Int. J. Dev. Biol. 45: 73-81 (2001)

Contrasting influences of the Organizer and induction concepts on the scientific activity of French embryologists

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Introduction

During the 19th century and before World War I, several significant German embryological textbooks had been translated into French. After World War I, such was not the case for Spemann's synthetic work on the analysis of embryonic development (1936), which was, on the other hand, almost immediately translated into English (1938). On the contrary, T.H. Morgan's book on "Embryology and Genetics" (1934) had been translated into French by J. Rostand in 1935. Curiously, Morgan's book did not mention the original reference of Spemann and Mangold's crucial experiments (1924), but only that of an English review published by Spemann one year later. In any event, the importance of the organizer concept was very quickly realized by French biologists (who read German), even though there were no French embryologists working in German laboratories at that time. Biologist Maurice Caullery, a well-known professor at Paris University, had spent a year in Germany, during his youth, visiting laboratories (Fischer, 1990). In 1939, he published a book on recent advances in experimental embryology, in which several chapters were devoted to the organizer and induction problems. But, as we shall see, no significant experimental work was then undertaken in France to expand our knowledge of the properties of the organizer in other animal models or to tackle the problem of the chemical nature of the supposed inducing factors, which already appeared disconcerting. Indeed, there were few embryologists in France at that time. The French-speaking Belgian embryologists were a reference for biologists. We shall see that French embryologists were involved in other original research work and did not change their activities. Moreover, another experimental field had been opened in the early 1930s. With the purification of sex hormones, known molecules were at last available to embryologists, who could start work on their effects on sex differentiation, i.e., on morphogenesis of gonads and genital tracts in Vertebrates. Two young French developmental biologists, after they had finished their thesis work, immediately started studying the effects of sex steroids on Vertebrate embryonic models, i.e., on the chick embryo (Etienne Wolff, who had been trained in Ancel's laboratory in Strasbourg) and on the frog tadpole (Louis Gallien, who first worked under Caullery's direction). Both of them were later to become the leading figures of French embryology (Beetschen, 1990; Fischer, 1990), but the beginning of their scientific career was marked for a long time by their fruitful work on experimental intersexuality, obtained by hormone treatment of embryos and larvae. Hormones were then considered as a special kind of inductor. Before we come back to later studies in Wolff's and Gallien's laboratories, directly relevant to the theme of organizer and embryonic inductions, we shall consider a few events related to research on the organizer, which occurred in France before World War II.

Abbreviations used in this paper: PGC, primordial germ cells.

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0214-6282/2001/$25.00

Printed in Spain
www.ijdb.ehu.es

Vintemberger's experiments on the frog egg (1932-1938)

Immediately after World War I, Professor Paul Ancel (1873-1961) was appointed to the Chair of Embryology at the Faculty of Medicine of Strasbourg. There, he started collaborating with Pierre Vintemberger as early as 1920. In 1924, they presented a series of communications to the "Société de Biologie", concerning their first results on the effects of X-rays on the development of chick and frog embryos. At this time, there was a controversial debate on the possible acceleration of development by X-rays (Ancel and Vintemberger, 1925). These studies were extended during the following years and gave birth to no less than 14 publications between 1924 and 1929. Embryonic cells were a good model to study the radio-sensitivity of diverse mitotic stages.

A consequence of this research work was the possibility to induce teratological defects by localized irradiation in the chick embryo. As a young student of Ancel, Etienne Wolff started working on this theme for several years, which resulted in a landmark thesis article (Wolff, 1936). Wolff much later reported that he would have been interested in working on the organizer and embryonic induction, but that Ancel told him that *"he [would] only be following in the footsteps of the Germans, and it [would] be difficult to come up with original findings"* (Dieterlen, 1990). Wolff then began working on the effects of sex hormones, as we have said.

At the same time, Ancel and Vintemberger had started another collaboration on activation and the determination of the bilateral symmetry plane and of dorsal-ventral polarity in the brown frog egg. The first two preliminary notes appeared in 1932 and were followed by many others (43 communications were presented at the "Société de Biologie" between 1933 and 1948 !). A long article, which is still frequently cited and is also considered a landmark publication, was finally issued (Ancel and Vintemberger, 1948). Thus, it seems clear that Ancel, who recognized the importance of the organizer, had decided not to work on it but rather to explore other fundamental and controversial fields.

Nevertheless, Vintemberger alone, in parallel with the above-mentioned studies, also worked on the organizer problem for a few years. His familiarity with the frog egg allowed him to design original experiments on the early determination and properties of the organizer in *Rana temporaria*. At this time, the German scientists had not yet much investigated the anuran embryo, having devoted most of their studies to urodeles.

Working on the 8-cell stage, Vintemberger first studied the developmental potencies of the four macromeres, which displayed rather high regulative properties. Then he examined the fate of the four micromere quartet, which was not able to organize a microembryo, though the dorsal micromeres contained a part of the grey crescent and of the presumptive chordal area. On the other hand, a rotation of 180° of the micromere quartet on the macromeres did not impede the formation of a normal single embryo. It thus appeared that the organizer area was not determined as early as believed by Brachet (1931) (Vintemberger, 1934, 1935 a,b). Dalcq (1933) performed a similar 180° "translocation" of the animal hemisphere on the vegetal one at the blastula stage, in the frog *Discoglossus,* after cutting the grey crescent area in two opposite parts, and he obtained a double embryo. Two years later, Vintemberger published the results of experiments in which he had associated the four micromeres of the 8-cell stage with a base of vegetal – most cells (presumptive endoderm) from a blastula (Fig. 1).

Small blastulae and abnormal gastrulae developed from these associations, giving rise to mesodermal and neural axial organs (notochord, somites, neural tube). This experiment showed that the micromeres were able to differentiate according to their presumptive significance, including the ability to form a functional organizer, when endodermal cells had exerted an influence on them (Vintemberger, 1936a). Vintemberger also transplanted the presumptive organizer area from a young frog blastula into the ventral marginal zone of another blastula and obtained double embryos (Vintemberger, 1936b). The presumptive chordal area of a young blastula was then heterotopically transplanted into the animal epiblastic ventral side of a recipient blastula and gave rise to various abnormal induced formations (Vintemberger, 1937). Finally, Vintemberger use Mangold's "Einsteckmethode" and inserted dorsal or ventral fragments of the equatorial area of young blastulae into the blastocoel of recipient blastulae (Vintemberger, 1936c). He also grafted more or less large pieces from the organizer area of a gastrula in the ventral animal side, i.e., in the epiblastic area, of a recipient blastula (Vintemberger, 1938 a,b).

All together, Vintemberger's results pointed to the lack of an early functional determination of the presumptive organizer area at the 8-cell stage, but to its presence at the early or mid-blastula stage, since the transplanted fragments were then able to induce gastrulation phenomena and the subsequent differentiation of axial organs. In any case, it was clear that the most animal part of the grey crescent area, which should give rise to a part of the presumptive chordal area, was not able to differentiate autonomously at the 8-cell stage. The stage at which the equatorial part of the grey grescent becomes functionally determined remained unknown, since it was not possible to isolate it from the macromeres, but it was shown than the endodermal vegetal-most cells might later interfere with this phenomenon. This was long before the role of endodermal cells in inducing mesoderm differentiation from ectodermal cap tissues was demonstrated by Nieuwkoop. Vintemberger also performed various experiments using X-ray irradiation to selectively kill blastomeres or the blastoporal area of frog embryos. They did not bring significant insight to the problems of organizer formation and role. After 1938, Vintemberger did not go further into his experiments on the frog organizer, and from then on devoted himself to the study of bilateral symmetry determination first in the frog egg (in collaboration with Ancel), then in the chick egg (in collaboration with Clavert).

A radical epigenesist: Paul Wintrebert (1867 – 1966)

Paul Wintrebert was first trained as a surgeon at the end of the 19th century, then turned himself into a vertebrate embryologist, being appointed to the Chair of Comparative Anatomy in Paris University in 1923. He worked both in Paris and in the marine biological laboratory of Banyuls-sur-Mer, near the Spanish border, where he had discovered, in 1906, the anuran *Discoglossus* living in the wild. From 1928 on and for many years, Wintrebert devoted himself to the study of *Discoglossus* development. He described the peculiar aspects of egg fertilization (at the animal pole), egg rotations, grey crescent formation, and cleavage until gastrula formation. He made large use of Vogt's vital staining techniques to

draw a fate map of the blastula and gastrula stages but his results were much different from those obtained by Vogt on *Bombinator*, although the latter is of the same family as *Discoglossus*. Thus, Wintrebert rapidly came into an open conflict with the Belgian embryologists (Dalcq, Pasteels), who worked on *Discoglossus* too, about the interpretation of the fate maps.

Wintrebert had very few or no collaborators and did not experiment with microsurgical techniques, which might appear as paradoxical for a surgeon. He was in fact a theorist, fighting against all aspects of preformationist influences (the so-called « germinal localizations » of the egg). He developed a theory of *physiological epigenesis* in which he insisted on the successive states of embryonic development as being purely physiological, the anlagen having no topographical preexistence or even true existence (Wintrebert, 1935a, b). Development thus is considered only as a chain of physiological states conditioned by the preceding steps, without a preorganised pattern (see also the article "Wintrebert", in Tort, 1996).

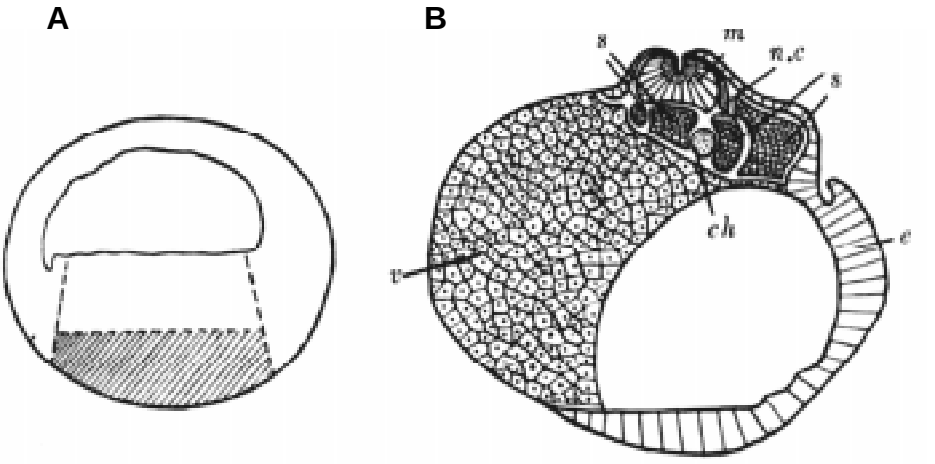

Fig. 1. Formation of axial organs after association of the micromere quartet (at 8-cell stage), with a base of vegetal endoderm cells from a frog blastula. (A) *Section through a young blastula showing the hatched endodermal region to be cut off from it, then associated with 4 micromeres.* **(B)** *Section through the obtained embryo: ch, notochord; e, epiblast; n, neural tube; n.c, neural crest; s, somites; v, endodermal yolk-laden cells. (modified from Vintemberger, 1936a).*

According to Wintrebert, the significance of the organizer was limited by the assumption of an "*initiator mitotic center*", appearing dorsally above the equator of the blastula, then moving towards and under the equator and inducing a mitogenic wave, giving rise to the organizing center itself at the beginning of the gastrula stage. According to this theory, the organizer does not exist before the invagination of the dorsal blastopore lip. It forms from the rolling up of the "mitogenic field". The main argument in favor of this mitogenic center was the presence of a dorsal area where the cells are smaller, interpreted as a consequence of an increased division rate. But this did not take into account the influence of the initially smaller size of the early dorsal blastomeres and no mitotic counts were made. Anyhow, Spemann's conception of the organizing center was considered by Wintrebert as a preformationist one and had to be discarded as such, though the importance of the organizer was of course recognized. Considering the "translocation" experiments on the *Discoglossus* blastula performed by Dalcq (1933), Wintrebert interpreted them as a proof of his theory, the dorsal rotated part of the organizer area (in Dalcq's meaning of the word) behaving as an initiator center on the ventral vegetal hemisphere to induce a second organizer in that ventral region (see also the figure of the translocation experiment in H. Alexandre's article, in this issue of the IJDB, Vol. 45, No. 1, 2001). Wintrebert was nevertheless right in considering the role of early displacements of the unsegmented egg axis as changing the final animal-vegetal polarity. He first described a 30° movement of the animal pole towards the ventral side of the egg, but he admitted that the egg rotation was *en masse* and rejected Ancel and Vintemberger's results on *Rana* egg. These authors had come to the conclusion that the "fertilization rotation" only affected the most superficial cytoplasmic layers and did not alter the position of the initial vegetal pole. Therefore, Wintrebert localized the appearance of the blastoporal slit at only 18° from the supposed final vegetal pole. This allowed him to place the presumptive chordal and neural territories *under* the blastula equator: consequently the grey crescent, lying *above* the equator, lost all significance as a "chordal area", being no longer a "germinal localization" but only a transitory epiblastic dorsal marker. The controversial debates between Wintrebert and the other embryologists, mainly Dalcq and Pasteels, lasted for years and sometimes occurred orally in scientific meetings. Pasteels (1936) repeated the vital staining experiments on *Discoglossus* and wrote a long article to contradict most of Wintrebert's assertions, concluding that Wintrebert's fate maps were erroneous, that the blastopore actually appears at 45° from the true vegetal pole, that the initiator center was a groundless and useless concept and that the organizer was preformed. Pasteels' conclusions have prevailed to this day among embryologists, but it might be interesting to reexamine the problem of the changes of egg axis in *Discoglossus*. In any case, Wintrebert never changed his views. At the age of 96, he published a large book on the self-development of the living being (Wintrebert, 1963), in which he developed his ideas on physiological epigenesis, and reproduced and extended the numerous results and figures that he had published (mainly in the "Comptes Rendus" of the "Société de Biologie" and of the Paris Academy of Sciences). He fought very explicitly against the "religious" attitude of his "preformationist" contradictors, i.e., most of the embryologists of that time. Wintrebert also published another book on evolutionism, where he envisaged a theory of *chemical Lamarckism* (Wintrebert 1949, 1962). We can only regret that so much work did not lead to a sound synthesis but mostly led to a dead end of embryological progress.

Secondary inductions: an embryological jungle

The discovery of the organizer should not conceal the importance of the discovery of lens induction by Spemann at the beginning of the century. Spemann himself considered the organizer as the origin of a chain of secondary inductions, giving rise to progressive organ determinations and differentiations. We have seen that Wintrebert himself, although he refused to consider the organizing center as anything more than a temporary physiological

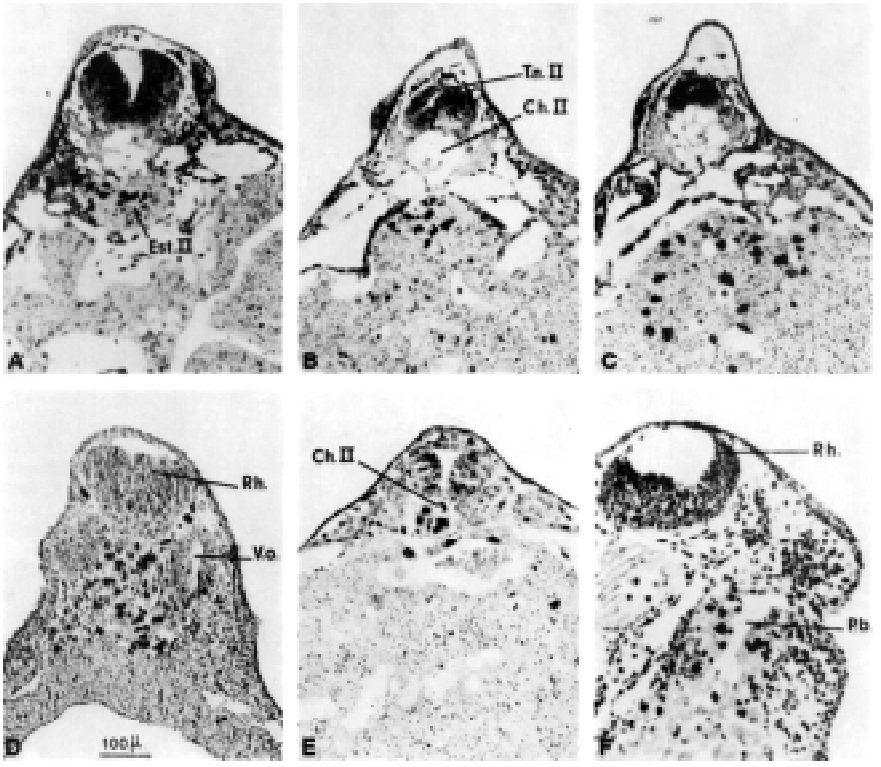

Fig. 2. Sections through secondary embryos induced after grafting a ^{3}H-thymidine labeled blastopore dorsal lip on a *Pleurodeles* gastrula. (A) *Marked cells in the secondary stomach (Est. II)* **(B)** *More posteriorly, in the same embryo, marked cells are still present under the axial organs (T.n. II, neural tube, Ch . II, notochord).* **(C)** *Still more posteriorly, a number of marked nuclei are present in the endoderm.* **(D)** *In another embryo, marked mesenchymal cells have migrated between the rhombencephalon (Rh.) and the otic vesicle (V.o.).* **(E)** *In a third embryo, in the anterior trunk, the notochord (Ch. II) and a few endodermal cells are marked.* **(F)** *At the rhombencephalon level (Rh.), a branchial pouch (P.b.) is marked (From Capuron, 1968b, with permission from CNRS Editions).*

property of the gastrula, recognized its initial role in development. But, here too, this embryologist had a negative attitude, refusing the idea of secondary inductors, which he considered as useless and as being purely verbal arguments. Nevertheless, Wintrebert's thoughts had no real influence outside his own laboratory.

After World War II, several young investigators published new examples of secondary inductions during urogenital organogenesis. In Bordeaux, in Avel's laboratory, Cambar (1948) demonstrated on the frog tadpole that the formation of the adult mesonephros is induced by the close proximity of the primary ureter. In Paris, in Gallien's laboratory, Houillon (1956) extended these results to the urodele embryo and, moreover, demonstrated a role of the "mesonephric blastema" in the organogenesis of the undifferentiated gonad in both sexes. In Wolff's laboratory, Calame also confirmed that the chick primary ureter is an inductor of the embryonic mesonephros (Bishop-Calame, 1966). Precisely, Wolff's school of embryology (see Dieterlen, 1990), from 1952 on, made large use of embryological recombinations in organ culture to demonstrate a considerable number of secondary inductions, giving rise to many definitive organs: liver, pancreas, lungs, heart, skin and its productions, etc. Most of these phenomena were and still are considered as basic examples of epithelial-mesenchymal interactions.

In spite of this successful wave of *in vitro* analyses, Wolff apparently did not give up his early desire to work on the organizer itself, when he was in Ancel's laboratory. He entrusted a young collaborator, Maryse Reyss-Brion, with the task of studying the effects of X-rays either on the organizer area, or on the competent reactive ectoderm of the urodele gastrula. It was shown that the irradiated ectoderm could not differentiate into neural tissue, but that the irradiated chordomesoderm was still able to induce a neural plate (Reyss-Brion, 1964).

The Spemann-Mangold experiment revisited: Capuron (1968)

As we have said, during the most active phases of Gallien's scientific career, his main interests lay in studies on sex determination and differentiation. At the end of the 1950s, Gallien chose a new collaborator, Alfred Capuron (1930-1997), to perform an extensive reinvestigation of Spemann and Mangold's famous experiment (1924), with the aim of studying the sexual organogenesis of the double embryo. This implied raising double larvae, capable of ingesting and digesting food for several weeks or months. On the other hand it had become possible to label embryonic nuclei for successive cell generations by incorporation of 3H-thymidine at the blastula stage. A marked organizer was thus excised at the very beginning of gastrulation (stage 8a of the developmental table of *Pleurodeles waltl* according to Gallien and Durocher, 1957) and grafted in the ventral marginal zone of an unlabeled recipient gastrula. This allowed much more precise cell marking than the pigment cell differences used by early investigators. Sections through double embryos or young hatched larvae were subjected to autoradiography, which made it possible to determine accurately the fate of the grafted blastopore lip and the precise origin of tissues in the induced secondary embryo.

Following two preliminary notes (Capuron 1962, 1963), Capuron published two extensive articles (Capuron 1968a, b) in which he analyzed the results of these major original research themes and compared them with those from several earlier authors. These articles were published in French in a newly launched journal, the "Annales d'Embryologie et de Morphogenèse", founded by Gallien himself, and that unfortunately appeared only for seven years. This is probably the main reason why Capuron's work was not cited later by authors who discussed the conditions of Spemann's initial experiments or repeated much later Capuron's experiments on *Xenopus* with labeled cells. Capuron did his experiments on *Pleurodeles* on a large scale. He performed a total of 805 grafting operations, 59 of which involved a marked organizer, and he obtained 634 induced secondary embryos, 43 of which were marked. Among the operated, non-marked double embryos, 242 developed and 67 were able to feed, but 14 only survived after metamorphosis.

Autoradiography was performed on 20 embryos. It was shown that the grafted blastopore lip had differentiated into anterior notochord, cephalic mesenchyme, anterior somites, anterior digestive tract and branchial pouches (Figs. 2 and 3). The major part of trunk tissues was formed from host cells, originating via « assimilating inductions ». The induced neural tube was entirely formed from the host ventral ectoderm. At variance with Spemann and Mangold's old results, Capuron did not find any case in which the ventral part of the induced neural tube contained cells originating from the graft. Later on, using *Xenopus laevis*, Recanzone and Harris (1985) grafted a blastoporal lip which was taken from *X. borealis* (fluorescent nuclei) or from 3H-thymidine labeled *X. laevis* gastrulae. These authors, like Spemann and Mangold, detected marked ventral cells in the induced neural tube. Thus, the question of a common origin for notochord and neural floor plate, recently demonstrated by Le Douarin (Le Douarin and Halpern, 2000) in the chick, remains open in the Amphibian embryo. But in 1968, Capuron interpreted Spemann and Mangold's results as being a consequence of an incomplete invagination of the grafted blastopore lip, placed on the invagination limit, in which case the external part of the graft becomes partly incorporated into ectoderm.

On young and older larvae, Capuron counted the primordial germ cells (PGC) and made reconstitutions of the genital crests and of the whole urogenital tract. A controversial issue was that of the primary origin of PGC in the Urodele embryo. Were they induced at a late stage in the somatic tissues of the secondary embryo, as claimed by Asayama and Amanuma (1957) for *Hynobius*, in which case there should be an increase of the overall number of PGC in the operated double embryos? Or were they already determined at the gastrula stage, before the operation, and in which case they should be distributed between the two pairs of gonads of the double embryo? Capuron favored the second idea. He did not find a significant increase of the overall number of PGC in double embryos, as compared to the number of PGC in a single control embryo.

Capuron also studied the various anatomical connections that arose between different parts of the genital and digestive tracts in double individuals and in the few adults that he obtained. His description was completely original, since nobody before him had been able to raise operated animals for such a long period. Capuron was later appointed to a professorship at the University of Lille, where he performed other studies dealing with the *in vitro* appearance of germ cells in mesodermal tissues that had been induced by ectoderm-endoderm associations, and with the *in vitro* behaviour of PGC in explants, etc.

An old concept of Holtfreter revisited: are there neural predispositions in the early gastrula ectoderm?

At the end of the 1970's, the mechanism(s) of neural induction remained poorly understood, in spite of the great amount of work performed during several decades, in different countries, to isolate and identify the inductive factor(s) from the Spemann-Mangold organizer (for review, see Saxen and Toivonen, 1962; Nieuwkoop *et al.,* 1985).

At that time, in Toulouse, Duprat and colleagues undertook new experiments on the neural inductive process, focusing their interest on the role of the target tissue itself (early gastrula ectoderm) in this neuralizing phenomenon.

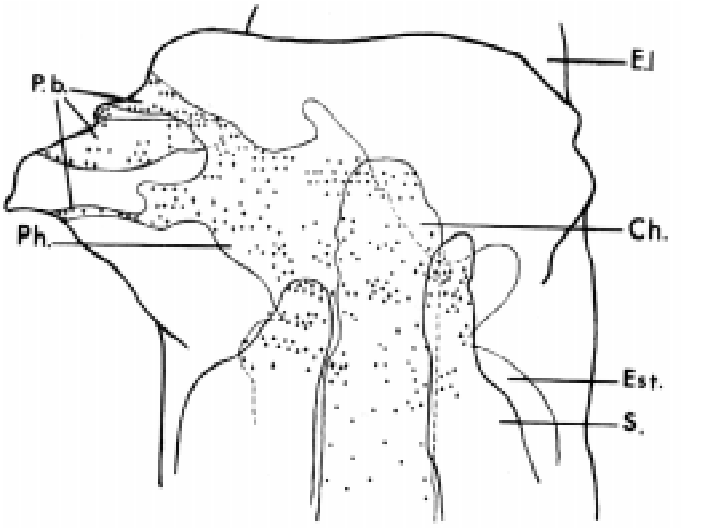

Fig. 3. Reconstitution of the head region induced by a labeled blastopore dorsal lip. *Marked cells are indicated by crosses in the endoderm, small black dots in the notochord and black triangles in the somites. Ch, notochord; E.I., primary embryo; Est. stomach; P.b. branchial pouches; Ph. pharynx; S. somites (From Capuron, 1968b, with permission from CNRS Editions).*

The idea was that this competent tissue could play an active role in the specificity of the neural inductive process, due to:1) results of their previous experiments on early neural differentiation in Urodeles, *Pleurodeles waltl, Ambystoma mexicanum* (Duprat, 1970; Duprat *et al.,* 1966, 1977); 2) the fact that various tissues and apparently very different non-specific factors could be neural inducers (cf. Saxen and Toivonen, 1962); 3) the fact that the temporally limited competence of the gastrula ectoderm to be neuralized highlighted its importance in the program of neural commitment; 4) the work of Tiedemann and Born (1978) showing that neural induction occurred even when a neuralizing protein-factor, isolated in their laboratory, was prevented from entering the competent ectoderm and so suggesting the important role played by the surface of the target tissue in the recognition of an entirely external signal, and 5) data reported by Barth and Barth (1941, 1969, 1974) indicating that mere ionic changes could initiate induction of neural cells from gastrula ectoderm in *Rana pipiens.*

Consequently, the concept proposed at the beginning of the eighties by Duprat and colleagues emphasized the role of the competent target tissue, rather than that of the inducer, in the neural inductive process. First they investigated the role of the structural organization of the target membrane of the ectoderm in neural commitment. They demonstrated that this neural process was intimately linked to the structure of the ectodermal plasma membrane. Experimental rearrangements, *in vivo* as well as *in vitro*, of the molecular structure of the target membrane using the FRAP technique (Fluorescence Recovery After Photobleaching) or treatments with lectins having specific affinities for different carbohydrates and glycoconjugates, prior to any association with the blastopore lip (the Spemann-Mangold organizer) or again using specific inhibitors of enzymes involved in glycosylation(s), strongly inhibited neural induction. This inhibition was reversible, the induction could proceed when the original membrane structure

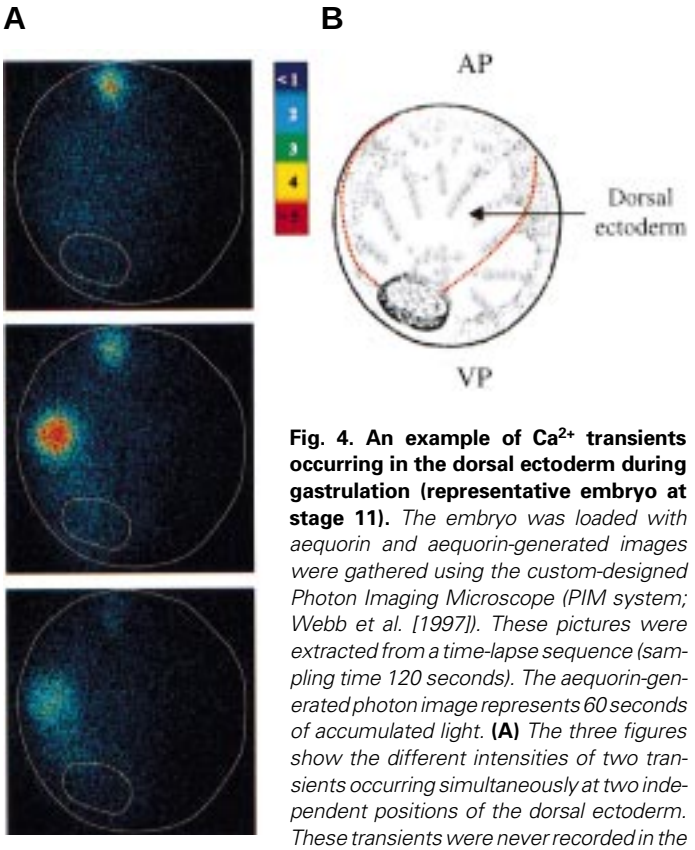

Fig. 4. An example of Ca^{2+} transients occurring in the dorsal ectoderm during gastrulation (representative embryo at stage 11). *The embryo was loaded with aequorin and aequorin-generated images were gathered using the custom-designed Photon Imaging Microscope (PIM system; Webb et al. [1997]). These pictures were extracted from a time-lapse sequence (sampling time 120 seconds). The aequorin-generated photon image represents 60 seconds of accumulated light.* **(A)** *The three figures show the different intensities of two transients occurring simultaneously at two independent positions of the dorsal ectoderm. These transients were never recorded in the ventral ectoderm.* **(B)** *A schematic of the whole embryo showing its orientation. AP, Animal Pole; VP, Vegetal Pole. Color scale indicates luminescent flux in photon per pixel. Scale bar, 0.3 mm.*

was restored by *de novo* synthesis of glycoconjugates (Duprat *et al.,* 1982; Gualandris *et al.,* 1985, 1991; Dupou *et al.,* 1987).These authors also demonstrated that the change in the membrane structure appeared to inhibit reception of an external signal but not its subsequent intracellular transduction if the signal was received prior to the membrane reorganization (Gualandris *et al.,* 1987). Experiments performed with Concanavalin A and succinylated-Con A strongly suggested the involvement of calcium influx in neuralization of the ectoderm (Gualandris *et al.,* 1985). It was also shown that the extensive extracellular matrix located beneath the competent ectoderm, and which plays a fundamental role in the morphogenetic movements during gastrulation, was not necessary for the transmission of the external signal to the target membrane and was not required for the inductive process itself (Duprat and Gualandris, 1984).

In 1985, with regard to the different results accumulated from 1981, these authors wrote:

> *« … whatever the mechanisms of action of the numerous inducing factors known until now, it is therefore quite possible that the competent target tissue itself contains the capacity and the specificity needed for neural induction. All that these neuralizing factors so far studied would have in common is the capability to initiate the same signal which sets in motion the machinery of neural determination » (Gualandris et al., 1985).*

Modifications in cell-cell interactions in the target membrane and / or initiation of ionic fluxes could be crucial factors in this process. We shall come back later to the point of involvement of ionic flux. Different sets of experiments were performed to address the question on the role of cell-cell contacts and interaction. In agreement with Holtfreter's pioneering observations (Holtfreter, 1945), they demonstrated with *Pleurodeles*, at the same time as Grunz and Tackle with *Xenopus* (see Grunz' contribution in this issue), that at the late blastula stage, disaggregation of the competent ectoderm into isolated cells commits some of them to the neural pathway (Saint-Jeannet *et al.,* 1989, 1990). This observation was in good agreement with the previous conclusion of these authors indicating that the competent ectoderm yet possessed some neural capacities. Another interesting finding was that the neural population that differentiated under dissociating conditions was composed of the two distinct neuronal and glial (astroglial) lineages. So throughout neuralization, despite the absence of organizer cues, cells from the gastrula ectoderm can differentiate into the two main neural lineages. This strongly suggests that the early gastrula ectodermal cells constitute a heterogeneous population presenting distinct predispositions (prepotentialities?) to be neuralized. If so, what is the molecular basis at the origin of the diverse potentialities of ectodermal cells?

Another important result that came from these experiments (induction by dissociation) was that only a limited number of generic neuronal characteristics were expressed (Saint-Jeannet *et al.,* 1993). Some specific neuronal genes (neurofilaments, tetanus toxin binding sites, …) were activated without any expression of genes involved in functional traits (genes encoding specific enzymes for biosynthesis or degradation of transmitters, transmitters themselves, etc…) whereas precursor neural cells isolated from the neurectoderm immediately after gastrulation, were committed to give rise to distinct subpopulations of mature cholinergic, dopaminergic, noradrenergic, GABAergic, peptidergic neurons (Duprat *et al.,* 1985; Pituello *et al.,* 1989a, 1989b, 1990).

These data (natural neural induction versus induction by dissociation) indicated that at the cell and at the gene level, neural induction was not an all-or-nothing event and that the expression of complete mature neuronal phenotypes involved a sequence of gene activation(s) that could be experimentally uncoupled. Induction by disaggregation (without the Spemann-Mangold organizer) allowed the first step of neural commitment to occur; mature, functional neuronal differentiation required some other factors or signals of chordomesodermal origin, that acted during gastrulation and later during embryogenesis, as shown *in vitro* when neural plate or neural fold neuroblasts isolated at the late gastrula stage, were cocultured with chordal and/ or mesodermal cells (Duprat *et al.,* 1985).

These authors, as did others throughout the world, undertook molecular approaches to study the role of such organizer factors (Zaraisky *et al.,* 1995; Ecochard *et al.,* 1995,1998). In this field of research, many major results have been obtained in US laboratories (for review cf. Harland and Gerhart, 1997; Gerhart's and also Gilbert's contributions in this issue). To make short this recent intensive international story, neuralization of ectoderm is currently considered as a default pathway (versus epidermal pathway). The organizer's secreted « neural » factors (noggin, chordin, follistatin, etc…) have the capacity, among others, to form high-affinity complexes with BMPs, ventral epidermal factors, thus inhibiting

the binding of these epidermal « inducers » to their ectodermal receptors and blocking their signaling. Neuralization appears to be the result of an inhibition of an epidermal induction and a derepression of inherent capacities of the competent ectoderm. In agreement with Holtfreter's pioneer concept, revisited in France by Duprat and colleagues (1985), all these recent experiments indicate that initial neural specificities reside in the target tissue itself.

The choice between neural and epidermal fates is controlled by calcium signaling

The first step of neural induction is probably a permissive event activated through different signal routes. One of them, the FGF signaling pathway, was studied in ParisVI- University in Boucaut's laboratory. Boucaut and his colleagues demonstrated, in whole embryos and also in isolated animal caps, that a truncated FGF receptor blocked neural induction by endogenous inducers. These authors proposed that the FGF receptor–related- signaling is required for the response of ectoderm to « inducing » signal and evidenced that FGF signaling plays a role in the patterning of the anteroposterior axis of the neurectoderm (Launay *et al.,* 1996; Riou *et al.,* 1998).

In Toulouse, Moreau, Duprat and colleagues were also interested in studies of neural signaling pathways. They demonstrated that the activation of L-type calcium channels (LTCs) followed by signal transduction via a calcium pathway, are sufficient to neuralize the ectoderm and activate both neuronal- and glial- specific genes in the ectodermal cell population (Moreau *et al.,* 1994). They have shown that an increase in $[Ca^{2+}]i$ occurring via LTCs is a direct cause of the triggering of neural induction *in vitro* in animal caps (Leclerc *et al.,* 1995a) and *in vivo* during gastrulation (Leclerc *et al.,* 1995b, 1997). No significant $[Ca^{2+}]i$ increase was recorded in the ventral ectoderm, which differentiates into epidermis. The characteristics of dynamic Ca^{2+} transients during gastrulation were located to specific domains in the dorsal ectoderm (Fig. 4).

These authors also demonstrated that calcium influx activates the expression of immediate early genes (IEG) such as *c-fos*, in neuralized ectoderm. They pointed out that noggin, one of the natural neural inducers, activated calcium-dependent pathways on isolated competent ectoderm and that it elicited the increase of intracellular calcium through activation of LTCs. Further there is an upregulation of a Fos- related protein (transcription factor). The calcium-dependent increase in the Fos-related protein upregulation is probably mediated by activation of a calcium calmodulin kinase (CaM kinase) which in turn phosphorylates CREB, a c-AMP response element binding protein (Leclerc *et al.,* 1999). Furthermore using the animal cap assay, they have shown that noggin, through the activation of L-type calcium channels, also induced the expression of m-RNA of *Xl POU 2*, a proneural gene, controlled by calcium signaling (personal communication). Thus, an activated intracellular calcium-dependent pathway controls the activation of immediate early genes that regulate downstream target genes involved in neural determination of the ectoderm.

Conclusion

After several decades of sparsely distributed activity, French embryologists tackled modern aspects of neural induction during the last 20 years. Few groups are still involved in this late consequence of the Spemann-Mangold organizer findings , but many problems remain to be solved at the cellular and molecular levels (what is the embryonic molecular basis of early different neural capacities of ectodermal cells?) and the French contribution might still be considered as significant in the international context.

Summary

Unlike biologists from several European countries, most French embryologists did not work from the onset on problems associated with the Spemann-Mangold organizer, though they were fully aware of the importance of the discovery. They preferred to stay on other original topics, but their later work was of course influenced by the induction concepts. The exploration of secondary inductions in various organ formations was flourishing after 1950. As far as primary induction is concerned, two exceptions must be stressed: Vintemberger, who, before World War II, worked on the frog organizer for a few years, and especially Capuron (1968), who repeated Spemann and Mangold's fundamental experiment on a large scale. Then, from 1980 on, a series of studies dealing with the neural induction concept focused on studies of the gastrula ectoderm itself, was undertaken, mainly in Toulouse University by Duprat and her colleagues, and in Paris-6 University by Boucaut and his colleagues.

KEY WORDS: *Spemann-Mangold organizer, neural induction, ectoderm, amphibians.*

Acknowledgements

We thank Professor V. Ruch (Strasbourg) who sent us a long list of Vintemberger's articles, B. Savelli for his help in preparing the illustrations, C. Mont and C. Michel for typing the manuscript and finally Professor J. Smith (Toulouse) who was kind enough to review the English text.

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