The roles of maternal α-catenin and plakoglobin in the early Xenopus embryo

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SUMMARY

Catenins (α, β and γ or plakoglobin) are cytoplasmic cadherin-associated proteins. Studies on cultured cells have suggested that both α-catenin and plakoglobin are important for the adhesive function of cadherins. α-catenin binds to both β-catenin and plakoglobin and may link the cadherin/catenin complex to actin filaments. Separate domains of plakoglobin bind to cadherin and α-catenin, suggesting it may act as a bridge between these molecules. However, plakoglobin may have other activities: it is expressed in both desmosomal junctions in association with desmogleins and the cytoplasm in conjunction with APC, and previous work suggests it may act in a dorsal signalling pathway when overexpressed in Xenopus embryos. Here, we have studied the roles of α-catenin and plakoglobin directly, by depleting the maternal mRNAs coding for each of them in developing Xenopus embryos. We find that depletion of maternal α-catenin causes the loss of intercellular adhesion at the blastula stage, similar to that reported previously for EP cadherin. Depletion of plakoglobin results in a partial loss of adhesion, and a loss of embryonic shape, but does not affect dorsal signalling.

Key words: α-catenin, plakoglobin, intercellular adhesion, cadherin, maternal α-catenin, Xenopus

INTRODUCTION

The catenins (α, β- and γ- or plakoglobin) are ubiquitous molecules first identified by their co-immunoprecipitation with adhesion molecules of the cadherin class (Kemler, 1992; Ozawa et al., 1989, 1990; Ozawa and Kemler, 1992). The first studies on catenins were carried out in cultured cells in vitro. These studies suggested that α-catenin is an essential requirement for cadherin-based adhesion, and that β-catenin and plakoglobin are necessary links between α-catenin and cadherin (reviewed in Kemler, 1993). More recently, this work has been complemented by investigations of the functions of the proteins in whole organisms. E-cadherin →− mice develop to the blastocyst stage but do not form a normal trophectoderm (Larue et al., 1994; Riethmacher et al., 1995), while β-catenin →− mice form a normal trophectoderm and progress to the egg-cylinder stage (Haegele et al., 1995). The increased expression of plakoglobin in β-catenin−/− blastocysts compared to wild-type levels suggest that plakoglobin may substitute for at least some of the functions of β-catenin in these embryos. Plakoglobin →− mice develop until 12-16 days of embryogenesis and die because of heart abnormalities (Ruiz et al., 1996). In Drosophila, the functions of armadillo (the homolog of β-catenin) have been shown to be more complex than was suggested by in vitro cell culture studies. It is required both for adherens junction function (Cox et al., 1996) and as a component of the wingless signalling pathway required for patterning of the early embryo (Wieschaus et al., 1984).

Here, and in previous work, we have undertaken functional studies of individual cadherins and catenins in Xenopus embryos by depleting the maternal stockpile of mRNA in oocytes and studying the effects on early development (Heasman et al., 1994a,b; Wylie et al., 1996). Xenopus embryos do not start transcription until the mid-blastula stage, making this a convenient approach to the study of intercellular adhesion in the blastula. Xenopus embryos are large and intercellular adhesion can be assayed easily by dissection and simple aggregation assays. Early stages in pattern formation, such as the formation of the mesoderm and the dorsal axis, can also be studied easily in Xenopus. We have shown previously that depletion of EP cadherin causes loss of intercellular adhesion at the blastula stage (Heasman et al., 1994b). In contrast, β-catenin-deficient embryos have no observable defect in adhesion but lack all dorsal axial structures (Heasman et al., 1994a), while overexpression of β-catenin dorsalizes Xenopus embryos (Funayama et al., 1995). Recently, the mechanism by which this occurs has been suggested by the finding that β-catenin binds to the DNA-binding protein XTcf-3, and together they are transported into cell nuclei (Molenaar et al., 1996). Furthermore, β-catenin has been reported to be localized in the nuclei on the dorsal side of Xenopus embryos at the midblastula stage (Schneider et al., 1996).

In this paper, we continue the analysis of the roles of the maternal catenins, specifically studying α-catenin and plakoglobin, in the early Xenopus embryo. Previous in vitro studies have suggested that α-catenin may only be involved in adhesion, but that plakoglobin may have a broader range of function. Plakoglobin, unlike the other catenins, is found both in
adherens junctions and desmosomes (Cowin et al., 1986), and is also found in the cytoplasm associated with the APC protein (Rubinfeld et al., 1995). In desmosomes, it binds directly to desmosomal cadherins and does not appear to interact with α-catenin (Heid et al., 1994; Roh and Stanley, 1995; Troyanovsky et al., 1994a,b). In some situations, the subcellular distributions of plakoglobin and β-catenin are different, which may also indicate that they have different functions (Nathke et al., 1994). However, β-catenin and plakoglobin are structurally related molecules (Peifer et al., 1992), and their overexpression results in nuclear localization and causes similar phenotypes, suggesting they may have similar functions, (Funayama et al., 1995; Karnovsky and Klymkowsky, 1995).

Here we report that depletion of maternal α-catenin causes loss of intercellular adhesion at the blastula stage, similar to that reported for EP cadherin. Depletion of plakoglobin results in a partial loss of adhesion, and a loss of embryonic shape, but does not affect the formation of the dorsal axis, unlike the phenotype caused by depletion of β-catenin.

MATERIALS AND METHODS

Oocytes and embryos

Full-grown stage VI oocytes were manually defolliculated, injected with antisense oligos or mRNA and cultured at 18°C. In rescue experiments, oocytes were injected with oligo on day 1, and with mRNA on day 2, after endogenous mRNA had been broken down (as shown in Fig. 1A, lanes 3 and 6) and after the injected oligo had degraded. Oocytes were stimulated to mature overnight using progesterone for approximately 10 hours and fertilised by transferring into the abdominal cavity of anesthetised frogs. The host frogs had been stimulated with human chorionic gonadotrophin 12 hours previously, to deplete the mRNAs in oocytes, by injecting 6 and 12 ng of untranslated mRNA (a gift from Dr P. Remy). The template was linearized with SfiI and transcribed in vitro using T3 RNA polymerase. Xenopus plakoglobin cDNA subcloned into SP64T was used as described previously (Karnovsky and Klymkowsky, 1995).

RNAs were ethanol precipitated and resuspended in sterile distilled water for injection. Injections were carried out using amounts of 1.2 to 2.0 ng.

Northern Blot analysis

Oocyte or embryo RNA was extracted as described previously (Gurdon et al., 1985). Electrophoresis and northern blotting were performed as described by previously (Hopwood et al., 1989) using two embryo equivalents per lane. The probes were synthesized by random priming of the excised inserts of α-catenin (EcoRI), plakoglobin (EcoRI and XbaI), MyoD (EcoRI and XhoI). Blots were stripped and rehybridized with probes for elongation factor EF1α as a loading control.

Aggregation assays

The aggregation assays were carried out on mid-blastula cells as described previously (Turner et al., 1992), except that aggregation was carried out in OCM, over a time course of 45 minutes to 2 hours. Briefly embryos were devitellined and allowed to disaggregate by a brief exposure to 67 mM phosphate pH 7.2, followed by dispersal in OCM on 2% agarose-coated plates. Groups of 40-80 cells were taken from the pool and blown together in OCM with a mouth pipette and allowed to aggregate for times of 1 or 2 hours without disturbance. The degree of aggregation was compared between experimental (oligo-injected) and control (uninjected, or injected with oligo on day 1 and rescuing mRNA on day 2).

Western blot analysis

Protein extracts were prepared either by the freon extraction method, for α-catenin, to remove yolk from protein lysates (Gurdon and Wickens, 1983) or as described previously, for plakoglobin (Detrick et al., 1990). Homogenates were cleared by centrifugation and the supernatants were combined with 5× SDS-DTT sample buffer and boiled for 5 minutes.

Samples were resolved by 8.5% SDS-PAGE, transferred to Immobilon-P membrane (Millipore) or nitrocellulose and blocked overnight as described elsewhere (Beckerle, 1986). Immunoblotting was performed using the electrochemiluminescence method (Amersham).

Polyclonal anti-α-catenin and anti-β-catenin antibodies was used at a dilution of 1:1000 and were gifts of Dr P. D. McCrea. Monoclonal anti-plakoglobin antibody (Transduction Laboratories) was used at dilution 1: 500. Anti-β1 integrin antibody (Gawantka et al., 1992; Developmental Studies Hybridoma Bank) was used at 1:20 of hybridoma supernatant.

Cell fractionation

Embryos were separated into insoluble and soluble fractions as described by Funayama et al. (1995). Briefly, five blastulae (stage 8) were homogenized in 250 µl of ice-cold 250 mM sucrose, 10 mM

Oligos: A3 5′-AGTCTCTCAACGGCTAGT3′

Oligo M7 5′-GGCTGTCGTTAGTCTGATG3′

Oligos were resuspended and stored at −80°C in sterile distilled water at 1 mg/ml concentration and injected in doses of 1.75-4 ng as described for each experiment.

RNA

Mouse α-catenin cDNA in pBluescript vector (Nagafuchi et al., 1991) was a gift of Dr M. Takeichi and was subcloned into pRN3 vector (a gift from Dr P. Remy). The template was linearized with SfiI and transcribed in vitro using T3 RNA polymerase. Xenopus plakoglobin cDNA subcloned into SP64T was used as described previously (Karnovsky and Klymkowsky, 1995).

RNAs were ethanol precipitated and resuspended in sterile distilled water for injection. Injections were carried out using amounts of 1.2 to 2.0 ng.
Hepes-NaOH, 2 mM MgCl₂, 1 mM EGTA, 0.5 mM EDTA, pH 7.4, and spun at 750 g for 5 minutes at 4°C in an Eppendorf microcentrifuge 5415C to remove the yolk platelets, pigment granules and nuclei. The supernatants were then spun at 100,000 g for 1 hour at 4°C in a tabletop ultracentrifuge TL-100 (Beckman Instruments Inc, Fullerton, CA) using a TLA 120.1 rotor. Soluble and sedimentable fractions were collected as the supernatant and pellet fractions, respectively.

RESULTS

Antisense oligos specifically deplete α-catenin and plakoglobin mRNAs

Fig. 1A,B shows the depletion of maternal mRNA for α-catenin and plakoglobin, respectively, after the injection of antisense oligos A3 (4 ng) for α-catenin, and M7 (1.7 ng) for plakoglobin into full-grown oocytes. The depletion of α-catenin mRNA was quantified as 75% of the original amount, while for plakoglobin 80% was depleted. In both cases, the depletion continued through the cleavage and blastula stages, and the level began to increase again by the gastrula stage, after the transcription of zygotic mRNAs began. Wild-type levels were restored by the neurula stage. The reduction of the RNAs was sufficient to cause substantial depletion of maternal α-catenin and plakoglobin protein (Fig. 1C,D).

The efficiency of the depletion of plakoglobin and α-catenin was further examined by comparing the amounts remaining in insoluble and supernatant fractions from mid-blastula-stage embryos developed from oligo-injected oocytes. In each experiment, sibling, oligo-injected embryos to those used in these analyses, were shown to have the characteristic phenotypes described in the following paragraphs. For both proteins, depletion occurred in both fractions compared to uninjected control samples (Fig. 1E). In contrast, embryos developed from β-catenin-depleted oocytes had substantial amounts of β-catenin remaining in the insoluble fraction (Fig. 1F), while most was depleted from the supernatant fraction. Sibling β-catenin-depleted embryos were allowed to develop and were completely ventralized, but did not show adhesion defects (data not shown). Fig. 1F (right-hand two lanes) shows control western blotting with a known membrane-associated protein, β-1-integrin, showing that it is concentrated in the insoluble fraction.

Maternal α-catenin is required for the adhesion of early embryonic cells

In a previous study, we showed that maternal EP cadherin is required for adhesion in the blastula-stage embryo, in both animal and vegetal cells. Here we asked whether α-catenin is also required for cell-cell adhesion. α-catenin-depleted oocytes were fertilized using the host-transfer method and allowed to develop. By the mid-blastula stage, all α-catenin-depleted embryos lacked adhesion (4 experiments; 37/37 cases). This was assessed in two ways. (1) Embryos were devitellined and the animal caps dissected to reveal the blastocoels. Fig. 2A shows a comparison between a control, uninjected embryo (brown) and a typical α-catenin-depleted embryo (blue). Both animal and vegetal cells were rounded and non-adhesive in depleted embryos. As with EP cadherin depletion, the most superficial layer of cells in α-catenin-depleted embryos was least affected. Secondly, in simple aggregation assays (see Materials and Methods), α-catenin-deficient cells failed to aggregate in comparison to wild-type cells (Fig. 2B, compare control aggregates of uninjected cells, LHS, with α-catenin-depleted aggregates, RHS). This disaggregated phenotype was rescued by the subsequent injection (24 hours after the oligo) of mouse α-catenin mRNA into α-catenin-depleted oocytes (Fig. 2C), showing that disaggregation was a specific effect (2 experiments; 10/10 cases). Sibling embryos to those dissected at the blastula stage were allowed to develop through gastrulation. Although gastrulation was delayed, embryos depleted of maternal α-catenin developed through the neurula stages and formed normal axial structures.

Plakoglobin deficiency affects both adhesion and the cytoarchitecture of blastulae

Studies of cell lines in vitro suggest that plakoglobin acts like β-catenin as a link between cadherin and α-catenin. If the same is true in the early embryo, either plakoglobin depletion or β-catenin depletion or both might be expected to cause a similar phenotype to that of EP cadherin and α-catenin depletion. It has been shown previously that β-catenin depletion in Xenopus does not result in disaggregation but prevents dorsal axis formation (Heasman et al., 1994a). However, the results presented above (Fig. 1F) show that a depletion sufficient to cause complete loss of dorsal structures does not affect membrane-bound β-catenin substantially. Overexpression of both plakoglobin and β-catenin causes dorsalization. Therefore we asked what phenotype would result from plakoglobin depletion. Plakoglobin-depleted embryos had a distinct and highly reproducible phenotype at the midblastula stage (Fig. 3). Firstly, devitellined embryos had a flattened morphology compared to controls, that was dose-dependent in its severity (11 experiments; 46/46 cases Fig. 3A). In Fig. 3A, typical plakoglobin-depleted and control embryos derived from one batch of oocytes were dissected and compared at the blastula stage. Control uninjected (brown, top left) and plakoglobin mRNA-injected embryos (red, bottom left) are shown on the left-hand side, and two doses of oligo-injected embryos, 2.0 ng (blue, top right) and 2.5 ng (mauve, bottom right) on the right. Plakoglobin-depleted embryos have a wider diameter than control embryos (ranging from a maximum of 140% wider, to a minimum of 110% wider in 20 pairs of sibling uninjected and plakoglobin-depleted embryos compared from several experiments), as a result of flattening in the animal/vegetal axis. Secondly, cell-cell adhesion was reduced, particularly between vegetal cells (Fig. 3A and 3F). This reduction of adhesion was confirmed in aggregation assays (Fig. 3B, compare uninjected control aggregates, LHS, with plakoglobin-depleted aggregates RHS).

To compare directly the phenotypes caused by α-catenin and plakoglobin depletion, we injected either M7 (plakoglobin) or A3 (α-catenin) oligos into a single batch of oocytes and compared their phenotypes at the blastula stage (Fig. 3C). Plakoglobin-depleted embryos had a flattened morphology unlike α-catenin-depleted embryos, even though, on dissection, α-catenin-depleted embryos were more completely disaggregated compared to plakoglobin-depleted embryos. The third characteristic of plakoglobin-deficient embryos was a significant delay in gastrulation (Fig. 3D, Table 1). Eventually
the embryos completed gastrulation, 1.5 hours later than controls and formed closed blastopores and neurulae with normal neural folds (Fig. 3E). In Fig. 3D,E, typical examples from the same batch of embryos are seen at the gastrula and neurula stage, respectively. In Fig. 3D, the upper two embryos were uninjected (brown) or plakoglobin mRNA (red, right)-injected, while the lower three embryos were plakoglobin depleted by 2.5 ng (mauve, left), 2.0 ng (blue, center) and 1.75 ng (green, right) of antisense oligo. Although these embryos were delayed compared to controls, they formed wild-type amounts of dorsal mesodermal tissue as judged by the expression of the dorsal mesodermal marker MyoD on a northern blot at the neurula stage (Fig. 4).
Fig. 2. Depletion of maternal $\alpha$-catenin mRNA and protein causes a disaggregated phenotype, which can be rescued using mouse $\alpha$-catenin mRNA. (A) A control embryo (left) together with an $\alpha$-catenin-depleted sibling embryo (right) dissected at the blastula stage to show the loss of interblastomere adhesion. (B) Midblastulæ disaggregated and allowed to aggregate for 45 minutes. Blastomeres from $\alpha$-catenin-deficient embryos (right) failed to aggregate in comparison to wild-type blastomeres (left). (C) Blastulæ derived from uninjected (middle) or injected as oocytes with 4 ng oligo A3 (right), and 4 ng oligo A3 plus 1 ng mouse $\alpha$-catenin mRNA (left) to show that mouse $\alpha$-catenin mRNA was able to restore a normal blastula morphology into $\alpha$-catenin-depleted embryos.

Fig. 4 shows that when sibling control embryos had reached early gastrula stage, zygotic MyoD was expressed in controls but not in delayed (stage 9) plakoglobin-depleted embryos. However, by the neurula stage, equivalent amounts of MyoD were expressed in both control and experimental embryos.

All aspects of the phenotype were partially rescued by the subsequent injection of Xenopus plakoglobin mRNA into plakoglobin-depleted oocytes (6 experiments, 29/29 cases). Fig. 3F shows sibling embryos derived from uninjected oocytes (top row), compared to plakoglobin-depleted embryos (middle row) and to embryos injected as oocytes with oligo to deplete plakoglobin and then (24 hours later) injected with Xenopus plakoglobin mRNA (bottom row). This figure illustrates the partial rescue of the flattened morphology by the appropriate RNA. Plakoglobin mRNA also rescued the delay in gastrulation as illustrated in Fig. 3G. Here the blue embryos lacked maternal plakoglobin, compared to the mauve plakoglobin+RNA-injected siblings. Embryos that were

Fig. 3. Depletion of maternal plakoglobin mRNA and protein causes a phenotype marked by a specific affect on cytoarchitecture, a mild adhesion defect most evident in the vegetal blastomeres and a delay of gastrulation. All aspects of this phenotype are at least partially rescued with Xenopus plakoglobin mRNA.

(A) A control embryo (top left, brown), a plakoglobin mRNA-treated embryo (bottom left, red) and two oligo doses of plakoglobin-depleted embryos, 2.0 ng (top right, blue) and 2.5 ng (bottom right, mauve). (B) Midblastulæ disaggregated and allowed to aggregate for 45 minutes. Blastomeres from plakoglobin-depleted embryos (right) aggregate less well than control blastomeres (left). (C) Comparison of devitellined plakoglobin-depleted (left, mauve) embryos to $\alpha$-catenin-depleted embryos (right, red). Plakoglobin-depleted embryos show a flattened morphology that is not evident in the $\alpha$-catenin-depleted embryos. (D) Plakoglobin-depleted embryos are delayed in gastrulation. A control embryo (top left, brown), a plakoglobin mRNA-treated embryo (top right, red), and three doses of plakoglobin oligo M7 (bottom left to right) 2.5 ng (mauve), 2.0 ng (blue), 1.75 ng (green). (E) Control and plakoglobin-depleted embryos form normal neural structures. Starting from left to right, plakoglobin-depleted embryos 2.5 ng (mauve), 2.0 ng (blue), 1.75 ng (green), control embryo (brown) and plakoglobin mRNA-treated embryo (red). (F) Partial rescue of oligo-mediated adhesion and cytoarchitectural defects at blastula stage with plakoglobin mRNA. Control embryos (top), plakoglobin-depleted embryos (middle) and plakoglobin-depleted embryos injected with Xenopus plakoglobin mRNA (bottom). (G) Plakoglobin mRNA can rescue the gastrulation delay of plakoglobin-depleted embryos. Plakoglobin-depleted embryos (left), depleted embryos injected with Xenopus plakoglobin mRNA (middle) and control embryos (right).
Injected either with oligo and then subsequently with the mRNA (Fig. 3G, mauve, center), or had mRNA only injected into them as oocytes (Fig. 3E, red, right), developed abnormally at the neurula stage with excessive dorsal mesodermal mRNA (Fig. 3G, mauve, center), or had mRNA only injected M7. The blot was stripped and reprobed with Ef1-α control for loading.

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<th>Oligo treatment</th>
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<th>Stage 10-10.5</th>
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Embryos derived from one batch of oocytes depleted of plakoglobin and/or β-catenin were staged when sibling uninjected control embryos reached the late gastrula stage (stage 12).

**DISCUSSION**

Considerable progress has been made in recent years in understanding the molecular architecture of the cadherin/catenin complex and in clarifying the relative roles of the catenins in morphogenetic processes. In this work, we have extended the functional analysis of α-catenin and plakoglobin by studying their roles in the earliest stages of development. We believe the phenotypes described are specific, because the defects can be rescued by the appropriate mRNAs. This analysis complements functional assays of maternal EP cadherin and β-catenin in *Xenopus* embryos (Heasman et al., 1994a,b), and of zygotic E cadherin, β-catenin and plakoglobin in mouse embryos (Haegel et al., 1995; Larue et al., 1994; Ruiz et al., 1996).

With regards to α-catenin, the predictions from studies with cultured cells were substantiated. A-catenin is essential for adhesion in the blastula. Nevertheless, cells lacking α-catenin divide normally, and eventually, as zygotic α-catenin is expressed, develop into relatively normal neurulae. Embryos depleted of maternal EP cadherin are also disaggregated at the blastula stage and yet develop into normal neurulae (Heasman et al., 1994b), suggesting that full adhesion at the blastula stage is not necessary for normal development. Although somewhat surprising, this finding is consistent with recent observations that cell-cell signalling events involved in mesoderm formation do not occur until after the mid-blastula transition (Wylie et al., 1996), and that specific mesodermal markers are expressed in embryos disaggregated through the cleavage and blastula stages (Lemaire et al., 1994).

The experiments described here suggest that maternal plakoglobin in the early *Xenopus* embryo may have a broader role than was revealed by studying zygotic plakoglobin in mouse embryos (Ruiz et al., 1996). In the mouse, loss-of-function studies, plakoglobin-depleted embryos developed normal tissue layers and axes but died at day 12-16 from defects in heart development involving abnormal adhesion plaques in cardiac muscle. Desmosomes in other tissues were not affected. Here we find that depletion of maternal plakoglobin affects the cytoarchitecture of the whole embryo as well as affecting adhesion and the timing of gastrulation. These defects may be due to interference with plakoglobin in classical cadherin adherens junctions, with desmosomal cadherins in desmosomal junctions or with cytoplasmic plakoglobin associated with APC protein and microtubules. Studies are underway to distinguish between these possibilities.

The results presented here suggest that one of the roles of maternal plakoglobin is to maintain adhesion in the blastula-stage embryo. We do not yet know whether this is due to an interaction of plakoglobin with desmosomal or classical cadherins in adhesion sites, or both. However, the degree of disaggregation of plakoglobin-depleted embryos was never as marked as that caused by α-catenin or EP cadherin depletion, even though the western analysis showed that the depletion of plakoglobin protein from the membrane was as efficient as that of α-catenin. One likely interpretation of this difference is that β-catenin is also involved in adhesion, particularly of animal cells, and is responsible for holding the animal cells together in plakoglobin deleted embryos. In previous experiments, we have shown that depletion of β-catenin does not affect adhesion, but does prevent dorsal signalling (Heasman et al., 1994a). However, we have shown here that the depletion of β-catenin, which is sufficient to cause a completely ventralized phenotype, does not significantly alter the levels of insoluble (including membrane-bound) β-catenin, where it is likely to be acting in an adhesive role. This is consistent with the conclusion of previous studies, that cytoplasmic β-catenin is involved in dorsal signalling when complexed to the transcription factor Xtcf 3 (Molenaar et al., 1996).

In a previous study, overexpression of plakoglobin mRNA resulted in the formation of extra dorsal axes, which led to the suggestion that plakoglobin may be involved in a signalling pathway in a dorsal signalling role (Karnovsky and
Klymkowsky, S. (1995; and reviewed in Hubler et al., 1996). However, loss-of-function studies do not support such a conclusion. β-catenin depletion causes embryos to develop without axes, even though maternal plakoglobin is expressed normally in these embryos (unpublished data), and studies on β-catenin null mice also suggest that plakoglobin is not able to rescue their phenotype completely (Haegel et al. 1995). This suggests that β-catenin and plakoglobin are not functionally redundant molecules. Secondly, the plakoglobin-depletion experiments described here support the view that plakoglobin is not essential in a signalling role. Although embryos were delayed in gastrulation, they developed with normal axes and normal dorsal mesodermal and neural structures. The most likely explanation for the ability of overexpressed plakoglobin to cause axis duplication is that the conserved armadillo repeats of this molecule mimic the signalling role of β-catenin. Mutaational analysis of β-catenin function has shown that the armadillo repeat region of the molecule is responsible for its signaling function (Funayama et al., 1995).

The conclusions from the experiments presented here is that α-catenin and plakoglobin have roles in adhesion in the Xenopus blastula, and that plakoglobin is also involved in maintaining the shape of the embryo. Although plakoglobin has similarities to Drosophila armadillo and β-catenin, plakoglobin is unlike β-catenin in not being essential as part of an Xwnt-signalling pathway patterning the dorsal axis. Further experiments are required to characterize both the cytoskeletal elements and adhesion complexes disrupted in these experiments.

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