ELSEVIER

Mechanisms of Development 67 (1997) 3–16

Review article

Gastrulation and homeobox genes in chick embryos

Lydia Lemaire, Michael Kessel*

Max-Planck-Institut für Biophysikalische Chemie, Am Faßberg, D-37077 Göttingen, Germany

Received 9 June 1997; accepted 29 June 1997

Abstract

We review the early stages of chick embryogenesis, in particular the formation of the hypoblast, and the ingression of endoderm and mesoderm through the primitive streak. The formation of a trilaminar embryo during gastrulation is accompanied by the specification of body axes. The first axis is already present in the unfertilized egg and runs from the cytoplasmatic animal to the yolk rich vegetal pole. Already within the uterus a second axis conveys bilateral symmetry to the embryo. It extends from a dorsal/anterior to a ventral/posterior position. These axial poles segregate during gastrulation to form the classical coordinates, a dorsal-ventral and an anterior-posterior axis. The establishment of axes is accompanied by the expression of specific combinations of homeobox genes during gastrulation in the chick, as in other metazoa. We review the avian specific information and compare it with findings in other species. A combinatorial homeobox code for the specification of identities during development is discussed. © 1997 Elsevier Science Ireland Ltd.

Keywords: Gastrulation; Homeobox gene; Chick embryo

1. Introduction

The landmarks of early metazoan development are the blastula and the gastrula. The blastula is the result of the first cleavages, which generate a sufficiently large number of omni- or pluripotent cells. The gastrula represents for the first time in ontogenesis a trilaminar embryo, consisting of the three germ layers ectoderm, endoderm and mesoderm. It is formed during gastrulation by extensive migrations of blastula cells, involving specific processes like epiboly, invagination, involution, ingression or delamination, i.e. the process of gastrulation. The juxtaposition of different cell layers during gastrulation offers the chance for many inductive interactions, with the possibility of initiating new developmental programs.

Accompanying the early steps of early embryogenesis is the fast generation of multiple cellular identities. The identity of a cell is a dynamic parameter, defined at a given time by cell lineage, specification, position, morphology, and, most precisely, by the subset of active genes. Among the many genes of a cell, the homeobox genes appear to stand in a special relation to cellular or regional identity. Their expression reflects the present developmental status of a cell, and allows the recognition of many aspects of identity. Moreover, homeobox genes can influence all features of cellular identity under experimental conditions, demonstrating their high position in the genetic hierarchy defining and controlling early embryogenesis. The unique information obtained from the investigation of homeobox genes has established them as ideal guides to the understanding of development, in particular of cellular identity.

In the present review we concentrate on the relation between gastrulation and homeobox genes in the avian embryo. We describe first the morphological aspects of development, the inductive potentials in the chick embryo, and the generation of the embryonic axes. We then summarize the information on homeobox genes in the early chick embryo, trying to describe the specification of identity during ontogeny.

* Corresponding author. Tel.: +49 551 2011560; fax: +49 551 2011504; e-mail mkessell@.gwdg.de

PII S0925-4773(97)00102-0

2. Early development of the chick

2.1. Cleavage and formation of the area pellucida

Development of the chick embryo proceeds for approximately 20 h in the uterus, until the calcareous shell is deposited around the egg (Romanoff, 1960). The early stages of chick embryogenesis have been systematically described by Eyal-Giladi and Kochav, who defined 13 stages, EK st.I (shortly after fertilization) to EK st.XIII (beginning of gastrulation; Eyal-Giladi and Kochav, 1976).

Approximately 5 h after fertilization, the extremely telolecithal avian egg enters the uterus and starts a process of discoidal cleavage, which lasts for about 11 h. The first vertical cleavage furrows divide the cytoplasmic disc, but leave the blastomeres open to the underlying yolk. After only 2 h of cleavage, the embryonic cells become separated from the yolk, and an embryo consisting of five to six cell layers is generated. In the second phase of intrauterine development the central area of the blastodisc becomes translucent and reduced to a thickness of only one cell layer. This 'area pellucida' remains surrounded by a thicker peripheral region of yolk-rich cells, the 'area opaca'. The embryo is separated from the uncleaved yolk by a fluid-filled space, the subblastodermal cavity. The distal region of the area pellucida is designated the marginal zone. Its polarization can be recognized by the position of Koller's sickle, a transient, crescent-shaped thickening at the anterior rim of the posterior marginal zone (Fig. 1A,C; Koller, 1882; Rauber, 1876). Koller's sickle is not always morphologically very obvious and can only be observed clearly in about 30% of examined blastoderms (Callebaut and Van Nueten, 1994). As further outlined below, Koller's sickle represents a focal point of early development.

2.2. Hypoblast formation

The freshly-laid, unincubated chicken egg (EK st.X) contains an embryo of approximately 2 mm in diameter and with about 60 000 cells. The area pellucida consists of an upper layer, the epiblast, and the first cells of a lower layer, the hypoblast. Hypoblast formation was initiated already in the late intrauterine period and persists during the first 6–8 h of egg incubation Two different populations of cells are involved in the process of hypoblast differentiation. First, small aggregates of 'primary' hypoblast cells are generated by polyingression of epiblast cells, and are randomly distributed over the lower surface of the epiblast. In the hours after laying, these aggregates receive a contribution of 'secondary' hypoblast cells, derived from the posterior marginal zone, advancing anteriorly via Koller's sickle (Eyal-Giladi et al., 1992). This layer incorporates the primary hypoblast cells and forms a continuous sheet, the hypoblast. It remains unclear, to what extent the hypoblast also receives a contribution from cells located below the posterior marginal epiblast, possibly derived from the germ wall (Stern, 1990). The front of the hypoblast cell sheet spreads from Koller's sickle forward, underlying the complete disc by EK st.XIII. Cells of the hypoblast do not contribute to the embryo as such, but will form the extraembryonic yolk sac. The chick embryo consisting of epiblast and hypoblast represents the blastula, the space between the two layers the blastocoel. All embryonic tissues, ecto-, neuroecto-, endo- and mesoderm will derive from the epiblast during gastrulation.

2.3. Primitive streak formation

Extrauterine development of the chick embryo is defined in stages according to Hamburger and Hamilton (1951). The first five stages (HH st.1 - HH st.5) describe development during the first 24 h, and cover the steps from blastula to gastrula (Fig. 1A; for review see Romanoff, 1960; Hamilton, 1965; Bellairs, 1986).

Gastrulation is a phase of extensive cellular convergence movements towards the midline of the epiblast (Khaner, 1993). Its beginning is indicated by a local thickening close to Koller's sickle, the primitive streak primordium. At this stage the ventral surface of the entire epiblast is still covered by a continuous basal lamina. Upon formation of an elongated primitive streak, the lamina disrupts along the midline of the streak as a prerequisite for de-epithelialization of epiblast cells. Cellular ingression correlates with the appearance of a median primitive groove, flanked by primitive ridges consisting of piled-up cells ready for gastrulation, the prospective endoderm and mesoderm cells (see below). The median 'opening' of the primitive streak represents the chicken blastopore, the primitive ridges the blastopore margin. In the anterior tip of the streak the primitive groove terminates in a depression, the primitive pit, which lies in the centre of the anterior fused thickening of the ridges, Hensen's node (Hensen, 1876). After 18–19 h of incubation (HH st.4) the streak has progressed to its maximal extension with an average length of 1.88 mm, extending about three-fifths of the length of the area pellucida (Spratt, 1946). With the regression of the primitive streak, Hensen's node is gradually shifted to a more posterior position (Spratt, 1947). Mesoderm ingression continues to occur for 30 h in the posterior part of the embryo, while the neural plate already elevates anteriorly. It comes to an end, when node and streak melt into a new structure, the tailbud. Only then, the ingression of cells ceases and is substituted by a budding mechanism allowing a further elongation of the body axis.

2.4. Endoderm formation

At mid-streak stages, the first cells of the epiblast ingress through the anterior part of the primitive streak (Fig. 1B), and insert into the hypoblast layer, which is displaced by this steadily enlarging endoderm patch (Bellairs, 1986; Garcia-Martinez et al., 1993; Rosenquist, 1972; Vakaet, 1962).

The ingressing cells form a new continuous layer beneath the epiblast, the definitive endoderm. The endoderm sheet grows concentrically by contributions from Hensen's node, so that earlier ingressed cells become more distally located. At its anterior end the displaced hypoblast forms the germinal crescent, which contains the primordial germ cells (Karagenc et al., 1996). With formation of the head fold (HH st.6), the early endoderm constitutes the ventral portion of the foregut, and will give rise to the thyroid gland and the lung. Endoderm which invaginated later will be found in the dorsal part of the foregut pocket, giving rise, e.g. to the dorsal pancreas. By HH st.4, the node will no longer generate bona fide endoderm, but a transitional, 'mesendodermal' cell type, which will go on to form the prechordal plate by HH st.5 (Fig. 1B).

2.5. Mesoderm formation

Ingressing cells which come to lie between endoderm and ectoderm are by definition mesodermal. In the chick, mesoderm cells ingress individually, and not as an infolding sheet. However, cells remain in contact via cytoplasmatic processes and migrate in the form of a coherent mobile cell reticulum until they have reached their final destinations (for review see Bellairs, 1986; Sanders, 1986; Winklbauer, 1994). Detailed fate maps and migratory routes of the mesodermal derivatives have been established by various methods, most recently by using the chick-quail chimera technique or by the use of lipophilic dyes (Selleck and Stern, 1991 and references therein, Schoenwolf et al., 1992; Psychoyos and Stern, 1996a). These studies have indicated the great complexity of fates present at various levels of the primitive streak. They have also allowed the deduction of a simplified rule, correlating the site of ingression with the medio-lateral position that a mesodermal cell will take up within the embryo. The closer a cell ingresses to the node, the closer it will be found to the midline (Fig. 1B).

The first mesoderm to be produced gives rise only to extraembryonic tissue, the blood islands and vessels necessary for supplying nutritive substances from the yolk to the embryo. It is followed by the prospective heart mesoderm, the first embryonic mesoderm constituting the early mesodermal wings ingressing at HH st.3, and reaching a very lateral and anterior position by HH st.5. (Garcia-Martinez and Schoenwolf, 1993). During HH st.3$^+$ the anterior streak also generates the paraxial head mesoderm, a cell population leading to mesenchyme and muscle formation in the head.

After endoderm formation is completed, midline axial tissue is derived from Hensen's node. First, the mesendodermal prechordal plate is formed (HH st.4), then the head process, and finally the notochord (HH st.5; Fig. 1B). The anterior primitive streak is the source of the paraxial mesoderm, i.e. the segmental plate and its derivatives, the somites. More specifically, the lateral node generates the medial part of the somites, whereas the anterior streak contributes to the lateral part of the somites (Selleck and Stern, 1991). Consecutively more posterior levels of the streak give rise to intermediate and lateral plate mesoderm, that will form the pronephric duct and the limbs. The most posterior part of the streak continues to contribute cells to the extraembryonic mesoderm.

2.6. Neural plate formation

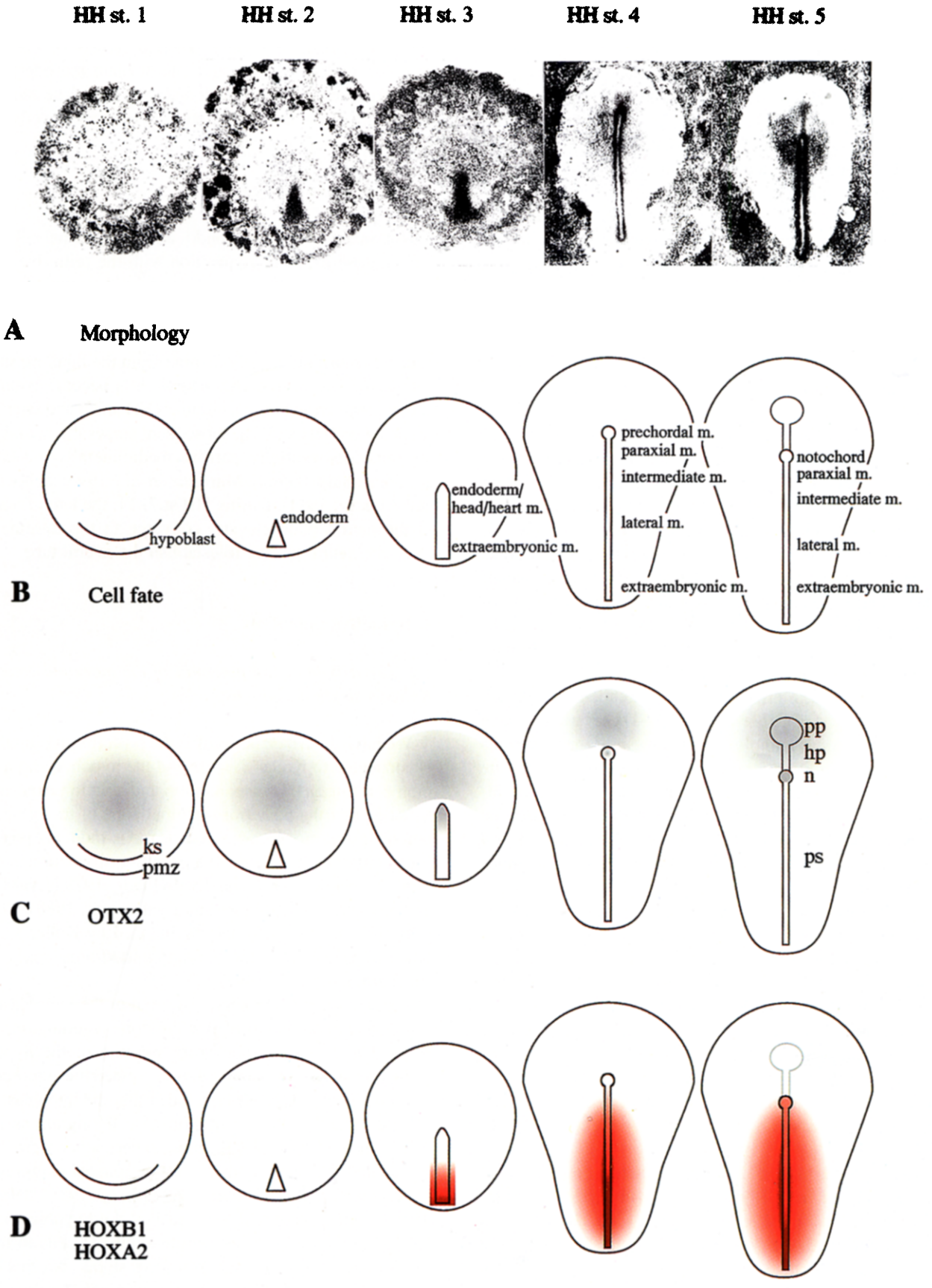
Not only the ingressing endo- and mesodermal cell populations arise in direct connection with the primitive streak, but also the neuroectoderm. Up to the mid-gastrulation stage (HH st.3) the entire epiblast is a flat epithelial sheet of cells. Already by HH st.3$^+$ a neural plate around Hensen's node becomes morphologically distinct from the flanking surface ectoderm. The prospective neural cells become columnar, and become arranged in a circular (HH st.3$^+$) and later pear-shaped area (HH st.5). In subsequent stages the neural plate thickens dorsoventrally, narrows mediolaterally and extends craniocaudally (Garcia-Martinez et al., 1993; Schoenwolf and Alvarez, 1991). During HH st.7–11, the lateral margins of the neural plate elevate, converge to the midline, and close the neural groove to establish the neural tube.

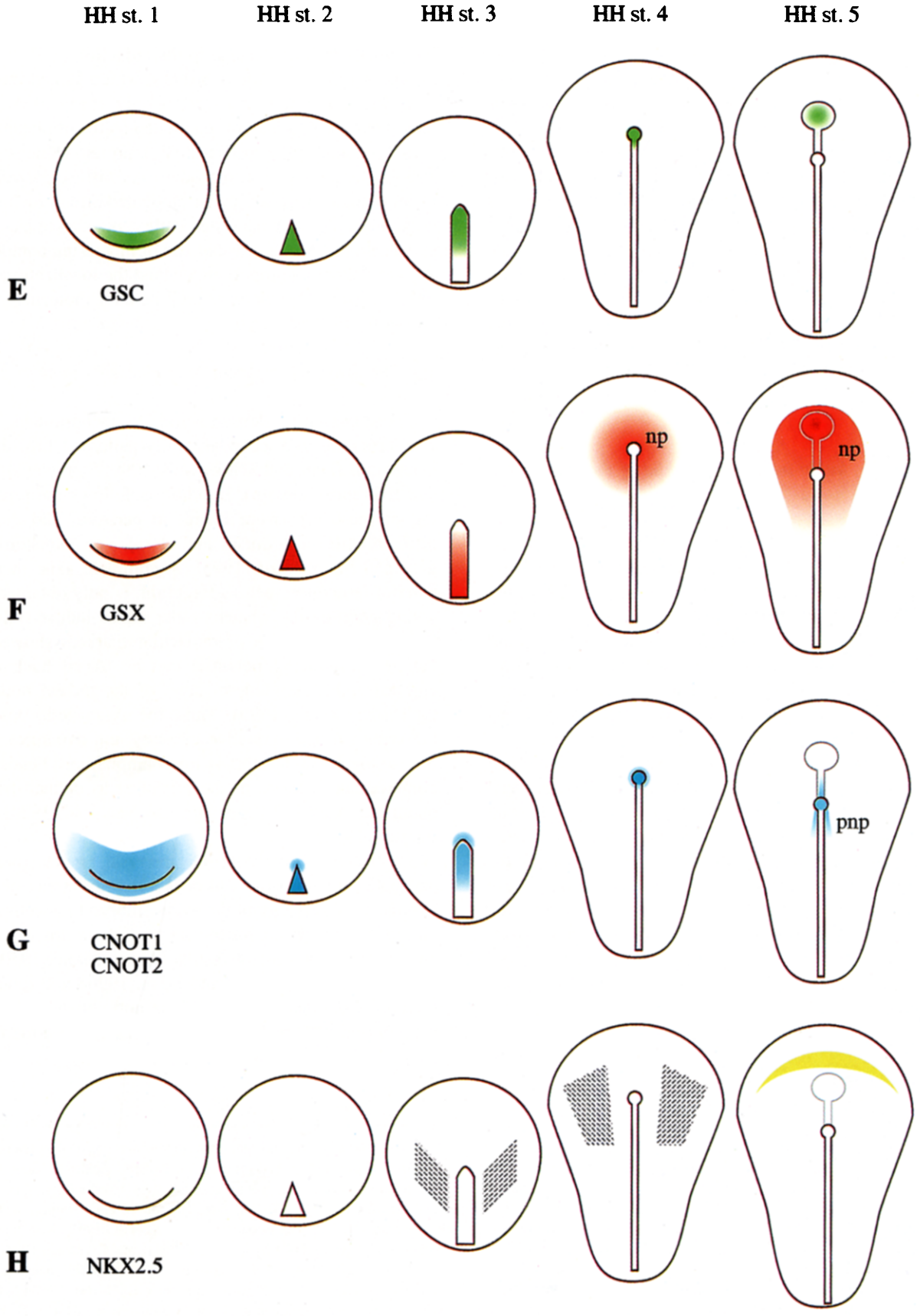
3. Inductive potentials

3.1. The axis-inducing potential of the marginal zone, Koller's sickle and the hypoblast

We have described above that the posterior marginal zone is connected to Koller's sickle and the hypoblast by a common cell lineage. Transplantation and ablation experiments revealed that the posterior marginal zone of the pre-gastrulation blastoderm possesses a strong axis-inducing potential. It can either induce de novo a primitive streak or can determine its origin (Azar and Eyal-Giladi, 1979; Eyal-Giladi and Khaner, 1989; Khaner and Eyal-Giladi, 1989). Ectopic primitive streaks could also be induced by Koller's sickle, when transplanted to the non-committed area opaca (Izpisúa-Belmonte et al., 1993).

Less clear is the inductive potential of the hypoblast. The pioneering experiments of Waddington (Waddington, 1933) showed that the anteroposterior rotation of the hypoblast during pre-primitive streak stages reversed the anteroposterior axis of the streak. He concluded that the hypoblast layer is able to define the orientation of cell migrations in the overlying epiblast. The original experiments were reexamined and extended by hypoblast or epiblast reaggregation experiments (Mitrani and Eyal-Giladi, 1981, 1984a,b). These studies promoted the idea that the hypoblast is able to change the fate of uncommitted epiblast cells to initiate the formation of an ectopic primitive streak, but that it can also repress the formation of the original streak in committed epiblast cells. It was also found that the epiblast


HH st. 1
HH st. 2
HH st. 3
HH st. 4
HH st. 5
A
Morphology
hypoblast
endoderm
endoderm/
head/heart m.
extraembryonic m.
prechordal m.
paraxial m.
intermediate m.
lateral m.
extraembryonic m.
notochord
paraxial m.
intermediate m.
lateral m.
extraembryonic m.
B
Cell fate
ks
pmz
pp
hp
n
ps
C
OTX2
D
HOXB1
HOXA2


HH st. 1
HH st. 2
HH st. 3
HH st. 4
HH st. 5
E
GSC
F
GSX
np
np
G
CNOT1
CNOT2
pnp
H
NKX2.5

alone developed a primitive streak according to the polarity of the EK st.XIII epiblast in the absence of any hypoblast layer. Recently, the experimental evidence and the interpretation derived from the hypoblast rotation studies was challenged (Khaner, 1995). Khaner did not confirm the axis-inducing potential ascribed to the hypoblast and attributed the direction of the embryonic axis solely to the basic polarity of the epiblast layer, i.e. Koller's sickle and the posterior marginal zone. The inductive potential of the hypoblast will continue to lie in the focus of future research, since it addresses one of the earliest inductive events during chick embryogenesis. In addition, the anterior front of the hypoblast or endoderm and its possible involvement in the patterning of the head deserves further attention (Thomas and Beddington, 1996). The secreted protein Cerberus, was recently identified as signaling molecule derived from dorsal/anterior endoderm in *Xenopus laevis* (Bouwmeester et al., 1996).

3.2. The gastrulation-inducing potential of the primitive streak

Grafts taken from different levels of the primitive streak have gastrulation-inducing potential, when transplanted into the peripheral area pellucida or into the area opaca of young host embryos. Middle parts of early to mid-streaks induce primitive streaks from uncommitted ectoderm (Gallera and Nicolet, 1969; Gallera, 1971; Lemaire et al., 1997). Similarly, a cell aggregation at the posterior end of the streak, the 'nodus posterior', is reported to promote gastrulation events (Vakaet, 1964, 1982).

When transplantations of streak parts were combined with the analysis of molecular markers, it turned out that the induced streaks did not normally express typical node markers, such as the homeobox gene GSC or the fork head transcription factor HNF3β, whereas they were positive for the pan-mesodermal marker brachyury (Lemaire et al., 1997). Thus, the induced structures closely resembled the inducing tissue, and represented cases of assimilatory inductions. Remarkably, even such disabled streaks went on to generate axial mesoderm and complete embryonic structures.

Activin-A and Vg1, two members of the TGFβ superfamily, are known for their mesoderm- and axis-inducing potential. The implantation of Activin-A expressing cells into chick blastoderms lead to the induction of gastrulation events or diffuse primitive streaks, which lacked the anterior, GSC expressing structures (Cooke et al., 1994). Complete secondary streaks were initiated by the implantation of cells producing the processed cVg1 protein (Seleiro et al., 1996). The induction of mesoderm, of diffuse gastrulation, of an elongated streak, of an elongated streak including a node, or finally of a patterned embryonic axis appear to be events along a regulatory cascade of increasing complexity. We are only beginning to understand the localization of the inducers and responders, as well as their molecular nature within the chick embryo.

3.3. The neuralizing potential of Hensen's node

Transplantion of Hensen's node to uncommitted, lateral ectoderm induces a secondary, fully-patterned, host-derived neural axis (Dias and Schoenwolf, 1990; Storey et al., 1992 and references therein). In addition follows the repatterning of mesoderm from lateral to paraxial, and the self-differentiation into dorsal mesoderm, i.e. notochord (Inagaki and Schoenwolf, 1993). A complete axis, including anterior structures such as forebrain, is only obtained from young node grafts, whereas older nodes induce only posterior levels. The use of molecular markers showed that the neural-inducing potential can be traced back to the epiblast and mesendoderm layer of the medial node sector (Storey et al., 1995). Thus, the avian node possesses the same lineage, inductive capacity and dynamics attributed to the dorsal blastopore lip of amphibians, hence is the homologous structure of the organizer as defined by Spemann and Mangold (Spemann and Mangold, 1924; Waddington, 1952).

Experiments involving the reversal, replacement, or extirpation of the node and/or the anterior streak revealed a remarkable capacity of the avian embryo to develop without its organizer, to regenerate and to return to normal development after major insults (Abercrombie, 1950; Grabowski, 1956; Psychoyos and Stern, 1996b and references therein). Complete removal of the node or the ablation of the anterior 40% of the primitive streak allowed the forma-

Fig. 1. Morphology, fate and homeobox gene expression in early chick embryos. Complete embryos (A) and schematic representations of the area pellucida (B–H) in the first five Hamburger-Hamilton stages. For further discussion see text. Abbreviations: hp, head process; ks, Koller's sickle; m, mesoderm; n, node; np, neural plate; pmz, posterior marginal zone; pp, prechordal plate; pnp, postnodal plate; ps, primitive streak. (A) Morphology. The original illustrations of the first five Hamburger-Hamilton stages are shown (Hamburger and Hamilton, 1951). (B) Cell fate. The fate of cells forming at Koller's sickle or ingressing at the various antero-posterior levels of the primitive streak are indicated. (C) OTX2 expression (Bally-Cuif et al., 1995). Note that a brief transient gap may exist in anterior ectoderm expression between HH st.4 and HH st.5. (D) HOXB1, HOXA2 expression (Prince and Lumsden, 1994; Sundin et al., 1990). Note the absence of HOX expression from HH st.1 and HH st.2. (E) GSC expression (Izpisúa-Belmonte et al., 1993). Note the focusing of GSC expression to the node. (F) GSX expression (Lemaire et al., 1997). Note that the anterior node at HH st.3 stops expressing GSX. (G). CNOT1, CNOT2 expression (Stein and Kessel, 1995; Stein et al., 1996b). Note focusing of the wide expression of prestreak embryos to the node at HH st.4. H. NKX2.5 expression (Schultheiss et al., 1995). Note that cardiac mesoderm does not express upon ingression from the streak (hatched areas), but only after arrival at the very anterior position. Fig. 1A was reprinted by permission of John Wiley & Sons, Inc.

tion of normal embryos, although the healed streaks did not regenerate GSC expression in reconstituted nodes (Psychoyos and Stern, 1996b). Substitution of the extirpated node with non-node cells resulted in the formation of a split embryonic axis with two notochords, demonstrating a suppressing effect of the midline on the flanking epiblast (Yuan et al., 1995a,b).

There is growing evidence to suggest that neural induction includes planar and vertical mechanisms, with a horizontal passage of signals within the ectodermal epithelium and a vertical passage of signals between different germ layers (for review see Hemmati-Brivanlou and Melton, 1997; Ruiz i Altaba, 1993). In chick embryos, neuralization starts before the primitive streak has reached its full extension and thus before the axial mesoderm has appeared. This suggests that the initial neuralizing signals are derived from Hensen's node and operate in a planar fashion. It does not exclude, however, additional vertical effects from direct derivatives of the node. Although the comparable sizes of the growing endoderm and the neural plate are suggestive of a vertical interaction, so far no evidence for a neuralizing potential of the prospective ventral endoderm has been obtained by transplantation. Molecular analyses of the *Xenopus* organizer have revealed that the various functions of the organizer go back to a generally dorsalizing effect, exerted on endoderm, mesoderm and ectoderm (Sasai et al., 1996 and references therein). The molecular mechanism of amphibian neuralization is the binding and thus inactivation of the secreted factor BMP4 by BMP-binding proteins, in particular chordin, noggin or follistatin (Hemmati-Brivanlou and Melton, 1997). In the HH st.4 chick embryo, BMP4 is expressed in the epiblast, excluding the neural plate and anterior streak (Schultheiss et al., 1997). This strongly suggests a conserved neuralization mechanism. However, BMP4 antagonism or antagonizing factors were so far not experimentally demonstrated.

4. Polarity, symmetry and embryonic axes

The unfertilized avian egg is radially symmetric around its animal-vegetal axis, extending from the cytoplasm to the yolk-containing part. The multicellular embryo develops from the cytoplasm, due to a discoidal cleavage modus, leaving the yolk uncleaved to become extraembryonal. The animal pole of the developing embryo is represented by the area pellucida. These cells are homologous to the animal half cells of a *Xenopus* blastula, and are the precursors for all cells of the three germ layers. Less clear is the location and functional significance of vegetal cells in the avian blastula. It is possible, however, that they are represented by the yolk-rich cells of the early area opaca. During gastrulation the animal-vegetal axis loses its importance, later becoming replaced by new axes, and finally disappearing.

Many observations and experiments have already revealed that during the intrauterine formation of the area pellucida a second embryonic axis is introduced, establishing the bilateral symmetry of the embryo (von Baer, 1828; Kochav and Eyal-Giladi, 1971; Eyal-Giladi and Fabian, 1980; Khaner, 1993). This process is dependent on the influence of gravity. During the rotation of the egg in the uterus the heavy yolk remains quiescent, the embryo is brought into an oblique position, and this asymmetry is transferred to the embryo. The new axis is morphologically concealed during late intrauterine development, before it becomes delineated first by the position of Koller's sickle, then by the direction of hypoblast spreading, and finally by the extension of the primitive streak. Commonly, in chick embryos this second axis is addressed as the antero-posterior axis. It appears, however, that initially dorsal-ventral values are also polarized along this axis. Dorsal structures develop from Hensen's node, and more ventral structures originate more closely to the base of the streak. Thus, it seems that mixed identities are specified at the beginning, so that the axis extends in a dorsal/anterior to ventral/posterior direction. Only with the extension of the primitive streak and the formation of embryonic mesoderm do the mixed identities segregate. By HH st.5, separate anterior to posterior and dorsal to ventral axial coordinates are clearly recognizable. Whereas the anteroposterior axis still follows the direction of the primitive streak, the dorsoventral axis runs from medial (axial) to lateral. The issue becomes even more complex, since also dorsal/posterior information is present from early on in the tip of the streak, correlating with the later formation of the notochord.

In the amphibian embryo the dorsoventral axis appears to be established before the anteroposterior axis (Slack and Tannahill, 1992). Thus, at first sight axial specification would follow a different order to that in birds. However, based on the above considerations, the amphibian and the avian system do not appear to be so different, with an intermediate axis preceding the final specification of the classical embryonic axes. A way out of the dilemma is in sight by following gene expression and function during early development. If axial parameters can be recognized by expression of, e.g. homeobox genes, then the traditional axial poles may be more precisely addressed as homeobox gene-encoded identities. In the next part of this review we will present such an attempt.

5. Homeobox genes

Homeobox genes have been found in unicellular eukaryotes and all metazoans (Duboule, 1994). So far we know of about 180 different homeobox genes from vertebrates, and around 70 chicken homeobox genes are described (Stein et al., 1996a). Function has been studied predominatly in mutants of the fruitfly *Drosophila melanogaster*, in the frog *Xenopus laevis* by RNA injections and in transgenic gain- or loss-of-function mutants of the mouse *Mus muscu-*

lus (McGinnis and Krumlauf, 1992; Dessain and McGinnis, 1993; Krumlauf, 1994; Harvey, 1996; Bally-Cuif and Boncinelli, 1997). We will not attempt to review these approaches here, since they were not undertaken in the chick embryo as an experimental system. Instead, we deduce the working hypothesis that homeobox genes are involved in the specification of regional or cellular identity. We postulate that the combination of expressed homeobox genes defines a molecular code organizing pattern and identity formation in the embryo. Below, we will specifically review and discuss the subset of avian homeobox genes involved in early embryogenesis of the chick embryo.

5.1. OTX2 identity: the animal cells of the chick embryo

Otx2 is one of the earliest homeobox genes expressed in vertebrate embryos. Chicken animal cells are characterized by expression of the OTX2 gene at least from the time of egg laying onwards, most probably also earlier (Fig. 1C; Bally-Cuif et al., 1995). Such an early Otx2 phase is also observed in the murine blastula, the egg-cylinder (Simeone et al., 1993). In the frog *Xenopus laevis*, the Otx2 protein is supplied maternally, and RNA is only weakly detectable in pre-gastrulation animal cells (Pannese et al., 1995). From the correlation between Otx2 and the omnipotent animal cell identity we suspect that Otx2 expression may define an embryonic groundstate, from which all later identities are diverging or elaborating.

With the onset of chick gastrulation, OTX2 expression remains only in the area rostral of the primitive streak and in Hensen's node. Signals produced in the context of gastrulation, from either the streak or the mesoderm, appear to be inhibitory for OTX2 expression. Exemplary is the negative response of the OTX2 gene to retinoic acid (Bally-Cuif et al., 1995). At HH st.4, the fully elongated streak has reached a position demarcating the level of the prospective rostral rhombencephalon, anterior of the prospective otic vesicle. A transverse line at this level divides the embryo into an anterior and a posterior territory. The anterior territory is an ontogenetic remnant of the animal pole of the blastula, i.e. the only embryonic area not directly reached by the blastopore. It remains characterized until late in development by OTX2 expression (Fig. 3). However, between the early and the later phase of OTX2 expression in the chick there is a short transient stage, when almost no ectodermal expression appears detectable in the anterior epiblast. Significant upregulation in all three germ layers occurs only upon the arrival of prechordal plate and head process cells beneath the anterior epiblast. The main structures identified by OTX2 are the prechordal plate, the head process, the paraxial head mesoderm, the first branchial arch, the trigeminal ganglion, the prosencephalon, and the mesencephalon (Fig. 3).

Characterization of the head region by an Otx gene, orthodenticle (otd), occurs also in *Drosophila melanogaster* (Finkelstein and Boncinelli, 1994). Conserved features are the location at the anterior pole, which is not directly reached by the blastopore, with a relatively sharp posterior demarcation from the rest of the axis at the level of the mouth, and the harboring of the anterior brain. Thus, a well conserved genetic system seems to maintain the original non-gastrulation identity, regardless of a dorsal-ventral inversion (gastro- versus notoneuralia) or the blastoporal fate (proto- versus deuterostomy; Arendt and Nübler-Jung, 1997).

5.2. Goosecoid identities: the establishment of dorsal/anterior values in chicken

The chicken goosecoid gene (GSC) is first expressed in the posterior blastoderm including and around Koller's sickle (Fig. 1E; Izpisúa-Belmonte et al., 1993). It then characterizes the primitive streak: first the complete streak (HH st.2), then only the anterior 40% (HH st.3), and finally it is restricted to Hensen's node (HH st.4). The GSC domain localized in Hensen's node of the chick gastrula at HH st.4 is comparable with the domain localized in the dorsal lip of the early *Xenopus laevis* gastrula. Both stages directly precede ingression of Gsc cells, which will eventually form the prechordal plate (Fig. 2A). The specification of 'dorsal/anterior' on the blastopore margin by Gsc homeobox genes appears to be a conserved feature in vertebrates, and coincides with the location of the organizer (see above). The close relation between GSC expression and the organizer could also be corroborated in functional studies, where the injection of GSC into ventral blastomeres of frog embryos led to secondary axis formation (Cho et al., 1991). However, the disruption of the murine gsc gene did not reveal a gastrulation or neurulation defect, prompting the suspicion of a duplicated gene or other redundancy mechanisms (Rivera-Peréz et al., 1995; Yamada et al., 1995).

Recently, a second homeobox gene of the goosecoid-type, GSX, was isolated from the chick genome (Lemaire et al., 1997). Its expression starts quite similar to that of GSC, however it is regulated differently in the elongating primitive streak (Fig. 1F). Its expression does not become concentrated to the anterior portion, but remains along the full length excluding the most anterior tip (HH st.3). Whilst GSC becomes confined to the node, GSX spreads around it in an ectodermal expression domain delineating the early neural plate (HH st.4 and 5; Fig. 2C). GSX cells stay ectodermal, while GSC cells leave the deep portion of the node to form the mesendodermal prechordal plate (Fig. 3).

The single goosecoid gene found in *Drosophila melanogaster* is expressed in the brain, the stomodaeum and parts of the stomatogastric nervous system (Goriely et al., 1996; Hahn and Jäckle, 1996). By being merely ectodermally expressed, D-gsc resembles the GSX more than the GSC pattern. The duplication of a single goosecoid gene to GSX and GSC, and the gastrulation of GSC cells would thus appear to be later achievements during evolution. The initial function of goosecoid in vertebrates appears to be the polar-

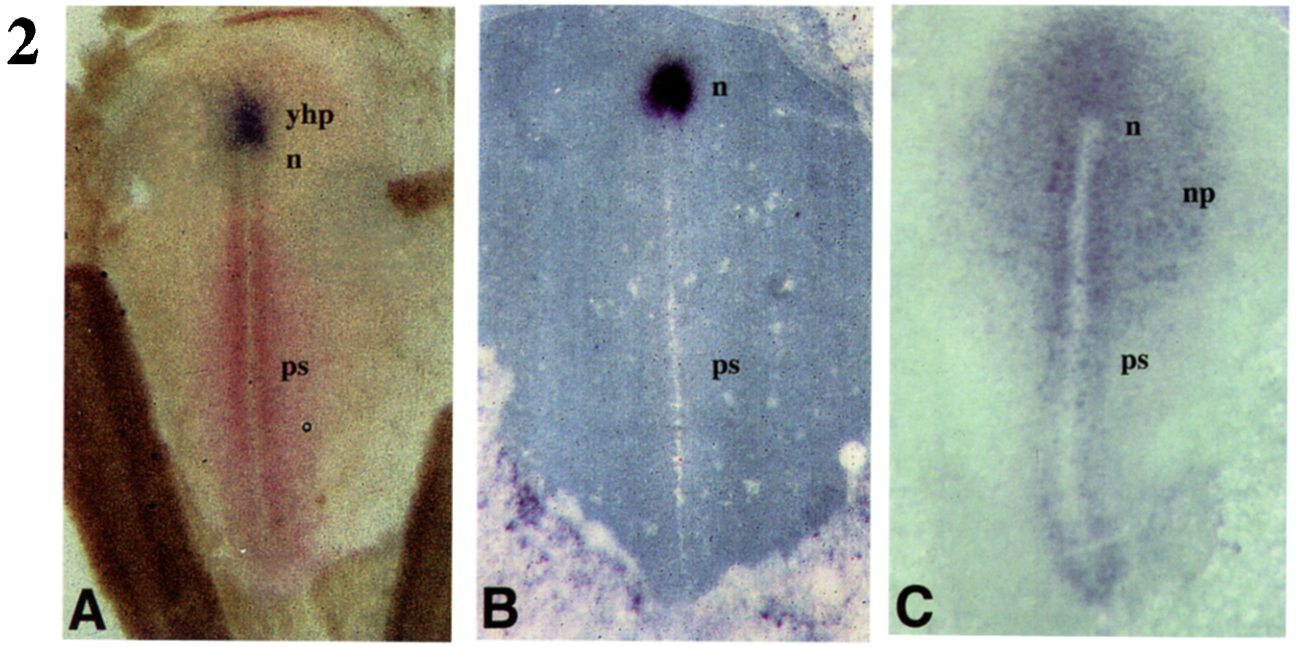


Fig. 2. Homeobox gene expression in the chick. (A) HH st. 4+ embryos shows GSC expression (blue) in the young head process (yhp), the prospective prechordal plate, which just ingressed from the node. HOXB1 expression (red) is visible in and around the primitive streak (ps). (B) CNOT1 expression restricted to the node (n). (C) GSX expression in the early neural plate (np).

ization of the blastopore, by the generation of a dorsal/anterior identity. The opposing identity is specified in *Xenopus laevis* by the two homeobox genes Xvent1 and Xvent2 (Gawantka et al., 1995; Onichtchouk et al., 1996). Extrapolating the information between frog and chick, the polarization of the vertebrate blastopore margin might function through combinatorial codes involving two goosecoid and two vent genes.

5.3. CNOT1/CNOT2 identity: specification of the posterior dorsal mesoderm

Dorsal mesoderm can be considered to consist of two distinct identities: the prechordal plate, underlying the developing forebrain, and the notochord, located at the level of the hindbrain and the spinal cord (Fig. 3). In between, at the level of the midbrain lies a transition struc-

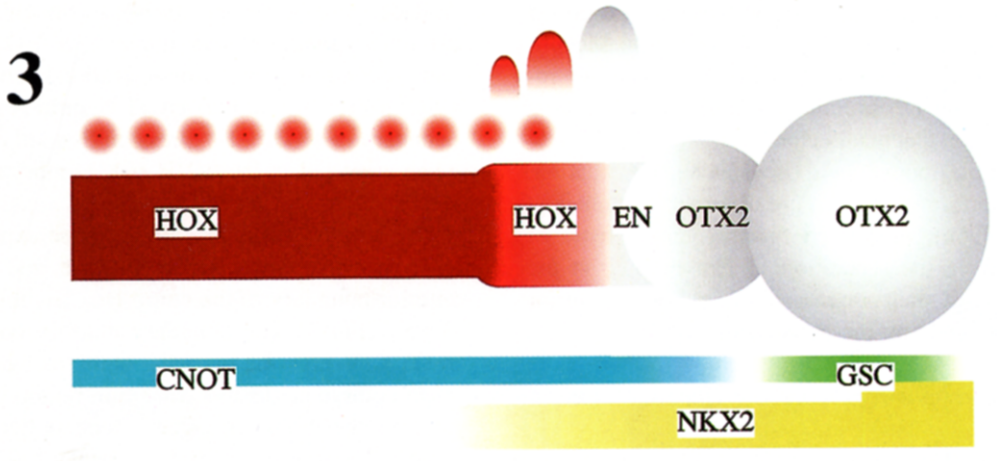


Fig. 3. Embryonic territories as defined by homeobox gene expression in post-gastrulation chick embryos. The central nervous system with flanking somites and branchial arches, and the axial mesoderm with ventral foregut/heart are schematically indicated. Expression of the OTX2 gene (gray) identifies fore- and midbrain regions (large and small circle) and the first branchial arch. EN expression (gray) characterizes the midbrain/hindbrain junction. Members of the HOX family (red) specify the trunk from the rostral hindbrain and the second branchial arch backwards, including all somites (red circles). The major boundary crossing the body axis between rhombomere 1 and 2 of the hindbrain, and between the first and second branchial arch is emphasized between red and gray. GSC expression (green) identifies the prechordal plate underlying the forebrain, whereas CNOT expression (blue) specifies the notochord underlying hindbrain and spinal cord. Members of the NKX2 family are expressed in the ventral endoderm and heart mesoderm (yellow).

ture, here defined as the head process, in order to point out a specific characteristic of the anterior notochord. The prechordal plate is characterized by the expression of the homeobox gene GSC, in addition to others such as OTX2. Two homeobox genes are expressed very specifically in the notochord, the clustered genes CNOT1 and CNOT2 (Knezevic et al., 1995; Ranson et al., 1995; Stein and Kessel, 1995; Stein et al., 1996b).

Before gastrulation both CNOT genes are active in large parts of the posterior epiblast around and in Koller's sickle (Fig. 1G). The upstream gene of the cluster, CNOT2, is activated earlier and more widespread than the downstream CNOT1 gene (Stein et al., 1996b). However, in general the two expression domains are very similar. The large CNOT domain in the pre-streak embryo includes the precursor cell population of the notochord. With formation of the primitive streak, CNOT expression becomes more focused in the anterior streak, and its surrounding epiblast (HH st.3). By HH st.4, CNOT1 and CNOT2 expressions are confined to the node, where they overlap with GSC expressing cells (Fig. 2B). While GSC positive cells leave the node immediately to form the prechordal plate (HH st.4), CNOT cells leave the node more slowly, their density increasing in the head process, before they become the only cells populating the notochord. Node and tailbud continue to express the CNOT genes until notochord generation ceases. It has been demonstrated that a stem cell-like notochord center is active in the most anterior node section (Rosenquist, 1983; Selleck and Stern, 1992). Interesting is the presence of a small, transient CNOT domain in the neural plate, which first surrounds the node, then is restricted to the postnodal plate, but always disappears with differentiation of the neural tube (Fig. 1G). Thus, each axial level of the neural tube has briefly expressed the CNOT genes at one stage of development (Stein and Kessel, 1995).

In *Drosophila melanogaster*, a CNOT related homeobox gene, 90Bre, is active in neuroblasts (Dessain and McGinnis, 1993). It is never found in gastrulated tissue and resembles in this respect the *Drosophila* goosecoid gene. We have discussed elsewhere, that neuroectodermal Not gene expression flanking the blastopore could be an anchestral situation, of which only transient remnants are observable in chicken (Stein et al., 1996b). A derived feature characteristic of chordates would be the separation of Not cells from the neuroectoderm, and their ingression as a new, posterior dorsal form of mesoderm, the notochord.

5.4. HOX identities: anteroposterior specification in the trunk

Different combinations of expressed Hox genes seem to specify anteroposterior cell identities in the trunk, from the rostral rhombencephalon backwards. Although not all avian Hox clusters are completely described, there is no doubt that they are organized in four clusters highly similar to the 39 murine genes. Hox genes are not expressed before primitive streak formation in chick embryos. Significant levels of expression were detected in HH st.4 embryos for genes of the lower paralog groups 1, 2 and 4. HOXB1 transcripts were found along the streak excluding the node (Fig. 2A), HOXA2 and HOXD4 are activated only in the posterior part of the streak (Fig. 1D; Sundin et al., 1990; Gaunt and Strachan, 1994; Prince and Lumsden, 1994). Thus, the temporal and structural colinearity rules first observed in murine embryos seem also to apply to chick embryogenesis (Krumlauf, 1994). With ongoing mesoderm formation, expression first spreads in a broad region of both ecto- and mesoderm, and then marks a distinct anterior boundary, which lies more anterior in the neuroectoderm as compared to the paraxial mesoderm. The most anterior boundary reached by a Hox gene, namely Hoxa-2, in birds as well as in mice is the second rhombomere and the second branchial arch (Fig. 3).

Gaunt and Strachan observed that the anterior spreading of HOXD-4 expression was not inhibited by the implantation of a glass barrier transversally to a HH st.4 primitive streak (Gaunt and Strachan, 1994). Thus, the wave-like anterior spreading of Hox gene expression is not the result of cell migration and does not require tissue continuity. Rather, the 3′- to 5′-activation of Hox genes appears to work as an intracellular cascade, temporally colinear to the organization of the Hox clusters. Expression of a Hox gene within any particular cell appeared as a direct consequence of the prior activation of a more 3′-located Hox gene. Such a mechanism poses the question as to how the cascade is initiated and how it is stopped. The best candidate for initiation is retinoic acid, which was demonstrated as an activator of Hox genes in many systems (e.g. Kessel and Gruss, 1991; Kessel, 1992, 1993). The functional RA receptor binding site 3′ of the murine Hoxb-1 gene gave further support to retinoic acid as an initiator of a Hox expression cascade (Marshall et al., 1994). In the chick, retinoic acid can quantitatively elevate the level of HOXB-1 expression and induce further anterior activation (Sundin and Eichele, 1992). In reverse, deficiency of vitamin A posteriorized the boundary of HOXB-1 expression in quail embryos, at least when analyzed in later (HH st.13) embryos (Maden et al., 1996).

The anterior boundary of Hox gene expressions appears to be of major functional importance, and in particular the anterior boundary of the entire Hox territory, delineated by Hoxa-2 (Fig. 3). This boundary abuts the posterior boundary of OTX2 expression in earlier embryos, whereas in somite-stage embryos a distinct transition region is created, specified by other homeobox genes such as EN1, EN2 or Gbx2 (Gardner et al., 1988; Bouillet et al., 1995). Experimental manipulation of the boundary was achieved by several approaches. It was shifted anteriorly by a Hoxa-7 transgene in transgenic mice or by retinoic acid exposure at the time of Hox gene activation (Balling et al., 1989; Kessel and Gruss, 1991; Sundin and Eichele, 1992; Kessel, 1993;). In these experiments the posterior Otx2 domain, the first branchial

arch, turned out as a major target. A posterior shift of the boundary was obtained by inactivation of the murine Hoxa-2 gene, which led to homeotic transformation of the second into a first branchial arch (Gendron-Maguire et al., 1993; Rijli et al., 1993).

In *Drosophila*, the boundary between Otd and Hox patterned parts divides the anteroposterior axis into a supraesophageal and a subesophageal region. Thus, we suspect that it originally represents the position of the dorsal/anterior pole of the blastopore, i.e. the mouth of the protostomia.

5.5. NKX2 identities: a ventral territory of the chick embryo

The early products of gastrulation are prospective foregut endoderm, as well as head and heart mesoderm (Fig. 1B). None of the homeobox genes discussed so far is expressed in these cells after their ingression, e.g. in the concentrically growing endoderm or the mesodermal wings. Key genes for ventral specification such as the two Xvent genes (Gawantka et al., 1995; Onichtchouk et al., 1996) have already been mentioned, and their chick homologs may be found in the prospective ventral mesoderm before or after ingression from the streak. Another family of homeobox genes relevant for early steps of pattern formation is characterized by homeodomains of the NK2-type (Harvey, 1996). In vertebrates, NK2 genes are expressed in overlapping domains of the ventral endoderm and its derivatives like the thyroid gland and the lung, and in the developing heart. Thus, they do not characterize the above mentioned cells directly upon ingression, but only when they have already migrated a considerable distance. A typical member of the NK2 family is the NKX2.5 gene. In the chick it is expressed in the cardiac mesoderm from HH st.5 onwards, probably in response to signals from the anterior endoderm (Fig. 1H; Schultheiss et al., 1995). A recently described chicken gene, NKX2.8, is initially expressed in cardiac mesoderm and ventral endoderm, and later characterizes the ventral portion of the branchial arches (Boettger et al., 1997). The ventral neuroectoderm also becomes an NKX2 domain, dependent on sonic hedgehog signaling (Ericson et al., 1995). The common denominator of the divergent expression domains of vertebrate NK2 genes at first sight is their restricted expression in the ventral part of the body. Therefore we have recently suggested a specific ventral territory going back to early ingressing endo- and mesodermal cell populations (Fig. 3; Boettger et al., 1997).

NK2 genes are known from invertebrates such as cnidaria, planaria, nematodes and arthropods. In *Hydra vulgaris*, a radially symmetric, mesoderm-free cnidarian, a NK2 gene is only active in the endoderm at the basal end of the axis, i.e. in an early gastrulation product located away from the blastopore (Grens et al., 1996). The *Drosophila NK2* gene tinman is essential for the early mesoderm forming the dorsal vessel, a heart-like structure again located most distally from the ventral blastopore (Bodmer, 1993). Thus, a common feature of NK-2 genes is the specification of early gastrulation products, which became located distally from the blastopore, a position referred to as ablastoporal in Cnidaria, dorsal in invertebrate bilateria, and ventral in vertebrates.

6. Conclusion

We have reviewed above the early development of the chick embryo and the expression of homeobox genes during gastrulation. We have described the consecutive establishment of identities, and the division of the embryo into large primary territories. Some of these can be correlated with homeobox gene expression in the avian embryo, supporting the evidence for a homeobox code represented by the combination of expressed homeobox genes. We describe an early OTX2 identity, which first equals all embryogenic cells, but then becomes restricted to the anterior head. Clearly identifiable is a GSC/GSX territory, first specifying a dorsal/anterior identity, and later separating into anterior and dorsal values. A ventral territory becomes established later, and is defined by combinations of NK2 gene expressions. The trunk territory is specified by overlapping domains of HOX gene expressions. No candidate genes could so far be found for other identities predictable from cell biological aspects. These could include a vegetal or an early ventral/posterior identity. It is well conceivable that the combinatorial nature of homeobox codes allows for the remarkable redundancy observed in vertebrate development. How such codes are established, imprinted, induced or changed remains a wide field for further exploration. It will be essential for our understanding of the mechanisms of development.

Acknowledgements

We thank T. Boettger, H. Knötgen, E. Pera, and T. Roeser for many discussions and suggestions. The authors are supported by the Max-Planck-Society and SFB271.

References

Abercrombie, M. (1950) The effects of antero-posterior reversals of lengths of the primitive streak in the chick. Phil. Trans. Roy. Soc. B. 234, 317–338.

Arendt, D. and Nübler-Jung, K. (1997) Dorsal or ventral: similarities in fate maps and gastrulation patterns in annelids, arthropods and chordates. Mech. Dev. 61, 7–21.

Azar, Y. and Eyal-Giladi, H. (1979) Marginal zone cells – the primitive streak-inducing component of the primary hypoblast in the chick. J. Embryol. Exp. Morphol. 52, 79–88.

Balling, R., Mutter, G., Gruss, P. and Kessel, M. (1989) Craniofacial abnormalities induced by ectopic expression of the homeobox gene Hox-1.1 in transgenic mice. Cell 58, 337–347.

Bally-Cuif, L., Gulisano, M., Broccoli, V. and Boncinelli, E. (1995) c-otx2 is expressed in two different phases of gastrulation and is sensitive to retinoic acid treatment in chick embryos. Mech. Dev. 49, 49–63.

Bally-Cuif, L. and Boncinelli, E. (1997) Transcription factors and head formation in vertebrates. Bioessays 19, 127–135.

Bellairs, R. (1986) The primitive streak. Anat. Embryol. 174, 1–14.

Bodmer, R. (1993) The gene tinman is required for specification of the heart and visceral muscles in Drosophila. Development 118, 719–729.

Boettger, T., Stein, S. and Kessel, M. (1997) The chicken NKX2.8 homeobox gene: A novel member of the NK-2 gene family. Dev. Genes Evol. 207, 65–70.

Bouillet, P., Chazaud, C., Oulad-Abdelghani, M., Dolle, P. and Chambon, P. (1995) Sequence and expression pattern of the Stra7 (Gbx-2) homeobox-containing gene induced by retinoic acid in P19 embryonal carcinoma cells. Dev. Dyn. 204, 372–82.

Bouwmeester, T., Kim, S., Sasai, Y., Lu, B. and De Robertis, E.M. (1996) Cerberus is a head-inducing secreted factor expressed in the anterior endoderm of Spemann's organizer. Nature 382, 595–601.

Callebaut, M. and Van Nueten, E. (1994) Rauber's (Koller's) sickle: the early gastrulation organizer of the avian blastoderm. Eur. J. Morphol. 32, 35–48.

Cho, K. W. Y., Blumberg, B., Steinbeisser, H. and De Robertis, E.M. (1991) Molecular nature of Spemann's organizer: the role of the *Xenopus* homeobox gene *goosecoid*. Cell 67, 1111–1120.

Cooke, J., Takada, S. and McMahon, A. (1994) Experimental control of axial pattern in the chick blastoderm by local expression of Wnt and activin: the role of HNK-1 positive cells. Dev. Biol. 164, 513–27.

Dessain, S. and McGinnis, W. (1993) *Drosophila* homeobox genes. In P.M. Wassarman (ed.), JAI Press Inc., Greenwich, Advances in Developmental Biochemistry Vol. 2, pp. 1–55.

Dias, M.S. and Schoenwolf, G.C. (1990) Formation of ectopic neuroepithelium in chick blastoderms: age related capacities for induction and self-differentiation following transplantation of quail Hensen's node. Anat. Rec. 229, 437–448.

Duboule, D. (1994) Guidebook to the Homeobox Genes. Oxford University Press, Oxford.

Ericson, J., Muhr, J., Placzek, M., Lints, T., Jessell, T.M. and Edlund, T. (1995) Sonic hedgehog induces the differentiation of ventral forebrain neurons: a common signal for ventral patterning within the neural tube. Cell 81, 747–56.

Eyal-Giladi, H., Debby, A. and Harel, N. (1992) The posterior area of the chicks area pellucida and its involvement in hypoblast and primitive streak formation. Dev. 116, 819–830.

Eyal-Giladi, H. and Fabian, B.C. (1980) Axis determination in uterine chick blastodiscs under changing spatial positions during the sensitive period for polarity. Dev. Biol. 77, 228–232.

Eyal-Giladi, H. and Khaner, O. (1989) The chick's marginal zone and primitive streak formation. II. Quantification of the marginal zone's potencies – temporal and spatial aspects. Dev. Biol. 134, 215–221.

Eyal-Giladi, H. and Kochav, S. (1976) From cleavage to primitive streak formation: a complementary normal table and a new look at the first stages of the development of the chick. I. General morphology. Dev. Biol. 49, 321–337.

Finkelstein, R. and Boncinelli, E. (1994) From fly head to mammalian forebrain: the story of otd and Otx.. Trends Genetics 10, 310–315.

Gallera, J. (1971) Primary induction in birds. Adv. Morphogenesis 9, 149–180.

Gallera, J. and Nicolet, G. (1969) Le pouvoir inducteur de l'endoblaste presomptif contenu dans la ligne primitive jeune de poulet. J. Embryol. Exp. Morphol. 21, 105–118.

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.

Garcia-Martinez, V. and Schoenwolf, G.C. (1993) Primitive-streak origin of the cardiovascular system in avian embryos. Dev. Biol. 159, 706–719.

Gardner, C.A., Darnell, D.K., Poole, S.J., Ordahl, C.P. and Barald, K.F. (1988) Expression of an engrailed-like gene during development of the early embryonic chick nervous system. J. Neurosci. Res. 21, 426–437.

Gaunt, S.J. and Strachan, L. (1994) Forward spreading in the establishment of a vertebrate Hox expression boundary: the expression domain separates into anterior and posterior zones, and the spread occurs across implanted glass barriers. Dev. Dyn. 199, 229–240.

Gawantka, V., Delius, H., Hirschfeld, K., Blumenstock, C. and Niehrs, C. (1995) Antagonizing the Spemann organizer: role of the homeobox gene Xvent-1. EMBO J. 14, 6268–6279.

Gendron-Maguire, M., Mallo, M., Zhang, M. and Gridley, T. (1993) Hoxa-2 mutant mice exhibit homeotic transformation of skeletal elements derived from cranial neural crest. Cell 75, 1317–1331.

Goriely, A., Stella, M., Coffinier, C., Kessler, D., Mailhos, C., Dessain, S. and Desplan, C. (1996) A functional homologue of goosecoid in Drosophila. Development 122, 1641–1650.

Grabowski, C.T. (1956) The effects of the excision of Hensen's node on the early development of the chick embryo. J. Exp. Zool. 133, 301–344.

Grens, A., Gee, L., Fisher, D.A. and Bode, H.R. (1996) CnNK-2, an NK-2 homeobox gene, has a role in patterning the basal end of the axis in hydra. Dev. Biol. 180, 473–488.

Hahn, M. and Jäckle, H. (1996) Drosophila goosecoid participates in neural development but not in body axis formation. EMBO J 15, 3077–3084.

Hamburger, V. and Hamilton, H. L. (1951) A series of normal stages in the development of the chick embryo. J. Morphol. 88, 49–92.

Hamilton, H.L. (1965) Lilie's Development of the Chick, An Introduction to Embryology. Holt, Rinehart and Winston, New York.

Harvey, R.P. (1996) NK-2 homeobox genes and heart development. Dev. Biol. 178, 203–216.

Hemmati-Brivanlou, A. and Melton, D. (1997) Vertebrate embryonic cells will become nerve cells unless told otherwise. Cell 88, 13–7.

Hensen, V. (1876) Beobachtungen über die Befruchtung und Entwicklung des Kaninchens und Meerschweinchens. Z. Anat. EntwGesch. 1, 353–423.

Inagaki, T. and Schoenwolf, G.C. (1993) Axis development in avian embryos: the ability of Hensen's node to self-differentiate, as analyzed with heterochronic grafting experiments. Anat. Embryol. 188, 1–11.

Izpisúa-Belmonte, J.C., De Robertis, E.M., Storey, K.G. and Stern, C.D. (1993) The homeobox gene goosecoid and the origin of organizer cells in the early chick blastoderm. Cell 74, 645–659.

Karagenc, L., Cinnamon, Y., Ginsburg, M. and Petitte, J.N. (1996) Origin of primordial germ cells in the prestreak chick embryo. Dev. Genet. 19, 290–301.

Kessel, M. (1992) Respecification of vertebral identities by retinoic acid. Development 115, 487–501.

Kessel, M. (1993) Reversal of axonal pathways from rhombomere 3 correlates with extra Hox expression domains. Neuron 10, 379–393.

Kessel, M. and Gruss, P. (1991) Homeotic transformations of murine vertebrae and concomitant alteration of Hox codes induced by retinoic acid. Cell 67, 89–104.

Khaner, O. (1993) Axis determination in the avian embryo. Curr. Top. Dev. Biol. 28, 155–80.

Khaner, O. (1995) The rotated hypoblast of the chicken embryo does not initiate an ectopic axis in the epiblast. Proc. Natl. Acad. Sci. USA 92, 10733–10737.

Khaner, O. and Eyal-Giladi, H. (1989) The chick's marginal zone and primitive streak formation. I. Coordinative effect of induction and inhibition. Dev. Biol. 134, 206–214.

Knezevic, V., Ranson, M. and Mackem, S. (1995) The organizer-associated chick homeobox gene, Gnot1, is expressed before gastrulation and regulated synergistically by activin and retinoic acid. Dev. Biol. 171, 458–470.

Kochav, S. and Eyal-Giladi, H. (1971) Bilateral symmetry in chick embryo determination by gravity. Science 171, 1027–1029.

Koller, C. (1882) Untersuchungen über die Blätterbildung im Hühnerkeim. Arch. Mikrosk. Anat. 20, 171–211.

Krumlauf, R. (1994) Hox genes in vertebrate development. Cell 78, 191–201.

Lemaire, L., Roeser, T., Izpisua-Belmonte, J.-C. and Kessel, M. (1997) Segregating expression domains of two goosecoid genes during the transition from gastrulation to neurulation in chick embryos. Development 124, 1443–1452.

Maden, M., Gale, E., Kostetskii, I. and Zile, M. (1996) Vitamin A-deficient quail embryos have half a hindbrain and other neural defects. Curr. Biol. 6, 417–26.

Marshall, H., Studer, M., Pöpperl, H., Aparicio, S., Kuroiwa, A., Brenner, S. and Krumlauf, R. (1994) A conserved retinoic acid response element required for early expression of the homeobox gene Hoxb-1. Nature 370, 567–571.

McGinnis, W. and Krumlauf, R. (1992) Homeobox genes and axial patterning. Cell 68, 283–302.

Mitrani, E. and Eyal-Giladi, H. (1981) Hypoblastic cells can form a disk inducing an embryonic axis in chick epiblast. Nature 289, 800–802.

Mitrani, E. and Eyal-Giladi, H. (1984a) Depolarized chick blastoderms fail to self-generate bilateral symmetry. Differentiation 27, 189–191.

Mitrani, E. and Eyal-Giladi, H. (1984b) Differentiation of dissociated-reconstitutes epiblasts of the chick under the influence of a normal hypoblast. Differentiation 26, 107–111.

Onichtchouk, D., Gawantka, V., Dosch, R., Delius, H., Hirschfeld, K., Blumenstock, C. and Niehrs, C. (1996) The Xvent-2 homeobox gene is part of the BMP-4 signalling pathway controling dorsoventral patterning of Xenpous mesoderm. Development 122, 3045–3053.

Pannese, M., Polo, C., Andreazzoli, M., Vignali, R., Kablar, B., Barsacchi, G. and Boncinelli, E. (1995) The *Xenopus* homologue of Otx2 is a maternal homeobox gene that demarcates and specifies anterior body regions. Development 121, 707–720.

Prince, V. and Lumsden, A. (1994) Hoxa-2 expression in normal and transposed rhombomeres: independent regulation in the neural tube and neural crest. Development 120, 911–923.

Psychoyos, D. and Stern, C.D. (1996a) Fates and migratory routes of primitive streak cells in the chick embryo. Development 122, 1523–1534.

Psychoyos, D. and Stern, C.D. (1996b) Restoration of the organizer after radical ablation of Hensen's node and the anterior primitive streak in the chick embryo. Development 122, 3263–3273.

Ranson, M., Tickle, C., Mahon, K.A. and Mackem, S. (1995) Gnot1, a member of a new homeobox gene subfamily, is expressed in a dynamic, region-specific domain along the proximodistal axis of the developing limb. Mech. Dev. 51, 17–30.

Rauber, A. (1876) Über die Stellung des Hühnchens im Entwicklungsplan. W. Engelsmann, Leipzig.

Rijli, F. M., Mark, M., Lakkaraju, S., Dierich, A., Dollé, P. and Chambon, P. (1993) A homeotic transformation is generated in the rostral branchial region of the head by disruption of Hoxa-2, which acts as a selector gene. Cell 75, 1333–1349.

Rivera-Peréz, J. A., Mallo, M., Gendron-Maguire, M., Gridley, T. and Behringer, R.R. (1995) Goosecoid is not an essential component of the mouse gastrula organizer but is required for craniofacial and rib development. Development 121, 3005–3012.

Romanoff, A.L. (1960) The avian embryo. The Macmillan Company, New York.

Rosenquist, G.C. (1972) Endoderm movements in the chick embryo between the early short streak and head process stages. J. Exp. Zool. 180, 95–103.

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

Ruiz i Altaba, A. (1993) Induction and axial patterning of the neural plate: planar and vertical signals. J. Neurobiol. 24, 1276–1304.

Sanders, E.J. (1986) Mesoderm migration in the early chick embryo. Dev. Biol. 2, 449–480.

Sasai, Y., Lu, B., Piccolo, S. and De Robertis, E.M. (1996) Endoderm induction by the organizer-secreted factors chordin and noggin in Xenopus animal caps. EMBO J. 15, 4547–4555.

Schoenwolf, G.C. and Alvarez, I.S. (1991) Specification of neuroepithelium and surface epithelium in avian transplantation chimeras. Development 112, 713–722.

Schoenwolf, G.C., Garcia-Martinez, V. and Dias, M.S. (1992) Mesoderm movement and fate during avian gastrulation and neurulation. Dev. Dyn. 193, 235–248.

Schultheiss, T.M., Xydas, S. and Lassar, A.B. (1995) Induction of avian cardiac myogenesis by anterior endoderm. Development 121, 4203–4214.

Schultheiss, T.M., Burch, J.B. and Lassar, A.B. (1997) A role for bone morphogenetic proteins in the induction of cardiac myogenesis. Genes Dev. 11, 451–462.

Seleiro, E.A.P., Connolly, D.J. and Cooke, J. (1996) Early developmental expression and experimental axis determination by chicken Vg1 gene. Curr. Biol. 6, 1476–1486.

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.

Selleck, M.A.J. and Stern, C.D. (1992) Commitment of mesodermal cells in Hensen's node of the chick embryo to notochord and somite. Development 114, 403–415.

Simeone, A., Acampora, D., Mallamaci, A., Stornaiuolo, A., D'Apice, M.R., Nigro, V. and Boncinelli, E. (1993) A vertebrate gene related to orthodenticle contains a homeodomain of the bicoid class and demarcates anterior neuroectoderm in the gastrulating mouse embryo. EMBO J. 12, 2735–2747.

Slack, J.M.W. and Tannahill, D. (1992) Mechanisms of anteroposterior axis specification in vertebrates. Development 114, 285–302.

Spemann, H. and Mangold, H. (1924) Über die Induktion von Embryoanlagen durch Implantation artfremder Organisatoren. Roux Arch. Entwiclungsmech. 100, 599–638.

Spratt, N.T.J. (1946) Formation of the primitive streak in the explanted chick blastoderm marked with carbon particles. J. Exp. Zool. 103, 259–304.

Spratt, N.T.J. (1947) Regression and shortening of the primitive streak in the explanted chick blastoderm. J. Exp. Zool. 104, 69–100.

Stein, S., Fritsch, R., Lemaire, L. and Kessel, M. (1996a) Checklist: vertebrate homeobox genes. Mech. Dev. 55, 91–108.

Stein, S. and Kessel, M. (1995) A homeobox gene involved in node, notochord and neural plate formation of chick embryos. Mech. Dev. 49, 37–48.

Stein, S., Niß, K. and Kessel, M. (1996b) Differential activation of the clustered homeobox genes CNOT2 and CNOT1 during notogenesis in the chick. Dev. Biol. 180, 519–533.

Stern, C.D. (1990) The marginal zone and its contribution to the hypoblast and primitive streak of the chick embryo. Development 109, 667–682.

Storey, K.G., Crossley, J.M., De Robertis, E.M., Norris, W.E. and Stern, C.D. (1992) Neural induction and regionalisation in the chick embryo. Development 114, 729–741.

Storey, K.G., Selleck, M.A.J. and Stern, C.D. (1995) Neural induction and regionalisation by different subpopulations of cells in Hensen's node. Development 121, 417–428.

Sundin, O. and Eichele, G. (1992) An early marker of axial pattern in the chick embryo and its respecification by retinoic acid. Development 114, 841–852.

Sundin, O.H., Busse, H.G., Rogers, M.B., Gudas, L.J. and Eichele, G. (1990) Region-specific expression in early chick and mouse embryos of Ghox-lab and Hox 1.6, vertebrate homeobox-containing genes related to Drosophila labial. Development 108, 47–58.

Thomas, P. and Beddington, R. (1996) Anterior primitive endoderm may be responsible for patterning the anterior neural plate in the mouse embryo. Curr. Biol. 6, 1487–1496.

Vakaet, L. (1962) Some new data concerning the formation of the definitive endoblast in the chick embryo. J. Embryol. Exp. Morphol. 10, 38–57.

Vakaet, L. (1964) Diversité fonctionelle de la ligne primitive du blastoderm de Poulet. C. R. Séanc. Soc. Biol. 158, 1964.

Vakaet, L. (1982) Experimental study of the ingression during gastrulation in the chick blastoderm. Verh. Konink. Acad. Geneesk. Belgie 44, 419–437.

von Baer, K.E. (1828) Entwicklungsgeschichte der Thiere: Beobachtungen und Reflexionen. Bornträger, Königsberg.

Waddington, C.H. (1933) Induction by the endoderm in birds. Wilh. Roux Arch. Entw. Mech. 128, 502–521.

Waddington, C.H. (1952) The epigenetics of birds. Cambridge University Press, Cambridge.

Winklbauer, R. (1994) Mesoderm cell migration in the vertebrate gastrula. Dev. Biol. 5, 91–99.

Yamada, G., Mansouri, A., Torres, M., Stuart, E.T., Blum, M., Schultz, M., De Robertis, E.M. and Gruss, P. (1995) Targeted mutation of the murine goosecoid gene results in craniofacial defects and neonatal death. Development 121, 2917–2922.

Yuan, S., Darnell, D.K. and Schoenwolf, G.C. (1995a) Identification of inducing, responding, and suppressing regions in an experimental model of notochord formation in avian embryos. Dev. Biol. 172, 567–584.

Yuan, S., Darnell, D.K. and Schoenwolf, G.C. (1995b) Mesodermal patterning during avian gastrulation and neurulation: experimental induction of notochord from non-notochordal precursor cells. Dev. Genet. 17, 38–54.