The avian organizer

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ABSTRACT **The development of avian embryos is characterized by the large amount of yolk present from the one-cell stage until late phases of organogenesis. In the chick, an axis of bilateral symmetry is established already before egg laying, when the egg rotates in the uterus. There is evidence for an active Wnt-catenin pathway in the vegetal cells in the periphery of the multi-cellular embryo. It overlaps with the posteriorly restricted expression of genes characterizing the vegetal hemisphere in amphibia. The zone of overlap bears several functional characteristics of a Nieuwkoop center, which is first apparent in the posterior marginal zone, but continues into the early primitive streak. Only the anterior part of the late streak is capable of direct neural induction, and only its tip, Hensen's node, can induce an anterior neural identity. This latter activity leaves the node together with the cells representing the anterior mesendoderm. Thus, although the constraints and dynamics of avian development make comparisons with the amphibian situation a complex undertaking, Hensen's node comes as close as possible to an organizer in Spemann and H. Mangold's definition.**

KEY WORDS: *Chick, node, streak, vegetal, induction.*

The discovery of the avian organizer

A wealth of experimental studies on the early embryology of the chick were performed after the groundbreaking work of K.E. von Baer (von Baer, 1828). The discovery of an organization center for the amphibian gastrula by Spemann and Mangold in 1924 (Spemann and Mangold, 1924) initiated a search for homologous structures or processes in other vertebrates, including birds. However, in 1924 not only work in amphibia, but also in the chick still suffered from the lack of culturing methods. These were introduced in the early thirties by J. Holtfreter, who developed a simple salt solution suitable for culturing amphibian embryos (Holtfreter, 1931), and by C.H. Waddington, who succeeded in cultivating chick embryos on plasma clots containing embryonic extract (Waddington, 1932). When Waddington transplanted a node or a piece of the anterior streak below the peripheral ectoderm of a cultivated embryo, he observed the induction of an ectopic neural plate, or the formation of a partial embryonic axis containing neural tube, notochord and somites (Waddington, 1932; Waddington and Schmidt, 1933; Waddington, 1934). It became clear that the avian primitive streak is homologous to the blastopore, and its tip, "Hensen's node", to the dorsal blastopore lip of an amphibian embryo. The three features of an organizer described by Spemann and Mangold, namely self differentiation towards axial mesoderm, reprogramming ("assimilation") of surrounding tissue and neuralization of ectoderm also applied to Hensen's node, which was therefore considered as the functional homolog of the amphibian organization center. Thus, the principal conservation of a major biological phenomenon was strongly suggestive.

Since these early experiments, the chick as an experimental system has come a long way. Methods were improved, detailed fate maps were established (Spratt, 1952; Schoenwolf and Sheard, 1990; Selleck and Stern, 1991; Psychoyos and Stern, 1996; and further references therein), and a precise staging system was introduced (Hamburger and Hamilton, 1951; Eyal-Giladi and Kochav, 1976). Normal development was manipulated *in ovo*, *in vitro* or in explants. Findings obtained in other experimental systems were systematically transferred and reinvestigated. The advent of molecular techniques renewed the interest in early vertebrate development and thus in a molecular interpretation of gastrulation and neurulation. Today, avian versions of most developmental control genes have been cloned and characterized, and are instrumental in pushing our knowledge about the gastrula to a new level.

In this review we summarize our current understanding of the avian organizer. Many aspects of our discussion would normally require also comparative descriptions of the situation in amphibia. We kept those as concise as possible and refer to published reviews instead. We follow the chick embryo through its early developmental steps, first describing the developmental processes leading towards the establishment of an organizer, and then the phenomenon itself. We will review the relevance of the posterior marginal zone, of

Koller's sickle, of the primitive streak and of Hensen's node, and will discuss molecules involved in the avian organization center.

Intrauterine establishment of symmetry axes in birds

The avian oocyte is extremely yolk rich. Thus, after fertilization the first cleavage furrows cannot separate the blastomeres completely, and the embryo develops as a disc of cells on top of the yolk (Eyal-Giladi and Kochav, 1976). In early stages of development, all blastomeres are open to the yolk, and only later the central cells become separated by tangential membranes. The animal pole of the intrauterine avian embryo lies in the center of the blastoderm, and the vegetal pole on the opposite side of the yolk sphere. Noteworthy, animal-vegetal polarity also exists along the meridians, so that the periphery of the embryo, where cells remain open to the yolk for the longest time, can be considered to be its most vegetal area (Fig. 1). Later, the periphery develops into extra-embryonic tissues.

The egg with the multi-cellular embryo rotates in the uterus around its longitudinal axis. Thereby, under the influence of gravity the blastoderm comes into an oblique position relative to the comparatively inert yolk (Fig. 1). The significance of this rotation for the formation of the embryonic axis was first observed by K.E. von Baer, who found that in most cases a chick embryo lies transversely to the long egg axis, with the head facing forward, if the pointed end of the egg faces leftward (von Baer, 1828). His rule was later confirmed under experimental conditions indicating that the upper part of a tilted
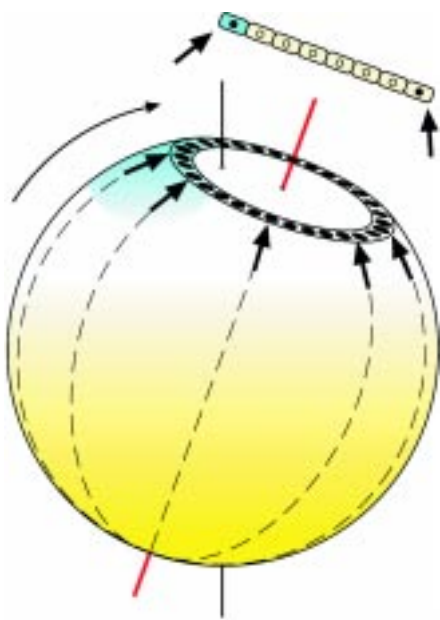

Fig. 1. Two influences on the axial specification of the early chick embryo. *1. Arrows indicate the vegetal-animal polarity along the meridians (dashed lines) of the yolk sphere. Black dots indicate the peripheral zone of the blastoderm where, by the time of egg laying, β-catenin is localized in the nucleus. Note the radial symmetry of these parameters. 2. The curved arrow indicates the direction of the intrauterin egg rotation. Note the tilting of the animal vegetal axis (red line). The oblique position of the embryo is a result of the rotation, during which the heavy yolk (yellow) tends to return to the vertical (black line). The blue color indicates that a different type of egg cytoplasm has access only to the prospective posterior pole of the blastoderm. Thus, bilateral symmetry is introduced under the influence of gravity.*

blastoderm becomes the posterior pole of the embryo (Kochav and Eyal-Giladi, 1971; Eyal-Giladi and Fabian, 1980).

In conclusion, two patterning aspects can be recognized in the intrauterine chick embryo. Firstly, the separation of a central and peripheral region of the embryo reflecting the animal-vegetal axis of the egg, explicitly an axis of radial symmetry. Secondly, the rotation of the egg providing an external cue introducing polarity to the disc, i.e. an axis of bilateral symmetry.

Two pathways necessary for the introduction of bilateral symmetry

Determinants from vegetal cells are important for the axial specification of echinoderms, fishes, amphibia and probably many more animals (Eyal-Giladi, 1997). On a molecular level, the specification of the early axes is best understood in the frog *Xenopus* laevis (Fig. 2; Harland and Gerhart, 1997; Heasman, 1997). The translocation of a vegetally derived signal to the dorsal hemisphere is critical for the introduction of bilateral symmetry. A key role is played by the Wnt/β-catenin pathway, which starts with a secreted Wnt-factor (or an equivalent signal), involves a downstream signal of the dishevelled gene, and culminates in the nuclear localization of the cell membrane protein β-catenin in the dorsal hemisphere. The nuclear localization of β-catenin can be achieved experimentally by exposure to lithium ions, which specifically inhibits the enzyme GSK3, a negative effector of the Wnt pathway and leads to hyperdorsalization of the embryo. A second important reaction system consists of the T-box factor VegT, the homeodomain proteins MIX.1 and/or Mixer, and the TGF-β related factor Vg1, all of which are finally involved in the specification of a vegetal identity in *Xenopus* laevis (Fig. 2; Lemaire *et al.*, 1998; Zhang *et al.*, 1998; Zorn *et al.*, 1999). The activities of this VegT/Vg1/MIX system overlap with the Wnt/β–catenin pathway in the dorso-vegetal blastomeres of the frog blastula. These cells possess a unique inductive potential, namely to induce an organizer without taking part in the induced structure themselves. The cell biological and molecular significance of this so-called "Nieuwkoop center" in amphibia is discussed in detail elsewhere (Harland and Gerhart, 1997; Heasman, 1997).

In chick embryos, there is no indication for a nuclear localization of β–catenin during intrauterine development. However, by the time of egg laying, nuclear β–catenin was found in the periphery of the blastoderm (Fig. 2; Roeser *et al.*, 1999). The nuclear localization could be induced by exposure to lithium ions, indicating a similarity to the amphibian Wnt pathway (Fig. 3). Before streak formation many putative developmental control genes are transcribed in a polarized fashion restricted to the prospective posterior part, most probably as a consequence of the tilting process described above (Fig. 1, Lemaire and Kessel, 1997). Among those are the *VegT* homologous gene *Tbx-6L* (Knezevic *et al.*, 1997), the homeobox gene *CMIX* (Peale *et al.*, 1998; Stein *et al.*, 1998) and the chicken *cVg1* gene (Seleiro *et al.*, 1996; Shah *et al.*, 1997; Fig. 2). Although not yet shown for the avian embryo, it can be suspected based on work in *Xenopus*, that these genes are involved in a common regulatory system.

In summary, there is evidence for an active Wnt/β–catenin pathway within the vegetal region of the chick embryo, which is radially distributed around the epiblast. In contrast, the Tbx6L/cVg1/CMIX system is restricted to the posterior vegetal region of the embryo. The activities of the two pathways overlap spatially in the posterior marginal zone (PMZ), which thus becomes a focal structure of early avian development.
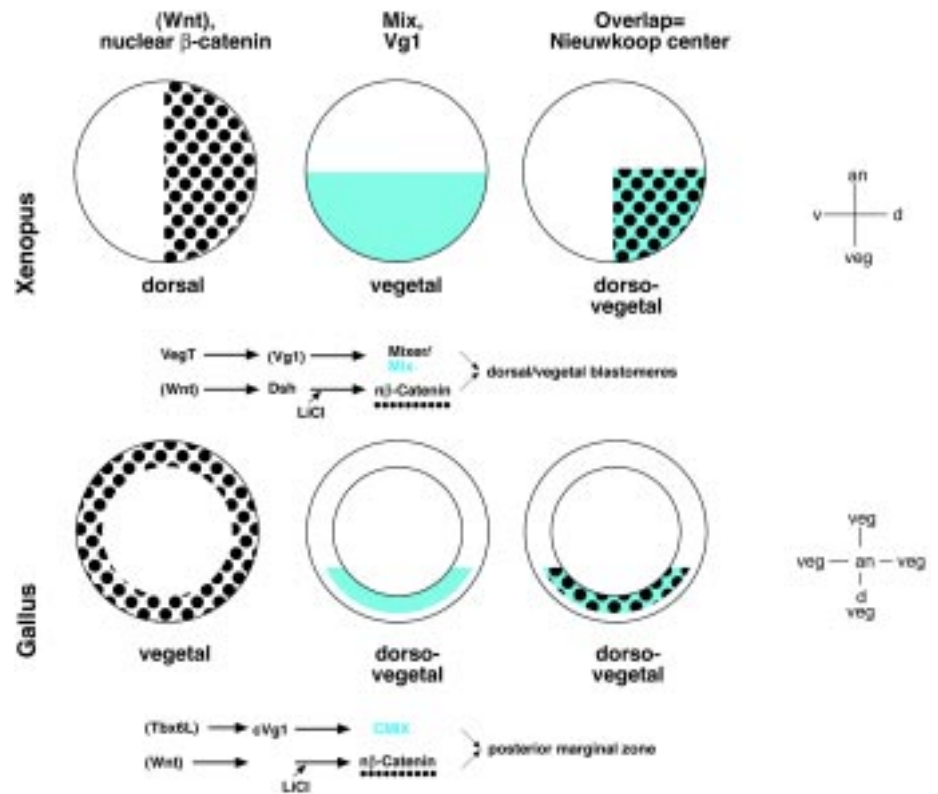
Fig. 2. The overlap of two pathways towards the organizer. *Xenopus laevis*. *The frog blastula is schematically depicted in a side view, with dorsal to the right and the animal pole to the top. Nuclear localization of β-catenin (black dots) occurs in the dorsal hemisphere, and transcripts for Mix.1, VegT and Vg1 are found in the vegetal hemisphere. Cells from the zone of overlap, the dorso-vegetal quadrant, represent the Nieuwkoop center.* **Gallus gallus**. *The avian blastoderm is depicted in a top view. Nuclear localization of β-catenin occurs in the periphery, and transcripts for CMIX, Tbx6L and cVg1 are found in the prospective posterior area of the blastoderm, where also putative dorsal genes begin to be expressed. The zone of overlap, the posterior marginal zone, is functionally equivalent to a Nieuwkoop center.*

The posterior marginal zone - an avian Nieuwkoop center?

At the time of egg laying, the avian blastoderm consists of a translucent area pellucida, surrounded by the area opaca. Between these two zones lies, morphologically more or less obvious, the marginal zone (Fig. 4). The posterior marginal zone is demarcated anteriorly by Koller's sickle (see discussion below), and posteriorly by the inner edge of the area opaca. Thus, an axis of bilateral symmetry, which will later be the antero-posterior axis, can already be recognized in these early embryos.

A transplanted PMZ is capable of inducing an ectopic primitive streak in epiblast tissue from an early pre-streak embryo lacking a PMZ of its own (Khaner and Eyal-Giladi, 1986; Khaner and Eyal-Giladi, 1989; Khaner, 1998). It could be demonstrated by cell labeling that PMZ cells do not take part in the formation of the axis they induce (Bachvarova *et al.*, 1998). An important secreted protein in the PMZ is the TGFβ related factor cVg1 (Seleiro *et al.*, 1996; Shah *et al.*, 1997). cVg1 secreting cells are able to promote the formation of a streak expressing organizer markers when transplanted to the epiblast of the marginal zone, but not of the central disc. The restriction to the marginal zone possibly correlates with the presence of nuclear β–catenin, and thus the activity of a Wnt pathway (Roeser *et al.*, 1999). However, the activity of a Wnt factor is difficult to prove, since these proteins cannot be produced easily in soluble form. Some evidence for the role of a Wnt factor in avian axis induction, organizer induction and cooperation with cVg1 could be obtained applying cells producing the Wnt1 protein (Joubin and Stern, 1999).

In order to understand the organizer inducing function of a Nieuwkoop center in zebrafish and *Xenopus*, it was of great help to identify the homeobox genes *dharma/nieuwkoid* (Koos and Ho, 1998; Yamanaka *et al.*, 1998) and *siamois* (Lemaire *et al.*, 1995),

respectively, which are direct targets of the Wnt/β–catenin pathway (Carnac *et al.*, 1996; Brannon *et al.*, 1997).The transcription of *siamois* and *dharma/nieuwkoid* is directly under the control of a transcription factor complex including β–catenin, and expressing cells induce non-cell-autonomously the expression of the organizer marker goosecoid. Corresponding avian homeobox genes for *siamois* as well as for *dharma/nieuwkoid* were so far not identified. At the moment, the only known PMZ specific homeobox gene is *CMIX*. Its polarized expression in the chick may indicate a different role from its frog homolog (*Mix.1*), which is not enough for axis formation on its own.

In conclusion, it appears that the avian PMZ fulfills major postulates for a functional Nieuwkoop center, namely the potential for organizer induction without itself contributing to the new structure and the involvement of genes homologous to the amphibian center.

Koller's sickle and the primitive streak primordium: the induction of gastrulation

Koller's sickle is the morphological landmark of the chick embryo before the onset of gastrulation (Fig. 4). While the epiblast is only one cell thick, a multi-cellular layer of yolk rich cells extends from the germ wall underlying the area opaca, to the marginal zone, and to the sickle, where they are particularly densely packed (Bachvarova *et al.*, 1998). Cells from the epiblast above and from middle layer of the sickle are fated to the primitive streak, the node, and later to the prechordal mesendoderm (Izpisúa-Belmonte *et al.*, 1993; Bachvarova *et al.*, 1998). The mesendoderm is also the fate of the bottle cells in the dorsal blastopore lip of the frog. Not only the fate, but also gene expression patterns indicate a role of the sickle as a part of the avian organizer. Thus, cells near the sickle and/or the PMZ express a whole
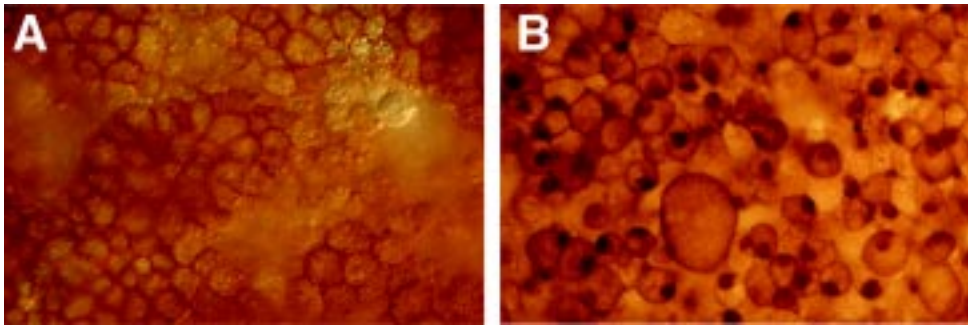
Fig. 3. The nuclear localization of β**-catenin in avian embryos.** *Detection of β-catenin by whole mount immunochemistry in the central epiblast of pre-streak chick embryos exposed to 100 mM NaCl* **(A)** *or LiCl* **(B)**. *Note the signal in the cytoplasma membrane (A) or in the nucleus (B). Data from (Roeser et al., 1999) with permission.*

battery of genes related to genes associated with the amphibian organizer, such as *Goosecoid* (*GSC*), *GSX*, *CNOT1*, *CNOT2*, *Hnf3β*, *OTX2* and *Chordin* (Bally-Cuif *et al.*, 1995; Ruiz i Altaba *et al.*, 1995; Stein and Kessel, 1995; Stein *et al.*, 1996; Lemaire *et al.*, 1997; Streit *et al.*, 1998). All these expression domains are initially sickle shaped, i.e. spread more or less broadly and transversely to the future longitudinal axis.

During the first hours of egg incubation these expression domains begin to converge and become longitudinally oriented, now representing the primordium of the primitive streak. It was recently suggested, that this must not necessarily occur due to posterolateral cell migrations (Wei and Mikawa, 2000). The formation of the early primitive streak could in essence be the result of polarized cell divisions from a spatially restricted area of the blastoderm adjacent to the PMZ. It is remarkable that the genes associated with the putative Nieuwkoop center, the PMZ, as well as with the organizer are represented in the early phase of the primitive streak. This could indicate a prolonged phase of organizer induction, still active while the first meso- and endodermal cells are already migrating.

Transplantation of sickle fragments to competent ectoderm induces the formation of a primitive streak, identifiable with the pan-mesodermal marker Brachyury (Ch-T), complete with a node, as identified by the expression of *GSC* or *Chordin* (Izpisúa-Belmonte *et al.*, 1993; Callebaut and Van Nueten, 1994; Bachvarova *et al.*, 1998). As expected from the behaviour of sickle cells in their endogenous situation, they also contribute to the induced streak or node in an ectopic position (Bachvarova *et al.*, 1998). In this respect, sickle mediated inductions differ significantly from the PMZ transplantations, where no contribution to the induced structures occurs. Remarkable is, however, that the sickle with its cells fated to the node and prechordal plate, is not sufficient to induce neural tissue directly.

Grafts of Koller's sickle contain cells producing Chordin, a factor expressed in epiblast cells anterior to the sickle and in the underlying cell layer (Streit *et al.*, 1998). Chordin is not capable of neural induction in avian ectoderm, but elicits the formation of a primitive streak expressing also organizer markers. (Streit *et al.*, 1998). This finding was at first sight unexpected after the identification of chordin as a bona fide neural inducer in *Xenopus* (Kessel and Pera, 1998). It appears that neural inducing factors are not yet expressed in or near Koller's sickle. Alternatively, they may already be present but are antagonized by other factors.

In summary, Koller's sickle contains precursor cells for the node and the prechordal plate, which also express classical organizer markers. Based on fate and gene expression the first aspects of an avian organizer can be recognized in Koller's sickle. However, the inductive capacity of the sickle and of the streak primordium is restricted to the induction of an organizer and a primitive streak, and does not include a capacity for neural induction. Thus, a major criterion for an organizer in the Spemann-Mangold sense, i.e. neural induction, is not fulfilled.

Hensen's node: the evolving capacity for neural induction

The primitive streak reaches its full length after the incubation of an egg for about nineteen hours. At the tip of the extended streak forms a thickened structure, where the three germ layers are tightly associated. Since its discovery by V. Hensen in a study on rabbit embryos (Hensen, 1876), the term "Hensen's node", abbreviated the node, is widely used. Some of the cells in the node are derived from Koller`s sickle or from the primitive streak primordium (Izpisúa-Belmonte *et al.*, 1993). However, many of these cells have left the

Fig. 4. Structural landmarks of early chick embryos. *Depicted is a pre-streak embryo (stage X; Eyal-Giladi and Kochav, 1976), an early streak embryo (stage HH2/HH3; Hamburger and Hamilton, 1951) and an extended streak embryo (HH3+/HH4). Indicated are the area opaca (ao), the area pellucida (ap), the marginal zone (mz), Koller's sickle (Ks), the primitive streak (ps) and the node (n). The blue color indicates the expression of molecular*

markers for the Nieuwkoop center in the posterior marginal zone, most the early streak and the posterior part of the extended streak. The red color indicates the expression of organizer markers in the sickle, the early streak, and the node.
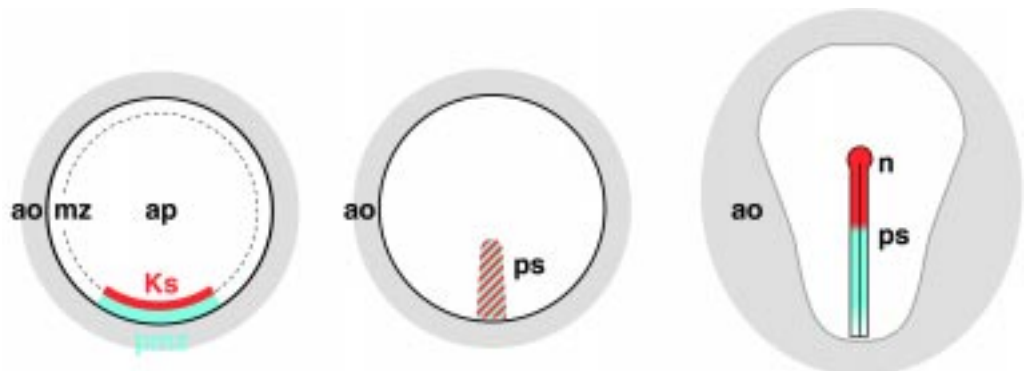
streak already by the time of node formation (Joubin and Stern, 1999). They are replaced by cells from the surrounding epiblast, which migrate towards the node and leave it readily, indicating a dynamical change of the cellular composition in the node. The cells derived from the tip of the streak are initially fated to the definitive endoderm, which spreads concentrically under the epiblast. The formation of Hensen's node in the second phase of primitive streak elongation is accompanied by molecular and functional changes. The expression of genes such as *cVg1, cWnt8c, CMIX*, or *GSX*, which characterized the complete streak in its early phase, now become restricted to its posterior portion (Hume and Dodd, 1993; Seleiro *et al.*, 1996; Lemaire *et al.*, 1997; Shah *et al.*, 1997; Stein *et al.*, 1998). Other early markers, such as Chordin, GSC, HNF3β, now become restricted to the anterior streak and finally to the node (Izpisúa-Belmonte *et al.*, 1993; Ruiz i Altaba *et al.*, 1995; Streit *et al.*, 1998).

Grafting of Hensen's node below competent ectoderm induces a neural plate, elevated neural folds, or even a closed neural tube (for an example see Fig. 5). This remarkable inductive potential of the node was first demonstrated by Waddington, and served to recognize the node as the equivalent of the dorsal blastopore lip in amphibia, the Spemann-Mangold organizer (Waddington, 1932). It has by now been investigated to a great detail both on a functional and a molecular level (e.g. Dias and Schoenwolf, 1990; Storey *et al.*, 1992; Lemaire *et al.*, 1997; Pera *et al.*, 1999). Molecular markers demonstrate readily that node-induced neural axes are regionally structured into fore-, mid-, hindbrain and spinal cord quality (Fig. 5). It is typical for such inductions that the basal membrane at the grafting site does not become interrupted, and therefore no mesoderm is formed. The potential for the induction of anterior neuroectoderm is restricted to the node at the extended streak stages, i.e. when it contains cells fated to the definitive endoderm or the mesendoderm. An efficient contribution of a node graft to the host endoderm normally is accompanied by efficient neural induction (Dias and Schoenwolf, 1990). The development of an endodermal layer appears to be essential for the formation of a neural plate (Pera *et al.*, 1999). The inductive potentials of different primitive streak segments are significantly different (Fig. 6). After transplantation to the anterior extraembryonic region (Fig. 5A), post-nodal grafts still induce neuroectoderm, but fail to induce anterior markers (Knoetgen *et al.*, unpublished). The potential of even further posterior streak grafts is restricted to the induction of secondary primitive streaks. These are more likely to express also anterior streak markers, i.e. the classical organizer markers (Chordin, GSC, HNF3β; Lemaire *et al.*, 1997), if they contain cells from the anterior middle portion of the streak (Fig. 6). In contrast, secondary streaks induced by fragments from the middle of the streak do not express these genes and are restricted to more general streak markers, in particular Brachyury (Ch-T; Gallera and Nicolet, 1969; Lemaire *et al.*, 1997).

Today, we know a number of secreted proteins, including chordin, noggin, and follistatin, which can induce neural ectoderm in amphibia (for review see Hemmati-Brivanlou and Melton, 1997). These products of the dorsal blastopore lip appear to function by binding BMP proteins and thus creating a zone free of unbound BMP4, the neural plate. In the chick, BMP4 is not expressed in the neural plate surrounding Hensen's node, whereas the expression of avian homologs of chordin and noggin was identified in the node (Connolly *et al.*, 1997, Streit, 1998 #3524; Schultheiss *et al.*, 1997). Thus, it is not evident how BMP antagonism could be the primary mechanism to induce neural ectoderm in the chick. Neither is BMP4 capable of suppressing neuralization completely, nor can neuralization be in-
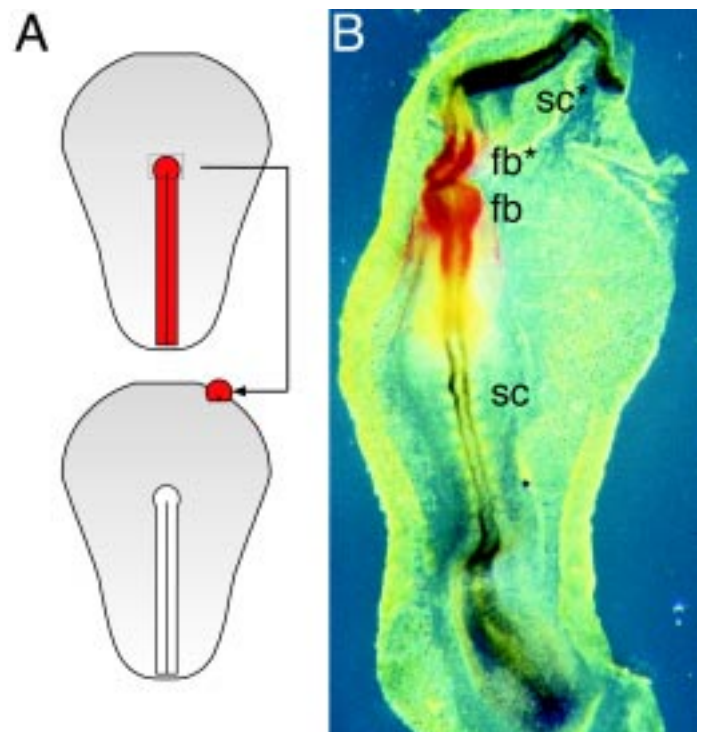

Fig. 5. A classical organizer transplantation experiment in the chick embryo. (A) *The transplantation of Hensen's node from a donor to the periphery of a cultivated chick embryo is depicted schematically.* **(B)** *The specimen is shown after an incubation for 18 hours, and subsequent whole mount in situ analysis with an OTX2 (red) and a HOXB1 (blue) probe. The primary embryo has developed to the 9-somite-stage. Its fore- and midbrain are characterized by OTX2, and the posterior embryo by HOXB1 expression. The secondary embryo developed head to head, with a prominent central nervous system, where the markers identify the antero-posterior patterning. fb, forebrain; sc, spinal cord.*

duced de novo by the application of chordin or noggin. BMP4 or the BMP-antagonist noggin merely affect the extent of the neural plate at its periphery (Knoetgen *et al.*, 1999b; Pera *et al.*, 1999).

Apart from its neural inducing capacity, the node possesses also the other features of an organizer in Spemann's definition, assimilation and self-differentiation. When a chick node is placed in an environment of lateral, i.e. ventral, mesoderm the induced embryonic axis will also contain somites, which result from a reprogramming or dorsalization of the surrounding mesoderm. A transplanted node will mainly differentiate into a chordoid or notochordal structure underlying the induced neural epithelium except for its anterior, forebrain-like region. It is remarkable that node transplants will not induce an organizer in return. On the one hand, this appears to be a consequence of the absence of organizer inducing factors (cVg1, possibly cWnt8C) from the tip of the elongated streak. In addition, an anti-organizer factor, the anti-dorsalizing morphogenetic protein ADMP, is expressed in the node and is responsible for restricting organizer activity to the node (Joubin and Stern, 1999).

In conclusion, pure neural induction is restricted to the tip and pure mesoderm induction to the middle of the elongated streak. In-between, there is an anteroposterior decrease of neural inducing, and an increase of a mesoderm inducing potential. In addition, more anterior grafts induce more anterior markers in the ectoderm or the mesoderm.
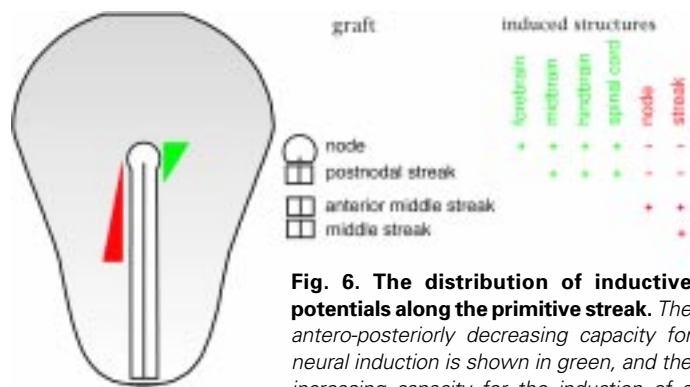
Fig. 6. The distribution of inductive potentials along the primitive streak. *The antero-posteriorly decreasing capacity for neural induction is shown in green, and the increasing capacity for the induction of a primitive streak in red. The summarized data were obtained by transplanting grafts to the periphery of cultured embryos as shown in Fig. 5A (Knoetgen, unpublished; Lemaire et al., 1997). Note that postnodal streak grafts do not induce forebrain. Note the absence of organizer induction by grafts from the middle of the streak, in contrast to grafts including more anterior cells of the streak. Note that node grafts do not induce organizer marker*s.

The prechordal mesendoderm: the separation of head and trunk organizer and the anteriorization of the neural plate

The cells leaving the node can be divided into three categories according to their fate (e.g. Selleck and Stern, 1991). The first to emigrate form the definitive endoderm. They are followed by a mesendodermal population of cells which migrates anteriorly to reach a position under the developing forebrain. These become on the one hand part of the definitive gut endoderm, but also constitute a mesodermal population, the prechordal mesoderm, between the anterior endo- and ectoderm (for discussion see Knoetgen *et al.*, 1999a). A third population generates the notochord which extends from the midbrain to the tail region.

The inductive potential of the node changes dramatically when the mesendodermal, GSC expressing cells have left the node. From now on anterior neural ectoderm can no longer be induced by the node, and the general, neural inducing potency decreases quickly (Dias and Schoenwolf, 1990; Storey *et al.*, 1992). However, the GSC expressing mesendodermal cells maintain the capacity to induce anterior neural ectoderm. After transplantation to the extraembryonic region they evoke the expression of forebrain markers such as the homeobox genes *GANF, CNOT1*, or *NKX2.1* (Pera and Kessel, 1997; Knoetgen *et al.*, 1999b). The change of inductive potential of the node closely mimics the features of the amphibian blastopore lip. Only the young lip can induce forebrain structures, and it loses this capacity fast. This was the key observation leading to the concept of separate head and trunk organizers (Spemann, 1931), and in this respect there appears to be no major difference between amphibia and birds. However, a major difference lies in the timing of neural induction in general and the anteriorization of the neuroectoderm. In the chick, the early neural plate forming around the node does not express anterior neural markers, such as GANF or NKX2.1 (Pera and Kessel, 1998; Knoetgen *et al.*, 1999b). In *Xenopus* laevis, the anterior markers are switched on as soon as evidence for neural induction is obtained (Harland and Gerhart, 1997). It appears that the chick neural plate is initially not regionalized and becomes so only after interacting with the prechordal mesendoderm.

In summary, we observe a separation of head and trunk organizers and a major role for the prechordal mesendoderm in birds reminiscent of the situation in amphibia. However, due to the prolonged phase of organizer generation, neural induction precedes anteriorization in birds.

The development of the avian organizer

Embryos from different vertebrate species are particularly different in the early stages of life. Despite this, the analysis of the organizer phenomenon has revealed numerous conserved aspects in fish, amphibia, birds and mammals. Most striking are the similarities in the molecular repertoire, and the majority of the key molecules was found in all model species. In the chick, gravity is the external cue creating a local asymmetry establishing the first patterning center of the chick embryo at the prospective posterior pole of the embryo. This center becomes reoriented along the future longitudinal axis with the formation of the primitive streak. The complex events leading to the establishment of the primitive streak require more than ten hours from the first initiation of organizer development to the final establishment of a bona fide organizer with a neural inducing potential. This prolonged induction phase indicates that developmental mechanisms related to the organizer must differ from those in amphibia. Indeed, several molecular concepts deduced from work on the frog could not directly be transferred to the chick. Thus, in spite of a fast growing knowledge, several questions concerning the avian organizer still remain unresolved. In the future two major induction processes, namely mesoderm and neural induction, still await an understanding on a molecular level.

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