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The Spemann-Mangold organizer: the control of fate specification and morphogenetic rearrangements during gastrulation in *Xenopus*

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ABSTRACT Vertebrate embryonic development is controlled by sequentially operating signalling centres that organize spatial pattern by inductive interactions. The embryonic body plan is established during gastrulation through the action of the Spemann-Mangold or gastrula organizer, a signalling source discovered 75 years ago by Hans Spemann and Hilde Mangold. Transplantation of the organizer to a heterotopic location in a recipient embryo results in the formation of a secondary embryonic body axis, in which several tissue types, most notably somites and the neural tube, are derived from ventral host cells. Because of these non-cell autonomous recruiting or inducing activities the organizer has become a paradigm for studying intercellular communication in the vertebrate embryo. Here, I review some of the recent advances in understanding 1) the initiation of the Spemann-Mangold organizer, 2) its function in pattern formation along the dorsal-ventral and anterior-posterior axes and 3) the integration of cell fate specification events and downstream execution of morphogenetic movements during gastrulation in *Xenopus laevis*.

KEY WORDS: *Spemann-Mangold organizer, cell fate specification, vegetal rotation, convergence extension, gastrulation, Xenopus.*

Introduction

Gastrulation is a dynamic developmental process during which a series of cell fate specification events and cellular rearrangements occur that transform a seemingly unpatterned early zygote into a stereotypical embryo with morphologically recognisable anterior-posterior (head-trunk), dorsal-ventral (back-belly) and left-right (asymmetric positioning of internal visceral organs) axes. Spatial pattern formation within the early vertebrate embryo is primarily established by the Spemann-Mangold or gastrula organizer, an evolutionarily conserved signalling centre, specific to the chordate lineage, that conveys positional information to its microenvironment (Spemann and Mangold, 1924; Harland and Gerhart, 1997; Nieto, 1999). An organizer graft has the capacity to induce a secondary embryonic body axis with well defined anteroposterior and dorsoventral axes, when transplanted to a heterotopic location in a recipient embryo (Spemann and Mangold, 1924; Harland and Gerhart, 1997; Nieto, 1999). The cellular constitution and inductive capacities of the Spemann-Mangold organizer change over time, such that developmentally older organizer grafts loose the capacity to induce secondary axes with heads, and instead only duplicate trunk/tail structures (Spemann, 1938). The organizer self differentiates into dorsal axial midline structures. Its progeny populates the prechordal plate, notochord, pharyngeal endoderm and variably the ventral midline of the spinal cord, the floorplate (Spemann, 1938, Harland and Gerhart, 1997). The other tissues of the secondary body axis, the somites and the basal anlage of the neural tube, are host-derived. These histotypes have been recruited and properly aligned into the supernumerary axis by dorsalizing signals that redirect the fate and morphogenetic behaviour of ventral cells (presumptive blood and ectoderm) to a more dorsal character (somitic mesoderm and neuroectoderm), hence the name organizer. Besides conveying positional information to surrounding cells, the Spemann-Mangold organizer also controls some of the complex morphogenetic rearrangements during gastrulation. Despite the identification in recent years of numerous gene products that participate in the inductive properties of the organizer, relatively little is known about the control of gastrulation movements. Here, I will highlight some of the recent discoveries in *Xenopus* that have provided new insights into the early molecular and morphogenetic events that take place during gastrulation.

Abbreviations used in this paper: MBT, mid-blastula transition; GFP, green fluorescent protein.

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Formation of the Spemann-Mangold organizer

The organizer in *Xenopus* is established on the dorsal side of the embryo through the combinatorial action of the "dorsalizing" Wnt/β-catenin signalling pathway and the "vegetal" TGF-β signalling cascade (Harland and Gerhart, 1997; Nieto, 1999). Both pathways are dependent on maternal cytoplasmic determinants that are localized in the vegetal hemisphere of the oocyte, which only displays animal-vegetal polarity. Radial symmetry in the unipolarized egg is broken by fertilization, which elicits a displacement of cortically attached organelles from a vegetal position to the future dorsal side, in a process referred to as cortical rotation (see Moon and Kimelman, 1998). This translocation results in the cytoplasmic enrichment of the Wnt signal transducer β-catenin on the presumptive dorsal side of the dividing embryo (Moon and Kimelman, 1998). The dorsal accumulation of β-catenin protein could in part be the consequence of directed vectorial transport, along cortically aligned microtubules, of Dishevelled (Dsh), another upstream intracellular Wnt pathway component (Miller *et al.*, 1999). Translocated Dsh antagonizes the activity of the constitutively active serine/threonine kinase glycogen synthase kinase-3β (GSK-3β), which is part of a multimeric β-catenin destruction complex, which in addition contains Axin and the tumor suppressor Adenomatous Polyposis Coli (APC). This ubiquitous kinase complex, when active in the absence of Wnt signal transduction, phosphorylates β-catenin and thereby targets it for proteolytic degradation by the proteasome (Moon and Kimelman, 1998; Peifer and Polakis, 2000). Besides the Dsh-dependent inhibition of GSK-3β activity, the depletion of GSK-3β protein on the dorsal side, by an as yet unknown mechanism, could also contribute to β-catenin stabilization (Dominguez and Green, 2000). Thus, the initial step leading to the induction of the Spemann-Mangold organizer, is the stabilization of β-catenin on the dorsal side. Cytosolic β-catenin translocates to the nucleus during cleavage stages, where it forms a heteromeric complex with the architectural HMG-box protein Tcf-3, to transactivate post-mid blastula transition (MBT) the expression of early zygotic target genes, such as Siamois and Twin. These homeodomain proteins in turn activate transcription of early Spemann-Mangold organizer genes in the dorsal marginal zone (Harland and Gerhart, 1997; Moon and Kimelman, 1998; Table 1).

The activation of Spemann-Mangold organizer genes in the dorsal marginal zone is in addition dependent on the specification of the mesodermal germ layer. Nieuwkoop originally demonstrated that the mesodermal germ layer arises in the equator or marginal zone of the gastrula embryo by inductive signals released from vegetal endoderm cells. Mesoderm induction was thought until recently to be solely mediated by maternal TGF-β and FGF signalling (Harland and Gerhart, 1997; Kimelman and Griffin, 1998). This concept has in part been revised with the demonstration that mesendoderm formation is dependent on maternally stockpiled VegT, a T-box DNA binding protein that acts as a transcriptional activator (Zhang *et al.*, 1998; Kimelman and Griffin, 1998). VegT mRNA is initially localized to the vegetal hemisphere of the oocyte, which is exclusively partitioned and translated in yolk-rich vegetal endoderm during subsequent cleavage stages (Stennard *et al.*, 1999). Embryos derived from *vegT*-depleted oocytes are impaired in blastopore formation and in large devoid of definitive endoderm and mesoderm. Vegetal endoderm explanted from such embryos no longer induces mesendoderm in responsive ectoderm cells. This observation strongly suggested that maternally provided VegT protein activates post-MBT the expression of zygotic mesoderm-inducing relay signals in vegetal endoderm (Zhang *et al.*, 1998; Kofron *et al.*, 1999). Several groups have recently shown that VegT directly activates members of the Nodal subclass of TGF-βs, Xnr-1, -2 and -4 and Derrière, a Vg-1-related TGF-β (Clements *et al.*, 1999; Kofron *et al.*, 1999; Sun *et al.*, 1999; Yasuo and Lemaire, 1999; Agius *et al.*, 2000; Chang and Hemmati-Brivanlou, 2000). Xnrs can efficiently rescue a complete embryonic axis in *vegT*-depleted embryos, whereas Derrière only rescues trunk/tail structures. The induction of *Xnrs* is likely to be direct as the *Xnr-1* promoter contains putative T-box binding sites which confer VegT-dependent activation in reporter assays (Kofron *et al.*, 1999; Hyde and Old, 2000). After the initial induction, Xnrs establish a positive autoregulatory feedback loop for maintenance of expression (Hyde and Old, 2000; Osada *et al.*, 2000). A model for Spemann-Mangold organizer formation is depicted in Figure 1 in which these recent observations have been integrated. Interestingly, *Xnrs* can be synergistically activated by the maternal VegT pathway and β-catenin, presumably accounting for the higher *Xnr* expression in dorsal vegetal endoderm (Agius *et al.*, 2000). This implies that β-catenin signalling is not strictly confined to the dorsal cortex but actually extends quite deep into the embryo. This notion is supported by the observation that expression of other dorsal anterior endoderm markers, such as *cerberus* and *Xhex*, also depends on the input of the β-catenin pathway (Jones *et al.*, 1999; Zorn *et al.*, 1999). This model is compatible with the original discovery that Activin, another TGF-β member can induce distinct cell fates as a function of its local concentration (Green *et al.*, 1992). Rather than Activin, Xnrs are likely to establish different cell fates *in vivo* (organizer progeny) in a

TABLE 1

EXPRESSION OF SPEMANN-MANGOLD ORGANIZER GENES IN PRESUMPTIVE PROGENY AT THE EARLY GASTRULA STAGE. THE EXPRESSION PROFILES ARE HIGHLY DYNAMIC AND CHANGE OVER TIME. TRANSCRIPTION FACTORS ARE LISTED IN BLACK AND SECRETED FACTORS IN RED

Dorsoanterior endoderm	*cerberus, dkk-1, Xhex, mix genes, Xblimp-1*
Prechordal plate	*dkk-1, frzb-1, chordin, noggin, goosecoid, Xotx-2, Xlim-1, Xanf-1*
Notochord	*chordin, noggin, follistatin, admp, Xnot, pintallavis, Xlim-1, Xbra, Xerg-1*
Floorplate	*Xfd-12', pintallavis*
Pharyngeal endoderm	
Organizer epithelium	*Xnr-3*

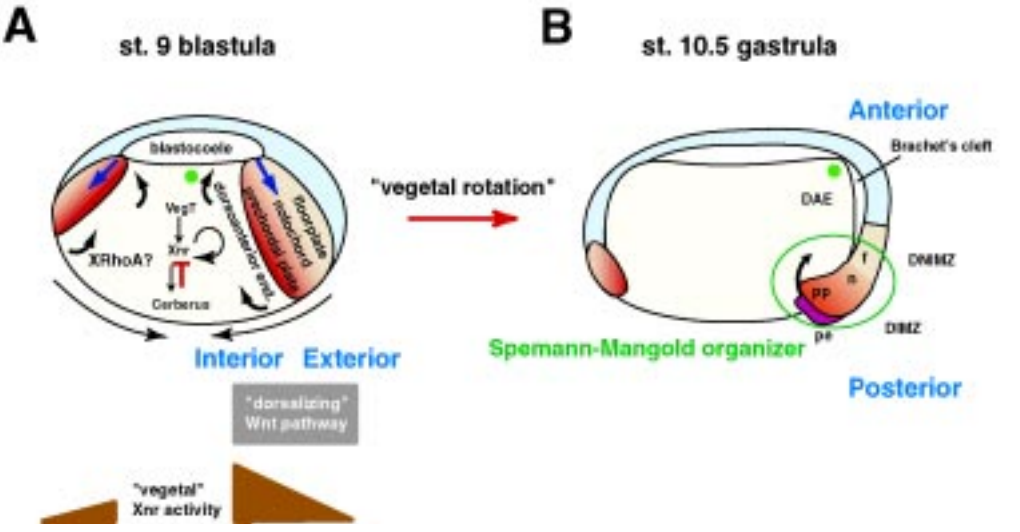

Fig. 1. Model for Spemann-Mangold organizer formation. *Schematic representation (lateral views through the dorsal midline) of mesoderm induction* **(A)** *and organizer regionalization (transition of A to B). Organizer progeny (dorsoanterior endoderm, prechordal plate, notochord and presumptive floorplate) is induced by the concerted action of the "vegetal" VegT/Xnr pathway and "dorsalizing" β-catenin cascade. Cerberus acts as a local feedback inhibitor of Xnrs in vegetal endoderm (red antagonistic arrow). The morphogenetic rearrangements in vegetal endoderm ("vegetal rotation") are indicated by black arrows (A) that constrict the vegetal outer surface and expand the blastocoel floor. This results in a passive displacement of organizer progeny to the marginal zone (indicated by blue arrows). Vegetal endoderm displacement is visualized by a hypothetical clone of cells (green) initially located in the blastocoel floor that is shifted to the dorsal side.* **(B)** *The Spemann-Mangold organizer is demarcated by a green circle . Abbreviations of organizer derivatives: pp, prechordal plate; n, notochord; f, floorplate; pe, pharyngeal endoderm; DAE, dorsoanterior endoderm. For details see main text.*

concentration dependent manner, with high levels emanating from the dorsal vegetal endoderm trailing of towards the dorsal side. The concerted action of Xnrs and β-catenin results in the diagonal alignment of dorsoanterior endoderm, prechordal plate, notochord and presumptive floorplate territories (Fig. 1A). Although Xnr ligands have emerged as essential generic mesendoderm inducers in virtually all species (Schier and Shen, 1999), the model depicted is grossly oversimplified as the other TGF-βs, Activin, Vg-1 and Derrière are also essential for some aspects of mesendoderm formation in *Xenopus*. Perhaps as maintenance factors as none of the abovementioned TGF-βs is able to rescue mesendoderm formation in *Xnr*-depleted embryos (Agius *et al*., 2000).

The Spemann-Mangold organizer progeny gives rise to the prechordal plate, notochord, pharyngeal endoderm and depending on the graft size, the floorplate and the dorsoanterior endoderm (Spemann, 1938; Fig. 1B). The recent identification of the forkhead transcription factor Xfd-12' indeed shows that precursors of the ventral midline of the spinal cord are specified in the organizer concomitant with determination of notochordal cell fate (De Robertis *et al*., 1997; Sölter *et al*., 1999; Fetka *et al*., 2000). A list of early Spemann-Mangold organizer genes, most of which encode transcriptional regulators or secreted factors is shown in Table 1, listed by virtue of their restricted expression in presumptive organizer derivatives at the onset of gastrulation. At present it is unclear where and when the pharyngeal endoderm actually arises and how it is induced, as no specific marker genes exist. It is beyond the scope of this review to describe the function of individual organizer genes, as this has been covered in other recent reviews (Harland and Gerhart, 1997; De Robertis *et al*., 1997). The epistatic relationship between genes expressed in the same lineage still needs to be determined in detail.

The position of the Spemann-Mangold organizer in the dorsal marginal zone is maintained by a cross regulatory network of ventralizing BMPs, Wnts and downstream homeodomain proteins that actively repress expression of organizer components throughout the rest of the embryo. Reciprocally, organizer-specific transcription factors in part act as repressors of ventral genes. Thus, the position and maintenance of the organizer depends on the balance between ventralizing "anti-organizer" factors and dorsalizing "organizer" antagonists (Harland and Gerhart, 1997).

Dorsal/ventral patterning

The Spemann Mangold organizer exerts its patterning activities at the onset of gastrulation. The main inductive properties are: 1) the dorsalization of ventral mesoderm, establishing zones of intermediate mesodermal histotypes, such as somitic mesoderm and 2) the induction of neuroectoderm in dorsal ectoderm (Spemann and Mangold, 1924; Harland and Gerhart, 1997; Nieto, 1999). Several secreted factors have been identified such as Chordin, Noggin and Follistatin, all expressed in chordamesoderm (prechordal plate and notochord), that can mimic these non-cell autonomous activities. This has provided a mechanistic understanding for this inductive aspect of organizer function. Rather surprisingly, based on the dominance of the organizer graft on the fate of ventral host cells, these secreted polypeptides were shown to act as permissive rather than as instructive intercellular signals (De Robertis *et al*., 1997; Graff, 1997; Harland and Gerhart, 1997). Although unrelated in sequence and presumably in structure, Chordin, Noggin and Follistatin function as specific antagonists of BMPs, which act as agonists of ventral cell fates. These dorsalizing factors physically trap BMP ligands in the extracellular space, sequestering them in inactive complexes, thereby preventing BMP receptor activation (De Robertis *et al*., 1997; Graff, 1997). Microinjection of BMP inhibitors or addition of recombinant proteins to ventral marginal zone explants induces the formation of somitic muscle in ventral cells, resulting in elongation of the ectopic secondary axis or dorsalized explant, respectively. Thus, BMP antagonism is sufficient to co-ordinately 1) induce paraxial somitic mesodermal cell fate and 2) initiate morphogenetic behaviour associated with this histotype (Graff, 1997; Harland and Gerhart, 1997). These observations have led to the proposal that BMP

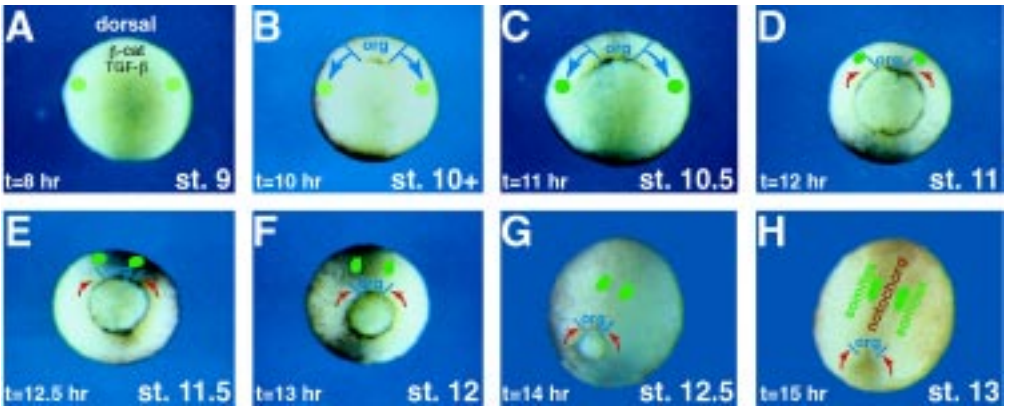

Fig. 2. Time lapse of *Xenopus* gastrulation. *Individual time frames from late blastula stage (st. 9) till the end of gastrulation (st. 13) (stages according to Nieuwkoop and Faber, 1967; time post-fertilization is indicated in the bottom corner to the left). The position of the Spemann-Mangold organizer during the course of gastrulation is indicated in blue. Blue arrows reflect the dorsalizing activities of secreted BMP antagonists emanating from the organizer that pattern the dorsolateral marginal zone (see text). Red arrows mark the onset and trajectory of convergence extension movements of paraxial mesoderm cells. Two hypothetical clones of cells at bilateral positions (light green) visualize this route, which converge and extend from st. 10.5, after paraxial cell fate has been established (indicated by colour change to dark green). This morphogenetic process requires signalling by the "planar polarity"-like Xwnt11/Dsh pathway (Tada and Smith, 2000; Wallingford et al., 2000). Embryos are viewed from* **(A-F)** *vegetal perspective or* **(G,H)** *represent a dorso-vegetal view.*

antagonism is the main inductive activity of the trunk organizer. It has remained elusive however, how these dorsalizing factors impinge on direct regulators of paraxial morphogenetic behaviour, which co-ordinate rearrangements of the actin cytoskeleton and/or microtubule network (see below).

Anterior/posterior patterning

Anteroposterior pattern within germ layers arises during gastrulation. Spemann and Mangold originally demonstrated the existence of head and trunk organizers at different developmental stages (Spemann, 1938). On a mechanistic level the difference between head and trunk organizer activities is currently best explained by a two-inhibitor versus one-inhibitor mechanism, as proposed by Niehrs and colleagues (Glinka *et al.*, 1997). In addition to BMP antagonists, that primarily pattern the trunk of the embryonic axis, the Spemann-Mangold organizer expresses several other secreted factors that antagonize the activity of Wnt glycoproteins, such as Cerberus, Dkk-1 and Frzb-1 (Niehrs, 1999). These Wnt antagonists are expressed in dorsoanterior endoderm and prechordal plate, but not in posterior chordamesoderm (Bouwmeester and Leyns, 1997; Niehrs, 1999). Combinatorial expression of Wnt antagonists in synergy with BMP antagonists results in induction of secondary axes with head structures (Glinka *et al.*, 1997, 1998; Piccolo *et al.*, 1999). The necessity for combinatorial inhibition of multiple growth factors is supported by the fact that sustained expression of either Wnt, BMP or Xnr ligands in dorsoanterior cells leads to suppression of head structures (Glinka *et al.*, 1997; Piccolo *et al.*, 1999). The activity of trunk inducing Xnr ligands is antagonized by Cerberus, a component of the head organizer which is a multifunctional growth factor antagonist (Bouwmeester *et al.*, 1996; Hsu *et al.*, 1998; Piccolo *et al.*, 1999). In the dorsoanterior endoderm Cerberus presumably acts as a cell-type specific feedback inhibitor, creating a zone that is free of Xnr signals because it disturbs the positive autoregulatory Xnr feedback loop (Hyde and Old, 2000; Osada *et al.*, 2000; Fig. 1A). The trunk organizer, in addition to Xnrs, expresses a BMP-related factor, anti dorsalizing morphogenetic protein (ADMP), which in turn represses head organizer formation (Dosch and Niehrs, 2000). Thus, the subdivision of head and trunk organizer compartments is established and/or maintained by a mutual inhibitory mechanism.

Spemann-Mangold Organizer regionalization

Recent observations have indicated that the Spemann-Mangold organizer is first regionalized into head and trunk organizer compartments at the onset of gastrulation even though cell fates are specified at the late blastula stage (Fetka *et al.*, 2000). Organizer progeny is initially aligned in regular diagonal stripes, without overt anteroposterior pattern, due to the morphogenetic activities of Xnr ligands acting in synergy with the β-catenin pathway (Fig. 1A). At the start of gastrulation anteroposterior pattern has become apparent; the dorsoanterior endoderm now occupies an anterior position with respect to the prechordal plate and notochord (Fig. 1B). Pregastrular movements have been observed (Keller, 1991) and clonal analysis has indicated a vegetal displacement of mesoderm from an equatorial region to a subequatorial position at the late blastula stage (Bauer *et al.*, 1994; Vodicka and Gerhart, 1995). Winklbauer and Schürfeld now demonstrated that these rearrangements are caused by an active pregastrular distortion of the vegetal cell mass, termed "vegetal rotation" (Winklbauer and Schürfeld, 1999). Support for an active displacement of vegetal endoderm cells has come from lineage tracer analysis, which showed that a labelled cell in the middle of the blastocoel floor is shifted to the dorsal side (Jones *et al.*, 1999; illustrated in Fig. 1). The net result of vegetal rotation is a constriction or narrowing of the outer vegetal surface and a concomitant expansion of the vegetal blastocoel floor, which passively displaces the definitive mesendoderm, with intrinsic anteroposterior pattern, to the marginal zone. This dynamic process is initiated in the Spemann-Mangold organizer, where it precedes dorsal blastopore lip formation and marks the onset of mesendoderm involution. It subsequently sweeps from dorsal to ventral preceding the propagation of the blastopore groove. On the dorsal side this vegetal expansion results in the formation of Brachet's cleft which separates the dorsoanterior endoderm and inwards migrating head mesendoderm from the presumptive neuroectoderm that will give rise to forebrain (Keller, 1991). Vegetal rotation seems to be driven by cell autonomous rearrangements of peripheral endoderm cells. This active process does not seem to be driven by microtubule rearrangements, as bottle cell formation and alignment of preinvoluted mesoderm are not disturbed in embryos treated with the microtubule depolymerizing drug nocodazole, even though consecutive move-

ments and blastopore closure are inhibited (Lane and Keller, 1997). Vegetal cells do form random protrusions, such as lamellipodial and filipodial extensions, suggesting that this process could result from rearrangements in the actin cytoskeletal architecture. Therefore, it is tempting to speculate that small GTPases of the Rho family, like Rho, Rac or Cdc42 could be regulators of this morphogenetic process. Rho GTPases act as intracellular switches that integrate diverse signalling events with changes in the actin cytoskeleton and have been implicated in a variety of morphogenetic processes during *Drosophila* development, such as gastrulation and dorsal closure (Settleman, 2000). Intriguingly, Cho and colleagues have recently isolated and functionally characterized a *Xenopus* Rho homologue, termed XRhoA (Wünnenberg-Stapleton *et al*., 1999). Ventral injection of XRhoA, but not constitutively active Rac, in synergy with a BMP antagonist, results in the induction of a secondary axis with complete head, akin to the combinatorial inactivation of BMP and Wnt signalling (Glinka *et al*., 1997). XRhoA alone has no detectable morphological effect. XRhoA does not exert its head-inducing activity by simply antagonizing Wnt or Xnr ligands, indicating that the mode of action is distinct from that of the Wnt or Xnr antagonists that participate in head organizer formation. XRhoA could have a signalling role thereby affecting cell differentiation, though this appears to be unlikely given the universal role of Rho GTPases in regulating morphogenesis (Settleman, 2000). An attractive alternative possibility is that XRhoA is actually involved in the process of vegetal rotation that spatially segregates head and trunk organizer territories. Expression of *XrhoA* is actually co-localized with *cerberus* in the dorsoanterior endoderm after vegetal rotation (Wünnenberg-Stapleton *et al*., 1999). XRhoA can increase cell adhesion acting as an antagonist of the constitutively active GTPase XRnd1. By regulating adhesion strength and actin dynamics XRhoA could control vegetal rearrangements that spatially separate the dorsoanterior endoderm (expressing the trunk antagonist *cerberus*) from the definitive mesendoderm (expressing the head antagonist *admp*), though this hypothesis awaits further investigations.

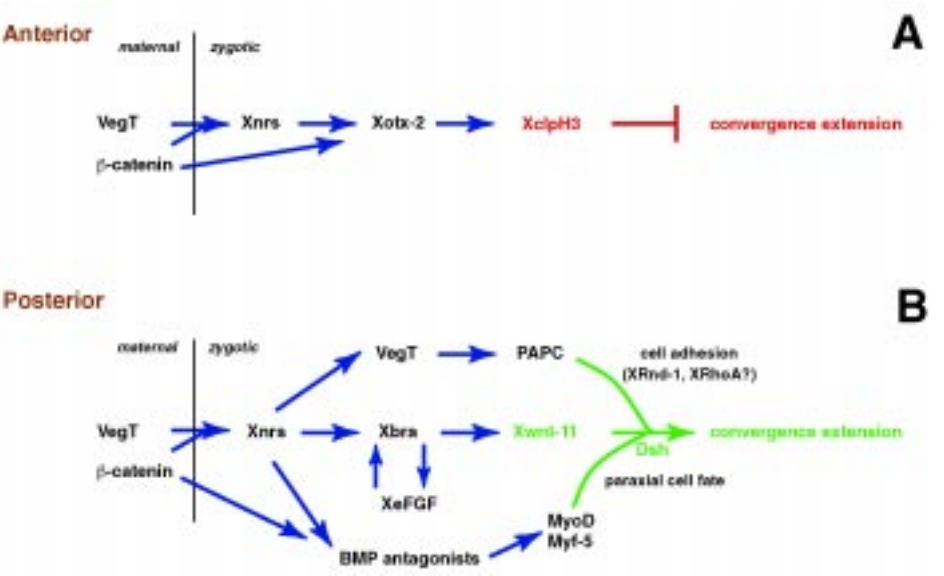

Fig. 3. Signal transduction pathways that spatially confine convergence and extension behaviour. *Schematic representation of signal transduction cascades that control the spatial regulation of convergence and extension movements during Xenopus gastrulation.* **(A)** *Mediolateral intercalations are actively repressed in anterior cells (head organizer derivatives) that express Xotx-2 and its downstream effector XclpH3.* **(B)** *Convergence extension movements are promoted in trunk histotypes (paraxial and axial mesoderm) that express Xbra and its effector Xwnt11. For details see main text.*

Morphogenetic movements of trunk histotypes

Morphogenesis of the embryonic body axis is primarily driven by mediolateral cell intercalations during convergence extension of trunk histotypes (Keller, 1991). These cellular rearrangements are confined to somite and notochord progenitors in the dorsal involuted marginal zone (DIMZ) and hindbrain and medial neural plate precursors in the dorsal non involuted marginal zone (DNIMZ) (Keller, 1991). Before involution radial intercalations result in reduced depth along the circumference, as cells intercalate perpendicular towards the outer surface. This is followed by mediolateral cell intercalations that drive involution of mesendoderm resulting in the elongation of trunk histotypes (Keller *et al*., 1992). Before gastrulation cells exert lamellipodial protrusions in random directions that become stabilized and directed along the mediolateral axis, resulting in a bipolar appearance from mid gastrula stage onwards (Shih and Keller, 1992; Lane and Keller, 1997). Mediolateral cell intercalations initiate in the vegetal alignment zone, a group of cells, that transverse the organizer, that exert bi-directional protrusive activity in a microtubule-dependent fashion, and subsequently propagate around the marginal zone (Shih and Keller, 1992; Lane and Keller, 1997). After stabilization of protrusions the bipolar cells align in the marginal zone, elongate and intercalate by traction forces on the dorsal midline (convergence) resulting in an elongation of the embryonic axis along the anteroposterior axis (extension). The trajectory of paraxial mesoderm cells is illustrated in Figure 2 during a time lapse analysis of gastrulation.

Despite the importance and universal nature of these morphogenetic rearrangements it has remained elusive how cell polarity and cell shape changes are initiated and coordinated with cell fate specification events. Recent work by Smith and colleagues and independently Harland and co-workers has brought new insights in this important aspect of gastrulation.

Smith and colleagues have been studying the function of Brachyury (Xbra); a T-box transcription factor, which is a key component for trunk morphogenesis as it is essential for posterior mesoderm formation and gastrulation movements (Conlon and Smith, 1999). In their ongoing efforts of unravelling the function of Xbra, they have now identified *Xwnt11* as a direct immediate response gene (Tada and Smith, 2000). *Xwnt11* was previously characterized as a maternally expressed Wnt, which can partially rescue axis deficiency in UV-ventralized embryos (Ku and Melton, 1993). *Xwnt11* is expressed in the circumblastoporal marginal zone during gastrulation in a pattern akin to that of *Xbra* (Ku and Melton, 1993; Tada and Smith, 2000). By employing a dominant-

negative strategy they show that Xwnt11 is essential for convergence extension movements of paraxial and axial cells without affecting dorsal-ventral patterning along the marginal zone. The dominant-negative *Xwnt11* construct specifically interferes with Xwnt11 function. It does not inhibit Xwnt8 activity, another member of the Wnt family, which specifies paraxial mesodermal cell fate, suggesting that both Wnts signal into divergent effector pathways. The *dn-Xwnt11*-induced phenotype is reminiscent of the morphological defects caused by overexpression of a truncated form of the intracellular Wnt transducer Dsh (Sokol, 1996). Dsh acts at the crossroads of two distinct Wnt/frizzled signalling pathways in *Drosophila*. It is an essential transducer of the canonical Wnt/β-catenin pathway as well as of the alternative "planar polarity" cascade, which controls the organization of epithelial structures, such as the orientation and chirality of ommatidia and the orientation of bristles and wing hairs (Boutros and Mlodzik, 1999). Interestingly, convergence extension movements can be restored by co-expression of *dn-Xwnt11* with truncated Dsh forms that can relay planar polarity signalling but which are defective for Wnt/β-catenin signal transduction. Conversely, a mutant form of Dsh that can only transduce the canonical Wnt pathway has a dominant inhibitory effect on convergence extension. These results imply that Xwnt11 is a downstream effector of Xbra, which initiates convergence extension behaviour of paraxial and axial cells via a "planar polarity"-like cascade involving Dsh (Fig. 3).

Harland and co-workers have come to the same conclusion while studying convergence extension behaviour from a more cellular perspective. By time lapse video imaging of GFP-tagged cells they demonstrate that cells in dorsal marginal zone explants indeed stabilize random protrusions along the mediolateral axis (Keller, 1991; Shih and Keller, 1992; Wallingford *et al.*, 2000). However, in the absence of Dsh activity random protrusions are generated and retracted continuously, without directional stabilization, indicating that paraxial and axial cells have lost the capacity to form bipolar protrusions even though they are intrinsically capable of generating random lamellipodia (Wallingford *et al.*, 2000). As a consequence of impaired cell polarity cells do not properly align and therefore do not elongate along the dorsal circumference. Like Tada and Smith, they show that mutant forms of Dsh that are only capable of relaying the canonical Wnt/β-catenin pathway can block convergence extension in a dominant-negative manner. By live imaging they furthermore demonstrate that a Dsh-GFP chimera translocates to the cell cortex in cells of dorsal marginal zone explants that undergo convergence and extension (Wallingford *et al.*, 2000). This is an important observation, as it allows for the visualization in live embryos of endogenous Wnt/frizzled signalling. Collectively, these data underpin the importance of the alternative Xwnt11/Dsh pathway in controlling cell polarity during *Xenopus* gastrulation (Fig. 3).

The non-canonical Wnt11/Dsh pathway appears to have a conserved role in the control of convergence extension during vertebrate gastrulation. It was demonstrated that the zebrafish mutant *silberblick* (*slb*) is caused by a null mutation in *wnt11* (Heisenberg *et al.*, 2000). The resulting mild cyclopia phenotype can be rescued by wnt11 as well as by the Dsh mutant that only transduces planar polarity signalling but is defective for relaying the canonical Wnt pathway (Heisenberg *et al.*, 2000). Interestingly, transplantation assays indicated that Wnt11 activity is required in a non-cell autonomous manner in paraxial tissues to drive elongation of axial structures.

Convergence extension movements are spatially regulated throughout the *Xenopus* embryo (Fig. 3). The homeodomain protein, Xotx-2 not only promotes development of anterior structures, such as the induction of the cement gland; it also prevents cells from engaging in convergence extension (Harland and Gerhart, 1997). Durston and colleagues have identified *calponin H3* (*clpH3*) as a downstream target that could mediate this inhibitory function (Morgan *et al.*, 1999). XclpH3 was identified in an expression screen devised to identify activities that suppress posterior development. *XclpH3* is an immediate early target of Xotx-2 as it is activated in the absence of de novo protein synthesis and its co-expression with *Xotx-2* depends on Xotx-2 activity. Overexpression of *XclpH3* in paraxial mesoderm, phenocopies the gastrulation defects (spina bifida) induced by ectopic *Xotx-2* expression. This inhibitory effect on convergence extension is cell autonomous and direct as cells in the marginal zone are correctly specified (Morgan *et al.*, 1999). The precise cellular mechanism by which XclpH3 exerts its activity remains to be elucidated. It is conceivable though that XclpH3 provides a direct link to the actin cytoskeleton network since these cytoplasmic proteins can bind actin and myosin and prevent movements of actin filaments in motility assays.

An important feature of cellular rearrangements is the continuous modulation of adhesivity of motile cells. Cells have to regulate their adhesivity in order to be able to move with respect to each other. XRnd1, a constitutively active small GTPase, which is expressed in a variety of cells that undergo morphogenetic rearrangements, including paraxial mesoderm, could regulate cell adhesion during convergence extension. Overexpression of XRnd1 in nascent ectoderm results in decreased adhesion of ectodermal blastomeres, which can be counteracted by XRhoA (Wünnenberg-Stapleton *et al.*, 1999). In paraxial mesoderm XRnd1 is co-expressed with paraxial protocadherin (PAPC), a divergent $Ca2^{+}$-dependent cadherin that is not linked to the cytoskeletal actin network via α- and β-catenin (Kim *et al.*, 1998). PAPC does engage in homotypic cell adhesion for which, in contrast to homophilic cell interactions between classical cadherins, the cytoplasmic domain is dispensable (Kim *et al.*, 1998). In fact, the absolute adhesion strength increases after truncation of the cytosolic tail suggesting that this domain could have a modulatory role in regulating adhesivity. A dominant-negative form of PAPC specifically blocks paraxial morphogenesis *in vivo* and in Activin-treated animal cap explants, indicating that PAPC-mediated adhesion is crucial for convergence extension behaviour of somitic precursors (Kim *et al.*, 1998). Notochordal cells express a related cell adhesion molecule, termed axial protocadherin (AXPC) suggesting that these two cadherin-like molecules could preserve histotype specificity during gastrular cellular rearrangements and indeed cells expressing either PAPC or AXPC sort out after random dispersion (Kim *et al.*, 1998). Classical cadherins, which are linked to the actin cytoskeleton, also play an essential role during convergence extension. The adhesive activity of C-cadherin is modulated, by an as yet unknown mechanism, in response to Activin resulting in reduced adhesion forces between mesodermal cells (Zhong *et al.*, 1999). Given that one of the components of the "planar polarity" cascade, Dsh also controls vertebrate gastrulation movements it is worth mentioning that the divergent *Drosophila* protocadherin Flamingo is essential for epithelial planar polarity. Wnt/frizzled signalling is thought to direct polarized Flamingo distribution along the proximal-distal axis of epithelial cells (Usui *et al.*, 1999). It is conceivable

that protocadherins or classical cadherins in *Xenopus* are likewise targets of the alternative Wnt/Dsh pathway that controls morphogenetic behaviour of paraxial and axial cells.

Conclusion

The work reviewed here reflects that important progress has been made in understanding some of the morphogenetic rearrangements that occur during gastrulation in *Xenopus*. In particular with the description of "vegetal rotation" as an active distortion of vegetal endoderm, that could account for the regionalization of the Spemann-Mangold organizer, and the identification of genes that co-ordinate cell polarity and cell shape changes during convergence extension movements of trunk histotypes. The identification of a "planar-polarity"-like Xwnt11/Dsh pathway that directly controls cellular behaviour is an important discovery that raises a number of intriguing questions that need to be addressed in the future. How do Xwnt11 or Dsh induce cell polarity? How do they modulate adhesivity of cells and last but not least how do they impinge on regulators of the actin cytoskeleton and/or microtubule network? In order to answer these issues it will be essential to determine the precise localization of endogenous Xwnt11/Dsh and perhaps PAPC or other cadherins in cells that undergo morphogenetic rearrangements. Live imaging using GFP technology will be a powerful tool to trace the dynamics (in spatial and temporal terms) of signalling events and actin and microtubule rearrangements. The elucidation of the non-canonical Xwnt11/Dsh pathway in *Xenopus* will certainly benefit from genetic studies on planar polarity signalling in *Drosophila*.

In order to understand organizer function from a cellular perspective it will be essential to further dissect the signalling pathways that co-ordinate cell fate specification and the mechanical aspects of gastrulation. The identification of direct target genes of other organizer-specific transcription factors will certainly aid to our understanding of how these factors integrate cell fate choices with cell-type specific morphogenetic behaviour. Ultimately, the continuing biochemical and cell biological dissection of the Spemann-Mangold organizer will undoubtedly result in an abstraction of the "Holy Grail of classical embryology" as a precisely regulated series of molecular and morphogenetic events.

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