Nuclear β-catenin and the development of bilateral symmetry in normal and LiCl-exposed chick embryos

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INTRODUCTION

The development of bilateral symmetry is highly divergent even among vertebrates (Eyal-Giladi, 1997), although the molecules involved in this process seem to be remarkably conserved. The mechanisms observed in various species are strongly correlated with size and yolk content of the ovum, and thus the formation of the first cleavages. Axis formation in a relatively yolk-poor embryo has been extensively characterized both on a morphological and a molecular level in the amphibian *Xenopus laevis* (Harland and Gerhart, 1997). The extremely yolk-rich avian embryos may serve as a model system to study the adaptation to different developmental constraints (Khaner, 1993).

The chick embryo develops as a disk on top of an extremely large yolk sphere (Eyal-Giladi and Kochav, 1976). The first cleavages occur superficially and leave the blastomeres open to the yolk. Still in the uterus, the central area of the embryo becomes separated from the yolk by horizontal cell membranes, so that two clearly distinguished regions appear, the translucent area pellucida, and the surrounding area opaca.

By the time of egg-laying the cell number has increased to about 60 000. The blastomeres are mostly forming the upper epithelial sheet of the epiblast, while only a few are in the lower layer, the primary hypoblast, which consists of dispersed cell aggregates. Incubation of the egg leads to the development of Koller’s sickle, and the generation of the secondary hypoblast, a continuous layer spreading below the epiblast anterior of the sickle and the posterior germ wall (Stern, 1990). Recent experimental analyses of hypoblast formation (Callebaut et al., 1996; Bachvarova et al., 1998) do not support a mechanism involving a change of layers from the epiblast of the posterior marginal zone (PMZ) to the lower layer (Eyal-Giladi et al., 1992). As soon as the secondary hypoblast is completed, the primitive streak, the chick blastopore, starts to extend from the posterior end of the embryo (Hamburger and Hamilton, 1951). The tip of the elongated streak represents the chick organizer, whereas the middle and posterior streak give rise to more ventral tissue (Kintner and Dodd, 1991; Psychoyos and Stern, 1996; Lemaire et al., 1997).

Although the bilateral symmetry of the avian embryo becomes morphologically obvious for the first time with the

Studies in *Xenopus laevis* and zebrafish suggest a key role for β-catenin in the specification of the axis of bilateral symmetry. In these organisms, nuclear β-catenin demarcates the dorsalizing centers. We have asked whether β-catenin plays a comparable role in the chick embryo and how it is adapted to the particular developmental constraints of chick development. The first nuclear localization of β-catenin is observed in late intrauterine stages of development in the periphery of the blastoderm, the developing area opaca and marginal zone. Obviously, this early, radially symmetric domain does not predict the future organizing center of the embryo. During further development, cells containing nuclear β-catenin spread under the epiblast and form the secondary hypoblast. The onset of hypoblast formation thus demarcates the first bilateral symmetry in nuclear β-catenin distribution. Lithium chloride exposure also causes ectopic nuclear localization of β-catenin in cells of the epiblast in the area pellucida. Embryos treated before primitive streak formation become completely radialized, as shown by the expression of molecular markers, CMIX and GSC. Lithium treatments performed during early or medium streak stages cause excessive development of the anterior primitive streak, node and notochord, and lead to a degeneration of prospective ventral and posterior structures, as shown by the expression of the molecular markers GSC, CNOT1, BMP2 and Ch-Thx6L. In summary, we found that in spite of remarkable spatiotemporal differences, β-catenin acts in the chick in a manner similar to that in fish and amphibia.

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Key words: Axis formation, Chick embryo, Gastrulation, β-catenin, LiCl, Dorsalization, Bilateral symmetry
The mechanism of axis formation has been studied most successfully in the African clawed frog, *Xenopus laevis*. Here, the axis of bilateral symmetry is determined in the unicellular embryo by the point of sperm entry which induces a rotation of the egg cortex. A network of microtubules transports ‘vegetal determinants’ to the future dorsal side of the embryo (Larabell et al., 1996; Sakai, 1996; Kageura, 1997; Rowning et al., 1997). The molecular nature of these determinants is still unknown. However, it is clear that in the following hours β-catenin accumulates in the cell nuclei on the dorsal side of the embryo, preceding the expression of the dorsal-specific genes (Schneider et al., 1996; Larabell et al., 1997). The nuclear localization of β-catenin appears to be a key event in the specification of the dorsal identity, and thus of the bilateral, prospective dorsoventral axis in amphibia (Heasman et al., 1994; reviewed by Heasman, 1997). Also in the zebrafish embryo, nuclear localization of β-catenin demarcates the prospective dorsal side of the embryo (Schneider et al., 1996).

Besides its activity as a cell adhesion molecule, β-catenin has been shown to be an important component of the Wnt pathway, together with other regulatory factors such as glycogen synthase kinase 3β (GSK-3β; He et al., 1995), GBP (Yost et al., 1998), the tumor suppressor protein adenomatous polyposis coli (APC; Munemitsu et al., 1995), axin (Zeng et al., 1997), and members of the LEF/Tcf family of proteins (Behrens et al., 1996; Huber et al., 1996; Molenaar et al., 1996; reviewed in Pennisi, 1998). The half-life of cytoplasmic β-catenin is directly regulated by phosphorylation through GSK-3β (Yost et al., 1996; Aberle et al., 1997). Unphosphorylated β-catenin is more stable and able to translocate together with LEF/Tcf into the cell nucleus (Fagotto et al., 1998), where the complex binds and activates transcription of its target genes, most remarkably the dorsal-specific homeobox gene *siamois* (Brannon et al., 1997; Fagotto et al., 1997).

Lithium chloride (LiCl), when supplied before the midblastula transition (MBT), has proved to be a convenient and potent experimental tool to induce dorsalization in amphibian and teleostean embryos (e.g. Kao et al., 1986; Stachel et al., 1993). The most prominent characteristics after pre-MBT LiCl exposure are a ubiquitous instead of polarized nuclear distribution of β-catenin (Schneider et al., 1996), a morphological radialization, a general dorsalization with hyper-development of organizer and notochord tissue (Cook and Smith, 1988; Kao and Elinson, 1988), and an unpolarized overexpression of dorsal markers (Cho et al., 1991). Mechanistically, this is most likely achieved by a direct inhibition of GSK-3β activity, and consequently an increase of nuclear β-catenin levels (Klein and Melton, 1996; Stambolic et al., 1996; Hedgepeth et al., 1997), although the inositol pathway can also be influenced by LiCl (Busa and Gimlich, 1989). In chick embryos, lithium treatments from late streak stage onwards caused a loss of anterior structures and cyclopia (Nicolet, 1961; Rogers, 1963; Nicolet, 1965) that are very similar to the malformations induced in amphibian and fish embryos when treated after MBT (Kao et al., 1986; Yamaguchi and Shinagawa, 1989; Stachel et al., 1993; Fredieu et al., 1997). However, the treatments were performed at low doses for relative long periods, resulting in weak and contradictory phenotypes (Nicolet, 1965).

We describe the subcellular distribution of β-catenin during development of the chick embryo. Nuclear localization was found during intrauterine and pre-streak stages in the periphery of the blastoderm, but not in the central epiblast. The distribution was radially symmetric until the beginning of secondary hypoblast formation. We show that lithium can cause ectopic nuclear localization of β-catenin, which is followed by radialization and dorsalization of the embryo. We discuss these results in the light of the different developmental constraints during formation of the bilateral axis in the chick as compared to the *Xenopus* embryo.

**MATERIALS AND METHODS**

**Staging of embryos**

Fertilized chick eggs (White Leghorn, obtained from Lohmann Tierzucht, Cuxhaven) were incubated at 38°C in a humidified incubator. Embryos were staged according to Eyal-Giladi and Kochav (roman numerals for pre-primitive streak stages; Eyal-Giladi and Kochav, 1976) and Hamburger and Hamilton (arabic numerals, after the appearance of the primitive streak; Hamburger and Hamilton, 1951). Intrauterine embryos were prepared from killed White Leghorn hens at Lohmann Tierzucht, Cuxhaven.

**Immunohistochemistry**

Embryos were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 20 to 40 minutes, transferred to Dent’s fixative (PBS containing 1% Tween-20, 1% DMSO) and incubated overnight. Storage for up to 10 days was done in methanol at −20°C. After rehydration in 75%, 50% and 25% methanol in PTB (1% Tween in PBS), the specimens were washed for 5 minutes in PBS and distilled H2O, preincubated for 30 minutes in NCS-PBDT (PBS containing 10% newborn calf serum, 0.05% Tween-20, 1% DMSO) and incubated overnight in NCS-PBDT containing 1/1000-1/2000 monoclonal antibody against chicken β-catenin (Mouse IgG1, clone 1B5B, Sigma C7207; Johnson et al., 1993). The embryos were washed twice in NCS-PBDT for 25 minutes, endogenous peroxidases inactivated for 40 minutes in NCS-PBD containing 6% H2O2, and washed in NCS-PBDT. Binding of the secondary antibody and diaminobenzidine (DAB) staining were done using the Vectastain Elite Kit (Vector), according to the manufacturer’s protocol.
Counterstaining with the DNA binding dye Hoechst 33342 (Molecular Probes) was done by incubation for 20 minutes in a 1 μg/ml solution in PBT.

Whole-mount in situ hybridization
Whole-mount in situ hybridization was essentially performed as described by Wilkinson (1992), except that no RNAse treatment was done. For paraffin sections (8 μm) embryos were dehydrated and embedded in Paraplast plus (Sherwood Medicals). Antisense riboprobes were synthesized from the following cDNAs: GSC (Izpisúa-Belmonte et al., 1993), CMIX (Stein et al., 1998), CNOT1 (Stein and Kessel, 1995), BMP2 (Francis et al., 1994), and Ch-Tbx6L (Knezevic et al., 1997).

Lithium treatments and New culture
Embryos together with large portions of the adjacent vitelline membranes, were removed from the eggs, transferred ventral side up to a watchmaker’s glass, and a glass ring was positioned around the blastoderm. Three types of lithium treatments were employed. Continuous treatment: embryos were incubated in cell culture medium containing 100 mM LiCl for 3 hours (Fig. 5). Strong treatment: embryos were incubated for 10-12 minutes in Tyrode’s solution containing 300 mM LiCl (Figs 6, 7). Weak treatment: embryos were incubated either for 5-6 minutes in 300 mM LiCl, or for 10-12 minutes in 100 mM (Fig. 8). After the treatments the embryos were washed at least three times with Tyrode’s medium and then transferred to a modified New culture (Stern, 1993). In short, the vitelline membrane with the embryo still attached to it was draped over the glass ring and placed in a 35 mm Petri dish on a fresh albumen substrate. All superficial liquid was carefully removed to allow incompletely attached embryos to adhere to the membrane again.

RESULTS

Perinuclear and nuclear localization of β-catenin in intrauterine chick embryos
We analyzed the subcellular distribution of β-catenin in intrauterine chick embryos using a monoclonal antibody directed against chicken β-catenin (Johnson et al., 1993). As expected for a ubiquitous cell membrane associated molecule, we generally observed strong staining of all cell membranes (Figs 1, 2, 3, 5).

At stage EK V, the chick embryo consists of about 1000 cells, a real blastoderm has not yet formed, and the axis of bilateral symmetry has not yet been specified. Such embryos did not show nuclear localization of β-catenin. However, we observed a remarkably strong, cytoplasmatic immunoreactivity in the perinuclear region (Fig. 1A). This pool of β-catenin was maintained during the different phases of mitosis, which were visualized by staining the DNA with Hoechst 33342 dye (Fig. 1B). Interphase nuclei were surrounded by a cytoplasmatic β-catenin accumulation in the perinuclear region (Fig. 1E,F). In

![Fig. 1. Perinuclear and nuclear localization of β-catenin in intrauterine chick embryos.](image-url)
prophase, β-catenin became localized to the poles of the nucleus (Fig. 1G,H), and in the metaphase, it co-localized with the mitotic spindle (Fig. 1I,J). Concomitant with the separation of the chromosomes during anaphase, it became distributed to the daughter cells in telophase (Fig. 1K,L).

After further intrauterine development, an area pellucida is recognizable, the medial part of the embryo is sealed by transverse cell membranes from the yolk, and only the peripheral cells are still in open contact to the yolk (EK VIII). It has been determined experimentally that in this phase axis specification commences (Kochav and Eyal-Giladi, 1971). We also observed a first indication for nuclear localization of β-catenin, beginning in cells at the margin of the blastoderm. It could be detected by the quenching of the Hoechst 33342 dye, and not clearly by direct observation (Fig. 1C, D). The validity of this indirect procedure is demonstrated in older embryos, where both quenching and direct detection indicates the presence of β-catenin (Fig. 3D; see also Schulte-Merker et al., 1992). We did not detect a significant asymmetry in the distribution of nuclear signals, either in the upper, or in the lower cell layers of intrauterine embryos.

**Domains of nuclear localization of β-catenin before the onset of gastrulation**

A massive nuclear localization of β-catenin could be detected in post-laying, unincubated blastoderms (EK X; Fig. 2). The distribution was radially symmetric and encompassed the entire area opaca and the marginal zone (Fig. 2B). In this belt-shaped area there was no sector with significantly stronger or weaker immunoreactivity. However, we observed in several cases a decreasing gradient of nuclear staining from the outer to the inner rim. Nuclear localization was detectable in cells of the upper, epiblast layer, and also in the lower layer underlying the marginal zone and the area opaca, consisting of cells from the germ wall. The central part of the area pellucida was devoid of nuclear staining (Fig. 2C). A few cells of the primary hypoblast showed weak immunoreactivity for β-catenin in their nuclei. We did not find any evidence for polarization in the lower layer.

At stage EK XI, Koller’s sickle becomes recognizable at the border between the posterior marginal zone and the central part of the area pellucida. From here, cells spread anteriorly and give rise to the secondary hypoblast (Stern, 1990; Eyal-Giladi et al., 1992). We found that the position of Koller’s sickle in relation to the domain of nuclearly localized β-catenin was remarkable. It was located at the border between the outer ring of the blastoderm, the cells of which contains β-catenin in their nuclei, and the central disk that is devoid of nuclear localization. All cells of the lower layer contained β-catenin in their nuclei, so that at stage EK XII and XIII the entire hypoblast was labelled, while the overlying epiblast remained free of nuclear localization. (Fig. 3A-C). When immunolabelled and Hoechst 33342-stained embryos were viewed from the lower layer, the visible parts of the epiblast overlying the hypoblast revealed Hoechst staining, whereas the described quenching effect inhibited fluorescence of the β-catenin-positive cells of the hypoblast itself, Koller’s sickle and the posterior marginal zone (Fig. 3D,E). The area opaca and the marginal zone at stage EK XIII still showed nuclear immunoreactivity, but in some embryos a progressive fading had occurred from the anterior marginal zone by stage EK XIII. In summary, we observed the first bilateral symmetry in nuclear β-catenin distribution at the onset of hypoblast formation (Fig. 4).

**LiCl treatment causes ectopic nuclear localization of β-catenin**

Treatments with LiCl are well established to manipulate the axis formation in frog and fish embryos, where they cause hyper-dorsal development and radialization (Kao et al., 1986; Cooke and Smith, 1988; Stachel et al., 1993). We examined the influence of LiCl on the subcellular distribution of β-catenin and on the axial specification in the chick embryo. Embryos from freshly laid eggs (EK X) were exposed in cell culture medium to 100 mM LiCl. After 3 hours incubation we observed massive nuclear localization of β-catenin in the entire blastoderm, including the central part of the area pellucida, which is normally free of nuclear localization (Fig. 5C,D). Control embryos incubated in 100 mM NaCl showed the normal pattern of stage EK X embryos (Fig. 5A,B). We also noticed that LiCl had an effect on the epithelial structure of the epiblast. In the lithium-treated embryos the cells rounded up and seemed to loosen the contact to their neighbour cells (Fig. 5C). These results demonstrate that LiCl is able to cause ectopic nuclear localization of β-catenin in chick embryos.
LiCl treatment at the pre-streak stage causes a radialization of the embryo

To study whether LiCl could influence the axis formation in chick embryos, we treated embryos from unincubated eggs (EK X) with 0.3 M LiCl for 10-12 minutes (strong treatment), washed at least three times, and incubated further in New culture. The strength of this treatment corresponds approximately to the treatments commonly used in frog and fish embryos (Kao et al., 1986; Yamaguchi and Shinagawa, 1989; Stachel et al., 1993).

After about 3-4 hours, an accumulation of hypoblast-like tissue was observed below the epiblast (n=23; Fig. 6E,F,M,N). Subsequently, the hypoblast contracted and coiled up in the middle of the embryo (Fig. 6G). Further incubation led, after about 12 hours, to a thickening and invaginations in the epiblast overlying the hypoblast (n=19; Fig. 6H,O). The resulting embryos appeared radially symmetric and did not develop any further (Fig. 6D,K,L). To analyze this process in more detail, we made use of the homeobox gene CMIX as a marker for the early bilateral symmetry in the chick embryo (Stein et al., 1998). In the early chick embryo, CMIX demarcates the region of the posterior marginal zone and Koller’s sickle (Fig. 6A; Stein et al., 1998). Already 3-4 hours after lithium treatment we observed an expansion of the sector in which CMIX is expressed (Fig. 6B). Further incubation led to expression also in the central region of the epiblast, until finally the entire central thickened epiblast became CMIX-positive (Fig. 6C,D). Thus, the radialized morphology of the Li-treated embryos is reflected in a radialized expression pattern of CMIX. During normal development the homeobox gene goosecoid (GSC) is first expressed in the posterior part of the secondary hypoblast, whereas the epiblast remains mostly free of GSC expression (Fig. 6J,M; Hume and Dodd, 1993; Bally-Cuif et al., 1995). Expression in the epiblast commences only at the beginning of primitive streak formation (Izpisúa-Belmonte et al., 1993). In LiCl-treated embryos, the entire thickened hypoblast became GSC-positive, whereas the
epiblast remained \textit{GSC}-negative, indicating that no primitive streak formation had occurred (Fig. 6K,L,N,O). In summary, lithium treatment of pre-streak embryos caused a radialization, recognizable both at a morphological and a molecular level.

**Lithium treatment of early streak stages causes hyper-development of dorsal/anterior and a loss of ventral/posterior structures**

To analyze the duration of LiCl sensitivity we treated embryos up to the stage of head process formation (HH5). Embryos treated just at the beginning of primitive streak formation showed very similar phenotypes to the pre-streak blastoderms described above \((n=6)\). The only difference was that they were not completely radialized, but exhibited a small streak that extended into the thickened epiblast anteriorly. Embryos treated during the medium streak stage (HH3) developed a thickening of the entire primitive streak after about 3-4 hours. However, those streaks soon thinned out in the posterior part (after 6-7 hours), and then degenerated further. In contrast, the tip of the streak increased significantly in size and developed...
into a massively thickened, node-like structure. The unnatural configuration of an extremely thick anterior, and an extremely thin posterior streak led in many cultures to a tearing, and thus destruction of the embryo.

Lithium treatment of frog and fish embryos before midblastula transition (MBT) leads to an expansion of the goosecoid-positive domain into the ventral region of the blastopore (Cho et al., 1991; Stachel et al., 1993). We never observed a major extension of the GSC-positive domain into the posterior part of the primitive streak ($n=20$). Instead, we found that the posterior part of the expression domain narrowed concomitantly with a drastic degeneration of the posterior primitive streak (Fig. 7D,G). The thickened anterior streak and surrounding mesodermal cells stained strongly positive with a GSC-probe (Fig. 7A-C). During further development the tip of the streak invaginated to form a pouch ($n=11$), at the tip of which GSC-positive cells left the epithelium (Fig. 7E,F).

We applied a BMP2 probe to similar embryos, which apparently could not reach the headfold stage ($n=11$). In normal HH5 embryos BMP2 expression domains are the posterior half of the primitive streak, an anterior arch of prospective ventral endoderm, a weak domain in the prechordal plate, and mesodermal domains underlying laterally the prospective epidermis flanking the neural plate (Fig. 7H; Andrée et al., 1998). After exposure to 0.3 M LiCl we observed a reduction of BMP2 expression at the degenerated posterior streak (Fig. 7J,M). The anterior arch of prospective ventral endoderm was not present, instead we observed a strong domain in and around the anterior, thickened streak (Fig. 7J).

Based on GSC (Fig. 7C) as well as BMP2 (Fig. 7K) expression we suspect that these cells correspond to the prechordal plate cells, a population of dorsal as well as anterior identity. The two BMP2 domains of lateral mesoderm were consistently found in contact with the primitive streak. We explain this finding of normally lateral mesoderm now close to the midline by a lack of mesoderm production from the post-nodal streak. This interpretation is corroborated by the irregular structure of the mesoderm, with significant constrictions at the paraxial levels (arrowheads). (M) Note the degeneration of the posterior streak (arrow).

Some of the more weakly treated embryos (0.1 M LiCl) were able to develop beyond the primitive streak stage (Fig. 8). They were analyzed with the late dorsal marker gene CNOT1, which demarcates the notochord and two posterior wings of prospective neural tube ectoderm flanking the primitive streak (Fig. 8A-C; Stein and Kessel, 1995). Also the posterior primitive streak was diminished or virtually absent in these embryos (Fig. 8F). Their notochords were thickened, apparently at the cost of paraxial mesoderm (Fig. 8D,E), whereas the posterior wings of the CNOT1 expression domain were missing (Fig. 8D; $n=5$). The absence of the posterior primitive streak and the corresponding mesoderm probe in less severely affected and thus further developed embryos was
detected using Ch-Tbx6L as a marker (n=15; Knezevic et al., 1997). The normal Ch-Tbx6L domain was significantly truncated by up to 50% (Fig. 8G-M).

In summary, lithium treatment during early gastrulation stages caused an enhanced dorsoanterior development and a posterior degeneration.

**DISCUSSION**

**The development from a radially to a bilaterally symmetric pattern of nuclear β-catenin localization**

In early intrauterine stages of development (EK V), we observed strong localization of β-catenin to the perinuclear region and colocalization with the mitotic spindle during cell divisions. This subcellular distribution suggests that the function of β-catenin as a transcription factor involved in axial patterning has not yet commenced. Although, so far, no similar pattern of in vivo β-catenin localization has been reported, it may be relevant to two earlier findings. On the one hand, the perinuclear distribution resembles the binding of β-catenin to the nuclear envelope that has been reported in vitro in the absence of ATP (Fagotto et al., 1998). On the other hand, β-catenin might be associated with the microtubule network surrounding the nucleus, an interpretation that is supported by the colocalization with the mitotic spindle. In principle, binding to microtubules could be mediated by APC (Rubinfeld et al., 1993; Munemitsu et al., 1994). A transport of β-catenin along microtubules during cortical rotation has been suggested for the *Xenopus* embryo (Rowning et al., 1997). Our results show that perinuclear localization and translocation into the nucleus can be differentially regulated in vivo.

Our immunohistochemical analysis reveals two different, radially symmetric domains in the prestreak chick blastoderm (EK X): a belt-like domain, comprising area opaca and marginal zone, that shows nuclear localization of β-catenin and...
the central part of the blastoderm that is devoid of nuclear localization. Koller’s sickle is located at the border between those two domains (Fig. 3). With the formation of a hypoblast anterior of Koller’s sickle below, the distribution of nuclear β-catenin becomes bilaterally symmetric. Cells that contain nuclear β-catenin become transported to a point on the midline, directly anterior of the sickle at stage EK XI. This stage and position is characterized by the first expression of GSC, chordin, and HNF-3β, which is quickly followed by the appearance of the primitive streak in the epiblast (Izpisúa-Belmonte et al., 1993; Kispert et al., 1995; Streit et al., 1998).

Conserved effects of LiCl in vertebrate embryos

Our experiments with pre- and early gastrulation chick embryos reveal strikingly similar consequences of LiCl exposure, but also some differences reflecting the different mechanisms of gastrulation between amphibia and birds. As in Xenopus, LiCl caused a ubiquitous nuclear localization of β-catenin, axial development was inhibited, radialization could be observed, in particular with CMIX as a molecular marker, and dorsal development was enhanced as evident by GSC and CNOT1 expression. At the same time we detected a degeneration of the posterior embryo by BMP2 and Ch-Thx6L, and found evidence for an underdevelopment of ventral cell populations, in particular the prospective ventral endoderm, by BMP2. There are differences between Xenopus and chick regarding the timing of gastrulation phases. Whereas frog embryos have to be exposed to LiCl before the midblastula transition, chick embryos also respond to LiCl when exposed during early to mid-primitive streak development, which is long after the onset of transcription (Zagris and Matthiopoulos, 1987). However, in both species the treatment has to occur before the ingression of the head process. The enlargement of the chick organizer which we observed in embryos treated at mid-streak stage did not extend into the prospective ventral mesoderm in the posterior portion of the streak, as would be the case in the Xenopus marginal zone. Instead, the posterior streak degenerated, reducing the blastopore to a radIALIZED invagination at the level of the node.

The enhanced nuclear distribution of β-catenin resulting from LiCl exposure influenced significantly the proximodistal positioning of Koller’s sickle and, consequently, of the hypoblast. The center of manipulated blastoderm became positive for CMIX, at this stage of development a marker for the posterior marginal zone and Koller’s sickle. The CMIX-negative periphery, the area opaca, was broadened and the hypoblast, marked by GSC, became contracted to the middle of the embryo. These observations indicate strongly that the proximodistal position of Koller’s sickle is linked to β-catenin signalling. They resemble the more equatorial positioning of the start of invagination in LiCl-treated Xenopus blastulae, due to an ectopic, low level of mesoderm induction extending to more animal levels (Cooke et al., 1989). Also fish embryos show a reduced epiboly after LiCl treatment (Stachel et al., 1993).

Conserved pathways towards bilateral axis formation?

Experimental evidence indicates that ‘vegetal determinants’ initiate the formation of the body axis in many animals (reviewed in Eyal-Giladi, 1997). In Xenopus this signal can be mimicked by a Wnt factor, and further downstream also by Li ions, which trigger a pathway culminating in the nuclear localization of β-catenin, which then acts as a transcription factor (see Introduction). Our demonstration of nuclear β-catenin in chick embryos and its response to LiCl suggests that such a pathway may not only be common to the early embryogenesis of Xenopus and zebrafish, but also be functional in avian embryos. On the other hand, no nuclear localization of β-catenin could be demonstrated in murine embryos so far (Pöpperl et al., 1997). It remains to be seen how maternal determinants, and/or Wnt related factors are involved upstream of nuclear β-catenin in the avian embryo.

As in the Xenopus embryo, also in the early chick embryo (EK X) a radially symmetric pattern of nuclear β-catenin is not enough for axis formation. However, a bilaterally symmetric distribution of nuclear β-catenin correlates with the appearance of an organizer and the onset of gastrulation in both species. In addition other, cooperating factors appear to be involved, among them the TGF-β factor Vg1. In the frog blastula, nuclear β-catenin is located in the dorsal half, and Vg1 RNA in the vegetal half, and thus an overlap exists in the dorsal-vegetal quadrant (Weeks and Melton, 1987; Schneider et al., 1996; Larabell et al., 1997). In the pre-streak chick embryo, nuclear β-catenin is located in the upper and lower layers of the complete marginal zone, whereas cVg1 is restricted to the epiblast layer of the PMZ, where both molecules overlap (Seleiro et al., 1996; Shah et al., 1997). In both species, cells in the zones of overlap include those involved in the induction of an organizer, without necessarily participating in its formation. Thus, PMZ transplants in the chick will induce a complete primitive streak (Bachvarova et al., 1998), and dorsovegetal cells in Xenopus will induce a blastopore lip (Harland and Gerhart, 1997), in both cases complete with dorsal structures. Only the ectopic application of processed cVg1 inside of the nuclear β-catenin domain leads to the formation of a secondary axis, while expression in the central part of the blastodisc, that is devoid of nuclear β-catenin, is not able to induce an axis (Shah et al., 1997). Thus, the localized synergy of nuclear β-catenin with an additional signal appears to be a conserved step also involved in axis development of the avian embryo.

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