Move it or lose it: axis specification in *Xenopus*

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Development 131, 3491-3499
Published by The Company of Biologists 2004
doi:10.1242/dev.01284

Summary

A long-standing question in developmental biology is how amphibians establish a dorsoventral axis. The prevailing view has been that cortical rotation is used to move a dorsalizing activity from the bottom of the egg towards the future dorsal side. We review recent evidence that kinesin-dependent movement of particles containing components of the Wnt intracellular pathway contributes to the formation of the dorsal organizer, and suggest that cortical rotation functions to align and orient microtubules, thereby establishing the direction of particle transport. We propose a new model in which active particle transport and cortical rotation cooperate to generate a robust movement of dorsal determinants towards the future dorsal side of the embryo.

Introduction

One of the major challenges in developmental biology is to understand how polarity is achieved in a developing embryo. For over a century, the amphibian embryo has played a major role in these investigations because its large size and external fertilization have facilitated the analysis of the early steps in the development of the embryonic axes. In the amphibian, the first body axis to be established is the dorsoventral (DV) axis, which ultimately also dictates the orientation of the anteroposterior (AP) axis. A large body of work, mostly from studies using the African clawed frog *Xenopus laevis*, has described how the initial specification of DV asymmetry depends on physical and biochemical events that occur shortly after fertilization.

Fertilization leads to the movement of maternally deposited dorsalizing factors from their original location at the bottom of the *Xenopus* embryo to a new location near the equator, on the side opposite the point of sperm entry (Fig. 1A,B). These factors cause a local stabilization of the Wnt pathway effector β-catenin, which is needed to activate zygotic genes of the dorsal organizer (Fig. 1C,D). As a result of organizer formation, the side opposite the sperm entry point is specified as dorsal (reviewed by Moon and Kimelman, 1998). Therefore, the creation of this DV asymmetry in β-catenin levels by the translocation of dorsal factors is a crucial event in the development of the amphibian embryo. However, we still have a poor understanding of how it occurs. In particular, there are two important questions that need to be answered: what is the moving dorsalizing activity, and how does it get to where it is going?

As we discuss in this review, although the molecular identity of the dorsalizing activity is still under investigation, there is evidence that the intracellular members of the classical Wnt signaling pathway are involved in this process: in particular, the β-catenin stabilizing proteins Glycogen Synthase Kinase 3 (GSK3)-binding protein (GBP) and Dishevelled (Dsh). We describe recent work showing that these proteins can be actively transported in the *Xenopus* egg.

The principal focus of this review is on the second question: how is the dorsalizing activity transported? The prevailing model has been that a physical displacement of the egg cortex, an event called cortical rotation, is responsible for the movement of cortically attached dorsalizing factors towards the future dorsal side. However, a closer examination of the early literature, in combination with recent data, points to an alternative mechanism, in which dorsal determinants travel on subcortical microtubules as the cargo of kinesin motor proteins. We discuss a potential alternative purpose for cortical rotation – to align the microtubule tracks on which the determinants travel. Finally, on the basis of previous models (Scharf and Gerhart, 1980; Vincent and Gerhart, 1987; Ziskind and Elinson, 1990; Kikkawa et al., 1996; Rowning et al., 1997; Miller et al., 1999b; Weaver et al., 2003), we propose a new integrated model in which both active transport by motor proteins and association with the rotating cortex contribute to the net movement of dorsal determinants towards the dorsal side.

The cell biology of dorsal specification

The unfertilized *Xenopus laevis* egg is radially symmetrical, with a darkly pigmented animal hemisphere and a lightly pigmented vegetal hemisphere. Sperm entry, which can occur anywhere in the animal hemisphere, causes the outer layer of the egg to loosen from the dense yolky core cytoplasm. This outer layer is called the cortex, and includes the plasma membrane of the egg, as well as cytoskeletal elements, the endoplasmic reticulum (ER) and other components (Houliston and Elinson, 1991a). The loosening of the cortex from the core creates a relatively yolk-free area called the shear zone (Fig. 1E). About midway through the first cell cycle, the cortex begins to rotate relative to the core in a process called cortical rotation, which continues until near the end of the first cell cycle and results in a ~30° displacement of the vegetal cortex away from the sperm entry site and towards the future dorsal region (Fig. 1F) (reviewed by Gerhart et al., 1989; Houliston and Elinson, 1992).

Cortical rotation coincides with the translocation of the maternal dorsalizing activity from the vegetal pole towards the prospective dorsal side of the embryo, in the same direction as

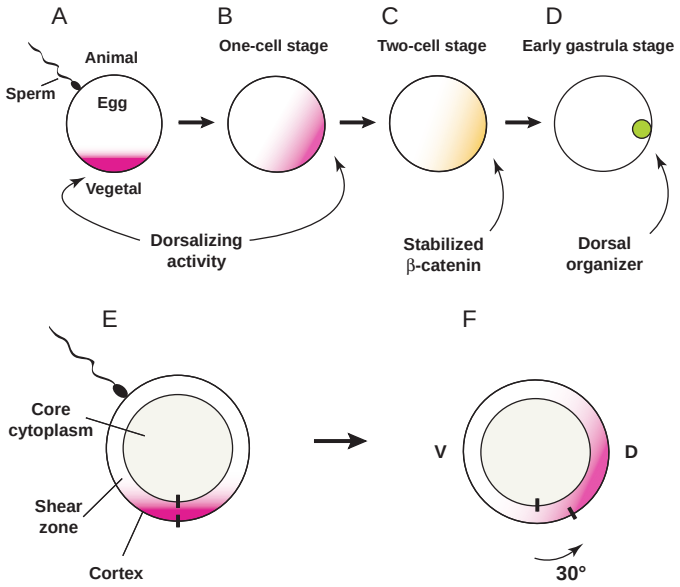

Fig. 1. Translocation of the maternal dorsalizing activity. (A-D) Translocation of the dorsalizing activity leads to β-catenin stabilization and the formation of the dorsal organizer. Between the time of fertilization and the first embryonic cell division, a maternally deposited dorsalizing activity (red) moves from (A) the vegetal pole to (B) the prospective dorsal region. (C) By the two-cell stage, maternal β-catenin (yellow) has become asymmetrically stabilized in the region that has received the dorsalizing activity. (D) Stabilized β-catenin activates genes of the dorsal organizer (green circle; also called the Nieuwkoop and Spemann organizers) in the dorsal equatorial region, as shown in an early gastrula embryo. (E,F) The dorsalizing activity translocates in the same direction as cortical rotation. (E) The dorsalizing activity (red) resides in the shear zone, an area of looser cytoplasm that forms between the outer cortex of the egg and the dense core cytoplasm following fertilization. The black bars at the vegetal pole mark the starting positions of the core and the cortex early in the first cell cycle. (F) During the first cell cycle, the cortex rotates relative to the core, moving about 30° towards the dorsal side in the same direction as the dorsalizing activity. This process is called cortical rotation, as represented by the displacement of the outer black bar. D, dorsal; V, ventral.

cortical rotation (Kikkawa et al., 1996; Sakai, 1996; Kageura, 1997). Cytoplasmic transplant experiments have shown that the dorsalizing activity resides in the subcortical cytoplasm of the shear zone (Fig. 1E) (Holowacz and Elinson, 1993; Kageura, 1997). Both cortical rotation and the translocation of the dorsal determinants depend on the assembly of a parallel array of microtubule (MT) bundles that appears in the vegetal shear zone of the egg about midway through the first cell cycle (Fig. 2A-C) (Elinson and Rowning, 1988) (reviewed by Houliston and Elinson, 1992; Chang et al., 1999). The MTs that form the parallel array appear to arise from several sources. Some of them are nucleated by the centriole of the sperm, which acts as a minus-end MT-organizing center (Fig. 2D). Other MTs extend towards the periphery from unknown sources deep in the cytoplasm and bend into the vegetal shear zone (Fig. 2E). Finally, some array MTs appear to polymerize spontaneously in the vegetal shear zone (Houliston and Elinson, 1991b; Schroeder and Gard, 1992).

Initially, MTs in the vegetal shear zone are disorganized and short, but as cortical rotation progresses, they lengthen into parallel bundles, which have their plus-ends oriented away from the site of sperm entry (Fig. 2C,F) (Houliston and Elinson, 1991a). The subcortical MTs depolymerize near the end of the first cell cycle, ending the process of cortical rotation (Schroeder and Gard, 1992; Marrari et al., 2003). During rotation, the cortex (and the dorsalizing activity) moves towards the plus-ends of the MTs, explaining why the direction of cortical rotation depends on the site of fertilization and why the dorsal organizer forms on the side of the embryo opposite the sperm entry point (reviewed by Houliston and Elinson, 1992).

The proper formation of the MT array is crucial for the development of the dorsal axis. If MT polymerization is blocked during the first cell cycle by exposure to UV irradiation or to the MT-depolymerizing drug nocodazole, or with inhibitory antibodies to the MT-associated protein XMAP230, the dorsal organizer does not form and the resulting embryos lack all dorsoanterior structures (Scharf and Gerhart, 1983; Elinson and Rowning, 1988; Cha and Gard, 1999). In the absence of a functional MT array, cortical rotation is prevented, and the dorsalizing activity remains stuck in the vegetal pole of the embryo (Vincent et al., 1987; Holowacz and Elinson, 1993). However, the inherent axis-inducing ability of the immobilized dorsalizing activity is unaffected: if subcortical cytoplasm is removed from the vegetal pole of a UV-treated embryo and transplanted into the marginal region of a host, the host develops secondary axial structures (Holowacz and Elinson, 1993). This result reveals that dorsal specification requires not just the dorsalizing activity, but also its successful translocation from the vegetal pole to the marginal zone.

The general view has been that the dorsal determinants associate with the moving cortex, which carries them up towards the margin during cortical rotation. The main rationale for this model is that the timing and direction of the movement of the dorsalizing activity correspond with those of cortical rotation. However, there is increasing evidence that cortical rotation is not the primary method used by the embryo to move the dorsalizing activity. In fact, the existence of an unknown underlying mechanism was first hypothesized by John Gerhart and colleagues almost 20 years ago, when they observed that cortical rotation could be uncoupled from the formation of the dorsal axis in some experimental situations (as discussed later). They therefore proposed that cortical rotation, rather than acting as the sole mechanism of axis determination, was instead simply a ‘cue’ that sensitized or oriented the response of the embryo to some underlying system (Black and Gerhart, 1985; Black and Gerhart, 1986; Vincent and Gerhart, 1987). The nature of this second system was a mystery, although they realized that, like cortical rotation, it required MTs.

While the cortical rotation-dependent model predominated, further clues about a possible alternative system accumulated. Cytoplasmic transplantation and removal experiments, which were used to map the location of the dorsalizing activity at different times, revealed that the dorsalizing activity is initially concentrated near the vegetal pole (Fig. 1A). During cortical rotation, this activity becomes broadly distributed along the dorsal side in a swath that reaches from the vegetal pole to near the equator (Fig. 1B) (Yuge et al., 1990; Fujisue et al., 1993;

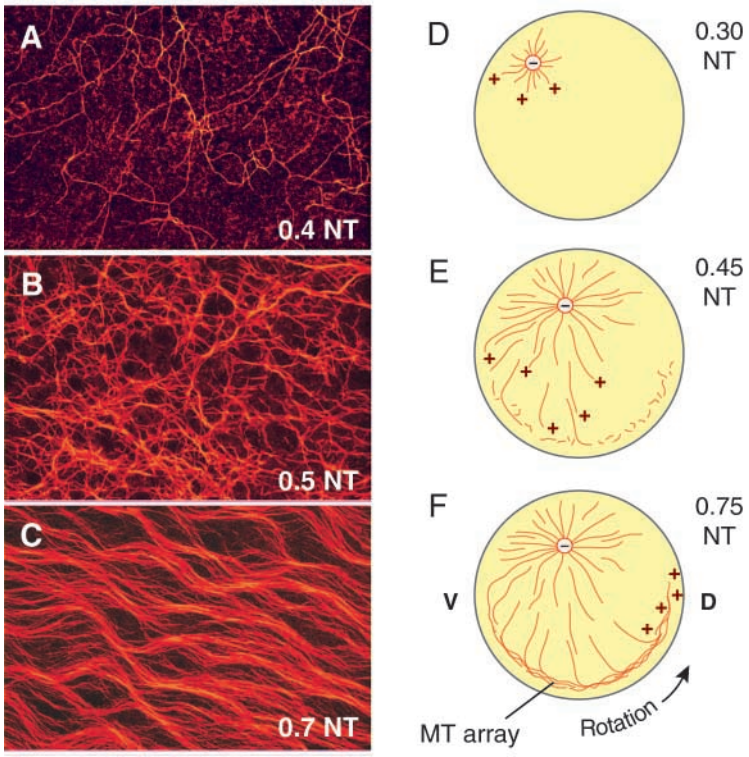

Fig. 2. Formation of the microtubule (MT) array. (A-C) Vegetal views of microtubule array formation during the first cell cycle. Times shown are normalized times (NT), with 0.0 NT representing fertilization and 1.0 NT representing the first cleavage division. (A) At 0.4 NT, short disorganized MT polymers have started to appear in the vegetal shear zone. (B) By 0.5 NT, more MTs are present, but are not yet aligned. (C) By 0.7 NT, during peak cortical rotation, the vegetal shear zone is populated by a parallel array of MT bundles that are aligned along the axis of rotation. (D-F) MTs of the vegetal array arise from several sources. (D) The sperm centriole introduces polarity by acting as a minus-end MT-organizing center (–). The resulting radial array of MTs is called the sperm aster. (E) MTs from the sperm aster grow toward the periphery of the egg, as do additional MTs from unknown sources in the core cytoplasm. In addition, short disorganized MT polymers arise in the vegetal shear zone. (F) During rotation, MTs from deep in the cytoplasm bend into the vegetal shear zone and align with peripheral MTs to form the parallel array, with the plus-ends (+) of the growing MTs pointing towards the future dorsal (D) side of the embryo. V, ventral. (A-C) Reproduced, with permission, from Cha and Gard (Cha and Gard, 1999). (D-F) Adapted, with permission, from Houliston and Elinson (Houliston and Elinson, 1991b).

Holowacz and Elinson, 1993; Kikkawa et al., 1996; Sakai, 1996; Kageura, 1997). This distribution suggested that the activity can somehow move faster than the speed of rotation, as the cortex moves only about 30° during the period of transport. It was therefore very intriguing when unidentified, membrane-bound organelles in the subcortical shear zone were discovered to translocate very quickly towards the prospective dorsal side, with velocities two to three times faster than that of cortical rotation (Rowning et al., 1997). Microinjected carboxylated beads are similarly capable of fast directed transport in the vegetal shear zone, and can spread more than 60° from their site of injection in the vegetal pole (Rowning et al., 1997). The velocities and behavior of the organelles and beads strongly suggested that their transport is mediated by MT motor proteins.

Although the significance of these organelles in the process of dorsal axis formation remains unknown, the observation that they translocate was the first evidence that cargo transport by MT motor proteins is likely to occur in the shear zone. The orientation of the MTs, with their plus-ends directed dorsalwards, implied the involvement of a plus-end-directed motor protein. All known plus-end-directed MT motors are members of the large superfamily of kinesin-related proteins (KRPs) (reviewed by Vale, 2003) (see also the Kinesin Home Page at http://www.proweb.org/~kinesin). A model was proposed in which the dorsal determinants, rather than being 'passively' carried by virtue of association with the cortex during rotation, might instead travel as cargo that is associated with kinesin motor proteins and that moves directly on the MT array (Kikkawa et al., 1996; Rowning et al., 1997).

In order to determine the validity of this model, it becomes essential to know two things. First, which molecules comprise the dorsalizing activity? And second, can a motor protein be identified that transports them? In the following section, we address the first of these questions, describing work that has been carried out to identify candidate dorsalizing proteins. Although this is still a work in progress, advances made in recent years indicate that these molecules are likely to be β-catenin regulatory proteins that act in the canonical Wnt signaling pathway.

The Wnt pathway during early frog development

The canonical Wnt signaling pathway is used by numerous organisms to make cell fate decisions in many developmental and cellular contexts. Its defining feature is the use of β-catenin as a downstream effector. In the absence of a Wnt signal, a host of proteins cooperate to target β-catenin for degradation by the ubiquitin-proteasome pathway. Active Wnt signaling causes the stabilization of β-catenin, allowing it to accumulate in the nucleus where it activates Wnt target genes as part of a complex with T-cell factor/Lymphoid enhancer factor (TCF/LEF) transcription factors. β-Catenin regulation by the Wnt pathway has been extensively reviewed elsewhere (Miller et al., 1999a; Dominguez and Green, 2001; Huelsken and Behrens, 2002).

The localized stabilization of β-catenin on the future dorsal side of the early *Xenopus* embryo (Fig. 1C) is a crucial event in the formation of the dorsal organizer; depletion of maternal β-catenin can cause a complete loss of dorsal structures in the embryo and ectopic expression of β-catenin ventrally leads to the formation of a secondary dorsal axis (Heasman et al., 1994; Funayama et al., 1995; Larabell et al., 1997). A key goal, therefore, is to elucidate the biochemical steps that connect the translocation of the dorsalizing activity to the stabilization of β-catenin. Movement of the dorsalizing activity is thought to be essential for β-catenin accumulation in the dorsal margin,

which does not occur if vegetal MT polymerization is blocked (Larabell et al., 1997). An obvious hypothesis is that the moving activity is β-catenin itself, which accumulates dorsally via active transport. However, as subcortical vegetal cytoplasm from β-catenin-depleted *Xenopus* embryos is still able to induce a secondary axis when transplanted into a normal host (Marikawa and Elinson, 1999), it appears more likely that the dorsalizing activity consists of an upstream factor or factors that move to the dorsal region in order to stabilize β-catenin there.

In the canonical Wnt pathway, the regulation of β-catenin stability is controlled by a complex of proteins called the β-catenin destruction complex. Central to this complex are two large proteins, Axin and Adenomatous polyposis coli (APC), which are thought to act by shuttling β-catenin in and out of the complex (reviewed by Bienz, 1999). In the absence of a Wnt signal, β-catenin is phosphorylated within the complex by two kinases, Casein Kinase 1α (CK1α) and GSK3, which marks β-catenin for ubiquitination and degradation (Fig. 3A) (reviewed by Dominguez and Green, 2001; Polakis, 2002). During active Wnt signaling, this process is inhibited through an only partially understood mechanism, which causes β-catenin to be stabilized.

In most biological contexts, an extracellular Wnt ligand is responsible for activating the upregulation of β-catenin stability. In *Xenopus* embryos, the microinjection of RNA that encodes canonical Wnts can indeed induce the formation of dorsal axial structures (McMahon and Moon, 1989; Smith and Harland, 1991; Sokol et al., 1991). However, on the basis of available expression data and known activities, no maternal Wnt ligand has yet emerged as a strong candidate dorsalizing factor in the early *Xenopus* embryo (Cui et al., 1995; Smith et al., 2000), although the demonstration that the Wnt receptor Frizzled 7 is somehow involved in *Xenopus* dorsal axis formation leaves open the possibility that Wnt-mediated signaling is used in this process (Sumanas et al., 2000). An interesting alternative idea is that a Wnt-independent mechanism is used to activate intracellular Wnt signaling components during dorsal axis specification. How Frizzled 7 functions in this process is still an important issue to be resolved.

Intracellular components of the Wnt pathway are clearly involved in *Xenopus* axis specification. One protein of particular interest is the vertebrate-specific GSK3-binding protein GBP, which is thought to act on the dorsal side to inhibit GSK3 function (Yost et al., 1998; Dominguez and Green, 2000). GBP binds directly to Dsh, a GSK3 inhibitor that is activated by a Wnt signal in the canonical pathway (Yost et al., 1998; Li et al., 1999; Salic et al., 2000). In the *Xenopus* embryo, Dsh is thought to act in part by recruiting GBP to the destruction complex (Salic et al., 2000). GBP binds GSK3 and removes it from Axin, and also causes GSK3 degradation (Dominguez and Green, 2000; Farr et al., 2000). GSK3 is thereby prevented from phosphorylating β-catenin (Fig. 3B), causing β-catenin levels to increase.

How strong is the evidence that GBP and Dsh are components of the endogenous dorsalizing activity? Like β-catenin and Wnt, GBP and Dsh are both capable of inducing the formation of a complete dorsal axis when their RNAs are microinjected into early *Xenopus* embryos (Sokol et al., 1995; Yost et al., 1998). Furthermore, overexpressed GBP mimics the endogenous dorsalizing activity in that it causes GSK3 degradation in the equatorial region (Dominguez and Green, 2000). Most importantly, GBP has been shown to be required for dorsal axis formation in *Xenopus* through the use of antisense oligonucleotides, which can be injected into the egg to target specific mRNAs for degradation prior to fertilization (Yost et al., 1998). The case is less clear for Dsh, as loss-of-

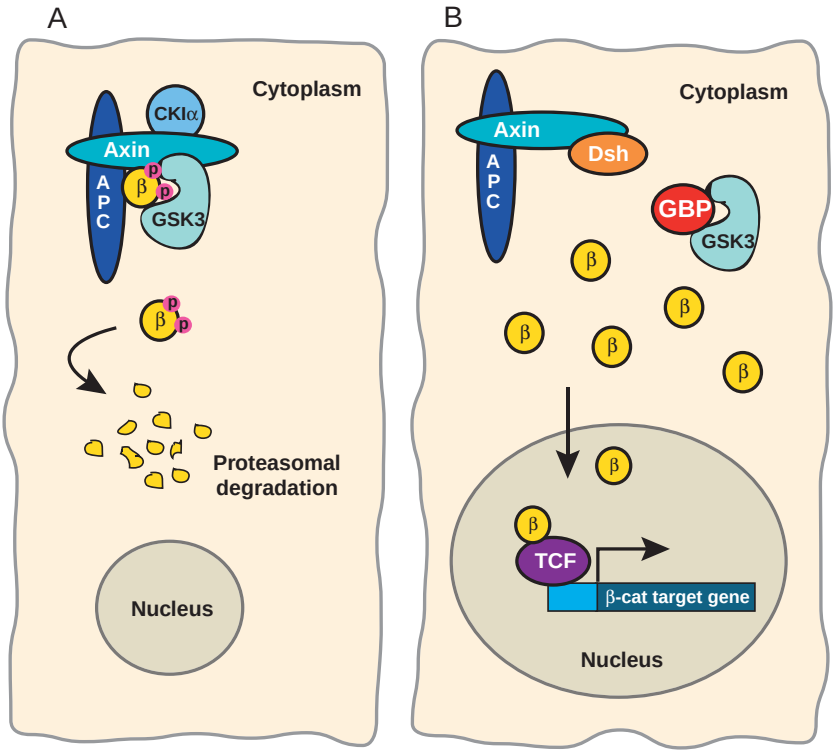

Fig. 3. The β-catenin destruction complex and its regulation by GBP and Dsh. (A) The destruction complex contains the large proteins Axin and APC, which bring β-catenin (β) into the complex and into close proximity to GSK3 and CK1α. These kinases phosphorylate (p) N-terminal residues in β-catenin and target it for degradation by the ubiquitin-proteasome pathway. (B) GBP and Dishevelled (Dsh) cooperatively inhibit the phosphorylation of β-catenin by the destruction complex. Dsh binds Axin and may help recruit its binding partner GBP to the destruction complex. GBP removes GSK3 from Axin, thereby disrupting the ability of GSK3 to phosphorylate β-catenin. Unphosphorylated β-catenin accumulates and enters the nucleus to activate the transcription of its target genes in a complex with TCF/LEF box transcription factors.

function experiments reducing Dsh levels in the egg prior to fertilization have not yet been reported. In embryos, the microinjection of RNA that encodes a dominant-negative Dsh had no effect on dorsal axis formation; however, the RNA was injected at the two-cell stage, too late to have an effect if Dsh has a required role during the translocation events of the first cell cycle (Sokol, 1996). A finding that supports a role for Dsh in this process is that endogenous Dsh protein is found at higher levels in the dorsal shear zone than in the ventral shear zone as early as the two-cell stage (Miller et al., 1999b). The distribution of endogenous GBP protein in the embryo is not yet known.

Experiments using various ventralizing molecules to block the activation of dorsal markers by transplanted vegetal cytoplasm suggest that neither GBP nor Dsh alone is the key β-catenin stabilizing factor in the vegetal shear zone (Marikawa and Elinson, 1999). One possibility is that GBP and Dsh synergize to stabilize β-catenin levels, as was recently shown in *Xenopus* egg extracts (Salic et al., 2000), or that they act in combination with other dorsalizing proteins (see Weaver et al., 2003). An alternative possibility is that GBP, Dsh, or both, are not part of the moving dorsalizing activity, but are instead somehow activated by it once it reaches the dorsal equatorial region. Therefore, two key questions need to be answered: (1) what is the relationship of GBP and Dsh to the moving dorsalizing activity; and (2) how is this activity transported to the future dorsal side?

Particles and motor proteins in the shear zone

An important breakthrough occurred when Miller and colleagues developed an assay to monitor the behavior of green fluorescent protein (GFP) fusion proteins in the vegetal shear zone of live *Xenopus* eggs during cortical rotation. Using this assay, both Dsh-GFP and GBP-GFP were found to form small particles in the vegetal shear zone that move quickly in the direction of cortical rotation, with roughly the same velocity and saltatory behavior that was earlier observed for translocating organelles in that region (Miller et al., 1999b; Weaver et al., 2003). The particle movements depend on the parallel MT array, as evidenced by the fact that the transport of Dsh-GFP was disrupted by UV irradiation. Conversely, treatment of embryos with D_2O, which causes precocious excessive polymerization of randomly oriented MTs, causes Dsh-GFP translocation to become randomized (Miller et al., 1999b). Some deletions within conserved regions of Dsh and GBP prevent them from assembling into particles, suggesting a requirement for specific protein-protein interactions (Miller et al., 1999b; Weaver et al., 2003). As further evidence of the specificity of the behavior, β-catenin, which is not a required component of the moving dorsalizing activity, does not form particles or translocate (Miller et al., 1999b). These results show that GFP-tagged GBP and Dsh are able to translocate to the presumptive dorsal side, supporting the idea that GBP and Dsh form part of the endogenous moving dorsalizing activity.

But what mediates the transport of GBP and Dsh? A yeast two-hybrid screen using murine GBP [called Frat1 (frequently rearranged in advanced T-cell lymphomas)] as bait provided the first insight into this question when it revealed that GBP binds to a kinesin light chain (KLC) (Weaver et al., 2003). This interaction was confirmed by co-immunoprecipitation of tagged mouse and *Xenopus* proteins from cultured mammalian cells and *Xenopus* embryos, respectively (Weaver et al., 2003). KLCs are cargo-binding subunits of conventional kinesin, a heterotetrameric MT motor. Conventional kinesins, which also contain two motor-containing heavy chain subunits, move along MTs from the minus- to plus-ends (reviewed by Verhey and Rapoport, 2001), which fits well with the fact that the dorsalizing activity moves towards the plus-ends of oriented MTs. A *Xenopus* KLC, fused to GFP, was shown to exhibit the same behavior as GBP and Dsh, forming particles that translocate quickly in the direction of cortical rotation (Fig. 4; see movies at http://dev.biologists.org/cgi/content/full/130/22/5425/DC1). Small deletions in a conserved domain of GBP-GFP disrupt both its ability to form translocating particles and to bind KLC, supporting the idea that GBP depends on its interaction with KLC in order to move (Weaver et al., 2003). As GBP also binds Dsh, a preliminary model has emerged in which GBP mediates the transport of dorsalizing particles by binding kinesin.

The most significant impact of the live imaging studies with GBP and Dsh is confirmation that dorsalizing proteins are capable of moving directly on the MT array, which provides an explanation for the ‘underlying system’ that was first proposed by Gerhart and colleagues to be the real mechanism of dorsal axis specification. It is now worth re-examining one of the original experiments that led to the proposal of this underlying system. If *Xenopus* embryos are treated with D_2O prior to cortical rotation, which causes precocious and randomized MT polymerization, the development of hyperdorsalized embryos results. In the most extreme cases, the embryos are completely dorsalized, despite a severe reduction in cortical rotation that occurs because of the misalignment of subcortical MTs (Vincent and Gerhart, 1987; Vincent et al., 1987; Scharf et al., 1989). This observation argues that cortical rotation is not the principal, or at least not the only, mechanism for dorsalizing activity transport, as the two phenomena can be experimentally uncoupled (Rowning et al., 1997). On the basis of recent findings, we suggest that D_2O treatment hyperdorsalizes embryos because the randomized MT bundles allow kinesin/GBP/Dsh particles to travel from the bottom of the embryo towards more than one location at the equator, resulting in ectopic β-catenin stabilization at the equator and the formation of an increased amount of dorsal organizer tissue (Miller et al., 1999b; Weaver et al., 2003).

Interestingly, there is evidence that the motor-based transport of dorsal determinants may also occur in other species. For example, in the zebrafish embryo, there appears to be one or more transient parallel MT arrays used to transport dorsal determinants from the vegetal cortex of the large yolk cell up to the embryonic blastomeres in the animal hemisphere. As in the frog, microinjected beads can translocate upwards in the periphery of the zebrafish yolk cell in a manner that suggests active transport by motor proteins (Jesuthasan and Stahle, 1997) (reviewed by Pelegri, 2003). There is also evidence of a motor-based transport system in one-cell medaka embryos. In this species, a parallel array of MTs forms in the vegetal region that are aligned with the DV axis and appears to act as tracks for the movement of cargo towards the future dorsal side (Abraham et al., 1995; Trimble and Fluck, 1995). Despite these similarities to events in the amphibian embryo, embryos of teleost fish, such as zebrafish and medaka, do not undergo cortical rotation (Ho, 1992). Therefore, it appears to

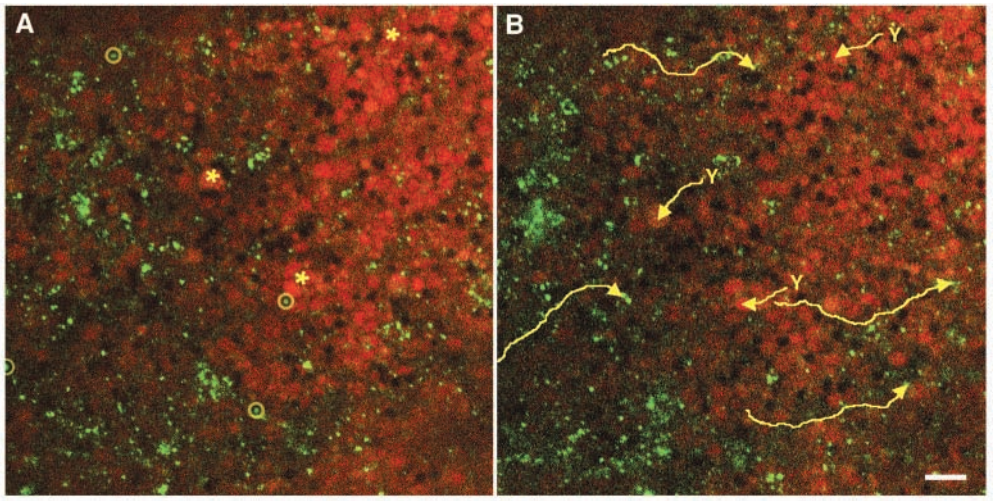

Fig. 4. Translocation of kinesin light chain (KLC)-GFP particles during cortical rotation. *Xenopus* KLC-GFP was expressed and imaged with a scanning confocal microscope in the vegetal shear zone of immobilized live eggs during the first cell cycle. (A) KLC-GFP particles (green) are observed in the vegetal shear zone during peak rotation. Yolk platelets of the core cytoplasm are stained in red. The starting positions of four KLC-GFP particles are circled, and three neighboring yolk platelets are marked with asterisks. (B) Time-lapse image showing the same field of view ~38 seconds later than in A. Owing to the immobilization of the egg, the core rotates opposite to the normal direction of cortical rotation, as seen by the displacement of the three core yolk platelets (Y) from right to left. The KLC-GFP particles have translocated a longer distance in the opposite direction (i.e. in the same direction cortical rotation would normally move), from left to right. Scale bar: 5 μm. Reproduced, with permission, from Weaver et al. (Weaver et al., 2003).

be possible to achieve the transport of dorsal determinants solely by translocating the dorsalizing activity directly on MTs. It is not clear how far the parallels go, but as zebrafish also have maternally deposited GBP and Dsh, and depend on dorsally stabilized β-catenin, much of the dorsalizing machinery may be conserved between fish and frogs (Sumoy et al., 1999; Kimelman and Schier, 2002) (J. Waxman and R. T. Moon, personal communication). Interestingly, embryos of more primitive fish, such as sturgeon, appear to undergo cortical rotation (Clavert, 1962), suggesting that perhaps it is an ancient process that has been lost in more derived fish species, while the MT-based transport of determinants has been conserved.

Cortical rotation and the alignment of the microtubule array

Cortical rotation is a conserved process that demands energy expenditure by the embryo during a crucial time in its development (Vincent et al., 1987). But if the dorsalizing activity can travel directly on the MT array, what purpose does the rotation serve? Evidence supports at least two possible functions. First, cortical rotation might serve to align the polymerizing MTs into parallel bundles and to orient their plus-ends towards the dorsal side. Second, as will be discussed later, cortical rotation might directly contribute to the overall dorsalwards movement of the dorsalizing activity.

How would rotation align the MTs? Although several scenarios can be imagined (see Marrari et al., 2003), the simplest model is that plus-end-directed motor proteins attached to the cortex move along the MTs and align them as the cortex moves towards their plus-ends, analogous to a comb moving through hair. The action of these KRP motors on the subcortical MTs could simultaneously align the MTs and generate the force required to translocate the cortex relative to the core cytoplasm. In support of this model, the injection of antibodies that inhibit KRPs into the vegetal shear zone causes MT flailing, which disrupts the organization of the MT array and causes a local arrest in cortical rotation movements (Marrari et al., 2000). Interestingly, very recent results have shown that the inhibition of the minus-end-directed MT motor dynein early in the first cell cycle of *Xenopus* development also leads to defects in cortical rotation, but for different reasons. In contrast to KRPs, dynein is not required for MT alignment in the shear zone, nor for generating force during cortical rotation; instead, dynein appears to contribute to the initial formation of the MT array by pushing MTs from the inner cytoplasm out towards the cortex (Marrari et al., 2004). As KRPs and dyneins make up the only two known classes of MT motor proteins, these results indicate that members of the KRP superfamily – the majority of which are plus-end-directed motors – must be responsible for organizing and aligning the MT array in the shear zone (Vale, 2003). Interestingly, Marrari and colleagues recently observed that the vegetal MT bundles, although initially thick and wavy in appearance, take on a straighter, more ‘fine-combed’ appearance towards the end of cortical rotation (Marrari et al., 2004). It is possible that this fine-combing effect is the result of the continual action of cortically attached motors moving along the length of the MTs.

Does cortical rotation help transport dorsal determinants?

On the basis of the results described above, it is possible to imagine a scenario in which cortical rotation serves only to align MTs, which are then used as tracks by kinesin motors that directly transport the dorsalizing activity to the equatorial region. But does cortical rotation also have a more direct role

in moving dorsal determinants, as the traditional view of its purpose holds? As already described, cortical rotation can be experimentally uncoupled from axis formation by D_2O treatment, which indicates that dorsal determinant transport can occur robustly in the absence of rotation. Yet, some evidence suggests that cortical rotation contributes to the overall movement of dorsal determinants towards the equator. In UV-treated embryos, where motor-based cargo transport is unlikely to occur because of a lack of polymerized MTs, dorsal axis formation can be rescued by centrifugation or by tilting the embryo, which mimics cortical rotation by forcing a displacement of the core cytoplasm relative to the immobilized cortex (Scharf and Gerhart, 1980; Black and Gerhart, 1986; Ziskind and Elinson, 1990). This rescue could occur if some dorsal determinants were associated with the vegetal cortex, causing their relative displacement towards the equatorial region of the core during centrifugation. Furthermore, although the most potent dorsalizing activity is concentrated tightly in a region at the vegetal pole, weak dorsalizing activity can be found in subcortical cytoplasm as far as 60° from the vegetal pole prior to cortical rotation (Kikkawa et al., 1996). It is therefore possible that a 30° rotation would be sufficient to transport enough dorsal determinants to the equator to induce axis formation. Thus, although there is good evidence for the particle model of transport, the traditional model in which cortical rotation moves dorsal determinants must still be taken into consideration. One way to reconcile the evidence that supports each of these models is to propose that dorsal determinant transport relies on a combination of both transport mechanisms, which together are more robust than either one acting alone. In support of this idea, translocating GBP-GFP particles in live eggs frequently appear to jump on and off of the MT array, interspersing periods of fast transport with slower periods moving at the speed of the cortex (see movie at http://dev.biologists.org/cgi/content/full/130/22/5425/DC1) (Weaver et al., 2003). Thus, cortical rotation might help keep determinants moving in the right direction.

An improved model for axis determination

To bring together the results discussed in this review, we suggest an updated model for how the dorsal determinants

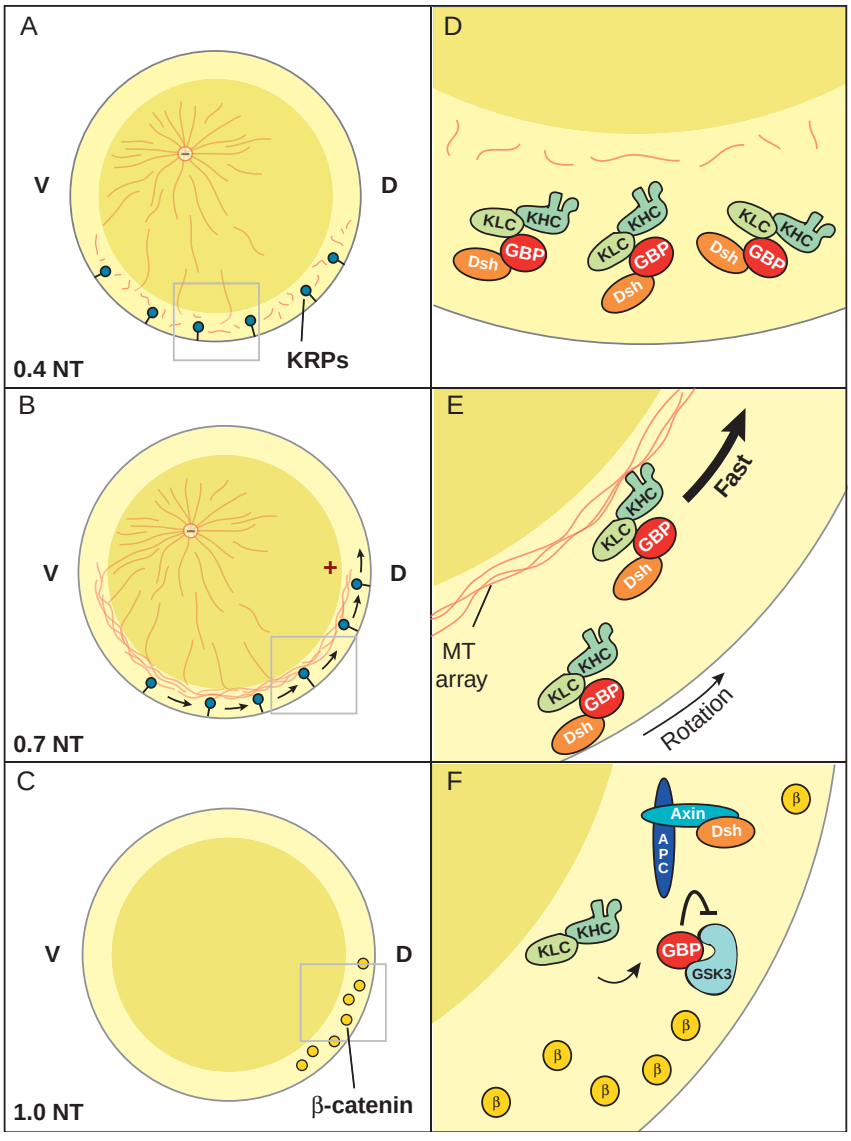

Fig. 5. Model for alignment of the MT array and the transport of the dorsalizing activity. (A) The sperm centriole (–) organizes microtubules (MTs) near the sperm entry point in the animal hemisphere. As cortical rotation initiates, disorganized MTs populate the vegetal shear zone, arising from both deep and peripheral sources. MTs are shown as red lines, and plus-end directed kinesin-related motor proteins (KRPs) attached to the cortex are shown as dark blue circles. (D-F) Enlargements of boxed regions shown in A-C. (D) Early in the first cell cycle, dorsalizing particles nucleate in the vegetal shear zone of the embryo. GBP, which is perhaps associated with other dorsalizing proteins such as Dishevelled (Dsh), interacts with the plus-end-directed motor protein kinesin by binding its cargo-carrying subunit, kinesin light chain (KLC). (B) As cortical rotation progresses, plus-end-directed KRP motor proteins tethered to the moving cortex associate with the MTs and move along their length, which serves to align them so that their plus-ends grow in the same direction that the cortex is moving. (E) As the MT array aligns, kinesin carries particles quickly towards the MT plus-ends, which are directed towards the future dorsal region near the equator. Some particles are transported more slowly in the same direction by associating with the rotating cortex. (C) Towards the end of the first cell cycle, the MT array depolymerizes, and cortical rotation and kinesin-based transport cease. (F) In the dorsal region, GBP dissociates from kinesin in favor of binding to GSK3. The interaction of GBP and GSK3 may be facilitated by Dsh binding to Axin, which would bring GBP to the destruction complex. GBP removes GSK3 from Axin, allowing β-catenin (β) to be stabilized in the dorsal region (also indicated in C). Stabilized β-catenin enters dorsal nuclei and activates transcription of dorsal organizer genes, ultimately resulting in the formation of the DV and AP axes. D, dorsal side; KHC, kinesin heavy chain; NT, normalized time; V, ventral.

cause β-catenin stabilization on the dorsal side of the *Xenopus* embryo. During oogenesis, particles are assembled at the vegetal pole, which contain kinesin, GBP, Dsh and, most probably, other components (Fig. 5D). When the sperm fertilizes the egg, it activates embryogenesis and introduces a centriole, which acts as an initial minus-end MT-organizing center (Fig. 5A). A combination of MT polymerization and cortical rotation acts to orient the MTs throughout the vegetal shear zone, such that midway through the first cell cycle most of the cortical MTs are oriented in the same direction (Fig. 5B). The MTs serve as tracks on which the particles can move. We suggest that the particles move by alternating periods of fast transport on the MTs with slower transport, when they are associated with the cortex (Fig. 5E). Cooperation between these two methods of transport may be important to ensure that the bulk of the dorsalizing particles undergo a net dorsalwards movement. Once the particles have moved to their new location, the dorsalizing proteins disassemble from the particles to inhibit the phosphorylation of β-catenin by GSK3, thus allowing β-catenin to escape degradation and accumulate locally (Fig. 5C,F). β-catenin later activates the transcription of dorsal organizer genes, ultimately resulting in the formation of the DV and AP axes.

Conclusions

Evidence is accumulating to support the idea that the early dorsal organizer in frogs is activated by the kinesin-dependent transport of particles containing regulators of the Wnt intracellular pathway. Although many questions remain to be answered (Box 1), this view ties together the 'classical' embryological studies of Gerhart and colleagues with more recent molecular studies. Although some aspects of the proposed particle transport process are specific to amphibian development, particularly the phenomenon of cortical rotation, this mechanism fits well with the emerging view that a wide range of biological systems use MT motor-dependent transport to move signaling proteins and other developmentally important molecules asymmetrically within embryos, oocytes and cells (Cohen, 2002; Schnapp, 2003; Betley et al., 2004; Gunawardena and Goldstein, 2004).

Box 1. Key unanswered questions regarding the transport of dorsal determinants in *Xenopus* embryos

- Which proteins make up the dorsalizing determinants? Although a few candidates have been identified, it is likely that the particles contain other components.
- How (and when) are dorsalizing particles assembled and disassembled? The particles need to be assembled at the vegetal pole prior to cortical rotation and then disassembled near the equator. How this is regulated is unknown.
- How does the Wnt receptor Frizzled 7 contribute to dorsal axis formation, and how does it relate to the moving dorsalizing activity? Is a Wnt signal involved?
- Which motors move the particles and which motors move the cortex?

We thank Wilson Clements and Tom Reh for comments on the manuscript, and David Gard for supplying pictures and for helpful suggestions. We also thank Josh Waxman and Randall Moon for sharing unpublished results, and Carolyn Larabell for help with Fig. 4.

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