Abstract. The tumor suppressor adenomatous polyposis coli (APC) negatively regulates Wingless (Wg)/Wnt signal transduction by helping target the Wnt effector β-catenin or its Drosophila homologue Armadillo (Arm) for destruction. In cultured mammalian cells, APC localizes to the cell cortex near the ends of microtubules. Drosophila APC (dAPC) negatively regulates Arm signaling, but only in a limited set of tissues. We describe a second fly APC, dAPC2, which binds Arm and is expressed in a broad spectrum of tissues. dAPC2’s subcellular localization revealed colocalization with actin in many but not all cellular contexts, and also suggested a possible interaction with astral microtubules. For example, dAPC2 has a striking asymmetric distribution in neuroblasts, and dAPC2 colocalizes with assembling actin filaments at the base of developing larval denticles. We identified a dAPC2 mutation, revealing that dAPC2 is a negative regulator of Wg signaling in the embryonic epidermis. This allele acts genetically downstream of wg, and upstream of Arm, dTCF, and, surprisingly, Dishevelled. We discuss the implications of our results for Wg signaling, and suggest a role for dAPC2 as a mediator of Wg effects on the cytoskeleton. We also speculate on more general roles that APCs may play in cytoskeletal dynamics.

Key words: Drosophila • adenomatous polyposis coli • Armadillo • β-catenin • Wingless

Human β-catenin (βcat)1 and its Drosophila homologue Armadillo (Arm) are key effectors of the conserved Wingless (Wg)/Wnt signal transduction pathway (for review see Gumbiner, 1998). In the absence of Wg signal, Arm in the cytoplasm is targeted for destruction by a multiprotein complex. Wg signal inactivates the destruction machinery, permitting accumulation of Arm in the cytoplasm and nucleus. Arm forms a complex with the DNA-binding protein, dTCF, to alter expression of Wg-responsive genes. Wnt signaling was implicated in colon cancer due to an increase in the frequency of benign colon polyps. These patients are heterozygous for a mutation in the adenomatous polyposis coli (APC) tumor suppressor; somatic disruption of the second copy of APC initiates polyp development (for review see Polakis, 1999). APC is part of the Arm/βcat destruction complex, along with Axin and the kinase Zeste-white 3 (Zw3)/glycogen synthase kinase 3β (GSK). In cells of a colon polyp, loss of APC function disables the destruction complex, leading to βcat accumulation, formation of βcat–TCF complexes, and activation of Wnt target genes such as the oncogene c-myc. APC is also important during normal development: mice homozygous for APC mutations die before gastrulation (Moser et al., 1995). A Drosophila homologue of APC, dAPC (Hayaishi et al., 1997), acts as a negative regulator of Arm signaling in the photoreceptors of the developing adult eye (Amed et al., 1998).

A PC’s biochemical function in the destruction complex remains somewhat mysterious. It is thought to be a protein scaffold that binds numerous protein partners at distinct sites along its length (see Fig. 1) (for review see Polakis, 1999). A PC was thought to facilitate interaction between βcat and GSK. However, Axin can also link βcat, GSK, and APC, raising questions about A PC’s role. Work in the nematode Caenorhabditis elegans suggests that A PC’s role

1. Abbreviations used in this paper: AEL, after egg laying; APC, adenomatous polyposis coli; Arm, Armadillo; βcat, β-catenin; BicD, Bicaudal D; Dsh, Dishevelled; dAPC, Drosophila APC; Fz, Frizzled; GSK, glycogen synthase kinase 3β; hAPC, human APC; Insc, Inscoluable; IP, immunoprecipitate; MT, microtubule; PP2A, protein phosphatase 2A; Wg, Wingless; Zw3, Zeste-white 3.
in Wnt signaling is more complex. Disruption of the closest nematode APC relative, apr-1 (by double-stranded RNA interference), unexpectedly led to a phenotype similar to that of loss-of-function mutations in Wnt and Arm relatives, suggesting that APR-1 is a positive effector of Wnt signaling (for review see Han, 1997).

A PC may also have additional cellular roles. When human A PC (hAPC) is overexpressed in cultured cells, it decorates microtubules (MTs) and can bind and bundle MTs in vitro (M unemitsu et al., 1994; Smith et al., 1994). In cultured cells, A PC localizes at the cell cortex in membrane puncta where bundles of MTs often terminate (Näthke et al., 1996). If one expresses a stabilized form of βcat (which cannot be phosphorylated by GSK) in D C K cells, mutant βcat accumulates with A PC in membrane puncta, and these cells display altered migratory behavior (for review see Barth et al., 1997). These data prompted the suggestion that A PC may regulate cell migration via its interaction with MTs, and that this role is modulated by βcat. A PC may also influence cytoskeletal dynamics by binding to EB1 (Su et al., 1995), which associates with the MT cytoskeleton in mammalian cells (Berrueta et al., 1998; Morrison et al., 1998). Yeast EB1 homologues contribute to MT function and may form part of a cytokinesis checkpoint (Beinhauer et al., 1997; Schwartz et al., 1997; Muhua et al., 1998). In addition to connections with MTs, A PC may associate with the actin cytoskeleton via β- and α-catenin.

The actin and MT cytoskeletons are both targets of the Wg/Wnt pathway. Signaling by Wnt family members or by their Frizzled (Fz) receptors is required to orient certain cell divisions in both nematodes and flies. In C. elegans, Wnt signaling directs the orientation of mitotic spindles in specific early embryonic blastomeres and orients postembryonic asymmetric cell divisions (for review see Han, 1997), whereas in Drosophila Fz is required for orientation of the mitotic spindles of bristle precursor cells (Gho and Schweiguth, 1998). Fz also plays a key role in orienting the cytoskeleton during formation of hairs and bristles, polarized outgrowths of the cell membrane (for review see Shulman et al., 1998). Both the actin and MT cytoskeletons are required for hair positioning and growth (Wong and A dler, 1993; Turner and A dler, 1998).

Whereas A PC regulates Arm signaling in the Drosophila larval photoreceptors (A hmed et al., 1998), two features of A PC were surprising given the widespread expression and essential function of mouse A PC. Embryonic expression of A PC is largely confined to the central nervous system (H ayashi et al., 1997), and null mutations in A PC are viable and fertile, with strong effects only in the larval photoreceptors (A hmed et al., 1998). These observations suggested the existence of a second A PC gene in flies; a second mammalian A PC has been identified (Nakagawa et al., 1998; van E s et al., 1999).

Materials and Methods

Biochemistry and Two-Hybrid Analysis

Extract preparation, immunoprecipitations (IPs), cell fractionation, and analysis of phosphorylation were as in Peifer (1993); anti-A rm IPs were at 1:40 and anti-dA PC 1 IPs at 1:50. Samples were analyzed by 6% acryl-
there is no correspondence between individual human and fly proteins, even though both phyla show neural-enriched isoforms, DPC and hAPC2, suggesting independent gene duplications. All APCs have six ARM repeats; a putative seventh ARM repeat is much more divergent and is not identifiable in dAPC2. The NH2-terminal conserved region (61% identity to dAPC vs. 44% identity to hAPC) distantly resembles the ARM repeat consensus and may form one or two degenerate ARM repeats. A PC family members also share similarity COOH-terminal to the ARM repeats. hAPC has two sets of repeated βcat binding sites, the 15 and 20 amino acid repeats (for review see Polakis, 1999; dAPC features are from Hayashi et al., 1997; hAPC lacks 15 amino acid repeats). dAPC shares two of the three 15 amino acid repeats of dAPC. hAPC and dAPC2 have five 20 amino acid repeats, among which are interspersed SAMP repeats (Fig. 1 C). dAPC2 has four SAMP repeats, whereas dAPC2 has two. dAPC2 ends 40 amino acids after the last SAMP repeat.

dAPC2 Protein

We generated antisera to a DAPC2 fusion protein (amino acids 491–1061); antisera from two independent rats immunized with this antigen both recognize a single set of protein isoforms of ~155–170 kD in embryonic extracts (Fig. 2 A) (they occasionally weakly cross-react with proteins of ~120 and > 200 kD). In contrast, the preimmune sera do not recognize any proteins on immunoblots of embryonic extract, supporting the specificity of the antisera. Further, as we show below, the migration on SDS-PAGE of the putative dAPC2 protein is altered in a dAPC2 mutant, consistent with these protein isoforms representing the genuine DAPC2 protein. The predicted molecular mass of DAPC2, 117 kD, is smaller than the observed molecular mass. However, an epitope-tagged version of the DAPC2 open reading frame expressed in human SW480 colon carcinoma cells also migrated at much higher apparent molecular mass than predicted from the sum of the predicted molecular mass of the DAPC2 coding sequence plus that of the epitope (Fig. 2 A). This suggests that the large apparent molecular mass of DAPC2 is a property of its migration on SDS-PAGE. We examined the developmental profile of DAPC2 expression during embryogenesis (Fig. 2 B). DAPC2 is present in the preblastoderm embryo (presumably maternally contributed), and levels remain relatively constant through the first half of embryogenesis, then drop sharply. As hAPC is phosphorylated (e.g., Rubinfeld et al., 1996), we suspected that the DAPC2 isoforms might be phosphorylation variants. To test this, we immunoprecipitated (IPed) DAPC2 from embryos and treated the IP with protein phosphatase 2A (PP2A), a serine/threonine-specific phosphatase. PP2A treatment reduced the apparent molecular mass of DAPC2; this effect was abolished if the PP2A inhibitor okadaic acid was included during incubation (Fig. 2 C, left panel). Further, if embryonic cells were dissociated and incubated in tissue culture medium, the apparent molecular mass of DAPC2 decreased (Fig. 2 C, right panel); this effect was also abolished by okadaic acid, suggesting that it is mediated by endogenous phosphatases. Parallel alterations in ARM phosphorylation support this hypothesis (Fig. 2 C, right panel) (Peifer, 1993). Taken together, these data suggest that the DAPC2 isoforms reflect, at least in part, differential phosphorylation.

Table I. Drosophila Melanogaster Stocks Used in Epistasis Tests

| Gene | Cross scheme | Results |
|------|--------------|---------|
| wg   | wg13/Cyo; dAPC2 females × males | All wg progeny show the suppressed phenotype (n > 100). |
|      | wg13/Cyo; dAPC2/Tdr(3R)crb87-4 females × males | 1/3 of wg progeny show the suppressed phenotype (n = 40). |
|      | wg13/Tdr; dAPC2 males | Df homozygotes show crumbs phenotype. |
| arm  | arm FRT101/289 females ×× dAPC2 males | 1/4 of the progeny show the arm zygotic phenotype (presumably arm 6/6; DAPC2 male embryos). |
| dTCF | dAPC2; dTCF/ey females × males | 3/4 show excess naked cuticle like DAPC2 single mutants (n > 200). |
| dsh  | dTCF/igo females ×× dAPC2 males | 1/4 of the progeny show the dTCF zygotic phenotype, and the remainder have excess naked cuticle (n > 200). |
|   1. dsh75 FRT101/TM3 females | Zygotic loss of dsh does not modify the DAPC2 mutant phenotype. |
|   2. Heat-shock progeny as third instar larvae twice to induce recombination | In the first (n > 30) and second (n = 23) crosses, 1/2 the progeny show the dsh germline clone mutant phenotype (presumed to be the dsh75/Y; dAPC2 male embryos) and 1/2 show a partially suppressed dAPC2 mutant phenotype, due to paternal rescue of dsh (n > 30). The partial suppression of the DAPC2 mutant phenotype is more dramatic ventrally, with most denticles belts fully formed; the dorsal surface is largely unrescued. In the cross to Df(3R)crb87-4/TM3 males, some progeny hatch (presumably those that are paternally wild-type), but dead embryos show phenotypes identical to those in the other crosses (n = 19). |
**dAPC2 Interacts Directly with Arm**

hAPC and dAPC (Hayashi et al., 1997) bind to βcat and Arm, respectively. We tested whether dAPC2 also interacts with Arm in vivo. We immunoprecipitated Arm from embryonic extracts, and, in parallel, IPed proteins with a control mAb, anti-myc. dAPC2 specifically co-IPed with Arm from both early and older embryos (Fig. 2 D), but did not co-IP with the control anti-myc antibody. We were unable to detect Arm in anti-dAPC2 IPs (data not shown); because the antigen for the dAPC2 antisera includes the Arm binding region, these sera might not recognize a dAPC2–Arm complex. We also found that a dAPC2 fragment containing the putative βcat binding sites co-IPed with βcat when expressed in the human colorectal cancer cell line SW 480 (data not shown).
The hAPC–bcat interaction is direct, and is mediated by the 15 and 20 amino acid repeats of hAPC and the Arm repeats of bcat (for review see Polakis, 1999); the analogous region of dAPC binds Arm (Hayashi et al., 1997). To test whether dAPC2 directly interacts with Arm, we used the yeast two-hybrid system (Fig. 2 E), examining whether dAPC2’s 15 and 20 amino acid repeats interact with the full set of Arm repeats of Arm (R1–13), or with the centralmost Arm repeats (R3–8; the binding site for Drosophila E-cadherin and dTCF). For comparison, we tested the 15 and 20 amino acid repeats of dAPC (Hayashi et al., 1997). The full 15 and 20 amino acid repeat regions of both dAPC and dAPC2 strongly interact with the entire Arm repeat region and with R3–8. We also tested 31–34 amino acid fragments carrying individual 15 or 20 amino acid repeats of dAPC and dAPC2 (selected as good matches to the consensus). Individual 15 amino acid repeats of either dAPC or dAPC2 interacted with both Arm repeat regions and with R3–8. An individual 20 amino acid repeat of dAPC also interacted with both Arm repeat regions.
frequently stain any structures in situ, we determined that preimmune sera do not specifically stain the actin cytoskeleton. To demonstrate that our anti-dAPC2 antisera are specific, we observed whether or not the preimmune sera act strongly with Arm repeats 1–13; its interaction with these repeats occurs within 0.3–0.5 μm from the surface of the embryo. The specificity of staining in situ is further supported by the change in intracellular localization seen in a dAPC2 mutant (see below), and by the fact that antisera from a second rat immunized with this antigen recognize a similar set of cellular structures (at least during midembryogenesis, the stage we examined).

Thus, we used our anti-dAPC2 antisera to characterize its expression and subcellular localization. During nuclear division cycles 10–13, which take place without cytokinesis in the peripheral cytoplasm of the embryo, dAPC2 shows dynamic changes in subcellular localization, coincident with those of actin (Fig. 3). Sequential changes in MT organization as nuclei proceed through mitosis direct reorganization of the cortical actin cytoskeleton (for review see Foe et al., 1993). Before nuclei migrate to the periphery, actin is found at the cortex in a random reticulum. When nuclei reach the periphery, actin condensations appear in interphase and prophase above each nucleus, forming an actin bud which overlays a cytoplasmic bud. This separates the mitotic apparatus of one nucleus from that of its neighbor. As division proceeds to metaphase, actin redistributes from the crown of the bud to its lateral cortex, forming an oblong ring around each spindle. During anaphase, actin redistributes into discs above each newly formed nucleus. Centrosomes and their associated MTs direct the changes in actin distribution, although the mechanism responsible for this interaction is not known.

In cycle 10–13 embryos, dAPC2 colocalizes with actin at all stages of mitosis (we could not test for colocalization with Arm, as its levels at these stages are too low to detect its localization). The dAPC2/actin colocalization is most prominent in the microvillar projections at the surface of the bud in interphase and prophase (Fig. 3, A–C). At metaphase and anaphase, dAPC2 and actin condensations are observed at the lateral cortex of the bud (Fig. 3, I–L); dAPC2 staining is somewhat less intense here relative to actin. Toward the base of the bud, condensations of actin and dAPC2 are also found in the region of the centrosome and asters (Fig. 3, E–H, arrows). These dAPC2 condensations occur within 0.3–0.5 μm of the surface of the embryo (data not shown), and thus are most prominent above the spindle apparatus; kinetochore MTs are not in uniform focus until ~1.25 μm from the surface of the embryo. The location of these dAPC2/actin condensations above the plane of the spindle places them in a position to interact with the asters. During later nuclear cycles when pseudocleavage furrows are present, more defined dots of actin and dAPC2 staining are sometimes observed (Fig. 3, I–P, arrows) in the region of the centrosomes. In one of our wild-type stocks, which was infected with the bacterial endosymbiont Wolbachia (visible as small propidium iodide–positive bodies), we observed an additional dAPC2 localization. Wolbachia associate with astral MTs in Drosophila and thereby disperse into newly formed cells (Callaini et al., 1994; Kose and Karr, 1995). In infected embryos, dAPC2 localizes with the actin cytoskeleton as in uninfected stocks, and also associates with bacteria at the asters (Fig. 3 D). A notable astral MT-associated protein, the kinesin-like protein KLP67A, is also reported to associate with bacteria (Pereira et al., 1997). EM studies have shown that the bacteria are encapsulated within a cytoplasmic vacuole attached to astral MTs via an electron-dense bridge, possibly composed of cellular MT-associated proteins (Callaini et al., 1994). dAPC2’s localization to the aster region of noninfected embryos and its association with bacteria suggest that dAPC2 may contribute to the binding of the vacuole to the asters.

After cellularization, dAPC2 is still enriched in the region of MTs. Increased levels of cytoplasmic dAPC2 are observed in mitotic domains (groups of cells undergoing synchronous mitosis) (Fig. 4 D). Here, cytoplasmic condensations of dAPC2 are observed in the region of the spindle in metaphase and anaphase (Fig. 4, E and F, arrows), but are absent in prophase or telophase (the other cells in the mitotic domain in Fig. 4, E and F, are in prophase); serial sections revealed that these cytoplasmic condensations are most prominent within 2–4 μm of the cell apex. In mitotic domains of a Wolbachia-infected strain, we observed punctate condensations of dAPC2 near the spindle poles, presumably astrally associated bacteria (Fig. 4 G), consistent with dAPC2 localization to bacteria associated with preblastoderm asters.

dAPC2 is also expressed in dividing cells of the larval brain (Fig. 5). The optic lobes contain two proliferative regions, the inner and outer proliferative zones. dAPC2 is highly expressed in dividing cells of the proliferative zones and in their immediate progeny, but not in differentiated neurons (Fig. 5, A and C). In contrast, Arm is not enriched in the proliferative zones (Fig. 5, B and D) but is enriched in axons. In the ventral nerve cord, Arm is found in axons, whereas dAPC2 is found in midline glial cells (Fig. 5, C and D). In contrast, dAPC2 localizes to axons, at least in embryos (Hayashi et al., 1997).

However, in larval neuroblasts (neural stem cells) dAPC2 and Arm share a striking asymmetric distribution. Neuroblasts divide asymmetrically to produce a large neuroblast and a smaller ganglion mother cell, which will divide symmetrically to produce two neurons (for review see Fuerstenberg et al., 1998). The asymmetric division requires specific orientation of the mitotic spindle. Insoluble (Ins), localized in a crescent opposite the future daughter cell during prophase and metaphase, is required for both spindle orientation and localization of the neural determinants Prospero and Numb (Kraut et al., 1996). In larval neuroblasts, both dAPC2 (Fig. 5 E, arrow) and Arm (Fig. 5 F, arrow) colocalize to a cortical crescent next to the future daughter cell; this crescent also includes the neural determinant Prospero (Fig. 5, H and I, arrow). In contrast to other asymmetric neuroblast components (for
review see Fuerstenberg et al., 1998), the dAPC2 and Arm crescents are present even at interphase (Fig. 5 E, lower neuroblast). In some neuroblasts, cortical actin also accumulates in a crescent with dAPC2 (Fig. 5, J and K, arrows), whereas in others this association is less apparent (Fig. 5, L and M, arrows). To examine the relationship between dAPC2 and the spindle, we triple-labeled neuroblasts with antibodies against phosphohistone, β-tubulin, and dAPC2 (Fig. 5, N–P). One pole of the spindle apparatus colocalizes with the dAPC2 crescent; dAPC2 is enriched at this point relative to the rest of the crescent (Fig. 5, O and P, arrows). We also observed low levels of dAPC2 at the opposite cortex at this stage of the cell cycle, the position of which often coincided with the other spin-
dle pole (Fig. 5, O and P, arrows). Whereas cortical dAPC2 associated with spindle poles, neuroblasts did not have cytoplasmic condensations of dAPC2 around the central spindle as were observed in epidermal cells. dAPC2 is also asymmetrically localized in embryonic neuroblasts (Fig. 5 G, arrows).

In nondividing cells, dAPC2 also associates with the cell cortex, and colocalizes with actin. In the embryo, dAPC2 is most strongly expressed in the epidermis and other epithelial cells. In the epidermis, dAPC2 is enriched at the cell cortex and is also found throughout the cytoplasm in a punctate distribution (Fig. 4 A). At the cortex, dAPC2 appears as numerous punctate condensations of protein (Fig. 4 A) which are most prevalent at the apical end of the lateral cell surface but are also found more basally. The most intense staining of dAPC2 appears at points of contact between multiple epidermal cells (Fig. 4 A, arrows). dAPC2 condensations often colocalize with condensations of actin (Fig. 4, A and B, arrows) and phosphotyrosine (data not shown), although actin and phosphotyrosine associate with the cortex more continuously. In fully polarized epithelial cells like the embryonic hindgut (Fig. 6, A and B) or the larval imaginal discs (Fig. 6, C and D), dAPC2 is enriched in adherens junctions, where it colocalizes with Arm; dAPC2 also accumulates on the apical plasma membrane (Fig. 6, A and C). The intracellular distribution of dAPC2 (Fig. 6 E), in contrast to that of Arm (Fig. 6 F), is not modulated in a segmental fashion. A strikingly different localization of dAPC2 occurs in the epidermis after stage 15. dAPC2 becomes organized into very large apical structures in segmentally repeated subsets of ventral epidermal cells (Fig. 6, G and H), just before the stage at which these cells initiate denticle formation. The dAPC2 structures occur specifically in anterior epidermal cells of each segment and colocalize with similar actin structures (Fig. 6, I–K), which likely represent larval denticle precursors.

Although dAPC2 colocalizes with actin in many tissues, it does not colocalize with actin in all contexts. For example, during cellularization, actin is prominent at the cellularization front, whereas dAPC2 is enriched at the apical cortex (data not shown). In addition, as we noted previously, at the cortex of epidermal cells actin is present at the membrane in a continuous fashion, whereas dAPC2 is restricted to regions of most intense actin staining. Finally, dAPC2 is not found with actin in cytokinesis furrows (Fig. 4, A and C). Thus, although dAPC2 associates with the actin cytoskeleton, the context-dependent nature of this association suggests that it is regulated.

Biochemical Properties of dAPC2

Biochemical analyses also suggest that dAPC2 associates with the cell cortex. When we fractionated 0–6-h-old embryos into soluble (S100) and membrane-associated (P100) fractions, dAPC2 partitioned almost equally into these two fractions (Fig. 7 A). In contrast, Arm was almost exclusively in the membrane fraction at this stage. The isoforms of dAPC2 in the membrane fraction migrated more rapidly on SDS-PAGE than those in either the soluble fraction or the total cell lysate (Fig. 7 A); because these isoforms are not detectable in total lysate, we suspect that they may arise during fractionation by dephosphorylation. To examine whether dAPC2 might associate with the membrane via a glycoprotein, we used Con A-Sepharose, which can be used to isolate membrane glycoproteins as well as proteins associated with them (e.g., Arm) (Peifer, 1993). A subset of dAPC2 specifically bound to Con A in extracts from 0–6-h embryos (Fig. 7 A; BicD was a negative control). Thus, dAPC2 may be anchored to the cortex via a transmembrane glycoprotein.

Identification of a dAPC2 Mutation

We mapped dAPC2 to polytene region 95F1–2 on the
third chromosome by in situ hybridization to wild-type and deficiency chromosomes. 

**Figure 5.** dAPC2 localization in dividing cells of the larval brain. (A–D) Third instar larval brain and ventral nerve cord double-labeled for dAPC2 (A and C) and Arm (B and D). OPZ, outer proliferative zone; IPZ, inner proliferative zone; NB, neuroblasts; MG, midline glia; AX, axons. (E, F, H, I, and J–M) Larval neuroblasts double-labeled for dAPC2 (E, H, J, and L), Arm (F), Prospero (I), and actin (K and M). dAPC2, Arm, and Prospero are asymmetrically localized at the cortex of neuroblasts. A ctn is sometimes observed in crescents (K and M). (G) Embryonic neuroblasts labeled for dAPC2 reveal an asymmetric distribution (arrows). (N–P) Larval neuroblasts triple-labeled for β-tubulin (N and P, green), dAPC2 (O and P, red), and phosphohistone (P, blue). Condensations of dAPC2 occur in the region of the spindle poles (arrows). Bars: (A–D) 50 μm; (E–P) 10 μm.

The mutant and parental chromosomes share 33 polymorphisms relative to the wild-type Canton S; only 8 altered the protein, and most changes are conservative (Fig. 1D). There is only a single difference between the parental chromosome and the mutant: deletion of three nucleotides, leading to deletion of serine 241. This serine residue...
falls within an alpha-helix in the third Arm repeat (by analogy to the Arm repeats of β-catenin) (Fig. 1 D). The length of this alpha-helix is invariant among APC family members, and this residue is either serine or alanine (a conservative change) in all APCs. Thus, we refer to this allele as dAPC2D.

Whereas homozygous mutant embryos accumulate normal levels of dAPC2, mutant dAPC2 migrates more rapidly on SDS-PAGE than wild-type protein (Fig. 8 M). A portion of dAPC2 in heterozygous mutants, which are wild-type in phenotype, also migrates abnormally (data not shown), suggesting that this is an intrinsic property of mutant dAPC2 rather than a consequence of the mutant phenotype. The subcellular localization of dAPC2 in dAPC2D mutants was dramatically altered at both the permissive (18°C) and restrictive (25°C) temperatures. At the restrictive temperature, dAPC2 association with the cell cortex is essentially abolished, rendering the protein almost completely cytoplasmic (Fig. 8 A vs. Fig. 8 D). At the permissive temperature, some cortical dAPC2 remains (Fig. 8 C). In heterozygotes, dAPC2 protein localization is intermediate between mutant and wild-type, as if mutant protein localizes incorrectly despite the presence of wild-type protein (Fig. 8 B). The loss of phosphorylated dAPC2 isoforms observed above (Fig. 8 M) may be a consequence of the loss of cortical association.

We also examined the localization of dAPC2D mutant protein at the restrictive temperature in other tissues. Although dAPC2D is found in apical buds in the preblastoderm embryo (Fig. 8, E and F), it no longer associates with actin structures as does the wild-type protein (Fig. 3, A–C). Furthermore, dAPC2D (Fig. 8 G) does not associate with the apical plasma membrane in the wing imaginal epithelia, marked by the presence of cortical actin (Fig. 8 H). In the larval neuroblasts, dAPC2D is largely cytoplasmic (Fig. 8, I and J), although an association with the cortex is sometimes observed (Fig. 8 I, arrow).
**dAPC2 Is a Negative Regulator of Wg Signaling in the Embryonic Epidermis**

dAPC2 is viable and fertile at the permissive temperature (18°C). At the restrictive temperature (25°C), dAPC2 homozygous mutants derived from heterozygous mothers are viable, indicating that maternal contribution of dAPC2 is sufficient for embryonic development. Heterozygous embryos derived from homozygous mutant mothers are wild-type and survive to adulthood, suggesting that zygotic function is also sufficient. Mutant embryos derived from mutant mothers (referred to below as dAPC2 maternal/zygotic mutants) have severe abnormalities in their embryonic body plan. On the ventral surface, wild-type embryos show segmentally repeated denticle belts interspersed with naked cuticle (Fig. 9 A). In dAPC2 maternal/zygotic mutants, denticle belts are replaced with an almost uniform expanse of naked cuticle (Fig. 9 B), as is observed when wg is ubiquitously expressed (Fig. 9 D). The dorsal surface also has an array of pattern elements marking specific cell fates (Fig. 9 E); cells receiving Wg signal secrete fine hairs. On the dorsal surface of dAPC2 maternal/zygotic mutants, many more cells secrete fine hairs (Fig. 9 F), as they do when wg is ubiquitously expressed (data not shown). Thus, maternal/zygotic loss of dAPC2 function activates Wg signal transduction both dorsally and ventrally, suggesting that wild-type dAPC2 helps negatively regulate this pathway.

Perturbing dAPC2 function at defined developmental time points supports this hypothesis. At the permissive temperature, dAPC2 mutant embryos develop normally into adults and a homozygous mutant stock can be maintained. When we shifted homozygous mutant embryos up to the restrictive temperature at 4 h after egg laying (AEL), they secreted uniform naked cuticle, like animals at the restrictive temperature throughout development. Progressively later upshifts result in intermediate cuticle defects, with increasing numbers of denticles secreted, until by 10 h AEL the pattern is essentially wild-type (data not shown).
not shown). Conversely, shifts from the restrictive temperature down to the permissive temperature at 4 h AEL fully rescue the pattern, whereas progressively later downshifts result in more and more naked cuticle replacing the ventral denticles belts. Thus, dAPC2 function may be dispensable for adult patterning; mutant embryos shifted up to the restrictive temperature after 10 h and cultured continuously at this temperature develop into apparently normal adults. This could be the result of partial activity of the dAPC2<sup>Ds</sup> allele. However, we suspect that dAPC2<sup>25</sup> is at least a strong hypomorph, as placing this allele over a deficiency for the region both in the mother and the zygote, does not increase the severity of the embryonic mutant phenotype at restrictive temperature (Fig. 9 C).

We carried out epistasis analysis to position dAPC2 with respect to other components of the signal transduction pathway. wg; dAPC2<sup>25</sup> maternal/zygotic mutants show a partial rescue of the wg phenotype, with restoration of the normal diversity of cuticular pattern elements and small expanses of naked cuticle (Fig. 9 G and H), suggesting that dAPC2 is downstream of wg. There are two possible explanations for the fact that the double mutant does not show the same phenotype as the single mutant: either dAPC2<sup>25</sup> is not null, or the negative regulatory machinery remains partially active in the absence of dAPC2. If dAPC2<sup>25</sup> is not null, we reasoned that repeating the epistasis test with dAPC2<sup>25</sup> in trans to a deficiency removing dAPC2 (Df(3R)crb87-4) might further reduce dAPC2 function, producing a double mutant phenotype more similar to that of dAPC2<sup>25</sup> alone. However, when we did this, there was no change in the double mutant phenotype (Fig. 9 I), sug-
gesting that dAPC2<sup>AS</sup> may be genetically null for this function. Other components of the Wg signal transduction pathway act downstream of dAPC2. Embryos maternally and zygotically mutant for both dishevelled (dsh) and dAPC2 (Fig. 9 K) show a phenotype indistinguishable from the dsh single mutant (Fig. 9 J), as do embryos maternally mutant for both dsh and dAPC2 that are zygotically dssh<sup>Y</sup>; dAPC2<sup>AS</sup>/Df(3R)crb87-4 (Fig. 9 L). Likewise, arm; dAPC2 and dAPC2; dTCF double mutants (derived from dAPC2 homozygous mothers) (Fig. 9, N and P) are indistinguishable from arm or dTCF single mutants (Fig. 9, M and O). Thus, dsh, arm, and dTCF all act genetically downstream of dAPC2; this was expected for arm and dTCF, but was surprising for dsh.

Loss of dAPC2 also leads to ectopic activation of Wg-responsive genes. One target is Wg itself. If the Wg pathway is constitutively activated by removing zw3 function (Siegfried et al., 1992) or by expressing constitutively active Arm (Pai et al., 1997), an ectopic stripe of Wg RNA is induced in each segment. A similar ectopic stripe of Wg RNA is seen in dAPC2<sup>AS</sup> maternal/zygotic mutants (Fig. 10, A and B). Similarly, the domain of expression of a second Wg target gene, en, is expanded relative to wild-type (Fig. 10, G and H), as it is in zw3 mutants or in the presence of activated Arm. In addition, a novel phenotype was observed. In dAPC2<sup>AS</sup> maternal/zygotic mutants (Fig. 10, D and E), the levels of Wg protein are higher and Wg extends more cell diameters away from wg-expressing cells than in wild-type (Fig. 10 F). These effects on Wg protein do not appear to be accounted for solely by ectopic activation of Wg RNA, as they are detected beginning at stage 9 before induction of ectopic wg, and they are not observed in embryos expressing activated Arm (Fig. 10 F). Thus, the efficiency of Wg protein transport (Dierick and Bejsovec, 1998) appears to be enhanced in dAPC2 mutants.

dAPC2 mutant embryos still respond to Wg signaling, as segmental stripes of stabilized Arm remain (Fig. 10, I and J). In dAPC2<sup>AS</sup> maternal/zygotic mutants, levels of cytoplasmic Arm in all cells are elevated, but cells receiving Wg signal continue to accumulate more Arm than their neighbors (Fig. 10 J). In contrast, zw3 loss of function results in uniform accumulation of cytoplasmic Arm in all cells, eliminating the Arm stripes (Peifer et al., 1994). Immunoblot analysis of Arm protein from dAPC2<sup>AS</sup> maternal/zygotic mutants revealed an accumulation of hypophosphorylated Arm (Fig. 8 M). This effect was not as dramatic as that seen in a zw3 mutant (Fig. 8 M), but was similar to that seen upon ubiquitous expression of Wg using the e22c-GAL4 driver (data not shown). Thus, the effect of dAPC2<sup>AS</sup> on Arm levels is intermediate between that of wild-type and that of zw3 loss of function, suggesting that negative regulation of Arm is reduced but not completely abolished in dAPC2<sup>AS</sup>

As dAPC2<sup>AS</sup> activates Wg signaling, we examined whether the change in its localization was simply a consequence of pathway activation. When we activated Wg signaling by ubiquitous Wg expression (via the e22c-GAL4 driver) or by removing zw3 function, the localization of dAPC2 was essentially unchanged, suggesting that pathway activation is not sufficient to eliminate cortical dAPC2 (Fig. 8, K and L; data not shown). There was also no apparent change in dAPC2 protein levels or isoforms in zw3 mutants relative to wild-type (Fig. 8 M); this was somewhat surprising as GSK phosphorylates hAPC (Rubinfeld et al., 1996), and suggests that dAPC2 can be phosphorylated by another kinase.

**Discussion**

**dAPC2 and Wg Signaling**

The current model for hAPC function suggests that it is part of the destruction machinery for βcat and thus, negatively regulates Wnt signaling. dAPC negatively regulates the Wg pathway in the Drosophila eye (Ahmed et al., 1998), although surprisingly not in other tissues. We examined the function of dAPC2, which shows a broader pattern of expression. dAPC2 interacts directly with Arm and negatively regulates Wg signaling in the embryonic epidermis, helping trigger Arm destruction. dAPC2 mutant embryos resemble zw3 mutants in cuticle phenotype and in ectopic activation of Wg target genes. One novel pheno-
type of dAPC2 is a broadening of the stripes of Wg protein, suggesting an effect on Wg transport (Dierick and Bejsovec, 1998). This is not observed when Wg signaling was activated by other means, suggesting that dAPC2 may have novel roles in Wg signaling.

In zw3 mutants, cytoplasmic Arm levels rise sharply (Peifer et al., 1994). The effect of the dAPC2 mutation on Arm was similar but less severe. The current model for the destruction machinery is that Zw3, Axin, and APC function as a complex, facilitating Arm phosphorylation by Zw3 and thus targeting it for destruction (for review see Polakis, 1999). Axin can bind GSK/Zw3 and βcat/Arm independently of APC. Perhaps in the absence of dAPC2, Zw3 may still phosphorylate Arm, but not as effectively, explaining why loss of dAPC2 affects Arm stability less severely than does loss of Zw3. However, this conclusion is tempered by the fact that dAPC2 is not a protein-null, and in addition, other APC family members may play redundant roles.

Our epistasis tests between dAPC2 and other components of the Wg pathway generally conform to earlier models of APC function, but also suggest further complexity. As expected, dAPC2 acts downstream of Wg and upstream of Arm and dTCF. However, the suppression of Wg by dAPC2 is incomplete. As above, this may be because dAPC2 is not null, because dAPC2 is not completely essential for Arm downregulation, or because of redundancy. In contrast to zw3 (Siegfried et al., 1992), dAPC2 is genetically upstream of dsh. However, the relative positioning of dAPC2 and Dsh will not be definitive until a protein-null allele of dAPC2 is available. Most current models place Dsh upstream of the destruction machinery, but the recent discovery that Dsh, along with Axin, APC, and Zw3/GSK, is a component of the destruction complex (Fagotto et al., 1999; Kishida et al., 1999; Smalley et al., 1999) reveals that these proteins may function as a network rather than as a linear series, making the results of epistasis tests more difficult to interpret. For example, the epistasis relationships might be explained if dAPC2 regulated assembly of Dsh into the destruction complex. In the absence of dAPC2, Dsh might constitutively turn off the destruction complex, activating signaling; thus, loss of dAPC2 would have no effect if Dsh is also absent.

The localization of dAPC2 to large membrane-associated structures is intriguing. Axin and Dsh also accumulate in large punctate, often cortical structures when overexpressed in vertebrate cells, and like dAPC2, a fraction of Axin associates with a glycoprotein (A Xelrod et al., 1998; Fagotto et al., 1999; Kishida et al., 1999; Smalley et al., 1999). Colocalization experiments will reveal whether cortical dAPC2 puncta contain other components of the destruction machinery. In light of these data, the inability of dAPC2 to associate with the plasma membrane may be informative. Loss of serine 241 likely affects the secondary structure of the Arm repeats, which may affect dAPC2 binding to a protein partner at the membrane. A membrane-bound localization of the destruction complex, perhaps via dAPC2, could be essential for optimal function of the Wg pathway. Both mislocalization of mutant dAPC2 protein and the slight residual activity of the destruction complex in these mutants could be explained if Arm destruction continues, albeit at greatly reduced levels, in the cytosol. These speculative ideas can be tested in the future by examining colocalization of dAPC2 and other components of the destruction complex in wild-type embryos and in the various mutant backgrounds.

Although dAPC (Ahmed et al., 1998) and dAPC clearly negatively regulate the Wg pathway, misexpression of APC in Xenopus suggested an apparent positive role in Wnt signaling (Vleminkx et al., 1997). A PR, the closest C. elegans APC relative, also appears to be a positive effector of Wnt signaling (Rochelleau et al., 1997). However, A PR is very distantly related to the A PC family. A PR's Arm repeats are only slightly more similar to those of APC than to the Arm repeats of Arm (Fig. 1 B). Whereas A PR has two highly divergent SAMP repeats (Rochelleau et al., 1997), it does not contain the conserved NH2-terminal region or recognizable 15 or 20 amino acid repeats. Perhaps A PR is not an APC homologue, but instead plays a distinct role in the pathway.

dAPC2 adults are viable and morphologically normal, suggesting that dAPC2 may not be required for critical functions such as patterning imaginal discs. Since the phenotypic severity of dAPC2 homozygotes is similar to that of dAPC2/D deficiency, this allele is likely to be at least a strong hypomorph for Wg signaling in the embryonic epidermis. Although it is possible dAPC2 only functions there, its widespread expression at other stages suggests otherswise. Whether or not dAPC2 is a null, dAPC2 may still serve other functions. The specific effects of dAPC2 (these data) and dAPC (Ahmed et al., 1998) mutations suggest that in some contexts they may be redundant. The possible other functions of dAPC2 remain to be tested by examining the effect of dAPC2 mutations on processes such as neuroblast divisions, and by characterizing dAPC2 double mutants.

dAPC2 and the Cytoskeleton

Previous studies of APC in vertebrate cultured cells revealed that APC localizes to the membrane and cytoplasm (e.g., Näthke et al., 1996), where it can associate with MTs (Munemitsu et al., 1994; Smith et al., 1994; Näthke et al., 1996). Our biochemical and localization studies of dAPC2 reveal a complex relationship between dAPC2 and the actin and MT cytoskeletons, suggesting potential functions for dAPC2 in regulation of the cytoskeleton.

dAPC2 colocalizes with actin in many but not all cell types, suggesting a regulated interaction. The association between the actin cytoskeleton and dAPC2 may occur via A rm and α-catenin, although in some places where dAPC2 and actin colocalize, there is little or no detectable Arm. The colocalization of dAPC2 and actin is intriguing given the effects of Wnt/Fz signaling on planar polarity in Drosophila (for review see Shulman et al., 1998; for possible effects of Wg see Tomlinson et al., 1997). In the wing, the best studied example, Fz signaling triggers asymmetric polymerization of actin, leading to development of an actin-based wing hair in the distal vertex of each hexagonal wing cell (Wong and A dler, 1993). The colocalization of actin and dAPC2 during the onset of denticle formation was particularly striking in this context, because the process of denticle formation is very similar to that of wing hair formation in the nature of the structure, its strict ori-
orientation in the plane of the tissue, and in its cell biological and genetic bases. This raises the possibility that Wg/Wnt signaling directly affects the actin cytoskeleton and thus tissue polarity, using dAPC2 as an effector.

Although dAPC2 does not contain the basic region thought to mediate MT association of hAPC, our data are consistent with the possibility that dAPC2, like hAPC (Munemitsu et al., 1994; Smith et al., 1994; Näthke et al., 1996), may associate with MTs under certain circumstances. The data for a microtubule association of dAPC2 are less robust than those suggesting association with actin. Whereas dAPC2 does not prominently localize to most microtubule-based structures (nor does hAPC, unless overexpressed), dAPC2 localized to several places consistent with a role in anchoring microtubules. In preblastoderm embryos, when actin is essential for tethering the spindle to the membrane (for review see Foe et al., 1993), dAPC2 colocalizes with cortical actin and subcortical actin puncta. Subcortical dAPC2 is concentrated just above the spindle, placing it in a position to interact with astral MTs as they reach toward the cortex. Both dAPC2 and actin also localize to a dot-like structure which may be the centrosome. In postblastoderm embryos, dAPC2 is subtly enriched in the vicinity of the spindle.

The asymmetric localization of dAPC2 in dividing neuroblasts is also consistent with a possible role for dAPC2 in linking the spindle to the cortex. During neuroblast mitosis, the spindle is specifically oriented (for review see Fuerstenberg et al., 1998). Insc, which localizes to a crescent opposite the future daughter cell from late interphase through metaphase, coordinates the neuroblast asymmetric cell division (Kraut et al., 1996). Other proteins are likely to act in this process; e.g., Bazooka acts upstream of Insc (Kuchinke et al., 1998). dAPC2 associates with the actin cytoskeleton in contexts where no Wg signaling is thought to occur, such as in preblastoderm embryos, dAPC2 may play more fundamental roles in cytokinetic regulation. Such functions may be revealed by further genetic analyses of dAPC and dAPC2.

We thank S. Tiong, C. Southern, M. Teachey, and N. Vo for assistance; C. Doe, B. Duronio, T. Karr, P. Polakis, L. Rose, S. Selleck, and B. Theurkauf for tutorials and discussions; and S. Hayashi, E. Wieschaus, U. Tempas, P. Polakis, C. Doe, the Bloomington Drosophila Stock Center, the Berkeley Drosophila Genome Project, and R. Fehon and the Duke University Comprehensive Cancer Center’s Shared Confocal Facility for essential reagents or equipment.

This work was funded by National Institutes of Health grant GM 47857, a U.S. Army Breast Cancer Research Program Career Development Award and the Human Frontier Science Program (to M. Peifer), a National Science Foundation Career Award IBN-9734072 and National Science Foundation grant IBN-96-00539 (to A. Bejsovec), a National Research Service Award grant 1 F32 CA 79172-01 (to B. McCartney), the National Cancer Institute of Canada (C. Kirkpatrick), and the Netherlands Kankerbestrijding (A. Baas).

Submitted: 2 June 1999
R evised: 29 July 1999
A ccepted: 9 August 1999

Note Added in Proof: While this manuscript was in review, a related work was published by Yu, X., L. Waltzer, and M. Bienz. 1999. Nature Cell Biol. 1:144–151.

References
Ahmed, Y., S. Hayashi, A. Levine, and E. Wieschaus. 1998. Regulation of Armadillo by a Drosophila APC inhibits neuronal apoptosis during retinal development. Cell. 93:1171–1182.
A xelrod, J. D., J. R. Miller, J. M. Shulman, R. T. Moon, and N. Perrimon. 1998. The dual role of Drosophila Dcc in regulating cell-cell recognition. Cell 93:1361-1371.

Barth, A. I., I. S. Nathke, and W. J. Nelson. 1997. Cadherins, catenins and APC. Curr. Opin. Cell Biol. 9:596-600.

Bhatia, A. S., N. J. Solomon, and J. C. Wolfe. 1998. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 140:615-620.

Beinhauer, J. D., I. M. Hagan, J. H. Hegemann, and U. Fleig. 1997. Mal3, the fission yeast homologue of the human APC tumor suppressor. J. Cell Biol. 139:479-486.

Birchmeier, W. 1995. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 130:1317-1321.

Blaschuk, O. F., R. W. Gardner, P. J. Bogen, and R. D. Waterston. 1994. The E-box binding protein E2-2 regulates the expression of the Drosophila engrailed gene in the developing central nervous system. J. Cell Biol. 125:19-26.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.

Boman, H. 1997. The role of cadherins and catenins in the regulation of cell-cell adhesion. J. Cell Biol. 138:1147-1149.