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Dynamin-dependent endocytosis is necessary for convergent-extension movements in *Xenopus* animal cap explants

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ABSTRACT Cadherin cell-cell adhesion molecules are important determinants of morphogenesis and tissue patterning. C-cadherin plays a key role in the cell-upon-cell movements seen during *Xenopus* gastrulation. In particular, regulated changes in C-cadherin adhesion critically influence convergence-extension movements, thereby determining organization of the body plan. It is also predicted that remodelling of cadherin adhesive contacts is important for such cell-on-cell movements to occur. The recent demonstration that Epithelial (E-) cadherin is capable of undergoing endocytic trafficking to and from the cell surface presents a potential mechanism for rapid remodelling of such adhesive contacts. To test the potential role for C-cadherin endocytosis during convergence-extension, we expressed in early *Xenopus* embryos a dominantly-inhibitory mutant of the GTPase, dynamin, a key regulator of clathrin-mediated endocytosis. We report that this dynamin mutant significantly blocked the elongation of animal cap explants in response to activin, accompanied by inhibition of C-cadherin endocytosis. We propose that dynamin-dependent endocytosis of C-cadherin plays an important role in remodelling adhesive contacts during convergence-extension movements in the early *Xenopus* embryo.

KEY WORDS: *C-cadherin, Endocytosis, dynamin, Xenopus, convergence-extension*

Introduction

Cell movements drive the morphogenetic processes that determine body plan and tissue organization in metazoan organisms (Bard, 1990). These encompass both cell-upon-cell and cell-on-matrix forms of cell locomotion. Although in post-developmental life cells often locomote on extracellular matrices, many important forms of morphogenetic movement involve the locomotion of cells upon one another (Gumbiner, 1992). Such cell-on-cell locomotion is exemplified by the convergent-extension movements that occur in many vertebrate embryos (Keller, *et al.*, 2000). Here intercalation of cells upon one another reorganizes whole populations of cells during gastrulation and neurulation. Importantly, such convergent-extension movements are subject to strict cellular regulation that is essential for development to occur normally (Keller, *et al.*, 2000). Any comprehensive understanding of morphogenesis therefore requires a proper description of the cellular mechanisms responsible for cell-on-cell locomotion.

In contrast to recent advances in understanding matrix-based cell locomotion, the cellular basis of cell-upon-cell locomotion is poorly understood. Minimally, such cell movement likely entails mechanisms for surface adhesion that are coupled to the actin cytoskeleton (Lauffenburger and Horwitz, 1996; Mitchison and Cramer, 1996). Surface adhesion supplies the traction for locomotion, while productive coupling to the actin cytoskeleton is envisaged to provide the protrusive and contractile forces necessary for cells to locomote.

Studies in a number of model systems suggest that cadherin cell adhesion molecules play key roles in cell-upon-cell locomotion. The cadherins are a large family of cell surface glycoproteins that mediate homophilic cell-to-cell adhesion (Yap, *et al.*, 1997a; Tepass, *et al.*, 2000). Classical cadherins are major determinants of intercellular adhesion in most solid tissues, which play important roles in cell sorting, tissue patterning, and morphogenesis. For example, DE-cadherin is essential for tracheal morphogenesis in Drosophila (Tepass, *et al.*, 1996; Tepass, *et al.*, 2000), whilst misexpression of the cadherin-associated regulator, p120-catenin, disturbs development in *Xenopus* (Paulson, *et al.*, 1999). Classical cadherins

Abbreviations used in this paper: ANOVA, analysis of variance; ARF6, ADP-ribosylation factor 6; DN, dynamin dominant-negative (S45N) dynamin1; GFP, green fluorescent protein.

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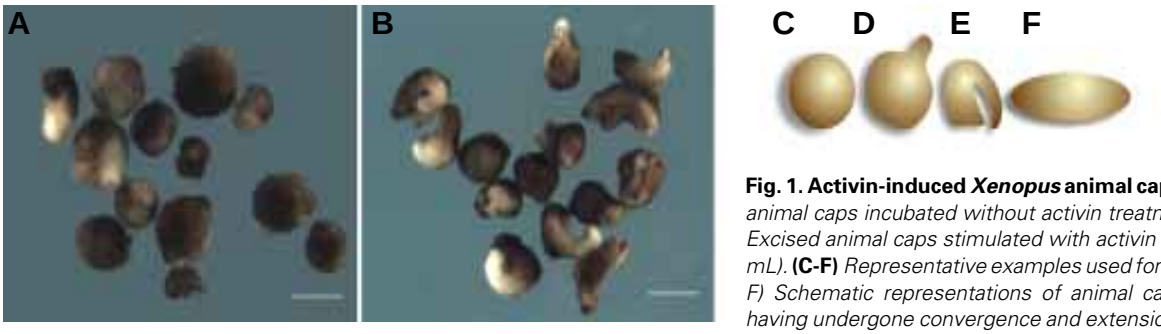

Fig. 1. Activin-induced *Xenopus* animal cap elongation. (A) *Excised animal caps incubated without activin treatment did not elongate.* **(B)** *Excised animal caps stimulated with activin showed elongation (5 ng/mL).* **(C-F)** *Representative examples used for scoring elongation. (E and F) Schematic representations of animal caps that are classified as having undergone convergence and extension movements (length = 2 x width). Animal caps that remained spherical (C) and those that showed some stump development but did not meet the length/width requirements (D) were not counted as having undergone convergence and extension movements. Scale bars in photographs A and B represent 1 mm.*

function as membrane-spanning macromolecular complexes, that physically associate with, and regulate, the actin cytoskeleton (Adams and Nelson, 1998; Kovacs, *et al.*, 2002). Cadherins are thus prime mechanisms to mediate cell-on-cell locomotion.

A role for cadherins in convergent-extension movements has been most clearly characterized in the early *Xenopus* embryo, where cell-cell adhesion is mediated by C-cadherin (Lee and Gumbiner, 1995). Animal cap explants from early *Xenopus* embryos elongate when stimulated with the mesoderm-inducing factor, activin, a morphogenetic phenotype which arises from convergence-extension movement (Symes, *et al.*, 1988; Smith, *et al.*, 1989). Using this convenient assay, Brieher and Gumbiner (1994) demonstrated that convergent-extension movements were accompanied by a regulated decrease in cell-cell adhesiveness mediated by C-cadherin while expression of dominantly-inhibitory cadherin mutants blocked animal cap elongation (Lee and Gumbiner, 1995). Importantly, convergence-extension was inhibited when animal caps were treated with an antibody that stimulates the higher-adhesive state of C-cadherin, preventing activin-induced decreases in adhesion (Zhong, *et al.*, 1999). Taken together, these data suggest strongly that C-cadherin and its regulated activity is essential for convergence-extension in *Xenopus* animal caps.

For cells to translocate upon one another, there must also be mechanism(s) to remodel the cell surface, allowing adhesive contacts to be broken and re-formed. Recent reports indicate that cadherins are capable of undergoing internalization and trafficking to and from the cell surface (Kamei, *et al.*, 1999; Le, *et al.*, 1999). As has also been demonstrated for integrin adhesion receptors (Ng, *et al.*, 1999), such trafficking provides a potential mechanism to remodel adhesive contacts during cell-on-cell locomotion. Internalization of cadherins would facilitate disassembly of contacts, thereby making cadherins available for adhesion to be established in other regions of the cell surface. A key step in this trafficking pathway is the initial step of cadherin endocytosis, a process which appeared to be mediated by a clathrin-dependent pathway (Le, *et*

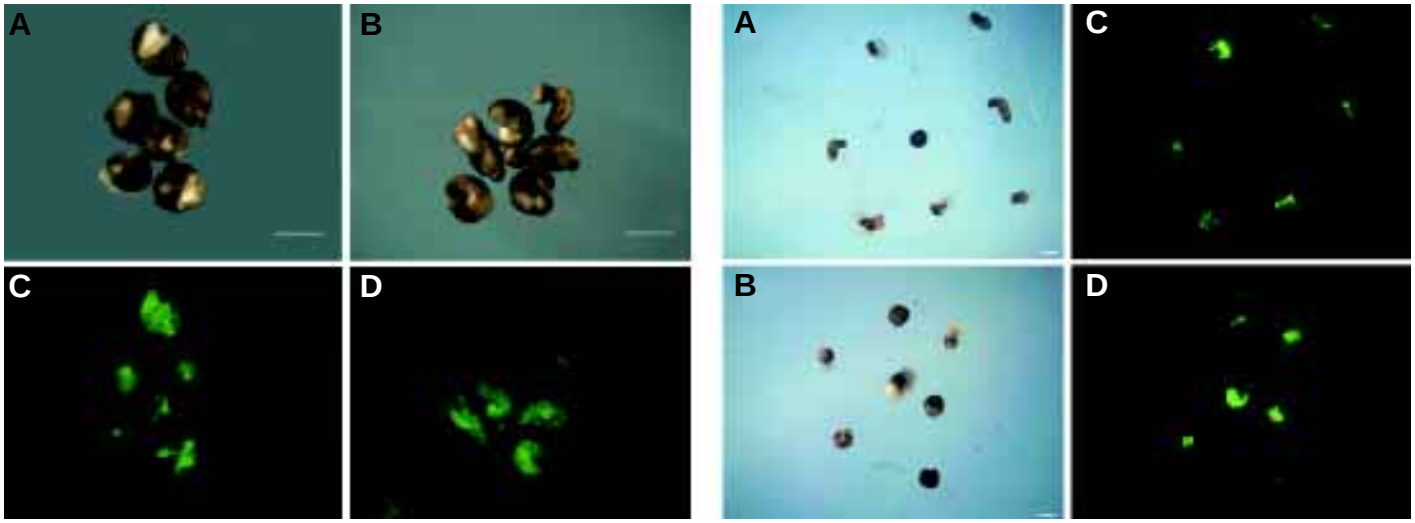

Fig. 2. (Left) Injection of GFP RNA alone does not affect animal cap elongation. *Animal caps excised from GFP-injected embryos treated without activin* **(A,C)** *and with activin* **(B,D)**. *Panels C and D show GFP fluorescence of animal caps shown in panels A and B respectively. The animal cap on the bottom left of B rolled to the bottom left of the image in D.*

Fig. 3. (Right) Expression of DN-dynamin inhibits activin-induced animal cap elongation. *Animal caps were excised from* Xenopus *embryos co-injected with GFP RNA and DN-dynamin RNA. Embryos injected with 3 pg DN-dynamin RNA into each blastomere at the two-cell stage, resulted in animal caps that maintained their structural integrity in both activin treated* **(A,C)** *and activin untreated* **(B,D)** *groups. (C and D) show GFP expression of embryos in panels A and B respectively. Scale bars represent 1 mm.*

al., 1999). Dynamin is a GTPase which plays an important role in clathrin–mediated endocytosis and synaptic vesicle recycling (Warnock and Schmid, 1996; Mukherjee, *et al.*, 1997) and dynamin mutants are well-described inhibitors of clathrin-dependent internalization (Herskovits, *et al.*, 1993; Van Der Bliek, *et al.*, 1993; Damke, *et al.*, 1994; Damke, *et al.*, 2001; Marks, *et al.*, 2001). In this report we therefore expressed dominant-negative dynamin mutants in *Xenopus* animal cap explants to test the potential role for C-cadherin endocytosis in convergence-extension movements.

Results

Animal Cap Elongation Assays

To identify a role for clathrin-mediated endocytosis during convergent-extension movements, we expressed a dominantly-inhibitory (S45N) mutant of dynamin1 (DN-dynamin) in early *Xenopus* embryos and tested the effects of the transgene on animal cap elongation. We studied three different groups of embryos: (1) control uninjected embryos; (2) embryos injected with GFP RNA alone as a marker; and (3) embryos injected with DN-dynamin RNA together with GFP RNA. Animal caps were excised from Stage 8 embryos and elongation assays were performed three times for each injection group. Half of the excised animal caps in each group were treated with the growth factor activin (5ng/mL) while the other half were left untreated. For quantitation animal cap explants that elongated so that their length was twice that of their width were scored as having undergone convergent-extension movements (Fig. 1).

Animal caps excised from uninjected embryos elongated when treated with activin (Figs. 1,2,4), as previously reported with this assay system. Compared with uninjected controls, expression of GFP alone did not affect the ability of explants to undergo convergence-extension when stimulated with activin (Tukey test, p=0.791). Animal caps injected with GFP and DN-dynamin (3.0 pg RNA) remained intact. However, animal caps expressing DN-dynamin (Figs. 3,4) did not elongate in response to activin to anything like the same degree as either control animal caps (p=0.01, Tukey test) or animal caps expressing GFP alone (p=0.005, Tukey test). This robust inhibition of activin-induced animal cap elongation therefore suggested a key role for clathrin-mediated endocytosis in convergence-extension movement.

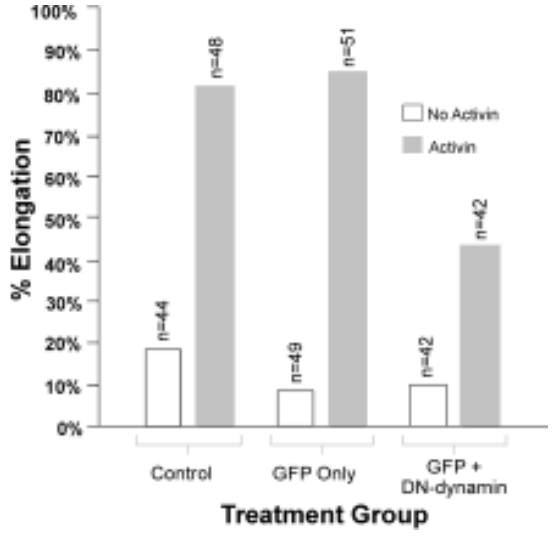

Fig. 4. Quantitation of activin-induced animal cap elongation. *Animal caps from uninjected control embryos and embryos injected with GFP RNA alone or with GFP RNA and DN-dynamin RNA were scored for elongation in the absence or presence of activin.*

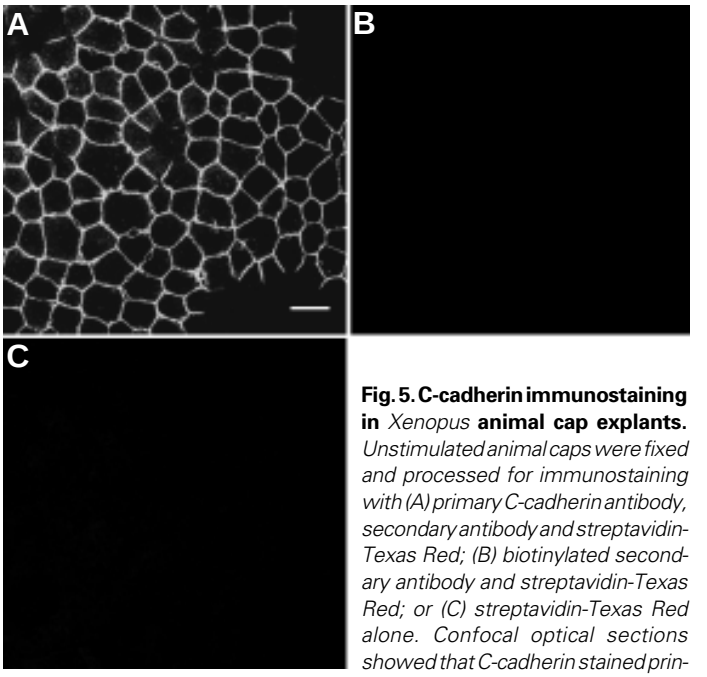

Fig. 5. C-cadherin immunostaining in *Xenopus* animal cap explants. *Unstimulated animal caps were fixed and processed for immunostaining with (A) primary C-cadherin antibody, secondary antibody and streptavidin-Texas Red; (B) biotinylated secondary antibody and streptavidin-Texas Red; or (C) streptavidin-Texas Red alone. Confocal optical sections showed that C-cadherin stained principally at cell-cell borders. There was no non-specific binding of secondary antibody or texas red to cellular components. Scale bars represent 20μm.*

C-cadherin Immunolocalization during Animal Cap Elongation

If cadherin endocytosis participated in convergent-extension movements we predicted that animal cap elongation would be accompanied by an increase in the intracellular pool of cadherin. Therefore, in order to assess whether activin treatment affected the subcellular distribution of C-cadherin, animal caps from the elongation assays were fixed and immunostained for C-cadherin. The surfaces of ectodermal cells were clearly stained in optical sections of the animal caps collected using a confocal laser scanning microscope (Fig. 5A). Control sections treated in the absence of the C-cadherin antibody revealed no non-specific background fluorescence (Fig. 5 B,C).

Activin treatment appeared to increase the intracellular staining of C-cadherin in ectodermal cells from both control uninjected and GFP-injected animal caps (Fig. 6 A-D), consistent with internalization of C-cadherin. To quantitate the extent of internalisation of C-cadherin we measured the intensity of cytoplasmic fluorescence staining per unit area in ectodermal cells of animal caps. Five optical sections were obtained randomly from each injection and treatment group. Each image had a 6x6 grid overlaid on it and, using a random number generator for grid coordinates, a random sample of cells was analysed and an average cytoplasmic fluorescence intensity for each cell calculated (Fig. 7). Activin induced an increase in intracellular total fluorescence intensity in the ectodermal cells of control uninjected embryos (P<0.001) as well as GFP-injected embryos (P<0.001). One way analyses of variance

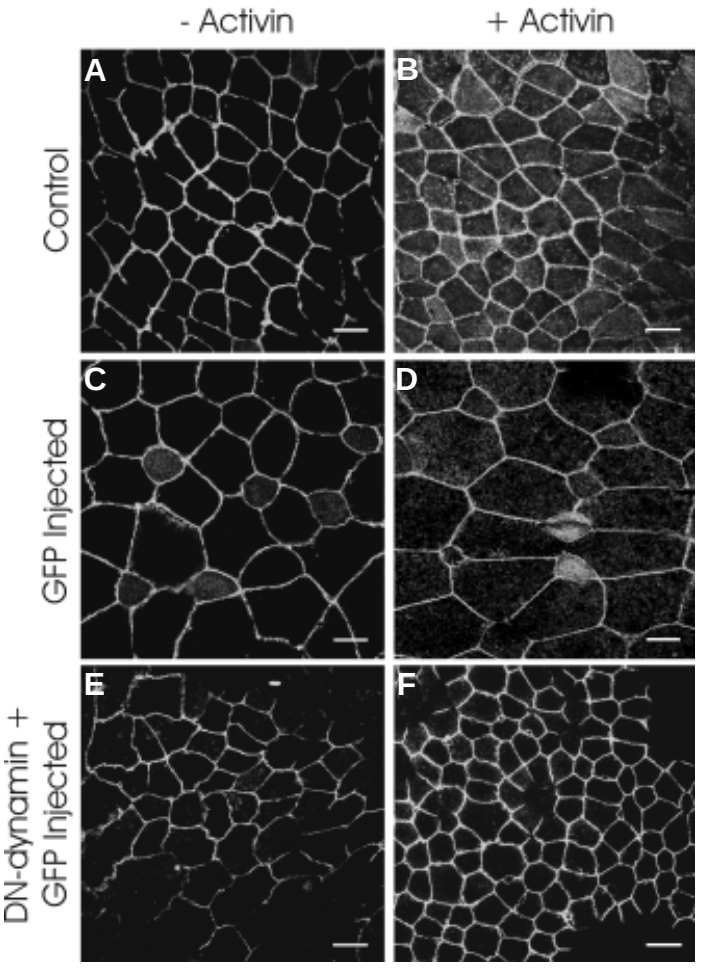

Fig. 6. C-cadherin immunostaining in *Xenopus* animal caps expressing DN-dynamin. *Animal caps were taken from control uninjected embryos (A,B), embryos injected with GFP RNA alone (C,D) and embryos injected with DN-dynamin RNA (E,F). Animal caps were incubated in the absence (A,C,E) or presence of activin (B,D,F) then processed and immunostained for C-cadherin. Activin treatment increased cytoplasmic C-cadherin staining in uninjected embryos (B) and embryos injected with GFP RNA alone (D), but to a much less marked extent in embryos injected with DN-dynamin RNA (F). Scale bars represent 20μm. Differences in cell size are due to slight variations in age between animal caps, since a cell division takes ~15 minutes. Cells in* **(C)** *that show cytoplasmic staining have probably been induced by residual mesoderm.*

(ANOVA) between injection groups in the activin-treated and -untreated groups revealed that the injection of GFP did not significantly increase total fluoresence intensity in the cytoplasm of activin-treated ectodermal animal cap cells (P=0.762). Activin-induced convergence-extension was therefore accompanied by an increase in the cytoplasmic pool of C-cadherin.

In contrast, the degree of cytoplasmic C-cadherin staining was substantially reduced in animal caps from embryos injected with DN-dynamin (Fig. 6 E,F; Fig. 7). Quantitation revealed that while activin increased the intracellular pool of C-cadherin in animal caps expressing DN-dynamin (P=0.011), the degree of internalization was much less than that observed in either control uninjected embryos (P=0.008) or in embryos injected with GFP alone (P=0.004; Fig. 7). A one-way ANOVA revealed that activin-treated animal caps excised from embryos expressing DN-dynamin showed no significant difference in cytoplasmic fluoresence intensity compared with activin-untreated animal caps in the control and GFP-only injection groups (P=0.330; Fig. 7). Expression of DN-dynamin therefore significantly reduced C-cadherin internalization in response to activin.

Discussion

Remodelling of adhesive contacts is a key process in cell locomotion. Translocation requires that cells make new adhesive contacts with their environment at their leading edges, as well as breaking and clearing older contacts at the rear (Lauffenburger and Horwitz, 1996; Mitchison and Cramer, 1996). Endocytosis is a ubiquitous process which eukaryotic cells utilize to remodel their cell surfaces, allowing the turnover and redistribution of cell surface proteins and lipids (Mukherjee, *et al.*, 1997). Increasing evidence that cell adhesion molecules (integrins, cadherins) undergo endocytosis provides the potential for this mechanism of receptor-driven internalization to be used to remodel adhesive contacts (Le, *et al.*, 1999; Ng, *et al.*, 1999). In this report we have identified a key role for dynamin-dependent endocytosis during convergent-extension movements in the early *Xenopus* embryo. Further, we found a strong relationship between the inhibition of C-cadherin internalization and inhibition of cell-upon-cell movement which suggests that endocytosis of C-cadherin makes a significant contribution to this form of morphogenetic movement.

In these experiments we utilized the activin-induced elongation of *Xenopus* animal caps, a well-characterized model for convergence-extension movements (Symes, *et al.*, 1988; Smith, *et al.*, 1989; Brieher and Gumbiner, 1994; Keller, *et al.*, 2000). A model that has, moreover, been used to identify a requirement for regulated cadherin activity during convergence-extension (Brieher and Gumbiner, 1994; Zhong, *et al.*, 1999). We noted that stimulation with activin quite rapidly induced a significant increase in cytoplasmic staining for C-cadherin. Taken with earlier evidence

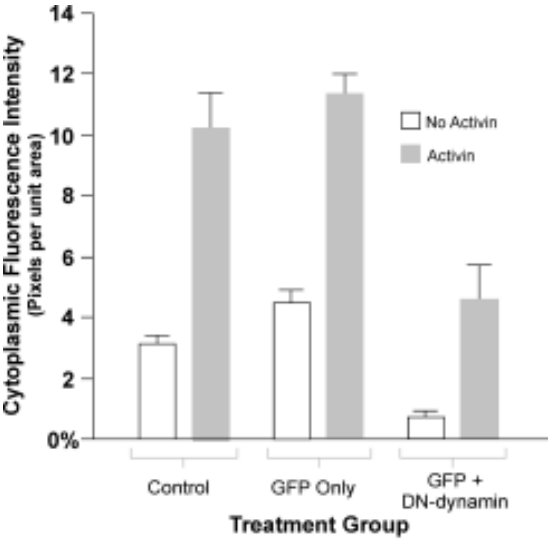

Fig. 7. Quantitation of cytoplasmic C-cadherin fluoresence intensity. *Animal caps were stained for C-cadherin and examined by confocal microscopy. Cytoplasmic C-cadherin fluoresence intensity per unit area was calculated as described in Methods.*

that activin does not affect the total cellular content of C-cadherin (Brieher and Gumbiner, 1994), this suggested that C-cadherin internalization increased in response to activin. Internalization of cadherins has been increasingly documented in cell culture (Kamei*, et al.*, 1999; Le*, et al.*, 1999; Palacios*, et al.*, 2001), typically under conditions that entail reorganization or disassembly of adhesive junctions. Increased cytoplasmic cadherin localization was also observed during epithelial-to-mesenchymal transitions in sea urchin embryos (Miller and Mcclay, 1997), suggesting that cadherin internalization may commonly accompany morphogenetic movements during development. Furthermore, cadherin internalization is subject to cellular regulation, being stimulated by a range of growth factors and intracellular signaling pathways (Kamei*, et al.*, 1999), including the ARF6 GTPase (Palacios*, et al.*, 2001). Of note, a reduction in the surface pool of C-cadherin would be predicted to accompany the activin-induced internalization of C-cadherin observed in our experiments, with a concomitant fall in cell surface cadherin adhesiveness (Angres*, et al.*, 1996; Yap*, et al.*, 1997b). Increased cadherin internalization therefore provides a potential mechanism for the regulated decrease in C-cadherin activity documented to participate in convergence-extension movements.

Expression of a dominantly-inhibitory mutant of dynamin substantially reduced the ability of animal cap explants to undergo convergence-extension when stimulated by activin, accompanied by decreased internalization of C-cadherin. Since the dynamin mutant used in our experiments is well-documented to inhibit clathrin-mediated endocytosis (Herskovits*, et al.*, 1993; Van Der Bliek*, et al.*, 1993; Damke*, et al.*, 1994; Damke*, et al.*, 2001; Marks*, et al.*, 2001), this provides evidence that activin-induced C-cadherin internalization may occur via a clathrin-mediated pathway. It is also, to our knowledge, the first evidence that dynamin-dependent endocytosis participates in convergence-extension. In particular, inhibition of C-cadherin endocytosis is likely to have prevented the activin-induced reduction in surface cadherin adhesiveness necessary for cell-on-cell movements to occur (Brieher and Gumbiner, 1994; Zhong*, et al.*, 1999). Of note, application of an antibody that promoted the high-adhesive state of C-cadherin effectively blocked activin-induced animal cap extension (Zhong*, et al.*, 1999), achieving results similar to those we observed upon expression of mutant dynamin.

Taken together, our findings suggest an important contribution of C-cadherin endocytosis to activin-stimulated convergence-extension movements. Although dynamin-dependent endocytosis was necessary for C-cadherin to redistribute into a cytoplasmic pool upon stimulation with activin, it is important to note that the effect of activin may not have occurred principally through changes in the rates of cadherin endocytosis. In the post-Golgi pathway of E-cadherin trafficking identified in MDCK cells, endocytosed E-cadherin was rapidly recycled back to the cell surface (Le*, et al.*, 1999). A net redistribution of cadherin to the cytoplasmic pool may therefore also occur if internalized C-cadherin cannot be recycled to the cell surface, independently of any change in the rate of cadherin internalization. By either mechanism, however, a net increase in the cytoplasmic pool of C-cadherin, and concomitant reduction in surface cadherin, provides an attractive mechanism for activin to down-regulate cadherin activity. As has been reported for integrins (Lawson and Maxfield, 1995), internalization and recycling of cadherins may also allow redundant adhesive contacts to be cleared from the rear ends of locomoting blastomeres, and facilitate recycling of cadherins to newly-forming adhesive contacts at their leading edges, thereby providing a mechanism for adhesive turnover during translocation.

While our findings point to an important role in C-cadherin endocytosis and recycling, dynamin may play additional roles in activin-induced convergence-extension movements. Clathrin-mediated endocytosis of growth factor receptors has been implicated in their signaling activity (Vieira*, et al.*, 1996), with the potential to influence activin signaling (Garamszegi*, et al.*, 2001). Also, there is emerging evidence that dynamin can affect actin assembly and regulation of the cytoskeleton (McNiven*, et al.*, 2000; Ochoa*, et al.*, 2000), that may be important in cell locomotion. It is consequently possible that the potent inhibition of animal cap extension by mutant dynamin also reflected perturbations in cell mechanisms additional to C-cadherin trafficking. Cadherin endocytosis may therefore constitute one of several key aspects of the cell surface remodelling necessary for cell-upon-cell locomotion to occur during development.

Materials and Methods

RNA Preparation and Injection

The dominant-negative (S45N) dynamin1 (DN-dynamin) mutant cDNA (Damke*, et al.*, 1994) was a kind gift from Dr. S. Schmid (Scripps). The coding region of DN-dynamin cDNA was excised from its original vector and inserted into the pCS2+ expression vector. RNA was transcribed using an Ambion SP6 Message Machine Kit according to the manufacturer's instructions. Two cell stage *Xenopus* embryos were placed into microinjection dishes in 0.1xMBS and 5% Ficoll and viewed using an SZX12 stereomicroscope (Olympus). Microinjection needles were prepared using a PUL-1 micropipette puller (World Precision Instruments Inc., Florida, USA) and fitted to a displacement microinjection apparatus (World Precision Instruments Inc., Florida, USA). The microinjection apparatus was mounted on a 3-dimensional micromanipulator (Narishige). For control experiments 9pL of capped GFP RNA (~1 μg/μL; a generous donation from Robin Connor and Nicole Wilson) was injected into both blastomeres of 2 cell stage *Xenopus* embryos. Experimental animals were co-injected with 9 pL of capped DN-dynamin RNA (~1 μg/μL) and capped GFP RNA (~1 μg/μL) at a ratio of 1 part DN-dynamin to 2 parts GFP (~33% DN-dynamin). Injected embryos were allowed to recover in 0.1xMBS + 5% Ficoll for one hour before being transferred to 1XMMR (100 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM Hepes). GFP expression was visualised using the same stereomicroscope with a fluorescence attachment (Olympus, Japan).

Animal Cap Explants Assays

Embryos were staged according to the normal stage of *Xenopus laevis* development (Nieuwkoop and Faber, 1967). Animal caps were isolated from de-jellied stage 8 control and experimental embryos in 1xMMR using two pairs of curved forceps and a dissection microscope (Olympus SZ11, Japan). Blastomeres were incubated in the presence or absence of 5 ng/mL human recombinant activin A (R&D Systems, MN, USA) in 1xMMR for 12 hours at room temperature. Convergent and extension movements were considered to have occurred when an animal cap had elongated so that its length was at least twice that of its width. Animal caps were fixed in 4% paraformaldehyde for one hour and then stored in 1xTBS (100 mM Tris-HCl, 0.9% NaCl, pH 7.4) and 0.02% sodium azide. Photographs of animal caps were obtained using a stereomicroscope (Olympus SZX12) with a fluorescence attachment (Olympus, Japan) and a Spot digital camera (Diagnostic Instruments Inc., MI, USA). All images were collected using Spot Advanced software (Diagnostic Instruments Inc., MI, USA).

Immunofluorescence Analysis

Animal caps were blocked in 2% bovine serum albumin (BSA) and 0.3% Triton X-100 in 1xTBS. Animal caps were then incubated in primary

antibody (1:400) in 1xTBS and 0.3% Triton X-100 for 15 hours at 4°C. The rabbit anti-C-cadherin polyclonal antibody was generated against the ectodomain of C-cadherin and is a kind gift from Drs. Bill Brieher and Barry Gumbiner (Brieher, *et al.*, 1996). Following three washes in 1xTBS and 0.3% Triton X-100, animal caps were incubated in a biotinylated goat, anti-rabbit IgG secondary antibody (1:400; Vector Laboratories) in 1xTBS and 0.3% Triton-X for one hour at room temperature. Animal caps were again washed three times in 1xTBS and 0.3% Triton X-100 and incubated in Streptavidin-Texas red (1:200) (Vector Laboratories) in the same buffer for one hour at room temperature. Two negative controls were used: omission of (1) primary antibody and (2) primary and secondary antibodies. Specimens were mounted between two coverslips in fluorescence mounting media (5% propyl gallate 5% ethanol, 25mM sodium bicarbonate, pH 9.6) and stored in the dark at 4°C. Optical sections were obtained using a Bio Rad MRC 1204 scanning laser confocal system, with a Krypton/Argon laser (American Laser Corp., USA) fitted on an Olympus BX60 Microscope. All images were prepared using Adobe Photoshop.

Levels of cytoplasmic C-cadherin staining were quantified using Image J (National Institute of Health, USA). Cytoplasmic staining for C-cadherin was used as an index of C-cadherin internalization. Five confocal optical sections were obtained for each injection and treatment group, and a 6x6 grid overlaid on each. Two die were rolled to generate a random grid coordinate and any blastomere that lay either wholly or partially within this square and whose cell surface was completely identifiable had its internal border outlined manually. Fluorescence intensity within this region of interest was measured and represented as pixels/unit area (arbitrary units). This was repeated ten times and an average of the fluoresence intensity was calculated for each image. In this analysis, the fluoresence intensity of C-cadherin staining per unit area of an optical section was taken to represent the level of endocytic activity per unit-volume of a cell, assuming that the distribution of cytoplasmic C-cadherin was throughout a cell. This method of analysis was not dependent on cell shape, since optical sections taken through the centre of two cells with equal volume and equal amounts of cytoplasmic C-cadherin have the same fluoresence intensity per unit area even if one cell was squashed, resulting in a larger cross sectional area. Differences in cell volume did not influence this quantification technique either, since fluoresence intensity was being used as a measure of endocytic activity levels, rather than absolute levels endocytosed C-cadherin.

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References

ADAMS, C.L. and NELSON, W.J. (1998). Cytomechanics of cadherin-mediated cell-cell adhesion. *Curr Opin Cell Biol.* 10: 572-577.

ANGRES, B., BARTH, A. and NELSON, W.J. (1996). Mechanism for transition from initial to stable cell-cell adhesion: kinetic analysis of E-cadherin-mediated adhesion using a quantitative adhesion assay. *J Cell Biology.* 134: 549-557.

BARD, J. (1990). Morphogenesis. Cambridge University Press, Cambridge.

BRIEHER, W.M. and GUMBINER, B.M. (1994). Regulation of cadherin function during activin induced morphogenesis of *Xenopus* animal caps. *J Cell Biology.* 126: 519-527.

BRIEHER, W.M., YAP, A.S. and GUMBINER, B.M. (1996). Lateral dimerization is required for the homophilic binding activity of C-cadherin. *J. Cell Biology.* 135: 487-489.

DAMKE, H., BABA, T., WARNOCK, D.E. and SCHMID, S.L. (1994). Induction of mutant dynamin specifically blocks endocytic coated vesicle formation. *J Cell Biol.* 127: 915-934.

DAMKE, H., BINNS, D.D., UEDA, H., SCHMID, S.L. and BABA, T. (2001). Dynamin gtpase domain mutants block endocytic vesicle formation at morphologically distinct stages. *Mol Biol Cell.* 12: 2578-2589.

GARAMSZEGI, N., DORE, J.J., JR., PENHEITER, S.G., EDENS, M., YAO, D. and LEOF, E.B. (2001). Transforming Growth Factor beta Receptor Signaling and Endocytosis Are Linked through a COOH Terminal Activation Motif in the Type I Receptor. *Mol Biol Cell.* 12: 2881-2893.

GUMBINER, B.M. (1992). Epithelial morphogenesis. *Cell.* 69: 385-387.

HERSKOVITS, J.S., BURGESS, C.C., OBAR, R.A. and VALLEE, R.B. (1993). Effects of mutant rat dynamin on endocytosis. *J Cell Biol.* 122: 565-578.

KAMEI, T., MATOZAKI, T., SAKISAKA, T., KODAMA, A., YOKOYAMA, S., PENG, Y.-F., NAKANO, K., TAKAISHI, K. and TAKAI, Y. (1999). Coendocytosis of cadherin and c-Met coupled to disruption of cell-cell adhesion in MDCK cells - regulation by Rho, Rac and Rab small G proteins. *Oncogene.* 18: 6776-6784.

KELLER, R., DAVIDSON, L., EDLUND, A., ELUL, T., EZIN, M., SHOOK, D. and SKOGLUND, P. (2000). Mechanisms of convergence and extension by cell intercalation. *Philos Trans R Soc Lond B Biol Sci.* 355: 897-922.

KOVACS, E.M., GOODWIN, M., ALI, R.G., PATERSON, A.D. and YAP, A.S. (2002). E-cadherin physically associates with the Arp2/3 complex to direct actin assembly in nascent adhesive contacts. *Curr Biol.* 12: 379-382.

LAUFFENBURGER, D.A. and HORWITZ, A.F. (1996). Cell migration: a physically integrated molecular process. *Cell.* 84: 359-369.

LAWSON, M.A. and MAXFIELD, F.R. (1995). Ca(2+)- and calcineurin-dependent recycling of an integrin to the front of migrating neutrophils. *Nature.* 377: 75-79.

LE, T.L., YAP, A.S. and STOW, J.L. (1999). Recycling of E-cadherin: a potential mechanism for regulating cadherin dynamics. *J. Cell Biol.* 146: 219-232.

LEE, C.-H. and GUMBINER, B.M. (1995). Disruption of gastrulation movements in *Xenopus* by a dominant-negative mutant for C-cadherin. *Dev. Biol.* 171: 363-373.

MARKS, B., STOWELL, M.H., VALLIS, Y., MILLS, I.G., GIBSON, A., HOPKINS, C.R. and MCMAHON, H.T. (2001). GTPase activity of dynamin and resulting conformation change are essential for endocytosis. *Nature.* 410: 231-235.

MCNIVEN, M., KIM, L., KRUEGER, E.W., ORTH, J.D., CAO, H. and WONG, T.W. (2000). Regulated interactions between dynamin and the actin-binding protein cortactin modulate cell shape. *J. Cell Biol.* 151: 187-198.

MILLER, J.R. and MCCLAY, D.R. (1997). Changes in the pattern of adherens junction-associated beta-catenin accompany morphogenesis in the sea urchin embryo. *Dev Biol.* 192: 310-322.

MITCHISON, T.J. and CRAMER, L.P. (1996). Actin-based cell motility and cell locomotion. *Cell.* 84: 371-379.

MUKHERJEE, S., GHOSH, R.N. and MAXFIELD, F.R. (1997). Endocytosis. *Physiol. Reviews.* 77: 759-803.

NG, T., SHIMA, D., SQUIRE, A., BASTIAENS, P.I.H., GSCHMEISSNER, S., HUMPHRIES, M.J. and PARKER, P.J. (1999). PKCa regulates b1 integrin-dependent cell motility through association and control of integrin traffic. *EMBO J.* 18: 3909-3923.

NIEUWKOOP, P. and FABER, J. (1967). Normal tables of *Xenopus laevis* development. Elsevier North-Holland, Amsterdam.

OCHOA, G.-C., SLEPNEV, V.I., NEFF, L., RINGSTAD, N., TAKEI, K., DANIELL, L., KIM, W., CAO, H., MCNIVEN, M., BARON, R. and DECAMILLI, P. (2000). A functional link between dynamin and the actin cytoskeleton. *J. Cell Biol.* 150: 377-389.

PALACIOS, F., PRICE, L., SCHWEITZER, J., COLLARD, J.G. and D'SOUZA-SCHOREY, C. (2001). An essential role for ARF6-regulated membrane traffic in adherens junction turnover and epithelial cell migration. *EMBO J.* 20: 4973-4986.

PAULSON, A.F., FANG, X., JI, H., REYNOLDS, A.B. and MCCREA, P.D. (1999). Misexpression of the catenin p120^{ctn}1A perturbs *Xenopus* gastrulation but does not elicit Wnt-directed axis specification. *Develop. Biol.* 207: 350-363.

SMITH, J.C., COOKE, J., GREEN, J.B., HOWES, G. and SYMES, K. (1989). Inducing factors and the control of mesodermal pattern in *Xenopus* laevis. *Development.* 107: 149-159.

SYMES, K., YAQOOB, M. and SMITH, J.C. (1988). Mesoderm induction in *Xenopus* laevis: responding cells must be in contact for mesoderm formation but suppression of epidermal differentiation can occur in single cells. *Development.* 104: 609-618.

TEPASS, U., GRUSZYNSKI-DEFEO, E., HAAG, T.A., OMATYAR, L., TOROK, T. and HARTENSTEIN, V. (1996). shotgun encodes Drosophila E-cadherin and is preferentially required during cell rearrangement in the neurectoderm and other morphogenetically active epithelia. *Genes Dev.* 10: 672-685.

TEPASS, U., TROUNG, K., GODT, D., IKURA, M. and PEIFER, M. (2000). Cadherins in embryonic and neural morphogenesis. *Nat Rev. Mol. Cell Biol.* 1: 91-100.

VAN DER BLIEK, A.M., REDELMEIER, T.E., DAMKE, H., TISDALE, E.J., MEYEROWITZ, E.M. and SCHMID, S.L. (1993). Mutations in human dynamin block an intermediate stage in coated vesicle formation. *J Cell Biol.* 122: 553-563.

VIEIRA, A.V., LAMAZE, C. and SCHMID, S.L. (1996). Control of EGF receptor signaling by clathrin-mediated endocytosis. *Science.* 274: 2086-2089.

WARNOCK, D.E. and SCHMID, S.L. (1996). Dynamin GTPase, a force-generating molecular switch. *Bioessays.* 18: 885-893.

YAP, A.S., BRIEHER, W.M. and GUMBINER, B.M. (1997a). Molecular and functional analysis of cadherin-based adherens junctions. *Annual Review of Cell and Developmental Biology.* 13: 119-146.

YAP, A.S., BRIEHER, W.M., PRUSCHY, M. and GUMBINER, B.M. (1997b). Lateral clustering of the adhesive ectodomain: a fundamental determinant of cadherin function. *Current Biology.* 7: 308-315.

ZHONG, Y., BRIEHER, W.M. and GUMBINER, B.M. (1999). Analysis of C-cadherin regulation during tissue morphogenesis with an activating antibody. *J. Cell Biol.* 144: 351-359.