Int. J. Dev. Biol. 42: 909-916 (1998)

Neurulation in amniote vertebrates: a novel view deduced from the use of quail-chick chimeras

NICOLE M. LE DOUARIN*, MARIE-AIMÉE TEILLET and MARTIN CATALA

Institut d'Embryologie Cellulaire et Moléculaire du CNRS et du Collège de France, Nogent-sur-Marne Cedex, France

ABSTRACT Two apparently different mechanisms successively contribute to the formation of the neural tube in the avian embryo: bending of the neural plate during the primary neurulation in the cephalo-cervico-thoracic region and cavitation of the medullary cord during the secondary neurulation in the lumbo-sacral region. During both these processes, gastrulation continues by the caudal regression of Hensen's node – also called cordoneural hinge in the secondary neurulation. Labeling of Hensen's node or cordoneural hinge by the quail chick marker system revealed that this structure, which is the equivalent of the dorsal blastoporal lip of the Amphibian embryo, i.e., of the Spemann's organizer, gives rise to the midline cells of the three germ layers: the floor plate of the neural tube, the notocord and the dorsal cells of the intestinal endoderm. Caudally to the organizer, both in primary and secondary neurulation, the presumptive territory of the alar plates of the future neural tube overlies the precursors of the paraxial mesoderm. Regression of Hensen's node bisects the ectoderm in two bilateral neural plates leaving in its wake the floor plate, the notocord and the dorsal endoderm.

KEY WORDS: *neurulation, organizer, floor plate, notocard, midline cells*

Primary and secondary neurulations

Neurulation in Amniotes proceeds in two temporally and spatially distinct steps that have been clearly recognized in the avian embryo. In the anterior part of the body, corresponding to the cephalic and cervico-truncal regions of the neural primordium, neurulation first involves the formation of the neural plate limited laterally and anteriorly by the neural folds. The latter constitute a boundary between the neural epithelium and the presumptive superficial ectoderm lying latero-ventrally to the neural plate and fated to become the epidermis. The neural plate is the site of morphogenetic movements leading to the formation of the neural tube by the apposition and fusion of the bilateral neural folds on the mid-dorsal line (Fig. 1).

The fate of the neural fold cells is to give rise to the neural crest from the level of the mid-diencephalon caudalward. The neural fold of the anteriormost area of the neural plate, however, has different and much more diversified fates. As shown by Couly and Le Douarin (1987) it yields glandular structures (adenohypophysis and epiphysis), sensory epithelium of the olfactory system, mucous epithelium of the olfactory cavities and of the roof of the mouth and the epidermis covering the upper lip (the beak in birds) and the frontal area (see Fig. 2).

Folding of the preexisting neural plate according to the process of *primary neurulation* leads to the formation of an anterior and a posterior neuropore respectively located ventrally to the telencephalon (Puelles *et al.*, 1987) and at the level of somite 27 (Catala *et al.*, 1995) in the chick and quail embryos. Somite 27 participates in the formation of the last thoracic and the first lumbar vertebrae (Fig. 3) and therefore corresponds to the transition from the truncal to the lumbar spinal cord. The part of the body located caudally to the posterior neuropore forms from an apparently homogenous mass of mesenchymal cells, the tail bud. From embryonic day 3 (E3) onward, in quail and chick embryos, the tail bud or "bourgeon troncocaudal" according to Pasteels (1937), is the site of intensive growth and morphogenesis resulting in its elongation and notably in the formation of a neural tube by a process referred to as *secondary neurulation* (Pasteels, 1937; Holmdahl, 1938; see Fig. 4).

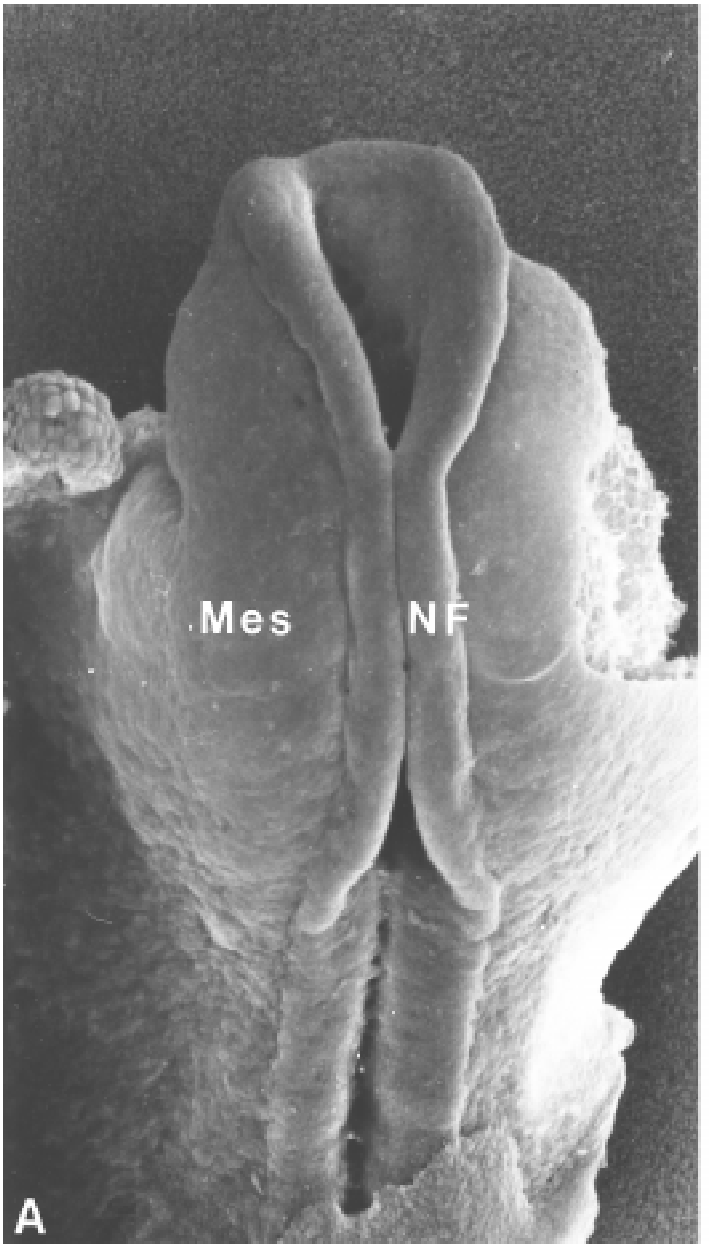
Cells of the tail bud aggregate on the midline forming a cellular cylinder of densely packed cells by a transition from a mesenchymal to an epithelial state (Fig. 4B,C). The neural tube differentiates through a process called cavitation which involves the formation of a central cavity inside the initially compact neural cylinder (Fig. 4D,E). Close to the level of the posterior neuropore, primary and secondary neurulations overlap for a short period of

Abbreviations used in this paper: CNH, cordo neural hinge; DBL, dorsal blastoporal lip; E, embryonic day; HN, Hensen's node; ss, somite stage.

***Address for reprints:** Institut d'Embryologie Cellulaire et Moléculaire du CNRS et du Collège de France, 49bis, Avenue de la Belle-Gabrielle, 94736 Nogent-sur-Marne Cedex, France. FAX: 33 1 48734377. e-mail : Nicole.le-douarin@infobiogen.fr

0214-6282/98/$10.00

Printed in Spain



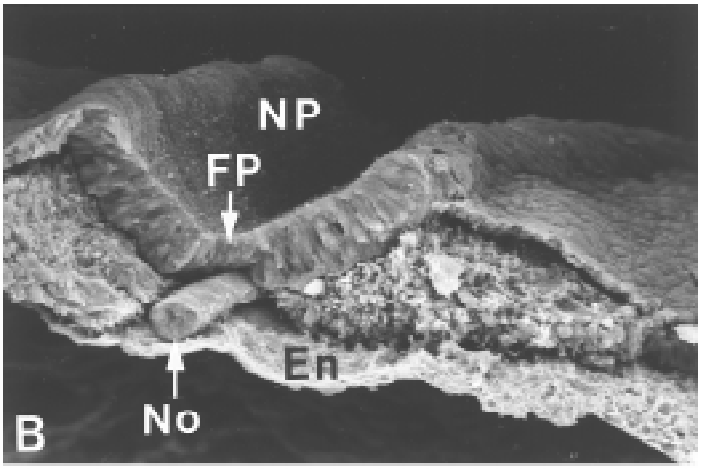


Fig. 1. Neural tube closure. *Scanning electron microscopy.* **(A)** *Cephalic region of a 6-somite stage embryo. Neural folds (NF) are close together at the mesencephalic level (Mes) while they are still separated posteriorly and anteriorly.* **(B)** *Troncal region of a 10-somite stage chick embryo. The neural plate (NP) is still largely open in the non-segmented region. Floor plate territory (FP), notocord (No) and medial endoderm (En) are in close contact one with the other.*

time and over a short anteroposterior distance. Later on, the secondary neural tube is undistinguishable from its anterior counterpart formed by folding of the preexisting neural plate (Fig. 4A).

Dorsally, neural crest cells delaminate from the most dorsal aspect of the medial cellular cylinder, according to a rostrocaudal direction. At the same time, the paraxial mesoderm becomes organized in somites and the coelomic cavities and ventral endomesodermal structures are formed according to morphogenetic processes that have not attracted much attention in the recent years (see Le Douarin, 1964 for the formation of the hindgut). The main characteristic of this secondary neurulation is that it leads to the formation of a neural tube in absence of a preexisting ectodermal germ layer and of a neural plate. Thus, tail bud organogenesis does not involve the initial step usually encountered in embryogenesis of most triblastic organisms whereby the three germ layers are clearly individualized before organogenesis begins.

The mechanisms through which the tail bud becomes organized have been the subject of controversies in the past. For Holmdahl (1925,1938) and more recently Griffith *et al.* (1992), the tail bud can be assimilated to a regeneration blastema in which the various tissues are formed by apposition of differentiating cells at the caudal end of already formed structures. By contrast, Pasteels (1937) considered that tail bud development proceeds according to rules similar to those controlling gastrulation. Such a view was demonstrated to be valid in the amphibian embryo (Bijtel, 1958; Gont *et al.,* 1993).

Analysis of tail bud development by the quail-chick chimera system

The quail-chick marker system seemed appropriate to study this problem and solve this controversy since it could allow the relative movements of the cells forming the tail bud to be followed, thus eventually disclosing a subjacent prepattern and possibly the predetermination of certain territories within this apparently homogenous structure.

The experimental design used in this study consisted in the substitution of defined territories of the 25-somite stage (ss) chick tail bud by their counterpart from stage-matched quails and *vice versa*. The various operations performed (Catala *et al.*, 1995) are indicated in Figure 6A.

Pasteels (1937) noticed that at the anterior region of the tail bud, the posterior ends of the notocord and of the ventromedial neural tube are joined together by a mass of densely packed cells that he designated as *"charnière chordo-neurale"* or cordoneural hinge (CNH) that he considered as the remnant of Hensen's node (HN), the homolog of the dorsal blastoporal lip (DBL) or organizer region of the amphibian embryo.

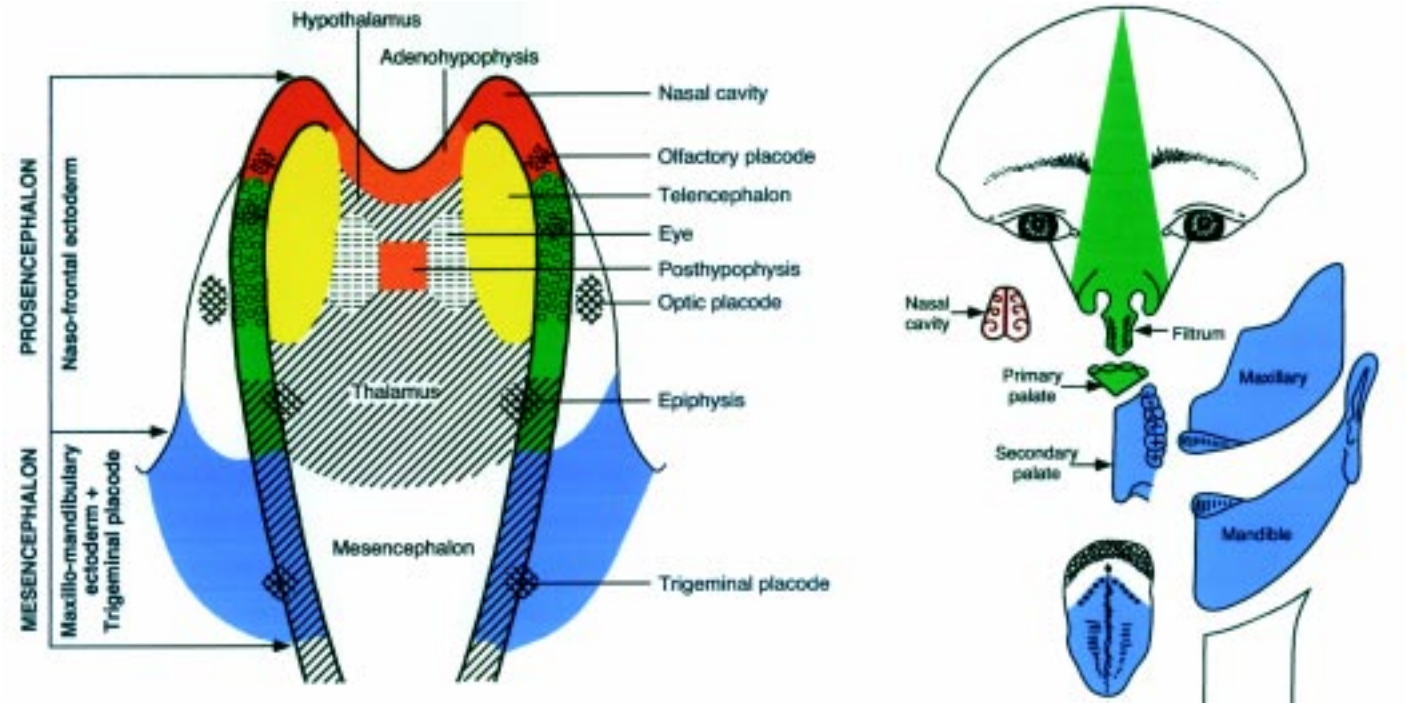


Fig. 2. Derivatives of the cephalic neural crest and human congenital abnormalities. *Different territories of the cephalic neural folds and associated ectoderm, and their derivatives in human head are schematized.*

In fact, Pasteels' view on the nature of this mass of densely packed cells was fully justified. Molecular markers allow now to show that HN as seen at the anterior tip of the early primitive streak in the gastrulating embryo and the CNH share several gene activities such as $HNF_3\beta$ (Fig. 5A,B,C), $CNot_2$ (Stein *et al.*, 1996) and chordin (Fig. 5D).

Some of these genes have been shown to be critical in the formation of axial structures. Such is the case for $HNF_3\beta$ which is expressed in the organizer in the frog (Ruiz i Altaba and Jessel, 1992) and the null mutation of which prevents neurulation in the mouse (Ang and Rossant, 1994). Moreover, $XNot_2$ is also expressed in the DBL and in the notocordal material extending down to the tail during neurulation in the *Xenopus* tail bud (Gont *et al.*, 1993).

Region 1, as defined in our experiments (Fig. 6A) includes the posterior end of the neural tube formed by the *primary neurulation* process and the CNH. The experiment thus consisted in grafting either the neural tube alone or a territory including also the CNH. It should be noted that the endodermal epithelium was included in neither the graft nor the excised territories.

Grafts of the terminal part of the neural tube produced the entire spinal cord at the level of sacral nerves 3 to 6 and the corresponding neural crest derivatives (Catala *et al.*, 1995). When the graft included the totality of the CNH located at the tip of the notocord (entire region 1 at the exclusion of the endoderm), besides the already noticed structures, all the cells of the notocord and floor plate down to the tail were of the quail type while the rest of the neural tube of the same region was made of chick cells (Fig. 6B,C). This means that the cell population forming the CNH yields both the floor plate and the notocord of the entire caudal region. The neural tube is thus formed through complex morphogenetic movements since its lateral walls and the floor plate joining them have a different origin.

The graft of *region 2,* located caudally to *region 1* in the tail bud, confirms this view. It gives rise to the lateral and dorsal parts of the

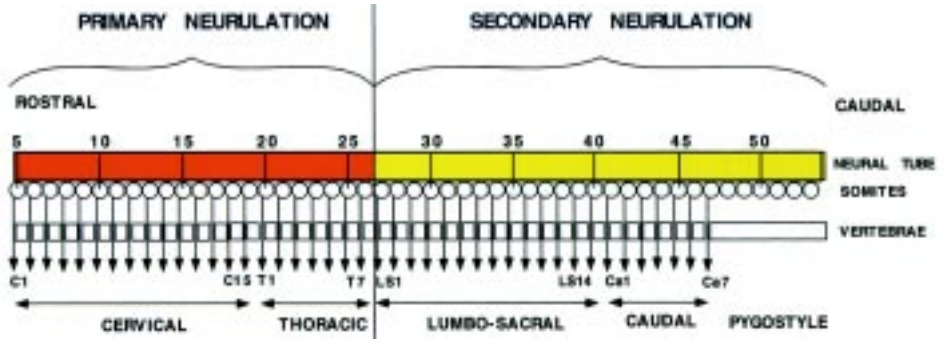


Fig. 3. Localization of the primary and secondary neurulations along the rostrocaudal axis. *The limit between primary and secondary neurulation is situated at the level of the 27th pair of somites. This is also the limit between thoracic and lumbo-sacral vertebral segments.*

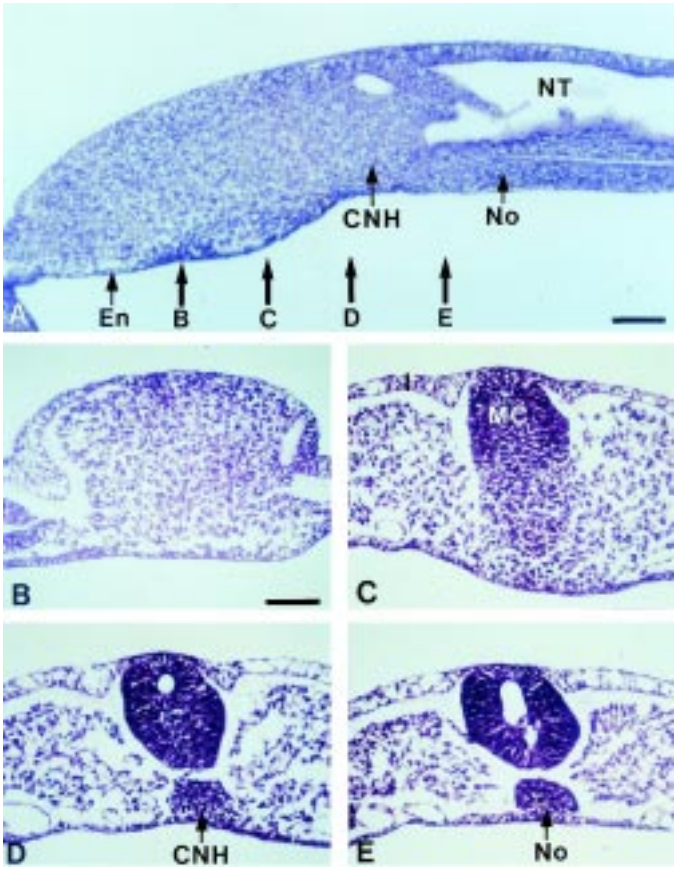


Fig. 4. Tail bud of the chick embryo at the 25-somite stage. (A) *On a sagittal section, the cordo neural hinge (CNH) is located at the point where the neural tube (NT) and the notocord (No) merge in the tail bud. Endoderm (En) limits ventrally the tail bud. Arrows (B-E) indicate caudorostral localization of transverse sections. At the caudalmost level* **(B)** *the medial region of the tail bud is denser than the lateral ones but no organized structure can be distinguished. More rostrally* **(C)** *medial cells aggregate dorsally into a solid cord forming the so-called medullary cord (MC). Cavitation of the medullary cord is initiated.* **(D)** *At the transverse level of the CNH, cavitation of the medullary cord is in progress.* **(E)** *Rostrally to the CNH the notocord (No) is lying ventrally to the nearly formed neural tube. Cresyl violet staining. Bars, 120 μm (A); 100 μm (B-E). (According to Catala et al., 1995).*

neural tube (including neural crest derivatives) caudal to the level of sacral nerve 6. As expected, however, the floor plate and notocord are of host origin along the entire posterior axis of the body in these chimeras observed at E4 and E10 (Fig. 6D). Although the exchanged territories are located medially, *region 2* also provides the host with somitic cells which arise from the ventralmost cells of the medial region of the tail bud at this stage, whereas the dorsalmost ones forming a pseudoepithelium yield the neural tube.

In fact, the graft of the terminal part of the tail bud designated here as *region 3* also yields paraxial mesoderm which cooperates with the deep cells of *region 2* to constitute the somitic derivatives of the sacral, lumbar and caudal regions. These cells, located in the medial region of the tail bud, thus correspond to the anteriormost part of the primitive streak of the early gastrula which has been shown to yield the paraxial mesoderm (Selleck and Stern, 1991).

Region 4 corresponding to the caudalmost part of the lateral regions of the tail bud yields the paraxial mesoderm (i.e., the somitic derivatives) of somites 34 to 36 included as indicated in Figure 6D,E,F. It is thus remarkable that the tail bud does not contain progenitors of the intermediate and ventral mesoderm meaning that these progenitors have migrated earlier, i.e. during the primary neurulation process.

One can deduce from these studies that tail bud development corresponds to the continuation of the gastrulation process in which the axial and paraxial structures of the lumbosacral and caudal parts of the body are laid down.

It appears that, in the secondary neurulation, formation of the neural tube involves the contribution of two territories spatially separated in the tail bud: one, located cranially in the CNH, undergoes a craniocaudal extension to form the floor plate/notocordal complex. The superficial and dorsalmost layer of cells located posteriorly to the level of the CNH is fated to form the lateral and dorsal walls of the whole neural tube that will emerge during the secondary neurulation. While the CNH regresses, the floor plate is inserted ventrally inside the epithelial medullary cord of the tail bud. This process of ventral insertion demonstrated by labeling of the CNH by quail cells in the chick embryo can also be seen in chick or quail intact embryos in which the CNH is labeled by HNF3β (Fig. 5C).

It is interesting to underline that these findings are in agreement with those obtained in *Xenopus* by Gont *et al.* (1993). In frogs, the anterior notocord is produced by the invagination of cells of the marginal zone of the blastopore. From stage 13 of Nieuwkoop and Faber (1967), however, this process of invagination ends and the notocord is then formed by elongation of the former DBL that Gont *et al.* (1993) assimilated to the CNH. These authors showed, by using cell tracers or by following the expression of the gene *Xnot2* in the CNH during tail bud extension, that the CNH remains at the tip of the tail while laying down the notocord and the floor plate. This means that, in the tail bud, gastrulation (i.e., formation of the notocord and of paraxial mesoderm as well as delimitation of the posterior endoderm and ectoderm) and neurulation are concomitant. In contrast, at earlier stages gastrulation precedes neurulation, and is considered as a prerequisite to neurulation since the neural plate is, in this view, induced by a vertical signal arising from the notocord. This view, however, was challenged by the following experiments.

Mode of the formation of floor plate and notocord in primary neurulation

In the avian embryo, at the primitive streak stage, the presumptive notocordal material has been localized in Hensen's node (Pasteels, 1937; Spratt, 1955; Nicolet, 1971; Rosenquist, 1983; Selleck and Stern, 1991; Schoenwolf *et al.,* 1992 and Garcia-Martinez *et al.,* 1993). Very few studies, however, have been devoted to the mechanisms controlling notocord elongation. By using carbon particles as a marker of embryonic territories, Spratt (1957) suggested that cells located in the primitive streak caudally to HN contribute to notocord elongation. Similarly, Sausedo and Schoenwolf (1993,1994), who studied gastrulating chick and mouse embryos in *in vitro* culture, concluded that extension of the notocord occurs principally through accretion of cells to its caudal end, together with active cell rearrangement and proliferation within the already formed notocord. This implies that formation of the notocord involves recruitment of cells that do not initially belong to HN but rather to more caudally located territories. We decided to

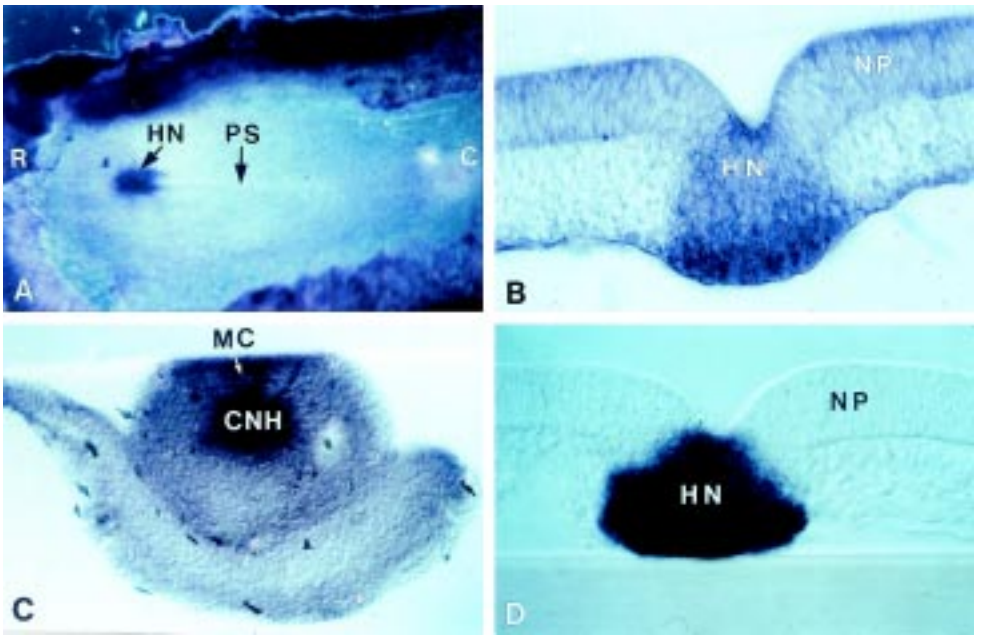


Fig. 5. Gene expression in the organizer. *$HNF_3\beta$ transcripts are present* **(A)** *in Hensen's node (HN) in a stage 4 (Hamburger and Hamilton, 1951) chick embryo;* **(B)** *in HN of a 5-somite stage chick embryo in transverse section;* **(C)** *in the cordo neural hinge (CNH) of a 30-somite stage chick embryo in transverse section.* **(D)** *Chordin transcripts are similarly shown in the HN of a 5-somite stage chick embryo in transverse section. R, rostral; C, caudal; PS, primitive streak; NP, neural plate; MC, medullary cord. In situ hybridizations.*

reinvestigate this question by replacing chick HN by its quail counterpart in the embryo *in ovo*. The idea was to perform an experiment compatible with long term survival of the embryo, a prerequisite to test the hypothesis that, during primary neurulation, HN material has a similar role as CNH as it stands in the secondary neurulation process. Ideally, the experiment should be done as early as possible during gastrulation.

Isotopic and isochronic transfer of quail HN to chick followed by normal development of the chimera up to E9 was carried out at 5- to 6-ss (see Fig. 7). At that stage the transverse level of HN corresponds to the future brachial level meaning that the axial organs (notocord, floor plate, neural tube and paraxial mesoderm) are already positioned in the cephalic and cervical parts of the body.

The quail cells implanted in these conditions included not only the mass of cells forming HN but also the posterior end of the already formed notocord and the underlying endoderm (Fig. 7A, *region 1*). In the operated embryos, examined in the following days, the floor plate and the notocord were derived from the quail transplant from the level of the graft down to the tail end (Fig. 7B,C). Moreover, quail endodermal cells were found mediodorsally in the intestine in the same region (Fig. 7B) This shows that the region of HN contains not only floor plate and notocord precursors but also progenitors of the dorsalmost endoderm of the gut.

In all embryos, the host notocord ended in a swelling at the upper-thoracic level which corresponds to the rostral level of the excision of host's HN. Caudally, the notocord was absent on a

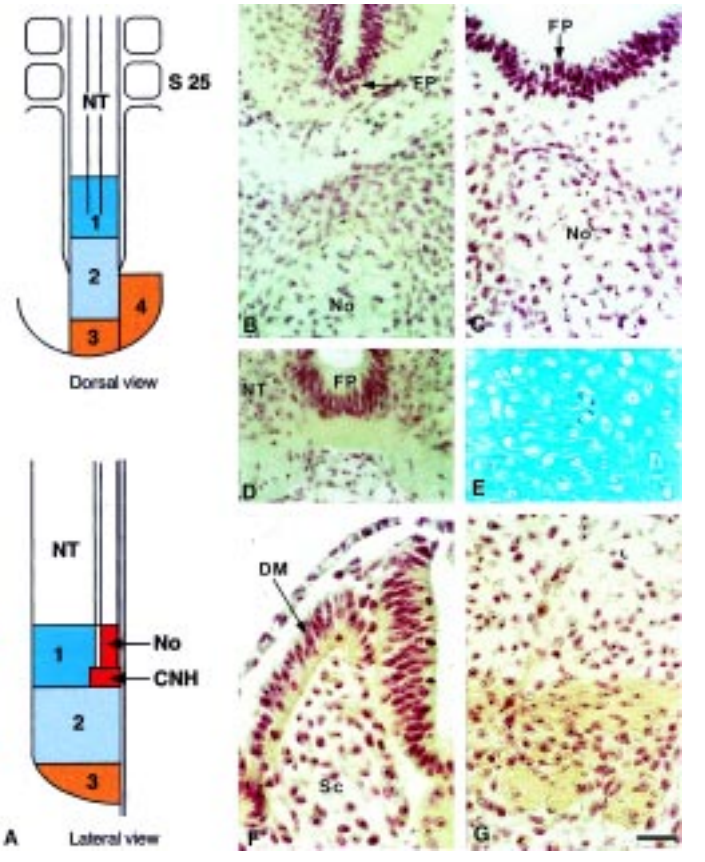


Fig. 6. Mapping of the tail bud at the 25-somite stage. (A) *Schematic representation of the quail-chick grafting experiments.* **(B** *and* **C)** *Graft of quail region 1 including the cordo neural hinge (CNH): the floor plate (FP) and the notocord (No) are made of quail cells from the lombar region (B) down to the tip of the tail (C).* **(D)** *Graft of region 2 : lateral and dorsal parts of the neural tube are made of quail cells in the lumbar region and the FP is of the host (chick) type.* **(E-G)** *Grafts of regions 3 or 4 : sclerotome (Sc) and dermomyotome (DM) cells of lumbar somites are of the donor type at E4 (E) and participate to vertebral cartilage (E) and dorsal muscles and connectif tissues (G) at later stages. B-G: Feulgen-Rossenbeck staining. E: Alcyan blue staining. Bars, 80 μm. (According to Catala et al., 1995).*

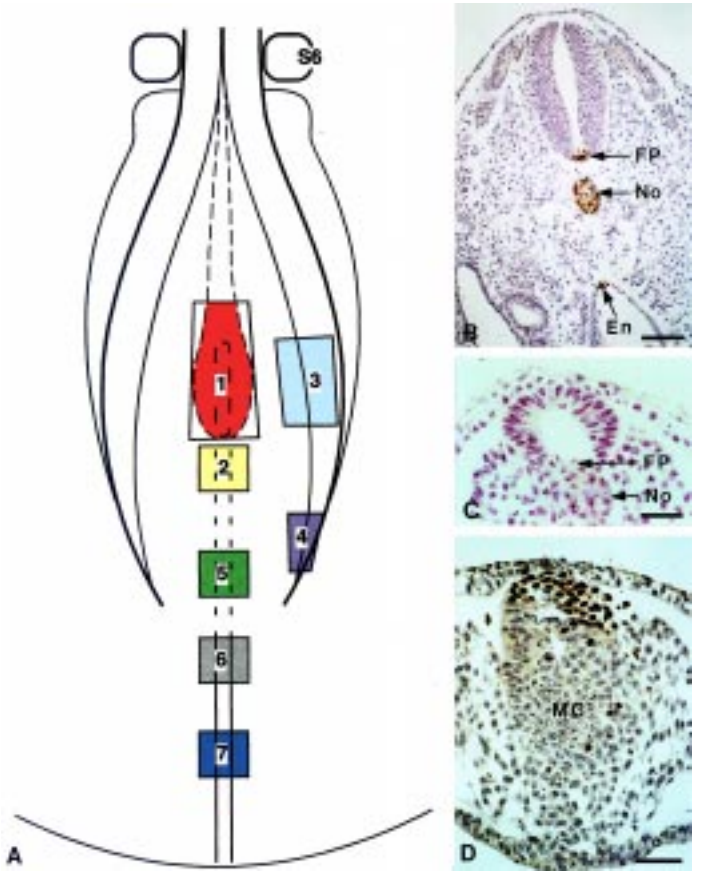


Fig. 7. Mapping of the chick sinus rhomboïdalis at the 5-6-somite stage. (A) *Schematic representation of the quail-chick grafting experiments.* **(B** *and* **C)** *Graft of region 1 : At E4, the floor plate (FP), notocord (No) and dorsal endoderm are made of quail cells, from the thoracic region (B) to the tip of the tail (C).* **(D)** *Graft of region 4 : At E3 the dorsal part of the medulary cord (MC) is made of quail cells in the tail bud. QCPN monoclonal antibody (B and D). Feulgen-Rossenbeck staining (C). Bars, A: 200 μm; B-C: 80 μm. (See Catala et al., 1996 for more details).*

length corresponding to several segments. From this level down to the tail, the floor plate, notocord and dorsal endoderm were derived from the grafted material. Such a gap between host and donor HN derived material did not exist when the operation did not include the endoderm. Thus, in certain experiments, the material located in the median pit and rostrally to it was removed as before but the endoderm was left *in situ*. The excised material was replaced by its quail counterpart. In this case also, the graft gave rise to floor plate and notocord, these labeled tissues extending to the most caudal part of the embryo. In this series, no gap was observed between the host and the labeled notocord, showing that, in presence of the host intact endoderm, normal rostrocaudal growth of the preexisting notocord could take place. Moreover, there were no indications of any morphological change marking the transition between primary and secondary neurulation. In embryos examined at 25-ss, quail cells were found to constitute the CNH located in the ventrorostral part of the tail bud where precursors of the caudalmost notocord have been localized at this stage (Catala *et al.,* 1995). In the chimaeras examined at E4, quail floor plate and notocord extended to the *ventriculus terminalis* (Fig. 7C).

The fate map of the superficial germ layer of the *sinus rhomboidalis* (i.e., the neural plate) was established by the same quail to chick substitution paradigm (see Fig. 7). Two important notions came out of these experiments. i) First, the ectoderm located just caudally to HN *(region 2)* is destined to yield the basal plate of the truncal neural tube and will be cut into two lateral parts by the regression of HN material which leaves on its wake the floor plate and the notocord. The floor plate thus becomes incorporated into the ventralmost part of the neural tube according to a mechanism similar to that going on in secondary neurulation. ii) Albeit caudally located with respect to HN, the ectodermal germ layer is well in the process of being individualized in the caudal part of the *sinus rhomboidalis* (see section level D on Fig. 1 in Catala *et al.*, 1996). More posteriorly in E such is not the case since the midline cells still constitute the primitive streak. The fate map of this region (see Fig. 7A) including either the superficial or the deep layers of

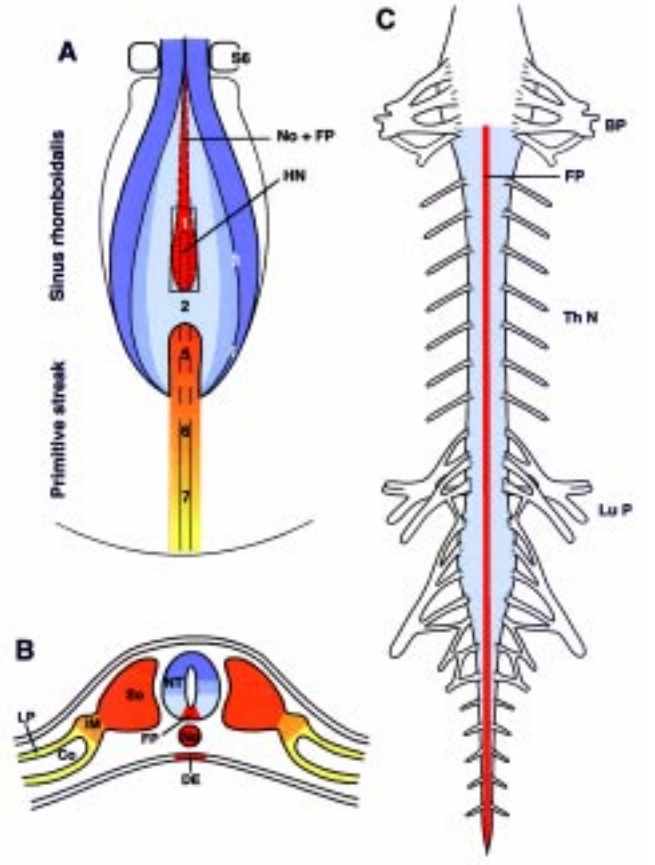


Fig. 8. Mapping of the chick sinus rhomboïdalis at the 5-6-somite stage. **(A)** *Schematic representation of the grafting experiments. The grafted territories are represented with numbers from 1 to 7. Their derivatives are shown with the same colors on a transverse section of an avian embryo after neurulation at E3* **(B)** *and on a ventral view of the spinal cord at birth* **(C)**. *Hensen's node (HN) gives rise to both notocord (No) and floor plate (FP) from the thoracic level down to the extremity of the tail. The rest of the neural tube (NT) originates from regions located caudally and laterally to the HN. Mesodermal precursors are arranged rostrocaudally in the primitive streak remnant, according to their future mediolateral disposition. BP, brachial plexus; Co, coelomic cavity; DE, dorsal endoderm; IM, intermediate mesoderm; LP, lateral plate; LuP, lumbar plexus; So, somite; ThN, thoracic nerves.*

cells (see Catala *et al*., 1996 for details) shows that the totality of the neural primordium is included in the ectoderm of the *sinus rhomboidalis*. Thus grafting of the posteriormost regions of this structure results in labeling the posterior neural tube which is built up according to the secondary neurulation process (Fig. 7D).

In certain experiments HN was removed in the 5-ss chick embryo and was not replaced by its counterpart from a quail. In this case a neural tube formed posteriorly to the excision that, as expected, was devoid of floor plate. It however extended down to the tail indicating that neurulation can proceed, at that stage, in total absence of contact of the ectoderm with a notocord. The ectoderm of the *sinus rhomboidalis* is thus committed to the neural differentiation pathway at 5-ss by a process that excludes vertical induction and can only be accounted for by planar induction.

Conclusions

These experiments bring about a novel view on the process of neurulation in Amniotes. They show that the cells forming HN homologous to the Amphibian DBL and corresponding to the Spemann's organizer are at the origin of three parallel dorsal structures extending over the whole length of the embryo and included respectively in each of the three germ layers: the floor plate in the ectoderm, the notocord in the mesoderm and the dorsal endoderm (Fig. 8). All these three structures are made up by the midline cells which, from the time they are included in HN and CNH, express genes that play a key role in patterning the embryonic anteroposterior (AP) axis such as $HNF_3\beta$ (Ang and Rossant, 1994) and chordin (Sasai *et al.,* 1994).

Whether, later in the development, the expression of specific floor plate genes depends upon an induction arising from the more ventral material of the midline cells (destined to form the notocord and ventral endoderm) cannot be excluded by these experiments. An alternative view, however, to explain the different fates of the ectodermal (floor plate), mesodermal (notocord) and endodermal components of the midline cells is to consider that after they have split and become incorporated into the three germ layers (see Fig. 9), they are subjected to different environments from which they receive different signals which lead to the expression of different genes.

Lateral grafts of an extra notocord or of an extra floor plate induce floor plate-like morphogenesis and gene expression in the neural tube (van Straaten *et al.,* 1988; Placzek *et al.,* 1990 and Yamada *et al.,* 1991). This phenomenon can be accounted for by the fact that both notocord and floor plate express several genes, such as chordin and noggin, which are responsible for the physiological functions of the organizer the most prominent of which is to induce a secondary axis laterally or ventrally. According to this view, induction of a secondary floor plate by notocord or floor plate grafts is just a manifestation of organizer activity and does not mean that the notocord has a special role in inducing a floor plate in normal development as it has been proposed (see Tanabe and Jessell, 1996).

The fact that a role was attributed to the notocord in the induction of the floor plate was essentially based on ablation experiments carried out in the chick embryo (van Straaten and Hekking, 1991; Yamada *et al.,* 1991). Ablation of the notocord in the non-segmented region of 10- to 15-ss chick embryos was described as resulting in an absence of floor plate. It was concluded that the midline cells of the neural plate were induced by a signal of notocord origin.

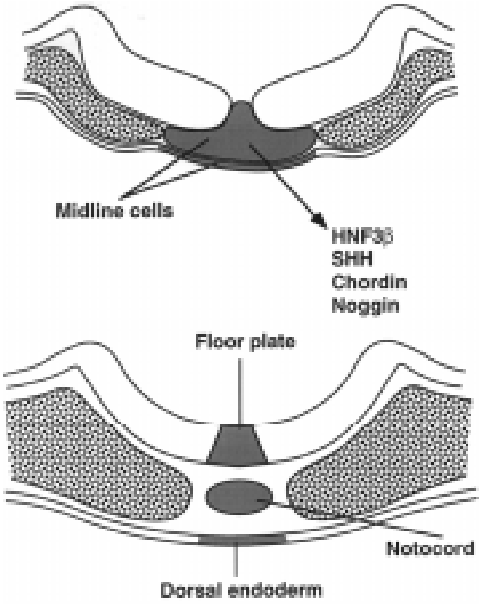


Fig. 9. Midline cells fate. *Hensen's node (HN) midline cells constitute a homogenous mass of cells expressing specific genes ($HNF_3\beta$, chordin). By regressing caudally and dilaminating this mass of cells gives rise to the floor plate and notochord of the whole caudal body underlined by dorsal endoderm originating in the same area.*

In fact, if the neural plate is separated from the notocord at this stage and is subsequently cultured in a three dimensional gel *in vitro* culture system, it gives rise to a neural tube with a normal expression pattern of *Pax3* and *Pax6* and showing a floor plate (Pituello *et al.,* 1995) expressing $HNF_3\beta$ and *Shh* genes (Pituello and Teillet, unpublished observations).

Moreover, in such ablation experiments carried out *in ovo* the neural tube was devoid of floor plate only at its posterior end corresponding to the few hundred micrometers rostral to HN at operation time. Careful histological examination of normal embryos shows that this posterior region of the neural primordium corresponds to a zone where delamination of CNH into notocord and floor plate is not yet completed. This means that ablation of the notocord also involves presumptive floor plate material in this region. Thus, absence of a floor plate in the more caudal part of the AP axis where the notocord is excised is due to the removal of the floor plate and not to a lack of induction of a floor plate in the neural plate. This view has been fully substantiated experimentally by Teillet *et al.* (1998).

Regression of HN/CNH in the avian embryo thus follows a mechanism similar to that operating in *Xenopus* from stage 13 of Nieuwkoop and Faber (Gont *et al.,* 1993). The experiments described above have been carried out at 5- to 6-ss and later. At 5-6-ss, HN is located at the presumptive brachial level of the embryo. Therefore, the conclusions drawn cannot necessarily be extrapolated to more anterior regions. It is however likely that the rostrocaudal regression of HN described here also takes place in the cephalic and cervical regions of the body where there is a notocord, that is up to the anterior mesencephalon. If this is the case, only formation of the precordal plate mesoderm would result from a caudorostral movement of cells as previously proposed (Selleck and Stern, 1991).

Acknowledgements

We thank A. Ruiz i Altaba for the gift of the HNF$_3\beta$ probe and E. de Robertis for the chordin probe. QCPN antibody was obtained from the Developmental Studies Hybridoma Bank maintained by the Department of Pharmacology and Molecular Sciences, John Hopkins University School of Medicine, Baltimore, MD, and the Department of Biological Sciences, University of Iowa, Iowa City, IA, under contract N01-HD-6-2915 from NICHD. This work was supported by the Centre National de la Recherche Scientifique (CNRS) and by grants of the following French charity funds: Association pour la Recherche contre le Cancer (ARC) and Ligue Nationale Française Contre le Cancer (LNFCC). The authors are grateful to Françoise Lapointe for in situ hybridization, Françoise Viala, Francis Beaujean, Hélène San Clemente and Sophie Gournet for preparing the illustrations and to Chrystèle Guilloteau for preparing the typescript.

References

ANG, S.-L. and ROSSANT, J. (1994). *HNF-3β* is essential for node and notochord formation in mouse development. *Cell 78*: 561-574.

BIJTEL, J.H. (1958). The mode of growth of the tail in Urodele larvae. *J. Embryol. Exp. Morphol. 6*: 466-478.

CATALA, M., TEILLET, M.-A., de ROBERTIS, E.M. and LE DOUARIN, N.M. (1996). A spinal cord fate map in the avian embryo: while regressing, Hensen's node lays down the notochord and floor plate thus joining the spinal cord lateral walls. *Development 122*: 2599-2610.

CATALA, M., TEILLET, M.-A. and LE DOUARIN, N.M. (1995). Organization and development of the tail bud analyzed with the quail-chick chimaera system. *Mech. Dev. 51:* 51-65.

COULY, G.F. and LE DOUARIN, N.M. (1987). Mapping of the early neural primordium in quail-chick chimeras. II. The prosencephalic neural plate and neural folds: implications for the genesis of cephalic human congenital abnormalities. *Dev. Biol. 120*: 198-214.

GARCIA-MARTINEZ, V., ALVAREZ, I.S. and SCHOENWOLF, G.C. (1993). Locations of the ectodermal and nonectodermal subdivisions of the epiblast at stages 3 and 4 of avian gastrulation and neurulation. *J. Exp. Zool. 267*: 431-446.

GONT, L.K., STEINBEISSER, H., BLUMBERG, B. and DE ROBERTIS, E.M. (1993). Tail formation as a continuation of gastrulation: the multiple cell populations of the *Xenopus* tailbud derive from the late blastopore lip. *Development 119*: 991-1004.

GRIFFITH, C.M., WILLEY, M.J. and SANDERS, E.J. (1992) The vertebrate tail bud: three germ layers from one tissue. *Anat. Embryol. 185*: 101-113.

HAMBURGER, V. and HAMILTON, H.L. (1951). A series of normal stages in the development of the chick embryo. *J. Morphol. 88*: 49-92.

HOLMDAHL, D.E. (1925). Experimentelle Untersuchungen über die Lage der Grenze zwischen primärer und sekundärer Körperentwicklung beim Huhn. *Anat. Anz. 59*: 393-396.

HOLMDAHL, D.E. (1938). Die Morphogenese des Vertebratorganismus vom formalen und experimentellen Gesichtspunkt. *Roux's Arch. Dev. Biol. 139*: 191-226.

LE DOUARIN, N. (1964). Etude expérimentale de l'organogenèse du tube digestif et du foie chez l'embryon de poulet. *Bull. Biol. 98*: 544-674.

NICOLET, G. (1971). Avian gastrulation. *Adv. Morphogen 9*: 231-262.

NIEUWKOOP, P. and FABER, J. (1967). *Normal table of Xenopus laevis.* Amsterdam: North Holland.

PASTEELS, J. (1937). Etudes sur la gastrulation des vertébrés méroblastiques. III. Oiseaux. IV Conclusions générales. *Arch. Biol. 48*: 381-488.

PITUELLO, F., YAMADA, G. and GRUSS, P. (1995). Activin A inhibits *Pax-6* expression and perturbs cell differentiation in the developing spinal cord *in vitro. Proc. Natl. Acad. Sci. USA 92*: 6952-6956.

PLACZEK, M., TESSIER-LAVIGNE, M., YAMADA, T., JESSELL, T.M. and DODD, J. (1990). Mesodermal control of neural cell identity: floor plate induction by the notochord. *Science 250*: 985-988.

PUELLES, L., DOMENACH-RATTO, G. and MARTINEZ-DE-LA-TORRE, M. (1987). Location at the rostral end of the longitudinal brain axis: review of an old topic on the light of marking experiments on the closing rostral neuropore. *J. Morphol. 194*: 163-171.

ROSENQUIST, G.C. (1983). The chorda center in Hensen's node of the chick embryo. *Anat. Rec. 207*: 349-355.

RUIZ I ALTABA, A. and JESSELL, T.M. (1992). *Pintallavis*, a gene expressed in the organizer and midline cells of frog embryos: involvement in the development of the neural axis. *Development 116*: 81-93.

SASAI, Y., LU, B., STEINBEISSER, H., GEISSERT, D., GONT, L.K. and de ROBERTIS, E.M. (1994). *Xenopus* chordin: a novel dorsalizing factor activated by organizer-specific homeobox genes. *Cell 79*: 779-790.

SAUSEDO, R.A. and SCHOENWOLF, G.C. (1993). Cell behaviors underlying notochord formation and extension in avian embryos - Quantitative and immunocytochemical studies. *Anat. Rec. 237*: 58-70.

SAUSEDO, R.A. and SCHOENWOLF, G.C. (1994). Quantitative analyses of cell behaviors underlying notochord formation and extension in mouse embryos. *Anat. Rec. 239*: 103-112.

SCHOENWOLF, G.C., GARCIA-MARTINEZ, V. and DIAS, M.S. (1992). Mesoderm movement and fate during avian gastrulation and neurulation. *Dev. Dynamics 193*: 235-248.

SELLECK, M.A. and STERN, C.D. (1991). Fate mapping and cell lineage analysis of Hensen's node in the chick embryo. *Development 112*: 615-626.

SPRATT, N.T. (1955). Analysis of the organizer center in the early chick embryo. I. Localization of prospective notochord and somite cells. *J. Exp. Zool. 128*: 121-163.

SPRATT, N.T. (1957). Analysis of the organizer center in the early chick embryo. II. Studies of the mechanics of notochord elongation and somite formation. *J. Exp. Zool. 134*: 577-612.

STEIN, S., NIß, K and KESSEL M. (1996). Differential activation of the clustered homeobox genes *CNOT2* and *CNOT1* during notogenesis in the chick. *Dev. Biol. 180*: 519-533.

TANABE, Y. and JESSELL, T.M. (1996). Diversity and pattern in the developing spinal cord. *Science 274*: 1115-1123.

TEILLET, M.A., LAPOINTE, F. and LE DOUARIN, N.M. (1998). The relation ships between notocord and floorplate in vertebrate development revisited. (Submitted).

VAN STRAATEN, H.W.M. and HEKKING, J.W.M. (1991). Development of floor plate, neurons and axonal outgrowth pattern in the early spinal cord of the notochord-deficient chick embryo. ANAT. EMBRYOL. 184: 55-63.

VAN STRAATEN, H.W.M., HEKKING, J.W.M., WIERTZ-HOESSELS, E.L., THORS, F. and DRUKKER, J. (1988). Effect of the notochord on the differentiation of a floor plate area in the neural tube of the chick embryo. *Anat. Embryol. 177* : 317-324.

YAMADA, T., PLACZEK, M., TANAKA, H., DODD, J. and JESSEL, T.M. (1991). Control of cell pattern in the developing nervous system: polarizing activity of the floor plate and notochord. *Cell 64*: 635-47.