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What can humans learn from flies about adenomatous polyposis coli?

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Summary

Somatic or inherited mutations in the *adenomatous polyposis coli* (APC) gene are a frequent cause of colorectal cancer in humans. APC protein has an important tumor suppression function to reduce cellular levels of the signaling protein β -catenin and, thereby, inhibit β -catenin and T-cell-factor-mediated gene expression. In addition, APC protein binds to microtubules in vertebrate cells and localizes to actin-rich adherens junctions in epithelial cells of the fruit fly *Drosophila* (Fig. 1). Very little is known, however, about the function of these cytoskeletal associations. Recently, Hamada and Bienz have described a potential role for *Drosophila* E-APC in cellular adhesion,⁽¹⁾ which offers new clues to APC function in embryonic development, and potentially colorectal adenoma formation and tumor progression in humans.

Introduction

APC protein is part of a multi-protein machinery that regulates the intracellular levels of β -catenin, an effector of the canonical Wnt signaling pathway that regulates many developmental processes.⁽²⁾ The proteins APC and axin mediate phosphorylation of β -catenin by glycogen synthase kinase (GSK) -3 β leading to β -catenin ubiquitination and degradation by the proteasome. Wnt signaling inhibits the APC–axin–GSK-3 β complex and causes cytoplasmic and nuclear accumulation of β -catenin. Nuclear β -catenin acts as a co-activator for transcription factors of the T-cell factor family and induces the expression of TCF-regulated genes. Tight regulation of intra-cellular β -catenin levels is a requirement for normal embryonic development and, correspondingly, mutations in APC or β -catenin that stabilize β -catenin are an early event in cancer formation.⁽²⁾

In addition to its role as a Wnt effector, β -catenin has another important role as an adapter protein that links cadherins, intercellular adhesion proteins, to the actin cytoskeleton.⁽³⁾ However, the connection between the Wnt effector (signaling) and structural adapter (adhesion) functions of β -catenin is poorly understood. Beta-catenin bound to cadherins in adherens junctions seems to be sequestered from degradation and is the main cellular pool of β -catenin in the absence of Wnt signaling. Wnt has been shown to increase the cadherin/catenin level at the cell surface by increasing the pool of free β -catenin in the cell.^(4,5) However, the prevalent model is that the APC–axin–GSK-3 β complex regulates the levels of free cytoplasmic β -catenin independent from the cadherin-associated β -catenin pool.⁽⁶⁾ The results by Hamada and Bienz indicate a new connection between cadherin-mediated adhesion and regulators of the Wnt pathway.⁽¹⁾

Mutation of *Drosophila* E-APC decreases intercellular adhesion

Both vertebrates and *Drosophila* have two APC genes, one of which is expressed in specific tissues whereas the other is expressed ubiquitously.^(7–11) The products of both of these APC genes are able to regulate β -catenin levels.^(7,8,11) In *Drosophila*, the ubiquitously expressed E-APC (dAPC2) localizes to adherens junctions in epithelial cells of the embryo and in ovarian germ cells.^(9,10,12) This localization is actin dependent and seems to be mediated by the armadillo domain in E-APC because single amino acid deletion (APC ^{Δ S}) or exchange (APC^{N175K}) in the APC armadillo domain results in delocalization of E-APC from the plasma membrane.^(1,10,12)

E-APC is important regulating the Wnt signal during embryonic development and maternally provided E-APC is sufficient for most of this activity.^(9,11) E-APC^{N175K} homozygous flies derived from heterozygous parents are viable but sterile and lay few eggs of which only some develop through late embryonic stages.⁽¹⁾ These “escaper embryos” are maternally and zygotically deficient for wild-type E-APC and show hyperactivation of the Wnt pathway, although levels of β -catenin are not increased in early embryos homozygous for the E-APC^{N175K} mutation indicating that E-APC^{N175K} may still mediate β -catenin degradation.⁽¹⁾ However, delocalization of E-APC from adherens junctions coincides with delocalization of β -catenin from adherens junctions. The early relocation of β -catenin from a signaling-inactive cadherin-bound pool into a soluble and potentially signaling-active pool seems to be sufficient to derepress the Wnt pathway in later stages of embryonic development.⁽¹⁾ Since Wnt itself is one of the targets of Wnt signaling, full derepression may be caused by positive Wnt autoactivation.⁽¹⁾

E-APC^{N175K} mutant embryos also show ultrastructural defects in the adhesive membranes of embryonic epithelium and egg chambers.⁽¹⁾ Since neither DE-cadherin localization nor DE-cadherin levels seem to be significantly affected in these cells, adhesion defects may be caused by loss of β -catenin from adherens junctions.

In some E-APC^{N175K} mutant egg chambers, oocytes are mislocalized and the penetrance of this phenotype is considerably enhanced in E-APC^{N175K} egg chambers that are also deficient for the second *Drosophila* APC.⁽¹⁾ Cadherin-mediated adhesion between germ cells and follicle cells is important for the correct positioning of the oocyte at the posterior pole of the egg chamber,⁽¹³⁾ and DE-cadherin and β -catenin mutants show early and severe mislocalization of the oocyte in the egg chamber.⁽¹³⁾ The data of Hamada and Bienz indicate that *Drosophila* APC proteins may be important for cadherin-mediated adhesion between ovarian germ cells and that they have some redundancy for this function.⁽¹⁾

The mechanism by which E-APC regulates adhesion remains to be determined. Since binding of cadherin and APC to β -catenin is mutually exclusive,⁽¹⁴⁾ how can E-APC affect the amount of β -catenin associated with cadherin in adherens junctions? It has been shown that β -catenin associates with newly synthesized E-cadherin before E-cadherin is transported to the cell surface.^(15,16) Therefore, E-cadherin rather than APC seems to mediate translocation of β -catenin to adherens junctions. Hamada and Bienz suggest that E-APC maintains junctional complexes rather than promoting their initial assembly.⁽¹⁾ There are some observations, however, that appear to refute this suggestion. First, the β -catenin that binds to the APC complex seems to be rapidly degraded.⁽²⁾ Second, Wnt signaling induces increased levels of cadherin–catenin complex and increased adhesion in some mammalian cells by inhibiting β -catenin degradation.^(4,5) Further research is needed to resolve these discrepancies.

Decreased intercellular adhesion in epithelial cells of E-APC^{N175K} mutant embryos and in egg chambers of E-APC^{N175K} mutant flies is an important observation that may have

implications for interpreting other E-APC mutant embryo phenotypes.^(17,18) McCartney et al. described a role of E-APC in linking spindles to cortical actin at pseudocleavage furrows in the early syncytial embryo. In E-APC mutant embryos an increased number of nuclei are lost from the cortex into the syncytial cytoplasm because of attachment defects of spindles to pseudocleavage furrows.⁽¹⁷⁾ Since both DE-cadherin and β -catenin localize to pseudocleavage furrows, functional adhesion may be important for spindle attachment to the furrow. Spindle orientation in the neuroepithelium of later stage *Drosophila* embryos is a tightly regulated process that is important for the maintenance of the epithelium as well as for the generation of neuroblasts, which derive from a low frequency of asymmetric cell divisions in the epithelium. Disruption of E-APC function by RNAi significantly increases the frequency of asymmetric spindle orientation and cell division.⁽¹⁸⁾ Since adherens junction integrity is required for spindle orientation and the maintenance of symmetric divisions,⁽¹⁸⁾ inhibiting E-APC function may affect spindle orientation indirectly by decreasing intercellular adhesion.

The role of APC in colorectal cancer

The human *adenomatous polyposis coli* (APC) gene product is mutated in an inherited predisposition to colorectal cancer, familial adenomatous polyposis (FAP), and in most sporadic colorectal cancers. FAP patients carry a mutation in one of the two APC alleles and develop hundreds of small benign colorectal adenomas, some of which progress to malignancy. The smallest detectable adenomas show mutational inactivation of the second APC allele indicating that adenoma formation requires loss of function in both APC alleles.⁽²⁾ Adenomatous polyps in FAP patients and in mouse model systems for FAP form as outpockets of intestinal epithelium above the crypt. These polyps initially have normal proliferation and apoptosis rates but eventually develop additional mutations in other genes that cause their transformation to malignancy.^(2,19)

A specific feature of the intestinal epithelium is its continuous regeneration from the crypts, which are stem cell compartments at the bases of the intestinal villi.⁽²⁰⁾ New epithelial cells are generated in the crypt and migrate as an epithelial sheet in a regulated and directed way from the bottom to the top of the villus where they are shed off into the intestinal lumen. Since intestinal epithelial cells are exposed to environmental mutagens, permanent renewal of these cells may protect against the accumulation of transforming mutations.⁽²⁰⁾ Targeted inhibition of cadherin function in intestinal epithelium results in adenoma formation whereas forced expression of cadherin or APC result in slowed or disorderly migration of cells along the crypt villus axis, respectively.^(20–23) These results indicate that defects in migration and adhesion of epithelial cells could contribute to intestinal polyp formation.^(20,24) The more ubiquitously expressed of the two vertebrate APC proteins, which is also the product of the *adenomatous polyposis coli* gene locus, binds to micro-tubules and accumulates in cortical clusters associated with the distal tips of microtubules.^(25–27) Cortical APC clusters form in areas of actively extending membranes and APC has been shown to promote microtubule assembly and stability in vitro.^(25,26,28)

Taken together these data suggest a role of APC in cell migration.⁽²⁴⁾ Although it remains to be shown whether vertebrate APC has a function in adhesion (see below), the observed adhesion defects in DE-APC mutants⁽¹⁾ may point to an additional mechanism by which APC mutations could contribute to intestinal polyp formation in humans.

What can vertebrates and *Drosophila* learn from each other?

Vertebrate APC has a large C-terminal domain that is missing in *Drosophila* E-APC.^(9,10) This C-terminal region encodes binding sites for microtubules, the microtubule-associated protein EB1 (“EB” for APC end binding) and a C-terminal peptide motif that binds to the

PDZ regions of the cortical protein Discs Large (DLG) tumor suppressor product.^(25,29,30) These additional protein binding sites may indicate functions of vertebrate APC that are different or additional to those of *Drosophila* E-APC.

Although vertebrate APC shows a predominant localization along microtubules and to cortical clusters at the tip of microtubules,^(26,27) localization to adherens junctions of epithelial cells has been observed and seems to be increased after treatment of cells with the microtubule drugs taxol or nocodazol.^(26,31) Therefore, it is important to investigate whether vertebrate APC has a function in intercellular adhesion.

It remains to be determined whether DE-APC binds microtubules indirectly through other microtubule-binding proteins. If DE-APC is able to mediate the interaction of microtubules with adherens junctions, it may play a more direct role in controlling spindle attachment and orientation in the *Drosophila* epithelium.^(17,18) In addition, it needs to be determined whether vertebrate APC plays a role in spindle attachment and orientation, and whether its microtubule or EB1-binding domains are essential for this role.

The armadillo domain in APC seems to be important for the localization of both DE-APC and vertebrate APC. This conserved domain is located in the N terminus of APC⁽¹⁾ and, in vertebrates, it mediates localization of APC to cortical clusters,⁽³²⁾ and binds to the kinesin-superfamily associated protein (KAP) 3.⁽³³⁾ Binding to KAP3 may mediate the plus-end-directed transport of vertebrate APC along microtubules towards the cell cortex.⁽²⁷⁾ Furthermore, the vertebrate APC armadillo domain binds to a Rac-specific guanine nucleotide exchange factor, Asef (APC stimulated exchange factor) which is activated by binding to APC.⁽³⁴⁾ Thus, APC may regulate actin dynamics via Asef and Rac.

The discovery of these new APC-binding partners raises some interesting questions for the adhesion defect observed by Hamada and Bienz in the DE-APC mutants. Is there a *Drosophila* KAP3 homolog that mediates transport of E-APC along microtubules to the cell cortex, and is the defect in cortical localization of DE-APC^{ΔS} and E-APC^{N175K} caused by the inability of these mutant proteins to bind to a KAP3 homolog? Is there an Asef homolog in *Drosophila*, and is the defect in intercellular adhesion caused by the inability of DE-APC mutant proteins to bind, localize and activate Asef? Answers to these questions could help to define the mechanism underlying the adhesion defect observed by Hamada and Bienz and the spindle attachment and orientation defects observed by McCartney et al. and Lu et al.^(1,17,18) More generally, it may then be possible to explain how the phenotypes observed in *Drosophila* E-APC mutants are related to the functions of vertebrate APC.

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Figure 1.

Cytoskeletal associations of adenomatous polyposis coli (APC) in epithelial cells. APC binds to microtubules (MT) and accumulates in cortical clusters at the tip of microtubules at the basal cell membrane. These APC clusters may be involved in membrane extension and cell migration. APC also colocalizes with the cadherin–catenin complex at adherens junctions close to the apical cell surface, and this localization is actin dependent. Mutations in APC that disrupt its localization to adherens junctions coincide with defects in intercellular adhesion. APC is part of a multi-protein machinery that regulates cytoplasmic levels of β -catenin by mediating β -catenin degradation. Wnt signaling or mutations in APC or β -catenin that inhibit β -catenin degradation cause nuclear accumulation of β -catenin and T-cell factor (TCF)/ β -catenin-mediated gene expression. Further research is needed to define how the cytoskeletal associations of APC affect its function to regulate β -catenin degradation.