; Meersman et al., 1997). These genes may not be part of the synexpression group because they function in other pathways as well, as is the case for *Smad4* which is required for signalling not only of BMPs but also of other TGF-β members (Massagué, 1998). Other synexpression groups identified
striking formal parallel to the prokaryote operon. With the advent of co-regulated gene clusters identified by DNA chip analysis, the idea of co-expression modules is now commonplace (Eisen et al., 1998; Niehrs and Pollet, 1999; Tavazoie et al., 1999; Lockhart and Winzeler, 2000).

**Outlook**

Spemann and Mangold’s dreams have come true. We are understanding embryonic induction at the molecular level to a degree that allows the production of vertebrate embryos with extra heads, tails and limbs. A surprisingly small number of signalling molecules regulate, in a combinatorial fashion, a vast array of patterning processes during axis formation and organogenesis. With the big picture becoming reasonably clear, the field is moving increasingly towards biochemical and cell biological analysis of the inducing factors. However, these signalling molecules carry rather little information per se. After all, there is a big difference between fly blastoderm and frog mesoderm, yet they are patterned by an analogous extracellular signalling system. Similarly, the decision to form a fore- or hindlimb is a function of the competence of the responding tissue, not of the inducer, and this is controlled by the particular set of transcription factors expressed in the cells. Hence, we will probably see research moving towards understanding the control of the transcriptional programme of embryonic cells and the intracellular network regulating it. In the age of whole sequenced genomes, global gene expression profiling, as well as whole genome comparisons to study relevant promoters, will probably play an eminent role in deciphering these processes.

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