Cinderella no longer: α-catenin steps out of cadherin's shadow

Jeanie A. Scott and Alpha S. Yap*

Division of Molecular Cell Biology, Institute for Molecular Bioscience, The University of Queensland, St Lucia, Brisbane, Queensland, 4072, Australia

*Author for correspondence (e-mail: a.yap@imb.uq.edu.au)

Accepted 19 September 2006
Journal of Cell Science 119, 4599-4605 Published by The Company of Biologists 2006
doi:10.1242/jcs.03267

Summary

To date, α-catenin has been best understood as an important cytoplasmic component of the classical cadherin complex responsible for cell-cell adhesion. By virtue of its capacity to bind F-actin, α-catenin was commonly envisaged to support cadherin function by coupling the adhesion receptor to the actin cytoskeleton. But is α-catenin solely the cadherin's handmaiden? A range of recent developments suggest, instead, that its biological activity is much more complex than previously appreciated. Evidence from cellular systems and model organisms demonstrates a clear, often dramatic, role for α-catenin in tissue organization and morphogenesis. The morphogenetic impact of α-catenin reflects its capacity to mediate functional cooperation between cadherins and the actin cytoskeleton, but is not confined to this. α-Catenin has a role in regulating cell proliferation and cadherin-independent pools of α-catenin may contribute to its functional impact.

Key words: α-Catenin, Cadherin, Actin cytoskeleton, Adhesion, Morphogenesis

Introduction

Spouses and siblings know well the neglect that can arise from too close identification with famous figures. Similar fates can even occur in biology. Take the example of α-catenin: first identified as a cytoplasmic protein that binds indirectly to classical cadherin cell adhesion molecules, its function and mechanism(s) of action have long been defined in terms of this interaction. A commonplace concept is that a trimolecular complex of classical cadherin, β-catenin and α-catenin associates with the actin cytoskeleton to determine cell-cell adhesion and hence tissue organization (Fig. 1A). One consequence of this gestalt is that the biological impact of α-catenin has often been thought to be encompassed by its contribution to cadherin activity. There is, however, increasing evidence that α-catenin has more complex and diverse functions then previously supposed. Our aim in this commentary is to survey some of these new developments that suggest a distinct cellular identity for this too-long neglected molecule.

α-Catenin is a morphogenetic regulator in its own right.

The functional importance of α-catenin was first directly tested in cultures of tumor cells lacking this protein (Hirano et al., 1992; Watabe et al., 1994). These cells aggregate poorly, reflecting a deficiency in cadherin-based cell-cell adhesion that can be overcome by re-expression of α-catenin (Hirano et al., 1992). Interestingly, such α-catenin-deficient cells possess the other core components of the cadherin molecular complex, but fail to concentrate them on the cell surface at adhesive contacts, which suggested that α-catenin exerted its important influence on adhesion by helping to ensure that cadherin was present at cell-cell contacts (Watabe et al., 1994). Consistent with this idea, restoration of α-catenin allowed these cells to polarize, establish specialized epithelial cell-cell junctions and undergo three-dimensional organization to form cysts (Watabe et al., 1994), properties of cultured epithelial cells that also require E-cadherin (Gumbiner et al., 1988; Troxell et al., 2001). Together, such observations indicated a necessary role for α-catenin in E-cadherin adhesion and in the morphogenetic consequences of cadherin activity.

To an extent, this conclusion from cultured cells was corroborated in organismal studies. Specific disruption of α-catenin in a variety of species compromises tissue integrity, which is consistent with disruption of cell-cell adhesion. Characteristically these phenotypes involve morphogenetic abnormalities, because cell differentiation is generally, but not always (Nemade et al., 2004), unaffected (Costa et al., 1998; Lien et al., 2006; Tinkle et al., 2004). These phenotypes often also resemble the changes associated with cadherin dysfunction. Thus, depletion of α-catenin causes disaggregation of early *Xenopus* embryos (Kofron et al., 1997), as does disrupting EP/C-cadherin, the predominant cadherin found at this stage of development (Heasman et al., 1994). In the early fly embryo, depletion of α-catenin by RNAi compromises morphogenetic movement during gastrulation (Magie et al., 2002), and an α-catenin mutation in mice blocks embryonic development at the blastocyst stage (Torres et al., 1997), as does targeted disruption of E-cadherin (Larue et al., 1994). Even more dramatic effects are seen later when α-catenin is disrupted in specific mouse tissues. Targeted deletion of α-catenin in neural precursors (Lien et al., 2006) or skin (Vasioukhin et al., 2001) dramatically compromises tissue cohesion and there is concomitant loss of adherens junctions. Overall, these phenotypic changes appear very consistent with disruption of

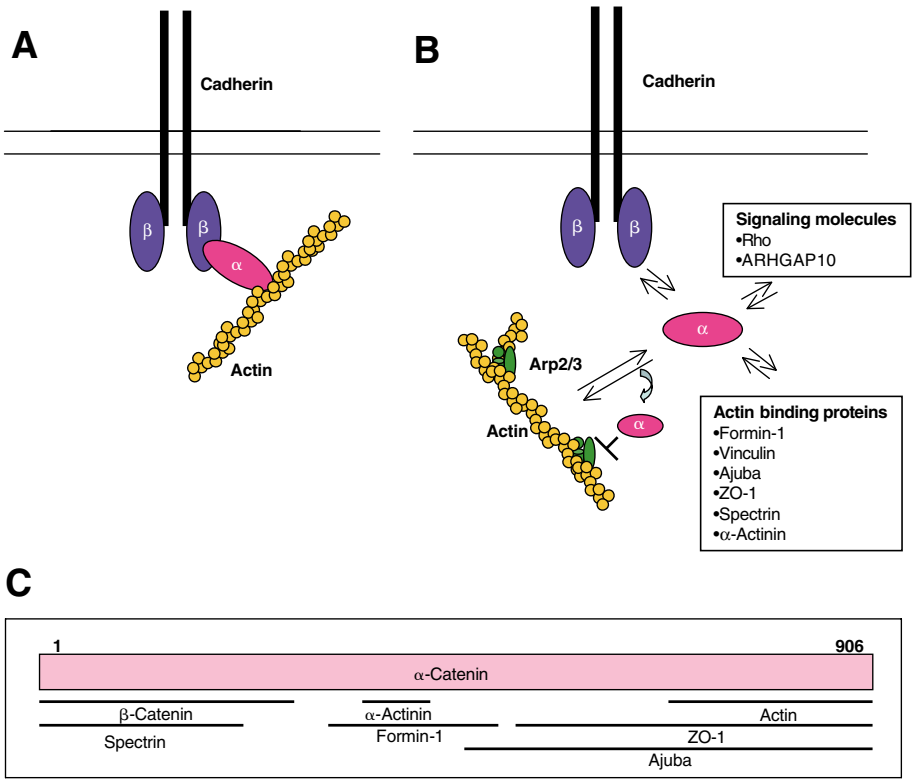

Fig. 1. Possible mechanisms for α-catenin-mediated cooperation between classical cadherins and the actin cytoskeleton. (A) A popular earlier model posited a quaternary complex comprising classical cadherins, β-catenin, α-catenin and cortical actin filaments. In this model, α-catenin directly binds to both actin filaments and β-catenin, thereby coupling stable actin filaments to the cadherin adhesion molecule. (B) The more complex current possibilities. α-Catenin, acting in the cytosol or bound through β-catenin to the cadherin molecular complex has the capacity to regulate the actin cytoskeleton by several mechanisms: binding directly to actin filaments, potentially thereby inhibiting Arp2/3-mediated actin nucleation; interacting with a range of actin regulators, including filament nucleators and binding proteins; and interacting with cell signalling pathways that can affect actin dynamics and organization. (C) The regions of α-catenin responsible for association with some actin-binding proteins have been mapped.

cell-cell adhesion, as one would predict if α-catenin critically supports cadherin function.

In several striking instances, however, the phenotypes associated with disruption of α-catenin differ significantly from those seen when cadherin itself is perturbed. For example, conditional deletion of α-catenin in skin disrupts tissue integrity so severely as to cause overt loss of the epidermis in newborn pups (Vasioukhin et al., 2001). E-cadherin deletion, by contrast, produced mild hyperproliferation with changes in hair follicle development in one study (Tinkle et al., 2004) and a specific tight junction defect in another (Tunggal et al., 2005). In neither case do the E-cadherin-null animals show the extensive and dramatic disruption of tissue architecture seen upon loss of α-catenin.

In mouse skin, upregulation of P-cadherin may compensate for loss of E-cadherin. However, this is unlikely to be the case in *Caenorhabditis elegans*, which has only one classical cadherin gene (*Hammerhead*, *Hmr-1*). Although worms have two α-catenin genes, only deletion of *Humpback* (*Hmp-1*) has a morphogenetic impact. Loss of *Hmp-1* produces a distinctive *humpback* phenotype, characterized by abnormal bulges on the dorsal side of the embryo (Costa et al., 1998). Interestingly, few animals possessing cadherin mutations display the *humpback* phenotype (~2%); instead, the majority show the *hammerhead* phenotype, in which the hypodermis, the covering epithelial layer of the embryo, fails to envelop the organism. These interesting phenotypic disparities suggest the possibility that α-catenin exerts cellular effects that are distinct from its undisputed contribution to cadherin function.

The challenges, then, are to elucidate the cellular mechanisms by which α-catenin supports cadherin activity and to identify other mechanisms that may account for additional morphogenetic effects. In this commentary we highlight three issues: the role of α-catenin in functional cooperation between cadherin adhesion and the actin cytoskeleton; its capacity to influence cell proliferation; and emerging evidence that α-catenin also acts independently of the cadherin molecular complex. We will discuss possible molecular mechanisms involved in each case, but one core theme is the idea that α-catenin functions as a dynamic molecular scaffold, that can select binding partners in a tightly regulated fashion.

α-Catenin, cadherins and the actin cytoskeleton

The notion that α-catenin facilitates cooperation between cadherins and the actin cytoskeleton is attractive for several reasons. Both cadherin adhesion and cytoskeletal activity are key determinants of tissue integrity and morphogenesis. Moreover, it has long been recognized that the biological function of cadherins requires intact actin filaments. Indeed, an increasing range of actin regulatory proteins can affect cadherin adhesion or its morphogenetic consequences. These include actin nucleators, such as formins (Kobielak et al., 2004) and Arp2/3 (Verma et al., 2004), scaffolding proteins such as cortactin (Helwani et al., 2004) and regulators of post-nucleation actin dynamics such as Ena/VASP proteins (Scott et al., 2006). Conversely, the actin cytoskeleton must be mechanically coupled to sites of adhesion if its dynamic activity is to affect cell-cell cohesion and patterning. One example of such coupling comes from the observation that N-cadherin molecules display retrograde flow on the dorsal surfaces of cells (Lambert et al., 2002). Not only do cadherins move rearward at rates similar to those reported for the cortical actin cytoskeleton, but retrograde flow of cadherin is abolished by disrupting actin integrity. This implies that N-cadherin

molecules are moved by coupling to retrograde actin flow, as has been observed for other transmembrane proteins (Felsenfeld et al., 1996). Molecular mechanisms that functionally and physically couple the cadherin adhesion apparatus to the actin cytoskeleton must then exist.

The notion that α-catenin fulfils this role arose from several early observations. During the biogenesis of cell-cell adhesions, the association of α-catenin with E-cadherin and β-catenin at the cell membrane coincides with the complex becoming Triton-insoluble (Hinck et al., 1994; Nathke et al., 1994). Moreover, E-cadherin is readily extracted with non-ionic detergents in tumor cells lacking α-catenin, but becomes more detergent-resistant upon expression of α-catenin (Watabe et al., 1994). While detergent-extractability has often been taken as evidence for cytoskeletal association, many other factors influence the detergent sensitivity of membrane proteins (Schuck et al., 2003). More directly, Rimm and colleagues (Rimm et al., 1995) used co-sedimentation assays to demonstrate that recombinant α-catenin can directly bind to actin filaments in vitro. These data supported the popular model in which α-catenin represents the crux of a quaternary complex coupling the cadherin molecular complex to cortical actin filaments and thereby linking the adhesion system to the actin cytoskeleton (Fig. 1A).

The existence of such a quaternary complex was not directly tested until recently (Yamada et al., 2005) and this yielded surprising results. Using in vitro binding assays, Yamada et al. (Yamada et al., 2005) confirmed that recombinant α-catenin can bind to F-actin; however, they detected no interaction when α-catenin was incorporated into a soluble complex with β-catenin and the cadherin cytoplasmic tail (Yamada et al., 2005). In fact, its binding to β-catenin and its binding to F-actin were apparently mutually exclusive (Drees et al., 2005). Nor could F-actin binding be detected when the ternary cadherin-catenin complex was reconstituted on stripped membrane patches. Finally, the protein dynamics of actin differed significantly from those of E-cadherin or catenins at cell-cell contacts (Yamada et al., 2005): turnover of GFP-tagged actin at cell-cell contacts was much more rapid than that of E-cadherin, β-catenin or α-catenin itself. Thus, a simple quaternary complex linking E-cadherin to stable actin filaments does not readily account for the functional coupling of cadherins to actin.

Does this mean that α-catenin has no role to play in cooperation between cadherins and actin? Although the biochemical basis for such cooperation is a more open question than it was previously, other observations suggest that it would be premature to dismiss a role for α-catenin in this process. The actin cytoskeleton at cadherin cell-cell adhesions may take several forms, which differ in their organization and dynamics (Scott et al., 2006; Zhang et al., 2005). These forms include cortical actin often found closely apposed to cadherin adhesions as well as prominent bundles that appear to terminate in adhesions.

Interestingly, genetic ablation studies in worms and mammalian cells suggest that α-catenin preferentially supports cadherin-based actin cables. During elongation of the *C. elegans* embryo, the epithelial cells of the hypodermis display an array of actin cables that are orientated parallel to the circumferential contour of the body and also appear to extend into the subapical adherens junctions (Costa et al., 1998). As elongation commences, the actin filament bundles thicken and shorten, decreasing the circumference of the worm while increasing its body length. In *hmp-1* mutant embryos, by contrast, the contracting actin bundles detach from the adherens junctions and retract towards the dorsal midline, failing to draw opposing edges of the cell together. This suggests that α-catenin may support morphogenetic cell shape changes by coupling contractile actin bundles to cell adhesions (Costa et al., 1998).

Similarly, two patterns of actin cables are found near contacts between mammalian epithelial cells. Perijunctional cables that run parallel to the zones of contact are most apparent in cohesive monolayers (Yonemura et al., 1995; Zhang et al., 2005). Additionally, shorter radial bundles that are orientated outwards to extend into cell-cell contacts often appear to terminate in prominent punctate cadherin adhesions (Scott et al., 2006; Vaezi et al., 2002). These radial actin cables are most prominent as cells establish contacts with one another but may also anchor the perijunctional actin cables. Interestingly, radial actin cables often appear to line up across cell-cell contacts (Sahai and Marshall, 2002; Vaezi et al., 2002; Yap et al., 1995), which suggests that, as in the worm embryo, they might identify lines of contractile force that extend across epithelial sheets. In keratinocytes from α-catenin-knockout mice, however, radial actin cables are much less evident and fail to line up across the cell contacts, which suggests that coupling between adhesions and actin cables is perturbed (Vaezi et al., 2002).

If α-catenin does not directly couple actin filaments to the cadherin molecular complex, are there other ways in which it might support cooperation between cadherins and the actin cytoskeleton, particularly actin bundles? Again, this remains an open question, but there are several possibilities (Fig. 1B). First, α-catenin may regulate actin filament dynamics. Recent work from the Fuchs lab reported that α-catenin can bind to, and recruit, formin 1, one of a family of actin nucleators that support barbed-end assembly of filaments in bundles, including the radial bundles that extend into cadherin contacts (Kobielak et al., 2004). Additionally, α-catenin can inhibit the activity of the Arp2/3 actin nucleator in vitro, perhaps by competing for F-actin association (Drees et al., 2005). Arp2/3 is also found at cadherin contacts (Kovacs et al., 2002; Yamada et al., 2005), where it contributes to actin assembly (Verma et al., 2004). Given that Arp2/3 is commonly believed to contribute to the formation of branched actin networks that drive cell surface protrusion, this suggests that α-catenin might regulate the balance between formation of branched actin networks (via Arp2/3) and formation of actin bundles (via formins), promoting the latter while inhibiting the former. If α-catenin can coordinate the activity of these two actin nucleators and consequently regulate the cytoskeleton, it could be instrumental in orchestrating the changes to cell shape that occur throughout development in response to cadherin ligation.

Second, α-catenin might promote actin bundling either directly or indirectly by recruiting other actin-regulatory proteins. Indeed, α-catenin can associate with several other actin-binding proteins (outlined in Fig. 1B,C), such as vinculin (Watabe-Uchida et al., 1998; Weiss et al., 1998), spectrin (Pradhan et al., 2001) and ZO-1 (Imamura et al., 1999). Whether such indirect interactions critically affect actin regulation at cadherin contacts remains to be directly tested.

Third, α-catenin may affect signalling pathways that

regulate the actin cytoskeleton. Notably, *Drosophila* Rho binds to α-catenin both in vitro and in vivo (Magie et al., 2002) and overexpression of α-catenin alters the phenotype of *Rho* mutant embryos. Interestingly, in vitro binding of Rho to α-catenin was independent of its nucleotide status, which suggests that this interaction influences the subcellular localization of Rho. Consistent with this is the observation that Rho fails to accumulate at cell-cell contacts in *Drosophila* embryos depleted of α-catenin by RNAi. Whether a similar biochemical and functional interaction occurs in vertebrates has yet to be reported. In mammalian cells, however, α-catenin also binds to ARHGAP10, a GTPase-activating protein that catalyses GTP hydrolysis on both Rho and Cdc42 (Sousa et al., 2005). Again, it remains to be determined whether this interaction affects Rho signalling at cell-cell contacts.

Overall, then, there are interesting alternative ways for α-catenin to regulate the actin cytoskeleton. And these alternatives may not be mutually exclusive. One example is the LIM-domain protein, Ajuba, whose recruitment into cell-cell contacts is regulated by α-catenin (Marie et al., 2003). Not only can Ajuba bind to α-catenin and F-actin, but in migrating cells it is capable of influencing a range of signalling molecules that regulate the cytoskeleton, including Rac1 and PtdIns(4,5)P_2 (Kisseleva et al., 2005).

Finally, can a specific role in actin bundling explain the impact of α-catenin on tissue cohesion and cadherin adhesion? We can envisage several ways in which this might be so. First, coupling of cell-cell adhesions to the contractile apparatus of the cytoskeleton may mechanically integrate cells across a population or monolayer. Such integration might facilitate resistance to detachment forces as well as coordinate morphogenetic movements in a cell population. Loss of integration would then sensitize tissues to mechanical disruption and compromise morphogenetic rearrangements. Second, if the cadherin puncta seen at the termini of actin cables represent lateral clusters, then assembly of actin cables may strengthen adhesion by stabilizing clusters, as has been suggested for focal adhesions (Chrzanowska-Wodnicka and Burridge, 1996). The fact that disruption of Ena/VASP proteins (Scott et al., 2006) or myosin II activity (Shewan et al., 2005) both perturb cadherin-based actin bundles and prevent generation of large cadherin clusters supports this idea. Again it must be emphasized that these possibilities, interesting though they might be, have yet to be tested directly. Clearly, there is much left to do.

α-Catenin and cell population control

Cell-cell cohesion and adherens junctions are not the only things affected by disruption of α-catenin. Altered cell population number is another distinctive feature of several α-catenin-deficiency phenotypes. In mice, targeted deletion of α-catenin in either the skin or in neural progenitor cells of the cerebral cortex leads to hyperproliferation, commonly manifested as poorly polarized cells that form hyperplastic masses (Vasioukhin et al., 2001). The hyperplasia of murine neural progenitors is attributable both to shortening of the cell cycle and decreased apoptosis (Lien et al., 2006). Conversely, re-expression of α-catenin slows the proliferation of tumor cells previously lacking this protein (Matsubara and Ozawa, 2004; Watabe et al., 1994).

The potential for α-catenin to influence population dynamics has direct morphogenetic relevance, because control of cell number can affect tissue size and organization (Chenn and Walsh, 2002; Lien et al., 2006). Moreover, it is potentially relevant to cancer. Although E-cadherin dysfunction is well known to promote invasiveness and metastasis in many types of carcinoma (Birchmeier et al., 1996), there are also cases where tumour progression correlates with reduced levels of α-catenin but not of E-cadherin (Matsubara and Ozawa, 2004; Watabe et al., 1994). If α-catenin can regulate cell population dynamics, its loss may contribute to tumorigenesis not only by affecting cell-cell adhesion, but also by promoting cell proliferation.

How, then, might α-catenin exert such effects? One likely avenue is by regulating signalling pathways that control cell proliferation. Changes in α-catenin levels can affect a number of cell cycle regulators, including expression of the p27 cyclin-dependent kinase inhibitor (Matsubara and Ozawa, 2001) and phosphorylation of p130, a member of the 'pocket protein' family of cell cycle regulators that trigger progression through the G0-G1 and G1-S transitions of the cell cycle (Cobrinik, 2005). Interestingly, hyperproliferative mouse keratinocytes lacking α-catenin display a striking upregulation of mitogenic signalling, resulting in sustained activation of the Ras-ERK pathway and increased responsiveness to growth factor stimulation (Vasioukhin et al., 2001). This is consistent with emerging evidence that the cadherin molecular complex can regulate growth factor signalling (Bryant et al., 2005). For example, E-cadherin was reported to inhibit cell growth by forming a complex with the EGF receptor, decreasing receptor mobility and its affinity for ligand (Qian et al., 2004). Whether α-catenin might affect such lateral sequestration has yet to be tested. Interestingly, α-catenin-deficient keratinocytes are hyper-responsive to a range of growth factors, which suggests that a downstream component common to multiple individual pathways might be affected. Indeed, insulin stimulation causes the E-cadherin–β-catenin complex to associate with the insulin receptor substrate-1 (IRS-1) adaptor in α-catenin-deficient cells, and this interaction appears to activate the Ras-MAP kinase pathway (Vasioukhin et al., 2001).

α-Catenin may also affect other signalling pathways that regulate cell proliferation, including the canonical Wnt signalling pathway (Kioussi et al., 2002), in which β-catenin plays a central role as a transcriptional regulator (Harris and Peifer, 2005). α-catenin can inhibit the ability of β-catenin to regulate transcription (Hwang et al., 2005; Takahashi et al., 2000). Blocking proteosomal degradation of α-catenin in chondrocytes causes an α-catenin–β-catenin heterodimer to accumulate and concomitantly suppresses Tcf/Lef transcriptional activity in response to Wnt-7a (Hwang et al., 2005). Similarly, co-expression of α-catenin blocks axis duplication in *Xenopus* embryos expressing β-catenin and Wnt-8, a classic assay of canonical Wnt signalling (Sehgal et al., 1997). It is therefore possible that loss of α-catenin may promote cell proliferation and perhaps tumor progression by failing to inhibit β-catenin signalling.

The hyperproliferative phenotype displayed by α-catenin-deficient mouse brains is not, however, typical of that associated with canonical Wnt signalling (Lien et al., 2006). Instead, microarray analysis identified upregulated expression of *Fgf15* and *Gli1*, transcriptional targets of the Hedgehog signalling pathway in this system. Moreover, inhibition of

Hedgehog signalling by cyclopamine prevents hyperproliferation in α-catenin-deficient brains, without affecting the disruption of cell-cell cohesion. This observation carries two important implications. First, it suggests that α-catenin may regulate cell-cell cohesion and cell proliferation through distinct cellular mechanisms. Second, it implies α-catenin can regulate cell proliferation in neural precursors by inhibiting Hedgehog signalling, through a mechanism that remains unknown. However, since the Hedgehog and Wnt signalling pathways can potentially interact in epithelial tissues (Watt, 2004), it would be fascinating to investigate a potential role for α-catenin in coordinating the activity of these two pathways.

Can α-catenin work without a cadherin?

Interestingly, the ability of α-catenin to regulate β-catenin signalling may involve protein pools that are independent of the cadherin itself (Fig. 2), and these could be present both in the cytoplasm (Drees et al., 2005; Gottardi and Gumbiner, 2004) and in the nucleus (Giannini et al., 2000). Association of α-catenin with β-catenin in the cytoplasm potentiates the binding of β-catenin to the cadherin (Castano et al., 2002), which would promote the adhesive function of β-catenin over its transcriptional role in canonical Wnt signalling (Gottardi and Gumbiner, 2004). Specific phosphorylation of Tyr142 in β-catenin blocks the interaction with α-catenin, lending support to the notion that this interaction in the cytoplasm may be physiologically regulated (Brembeck et al., 2004). Moreover, nuclear expression of α-catenin also antagonizes the transcriptional activity of β-catenin (Giannini et al., 2000).

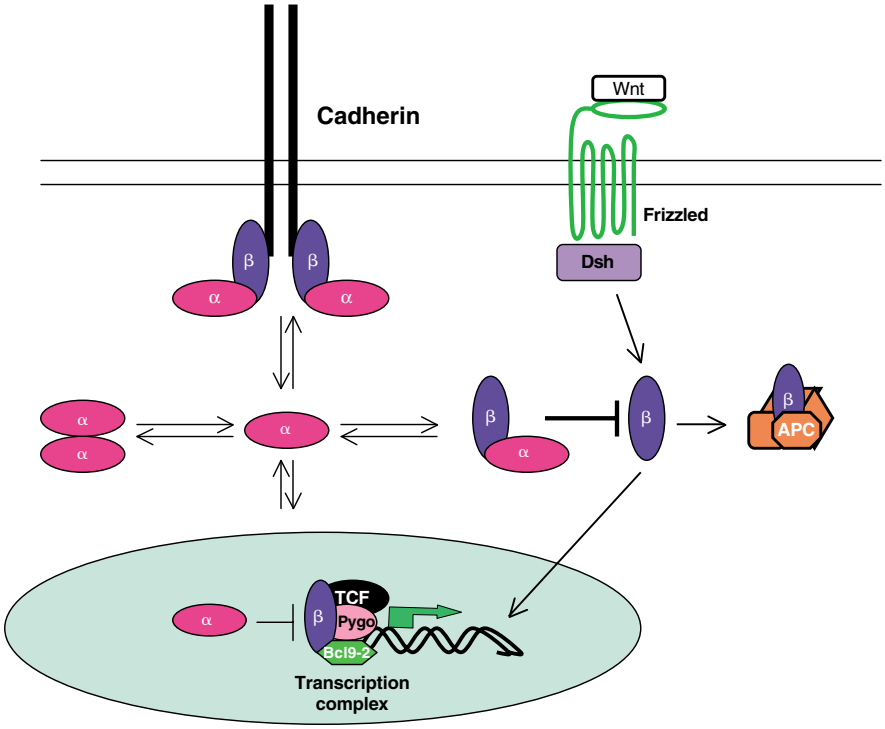

Fig. 2. Multiple cellular pools of α-catenin. α-Catenin exists in multiple cellular pools: incorporated into the cadherin complex at the plasma membrane; as monomers, homo- and hetero-dimers in the cytosol; and potentially also in the nucleus. Both in the cytosol and in the nucleus, cadherin-independent α-catenin may regulate β-catenin signalling in the canonical Wnt pathway by forming heterodimers with β-catenin. α, α-catenin; β, β-catenin; Dsh, dishevelled; Pygo, Pygobus; TCF, T-cell factor.

Nor may these be the only roles for cadherin-independent α-catenin. Recombinant α-catenin monomers and dimers inhibit Arp2/3-mediated actin nucleation in vitro (Drees et al., 2005), suggesting that in the cytosol these species might exert a similar effect on actin assembly. α-catenin has also been identified in a cytosolic complex with APC and β-catenin (Su et al., 1993) and it has been suggested that α-catenin cooperates with APC and armadillo to support cortical tethering of mitotic spindles during the syncytial blastoderm stage in fly embryos (McCartney et al., 2001). Thus, although mechanistic analysis of α-catenin has been dominated by the concept that it acts as part of the cadherin molecular complex, this may not be exclusively so.

Issues for the future

Clearly, we are moving away from any simple model for α-catenin function. Instead, we suggest that α-catenin's substantial biological impact arises from its capacity to participate in several fundamental cellular processes. We have highlighted roles in cooperation between cadherins and the actin cytoskeleton as well as in regulation of cell proliferation. In the latter case, rather than being solely active when it is complexed with the cadherin, α-catenin also exists in cadherin-independent pools that are likely to be functionally significant. Nor may the processes that we have highlighted be the only relevant ones. Cell polarity is often altered in α-catenin-null tissues, and α-catenin can bind to molecules such as ZO-1 (Itoh et al., 1997) that participate in other specialized junctions involved in biogenesis of the polarized epithelial phenotype. α-Catenin is also found in many non-epithelial tissues, including neurons where it regulates synaptic stability (Abe et al., 2004; Park et al., 2002). Understanding the signals that control where and when α-catenin works in the cell, and identifying how it chooses amongst a repertoire of potential partners, will provide invaluable insights into this versatile molecule.

We thank all our laboratory colleagues for their continued support, advice and generous intellectual stimulation. The authors were supported by the National Health and Medical Research Council of Australia.

References

Abe, K., Chisaka, O., Van Roy, F. and Takeichi, M. (2004). Stability of dendritic spines and synaptic contacts is controlled by alpha N-catenin. *Nat. Neurosci.* **7**, 357-363.

Birchmeier, C., Birchmeier, W. and Brand-Saberi, B. (1996). Epithelial-mesenchymal transitions in cancer progression. *Acta Anat. Basel* **156**, 217-226.

Brembeck, F. H., Schwarz-Romond, T., Bakkers, J., Wilhelm, S., Hammerschmidt, M. and Birchmeier, W. (2004). Essential role of BCL9-2 in the switch between beta-catenin's adhesive and transcriptional functions. *Genes Dev.* **18**, 2225-2230.

Bryant, D. M., Wylie, F. G. and Stow, J. L. (2005). Regulation of endocytosis, nuclear translocation, and signaling of fibroblast growth factor receptor 1 by E-cadherin. *Mol. Biol. Cell* **16**, 14-23.

Castano, J., Raurell, I., Piedra, J. A., Miravet, S., Dunach, M. and Garcia de Herreros, A. (2002). Beta-catenin N- and C-terminal tails modulate the coordinated binding of adherens junction proteins to beta-catenin. *J. Biol. Chem.* **277**, 31541-31550.

Chenn, A. and Walsh, C. A. (2002). Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. *Science* **297**, 365-369.

Chrzanowska-Wodnicka, M. and Burridge, K. (1996). Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. *J. Cell Biol.* **133**, 1403-1415.

Cobrinik, D. (2005). Pocket proteins and cell cycle control. *Oncogene* **24**, 2796-2809.

Costa, M., Raich, W., Agbunag, C., Leung, B., Hardin, J. and Priess, J. R. (1998). A putative catenin-cadherin system mediates morphogenesis of the Caenorhabditis elegans embryo. *J. Cell Biol.* **141**, 297-308.

Drees, F., Pokutta, S., Yamada, S., Nelson, W. J. and Weis, W. I. (2005). α-catenin is a molecular switch that binds E-cadherin-beta-catenin and regulates actin-filament assembly. *Cell* **123**, 903-915.

Felsenfeld, D. P., Choquet, D. and Sheetz, M. P. (1996). Ligand binding regulates the directed movement of beta1 integrins on fibroblasts. *Nature* **383**, 438-440.

Giannini, A. L., Vivanco, M. and Kypta, R. M. (2000). alpha-catenin inhibits beta-catenin signaling by preventing formation of a beta-catenin*T-cell factor*DNA complex. *J. Biol. Chem.* **275**, 21883-21888.

Gottardi, C. J. and Gumbiner, B. M. (2004). Distinct molecular forms of {beta}-catenin are targeted to adhesive or transcriptional complexes. *J. Cell Biol.* **167**, 339-349.

Gumbiner, B., Stevenson, B. and Grimaldi, A. (1988). The role of the cell adhesion molecule uvomorulin in the formation and maintenance of the epithelial junctional complex. *J. Cell Biol.* **107**, 1575-1587.

Harris, T. J. and Peifer, M. (2005). Decisions, decisions: beta-catenin chooses between adhesion and transcription. *Trends Cell Biol.* **15**, 234-237.

Heasman, J., Ginsberg, D., Geiger, B., Goldstone, K., Pratt, T., Yoshida-Noro, C. and Wylie, C. (1994). A functional test for maternally inherited cadherin in Xenopus shows its importance in cell adhesion at the blastula stage. *Development* **120**, 49-57.

Helwani, F. M., Kovacs, E. M., Paterson, A. D., Verma, S., Ali, R. G., Fanning, A. S., Weed, S. A. and Yap, A. S. (2004). Cortactin is necessary for E-cadherin-mediated contact formation and actin reorganization. *J. Cell Biol.* **164**, 899-910.

Hinck, L., Nathke, I. S., Papkoff, J. and Nelson, W. J. (1994). Dynamics of cadherin/catenin complex formation: novel protein interactions and pathways of complex assembly. *J. Cell Biol.* **125**, 1327-1340.

Hirano, S., Kimoto, N., Shimoyama, Y., Hirohashi, S. and Takeichi, M. (1992). Identification of a neural alpha-catenin as a key regulator of cadherin function and multicellular organization. *Cell* **70**, 293-301.

Hwang, S. G., Yu, S. S., Ryu, J. H., Jeon, H. B., Yoo, Y. J., Eom, S. H. and Chun, J. S. (2005). Regulation of beta-catenin signaling and maintenance of chondrocyte differentiation by ubiquitin-independent proteasomal degradation of alpha-catenin. *J. Biol. Chem.* **280**, 12758-12765.

Imamura, Y., Itoh, M., Maeno, Y., Tsukita, S. and Nagafuchi, A. (1999). Functional domains of alpha-catenin required for the strong state of cadherin-based cell adhesion. *J. Cell Biol.* **144**, 1311-1322.

Itoh, M., Nagafuchi, A., Moroi, S. and Tsukita, S. (1997). Involvement of ZO-1 in cadherin-based cell adhesion through its direct binding to alpha catenin and actin filaments. *J. Cell Biol.* **138**, 181-192.

Kioussi, C., Briata, P., Baek, S. H., Rose, D. W., Hamblet, N. S., Herman, T., Ohgi, K. A., Lin, C., Gleiberman, A., Wang, J. et al. (2002). Identification of a Wnt/Dvl/beta-Catenin → Pitx2 pathway mediating cell-type-specific proliferation during development. *Cell* **111**, 673-685.

Kisseleva, M., Feng, Y., Ward, M., Song, C., Anderson, R. A. and Longmore, G. D. (2005). The LIM protein Ajuba regulates phosphatidylinositol 4,5-bisphosphate levels in migrating cells through an interaction with and activation of PIPKI alpha. *Mol. Cell. Biol.* **25**, 3956-3966.

Kobielak, A., Pasolli, H. A. and Fuchs, E. (2004). Mammalian formin-1 participates in adherens junctions and polymerization of linear actin cables. *Nat. Cell Biol.* **6**, 21-30.

Kofron, M., Spagnuolo, A., Klymkowsky, M., Wylie, C. and Heasman, J. (1997). The roles of maternal alpha-catenin and plakoglobin in the early Xenopus embryo. *Development* **124**, 1553-1560.

Kovacs, E. M., Goodwin, M., Ali, R. G., Paterson, A. D. and Yap, A. S. (2002). Cadherin-directed actin assembly: E-cadherin physically associates with the Arp2/3 complex to direct actin assembly in nascent adhesive contacts. *Curr. Biol.* **12**, 379-382.

Lambert, M., Choquet, D. and Mege, R. M. (2002). Dynamics of ligand-induced, Rac1-dependent anchoring of cadherins to the actin cytoskeleton. *J. Cell Biol.* **157**, 469-479.

Larue, L., Ohsugi, M., Hirchenhain, J. and Kemler, R. (1994). E-cadherin null mutant embryos fail to form a trophectoderm epithelium. *Proc. Natl. Acad. Sci. USA* **91**, 8263-8267.

Lien, W. H., Klezovitch, O., Fernandez, T. E., Delrow, J. and Vasioukhin, V. (2006). alphaE-catenin controls cerebral cortical size by regulating the hedgehog signaling pathway. *Science* **311**, 1609-1612.

Magie, C. R., Pinto-Santini, D. and Parkhurst, S. M. (2002). Rho1 interacts with p120^{ctn} and a-catenin, and regulates cadherin-based adherens junctions in Drosophia. *Development* **129**, 3771-3782.

Marie, H., Pratt, S. J., Betson, M., Epple, H., Kittler, J. T., Meek, L., Moss, S. J., Troyanovsky, S., Attwell, D., Longmore, G. D. et al. (2003). The LIM protein Ajuba is recruited to cadherin-dependent cell junctions through an association with alpha-catenin. *J. Biol. Chem.* **278**, 1220-1228.

Matsubara, S. and Ozawa, M. (2001). Expression of alpha-catenin in alpha-catenin-deficient cells increases resistance to sphingosine-induced apoptosis. *J. Cell Biol.* **154**, 573-584.

Matsubara, S. and Ozawa, M. (2004). Expression of alpha-catenin in alpha-catenin-deficient cells results in a reduced proliferation in three-dimensional multicellular spheroids but not in two-dimensional monolayer cultures. *Oncogene* **23**, 2694-2702.

McCartney, B. M., McEwen, D. G., Grevengoed, E., Maddox, P., Bejsovec, A. and Peifer, M. (2001). Drosophila APC2 and Armadillo participate in tethering mitotic spindles to cortical actin. *Nat. Cell Biol.* **3**, 933-938.

Nathke, I. S., Hinck, L., Swedlow, J. R., Papkoff, J. and Nelson, W. J. (1994). Defining interactions and distributions of cadherin and catenin complexes in polarized epithelial cells. *J. Cell Biol.* **125**, 1341-1352.

Nemade, R. V., Bierie, B., Nozawa, M., Bry, C., Smith, G. H., Vasioukhin, V., Fuchs, E. and Hennighausen, L. (2004). Biogenesis and function of mouse mammary epithelium depends on the presence of functional alpha-catenin. *Mech. Dev.* **121**, 91-99.

Park, C., Falls, W., Finger, J. H., Longo-Guess, C. M. and Ackerman, S. L. (2002). Deletion in Catna2, encoding aN-catenin, causes cerebellar and hippocampal lamination defects and impaired startle modulation. *Nat. Genet.* **31**, 279-284.

Pradhan, D., Lombardo, C. R., Roe, S., Rimm, D. L. and Morrow, J. S. (2001). a-catenin binds directly to spectrin and facilitates spectrin-membrane assembly in vivo. *J. Biol. Chem.* **276**, 4175-4181.

Qian, X., Karpova, T., Sheppard, A. M., McNally, J. and Lowy, D. R. (2004). E-cadherin-mediated adhesion inhibits ligand-dependent activation of diverse receptor tyrosine kinases. *EMBO J.* **23**, 1739-1748.

Rimm, D. L., Koslov, E. R., Kebriaei, P., Cianci, C. D. and Morrow, J. S. (1995). Alpha 1(E)-catenin is an actin-binding and -bundling protein mediating the attachment of F-actin to the membrane adhesion complex. *Proc. Natl. Acad. Sci. USA* **92**, 8813-8817.

Sahai, E. and Marshall, C. J. (2002). ROCK and Dia have opposing effects on adherens junctions downstream of Rho. *Nat. Cell Biol.* **4**, 408-415.

Schuck, S., Honsho, M., Ekroos, K., Shevchenko, A. and Simons, K. (2003). Resistance of cell membranes to different detergents. *Proc. Natl. Acad. Sci. USA* **100**, 5795-5800.

Scott, J. A., Shewan, A. M., den Elzen, N. R., Loureiro, J. J., Gertler, F. B. and Yap, A. S. (2006). Ena/VASP proteins can regulate distinct modes of actin organization at Cadherin-adhesive contacts. *Mol. Biol. Cell* **17**, 1085-1095.

Sehgal, R. N., Gumbiner, B. M. and Reichardt, L. F. (1997). Antagonism of cell adhesion by an alpha-catenin mutant, and of the Wnt-signaling pathway by alpha-catenin in Xenopus embryos. *J. Cell Biol.* **139**, 1033-1046.

Shewan, A. M., Maddugoda, M., Kraemer, A., Stehbens, S. J., Verma, S., Kovacs, E. M. and Yap, A. S. (2005). Myosin 2 is a key Rho kinase target necessary for the local concentration of E-Cadherin at cell-cell contacts. *Mol. Biol. Cell* **16**, 4531-4532.

Sousa, S., Cabanes, D., Archambaud, C., Colland, F., Lemichez, E., Popoff, M., Boisson-Dupuis, S., Gouin, E., Lecuit, M., Legrain, P. et al. (2005). ARHGAP10 is necessary for alpha-catenin recruitment at adherens junctions and for Listeria invasion. *Nat. Cell Biol.* **7**, 954-960.

Su, L.-K., Vogelstein, B. and Kinzler, K. W. (1993). Association of the APC tumor suppressor protein with catenins. *Science* **262**, 1734-1737.

Takahashi, N., Ishihara, S., Takada, S., Tsukita, S. and Nagafuchi, A. (2000). Posttranscriptional regulation of alpha-catenin expression is required for Wnt signaling in L cells. *Biochem. Biophys. Res. Commun.* **277**, 691-698.

Tinkle, C. L., Lechler, T., Pasolli, H. A. and Fuchs, E. (2004). Conditional targeting of E-cadherin in skin: insights into hyperproliferative and degenerative responses. *Proc. Natl. Acad. Sci. USA* **101**, 552-557.

Torres, M., Stoykova, A., Huber, O., Chowdhury, K., Bonaldo, P., Mansouri, A., Butz, S., Kemler, R. and Gruss, P. (1997). An *a-E-catenin* gene trap mutation defines its function in preimplantation development. *Proc. Natl. Acad. Sci. USA* **94**, 901-906.

Troxell, M. L., Loftus, D. J., Nelson, W. J. and Marrs, J. A. (2001). Mutant cadherin affects epithelial morphogenesis and invasion, but not transformation. *J. Cell Sci.* **114**, 1237-1246.

Tunggal, J. A., Helfrich, I., Schmitz, A., Schwarz, H., Gunzel, D., Fromm, M., Kemler, R., Krieg, T. and Niessen, C. M. (2005). E-cadherin is essential for in vivo epidermal barrier function by regulating tight junctions. *EMBO J.* **24**, 1146-1156.

Vaezi, A., Bauer, C., Vasioukhin, V. and Fuchs, E. (2002). Actin cable dynamics and Rho/Rock orchestrate a polarized cytoskeletal architecture in the early steps of assembling a stratified epithelium. *Dev. Cell* **3**, 367-381.

Vasioukhin, V., Bauer, C., Degenstein, L., Wise, B. and Fuchs, E. (2001). Hyperproliferation and defects in epithelial polarity upon conditional ablation of alpha-catenin in skin. *Cell* **104**, 605-617.

Verma, S., Shewan, A. M., Scott, J. A., Helwani, F. M., den Elzen, N. R., Miki, H., Takenawa, T. and Yap, A. S. (2004). Arp2/3 activity is necessary for efficient formation of E-cadherin adhesive contacts. *J. Biol. Chem.* **279**, 34062-34070.

Watabe, M., Nagafuchi, A., Tsukita, S. and Takeichi, M. (1994). Induction of polarized cell-cell association and retardation of growth by activation of the E-cadherin-catenin adhesion system in a dispersed carcinoma line. *J. Cell Biol.* **127**, 247-256.

Watabe-Uchida, M., Uchida, N., Imamura, Y., Nagafuchi, A., Fujimoto, K., Uemura, T., Vermeulen, S., van Roy, F., Adamson, E. D. and Takeichi, M. (1998). alpha-Catenin-vinculin interaction functions to organize the apical junctional complex in epithelial cells. *J. Cell Biol.* **142**, 847-857.

Watt, F. M. (2004). Unexpected Hedgehog-Wnt interactions in epithelial differentiation. *Trends Mol. Med.* **10**, 577-580.

Weiss, E. E., Kroemker, M., Rudiger, A. H., Jockusch, B. M. and Rudiger, M. (1998).

Vinculin is part of the cadherin-catenin junctional complex: complex formation between alpha-catenin and vinculin. *J. Cell Biol.* **141**, 755-764.

Yamada, S., Pokutta, S., Drees, F., Weis, W. I. and Nelson, W. J. (2005). Deconstructing the cadherin-catenin-actin complex. *Cell* **123**, 889-901.

Yap, A. S., Stevenson, B. R., Waters, M. J., Keast, J. R. and Manley, S. W. (1995). Vinculin localization and actin stress fibers differ in thyroid cells organized as monolayers or follicles. *Cell Motil. Cytoskeleton* **32**, 318-331.

Yonemura, S., Itoh, M., Nagafuchi, A. and Tsukita, S. (1995). Cell-to-cell adherens junction formation and actin filament organization: similarities and differences between non-polarized fibroblasts and polarized epithelial cells. *J. Cell Sci.* **108**, 127-142.

Zhang, J., Betson, M., Erasmus, J., Zeikos, K., Bailly, M., Cramer, L. P. and Braga, V. M. (2005). Actin at cell-cell junctions is composed of two dynamic and functional populations. *J. Cell Sci.* **118**, 5549-5562.