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Paradigm Shifts In Neural Induction

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Abstract

SUMMARY. — The molecularization of developmental biology was originally seen as a challenge to the integrity of that discipline. However, important new insights from the analysis of gene expression soon transformed the field from one of experimental anatomy to one of developmental genetics. One of the main areas to be transformed from an anatomical to a molecular study was « primary embryonic induction ». The molecular analyses showed that some of the fundamental concepts concluded from the experimental embryological approach to primary embryonic induction were false. First, the neural fate of cells was not being induced. Rather, the epidermal fate was induced and the neural state was the default, uninduced, fate of ectodermal tissues. Second, primary embryonic induction was not something unique to vertebrates. Rather, the ventral neural cord of insects formed using the same mechanisms as the dorsal neural tube of vertebrates. Third, the brain formed in a matter distinctly different from that of the spinal cord. Despite these differences, there has been a clear and strong continuity between the experimental embryological tradition and the molecular genetic tradition, and these new results are seen by many contemporary developmental geneticists as strengthening, rather than destroying, the older science.

Résumé

RÉSUMÉ. — La « molécularisation » de la biologie du développement a d'abord été considérée comme une menace pour la discipline. Cependant, des aperçus nouveaux, issus de l'analyse de l'expression des gènes transformèrent bientôt le domaine d'une anatomie expérimentale en celui de l'expression des gènes. Un des principaux domaines à être transformé, d'une étude anatomique en une étude moléculaire, fut celui de l'« induction embryonnaire primaire ». Les analyses moléculaires montraient que certains concepts fondamentaux tirés d'une approche de l'induction embryonnaire primaire par l'embryologie expérimentale se révélaient faux. Tout d'abord, le destin neural des cellules n'était pas induit. Ce qui était induit était le destin épidermique. L'état neural était le destin défaillant et non induit des tissus ectodermiques. En second lieu, l'induction embryonnaire primaire n'était pas réservée uniquement aux vertébrés. La corde neurale ventrale des insectes est formée en utilisant les mêmes mécanismes que ceux employés par le tube neural dorsal des vertébrés. En troisième lieu, la formation du cerveau est une affaire distincte, différente de celle de la colonne vertébrale. En dépit de ces différences, il existe une continuité claire et forte entre la tradition de l'embryologie expérimentale et celle de la génétique moléculaire ; ces données nouvelles sont considérées par de nombreux généticiens du développement comme un renforcement plutôt qu'une destruction de la science antérieure.

Paradigm shifts in neural induction

Scott F. GILBERT (*)

RÉSUMÉ. — La « molécularisation » de la biologie du développement a d'abord été considérée comme une menace pour la discipline. Cependant, des aperçus nouveaux, issus de l'analyse de l'expression des gènes transformèrent bientôt le domaine d'une anatomie expérimentale en celui de l'expression des gènes. Un des principaux domaines à être transformé, d'une étude anatomique en une étude moléculaire, fut celui de l' « induction embryonnaire primaire ». Les analyses moléculaires montraient que certains concepts fondamentaux tirés d'une approche de l'induction embryonnaire primaire par l'embryologie expérimentale se révélaient faux. Tout d'abord, le destin neural des cellules n'était pas induit. Ce qui était induit était le destin épidermique. L'état neural était le destin défaillant et non induit des tissus ectodermiques. En second lieu, l'induction embryonnaire primaire n'était pas réservée uniquement aux vertébrés. La corde neurale ventrale des insectes est formée en utilisant les mêmes mécanismes que ceux employés par le tube neural dorsal des vertébrés. En troisième lieu, la formation du cerveau est une affaire distincte, différente de celle de la colonne vertébrale. En dépit de ces différences, il existe une continuité claire et forte entre la tradition de l'embryologie expérimentale et celle de la génétique moléculaire ; ces données nouvelles sont considérées par de nombreux généticiens du développement comme un renforcement plutôt qu'une destruction de la science antérieure.

MOTS-CLÉS. — Induction neurale ; induction primaire ; Spemann ; organisateur ; changement de paradigme ; molécularisation ; biologie du développement.

SUMMARY. — The molecularization of developmental biology was originally seen as a challenge to the integrity of that discipline. However, important new insights from the analysis of gene expression soon transformed the field from one of experimental anatomy to one of developmental genetics. One of the main areas to be transformed from an anatomical to a molecular study was « primary embryonic induction ». The molecular analyses showed that some of the fundamental concepts concluded from the experimental embryological approach to primary embryonic induction were false. First, the neural fate of cells was not being induced. Rather, the epidermal fate was induced and the neural state was the default, uninduced, fate of

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ectodermal tissues. Second, primary embryonic induction was not something unique to vertebrates. Rather, the ventral neural cord of insects formed using the same mechanisms as the dorsal neural tube of vertebrates. Third, the brain formed in a matter distinctly different from that of the spinal cord. Despite these differences, there has been a clear and strong continuity between the experimental embryological tradition and the molecular genetic tradition, and these new results are seen by many contemporary developmental geneticists as strengthening, rather than destroying, the older science.

KEYWORDS. — Neural induction ; primary induction ; Spemann ; organizer ; paradigm shift ; molecularization ; developmental biology.

INTRODUCTION

The induction of the amphibian central nervous system has long been the model for those cell and tissue interactions forming the vertebrate body axis. The history of the hunt for organizers is a fascinating story of a large, international, field of science, and it includes great victories and great disappointments. From the late 1930s to the mid-1980s, the « primary induction problem » was considered a graveyard of biologists, a problem so fraught with non-specificity, uninterpretable results, and conflicting data, that a young biologist would be foolish to enter the morass. Joseph Needham (1) summarized the mood of those scientists who had been working on this problem :

> « In conclusion, it may be said that although the progress made in the past ten years in these fields has been very great, we can nevertheless see now that owing to the special difficulties of the subject [...], it may be more like fifty years before we can expect to have certain knowledge concerning the chemical nature of the naturally occurring substances involved in embryonic induction. »

His fifty years turned out to be a prescient prediction. Near the end of that span of time, other investigators (2) began to question the validity of the enterprise searching for these molecules :

> « More than fifty years of effort have failed to reveal the putative inductor substances, nor has any progress been made in discovering the

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(1) Joseph Needham, Biochemical aspects of organizer phenomena, *Growth*, suppl., 3 (1939), 45-52.

(2) Marcus Jacobson, Origin of the nervous system in amphibians, *in* Nicholas C. Spitzer (ed.), *Neuronal Development* (New York : Plenum, 1982), 45-99.

cellular mechanisms of release, transmission, reception, and interpretation of developmental signals that are supposed to result in regional differentiation. »

Even Saxén, Toivonen, and Nakamura (3), three of the few researchers whose laboratories continued to investigate primary embryonic induction during the 1970s, lamented :

> « Why do the scientists investigating embryonic induction lag behind their brilliant colleagues in many other areas of biology in which the 1960s and 1970s have witnessed many great victories and discoveries of fundamental importance ? »

The reasons turn out to be quite simple. First, developmental biology had pushed its biochemical techniques to the limit. The proteins responsible for induction are present in very low concentrations, and the embryos are not able to be obtained in enough volume to offset this disadvantage. Second, the amphibian embryo (on which experiments of neural induction were performed) contains large amounts of yolk and lipid that interfere with the purification procedures of traditional biochemistry (4). It would take the tools of a sophisticated brand of molecular biology to find the inducer molecules and to delineate what these factors were doing. However, once the techniques of gene cloning and *in situ* hybridization opened the floodgates, we have been deluged with information about how these processes occur.

In fact, within the past five years, these techniques have occasioned three major paradigm shifts in this area. First, as we shall see, the paradigm for neural induction (since 1924) has been that soluble molecules are secreted by the Organizer, a structure that comprises the pharyngeal mesendoderm that underlies the anterior head, the notochord that underlies the dorsalmost ectoderm of the remaining amphibian embryo, and the dorsal blastopore lip which gives rise to the notochord and pharyngeal mesendoderm. These soluble factors have been thought to actively instruct the ectodermal cells above it to become neurons. This was concluded by experiments involving both positive and negative inference.

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(3) Lauri Saxen, Sulo Toivonen, and Osamu Nakamura, Concluding remarks. Primary embryonic induction : An unsolved problem, *in* Osamu Nakamura and Sulo Toivonen (eds), *Organizer : A milestone of a half-century from Spemann* (Oxford : Elsevier-North Holland, 1978), 315-320.

(4) Horst Grunz, Neural induction in amphibians, *Current Topics on developmental biology*, 35 (1997), 191-228.

Removing notochord from beneath an area that would otherwise become neural tube causes the ectoderm to become epidermal, while adding notochord to areas that would usually produce epidermis caused these cells to produce a new neural tube. So the ectoderm was seen as having two major fates : neural if underlain by the notochord, and epidermal if it were not underlain by the notochord. The default state was for these ectodermal cells to become epidermis. New information concerning the identity and nature of these factors secreted by the Organizer now causes scientists to think that the default state of the ectoderm is to become *neural*, and that the ectoderm cells are actively induced by the ventral and lateral mesoderm to become *epidermal*. The Organizer is now thought to act by blocking these epidermal-inducing molecules, thereby preventing the induction of the ectoderm above it. In the absence of this induction to epidermis, the dorsal ectoderm above the Organizer becomes committed to a neural fate.

The second paradigm shift brought about by our knowledge of the factors inducing neural and epidermal specification is that the specification of the insect ventral nerve cord and the vertebrate dorsal neural tube are accomplished by the same set of instructions. The two types of nervous systems develop in very different manners, and prior to 1995, it had been thought that the instructions to form these two types of nervous systems were very different. We now know that the instructions for specifying which region of the ectoderm is to form neural tissue are remarkably similar between these two highly diverged groups of organisms.

The third paradigm has been that induction caused all the newly induced neural cells to initially assume the fate of forebrain tissue. Only after this initial « activation » would the « transformation » of this fate into midbrain, hindbrain, and spinochordal structures commence. This paradigm has also been called into question by the findings that different inducer molecules are produced by the cells in the region underlying the anterior head and that these molecules have functions distinct from those factors which induce the rest of the neural tube.

PRIMARY EMBRYONIC INDUCTION

The problem of « primary embryonic induction » can be dissected into three sets of inductive events :

a / What factors induce the formation of the Organizer ? It turns out the cells of the Organizer are themselves induced (by the endodermal cells underlying it).

b / What are the structures and functions of those molecules which are produced by the Organizer and which neuralize the ectoderm above it, dorsalize the mesoderm adjacent to it, and anterioralize the endoderm beneath it ?

c / How are the regions of the neural tube specified according to their respective anterior-posterior location along the axis ?

This article looks at the central problem of these three, the question that historically has been considered as « the induction problem ». We will therefore consider 1° how the « soluble inducing factors » have become purified molecules and 2° how knowledge of what these molecules do has changed our traditional ways of thinking about induction. We will focus our attention on the neuralizing property of the organizer, since, until recently, this function was the only one being extensively studied.

In 1924, Hans Spemann and Hilde Mangold revolutionized embryology with their discovery that a particular region of the embryo was responsible for the emergence of the central nervous system. They showed that when this region, the dorsal blastopore lip, was transplanted from one salamander embryo into the future ventral region of another such embryo, it invaginated completely. Moreover, the transplanted dorsal lip tissue (and no other transplant from the early-gastrula-stage embryo) produced a new notochord that caused the formation of a new neural tube and, subsequently, a secondary embryo (5). They called this region the

(5) Hans Spemann and Hilde Mangold, Über Induktion von Embryonanlagen durch Implantation artfremder Organisatoren, *Wilhelm Roux' Archiv für Entwicklungsmechanik der Organismen* [ci-après abrégé *Roux' Archiv*], 100 (1924), 599-638 ; Viktor Hamburger, *The Heritage of experimental embryology* (Oxford : Oxford Univ. Press, 1988) ; Peter E. Fässler, *Hans Spemann 1869-1941 : Experimentelle Forschung in Spannungsfeld von Empirie und Theorie* (Berlin : Springer, 1996). For a reprint and English translation of this paper, please see the forthcoming volume of the *International Journal of developmental biology* on *The Spemann-Mangold organizer*, edited by Eddy DeRobertis and Juan Arechaga (2001).

« organizer » of the embryo. The organizer, itself, did not contribute to the neural tissue. Rather, it formed the pharyngeal endoderm and dorsal mesoderm (notochord and somites) that lay beneath the cells that were to become the central nervous system. The cells of the new neural tube were derived primarily from the host ectoderm. Cells that otherwise would have remained epidermal were instructed to become neurons. The use of newts with differently pigmented eggs greatly facilitated their analysis of the five secondary embryos that resulted from their transplants. Of particular interest was transplant Um 132, wherein a dorsal blastopore lip from an advanced *Triturus cristatus* (unpigmented) gastrula was transplanted into the presumptive flank region of a more heavily pigmented, *Triturus taeniatus gastrula.* Sections taken from the tailbud larval stage showed that the donor cells became part of the notochord and somites of the secondary embryo. Many of the somites and most of the neural tube and other organs were derived from host tissues. Thus, a block of tissue from the dorsal blastopore lip was able to induce the formation of a secondary dorsal axis from host tissue. This induction subsequently initiated a cascade of other inductive events that led to the construction of the embryo (hence the use of the term « primary embryonic induction » to describe this event).

The published account (6), displaying the terms induction and organizer prominently in its title, was based on only the first two years of research, those encompassing the 1921 and 1922 breeding seasons. This publication refers to only six cases. Sander has shown that there later were a total of 275 transplants, of which fifty-five survived the operation (7). Twenty-eight of these had prominent secondary neural axes, and eleven of these secondary axes were flanked by somites. Spemann and Mangold coined the term « organizer » to emphasize the ability of this dorsal blastopore lip tissue to direct the development of the host tissue and to give these redirected cells a coherent, unified, organization.

« A piece of the upper blastoporal lip of an amphibian embryo undergoing gastrulation exerts an organizing effect on its environment in such a

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(6) Spemann and Mangold, *op. cit.* in n. 5.

(7) Klaus Sander, Hans Spemann, Hilde Mangold und der « Organisatoreffekt » in der Embryonalentwicklung, *Akademie Journal* (Januar 1993), 7-10.

way that, if transplanted to an indifferent region of another embryo, it causes there the formation of a secondary embryonic anlage. Such a piece can therefore be designated as an organizer (8). »

This tissue had the ability to invaginate and differentiate autonomously, to induce the neural plate and, by assimilative induction, to organize somites from the lateral plate mesoderm of the host.

This induction of the neural axis by the dorsal blastopore lip and its derivatives has also been demonstrated in *Xenopus laevis*, the amphibian of choice for most molecular studies (9). The *Xenopus* organizer appears to be similar in many ways to the « original » newt organizers, despite the difference in mesoderm formation between urodeles and anurans. Like the newt organizers, the *Xenopus* organizer field can regulate to induce secondary axes when split in half (10).

NEURAL INDUCTION BY DIFFUSIBLE MOLECULES

The mechanism of induction was controversial from the start. Basically, the argument has centered on whether the inductive signal from the dorsal blastopore lip was sent in a *cis*-fashion, anteriorly and horizontally through the plane of the ectodermal cells, or in a *trans*-fashion, from the dorsal mesoderm vertically to the overlying ectoderm. Spemann (11) was originally in favor of a hori-

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(8) Spemann and Mangold, *op. cit.* in n. 5, 637.

(9) Robert L. Gimlich and Jonathan Cooke, Cell lineage and the induction of nervous systems in amphibian development, *Nature*, 306 (1983), 471-473 ; J. C. Smith and J. W. M. Slack, Dorsalization and neural induction : Properties of the organizer in *Xenopus laevis*, *Journal of embryology and experimental morphology*, 78 (1983), 299-317 ; Marcus Jacobson, Clonal organization of the central nervous system of the frog. III. Clones starting from individual blastomeres of the 128-, 256-, and 512-cell stages, *Journal of neuroscience*, 3 (1938), 1019-1038 ; G. Recanzone and W. A. Harris, Demonstration of neural induction using nuclear markers in *Xenopus*, *Roux' Archiv*, 194 (1985), 344-354.

(10) R. M. Stewart and J. Gerhart, The anterior extent of dorsal development of the *Xenopus* embryonic axis depends on the quantity of organizer in the late blastula, *Development*, 109 (1990), 363-372.

(11) Spemann, *op. cit.* in n. 5 ; Hans Spemann, Über den Anteil von Implantat und Wirtskeim an der Orienterung und Beschaffenheit der induzierten Embryonanlage, *Roux' Archiv*, 123 (1931), 389-517 ; Otto Mangold und Hans Spemann, Über Induktion von Medullarplatte durch Medullarplatte im jüngeren Keim, *Roux' Archiv*, 111 (1927), 341-422.

zontally transmitted inducing signal that was sent by the dorsal blastopore lip from cell to cell through the ectoderm. However, evidence began pointing towards a diffusible inducer elaborated from the involuting mesoderm. First, Spemann's student Bruno Geinitz (12) showed that dorsal blastopore lips transplanted into the blastocoel induced excellent secondary neural tubes, and another student, Alfred Marx (13), showed that pure dorsal mesoderm from a late gastrula could induce the neural plate from the ectoderm, while pure ectoderm could not. This supported the view that signals were transmitted vertically from notochordal mesoderm to ectoderm. The article by Bautzmann *et alii* (14) – where each researcher presents his own rather preliminary set of experiments – showed that dead tissue (including not only dorsal blastopore lip-derived mesoderm, but also dead embryonic intestine and epidermis) could act as organizer and cause the ectoderm to form brain tissue. Moreover, Johannes Holtfreter (15) showed that when ectoderm was isolated in vitro and wrapped around notochord, the ectoderm will form brain structures. Surprisingly, not only did killed dorsal blastopore lip tissue induce, but so did killed endoderm, prospective epidermis and even boiled uncleaved salamander egg. This was confirmed using the « Einsteck-method » of implanting the potential inducing tissue inside the blastocoel directly beneath the ventral ectoderm. By these experiments, it appeared that the inducer was diffusible and that it could pass from an underlying inducing tissue. Holtfreter (16) soon followed these observations with his exogastrulation experiments demonstrating that neural tissue failed to form when the mesoderm failed to contact the ectoderm. He also showed that induction was prevented when a sheet of vitelline envelope was placed between the chordamesoderm and the ectoderm. Thus, the inducing signal appears to be transmitted vertically, from the dorsal mesoderm to the ectoderm above it.

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(12) Bruno Geinitz, Embryonale Transplantation zwischen Urodelen und Anuren, *Roux' Archiv*, 106 (1925), 357-408.

(13) Alfred Marx, Experimentelle Untersuchungen zur Frage der Determination der Medullarplatte, *Roux' Archiv*, 105 (1925), 20-44.

(14) Hermann Bautzmann, Johannes Holtfreter, Hans Spemann, and Otto Mangold, Versuche zur Analyse der Induktionsmittel in der Embryonalentwicklung, *Naturwissenschaften*, 20 (1932), 971-974.

(15) Holtfreter, *op. cit.* in n. 14.

(16) Johannes Holtfreter, Nachweis der Induktionsfähigkeit abgetöteter Keimteile, *Roux'iv.*, 128 (1933), 584-633.

The Bautzmann *et alii* article (17) initiated an enormous expansion of research which attempted to discover the identity of this inducing factor or factors. Løvtrup and his colleagues (18) remarked that « few compounds, other than the philosopher's stone, have been searched for more intensely than the presumed agent of primary induction in the amphibian embryo », and Harrison (19) referred to the amphibian gastrula as a « new Yukon to which eager miners were now rushing to dig for gold around the blastopore ». In 1953, Niu and Twitty (20) provided further evidence that diffusible factors from the dorsal mesoderm played a major role in primary induction. They put salamander dorsal mesoderm into a drop of culture medium. After conditioning this medium for several days, the mesodermal tissue was removed, and pieces of ectoderm were placed into the medium. These ectodermal explants became neural cells and pigment cells.

Such diffusible molecules were even more clearly demonstrated in a series of transfilter experiments. In 1961, Lauri Saxén demonstrated that neural induction could occur through a 150 micron thick, 0,8 micron pore size filter, strongly suggesting that the inducer was diffusible (21). Sulo Toivonen and coworkers (22) extended this work by showing that neural induction took place through a nucleopore filter even though electron microscopy failed to reveal any intercellular contact through the 0,05 um pores. The biochemical purification of this *Induktionsstoffe* had been part of the Finnish laboratory's program, starting with Toivonen's student, Taina Kuusi (23). It also became the focus of the Tiedemanns' ongoing

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(17) Bautzmann *et al.*, *op. cit.* in n. 14.

(18) Soren Løvtrup, U. Landstrom, and H. Løvtrup-Rein, Polarity, cell differentiation, and primary induction in the amphibian embryo, *Biological Reviews*, 53 (1978), 1-42, here 24.

(19) Quoted in Victor C. Twitty, *Of scientists and salamanders* (San Francisco : Freeman, 1966), 39.

(20) M. C. Niu and Victor C. Twitty, The differentiation of gastrula ectoderm in medium conditioned by axial mesoderm, *Proceedings of the National Academy of sciences* [États-Unis], 39 (1953), 985-989.

(21) Lauri Saxén, Transfilter neural induction of amphibian ectoderm, *Developmental Biology* [ci-après abrégé *Dev. Biol.*], 3 (1961), 140-152.

(22) Sulo Toivonen and J. Wartiovaara, Mechanism of cell interaction during primary induction studied in transfilter experiments, *Differentiation*, 5 (1976), 61-66 ; Sulo Toivonen, D. Tarin, L. Saxén, P. J. Tarin, and J. Wartiovaara, Transfilter studies on neural induction in the newt, *Differentiation*, 4 (1975), 1-7.

(23) Taina Kuusi, Über die chemische Natur der Induktionsstoffe im Implantatversuch bei *Triton*, *Experientia*, 7 (1951), 299-300.

research program (24) who showed that neuralizing factors from amphibian embryos may be isolated as large complexes and remain active when complexed to Sephadex beads (25). This suggests that the factor(s) act by binding to membrane rather than by entering the cell.

The Japanese program for the study of embryonic induction also concentrated efforts in finding inducer substances. Begun by professors Yo Okada and Osamu Nakamura, this work is being continued by professor Makato Asashima, himself a student of Heinz Tiedemann. Eventually, it was Asashima's work on activin which culminated the biochemical search for the organizer molecules. His work linked the Japanese studies to those of the German group by showing that the caudalizing factor isolated in the German laboratory was the same as the activin-like factor found by Japanese researchers (26).

Other laboratories, such as the Cambridge-based group of Conrad Waddington and Joseph and Dorothy Needham, attempted to find the inducer by seeing which natural substances could induce neural plate formation when added to competent ectoderm or implanted into the blastocoel (27). In their work of 1933 and 1935, Waddington and the Needhams (28) showed that ether extracts of

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(24) Heinz Tiedemann, U. Becker, and H. Tiedemann, Chromatographic separation of a hindbrain-inducing substance into mesodermal and neural inducing factors, *Biochimica Biophysica Acta*, 74 (1963), 557-560 ; Heinz Tiedemann and Hildegard Tiedemann, Das Induktionsvermögen gereinigter Induktionsfaktoren im Kombinationsversuch, *Revue suisse de zool.*, 71 (1964), 117-137 ; Heinz Tiedemann, F. Lottspeich, M. Davids, S. Knochel, P. Hoppe, and Ha. Tiedemann, The vegetalizing factor. A member of the evolutionarily highly conserved activin family, *FEBS Letters*, 300 (1992), 123-126.

(25) Jochen Born, J. Janeczek, W. Schwarz, H. Tiedemann, and Ha. Tiedemann, Activation of masked neural determinants in amphibian eggs and embryos and their release from the inducing tissue, *Cell Differentiation and development*, 27 (1989), 1-7 ; J. Janeczek, J. Born, P. Hoppe, and H. Tiedemann, Partial characterization of neural inducing factors from *Xenopus* gastrulae – evidence for a larger protein complex containing the factor, *Roux' Archiv*, 201 (1992), 30-35.

(26) Makato Asashima, H. Uchiyama, H. Nakano *et al.*, The vegetalizing factor for chicken embryos – its EDF (activin A)-like activity, *Mechanisms of development*, 34 (1991), 135-141.

(27) Pnina Abir-Am, The philosophical background of Joseph Needham's work in chemical embryology, *in* Scott F. Gilbert (ed.), *A conceptual history of modern developmental biology* (New York : Plenum Press, 1991), 159-180.

(28) Conrad H. Waddington, J. Needham, W. W. Nowinski, and R. Lemberg, Studies on the nature of the amphibian organization centre. I. Chemical properties of the evocator, *Proceedings of the Royal Society* [London] *B*, 117 (1935), 289-310 ; Conrad H. Waddington

adult newts could act as an organizer. Since this activity could turn presumptive epidermis into non-specific neural tissue, Waddington called this activity as the « evocator ». (The molecules specifying the type of neural tissue were referred to as the « individuators ».) The properties of the evocator fraction suggested that it was a steroid, and both natural and artificial steroids were found to induce neural plates. A steroid inducer made a great deal of sense (29), since sterols had been found to be the basis for male and female sex hormones, cancer-producing hydrocarbons, cardiac glycosides, and vitamin D. Moreover, such sterol compounds had been found in eggs. It was expected that steroid-like hormones would function in early development just like they did during later development.

THE PROBLEM OF NON-SPECIFICITY

However, sterols were not the only chemicals that induced neural development. One of the reasons for the lack of knowledge about these inducer molecules was the lack of a stringent assay system. It appeared that numerous totally unrelated compounds could induce neural development from the ectoderm of early salamander gastrulae. Following the Bautzmann *et alii* paper (30), the strategy during the 1930s was simple and straightforward : the normal target tissue, the competent ectoderm, was exposed to various candidate molecules, and the results were monitored as morphologically distinguishable secondary structures after prolonged cultivation. At first, progress was stimulating, and scientists in various laboratories reported successful induction with various purified compounds. The initial reports that natural lipid molecules could induce neural tubes initially caused great excitement, and Waddington and Joseph Needham spent over three years attempting to biochemically characterize the active agent in the ether extracts. However, some of the neural-inducing molecules

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and Dorothy M. Needham, Studies on the nature of the amphibian organization centre. II. Induction by synthetic polycyclic hydrocarbons, *Proceedings of the Royal Society* [London] *B*, 117 (1935), 310-317.

(29) Joseph Needham, *Order and life* (New Haven : Yale Univ. Press, 1936).

(30) Bautzmann *et al.*, *op. cit.* in n. 14.

were so unlike one another that there seemed to be no structural specificity. To further complicate matters, the German workers claimed that acids (oleic, linoleic and nucleic) initiated induction (31), and Barth's experiments (32) implicated a protein inducer. If this were not confusion enough, Waddington and colleagues (33) showed that unnatural compounds that did not even resemble naturally occurring molecules were able to induce neural formation in the ectoderm. Even a dye, methelene blue, induced neural tubes. As Waddington and Dorothy Needham end their discussion to one of their articles (34) :

> « Dodds has metaphorically spoken of these synthetic substances as skeleton keys, which can unlock several doors [...] Here the skeleton key is so unlike the householder's latchkey that one wonders whether the house has been entered through the back-door, or, in an even more unorthodox manner, through a window. »

In 1936, Waddington, Needham, and Brachet hypothesized that the evocator substance might be produced throughout the embryo, but it was just released or activated in one particular region (35). This fitted well with Holtfreter's discovery (36) that non-inducing regions of the amphibian gastrula could acquire the ability to induce when they were killed by ethanol treatment. Herrmann (37) has called this period « the biochemical Odyssey » of the 1930s and it is recounted in Needham (38) and in Saxén and Toivonen (39).

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(31) Else Wehmeier, Versuche zur Analyse der Induktionsmittel bei der Medullarplattenduktion von Urodelen, *Roux' Archiv*, 132 (1934), 384-423.

(32) L. G. Barth, The chemical nature of the amphibian organizer : III. Stimulation of the presumptive epidermis of Ambystoma by means of cell extracts and chemical substances, *Physiological zoology*, 12 (1939), 22-29.

(33) Conrad H. Waddington, J. Needham, and J. Brachet, Studies on the nature of the amphibian organization centre. III. The activation of the evocator, *Proceedings of the Royal Society* [London] *B*, 120 (1936), 173-198.

(34) Waddington and D. Needham, *op. cit.* in n. 28, 316.

(35) *Op. cit.* in n. 33.

(36) Johannes Holtfreter, Die totale Exogastrulation, eine Selbstrablösung des Ektoderms vom Entomesoderm, *Roux' Archiv*, 129 (1933), 669-793.

(37) Heinz Herrmann, Molecular mechanisms of differentiation : An inquiry into the protein forming system of developing cells, *in* W. W. Novinski (ed.), *Fundamental Aspects of normal and malignant growth* (Amsterdam : Elsevier, 1960), 494-545 on 520.

(38) Joseph Needham, *Biochemistry and morphogenesis* (Cambridge : Cambridge Univ. Press, 1959).

(39) Lauri Saxén and Sulo Toivonen, *Primary Embryonic Induction* (London : Academic Press, 1962).

THE MOLECULARIZATION OF THE ORGANIZER

In 1962, Waddington reinterpreted induction and inducers in terms of molecular biology. In particular, he linked embryonic induction to enzymatic induction (40). Inducible enzymes had been called adaptive enzymes until the early 1950s, and their relationship to development had been proposed by Jacques Monod as early as 1947. Monod (41) saw the phenomenon of enzymatic adaptation as a possible solution to the problem of how identical genomes could synthesize different « specific » molecules. That same year, another researcher in this field, Sol Spiegelman (42) redefined embryonic differentiation as « the controlled production of unique enzymatic patterns ». He altered the terminology of the adaptive enzyme studies, claiming that such enzymes were *induced.* He thus took the notion of « adaptive enzymes » out of the domain of evolution (where they seemed Lamarckian anyway) and into the domain of embryology. In 1953, the major researchers in this field agreed, signing a joint letter to *Nature* (43). The directed enzymatic synthesis would be known as « enzyme induction » and « any substance thus inducing enzyme synthesis is an enzyme "inducer" ».

When the Jacob and Monod model for the *lac* operon was reported, Waddington immediately saw the importance of enzymatic induction for studies of embryonic induction (44). He even made a diagram based on primary embryonic induction wherein the evocator (inducer) would diffuse from the mesoderm and either act directly on the structural genes or else combine with a regulator

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(40) Conrad H. Waddington, *New Patterns in genetics and development* (New York : Columbia Univ. Press, 1962).

(41) Jacques Monod, The phenomenon of enzymatic adaptation and its bearing on problems of genetics and cellular differentiation, *Growth Symposium*, 11 (1947), 223-289.

(42) Sol Spiegelman, Differentiation as the controlled production of unique enzymatic patterns, *in* J. F. Danielli and R. Brown (eds), *Growth in relation to differentiation and morphogenesis* (Cambridge : Cambridge Univ. Press, 1948), 286-325.

(43) M. Cohn, J. Monod, M. R. Pollock, S. Spiegelman, and R. Y. Stanier, Terminology of enzyme formation, *Nature*, 172 (1953), 1096.

(44) Waddington, *op. cit.* in n. 40. Scott F. Gilbert, Enzyme adaptation and the entrance of molecular biology into embryology, *in* Sahotra Sarkar (ed.), *The Philosophy and history of molecular biology : New perspectives* (Dordrecht : Kluwer Acad. Publishers, 1996), 101-123.

substance to make the factor that activated the genes by binding to a promoter region. However, the number, kind, and functions of these possible inducer molecules were totally unknown.

Understanding the biochemical mechanisms of induction would have to wait for the techniques of molecular biology. By the late 1980s, several developmental biologists felt that molecular biology had finally something to offer them. Fred Wilt (45) urged that the time had come for molecular biology to try explaining development, and John Gurdon (46) concluded : « Nucleic acid technology has probably now reached a sufficient level of precision and efficiency of operation to be usefully applied to the analysis of inductive responses... »

The first of these better techniques was the *Xenopus* assay systems that had been pioneered by Gurdon. Unlike the salamanders and toads used previously, the ectoderm of *Xenopus laevis* fails to respond to non-specific neural inducers (47). Thus, the problem of non-specificity was avoided when *Xenopus* was used. For a long while, it did not seem like anything induced neural tube formation in these frogs, and frustration mounted (see above). Indeed, it first appeared that neural induction in *Xenopus* did not take place through the vertical induction system at all. Rather, the evidence from *Xenopus* suggested that induction was through the plane of the ectoderm. In the 1980s and early 1990s, several laboratories had shown that the *Xenopus* ectoderm is heterogeneous with respect to its neural competency, and that this difference is generated both by cell autonomous differences between cleavage-stage blastomeres (48) and by a signal emanating in a cis-fashion from the

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(45) Fred H. Wilt, Determination and morphogenesis in the sea urchin, *Development*, 100 (1987), 559-575.

(46) John B. Gurdon, Embryonic induction : Molecular prospects, *Development*, 99 (1987), 285-306, here 302.

(47) Chris R. Kintner and Douglas A. Melton, Expression of *Xenopus* NCAM RNA in ectoderm is an early response to neural induction, *Development*, 99 (1987), 311-325 ; A. Ruiz i Altaba, Planar and vertical signals in the induction and patterning of the *Xenopus* nervous system, *Development*, 116 (1992), 67-80.

(48) H. Kageura, and K. Yamana, Pattern regulation in isolated halves and blastomeres of early *Xenopus laevis*, *Journal of embryology and experimental morphology*, 74 (1983), 221-234 ; Id., Pattern regulation in defect embryos of *Xenopus laevis*, *Dev. Biol.*, 101 (1984), 410-415 ; Rebecca M. Akers, C. R. Phillips, and N. K. Wessells, Expression of an epidermal antigen used to study tissue induction in the early *Xenopus laevis* embryo, *Science*, 231 (1986), 613-616 ; Cheryl London, R. M. Akers, and C. R. Phillips, Expression of epi1, an epidermis-specific marker in *Xenopus laevis* embryos, is specified prior to gastrulation, *Dev.*

newly formed dorsal blastopore lip (49). *Xenopus* exogastrulae (of the kind that Holtfreter made with newt embryos) express NCAM, NF3, and Xhox3, three antigens found within induced ectoderm but not in presumptive epidermal tissue (50). Using a modified sandwich technique that prevented the dorsal mesoderm from vertically contacting the ectoderm, Doniach and coworkers (51) and Ruiz i Altaba (52) both showed that four position-specific neural markers were expressed in the explant ectoderm in the appropriate anterior-posterior sequence. Similarly, Keller and coworkers (53) demonstrated that planar signals from the early gastrula dorsal blastopore lip are both necessary and sufficient to induce convergent extension and NCAM expression in the presumptive hindbrain-spinal cord ectoderm directly adjacent to it. However, this ectoderm did not roll into a tube or form the dorsal-ventral pattern typical of the normally induced neural tube. These latter functions have been ascribed to the notochord (54) and probably represent actions of the underlying mesoderm upon the overlying ectoderm.

Biol., 129 (1988), 380-389 ; Betty C. Gallagher, A. M. Hainski, and S. A. Moody, Autonomous differentiation of dorsal axial structures from an animal cap cleavage blastomere in *Xenopus*, *Development*, 112 (1991), 1103-1114.

(49) C. R. Sharpe, A. Fritz, E. M. De Robertis, and J. B. Gurdon, A homeobox-containing marker of posterior neural differentiation shows importance of predetermination in neural induction, *Cell*, 50 (1987), 749-758 ; Robert Savage and Carey Phillips, Signals from the dorsal blastopore lip region during gastrulation bias the ectoderm toward a non-epidermal pathway of differentiation in *Xenopus laevis*, *Dev. Biol.*, 133 (1989), 157-168.

(50) Kintner and Melton, *op. cit.* in n. 47 ; Jane E. Dixon and Chris R. Kintner, Cellular contacts required for neural induction in *Xenopus laevis* embryos : Evidence for two signals, *Development*, 106 (1989), 749-757 ; A. Ruiz i Altaba, Neural expression of the *Xenopus* homeobox gene Xhox 3 : Evidence for a patterning neural signal that spreads through the ectoderm, *Development*, 108 (1990), 595-604.

(51) Tabitha Doniach, C. R. Phillips, and J. C. Gerhart, Planar induction of anteroposterior pattern in the developing central nervous system of *Xenopus laevis*, *Science*, 257 (1992), 542-545.

(52) Ruiz i Altaba, *op. cit.* in n. 47.

(53) Ray Keller, J. Shih, A. K. Sater, and C. Moreno, Planar induction of convergence and extension of the neural plate by the organizer of *Xenopus*, *Developmental Dynamics*, 193 (1992), 218-234.

(54) Holtfreter, *op. cit.* in n. 36 ; Jodi Smith, and Gary C. Schoenwolf, Notochordal induction of cell wedging in the chick neural plate and its role in neural tube formation, *Journal of experimental zoology*, 250 (1989), 49-62 ; H. W. M. von Straaten, J. W. M. Hekking, J. P. W. M. Beursgens, E. Terwindt-Rouwenhorst, and J. Drukker, Effect of the notochord on proliferation and differentiation in the neural tube of the chick embryo, *Development*, 107 (1989), 793-803 ; T. Yamada, M. Placzek, H. Tanaka, J. Dodd, and T. M. Jessell, Control of cell pattern in the developing nervous system : Polarizing activity of floor plate and notochord, *Cell*, 64 (1991), 635-647.

These experiments were criticized by several groups (55). Using different procedures, they halted the migration of the mesoderm into the embryo at various stages of gastrulation. When dorsal mesoderm (notochordal) invagination was stopped at the onset of gastrulation, no dorsal axis was formed. However, when the invagination was halted midway through gastrulation, only the anterior structures were missing. Inhibiting the last movements of gastrulation had little or no effect on axis formation. This suggested that vertical, *trans*, signals from the mesoderm were indeed critical for the development of the dorsal axis. Doniach and her colleagues (56) hypothesized that while the planar signals might be most important early in gastrulation, the trans-inducing signals from the notochord might be essential in reinforcing this pattern and bringing the mesodermal and ectodermal axial patterns into register with one another. So in 1992 it looked like the paradigm of vertical induction from the notochord to the ectoderm had reached an impasse. No soluble factors had been found, and a different source of inductive agency had been proposed.

IDENTIFYING THE « INDUCERS »

This impasse was broken in 1993. Earlier, Smith and Harland (57) had isolated a gene whose product appeared to dorsalize the mesoderm. This gene, noggin, was found by constructing a cDNA plasmid library from dorsalized (lithium-treated) gastrulae. RNAs synthesized from sets of these plasmids were injected into

(55) John Gerhart, M. Danilchik, T. Doniach, S. Roberts, B. Rowning, and R. Stewart, Cortical rotation of the *Xenopus* egg : Consequences for the anteroposterior pattern of embryonic dorsal development, *Development* (suppl.), 107 (1989), 37-52 ; C. R. Sharpe and J. B. Gurdon, The induction of anterior and posterior neural genes in *Xenopus laevis*, *Development*, 109 (1990), 765-774 ; Ali Hemmati-Brivanlou, R. M. Stewart, and R. M. Harland, Region-specific neural induction of an engrailed protein by anterior notochord in *Xenopus*, *Science*, 250 (1990), 800-802.

(56) Doniach, *op. cit.* in n. 51.

(57) William C. Smith and Richard M. Harland, Injected *wnt-8* RNA acts early in *Xenopus* embryos to promote formation of a vegetal dorsalizing center, *Cell*, 67 (1991), 753-765 ; Id., Expression cloning of noggin, a new dorsalizing factor localized to the Spemann organizer in *Xenopus* embryos, *Cell*, 70 (1992), 829-840.

the ventralized embryos (having no neural tube) produced by irradiating early embryos with ultraviolet light. Such UV-treated embryos have no dorsal blastopore lip, no notochord, and no organizer activity. Those sets of plasmids whose RNAs rescued the dorsal axis were split into smaller sets, and so on, until single plasmid clones were isolated whose mRNAs were able to restore the dorsal axis in such embryos. One of these clones contained noggin. Smith and Harland (58) have shown that newly transcribed (as opposed to maternal) noggin mRNA was first localized in the dorsal blastopore lip region and then became expressed in the notochord. Moreover, if the early embryo were treated with lithium chloride (LiCl) so that the entire mesodermal mantle became notochord-like organizer tissue, then noggin mRNA was found throughout the mesodermal mantle. Treatment of the early embryo with ultraviolet light inhibited the synthesis of noggin mRNA. Injection of noggin mRNA into 1-cell, UV-irradiated embryos completely rescued the dorsal axis and allowed the formation of a complete embryo. The mRNA sequence for the noggin protein suggested strongly that it is a secreted protein.

In 1993, Smith and colleagues (59) found that noggin protein could accomplish two major functions of the organizer : it induced neural tissue from the dorsal ectoderm, and it dorsalized the mesoderm cells that would otherwise contribute to the ventral mesoderm. Moreover, the noggin protein was also able to induce neural tissue in gastrula ectoderm without the presence of any dorsal mesoderm (60). When noggin was added to gastrula (or animal cap) ectoderm, the ectodermal cells were induced to express forebrain-specific neural markers. Moreover, the gene products for notochordal or muscle cells were not induced by the noggin protein.

The second candidate was a protein called chordin (61). Chordin was also isolated by using dorsalized (lithium-treated) embryos. Here, duplicate filters containing members of a plasmid library

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(58) Smith and Garland (1992), *op. cit.* in n. 57.

(59) William C. Smith, A. K. Knecht, M. Wu and R. M. Harland, Secreted noggin mimics the Spemann organizer in dorsalizing *Xenopus* mesoderm, *Nature*, 261 (1993), 547-549.

(60) Teresa M. Lamb, A. Knecht, W. C. Smith *et al.*, Neural induction by the secreted polypeptide noggin, *Science*, 262 (1993), 713-718.

(61) Yoshiki Sasai, B. Lu, H. Steinbeisser, D. Geissert, L. K. Gont, and E. M. De Robertis, *Xenopus* chordin : A novel dorsalizing factor activated by organizer-specific homeobox genes, *Cell*, 79 (1994), 779-790.

constructed from normal dorsal blastopore lip cDNA were hybridized to radioactive probes from either dorsalized or vegetalized embryos. This technique isolated clones whose mRNAs were present in the dorsalized but not in the ventralized embryos. These clones were tested by injecting them into ventral blastomeres and seeing if they induced secondary axes. One of the clones capable of inducing a secondary neural tube contained the chordin gene. The chordin mRNA was found to be localized in the dorsal blastopore lip and later in the dorsal mesoderm of the notochord.

The third candidate for an inducer molecule was follistatin. This molecule was found through an unexpected result in an experiment designed to determine whether the growth factor activin was critical for mesoderm induction (62). Ali Hemmati-Brivanlou and Douglas Melton (63) constructed a dominant negative activin receptor and injected it into embryos. Remarkably, the ectoderm began to express neural-specific proteins. It appeared that the activin receptor (which also binds other structurally similar molecules such as the bone morphogenetic proteins) normally functioned to bind an inhibitor of neurulation. By blocking its function, all the ectoderm became neural. In 1994, Hemmati-Brivanlou and Melton (64) proposed a « default model of neurulation » whereby the organizer would produce inhibitors of whatever was inhibiting neurulation. This model was supported by and explained the cell dissociation experiments which had produced odd results. Here, three laboratories (65) had shown that when whole embryos or their animal caps are dissociated, they form neural tissue. This would be explainable if the « default state » was not epidermal, but neural, and that the tissue would have to be induced to have an epidermal phenotype. The organizer, then, would block this epidermalizing induction.

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(62) Asashima, *op. cit.* in n. 26.

(63) Ali Hemmati-Brivanlou and Douglas A. Melton, A truncated activin receptor inhibits mesoderm induction and formation of axial structures in *Xenopus* embryos, *Nature*, 359 (1992), 609-614 ; Id., Inhibition of activin signalling promotes neuralization in *Xenopus*, *Cell*, 77 (1994), 273-281.

(64) Hemmati-Brivanlou (1994), *op. cit.* in n. 63.

(65) Horst Grunz, and L. Tacke, Neural differentiation of *Xenopus laevis* ectoderm takes place after disaggregation and delayed reaggregation without inducer, *Cell Differentiation and development*, 32 (1989), 117-124 ; Sheryl M. Sato and Thomas D. Sargent, Development of neural inducing capacity in dissociated *Xenopus* embryos, *Dev. Biol.*, 134 (1989), 263-366 ; S. F. Godsave and J. M. W. Slack, Clonal analysis of mesoderm induction in *Xenopus*, *Dev. Biol.*, 134 (1989), 486-490.

Since mutated activin receptors caused neural tissue to form, it was thought that natural activin inhibitors might be used by the embryo in a similar manner to specify the neural ectoderm. One of these natural inhibitors of activin (and its related compounds such as bone morphogenesis proteins) is follistatin. Using *in situ* hybridization, Hemmati-Brivanlou and Melton (66) found the mRNA for follistatin in the dorsal blastopore lip and notochord.

So it appeared that there might be a neural default state and an actively induced epidermal fate. This was counter to the neural induction model that had preceded it for 70 years. But what proteins were inducing the epidermis, and were they really being blocked by the molecules secreted by the organizer ?

The leading candidate appeared to be bone morphogenesis protein-4 (BMP4). There appeared to be an antagonistic relationship between BMP4 and the dorsal mesoderm. If the mRNA for BMP4 were injected into 1-cell *Xenopus* eggs, all the mesoderm in the embryo became ventrolateral mesoderm, and no involution occurred at the blastopore lip (67). Moreover, when animal caps from embryos injected with *bmp4* mRNA were isolated and implanted into the blastocoels of young *Xenopus* blastulae, they caused the formation of an extra tail. Conversely, overexpression of a dominant-negative BMP4 receptor (which should block BMP4 reception) resulted in the formation of two dorsal axes (68). Thus, BMP4 seemed to be able to override the dorsal signals. In the mid-1990s, studies from the De Robertis laboratory showed that chordin specifically interfered with BMP4 (69).

(66) Hemmati-Brivanlou (1994), *op. cit.* in n. 63.

(67) L. Dale, G. Howe, B. M. J. Price, and J. C. Smith, Bone morphogenetic protein 4 : A ventralizing factor in early *Xenopus* development, *Development*, 115 (1992), 573-585 ; C. Michael Jones, K. M. Lyons, P. M. Lapan, C. V. E. Wright, and B. L. M. Hogan, DVR-4 (bone morphogenetic protein-4) as a posterior-ventralizing factor in *Xenopus* mesoderm induction, *Development*, 115 (1992), 639-647.

(68) Jonathan M. Graff, R. S. Thies, J. J. Song, A. J. Celeste, and D. A. Melton, Studies with a *Xenopus* BMP receptor suggest that ventral mesoderm-inducing signals override dorsal signals *in vivo*, *Cell*, 79 (1994), 169-179 ; Mitsugu Maeno, R. C. Ong, A. Suzuki, N. Ueno and H. F. Kung, A truncated bone morphogenesis protein-4 receptor alters the fate of ventral mesoderm to dorsal mesoderm : Role of animal pole tissue in the development of ventral mesoderm, *Proceedings of the National Academy of sciences* [États-Unis], 91 (1994), 10260-10264.

(69) Stefano Piccolo, Y. Sasai, B. Lu, and E. M. De Robertis, Dorsoventral patterning in *Xenopus* : Inhibition of ventral signals by direct binding of chordin to BMP-4, *Cell*, 86 (1996), 589-598 ; Scott A. Holley, P. D. Jackson, Y. Sasai, B. Lu, E. M. De Robertis, F. M. Hoffmann, and E. L. Ferguson, A conserved system for dorsal-ventral patterning in insects and vertebrates involving sog and chordin, *Nature*, 376 (1995), 249-253.

BMP4 is initially expressed throughout the ectoderm and mesodermal regions of the late blastula. However, during gastrulation, BMP4 transcripts are restricted to the ventrolateral marginal zone (70). The BMP4 protein induces the expression of several transcription factors (Xvent-1, Vox, Mix.1, Xom) that are key regulators of ventral mesodermal and ectoderm development. These transcription factors induced by BMP4 repress dorsal genes while at the same time activating ventrolateral mesodermal proteins (71). Wilson and Hemmati-Brivanlou (72) also showed that the addition of BMP4 to dissociated ectoderm cells prevented them from becoming neural. Thus, by 1996, it seemed that BMP4 was the active inducer of ventral ectoderm (epidermis) and ventral mesoderm (blood cells and connective tissue), and that chordin would prevent its function. The organizer worked by secreting an inhibitor of BMP4, not by directly inducing neurons.

This hypothesis obtained further credence from an unexpected source – the emerging field of evolutionary developmental biology. It was shown that the same chordin-BMP4 interaction that instructed the formation of the neural tube in vertebrates also formed the neural tube in flies (73). The dorsal neural tube of the vertebrate and the ventral neural tube of the fly appeared to be generated by the same set of instructions, conserved throughout evolution. De Robertis and Sasai (74) even resurrected E. Geoffroy Saint-

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(70) Ali Hemmati-Brivanlou and Gerald H. Thomsen, Ventral mesodermal patterning in *Xenopus* embryos : Expression patterns and activities of BMP-2 and BMP-4, *Developmental Genetics*, 17 (1995), 78-89 ; Jennifer Northrop, A. Woods, R. Seger, A. Suzuki, N. Ueno, E. Krebs, and D. Kimelman, BMP-4 regulates the dorso-ventral differences in FGF/MAPKK-mediated mesoderm induction in *Xenopus*, *Dev. Biol.*, 172 (1995), 242-252.

(71) Volker Gawantka, H. Delius, K. Hirschfeld, C. Blumenstock, and C. Niehrs, Antagonizing the Spemann organizer : Role of the homeobox gene Xvent-1, *EMBO Journal*, 14 (1995), 6268-6279 ; Stephanie H. B. Hawley, K. Wünnenberg-Stapleton, C. Hashimoto, M. N. Laurent, T. Watabe, B. W. Blumberg, and K. W. Y. Cho, Disruption of BMP signals in embryonic *Xenopus* ectoderm leads to direct neural induction, *Genes and development*, 9 (1995), 2923-2935 ; Paul E. Mead, I. H. Brivanlou, C. M. Kelly, and L. I. Zon, BMP-4 repressive regulation of dorso-ventral patterning by the homeobox protein Mix. 1, *Nature*, 382 (1996), 357-360 ; Jennifer E. Schmidt, G. van Dassow, and D. Kimelman, Regulation of dorsal-ventral patterning : The ventralizing effects of the novel *Xenopus* homeobox gene *Vox*, *Development*, 122 (1996), 1711-1721.

(72) Paul A. Wilson and Ali Hemmati-Brivanlou, Induction of epidermis and inhibition of neural fate by BMP-4, *Nature*, 376 (1995), 331-333.

(73) Holley, *op. cit.* in n. 69 ; Eddy M. De Robertis and Y. Sasai, A common plan for dorsoventral patterning in Bilateria, *Nature*, 380 (1996), 37-40.

(74) *Ibid.*

Hilaire's 1822 discussion (75) of the lobster being but the mouse upside-down and that all animals might be variations upon a common theme. This was the second paradigm shift occasioned by the newly acquired information on the molecular biology of induction.

Several laboratories found that noggin, follistatin, and chordin each prevented the BMP4 proteins from either maturing or binding to the prospective dorsal cells (76). The organizer was not so much an inducer as the structure that protected cells from being induced. The neural state was not that which was achieved by induction, but was that fate which was not induced.

HEAD FORMATION

The third paradigm concerned the nature of the induced neural tissue. It was thought that all the neural tissue induced by the organizer was of forebrain specificity and that the organizer initially used the same activator/evocator molecules throughout its length. But the most anterior portion of the organizer appears to be different from the rest. Whereas most of the dorsal ectoderm is underlain by notochord, the most anterior regions of the head and brain are underlain by anterior pharyngeal endomesoderm. This endomesoderm constitutes the first cells of the dorsal blastopore lip. Recent studies have shown that these cells not only induce the most anterior head structures, but that they do it using a mechanism distinct from blocking BMP4.

In 1993, Jan Christian and Randall Moon showed that Xwnt8, a member of the Wnt family of growth and differentiation factors, also inhibited neural induction (77). Xwnt8 was found to be syn-

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(75) Étienne Geoffroy Saint-Hilaire, Considérations générales sur la vertèbre, *Mémoires du Muséum d'histoire naturelle*, 9 (1822), 89-119.

(76) Yoshiki Sasai, B. Lu, S. Piccolo, and E. M. De Robertis, Endoderm induction by the organizer-secreted factors chordin and noggin in *Xenopus* animal caps, *EMBO Journal*, 15 (1996), 4547-4555 ; Lyle B. Zimmerman, J. De Jesús-Escobar, and R. M. Harland, The Spemann organizer signal noggin binds and inactivates bone morphogenetic protein 4, *Cell*, 86 (1996), 599-606.

(77) Jan L. Christian and Randall T. Moon, Interactions between Xwnt8 and Spemann organizer signaling pathways generate dorsoventral pattern in the embryonic mesoderm of *Xenopus*, *Genes and development*, 7 (1993), 13-28.

thesized throughout the marginal mesoderm – except in the region forming the dorsal blastopore lip. Thus, a second anti-neuralizing secreted protein had been found.

In 1996, Tewis Bouwmeester and colleagues (78) showed that the induction of the most anterior head structures could be accomplished by a secreted protein called Cerberus. Unlike the other secreted proteins, Cerberus promoted the formation of the cement gland, eyes, and olfactory placodes. When *cerberus* mRNA was injected into the vegetal ventral set of *Xenopus* blastomeres at the 32-cell stage, ectopic head structures were formed. These head structures were made from the injected cell as well as from neighboring cells. The *cerberus* gene was found to be expressed in the endomesoderm cells that arise from the deep cells of the early dorsal blastopore lip. It was not found throughout the notochord. Two things this protein did were to bind both BMPs and Xwnt8 (79).

Shortly thereafter, two other proteins, Frzb and Dickkopf, were discovered to be synthesized in the involuting endomesoderm. Frzb is a small soluble form of the Wnt receptor protein which is capable of binding Wnt proteins in solution (80). If embryos are made to synthesize excess Frzb, the Wnt signaling pathway fails to occur, and the embryos lack ventral posterior structures, becoming solely head. The Dickkopf protein also appears to interact directly with Wnt proteins extracellularly. Injection into the blastocoel of antibodies against Dickkopf protein causes the resulting embryos to have small deformed heads (81).

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(78) Tewis Bouwmeester, S.-H. Kim, Y. Sasai, B. Lu, and E. M. De Robertis, Cerberus is a head-inducing secreted factor expressed in the anterior endoderm of Spemann's organizer, *Nature*, 382 (1996), 595-601.

(79) Andrei Glinka, W. Wu, D. Onichtchouk, C. Blumenstock, and C. Niehrs, Head induction by simultaneous repression of BMP and Wnt signaling in *Xenopus*, *Nature*, 389 (1997), 517-519.

(80) Lue Leyns, T. Bouwmeester, S.-H. Kim, S. Piccolo, E. M. De Robertis, Frzb-1 is a secreted antagonist of Wnt signaling expressed in the Spemann organizer, *Cell*, 88 (1997), 747-756; Shouwen Wang, M. Krinks, K. Lin, F. P. Luyten, and M. Moos, Jr., Frzb, a secreted protein expressed in the Spemann organizer, binds and inhibits Wnt-8, *Cell*, 88 (1997), 757-766.

(81) Andrei Glinka, W. Wu, A. P. Monaghan, C. Blumenstock, and C. Niehrs, Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction, *Nature*, 391 (1998), 357-362.

Andrei Glinka and colleagues (82) have thus proposed a new model for embryonic induction. The induction of trunk structures may be caused by the blockade of BMP signaling from the notochord. However, to produce a head, one needs to block both the BMP signal and the Wnt signal. This blockade comes from the endomesoderm, now considered the most anterior portion of the organizer. Interestingly, in 1931, Spemann thought that there might be two organizers, one for the head and one for the trunk. After 1933, he did not push this view further. The following figure provides one current interpretation of neural induction in the amphibian embryo.

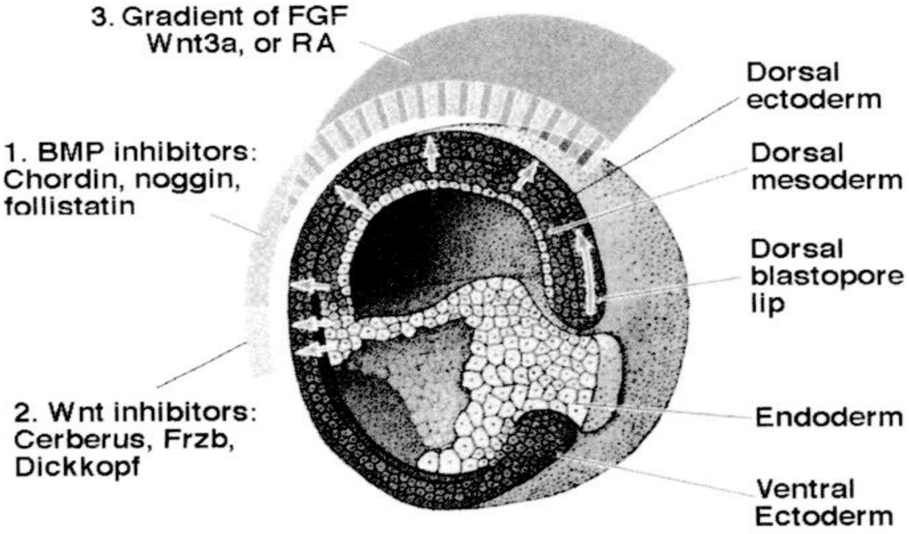

Fig. 1. — *Model for organizer function and axis specification in the* Xenopus *gastrula*

1 / BMP inhibitors from organizer tissue (dorsal mesoderm and pharyngeal mesendoderm) block the formation of epidermis, ventrolateral mesoderm, and ventrolateral endoderm.

2 / Wnt inhibitors in the anterior of the organizer (pharyngeal mesendoderm – the first cells of the dorsal blastopore lip that involute into the embryo) allow the induction of head structures.

3 / Gradients of caudalizing factors (eFGF, retinoic acid, and/or Wnt3a) specify the regional properties of the neural tube along the anterior-posterior axis. (Scott F. Gilbert, *Developmental Biology*, 6th ed. (Sunderland : Sinauer Associates, 2000), 333 ; with permission.)

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(82) Glinka, *op. cit.* in n. 79.

CONCLUSIONS

The past five years have seen the overturning of three major paradigms in the field of primary embryonic induction, one of the most central areas of all developmental biology. These paradigms have been replaced by new models that have yet to be fully tested. The first paradigm shift concerned the default state of the uninduced tissue. It had been thought that epidermis was the default state of the ectoderm. Now it appears that dissociated ectoderm cells naturally become neural. They have to be induced to become epidermal, and the organizer functions to block this induction. The second paradigm shift involved the perceived differences between insect and vertebrate nerve formation. What had been thought to be extremely different mechanisms of forming a nervous system appears now to be remarkably homologous. The third paradigm concerned the nature of the inducer. It was not thought that the most anterior region of the head was induced differently than the rest of the body. Now it appears that a second pathway has to be inhibited in order for the head to form properly.

Interestingly, all these signals for neural specificity appear to be blockers of epidermal induction. They appear to say : « You won't become epidermal. » Might we expect, though, to find other signals that say, « and you will become neural ». We find such pushes and pulls throughout the embryo. The instructions for cell division have both a « You will divide », and a « You won't not-divide » component. Similarly, the instructions to cells to form a testis also come with a « and you will not form an ovary » component. In order to make a *Drosophila* head, you must upregulate the head-forming genes and down-regulate everything else. As we predicted in an earlier paper (83), both positive and negative signals should coexist. But where are we finding the positive signals for neural induction ? These might be coming *via* the planar route mentioned earlier. This route appears to act early in development and may even be present in the early blastula. Given the evidence for the

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(83) Scott F. Gilbert and Lauri Saxén, Spemann's organizer : Models and molecules, *Mechanisms of development*, 41 (1993), 73-89.

existence of these planar signals, it would seem reasonable to expect that they would be ones promoting neural specification.

The research into primary embryonic induction provides a fascinating example of how molecular biology can challenge the core paradigms of a central portion of a discipline without disrupting that discipline. The field of developmental biology was not shaken to its foundations by the revelations of these molecular approaches. Rather, the molecular biology was seen to be at a deeper level, and it served to explain some of the outlying phenomenon (such as the neuralization of dissociated ectoderm). The experiments of Spemann and Mangold documenting primary embryonic induction generated the framework of modern experimental embryology. The reasons for this continuity are many (84) and probably include the fact that many of the experimental embryologists who had worked on the organizer problem are still alive and able to give counsel and instruction to the new generation of molecularly oriented investigators – as is the case with Hamburger for instance (85). Moreover, the young investigators could underscore the importance of their research by linking it to this classical and primary enigma of experimental embryology. The continuity of « the tradition of inquiry » was more important than the rhetorical stance of discontinuity. The fact that many of the molecularly oriented researchers were aware of and appreciative of the history of embryology most likely plays a major role, as well. Research into the molecular mechanisms of these phenomena are still providing new frameworks for investigating the development of organisms from eggs to adults.

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(84) Scott F. Gilbert. Continuity and change : Paradigm shifts in neural induction. *International Journal of developmental biology*, 45 (in press). This is a similar review to the present one, stressing the molecular aspects for a scientific audience. Also, Herbert Steinbeisser. The impact of Spemann's concepts on molecular embryology. *International Journal of developmental biology*, 40 (1996), 61-68.

(85) Hamburger (see footnote 5) writes explicitly to instruct molecular biologists about the problem posed by experimental embryology. The forthcoming edition of the *International Journal of developmental biology* is edited by a molecular embryologist and a classical embryologist and illustrates this continuity.