# The Fate of Spemann's Organizer

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**[REVIEW]**

# The Fate of Spemann's Organizer

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## 1. HISTORY OF THE PROBLEM

*The researches of many commentators have already thrown much darkness on the subject, and it is probable that, if they continue, we shall soon know nothing at all about it.*

*Mark Twain*

Up to now researches are far from understanding fully the mechanisms which realize the embryonic development of vertebrate animals. Sometimes it seems that to the end of XX century the embryologists are further from the original goal to explain how the development of an organism is accomplished than it seemed at the beginning of the century when H. Spemann and H. Mangold discovered the so-called "organizer" in amphibian embryos (Spemann and Mangold, 1924). They had observed that after transplantation of a dorsal blastopore lip of the early gastrula to the ventral side of another embryo a secondary embryonic axis developed on this side. Recent studies into the history of this discovery (Sander, 1993) have detected that the decisive experiment had been made by Hilda Mangold in 1921 when she transplanted a piece of dorsal lip of the ring-like blastopore of a *Triturus* embryo to the ventral side of another embryo at the same stage. As a result, a secondary complex of axial organs including head structures was formed at the place of a transplant. In another experiment, when the transplant was taken from a lightly pigmented embryo of *Triturus cristatus* and transferred to a heavily of pigmented *Triturus vulgaris*, it was shown that the secondary neural structures originated mainly from cells of the recipient.

The participation of a dorsal blastopore lip in the programme of development of a new embryo may be regarded in three significant aspects: 1/ Formation of axial structures and establishment of the plane of bilateral symmetry; 2/ Redetermination of fate for the party of host cells and their inclusion in the structures induced by the transplant; 3/ Induction of the nervous system. The dorsal lip, the influence of which causes the development of a new embryo, had been termed the **organizer**. Today researchers usually call it the **Spemann's organizer**. The formation of a neural plate which gives rise to the central nervous system (CNS) was consid-

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Abbreviation used in this paper: PCM, prechordal mesoderm, CNS, central nervous system; r1–r8, rhombomeres of hindbrain; AP, antero-posterior; DV, dorso-ventral; NCC, neural crest cells; dpc, days post coitum.

ered to be a result of an inductive influence from the underlying mesoderm (organizer property). This latter process was initially called the primary embryonic induction and is now more properly called neural induction.

A certain degree of regional differences among induced neural structures had already been observed by Spemann himself: some of them represented more cranial derivatives of CNS, others were more caudal ones. He soon after demonstrated that the fragment of dorsal lip taken from an early gastrula induced anterior neural structures, whereas the same region from a late gastrula led to the formation of caudal neural structures and that's why Spemann is credited with distinguishing between a "head" and "trunk" organizer respectively (Spemann, 1938; Hamburger, 1988).

Since Spemann's group (Bautzmann *et al.*, 1932) showed that dead tissues can also act as inducers and that the inductive signals can be transferred in cell-free conditions, it has inferred that inducing signals are of a chemical nature. During the 10 following years the leading scientists of the time studied a tremendous number of different substances in order to ascertain the chemical nature of inducers. However, the studies reached a deadlock on account of weakness in the available microchemical methods which existed at that time (reviews: see Saxen and Toivonen, 1962; Saxen, 1997).

In parallel with this initial period of chemical studies, investigations into the tissue specifity of inducing agents had begun. These proved that different tissues from adult organisms of various species of animals had unequal inducing effects giving rise to different regions of the nervous system. Subsequent efforts in the 40's–60's led to the formation of the "double-gradient hypothesis" according to which there are two basic inducing structures - "neural" and "mesodermal". It was supposed that both inducers may act jointly in various concentrations and form the complete spectrum of CNS derivatives (Saxen and Toivonen, 1962). This point of view about the existence of two basic inducers and about the possibility to obtain very different types of tissue and cellular differentiation by mixing two inductors in various proportions retains support today (Saxen, 1997). The model of "three signals" was proposed later with the aim of taking into account a greater number of experimental facts that had been discovered (Slack, 1983; Smith, 1989; Christian and Moon, 1993a). Additionally, the discovery that during early blastulation, the signals are required from a region vegetal to the prospective dorsal lip towards the animal pole in order to initiate development of the mesoderm or organizer (Nieuwkoop, 1969), have been included in the textbooks and model of "three signals". Later, this region designated as a Nieuwkoop's centre.

A new round of far-reaching investigations into Spemann's organizer began about 20 years ago in conjunction with the development of new molecular-biological and genetical approaches. The study of Lewis (1978) concerning the mapping of the homeotic gene complex *Bithorax* in *Drosophila* could be regarded as pioneering in this age of investigation. It was demonstrated that these genes are arranged in a single cluster and in the same sequence as they are disposed into the chromosome, they are expressed along the forming body axis in the antero-posterior (AP) direction during embryogenesis (Lewis, 1978; Scott *et al.*, 1983).

The study of Lewis was performed using classical genetical methods. Development of recombinant DNA technology and the cloning of genetic sequences in the early 80's greatly advanced the molecular methods of DNA analysis. This provided several laboratories with the opportunity to begin deciphering of the molecular structure of some homeotic genes in *Drosophila*. It had been revealed that there is common sequence in the cDNAs for homeotic genes. This wasn't unexpected since Lewis had proposed that homeotic genes arose by tandem duplication and therefore they were expected to share common DNA sequences. The more so as it is astonishing that the sense of this sequence, termed as "homeobox", was assessed correctly at once (McGinnis *et al.*, 1984; Gehring, 1997). Studies of the evolutionary aspects of the problem showed the presence of genes containing homeobox in other animals (Carrasco *et al.*, 1984).

The homeobox domain being a conserved region of all homeotic genes in *Drosophila* as well as in other animals, consists of an identical sequence containing 180 base pairs. It is located in the protein-coding region of the respective genes and encodes a 60 amino acid homeodomain. Further details of the studies of homeobox function are not within the scope of this review. I would like only to remind the reader that the homeodomain forms a characteristic helix-turn-helix structure which is capable of binding to the corresponding DNA sequence in the regulatory region of target genes thus activating or repressing their transcriptional activity.

Later, futher studies were performed with the aim of screening genes which are expressed specifically in the dorsal lip, i.e. in the organizer. The first gene identified contained a homeobox in its composition. This new gene *goosecoid* (*gsc*) was found to be expressed in the zone of the organizer in *Xenopus* and then in mouse (Blumberg *et al.*, 1991; Cho *et al.*, 1991). In order to reveal the role of *gsc* gene during the development of the embryo, microinjection of *gsc* mRNA in to the ventral blastomeres of 4-cell embryos was performed. The formation of secondary embryonic axis was observed with a large frequency in such embryos. During the following years a large range of molecular factors was discovered (*noggin*, genes of family *Wnt*, *Xlim1*) (Kessler and Melton, 1994; De Robertis, 1995), which were also capable to initiate the development of a secondary embryonic axes upon injection of their of mRNA into ectopic parts of an egg. Today, over 20 genes expressed in the organizer region, have been found: the majority are capable of inducing the development a new axial complex after their injection into the ventral part of the embryo (Lemaire and Kodjabachian, 1996).

Thus, one begins to see a rather composite and intricate situation with the chain of genes and their products initiating formation of a new embryo from the cells which were destined for another fate in the course of normal embryogenesis. All this reminds the history with the studies of the primary embryonic induction during the 30's when it seemed, as it

seems now too, that almost solved issue becomes more and more intricated and incomprehensible. The similar developmental effect of the overexpression of many genes from the organizer zone might be explained in a way that each of them functions as a single step in a multistage cascade of events which participates firstly in the induction of mesoderm and then of the neural plate. May this cascade be turned on irreversibly from many intermediate states?

## 2. FATE OF THE ORGANIZER DURING GASTRULATION

As had already been demonstrated by H. Spemann, the inducing properties of the dorsal blastoporal lip of the amphibian embryo change during the course of gastrulation. Ectopic transplantation of an early gastrula dorsal lip into the ventral part induces the development of head structures predominantly, while the same procedure with a late gastrula dorsal lip results in the appearance of trunk and caudal structures. However, in reality the early organizer initiates/directs not only the development of the head department, but also the development of more posterior regions. De Robertis (1995) explains this difference in terms of differential activities of organizers from the region of dorsal lip of early and late gastrula by the maximal activity of the first.

All these inductive influences occur against a background of morphogenetic movements that were described for amphibia in detail, particularly for *Xenopus* (Fig. 1). Gastrulation begins with the appearance of the black dorsal blastopore pigment line at 50° latitude from the vegetal pole. The cells, which are located in the blastopore lip of the early gastrula and which show their organizing abilities in the formation of a head department, involute into the embryo earlier than any other material and they constitute the leading edge of the whole invaginating cellular layer (Gerhart and Keller, 1986). These early invaginating cells form the prechordal mesoderm or prechordal plate. All cells that invaginate later become the chordomesoderm from which the notochord and somites are formed, i.e. the trunk and caudal structures. From these data, it seems quite evident that quite different cells are localized in the dorsal blastopore lip at the beginning and the end of gastrulation. As a result of morphogenetic movements of the originally common layer, these two kinds of cells migrate to be located in very different parts of the embryo. The fact that after ectopic transplantation cells of early and late organizers cause the development of head and trunk-caudal structures respectively could be interpreted as their having already become determined within the region of the dorsal lip or even before the morphogenesis of it. It confirms the regionalized gene expression in the progenitors of the blastopore tissue. It was detected into anterior (vegetal) and posterior (animal) domains respectively by the expression of *Gsc* (expressed later by the prechordal plate) and *Xnot* (later found in the notochord) (Harland and Gerhart, 1997). Therefore the segregation of patterning activity for both organizers may exist prior to the morphogenesis of the blastopore lip. The similar conclusion was made by Camus and Tam in the mouse (1999).

Structures, functionally equivalent to the amphibian organizer, have been found in other vertebrates on the basis of their ability to induce a secondary axis after heterotopic transplantation of these structures at the equivalent stage of an early gastrula. In fish this actually is localised to the germ shield (Shih and Fraser, 1996). In birds and mammals, the properties of the organizer are performed by the node (in birds it calls Hensen's node) which is a structure located at the anterior end of the primitive streak (Beddington, 1994; Psychoyos and Stern, 1996).

The above displacements of organizer cells have been confirmed by experiments employing molecular markers that are specific for the early organizer. As I remarked previously, the first identified marker gene *gsc* of the organizer region was discovered already in 1991. Onset of its expression begins at the pregastrula stage, but only in the region that is destined to become the head organizer. After this, the

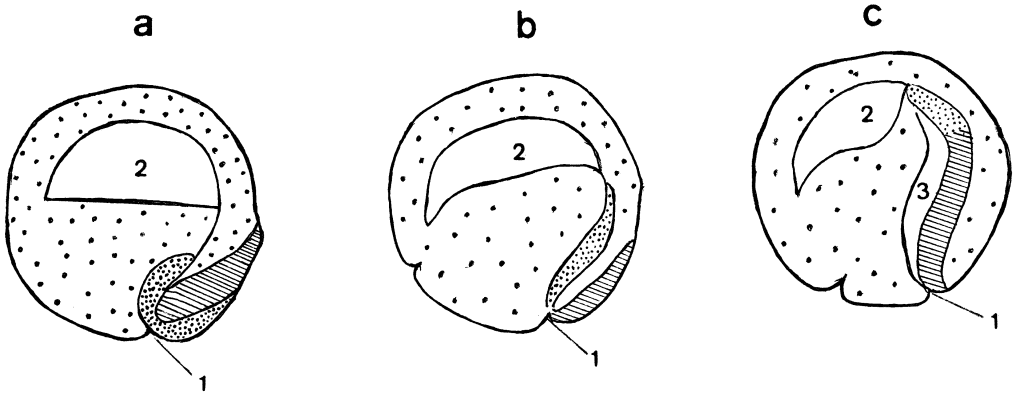


**Fig. 1.** The scheme of *Xenopus* gastrulation. (**a**) Stage 10 (by Nieuwkoop and Faber, 1967). The beginning of gastrulation or the early dorsal blastoporal lip. Organizers for the development of head region (Spemann's organizer property) and for the trunk-tail region marked by the densely dotted and hatched zones, respectively. (**b**) Stage 11. The Spemann's organizer invaginates inside of the egg, meanwhile the precursor of chordomesoderm is disposed just in the dorsal lip. (**c**) Stage 11, 5. The involution of mesoderm is completed. The material of the early dorsal lip (dense dots) is disposed in the prechordal zone, in front of chordomesoderm (hatches), the prospective notochord. 1– dorsal lip, 2– blastocoel, 3– archenteron.

expression of *gsc* is found in the early dorsal lip. When the involution movements are beginning, *gsc* expression gradually disappears from the lip. The expression of this gene in *Xenopus* and chick remains only in cells which leave early the dorsal lip (Steinbeisser and DeRobertis, 1993; Izpisua-Belmonte *et al*., 1993). Cells of the dorsal lip deep layers which express *gsc* migrate by means of crawling movements along the roof of archenteron and reach the limits of the prechordal plate (Niehrs *et al*., 1993). It seems, that owing to the expression of *gsc*, the dorsal lip cells receive the impulse which forces them to migrate to the prospective prechordal zone. Consistent with this idea it was demonstrated that injection of *gsc* mRNA into ventral cells causes them to migrate to the anterior part of embryo and settle in the space directly anterior to the notochord (Steinbeisser and De Robertis, 1993). Later, the cells expressing gsc will be included in the head mesoderm tissue (more exactly, in the prechordal part of it, the structure that underlies the forebrain).

In a similar way the expression of the *Xotx2* gene has been studied. Firstly, the expression is found in a layer of deep cells of the early dorsal lip. Later, these cells crawl along the roof of the blastocel and are finally incorporated into the prechordal mesoderm tissue (Pannese *et al*., 1995). Similar morphogenetic studies of cell migrating from Spemann's organizer to the prechordal mesoderm have been performed during the course of gastrulation in the analysis of the expression of the *Xlim-1* (Taira *et al*., 1992) and *Frizzled-8* (*xfz8*) genes (Deardorff *et al*., 1998).

Experiments with these markers have strengthened the afore-mentioned observations by Gerhard and Keller (1986) about the migration of organizer cells to the prechordal zone during gastrulation and have helped establish that the cells of the prechordal plate arise from the deep layers of the early dorsal lip (Keller *et al*., 1992). In the head part of a neurula embryo this prechordal plate takes up a comparatively small zone (Steinbeisser and De Robertis, 1993; Izpisua-Belmonte *et al*., 1993). Finally, when grafted into an early gastrula, the prechordal plate of a late gastrula can induce an ectopic head, similar to the induction after the ectopic grafting of an early dorsal lip (Gilbert, 1994; Lemaire and Kodjabachian, 1996). These data suggest that the dorsal lip of an early gastrula and the prechordal mesoderm of a late gastrula are one and the same population of cells that has concerved its inducing properties throughout gastrulation, hence the deep layer cells of Spemann's organizer shift from the early dorsal lip to the prechordal zone during of this period (Fig. 1).

In a similar way, Hensen's node, which is the chick equivalent of Spemann's organizer, loses the ability to induce the development of head structures of the CNS from the epiblast *area opaca* cells from the stage of definitive primitive streak (stage 4+ by Hamburger and Hamilton, 1951). Simultaneously the stage HH4+ is marked by the appearance of the group of cells within prechordal plate which migrate anteriorly from Hensen's node (Lemaire *et al*., 1997; Foley *et al*., 1997).

Recently new facts concerning the separate existence of head and trunk organizers in the mammalian embryo were presented. The mouse node, located at the anterior end of the primitive streak, has been considered to be an equivalent of the dorsal blastopore lip of amphibia. However, ectopic grafting is unable to induce a complete axis; secondary axes invariably have anterior truncations and lack, for example, forebrain (Beddington, 1994; Tam *et al*., 1997). 2 recent studies (Beddington and Robertson, 1999; Camus and Tam, 1999) present evidence that the mammalian head and trunk organizers belong to separate germ layers, the primitive endoderm (head organizer) and the epiblast (trunk). Anterior visceral endoderm (AVE) discloses its abilities of anterior organizer almost a day before the primitive streak is formed (Beddington, 1998). Knoentgen *et al*. (1999) confirmed the ability of the mammalian AVE, taken from the prestreak stage and transplanted into the *area pellucida* of HH3 chick blastoderm, to induce morphological features as well as markers characteristic for the anterior neuroectoderm. Similar abilities display the chick prechordal endomesoderm that develops only to the HH5 stage. The separation of two organizer subcompartments in mammals between different germ layers is the undoubted evidence that both organizer domains in other vertebrate classes (in amphibia and avians) predetermined at least in various cellular populations of a single layer.

The common elements of gastrulation in the different vertebrate classes are observable not only by the method of transplantation but also through expression analyses of various molecular markers. For example, the *Brachyury* (*T*) gene is a marker for the whole trunk mesoderm and the location of the prospective mesoderm tissues at early stages may be determined by *T* expression. In *Xenopus* and zebrafish, the prospective mesoderm may be seen as the ring around the spherical embryo: this is the so-called marginal zone. In mice, the primitive streak is a homologous structure of this ring (De Robertis *et al*., 1994). It has been proved that *gsc* which is a marker of the dorsal blastopore lip in amphibia is also present in the homologous structures of other mentioned groups of vertebrates such as fish and avians. This information represents additional evidence for functional conservation among all these structures (Lemaire *et al*., 1997). In *Xenopus* maximal expression of *gsc* is achieved just before the formation of the dorsal lip (stage 10) (Niehrs *et al*., 1994): the zone of expression is still above the prospective dorsal lip, where later the Spemann's organizer localized (Gerhart *et al*., 1989). In chick maximal expression of *gsc* has been observed before the primitive streak reaches the maximal elongation (stage 3+) (Izpisua-Belmonte *et al*., 1993), at stage of 50% epiboly in zebrafish (Stachel *et al*., 1993) and at 6.5 days *post coitum* (dpc) in the mouse (Blum *et al*., 1992). It has been proposed that these stages are equivalent during vertebrate gastrulation (De Robertis *et al*., 1994).

## 3. THE ORGANIZING PROPERTIES OF PRECHORDAL PLATE (MESODERM)

Prechordal plate is traditionally considered to be the mesendodermal structure which is located along the body axis immediately in front of the notochord (Adelmann, 1922; Meier,

1981; Seifert *et al*., 1993; Knoetgen *et al*., 1999). It exists in all vertebrates and is a temporal structure. In lower chordates, ascidians (subphylum *Urochordata*) and lancelets (subphylum *Cephalochordata*), the prechordal plate is probably absent (Pera and Kessel, 1997; Baker and Browner-Fraser, 1997; Yasui *et al*., 1998). Let me next use the term "*Protochordata*", like Gans and Northcutt (1983), to designate all lower chordates.

Spratt (1955) and Rosenquist (1983) studied the chick embryo and regarded the prechordal plate as a part of all mesoderm which arose by the invagination of cell layers through Hensen's node during the course of gastrulation. Jacob *et al*. (1984) have described the region anterior to the prospective notochord in chick embryo as a continuation of the mesodermal head process. They have offered the term "prechordal mesoderm" for this special axial mesoderm. The term "prechordal plate" is also used for the embryos of birds and other vertebrates in this context as well as in order to describe the median thickening of the endodermal epithelium in front of the prechordal mesoderm (Seifert *et al*., 1993).

The axial position of prechordal mesoderm (PCM) bordered by endo-, ecto- and neuroectoderm supports the idea it may play a role in the induction and patterning of the head structures. During the process of gastrulation PCM arises as the first direct derivative of the organizer and it is localized in front of the extending notochord. In birds, it initially arises from Hensen's node as a group of mesenchymal cells (Pera and Kessel, 1997). In the process of early forebrain development, the population of mesenchymal cells, which is PCM, is disposed in front of the notochord and dorsally to the preoral anterior intestine (Meier, 1981; Seifert *et al*., 1993).

It is noteworthy that the concept of the prechordal plate is different in the works of various authors. For example, Pera and Kessel (1997) determine the prechordal plate as the structure consisting of the PCM and the adjacent endoderm (without including the endodermal cells which lie in the roof of the anterior intestine). On the contrary, Seifert *et al*. (1993) regard these endodermal cells as a part of the prechordal plate (but not the prechordal mesoderm). Knoetgen *et al*. (1999) demonstrating histological pictures of chick embryo anterior region show that the cells of prechordal mesoderm are not separated from the endoderm and call this structure by the prechordal endomesoderm.

It should be noted that the cells having the organizing properties apparently comprise only one portion of the PCM; the other are used to form the occular muscles and some bones of the skull (Couly *et al*., 1992, 1993).

Removal of the PCM prompts a cyclopy and a considerable narrowing of the anterior neural tube (Pera and Kessel, 1997). However, the most striking result of PCM ablation, in my opinion, is the fact that it results in defects in the ventral part of the forebrain, namely a reduction of the hypothalamus and an entire absence of the hypophysis (Kimura *et al*., 1996). I will now explain why I consider it to be an essential event.

In amphibia, only the early lip of the blastopore shows the ability to induce the rostral parts and probably the whole nervous system (De Robertis, 1995). As a result of tissue movements during gastrulation, cells invaginate from the early blastopore lip, migrate through the archenteron and later differentiate into the PCM. As I have said above, the PCM retains the properties of an early dorsal lip, because in vivo and in vitro experiments have shown that, at the early gastrula stage, it is able to induce the rostral parts of CNS. There is indication that the transplantation of the PCM from a late gastrula to an early gastrula may induce the development of an ectopic head (Gilbert, 1994; Lemaire and Kodjabachian, 1996).

It has been shown recently that similar processes occur in the homologous structures of birds (Storey *et al*., 1992; Foley *et al*., 1997). The latter authors conclude that Hensen's node, the avian homolog of Spemann's organizer, loses the ability to induce the rostral parts of nervous system already at the stage 4-4+ (Hamburger and Hamilton, 1951), i.e. within a few hours of its appearance (Dias and Schoenwolf, 1990; Kintner and Dodd, 1991). During the same period the PCM is migrating anteriorly from Hensen's node (Hamburger and Hamilton, 1951). Foley *et al*. (1997) have studied whether the prechordal tissue retains the functions of the early Hensen's node. They made the transplantation of PCM using the system of chick-quail and analysed also the induction of a number of molecular markers, which are specific for different subdivisions of the nervous system. It proved that the prechordal tissue is capable after its grafting into the presumptive hindbrain to force these cells to express forebrain markers thus demonstrating the ability of the PCM to anteriorize prospective caudal tissue. For example, under the influence of recipient prechordal tissue, the host hindbrain cells begin to express the markers *tailless* and *Otx2* which are specific for the forebrain. Similar results were obtained by Dale *et al*. (1997).

In other experiments, the grafting of PCM into the different regions of the *area opaca* or *area pellucida* which consist of uncommitted ectoderm revealed the formation of forebrain-like structures that expressed forebrain-specific molecular markers (Pera and Kessel, 1997; Knoetgen *et al*., 1999).

The idea about interconnexion or unity of the early blastopore lip (as well as its homologs in fish, avians and mammals) and PCM seems to be reinforced by the fact that many genes are expressed in both structures. Among these genes are *follistatin*, *noggin*, *chordin* as well as several homeobox genes (*gsc*, *Lim1*, *Otx2*) (Blitz and Cho, 1995; De Robertis, 1995; Shawlot and Behringer, 1995; Pera and Kessel, 1997).

These data gained using avian embryos give us the possibility to see analogy in the morphogenetic processes occuring during the beginning of gastrulation not only between early dorsal lip in amphibia and Hensen's node in birds, but also between PCMs in both classes of vertebrate embryos. The ability to induce mainly structures of the head tissues in birds passes during the course of gastrulation from the Hensen's node (or from the dorsal lip in amphibia) to the PCM.

## 4. THE ROLE OF ORGANIZER IN THE FORMATION OF THE HEAD

The homeobox gene *Xlim-1* is also expressed in the organizer field (Taira *et al*., 1992). It belongs to the *LIM* class of homeobox genes which encode proteins with two tandemly repeated *LIM* domains disposed amino-terminally to a homeodomain. The *LIM* domain is determined by the cysteine-rich motif which binds iron and zinc ions and mediates protein-protein interactions (Taira *et al*., 1992; David *et al*., 1995; Glasgow *et al*., 1997). The gene *Xlim-1* is similar in the homeobox and *Lim* domains to *lin-11* of *Caenoharbditis elegans*. Injection of RNA coding for an activated form of *Xlim-1* into the ventral equatorial region of amphibian embryos induced a secondary axis (Taira *et al*., 1994). The mouse homologue of *Xlim-1* the *Lim1* has been cloned by Barnes *et al*. (1994).

*Lim1* is expressed in the node and the primitive streak. In order to define whether the *Lim1* gene product is required during embryogenesis, *Lim1* null mice were generated by gene targeting in embryonic stem (ES) cells. These cells were introduced into blastocysts that gave rise to chimeric mice capable of transmitting to the germ line. Homozygous *Lim1*–/– embryos lack head structures just anterior to the otic vesicles. The trunk and tail, however, appeared to be normal (Shawlot and Behringer, 1995).

To determine whether the anterior neural tissues are present in *Lim1*–/– mice, Shawlot and Behringer (1995) examined the expression of three neural markers: *Otx2*, which is expressed in the forebrain and midbrain region in wild-type embryos, *En*, which marks the presumptive boundary between the midbrain and hindbrain, and *Krox20*, expressed in the hindbrain in rhombomeres 3 and 5. In *Lim1*–/– embryos, only *Krox20* expression was found in rhombomeres 3 and 5. It is worth noting that the domain of r3 expression was located at the extreme anterior end of the embryo and was reduced in size. These experiments indicate that *Lim1*–/– embryos lack fore- and midbrain, but retained a portion of the hindbrain, therefore, the neural truncation occured just anterior to r3.

The mouse embryos with the mutation *Lim1*–/– at the neural plate stage (7, 5 dpc) have, compared with wild-type embryos, no morphologically distinct node-organizer, head process or PCM. Yet, expression of *gsc* (with unusual localization, however) was detected. The other gene-marker of the node region, *HNF3*-β was expressed only from 8, 5 dpc. During normal development this gene is expressed in the PCM region at 7. 5 dpc stage (Ang *et al*., 1993; Weinstein *et al*., 1994). Consequently, the absence of *HNF3*-β expression at this stage in the mutant suggests that the PCM is absent too. At present it is difficult to say if PCM is absent totally or it is only reduced. The expression of these genes (*gsc*, *HNF3*-β) seems to support the second possibility, since these genes are expressed not in the proper place and not at the proper time. In any case, these results point to a correlation between head formation anterior to r3 and the presence of the PCM. The role of the gene *Lim1* appears to be involved in the proper formation and of functioning of the PCM.

A similar phenotypic effect was observed after inactivation of the homeobox gene *Otx2* that is also expressed in the organizer region (Acampora *et al*., 1995; Ang *et al*., 1996; Lumsden and Krumlauf, 1996). In the homozygous null mouse *Otx2*–/– all structures of fore- and midbrain are absent while the hindbrain, judging by expression of the marker *Krox20*, is retained up to the level of r3. At the same time trunk and tail structures are normal (Matsuo *et al*., 1995). It should be also noted that in the *Otx2*–/– mice the heart and foregut are absent. It is noteworthy that the heart does not develop in the absence of the organizer (Sater and Jacobson, 1990; Yuan and Schoenwolf, 1999).

It should be indicated that both genes (*Lim1* and *Otx2*) are expressed not only in the node, but also in the PCM (Shawlot and Behringer, 1996). Meanwhile, it is not currently known how both genes interact in the process of head development.

The materials considered above enable us to conclude that the destruction of the organizer stops the development of the whole head region. The fact that this developmental arrest occurs owing to the mutation in one gene only gives an opportunity to suppose that phylogenetically PCM might arise in the result of a big macroevolutionary mutation as a saltatory event.

## 5. THE BOUNDARY BETWEEN HEAD AND TRUNK IN THE VERTEBRATES

The head is usually defined as the part of the body rostral to the first permanent vertebra (Horder *et al*., 1993). However, such a simplified notion is not in accordance with the facts as will be shown below.

From the position of the evolutionary morphology which separates the vertebrates from other chordates, the most significant peculiarity of the vertebrates is a powerful development of the head including, of course, compound brain subdivisions. Gans and Northcutt (1983) have defined this process as the evolution of a “new head”. Emphasizing the novelty of this formation, these authors suppose that the vertebrate head may be to a certain extent regarded as a new specialization added rostrally to the existing body of cephalochordates.

The basic novel acquirement of the vertebrates is a head skull composed of cartilage and bone tissues. The main feature of all chordates is a notochord. In the vertebrates, however, the notochord extends anteriorly only up to the level of basi- cranial fenestra of the skull, while in the cephalochordates it extends along the entire body up to the rostral tip of the head (Fig. 2).

Indeed, almost all novel acquirements of the vertebrates are connected with the development of structures which lie ahead of the anterior tip of the notochord (Fig. 2). There is one opinion which holds that the head brain, the sense organs and their surrounding and supporting elements seem to originate from the epidermal nerve plexus of protochordates (Gans and Northcutt, 1983; Holland and Garcia-Fernandez, 1996).

Recent experimental studies dedicated to the origin of

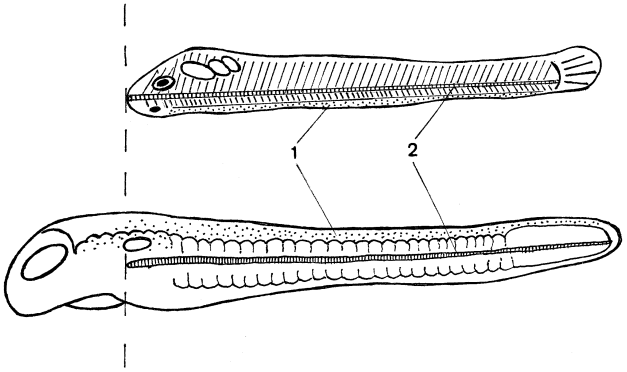


**Fig. 2.** Comparison of general body plans in the young larva of a lancelet (it takes top place by the back side downwards) and the embryo of a fish (in the bottom). The lancelet (*Branchiostoma*) belongs to the subphyla *Cephalochordata*, the representatives of which have the notochord that begins from the rostral part of the head and passes along the entire longitudinal axis of the body. In fish and other vertebrates the notochord begins at the level of the otic vesicles. The head department in whole (on the left part of figure from the vertical dotted line) is the innovation of vertebrates. 1– neural cord, 2– notochord.

head in the vertebrates and its cephalization revealed that the vertebrate head derives mainly from two structures: the neural crest and the epidermal neurogenic placodes. They participate in the formation of all sensory novelties and also in the development of all connective tissues and bones of the cranium (Gans and Northcutt, 1983; Northcutt and Gans, 1983; Hall, 1988). Neural crest is a population of mesenchymal cells originaling from ectoderm (Le Douarin, 1982; Hall, 1988) at the junction between the neural ectoderm and the epidermal ectoderm along the entire length of the neural tube. These cells migrate throughout the vertebrate cord and participate in the formation of many head and trunk structures. The epidermal placodes that are derivatives of ectoderm also appear as antero-dorsal thickenings on both sides of the neural tube, lying lateral to the neural crest in three main regions: otic, olfactory and epibranchial (Langille and Hall, 1989). Almost all structures that develop in the vertebrate head are novel formations, but not modifications of preceding structures. Among such novelty is hypophysis (Polenov, 1971).

The conception of a "new head" has got splendid confirmation in the subsequent works using the substitution quail-chick chimera technique to study the origin of skull in vertebrate embryos. Because of cytological peculiarities (immunochemical ones are added to them now), the quail embryonic cells may be distinguished from the chick cells. This allows one to trace the fate of grafted cells in chimeric embryos. The quail-chick marker system in the avian model demonstrates that in vertebrates the skeleton is entirely of neural crest origin (reviewed in Le Douarin, 1982). The important conclusions being that the skull by its origin has been shown to be divided into two main regions. The first region is localized in front of the extreme tip of the notochord which reaches the *sella turcica* and the second one is localized caudally to this boundary. The former ("**prechordal skull**") is derived entirely from the neural crest, the latter ("**chordal skull**") consists of bones which are the derivatives of prechordal mesoderm (*basipostsphenoid*, *supraoccipital*), the anterior somites (*basi-* and *exo- occipital*; *otic capsule* partly) and the neural crest (*parietal* and another part of *otic capsule*) (Couly *et al*., 1992, 1993; Kontges and Lumsden, 1996). Finally, it has been proven that the boundary between these two parts of the skull, which have different origins, passes through the middle of the *sella turcica* (Couly *et al*., 1993). It is interesting that this bone itself has a double origin: the rostral part is a derivative of neural crest cells and the caudal part originates from mesodermal cells. Thus, the middle of the *sella turcica* is the dividing boundary between two parts of the cranium with different tissue origins (Couly *et al*., 1993). It is worth noting here that the hypophysis is localized in a depression in the midpart of the *sella turcica*.

It should be emphasized that the anterior tip of the notochord reaches the basipostsphenoidal bone and therefore lies approximately at the level of midpart of the *sella turcica* (Couly *et al*., 1993). In reality, these striking coincidences again and again indicate the importance of the considered boundary delimiting head and trunk-tail parts of the vertebrate body. The organizer takes its position just at the prospective junction these parts. It evidently directs the formation of both parts of the body. From an evolutionary point of view, the boundary separates a "new head" of vertebrates from that part of body which they inherited from their protochordate ancestors.

The otic vesicles are considered by some to be the boundary of a division into head and trunk structures and in this case the vertebrate body is divided into the preotic (head) and postotic (trunk) departments (Kingsbury and Adelmann, 1924; Wachtler and Jacob, 1986; Horder *et al*., 1993). The morphological relations concerning the mutual disposition between otic vesicles, anterior tip of the notochord and hindbrain rhombomeres are easy to observe in transparent live fish embryos (Fig. 3). It is more hardly to receive the like information for the other classes of vertebrates owing to the opacity of their embryos. However the attention to these correlations didn't pay up to now. Apparently it is not accidental that a number of rhombomeres even in the same species is described as different. For example, Hamburger and Hamilton (1951) have discerned 5 neuro- or rhombomeres only, whereas later, using the cellular and molecular methods, have identified already 7 (Vaage, 1969; Lumsden and Keynes, 1989). Taking into account that the otic vesicles are still lying at the early stages just at the level of r4–r5 and that the rostral tip of notochord reaches up to the level of the otic vesicles (Fig. 3), it may be presumed that: 1) the notions a "preotic department" and a "new head" practically coincide; 2) the limit of influence of genes *Lim1* –/– and *Otx2* –/– when all head structures are absent, passes across r3/4 and in this sense these genes may be considered responsible for the formation of an entire "new head".

The developmental patterning of the head is apparently based on the use of mechanisms different from those for the trunk-tail part of body. In the latter the intercalations of cells

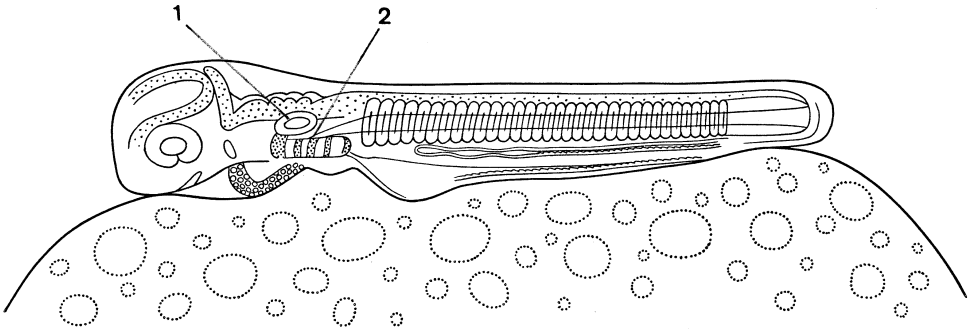


**Fig. 3.** An embryo of Atlantic salmon *Salmo salar L.* during the somitogenesis (stage of 40 pairs of somites) (from: Gorodilov, 1996). 1– otic vesicle, 2– rostral tip of notochord.

are observed. As a result, a narrowing and extension of the embryonic field occurs, in which the differentiation of an axial organ complex begins. Yamada (1994) have brought up the notion "caudalization" which is proposed to mediate by convergent extension of posterior cell motility; the border-line of caudalization is marked by the anterior end of the notochord. Migration of cells into the head region is of another nature (Keller *et al.*, 1992). The mechanism underlying hindbrain segmentation strengthens this hypothesis. It appeared that the first three rhombomeres and the subsequent more caudal ones are shaped by different mechanisms (Kulesa and Fraser, 1998). Differences in the mode of development of the preotic and postotic departments are also underlined in the literature. The segmental anatomy of the trunk-tail part of the vertebrate body is quite evident, but the ideas about segmental organisation of the head (Meyer, 1981; Jacobson, 1988) remain controversial topic (Wachtler and Jacob, 1986; Couly *et al.*, 1992; Kuratani *et al.*, 1999).

## 6. THE PARTICIPATION OF HOMEOBOX AND OTHER FAMILIES OF THE GENES IN THE FORMATION OF TRUNK-TAIL AND HEAD REGIONS OF THE BODY

### A. The complex of Hox homeobox genes

The early development of head and trunk-tail regions of the body requires first of all the participation of different subfamilies of the homeobox genes. The most numerous and well studied complex of *Hox* homeobox genes is related to the group of regulator genes expressing along AP axis and found in all *Metazoa* studied to date (Graham *et al.*, 1989; Duboule and Dolle, 1989; McGinnis and Krumlauf, 1992; Burke *et al.*, 1995; Holland and Garcia-Fernandez, 1996). This complex was first described in *Drosophila* (Lewis, 1978). It was found later that in vertebrates this complex exists as 4 copies appearing during the course of evolution by the way of two successive cycles of duplication of the ancestral cluster *HOM-C* type (Holland *et al.*, 1994). In four clusters of mouse and human there are 38 *Hox* genes divided into 13 paralogous groups. However, not all genes have 4 homologues. Successive comparisons of *Hox* genes in the four homologous sets has demonstrated that a part of each of the paralogues in every set was lost. For example, in mammals one gene of group 1 was lost while in fishes all 4 paralogues of this group are presented (Holland and Garcia-Fernandes, 1996). At the same time, *Abd-B* homologues, which had initially appeared in invertebrates, during the course of vertebrate evolution form a novel paralogous groups 9–13 (Manak and Scott, 1994).

The genes of the *Hox* family are expressed along the AP axis in the CNS and paraxial mesoderm, each with a precise anterior expression boundary. Conservation of this gene set recognized by the extent of homology and by the order of their location along the chromosome practically for all *Metazoa* from flatworms to higher vertebrates seems to be paradoxical against the background of tremendous diversity of phenotypes (forms) among all bearers of this cluster. Carrol (1995) thinks that *Hox* genes act to demarcate rather than to specify a relative position of different structures along the body axis (as though these genes outline a scheme of the main plan of body construction). The proteins encoded the *Hox* genes control the activity of many other genes. For instance, it has recently been estimated that the *Drosophila* genome contains 85 to 170 genes which are regulated by the product of the *Ultrabithorax* gene (Carroll, 1995).

The principle of colinearity between the order of genes in a cluster and their expression pattern along the rostro-caudal embryonic axis discovered by Lewis (1978) in the case of *Drosophila* HOM genes is mirrored for the *Hox-C* genes of vertebrates. It has been shown that the genes located at the 3' extremities of the each of the 4 complexes (such as group 1, 2 or 3 genes) have clear anterior limits of expression within the hindbrain. The genes of the group 1 have the anterior extremity of homeodomain at the boundary r3/r4 (Wilkinson, 1993; Couly *et al.*, 1998). Paralogues genes the 4$^{th}$ and 5$^{th}$ have an anterior limit at the level of prospective somites which demarcate the structures of cervical region, whereas genes located at 5' positions (e.g. groups 11–13 genes) are expressed at the level of the future sacral and caudal regions (Gaunt, 1994; Burke *et al.*, 1995).

Couly *et al.* (1998) showed that along the cephalic neural axis into neural crest cells (NCC) two domains can be distinguished: an anterior region corresponding to the mesencephalon and part of the metencephalon (r1–r2) in which no genes of the Hox cluster are expressed, and a posterior region corresponding to r3–r8 in which Hox genes of the first paralogous groups are expressed. It was ascertained that *Hox* gene-

expressing NCC cannot substitute for *Hox* gene non-expressing cells in construction of a lower jaw. The latter is colonized by NCC from the posterior mesencephalon and from r1 and r2 with a small contingent of cells from r3 (! - compare with the effect of *Lim1*–/– described in Section 4) that do not express any genes of the *Hox* clusters (Kontges and Lumsden, 1996; Couly *et al*, 1996, 1998). Moreover, the substitution of r4–r6 region by the fragments of the neural fold with the Hox gene non-expressing NCC yielded a typical duplication of the lower jaw, that is the structure of a "new head". These results underline the profound discrepancy between the head and the notochordal part of the vertebrate body once more.

According to Duboule (1994), one should distinguish between two aspects of colinearity, "spatial" and "temporal". In the first case it is a question of the topographical succession of gene expression, in the second it is the time of their successive activation. In reality, it is better to talk about different aspects of a single process of successive linear expression, where one aspect causes another. I think that the factor of time is a primary one; evidence of this idea have presented in my review (Gorodilov, 1992).

Thus, all complexes of Hox homeotic genes are expressed successively along the AP axis where the anterior limit of the expression of paralogous group 1 genes is between r3/r4. This practically coincides with the boundary between the influence of the *Lim1* and *Otx2* genes. The existence of both is necessary for the development of all head structures lying anterior to r4. The same boundary separates the "new head" of vertebrates - an absolutely novel acquirement - and the rest of the body inherited from the invertebrates and protochordates (Yamada, 1994; Holland *et al*., 1996). Is there a group or cluster of genes that is also of fundamental importance for head development in the vertebrates? This question will be considered in the next subsection.

**B. The complex of homeobox genes for the development of the vertebrate head**

The complex of *Hox* genes determines the spatial expression and is respectively a plan of establishment of the postotic body only, while early development of the preotic part, i.e. the head structures, also needs homeobox genes (but not those of the *Hox* cluster). In *Drosophila*, two genes of the type "gap"—*orthodenticle* (*Otd*) and *empty spiracles* (*Ems*) have been found. Mutational studies have shown that they are important for the segmentation of the head (Finkelstein *et al*., 1990; Cohen and Jürgens, 1990; Finkelstein and Perrimon, 1991). Both genes encode homeobox proteins that as shown for the case of *HOX* proteins are transcription factors as well. Their expression occurs in overlapping domains at the anterior pole of the blastodermal stage of the fly embryo.

Later, 4 genes expressed in the mouse head brain were revealed. They proved to be the homologs of *Otd* and *Ems*, each gene having 2 homologs. These mouse genes were designated as *Otx1*, *Otx2*, *Emx1* and *Emx2*. The expression areas of the genes in head brain were overlapping, like the Russian dolls (matreshka), in succession of *Emx1*< *Emx2*< *Otx1*< *Otx2* (Simeone *et al*., 1992, 1993; Finkelstein and Boncinelli, 1994). It is interesting that ectopic injections of *Otx* genes into posterior parts of the body inhibit the movements of convergent extension of the trunk-tail structures. Such movements are typical for the posterior part of the body. This causes a default of the trunk and tail, while the injection of these genes into the anterior domains does not produce any effect on their development (Andreazzoli *et al*., 1997).

When one compares the expression of *Hox* group genes with 4 *Otx* and *Emx* genes, a striking difference is seen. The anterior limit of *Hox* gene expression shifts posteriorly in accordance with the order of their arrangement along the cluster on the chromosome. Diagramic delineation of successive domains along the main body axis (Fig. 4) presents this peculiarity as a stepwise displacement of the anterior limit of their expression. Unlike the *Hox* complex, the genes *Otx* and *Emx* which are expressed anterior to the prechordal plate may have a similar step like expression profile but as though in mirror reflection, since the staggered borders for these genes are seen in their posterior limits of expression (Simeone *et al*., 1992). Thus, it is clear that the front of expression in the head region moves not antero-posteriorly but, on the contrary, postero- anteriorly. Indeed the onset of expression for all 4 genes is different and follows in succession *Otx2*, *Otx1*, *Emx2*, *Emx1* such that a reversed onset of expression occurs there being in a postero-anterior direction.

So, the vector of the successive expression of homeobox genes for the postotic part of the body occurs (moves) in an antero-posterior direction; on the contrary, for the preotic part, i.e. for the head, the vector of successively expressing homeobox genes is reversed to the opposite direction, in other words it displays a postero-anterior trend.

More and more facts have been accumulated in favour of the last statement. For example, Blitz and Cho (1995) have observed the wave of *Xotx2* expression moving through the ectoderm of gastrula stage embryos under the influence of anterior prechordal mesoderm. It has been found that the succession of expression of this gene from the midgastrula to early tail bud proceeds in the following order: midbrain, forebrain, anlage of cement gland; i.e. the wave of gene expression travels from the posterior structures of head to the anterior ones. This result is in accordance with earlier observations (Sive *et al*., 1989) which described during gastrulation a wave of inductive activity that also ended in the development of the cement gland. The *Xotx2* expression seems to be an early manifestation of this wave. By the similar mode *SHH* (*sonic hedgehog*) expression and ventralization of anterior neural plate extend from the prospective hindbrain to forebrain (Pera and Kessel, 1997). Recently Fredieu *et al*. (1997) have revealed that the treatment of *Xenopus* gastrula by lithium brings about the loss of a certain part of the anterior brain. The earlier the treatment, the more posterior the loss of head brain structures (at first diencephalon, then telencephalon).

It should be noted that the antero-posterior wave of differentiation that we observe along the trunk-tail part of verte-

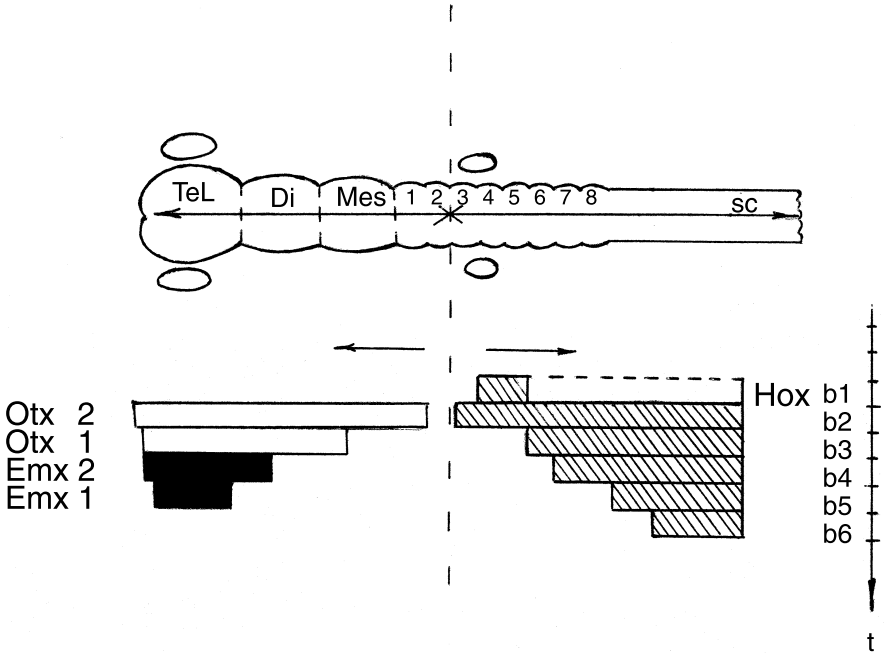


**Fig. 4.** The border of domains of expression for *Otx* and *Emx* and for the complex of *Hox* genes (cluster *b* is as an example) in head and spinal brain. The limits of expression for each gene (in the correlation with the central neural system subdivisions) are presented as the rectangle bars. (Adapted from: Holland *et al.*, 1992). *– the location of the organizer (prechordal mesoderm) after the gastrulation; *t*-temporal succession of beginning of gene expression; the arrows along the body axis indicate the directions of gene expression movement during the development. *Tel*, telencephalon; *Di*, diencephalon; *Mes*, mesencephalon; *1–8*, hindbrain rhombomeres; *Sc*, spinal cord.

brate embryos is evident. It is easy to observe the wave of somitogenesis, then the waves of the differentiation of somites and vacuolization of notochord, etc., which always follow from the anterior edge of the notochord in an antero-posterior direction. Probably the waves of differentiation are subject to the system owing to a transmission of positional information signals carried out along the trunk-tail part of the body. The Cartesian coordinates may be presented as such a system, where the directions AP and DV (dorso-ventral) could considered as two perpendicular axes of coordinates. Analysis of the destiny of cells after experimental rotation of neural plate parts (Simon *et al.*, 1955, cited from Lumsden and Krumlauf, 1996) showed that regional destiny is determined initially along the AP axis and is accomplished independently of the destiny of cells along the DV axis. Spreading of these waves is evidently connected with the longitudinal structures, in the first place in response to signals from the notochord (Cooke and Zeeman, 1975). It is more difficult to be sure of how similar process may occur in the head region.

If AP patterning of the axial mesoderm and neural tube is achieved significantly by the participation of homeobox gene family the initial step of DV patterning (along the second Cartesian axis) apparently depends on the determination of ventral midline in the floor of neural tube. It demands the expression of *Sonic hedgehog* (*Shh*) gene in underlying mesoderm along the entire rostrocaudal axis. In addition, the PCM co- expresses bone morphogenetic protein 7 (BMP7) to induce rostral diencephalic ventral midline cells (Chiang *et al.* 1996; Dale *et al.*, 1997).

### C. Meaning of BMPs, WNTs and other gene families for the laying of the head and trunk-tail regions of the vertebrate embryo

It should be borne in mind that not only the system of homeobox genes plays a part in the determination of the main plan of vertebrate body organization. In the last years, number of molecular interactions in the course of ontogenetic rise of the Spemann's organizer and the formation of the primary body axis were advanced owing to the identification of many molecular components of the processes considered.

According to the classical embryological concepts, Spemann's organizer produces the active signals that can convert ventral mesoderm to dorsal mesoderm (dorsalization) and ventral ectoderm to dorsal ectoderm (neural induction). Series of recent molecular studies seem to contradict the notion in which dorsal signaling is dominant. These studies rather suggest that the ventral position is dominant and needs to be antagonized for dorsal fate to develop. The organizer molecules, *chordin* and *noggin*, act by counteracting ventral signals rather than by directly promoting dorsal fates (Piccolo *et al.*, 1996; Zimmerman *et al.*, 1996). These two molecules block active, ventral-inducing bone morphogenetic protein (BMP) signals (Hemmati-Brivanlou and Melton, 1997; Graff, 1997) by sequestering BMP proteins into inactive complexes (Harland and Gerhart, 1997).

According to the novel concept the repression of BMP signaling leads to the formation of an organizer. However, inhibition of BMPs is not sufficient to create a fully functional early gastrula organizer: ventral expression of the BMP

inhibitors (*noggin*, *chordin*, *follistatin*, etc.) induces secondary axes that usually lack structures anterior to the hindbrain (Graff *et al.*, 1994; Lemaire *et al.*, 1995; Lemaire and Kodjabachian, 1996). This suggested that additional factors are necessary for head formation. The factors involved are likely to be WNT inhibitors. At least fifteen different Wnt genes are known in vertebrates (Nusse and Varmus, 1992; Cadigan and Nusse, 1997). In *Xenopus*, microinjection of several Wnts into the ventral cells of the early embryos leads to a complete duplication of the body axis (Cadigan and Nusse, 1997; Deardorff *et al.*, 1998). This duplication is believed to arise from formation of a second Nieuwkoop's centre, a group of dorsal vegetal cells of the early blastula which induces overlying tissue in order to it might become the Spemann's organizer.

During gastrulation WNTs as well as BMPs antagonize the organizer. The organizer secrets at least three potent WNT inhibitors: *cerberus*, *frzb-1* and *dickkopf-1*, to counteract the effect of genes encoding WNTs. All three WNT inhibitors are expressed predominantly in prechordal mesoderm, albeit in different pattern, but not in posterior chordamesoderm of the midgastrula, unlike trunk-inducing BMP antagonists. Their common function is to inhibit posteriorizing WNT signals and thus to promote head induction downstream of Nieuwkoop's centre signaling (Niehrs, 1999).

What is the effect of inhibiting both BMP and WNT signaling? It is ascertained that co-injection of mRNAs encoding WNT and BMP inhibitors induces secondary heads (Glinka *et al.*, 1997; Niehrs, 1999). The last author presented a two-inhibitor model according to which the function of Spemann's organizer is to express inhibitors of BMPs and WNTs, and thus to counteract instructive signals of Nieuwkoop's centre. Head induction results from the combined expression of WNT and BMP inhibitors while trunk induction requires the activation of BMP inhibitors only (Glinka *et al.*, 1997; Niehrs, 1999).

Apparently the ability of a large number of factors to carry out the basic functional test of the organizing potency of the early gastrula organizer to induce the second axis in heterotopic region (among them homeobox genes in the organizer, Wnt genes, *frizzled* proteins, etc.) suggests that the mechanism of this induction is either duplicated many times, or may start with the different intermediate steps. As an instance of duplicated dorsalising pathway is the homeobox gene *Siamois* which parallels antagonizing the BMP pathway (Carnac *et al.*, 1996). Another instance is *Smad 10* gene that generates ventrally complete dorsal axis, like the Spemann's organizer, but does not block BMP signals (LeSueur and Graff, 1999).

It is evident that molecular-genetic studies are provided valuable insights into the nature of Spemann's organizer. At the same time there is sensation that these molecular schemes should be superimposed on the distinct spatially-temporal structure in conditions of which the embryo form. I keep in mind that molecular interactions only can not explain as the morphogenetical patterns of organisms form. Unfortunately the spatial and temporal laws governing the development of organism are largely unknown.

## 7. THE SYSTEM OF A UNITED LONGITUDINAL AXIS OF THE VERTEBRATE BODY

As already mentioned, in cephalochordates the notochord is an axial rod that penetrates the entire body of the animal from the anterior tip of the head up to the posterior extremity (see Fig. 2). It is evident that the notochord is not only the most important element organizing the shape of the animal body entirely, but it also participates as a component of the system of coordinates used for the spreading of positional information signals in the rostro-caudal direction. The most important components of the last process are a complex of *Hox* homeobox genes and the availability of the structural vector (of the notochordal axis). As a result of this, the successive division of the trunk-tail body into a certain number of segments (regions) is accomplished with the including in every segment of an autonomic regional programme. It is likely that only after the separation of each region, the mechanisms of the DV patterning begin to function in this region. Structures which lie outside of the region influenced by the notochord may consider as independent from the control of any regional programme. In the cephalochordate ancestors, the immediate precursors of the vertebrates, the group of mesodermal cells could find itself, because of heterotopic translocation or other cause, in the position in front of the notochord. The escape out the rigid influence of the notochordal structures might provide prerequisites for the rapid and independent evolution of the new (prechordal) department. Further this outchordal mesoderm might become the initial point for the evolutionary process of a vertebrate "new head" formation.

In vertebrates, as we know, the notochord begins at the level of hindbrain r3/r4. Everything that lies anteriorly to the notochord, i.e. the region of a "new head", does not overlap with it. However, on closer inspection of the anatomical structure of vertebrate body one can come to the conclusion that the organization of a longitudinal axis as a "rod penetrating the entire body of animal" has place here too, although the basic axis contains several elements but not a single one as in *Amphioxus* (Cephalochordates). It may be seen on the schematic delineation of teleosts where the axial elements make

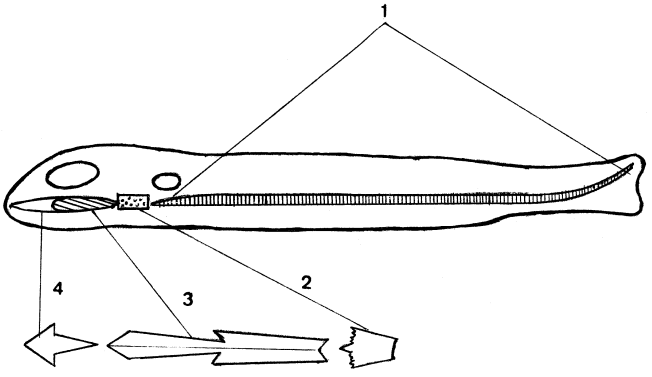


**Fig. 5.** Schematic delineation of the entire longitudinal axis of bilateral vertebrate body from the tail up to the rostral extremity. 1– notochord, 2– *basioccipital*, 3– *parasphenoid*, 4– *vomer*.

the entire long axis of the body (Fig. 5).

The fact that the trunk-tail region is penetrated by the notochord is well-known and does not require special comments. The cone-shaped anterior end of notochord becomes embedded into the *basioccipital* structure laying ahead of it. This bone in its midpart has a deepening (*sella turcica*) where a hypophysis is localized. In front the *basioccipital* is combined with the forked end of *parasphenoid*. The latter is the longest and straighted skull bone. It is included in composition of the cranial base where it disposes medially and lies on the united axis with the notochord. Considering its long, stretching, stiletto-shaped form, anyone may conclude that the *parasphenoid* is apparently destined to replace the notochord disposed in the head of cephalochordate ancestors. The

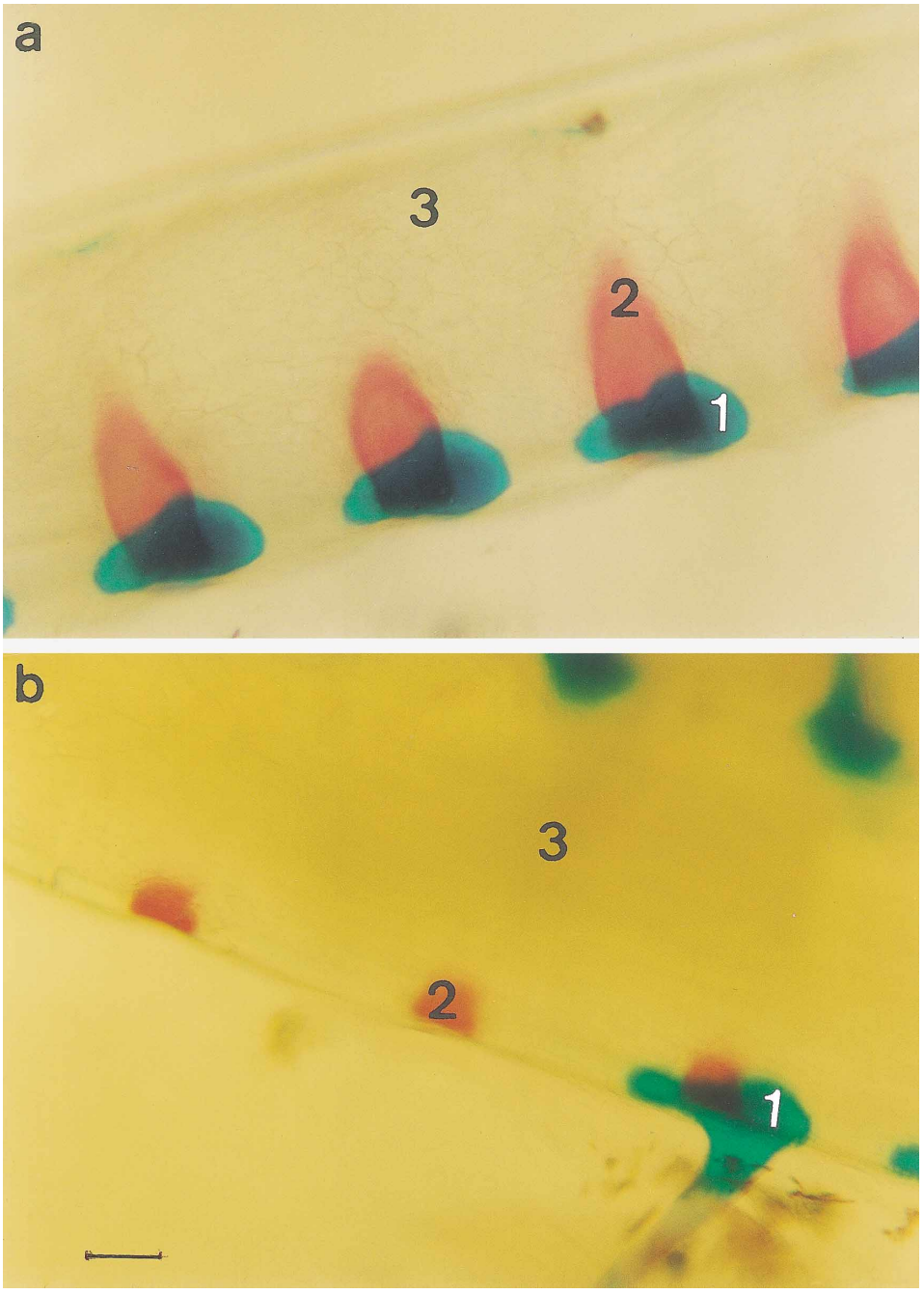


**Fig. 6.** Areas of total preparation of young specimen of the Arctic char *Salvelinus alpinus* (length of body 27 mm) at the exposure of ossificating process of vertebrae: (**a**) in anterior vertebrae (at the level of 10–15 vertebrae), (**b**) in caudal vertebrae (at the level 57–60). Staining with Alcian blue to reveal the cartilage tissue and Alizarin red to reveal the bone tissue. In this species the process of bone formation in vertebrae begins on the ventral side and spreads gradually along the lateral walls in dorsal direction. It is evident that the ossification is more advanced in the anterior vertebrae. The articulations between the spinous processes and the bodies of vertebrae are stained in blue color.Left side view, ventral to the down. 1– articular cartilage, 2– lateral ossification of the vertebra, 3– chorda. Bar, 300 μ.

existence of shoots and roughnesses along it underlines its second important function: to be the part of cranial construction. Anterior to *parasphenoid*, and slightly overlapping with it, a small stretching *vomer* completes the axis.

It was noted earlier that the anterior tip of notochord lies at the level of the midpart of the *sella turcica* (Couly and Le Douarin, 1988; Couly *et al.*, 1993). At the stages of late gastrula and neurula, PCM with the features of Spemann's organizer is localized there. The PCM organizing characteristics become apparent not only due to their ability to induce the structures of the head but also due to the onset of expression of the homeobox genes that begins from this point of embryo in the AP direction (*Hox* genes) and in the opposite PA direction (*Otx*, *Emx* genes) (Fig. 4). The notochord and adjoining

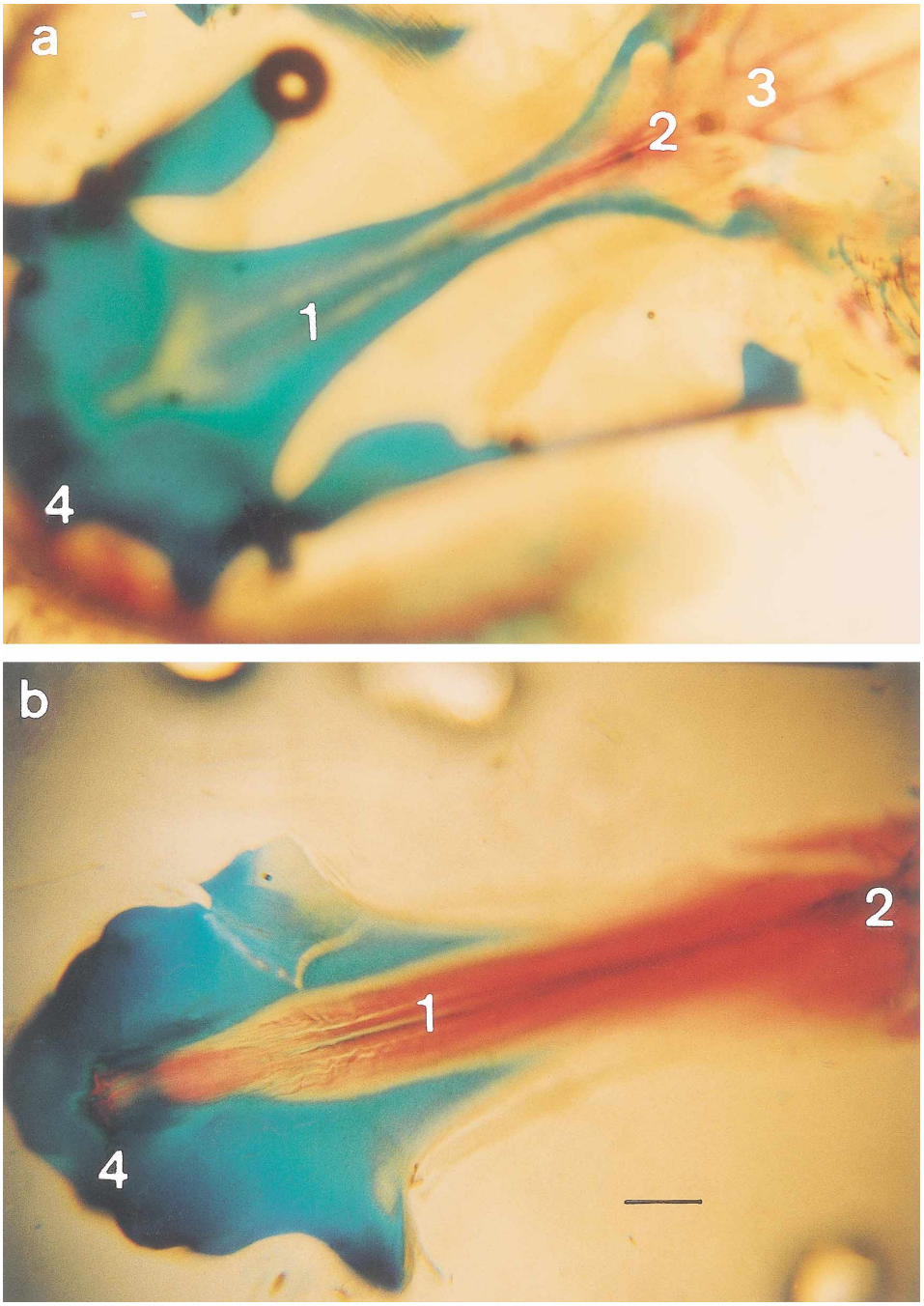


**Fig. 7.** Ossification of parasphenoid. Total preparations of young specimens of Arctic char *S. alpinus*. (**a**) *parasphenoid* of specimen with the body lenght 35 mm. *Parasphenoid* consists on the whole of the cartilage tissue (stained with Alcian blue); the ossification occured only in the small medial area on the border with the *basioccipital*. (**b**) *parasphenoid* of older (3 weeks) specimen with the body lenght 55 mm. Practically all axial zone of *parasphenoid* is stained by Alizarin red demonstrating that the process of ossification advanced in rostral direction. Anterior to the left. 1– *parasphenoid*, 2– *basioccipital*, 3– vertebra, 4– Meckel's cartilage. Bar, 400 μ.

axial structures may be considered as the structures destined for transmission of signals from the organizer to the trunk-caudal parts of embryo (Domingo and Keller, 1995), while in the rostral direction this process might be realized through the tissue that is a prospective *parasphenoid*. Regular succession of signals coming from the organizing centre can be controled by the way of a mechanism which counts the developmental time (Gorodilov, 1992).

Apart from the examples that I have presented above as an evidence of influences spreading into different directions from the boundary between head and the chordal part of the body, I would like to add my own data concerning the dynamics of the ossification process of the axial structures in the Arctic char *Salvelinus alpinus*. Ossification of vertebral bodies in this species begins with the appearance of a narrow osteal cross-piece between right and left spinous processes. Further it spreads from the articular cartilage of spinous processes along both lateral surfaces of the vertebrae in a dorsal direction. One can see the development of spine ossification in Fig. 6 where this process is shown for the anterior (Fig. 6a) and caudal (Fig. 6b) vertebrae of the same specimen. It is evident that the ossification is more advanced in the anterior vertebrae than in the caudal ones. Therefore the conclusion can be made about the rostro-caudal direction of this process of development.

In another case I have observed an ossification in the *parasphenoid* in young fishes of two different ages (Fig. 7). In younger specimens the ossification of the *parasphenoid* (Fig. 7a) begins only at the boundary with the *basioccipital* which has been shown to be more advanced rostrally in older individuals (Fig. 7b).

## 8. THE ONTOGENETIC ORIGIN OF HYPOPHYSIS

I tried to show above that the PCM continues to preserve its organizing features as a successor of amphibian dorsal blastopore lip or its corresponding analogous structures in other vertebrates. The PCM participates in the formation of trunk-tail and head regions apparently generating signals which spread in both caudal and rostral directions.

What is the further fate of this structure after its role in imparting information driving the formation of the main structures of the vertebrate embryo? Analysis of anatomical, histological and comparatively embryological materials points to a close connection between the PCM and the forming hypophysis. It is also necessary to take into account the exclusively important role which both these structures play in the corresponding periods of ontogeny.

The hypophysis is the most important endocrine gland providing the realization of normal ontogeny after embryogenesis. The anterior part of the hypophysis is the adenohypophysis or Rathke's pouch, as it was called in birds and mammals. It develops from an outpouching of the oral epithelium from the roof of the stomodeal cavity (Adelmann, 1922; Jacobson *et al*., 1979). The posterior part or neurohypophysis develops from the infundibulum, a down-growth from the floor of the primordial diencephalon (Jacobson *et al*., 1979; Kimura *et al*., 1996).

Researchers, who studied the development of adenohypophysis, have emphasized the close connection between Rathke's pouch and PCM. It was traced particularly thoroughly by Jacobson *et al*. (1979) in chick embryos. These authors found that the roof of Rathke's pouch and portions of its walls arose from the ventral base of the brain. The main interesting fact in this work is that Rathke's pouch is in direct contact with the prechordal plate (PCM). The point at which the ectoderm contacts the prechordal plate has been called the tip of the pouch. At this tip, there is a conjunction of the brain floor, ventral ectoderm, foregut, and prechordal plate. Jacobson *et al*. (1979) showed further that the tip is a pivotal point about which the surrounding tissues form the walls of the pouch. These walls form as a whole when a cranial flexure of brain arises, which is exactly the stage at which Rathke's pouch develops.

In chick at stages 10 to 12 (by Hamilton and Hamburger, 1951; there are 10–15 somites), the head of the embryo extends unbent over the proamnion (Fig. 8a). When the brain bulges begin to form and the head remains unbent, the prosencephalon is already directed ventrally at right angles to the long axis of the embryo as a result of its bulging (Fig. 8b). Most of the bending called cranial flexure is accomplished within the 5-hour period between stages 12 and 14. This is the time during which Rathke's pouch is transforming from a flat placode into an alongated pouch lying beneath the floor of the brain. Because the coincidence of cranial flexure and pouch formation was found in a variety of vertebrates, the flexure has been suggested to cause the folding of the ventral ectoderm, thereby producing Rathke's pouch (Fig. 8b, c) (reviewed in Doscočil, 1966, 1970). Thus, pouch formation is a passive result of shifts and movements of growing brain tissue (Denis'evskii and Bozhok, 1974; Jacobson *et al*., 1979).

This inference is confirmed indirectly by the results of the targeted disruption (knock-out) of the gene *Lhx3*, a *LIM*-homeobox gene expressed in the pituitary throughout development. In mice homozygous for the *Lhx3* mutation, all pituitary specific cell lineages, except the corticotropes, were not capable to secrete hormones. At the same time, Rathke's pouch itself formed, although later it failed to grow and differentiate, leading to the absence of the anterior and intermediate lobes of the pituitary (Sheng *et al*., 1996). The fact that formation of Rathke's pouch is not absolutely required for the subsequent development of the pituitary was also confirmed by observations on the formation of the zebrafish pituitary. In fish embryogenesis the obvious cranial flexure, similar to chick or mouse, is not observed and therefore probably a structure homologous to Rathke's pouch is lacking. Instead a first specification of the pituitary anlage is detected as two small areas lateral to the anteriormost neural tube. These two areas were identified as *Lim-3*-positive domains. It is known that *Lim-3* expression precedes the formation of a morphologically identifiable pituitary cluster. Later in development, the pituitary anlage cells do converge at the midline to form a structure that can be identified morphologically as a pituitary anlage

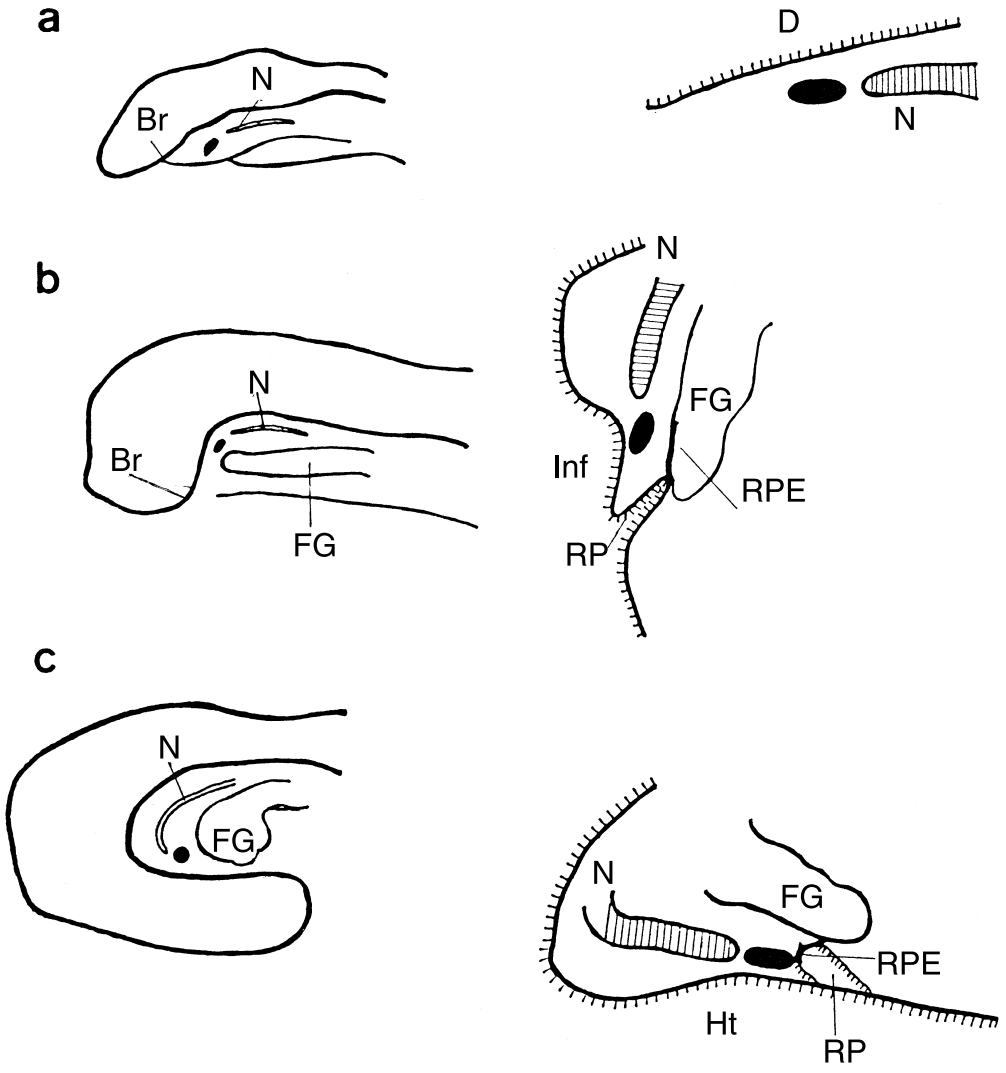


**Fig. 8.** The scheme of formation of the Rathke's pouch in result of the process of growth of brain bulges and cranial flexure in chick embryo (according to data of Denisjevskii and Bojok, 1974, Jacobson *et al*., 1979). (**a–c**) **On the left**: the common views of the chick head on the different stages of embryogenesis; **on the right**: the positions of the PCM and the ventral edge of diencephalon at the different states of the cranial flexure. (**a**) The head of the embryo before the beginning of the cranial flexure. (**b**) The ventral bulge of the brain and the cranial flexure at right angle to the embryonic axis coincide with the start of the formation of walls for the prospective Rathke's pouch. (**c**) The cranial flexure has already rotated the brain around ventrally and caudad to form Rathke's pouch. PCM indicated everywhere as blackened spot. *Br*, brain; *D*, diencephalon; *FG*, foregut; *Inf*, infundibulum; *Ht*, hypothalamus; *RP*, Rathke's pouch; *RPE*, ectoderm of Rathke's pouch; *N*, notochord.

(Glasgow *et al*., 1997).

Apparently that part of Rathke's pouch ectoderm which is joined with the prechordal mesoderm in the future tip of the pouch plays a role in the formation of the pituitary (Fig. 8b, c). Specifically this part of ectoderm gradually thickens into a placode (Jacobson *et al*., 1979) and, probably under the influence of the cells of prechordal mesoderm organizer, participates in adenohypophysis development. There are relatively recent reports that the ectoderm destined for the generation of adenohypophysis cells arises not from the stomodeum cavity, but from the epidermal placodes which appear in their turn from ectodermal thickenings located in the head part of the neurula on both sides of the neural tube (Kawamura and Kikuyama, 1992; Kikuyama *et al*., 1993).

Experimental transplantation of tissues taken from the region of anterior neuroectoderm and fate mapping studies in chick (Couly and Le Douarin, 1987), frog (Eagleson and Harris, 1989; Kawamura and Kikuyama, 1992) and mouse embryos (Osumi-Yamashita *et al*., 1994) have shown that the anlage of the adenohypophysis arises from the zone of the anterior ridge of the neural plate. It is unlikely that this anlage develops from the superficial tissue, more likely it arises from deeper layers, apparently from the mesoderm (Kawamura and Kikuyama, 1992).

As far as is known, in vertebrate embryos the pituitary is located in the *sella turcica*. As described above this bone has a double origin. It was also noted that the anterior tip of the notochord lies approximately at the level of the *sella* midpoint.

Since the PCM borders the anterior notochordal end it is evident that it occupies just the area where the pituitary is located in the definitive organism (Couly and Le Douarin, 1985, 1987, 1988).

The genes which probably play a principal role in the development of the hypophysis are also initially expressed in the region of the organizer. In the mouse embryo, a new homeobox gene has recently been isolated which is expressed firstly in PCM and later its transcripts become restricted to Rathke's pouch (Hermesz *et al.*, 1996). It has been termed as *Rpx*, Rathke's poucn homeobox. This gene is also refered to as *Hesx1* (Thomas *et al.*, 1995). Orthologues of *Hesx1/Rpx* are *Xanf* in amphibia and *Ganf* in chick (Zaraisky *et al.*, 1995; Kazanskaya *et al.*, 1997).

In mouse, Rpx transcripts are first detected by in situ hybridization at 6, 5 dpc, the onset gastrulation, in the endodermal layer. Later, more accurate definition showed that Rpx expression at this stage is seen in a very restricted region of the endoderm, in anterior visceral endoderm (AVE), that possesses the head organizing activity (Dattani *et al.*, 1998; Beddington and Robertson, 1999). At 7 dpc Rpx expression was detected in the midline endoderm/mesendoderm that forms the prechordal plate precursor. At 7, 5 dpc *Rpx* expression was also detected in the ectoderm which is juxtaposed to the endoderm/prechordal mesoderm and which will become the cephalic neural plate. Dramatic restriction of *Rpx* expression was seen at 9 dpc. The expression was no longer detectable in the neuroectoderm, but was exclusively restricted to the layer of ectodermal cells that will give rise to Rathke's pouch, the primordium of the adenohypophysis. The latter ultimately becomes the anterior and intermediate lobes of the pituitary.

It has been shown that in mice 9, 5–10 dpc the anterior wall of Rathke's pouch is in direct contact with the diencephalon base (Kimura *et al.*, 1996; Sheng *et al.*, 1996), the prospective hypothalamus. Subsequently, the neurohypophysis, derived from an outpocketing of the diencephalic neuroectoderm adjoining to the Rathke's pouch, gives rise to the posterior or neural lobe of the pituitary, which contains the axon terminals of neurosecretory neurons (Hermesz *et al.*, 1996).

At 11, 5 dpc *Rpx* is strongly expressed in virtually all cells of the pouch. 1–1.5 days later, the definitive pituitary cell types begin to differentiate into Rathke's pouch. During 3 days practically all definitive cell types of the adenohypophysis appear one after the other in successive temporal order: corticotropes (12, 5 days), thyreotropes (13, 5 days), melanotropes (14, 5 days), somato-, lacto- and gonadotropes (15, 5–16 days).

*Rpx* is the earliest known gene to be expressed in the pituitary primordium and apparently *Rpx* is involved in the initial determination of the pituitary cells (Hermesz *et al.*, 1996). *Rpx* activity first begins at the stage of early gastrula in AVE that is supposed to be a component of the early gastrula organizer for the induction of anterior neural tissues (Beddington and Robertson, 1999; Camus and Tam, 1999). After this Rpx activity appears in prechordal plate (endomesoderm) and then in region of adenohypophysis indicating of the likely interconnection and probably the succession between this structure and the pituitary.

*Rpx* is an important, but not the only gene participating in pituitary formation. At least 5 homeobox genes taking part in the formation of the adenohypophysis have been found to date

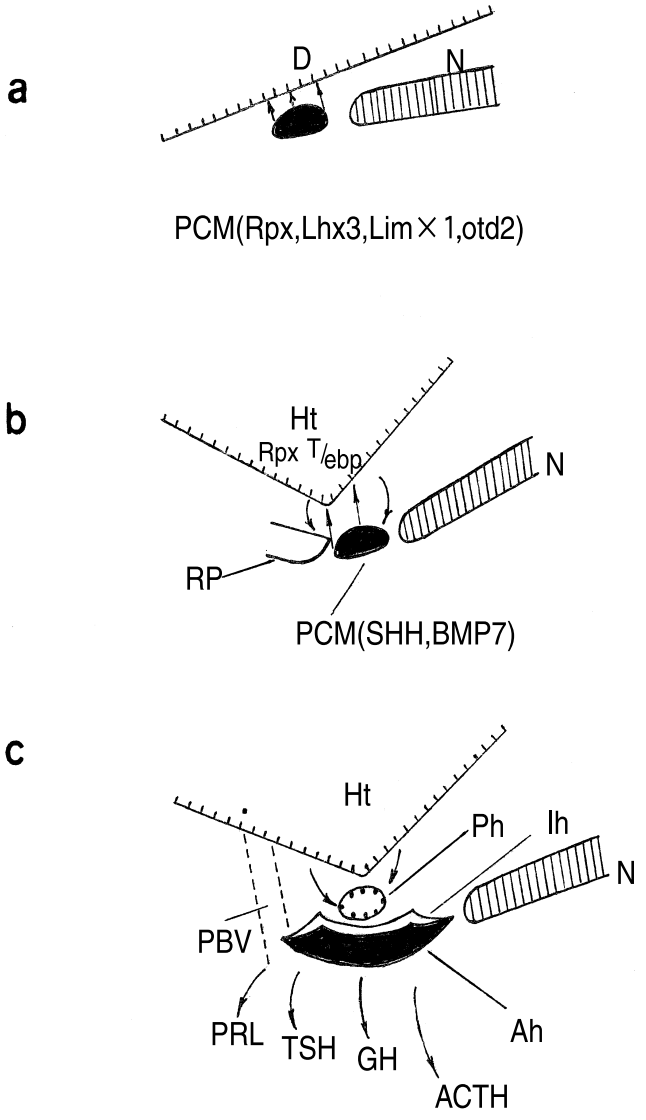


**Fig. 9.** Several steps of the PCM and hypophysis development. (**a**) The period of neurulation. The participation of the PCM in the formation of brain and head of the vertebrates. Some genes which are expressed within the PCM and have the influence on brain development are indicated in brackets. (**b**) The stage of the forebrain ventralization and the formation of hypothalamus. Ventralizating factors (e.g. *SHH*, BMP7) from the PCM effect on the brain; the return influence of *Rpx*, *T/ebp*, etc., expressing in the ventral brain, are directed to the PCM. (**c**) Substitution the PCM by adenohypophysis and formation of the pituitary gland with the cells secreting ACTH (adrenocorticotrope hormon), GH (growth hormon), TSH (tireo-stimulating hormon), PRL (prolactin) and other. *Ah*, adenohypophysis; *D*, diencephalon; *Ih*, intermediate hypophysis; *Ht*, hypothalamus; *N*, notochord; *PBV*, portal blood vessel; *Ph*, posterior (neuro) hypophysis; *RP*, Rathke's pouch. The black zones mark PCM (a, b) and adenohypophysis (c).

(Watkins-Chow and Camper, 1998). One of the *LIM*-homeobox family of genes *Lhx3* (also known as *mLim*-3 and P-*Lim*) has an important role in pituitary formation and the activity of *Rpx* (Bach *et al*., 1995;Sheng *et al*., 1997; Sheng and Westphal, 1999). This has been shown by targeted disruption of the *Lhx3* gene. In mice homozygous for the *Lhx3* 0–/– the gene *Rpx* is expressed normally up to the stage of 10.5 d.p.c., but then its activity decreases and ceases. As a result, the *Lhx3* mutant lacks the anterior and intermediate lobes of the pituitary and, naturally, all 5 types of hormonal cells are absent (Sheng *et al*., 1996, 1997). Gene *Lhx3* is required for the differentiation of cell populations that produce growth hormone, thyroid-stimulating hormone, prolactin, and luteinizing hormone, as well as the maintenance of these major cell lineages in the adult mouse pituitary (Glasgow *et al*., 1997).

Another gene, the expression of which is traced for the space of the whole history of the studied tissue from the Spemann's organizer to the hypophyseal anlage, has been recently investigated (Deardorff *et al*., 1998). I refer here to the gene *Xfz8* that was isolated in *Xenopus*. It belongs to the group of genes that produce *frizzled* proteins identified as likely receptors for *Wnt* ligands. Ectopic expression of *Xfz8* in ventral cells leads to complete secondary axis formation. It has been shown that *Xfz8* expression begins in the early blastopore lip (stage 10+) and become restricted to the midline cells extending from the lip to the leading edge of the migrating endomesoderm. During late gastrula and early neurula (stages 12 and 13) *Xfz8* is detected in the PCM. Finally, by tailbud stage 22 *Xfz8* is expressed in the stomodeal-hypophyseal anlage (Deardorff *et al*., 1998).

The influence of prechordal plate firstly on the development of the forebrain and later, on contrary, the influence already of the forebrain or, more exactly, its ventral region on the hypophysis has been studied by means of the observation of effect of the gene *T/ebp* or *Nkx2.1* (Kimura *et al*., 1996; Pera and Kessel, 1997). This gene demarcates the ventral part of the forebrain which will form then the hypothalamus and neurohypophysis (Lazzaro *et al*., 1991). It is expressed in the ventral diencephalon which lies just above the prechordal mesoderm. Such a position of expressed domain *T/ebp* supposes essential influence of vertical signals from the PCM upwards on the ventral part of forebrain with the aim of activating *T/ebp*. Later the zone of expressed *T/ebp* acts in the opposite direction, participating in hypophysis formation (Fig. 9a, b). In the *T/ebp* –/– mutants significant impairments take place in the ventral part of forebrain and the hypophysis is lacking entirely (Kimura *et al*., 1996).

## 9. SPEMANN'S ORGANIZER AND THE ORIGIN OF VERTEBRATES

I allow myself to sum up what principal innovations have originated in phylum *Chordata* in general and in subphylum *Vertebrata* specifically. It is recognized that the very general characters, which unite lower chordates (subphyla *Tunicata* and *Cephalochordata*) and vertebrates, are the notochord and neural dorsal cord. In Table 1 I have tried to collect together all the data about the very important innovations that became fundamental for the unification of a lower chordates and vertebrates in limits of the phylum Chordata. It is evident that first two characters (notochord and neural cord or tube) are inherent to all chordates. Other features indicated in Table 1 are characteristic for the vertebrates only. They listed in the sequence how they appear in ontogenesis. All of them somehow connected with the vertebrate head.

**Table 1.** Comparative list of new morphological characters of higher systematic status gained by the lower chordates and vertebrates in the evolutionary process. (+) as the existence of character, (–) as the absence of it

| Character | Lower chordates | Vertebrates |
|---|---|---|
| Notochord | + | + |
| Dorsal neural plate | + | + |
| Spemann's organizer | – | + |
| Prechordal mesoderm | – | + |
| Neural crest | – | + |
| Ectodermal neural placodes | – | + |
| Brain with subdivisions | – | + |
| Skull cartilaginous and bone | – | + |
| Hypophysis | – | + |
| Visceral cranium | – | + |
| Some organs of senses | single | paired |

The earliest event in this scenario is the formation of mesoderm by means of invagination. This is conventional process even for many invertebrates (Fig. 10a). Precisely the same process ensures the formation of mesodermal primordia in protochordates. However, more advanced step is the conversion of the mid-dorsal mesoderm into chordomesoderm (the presumptive notochord). The last induces the overlying ectoderm causing it to differentiate into a concentrated layer of neural tissue, the neural cord, which produces a neural tube (Fig. 10b). The formation of both structures, a notochord and a neural tube, is a prerequisite for the development of a bilaterally symmetrical body characteristic to all chordates. The description of the development of a notochord and a neural cord in amphioxus (Conklin, 1932) shows that the mode of their formation in protochordates and vertebrates differs very little from one another (Langille and Hall, 1989). Taking it into account, anyone can image that if the chordamesoderm of protochordates delineates the plane of bilateral symmetry and induces the neural system development, it plays indeed the same role that does the second part of Spemann' organizer, forming the trunk in vertebrate body. In order to say about likeness or even identity of them, one issue should be decided: is the ability to organize the axial organs of protochordates predetermined up to the beginning of gastrulation (as in vertebrates)? Apparently it is so. For example, in ascidians presumptive notochord cells are specified already during the cleavage (Jeffery, 1997). In such a situation from two parts of the heterogenous Spemann's organizer only one that is responsible for the induction of head is truly new and the very early character of vertebrates (Fig. 10c).

Further I will try shortly to review the succession of events connected with the appearance of Spemann's organizer and

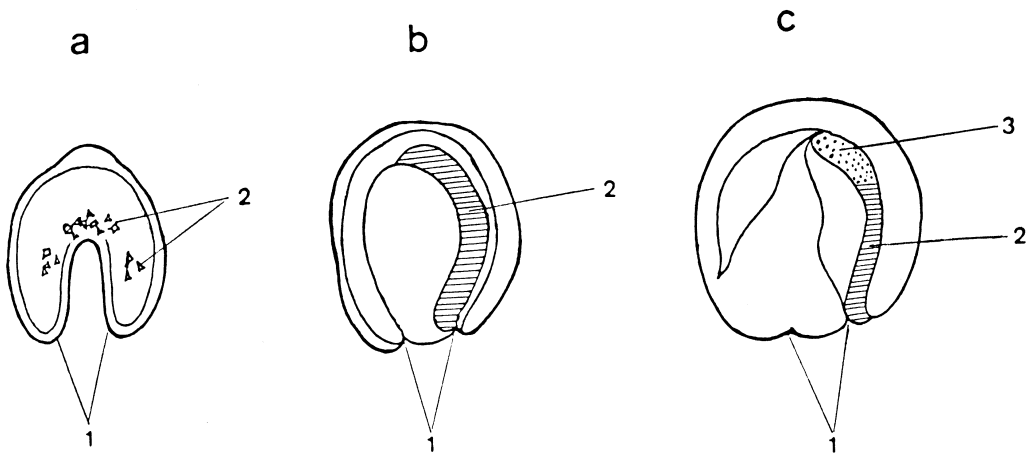


**Fig. 10.** The main stages of the Spemann's organizer evolution. (**a**) Invagination and formation of mesoderm. This process is constantly observed already in invertebrates. 1- blastopore; 2- mesoderm. (**b**) In protochordates the formation of notochord provides the bilateral symmetry of the body and induction of dorsal neural tube. Protochordates develop the trunk organizer (hatched zone), that is one from two basic components of heterogeneous Spemann's organizer. 1– blastopore; 2– chordomesoderm or the trunk component of Spemann's organizer. (**c**) In vertebrates the head organizer (dotted zone) is added to the trunk component (hatched zone). The former is the truly novel acquirement of vertebrates. 1– blastopore; 2– trunk (chordal) component of Spemann's organizer; 3– head component of Spemann's organizer.

its participation in the vertebrate head development (with amphibia as a paradigm).

1) The process preceding the formation of blastopore is the dorsalization of the embryo through the signal molecules (BMPs, WNTs). They emanate from the vegetal part of egg (from Nieuwkoop's centre in amphibia) to the marginal zone (Kimelman *et al*., 1992; Christian and Moon, 1993b; Lemaire and Kodjabachian, 1996; Hedgepeth *et al*. 1997) The last period the corrections were submitted in this scheme. They implies that the dorsalization of embryos is after the expression of noggin, chordin and apparently other genes which block the ventralising signals (Hemmati-Brivanlou and Melton, 1997; Graff, 1997)

2) The very first morphological structure that appears in embryos and that is connected with the development of head is the part of Spemann's organizer disposed in the dorsal lip of early gastrula.

3) In result of invagination, the cell population of early dorsal lip moves to the presumptive anterior region of embryo.The tissue of trunk organizer that moves after the head organizer is disposed in the same layer (Fig. 1). The former develops into the notochord, the latter arranges immediately ahead of it and designates as prechordal mesoderm. The PCM is a derivative or successor of early gastrula Spemann's organizer and presents itself the most important new structure of vertebrate embryos at late gastrula-early neurula stages.

4) PCM and notochord induce overlaying ectoderm, each on its level along the bilateral axis, to form the neural plate. The specifical features of neural system development in head and trunk regions depend on specifical pecularities of corresponding organizer that were described in previous sections. I remind that the head organizer ensures not only of the development of head structures, but it is also able to induce entire embryo axis. Positioning of the PCM in the prechordal zone, at the point from which the formation of the head begins, is the most favourable in a spatial respect: from this position it is able to exert control over the development of both head and trunk departments. The axial structures are apparently used for the endowing of signal transmission: a notochord complex in the posterior direction, a *parasphenoid* complex in the anterior one. Expression patterns of homeobox gene rows directed to opposite sides have a beginning just in the zone where PCM is located (Fig. 4).

5) The head brain formation begins under control of PCM. Necessity of PCM for the establishment of head has been shown by the mutation Lim1–/– (section 4), when the absence (or reduction) of PCM precedes the full absence of head tissues (Shawlot and Behringer, 1995). In protochordates, which have a notochord and neural tube, but have no PCM, the brain is developed so poorly that its various subdivisions and primary sensory organs are practically indistinguishable (Guthrie, 1975; Lovtrup, 1977; Jollie, 1977; Langille and Hall, 1989). In vertebrates these organs acquire powerful development. Experimental removal of PCM in chick (Pera and Kessel, 1997) have possibly been done of a time point when the head already began to develop. Nevertheless, it led to significant narrowing of the anterior neural tube and cyclopia. By the way, the existence of the single eye is characteristic for all lower chordates.

6) In the process of neural tube formation at the junction between neural ectoderm and the epidemal ectoderm along the entire length of it, a population of cells arises from the ectoderm. They compose the so- named neural crest incorporating initially into the neural tube to form its dorsalmost alar closure. At this stage of developmental process the formation of neural crest is a major cephalization event on the way to the definitive vertebrate. The neural crest is unique as an ectodermally-derived source of mesenchyme that plays exceptional role for the vertebrate head. It exhibits a vast number of developmental fates: it either give rise to, or participate

in, the development of all sensory innovations, as well as the neuro- dermato-, and visceral-crania and connective tissues of the vertebrate head (Gans and Northcutt, 1983; Hall, 1988; Couly *et al*., 1992, 1993; Kontges and Lumsden, 1996). At the same time it is necessary to underline that these unique cells are the innovations of the vertebrates since such cells have not been detected in *Amphioxus* (Holland *et al*., 1996; Baker and Browner-Fraser, 1997). Exactly as the epidermal placodes that in vertebrates present themselves the dorsal thickenings of ectoderm and participate in the development of paired sense organs, are absent in protochordates.

7) Cephalization of brain, the development of pharyngeal and jaw systems are fulfiled by the ectomesenchymal cells migrating ventrally from the neural crest. This migration begins rather early, in chick embryo at the 6- to 7-somite stage (Couly *et al*., 1992). Simultaneously, the PCM is gradually transformed in the adenohypophysis, apparently without ceasing its organizing functions. In the previous section I analyzed the data of morphological and molecular-genetical studies as evidence of this transformation. Moreover, in lower chordates, ascidians and lancelets, neither PCM nor hypophysis have been found (Conklin, 1932; Satoh, 1994). Taking into account my arguments in favour of succession of these structures and their interdependence, the lack of both structures in protochordates looks understandable.

Thus, it is undoubted that the very striking difference of the vertebrates from all preceding Metazoa, including the lower chordates, refers to the origin of a new head. The number of fundamental innovations connected with the origin and development of head (Table 1) is unparalleled for the evolutionary history of animals. Among them Spemann's organizer, the PCM and the hypophysis are different states of the same organizing system playing exceptional role for the regulation of development and live activities of vertebrate organism in whole during the various stages of ontogeny.

I have already tried above (Section 7) to delineate a possible evolutionary origin of the head in vertebrates. Initially in cephalochordate ancestor it might have been only a small cellular cluster segregated by some way from the notochord into an anterior extremity of the body (the case of heterotopic evolutionary changeability). Apparently this precursor of the PCM escaped from the rigid control of the notochord and paraxial structures which provide transmission of the positional information along the notochord in the AP direction. PCM is a vertebrate-derived feature and has been theoretically considered to be a part of the forward growth of the head (Jollie, 1977). The arising of PCM might induce the displacement of the neural plate into an anterior direction and further produce a stimulating influence on its development making it more composite. Experimental data that confirm the reality of the time history of such an evolutionary events appeared most recently. Ermakova *et al*. (1999) microinjected gene *Xanf*, the ortholog of *Rpx*, in *Xenopus* dorsal ectoderm. They demonstrated that ectopic *Xanf* can expand the neural plate at expense of adjacent epidermis. Consequently, this gene which plays the most important role for PCM and then for the development of adenohypophysis, has the ability for conversion to CNS fate of the prospective cells of epidermis. It is possible that owing to genes of this family the PCM could be essential for the development of the vertebrate head brain. In other work Knoetgen *et al*. (1999) demonstrated that *noggin* normally expressed in the organizer is able also to enlarge the anterior neural plate. It is possible that *noggin* causes this effect through the induction *Xanf* and its orthologs (Sasai *et al*., 1995). The anterior end of the notochord has remained where it was in the ancestral form of *Amphioxus*, whereas the brain has grown forward (Kingsbury and Adelmann, 1924; Jollie, 1977). The lack of segmentation of prechordal neural plate (Kuratani *et al*., 1999) might facilitate its outgrowth (Gans and Northcutt, 1983). Owing to the rather free and weakly controlled outgrowth of anterior mass of neuroectoderm, the cells with totipotent characters as precursors of neural crest ectomesenchym might have arisen. These cells were able subsequently to give rise for a vast number of developmental fates, including nerves, muscles, cartilages, bones. The interconnection between PCM and overlying outgrowing brain has gradually complicated and improved, the PCM controlling and organizing functions have also developed. *Rpx*, *T/ebp* and other genes may be examples of reciprocal inductive relationship between PCM and overlying brain. On account of the growth of head brain mass and dimensions, of the increase of the distance from an organizing PCM to the cells- and organs-targets the structure of the PCM cells properly has become to change. The new means of the connection between these cells and cells-targets have become necessary. The network of blood vessels could carry out this connection (Fig. 9c), while the role of signal mediators has been transfered to the hormones threw away from the cells in the blood. It was just that cause why the PCM structure could be transformed in the complex of grandular cells.

After the formation of the vertebrate head, the development of jaws became another powerful innovation during the evolution of vertebrates towards two broad groups: *Agnatha and Gnathostomata*. The fact that the elements of the jaws are branchial arch derivatives is inferred from developmental and morphological evidence (Maderson, 1987;Carroll, 1988). Among the series of pharyngeal pouches that form in *Agnatha*, the most anterior pouch was called the mandibular one, since it is a precursor of the lower jaw. Subsequent pouches form the hyoid, branchial skeleton and associated tissues (taken together, they may be called the visceral cranium or splanchnocranium) (Hall, 1988; Langille and Hall, 1989; Horigome *et al*., 1999).

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**10. SUMMARY**

In 1924 H. Spemann and H. Mangold discovered that a

piece of the dorsal lip of the blastopore from *Triturus cristatus*, after transplantation to the ventral side of another embryo was able to cause the neighbouring tissues to change their fate and participate in the formation of a new embryo. They called the dorsal lip *the organizer*. Since then, during 75 years attempts have been made to establish the intimate mechanisms of organizer activity. However, a real advance in the understanding of organizer mechanisms had not been achieved.

In the last 15–20 years, genetic and molecular techniques have been vastly improved. They have helped to trace the fate of many cell lineages and to compile more exactly fate maps for the different parts of the embryo. Using these data, I have made an attempt to trace the fate of Spemann's organizer after the early gastrula stage.

Analysis of data concerning the inductive abilities of organizer cells, of the using of markers and the observation of the expression of specific genes allows one to conclude that Spemann's organizer in amphibia, and its homologues in other vertebrates too are heterogeneous: they have in their composition at least distinct cell populations with the head organizer and trunk organizer activities. These populations is proposed to be determined to become the head and trunk organizers still being in the blastula stage and they might take their place either in single continuous cell layer (as in amphibia and birds) or to be separated to the different tissue germs (as in mammals). When the dorsally-ventral orientation of embryo is established and the organizer structure begins to develop the very early invaginating cells from the dorsal blastopore lip (in the case of amphibia) move in advance of the entire invaginating mesoderm and occupy the place just in front of the notochord by the end of gastrulation. It is supposed that the early dorsal lip and the prechordal mesoderm (PCM) are one and the same cell population, i.e. during gastrulation Spemann's organizer transfers from the lip of blastopore to the prechordal zone.

The PCM seems to play an exclusive role in the formation of the vertebrate head, because some mutations in genes expressed in the PCM result in the deletion of the entire head. It is supposed that the spreading of differentiating signals from the PCM occurs along the main body axis in both caudal and rostral directions. After the formation of the main body plan, the PCM becomes replaced by the adenohypophysis. This conclusion is drew not only from the same topological placing of both structures, but from the similarities of the set of specific genetical markers that express in both that supposes the existence of the deep connection and succession between them. The adenohypophysis seems to arise directly from the PCM or cells of the ectoderm that receive the influence of the PCM and are subsequently transformed into the humoral cells of the adenohypophyis. In such the interpretation PCM and Spemann's organizer properly as well, may consider as precursors of basic hormone-regulating system of an organism functioning during the postembryonic ontogeny. This inference is supported by the fact that all three structures origin first in vertebrate animals only.

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