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Drosophila APC2 and APC1 Play Overlapping Roles in Wingless Signaling in the Embryo and Imaginal Discs

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The regulation of signal transduction plays a key role in cell fate choices, and its disregulation contributes to oncogenesis. This duality is exemplified by the tumor suppressor APC. Originally identified for its role in colon tumors, APC family members were subsequently shown to negatively regulate Wnt signaling in both development and disease. The analysis of the normal roles of APC proteins is complicated by the presence of two APC family members in flies and mice. Previous work demonstrated that, in some tissues, single mutations in each gene have no effect, raising the question of whether there is functional overlap between the two APCs or whether APC-independent mechanisms of Wnt regulation exist. We addressed this by eliminating the function of both *Drosophila* APC genes simultaneously. We find that APC1 and APC2 play overlapping roles in regulating Wingless signaling in the embryonic epidermis and the imaginal discs. Surprisingly, APC1 function in embryos occurs at levels of expression nearly too low to detect. Further, the overlapping functions exist despite striking differences in the intracellular localization of the two APC family members.

Key Words: **APC; β-catenin; Armadillo; Wnt; Wingless; *Drosophila*; tumor suppressor.**

INTRODUCTION

Signal transduction plays a key role in setting cell fates in embryogenesis. When inappropriately activated by mutation, however, signal transduction pathways often help trigger oncogenesis. The Wnt pathway provides an excellent example of this. Wnt signaling regulates diverse developmental decisions in all animals studied, and inappropriate activation of Wnt signaling leads to colon and other cancers. The most common mechanism by which Wnt signaling is activated in tumors is by loss-of-function mutations in the tumor suppressor APC (reviewed in Polakis, 2000).

Transduction of Wnt signals occurs via regulation of the levels of cytoplasmic Arm/βcat (the pool not assembled into cell–cell adhesive junctions; reviewed in Polakis, 2000). In the absence of signal, cytoplasmic Arm/βcat is rapidly targeted for destruction in a two-step process. Arm/βcat is first captured by a multiprotein complex that includes APC and a second scaffolding protein Axin. This complex targets Arm/βcat for phosphorylation by Zeste-white3 (Zw3)/glycogen synthase kinase-3 (GSK3). Phosphorylated Arm/βcat is a substrate for a ubiquitin ligase, targeting it for proteasomal destruction. Wnt signaling turns off the APC/Axin/GSK3 complex by an unknown mechanism. This triggers the accumulation of Arm/βcat, which enters the nucleus and works with DNA-binding proteins of the TCF/LEF family to activate Wnt target genes. These data helped explain APC's tumor suppressor role. In the colon, the absence of functional APC allows βcat levels to rise, activating Wnt target genes such as *cyclinD1* and *c-myc*, promoting cell proliferation. This model fits the data well, though questions remain. For example, since APC is expressed in most tissues, why is the colon the primary tissue in which tumors arise in *APC* heterozygous people or mice?

APC's role in tumors led to the hypothesis that it is a key

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negative regulator of Wnt signaling in other contexts (APC family proteins also play Wnt-independent cytoskeletal roles; see accompanying paper for details). Supporting a key role in Wnt regulation, mammalian APC is broadly expressed, and mice homozygous for loss-of-function mutations in *APC* die as early embryos (Fodde *et al.*, 1994; Moser *et al.*, 1995). This hypothesis was further tested in *Drosophila*, where a firm connection to Wnt signaling was established. Loss-of-function mutations in fly *APC1* activate Wnt signaling in the photoreceptors of the eye, triggering their apoptosis (Ahmed *et al.*, 1998). Unlike mammalian APC, however, mutations in fly *APC1* are not lethal, and its protein product is not ubiquitous. In embryos, high level expression is limited to CNS axons and developing germ cells (Hayashi *et al.*, 1997), suggesting that APC1 is absent from many tissues where Wnt signaling is required.

One possible explanation for the limited effect of mutations in fly *APC1* as well as the limited tissue-spectrum of tumors resulting from mutations in mammalian *APC* was functional redundancy with other, as yet undiscovered APC family members. This led several labs to identify second *APC* relatives in both mammals (APC2 or APC-L; (Nakagawa *et al.*, 1998; van Es *et al.*, 1999) and flies (APC2 or E-APC; Hamada *et al.*, 1999; McCartney *et al.*, 1999; Yu *et al.*, 1999). Fly APC2 is broadly expressed in embryonic and postembryonic development, consistent with the redundancy hypothesis (McCartney *et al.*, 1999; Yu *et al.*, 1999). To test this further, mutations in *APC2* were characterized. Animals maternally and zygotically mutant for *APC2* die as embryos with inappropriate stabilization of Arm and activation of Wg signaling in the embryonic epidermis (McCartney *et al.*, 1999), supporting the idea that different APC family members function in different tissues.

However, there are many tissues where neither single mutant has an effect (Ahmed *et al.*, 1998; McCartney *et al.*, 1999). Most striking are the larval imaginal discs, precursors of the adult epidermis, which are affected by neither single mutant. Two hypotheses might explain this. First, APC proteins may not be essential for all Wnt regulation—in cultured cells, Axin overexpression partially compensates for loss of APC (von Kries *et al.*, 2000). Alternatively, in some *Drosophila* tissues, APC1 and APC2 may play redundant roles, and thus mutation in one would not disrupt function. We have tested these hypotheses by creating situations where animals or tissues are double mutant for both *APC* genes. These experiments reveal that APC1 and APC2 play redundant roles in both the embryonic epidermis and the larval imaginal discs, where they cooperate to regulate Wg signaling.

MATERIALS AND METHODS

Genetic and Phenotypic Analysis

Alleles used were: $APC2^{\Delta S}$ (McCartney *et al.*, 1999), $APC2^{d40}$ (McCartney *et al.*, 2001), and $APC1^{Q8}$ (Ahmed *et al.*, 1998). $APC2^{g10}$ was generated in an EMS mutagenesis screen, identified by failure to complement $APC2^{\Delta S}$ (unpublished data). Double mutant *APC2 APC1* chromosomes were generated by meiotic recombination. All stocks were kept at 25°C. Embryo collections were done at 27°C. Transgenic lines used for misexpression and overexpression studies were UAS-APC2-GFP (R. Rosin-Arbesfeld and M. Bienz), UAS-APC1 (E. Wieschaus). Transgenes were expressed by crossing to Engrailed-GAL4 at 27°C. Canton S was the wild type. To generate germline clones, larvae of genotype $FRT82B\ ovo^{D}/APC2^{d40}APC^{Q8}$ were γ−irradiated with 1000 rads at 32–48 h after egg-laying (AEL) at 25°C. Females were crossed to $APC2^{d40}\ APC^{Q8}/TM3actinGFPSer$ males at 27°C. Embryos produced from this cross were either maternally and zygotically mutant, or paternally rescued; these were distinguished by the presence of GFP. To generate imaginal disc clones, larvae of the genotype $FRT82B\ myc\ Sb^{63}/APC2^{d40}\ APC^{Q8}$ were γ−irradiated with 1100 rads at 24–36 h AEL at 25°C. Clones in the discs of wandering third instar larvae were identified by the absence of APC2 and myc. Adult wings with clones were mounted on slides in Faure's solution.

Immunolocalization

Imaginal discs were fixed in 4% paraformaldehyde for 20 min. Embryos were fixed in 1:1 3.7% formaldehyde in PBS:heptane for 20 min. All were blocked in 1% normal goat serum/0.1% Triton X-100 in PBS for at least 2 h. Primary antibodies were as follows: rat polyclonal anti-APC2 (1:1000), mouse monoclonal anti-Arm N27A1 (DSHB, 1:200), and rabbit polyclonal anti-Armadillo N2 (1:200) and anti-APC1 (1:1000).

RESULTS

APC1 and APC2 Play Overlapping Roles in Regulating Wg Signaling during Embryogenesis

Both APC1 and APC2 (McCartney *et al.*, 1999) have phenotypes that suggest that they act as regulators of Wg signaling, but each only affects a subset of the tissues where Wg signaling regulates development. Further, there are certain tissues, such as the imaginal discs, where neither gene has a phenotype. One possible explanation is partial redundancy between the two genes. We first addressed this issue in the embryo. Embryos maternally and zygotically mutant for *APC2* exhibit Arm stabilization and thus activation of Wg signaling in the epidermis (McCartney *et al.*, 1999; Hamada and Bienz, 2002). However, neither their cuticle phenotype nor the level of Arm accumulation are as drastic as those of *zw3* maternal and zygotic mutants (Peifer *et al.*, 1994; Siegfried *et al.*, 1992). Three hypotheses seemed possible: (1) The *APC2* allele tested retained some function, (2) APC1 might provide some function in the absence of APC2, or (3) APC proteins might not be absolutely essential for Arm destruction.

To test the redundancy hypothesis, we generated embryos that were maternally and zygotically $APC2^{d40}\ APC1^{Q8}$, and thus double mutant for both APCs (Figs. 1E and 1F). Wild-type embryos have a segmentally reiterated pattern of anterior denticles (Fig. 1A, arrow) and posterior naked cuticle (Fig. 1A, arrowhead) on their ventral surfaces. Dorsally, cells secrete different hair types at different posi-

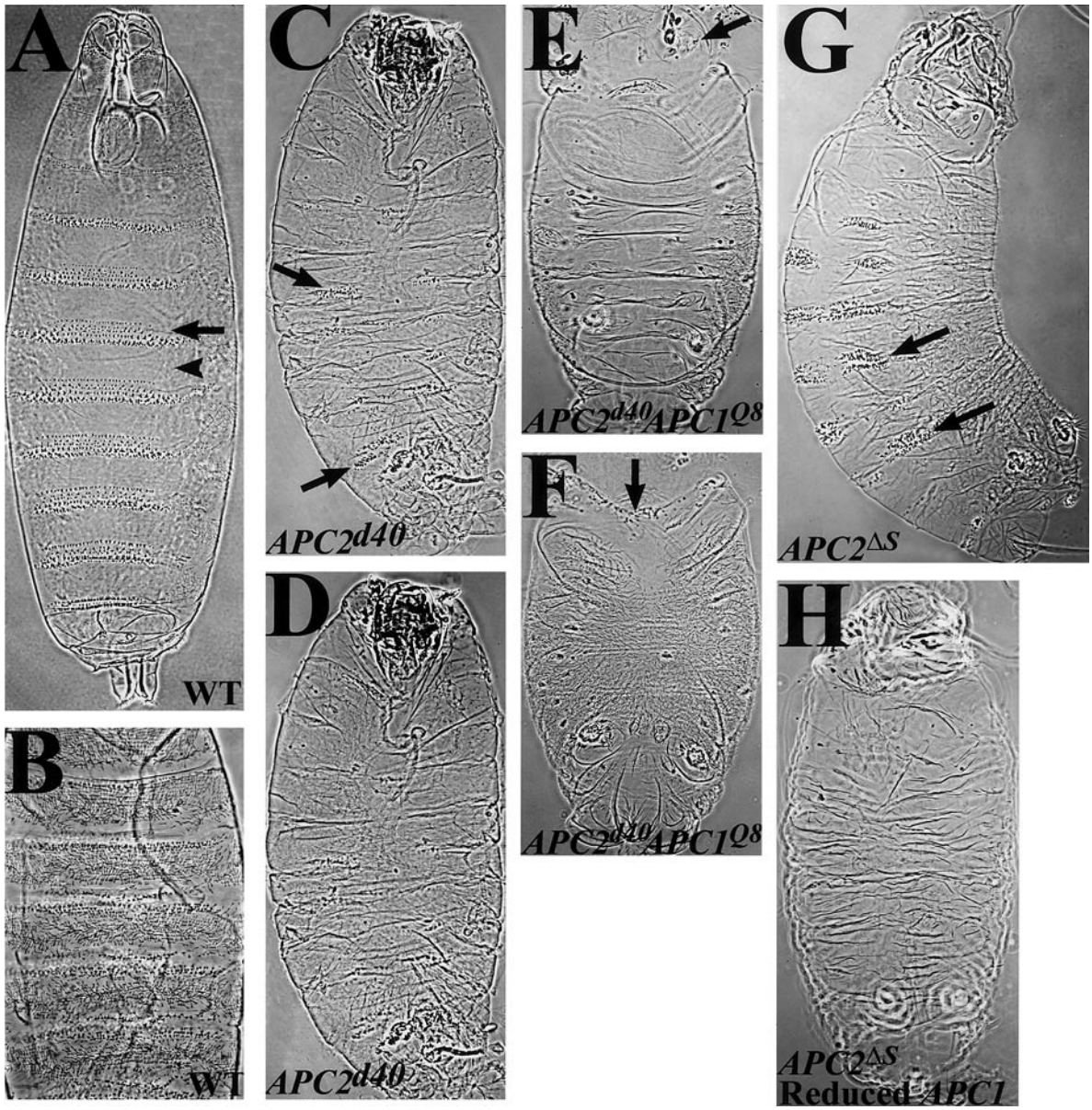

FIG. 1. *APC1* mutations enhance the embryonic phenotype of *APC2*. Cuticle preparations, anterior up. (A, B) Wild-type ventral (A) and dorsal (B) cuticles. Ventrally, note the segmentally reiterated anterior denticle belts (arrow) and posterior naked cuticle (arrowhead). Dorsally, different hair types are secreted at different positions along the anterior–posterior axis. (C, D) $APC2^{d40}$ maternal and zygotic mutant. Ventral (C) and dorsal (D) cuticles. Ventrally, most cells are converted to posterior fates but some cells still secrete denticles (e.g., arrows). (E, F) $APC2^{d40}$ $APC1^{Q8}$ maternal and zygotic double mutant. Ventral (E) and dorsal (F) cuticles. Ventrally, all cells secrete naked cuticle, and dorsally, all cells secrete fine hairs characteristic of posterior cells. Head involution is disrupted (E, arrow) and dorsal closure is partially disrupted (F, arrow). (G) $APC2^{\Delta S}$ maternal and zygotic mutant. Ventrally, most cells choose posterior fates but some cells still secrete denticles (arrows). (H) Progeny of $APC2^{\Delta S}$ $APC1^{Q8}/APC2^{\Delta S}$ females and males. All ventral cells secrete naked cuticle.

tions (Fig. 1B). In $APC2^{d40}$ maternal and zygotic single mutants, most cells take on posterior fates and secrete naked cuticle, indicative of excess Wg signaling (Fig. 1C). However, unlike *zw3* maternal and zygotic mutants, some cells still adopt anterior fates and secrete denticles. (e.g., Fig. 1C, arrows), In contrast, $APC2^{d40}$ $APC1^{Q8}$ maternal and zygotic double mutants exhibit a much stronger embryonic cuticle phenotype (Fig. 1E). Maternal and zygotic double mutant cuticles were shorter, and, unlike $APC2^{d40}$ maternal and zygotic single mutants, most embryos completely lack

denticles. Further, maternal and zygotic double mutants exhibit a complete failure of head involution (Fig. 1E, arrow). Dorsally, all cells are transformed to the fate normally adopted by posterior cells, and thus all secrete fine dorsal hairs (Fig. 1F), and maternal and zygotic double mutants exhibit slight abnormalities in dorsal closure not seen in the single mutant (Fig. 1F, arrow). Thus, the cuticle phenotype of the maternal and zygotic double mutant is much more similar to that of embryos maternally and zygotically mutant for *zw3* (Siegfried *et al.*, 1992), suggesting that APC1 partially compensates for loss of APC2 in the embryonic epidermis. Interestingly, embryos receiving paternal wild-type copies of *APC2* and *APC1* are rescued to viability.

We also looked at zygotic *APC2 APC1* double mutants, combining $APC1^{Q8}$ with $APC2^{d40}$, $APC2^{\Delta S}$, and $APC2^{g10}$. All of these zygotic double mutant combinations were embryonic viable and exhibited a wild-type cuticle pattern (data not shown). This suggests that maternally contributed APC1 and APC2 are sufficient for embryonic Wg signaling; this is similar to what we previously observed for *APC2* zygotic single mutants (McCartney *et al.*, 1999). Zygotic *APC2 APC1* double mutants die later as larvae, with defects in brain development (see accompanying paper).

APC1 and APC2 Both Regulate Arm Levels in Embryos

To further test the functional overlap between APC1 and APC2, we examined Arm accumulation in maternal and zygotic double mutants. In wild-type embryos, Arm accumulates at adherens junctions of all cells, but in cells that do not receive Wg signal, Arm levels in the cytoplasm and nucleus are low (Fig. 2I, arrowheads). Wg signal stabilizes cytoplasmic and nuclear Arm (Fig. 2I, arrows). In $APC2^{d40}$ maternal and zygotic single mutants, Arm levels are elevated (Fig. 2D), but do not become uniformly high as is observed in *zw3* maternal and zygotic mutants (Fig. 2G; Peifer *et al.*, 1994); in this, $APC2^{d40}$ resembles $APC2^{\Delta S}$ (McCartney *et al.*, 1999). In contrast, in $APC2^{d40}$ $APC1^{Q8}$ maternal and zygotic double mutants, Arm levels become extremely elevated (Figs. 2B and 2E), thus more precisely matching *zw3* maternal and zygotic mutants.

We next looked more closely at subcellular localization. In $APC2^{d40}$ maternal and zygotic single mutants, the levels of Arm are elevated but one can see further Arm stabilization by Wg signaling (Fig. 2L, arrows). In maternal and zygotic double mutants (when we turned down the brightness to compensate for increased Arm levels), we observed that Arm is highly elevated in both the cytoplasm and nuclei (Fig. 2K), with no differences seen between cells that receive Wg signal and those that do not. Further, in $APC2^{d40}$ $APC1^{Q8}$ maternal and zygotic double mutants, Arm becomes somewhat enriched in nuclei relative to the cytoplasm; this is most evident in cells of the amnioserosa (Figs. 2H and 2K, arrowheads). In this regard, $APC2^{d40}$ $APC1^{Q8}$ maternal and zygotic double mutants are more similar to *Axin* maternal and zygotic mutants (Tolwinski and Wieschaus, 2001) than to *zw3* maternal and zygotic mutants, in which levels of Arm in the cytoplasm and nuclei are equal (Peifer *et al.*, 1994). Tolwinski and Wieschaus (2001) previously suggested that Axin acts as a cytoplasmic anchor for Arm, and that this plays an important role in regulating Wg signaling. Our data suggest that APC proteins may act with Axin to form this cytoplasmic anchor. We also noted that in $APC2^{d40}$ $APC1^{Q8}$ maternally double mutant but zygotically rescued siblings, cells that do not receive Wg signal (Fig. 2J, arrowheads) have slightly elevated Arm levels relative to wild type. This may result from Arm stabilization before the onset of zygotic gene expression, as we previously observed in *zw3* (Peifer *et al.*, 1994).

APC1 Is Expressed at Very Low Levels in the Epidermis during Embryogenesis

These data, along with the lack of an embryonic phenotype of the *APC1* single mutant (Ahmed *et al.*, 1998), suggest that, while APC2 plays an essential role in the embryonic epidermis, APC1 plays an accessory role, ameliorating the *APC2* mutant phenotype. APC2 is uniformly expressed by all cells in the embryonic epidermis, accumulating at the apicolateral cell cortex (McCartney *et al.*, 1999; Figs. 3A and 3B). Previous analysis of APC1 suggested that in the embryo it accumulates to high levels only in the axons and the developing germline (Hayashi *et al.*, 1997), although uniformly expressed maternal mRNA is present. We thus reexamined APC1 expression in the epidermis, using the zygotic double mutant $APC2^{d40}$ $APC1^{Q8}$ as a negative control. While we could easily detect APC1 accumulation in CNS axons (see accompanying paper) and the germline (data not shown), the level of accumulation in the epidermis was relatively low, and it appeared diffusely cytoplasmic. At early stages of embryogenesis, the epidermal staining we observed in wild type and $APC2^{d40}$ $APC1^{Q8}$ was very similar, perhaps reflecting maternally contributed APC1, while in late embryos we could detect expression above background in the wild-type versus the mutant epidermis (Figs. 3C and 3D). Thus, a very low level of APC1 seems to be able to provide some residual function in Arm destruction.

This activity of APC1, despite its low level of expression, prompted us to examine whether there might be dose-sensitive interactions between the two *APC* genes. We thus reduced the dose of *APC1* in embryos mutant for *APC2*, by crossing males and females of the genotype $APC2^{\Delta S}$ $APC1^{Q8}/APC2^{\Delta S}$ $APC1^{+}$. The progeny of this cross (Fig. 1H) had a more severe cuticle phenotype than the progeny mutant only for $APC2^{\Delta S}$ (Fig. 1G). We saw similar dose-sensitive interactions using the $APC2^{d40}$ $APC1^{Q8}/APC2^{d40}$ genotype (data not shown).

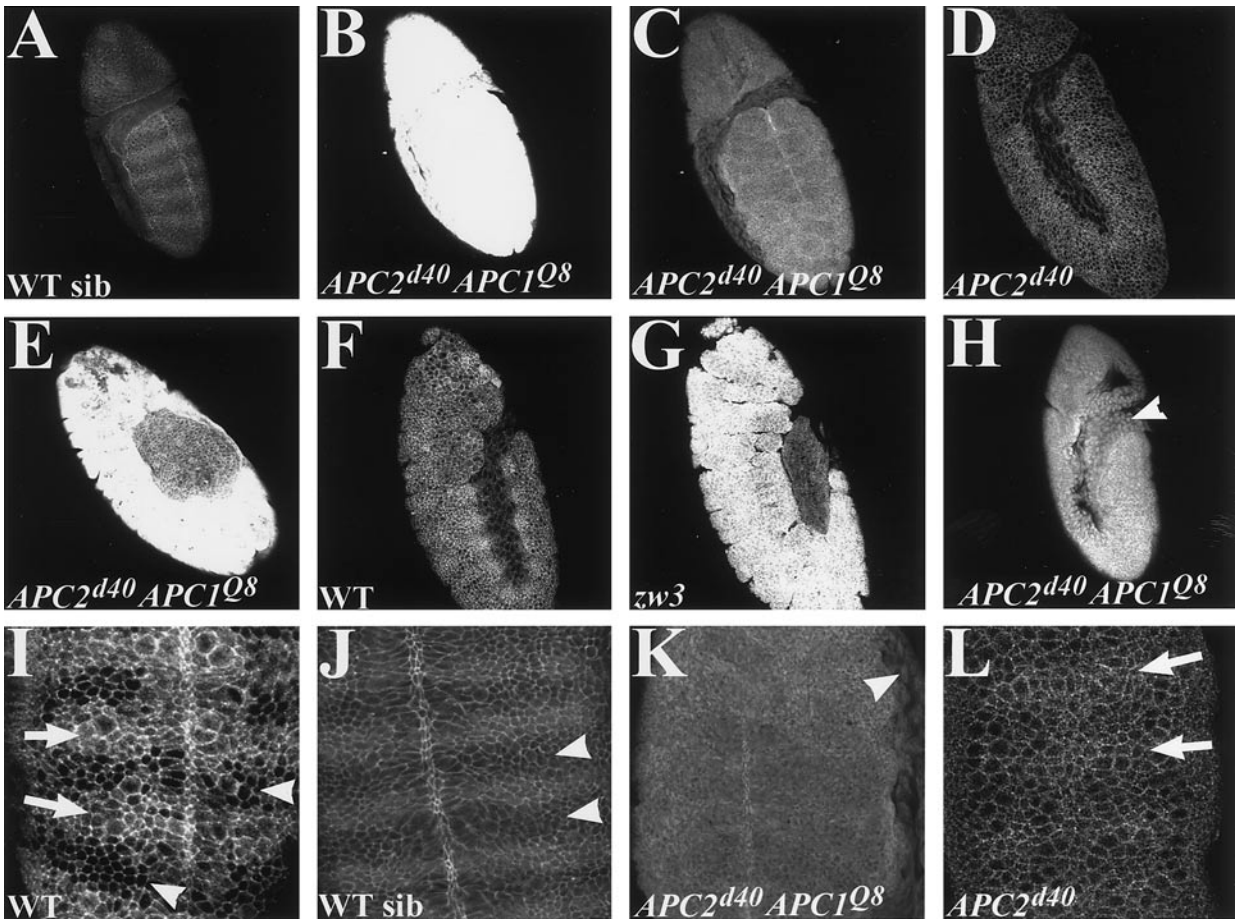

FIG. 2. In *APC2 APC*1 maternal and zygotic double mutant embryos, Arm levels are highly elevated. Embryos stained to reveal Arm. Anterior is up. (A–D) Stage 9. (A, B) Both are progeny of a mother whose germline was $APC2^{d40}$ $APC1^{Q8}$ double mutant. They were prepared for immunofluorescence and imaged together. (A) Zygotically rescued sibling. Stripes of cells in which Wg has stabilized Arm are clearly visible. (B) $APC2^{d40}$ $APC1^{Q8}$ maternal and zygotic double mutant. Arm levels are highly elevated. (C) Same as (B) with brightness reduced to allow visualization of subcellular localization. (D) $APC2^{d40}$ maternal and zygotic single mutant. Levels of Arm are somewhat elevated in all cells. (E) Stage 14 $APC2^{d40}$ $APC1^{Q8}$ maternal and zygotic double mutant. (F) Stage 11 wild-type. (G) Stage 11 *zw3* maternal and zygotic mutant. (H) Lateral view of stage 9 $APC2^{d40}$ $APC1^{Q8}$ maternal and zygotic double mutant. Arrowhead, nuclear enrichment in amnioserosa. (I–L) Close-ups, stage 9. Brightness in (K) was reduced to allow visualization of subcellular localization. (I) Wild-type. Arrowheads, cells not receiving Wg– Arm is only in adherens junctions. Arrows, cells that received Wg, have stabilized cytoplasmic and nuclear Arm. (J) $APC2^{d40}$ $APC1^{Q8}$ maternal mutant that was zygotically rescued. Cells that have not received Wg signal accumulate slightly elevated levels of Arm (arrowheads). (K) $APC2^{d40}$ $APC1^{Q8}$ maternal and zygotic double mutant. All cells accumulate high levels of Arm; some nuclear enrichment is observed (e.g., arrowhead). (L) $APC2^{d40}$ maternal and zygotic single mutant. While Arm levels are somewhat elevated in all cells, Wg signal still can further stabilize Arm (arrows).

APC1 and APC2 Accumulate at Very Different Intracellular Locations When Mis-Expressed in the Embryonic Epidermis

Endogenous APC2 localizes to the cell cortex in most cells throughout development, including those of the embryonic epidermis during the establishment of segment polarity (McCartney *et al.*, 1999). We thus have suggested that the cell cortex is the likely location of the destruction complex, and that the function of APC2 might be to recruit the destruction complex to this site. In support of this, the mutant proteins encoded by $APC2^{d40}$ and $APC2^{\Delta S}$, that lack function in Wg regulation, no longer localize to the cortex (McCartney *et al.*, 1999, 2001).

As the data above suggest that APC1 can contribute to destruction complex function, we suspected that it should localize similarly to APC2. As the endogenous level of expression of APC1 was too low to reliably assess intracellular localization, we mis-expressed both APC1 and APC2-GFP in stripes in the embryonic neurectoderm using en-

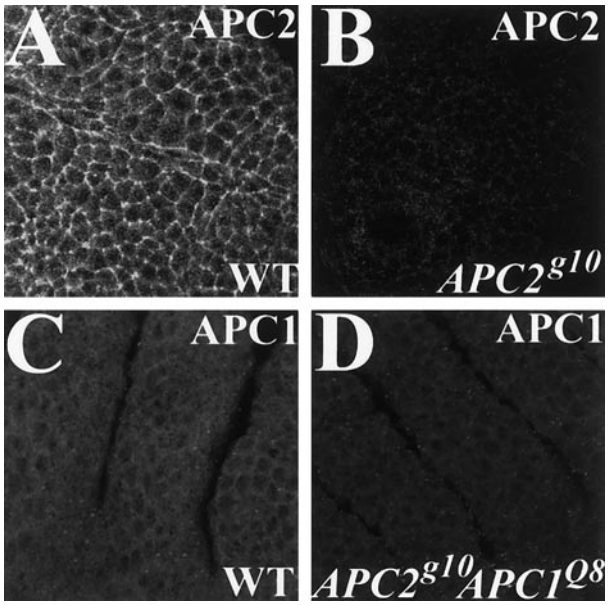

FIG. 3. APC1 and APC2 localization in embryos. (A, B) APC2 expression in the embryonic epidermis of stage 11 wild-type (A) and *APC2*^{g10} maternal and zygotic mutant (B; negative control) embryos. APC2 is enriched at the apicolateral cell cortex. (C, D) APC1 expression in the epidermis of stage 15 wild-type (C) and *APC2*^{g10}*APC1*^{Q8} zygotic double mutant (D; negative control) embryos. APC1 accumulates at low levels diffusely in the cytoplasm.

grailed (en)-GAL4. Expression levels exceeded those of endogenous APC2, as revealed by staining embryos expressing APC2-GFP with anti-APC2, which recognizes both endogenous and exogenous protein. Levels of staining were much higher in the segmental stripes where en-GAL4 is expressed (Fig. 4A). When overexpressed, APC2 localized to the cell cortex (Figs. 4A–4C), resembling endogenous APC2. In contrast, overexpressed APC1 was diffusely cytoplasmic, with strong enrichment at what appear to be centrosomes and associated microtubules of both epidermal cells (Figs. 4D–4G and 4I) and neuroblasts (Figs. 4J and 4K). We saw similar differences in localization of the two proteins in the larval brain (see accompanying paper). These data raise questions about the normal localization of the destruction complex, as the primary location of APC1 upon overexpression is different from that of APC2. We also made one additional observation that may be relevant to this discussion. When we overexpressed APC1 in the embryonic epidermis, we found that endogenous APC2 now became less cortical and more diffusely cytoplasmic (Figs. 4G and 4H, white bracket vs red bracket). We also observed recruitment of endogenous APC2 to centrosomes (Figs. 4G–4L, white arrowheads) in epidermal cells and neuroblasts overexpressing APC1. This suggests that the two APCs may each be able to recruit the other to new locations; this is further supported by similar observations after mis-expression in the larval brain (see accompanying paper).

APC1 and APC2 Play Redundant Roles during Imaginal Disc Development

The overlapping functions of APC1 and APC2 in the embryo raised the possibility that redundancy might help explain why neither *APC1* (Ahmed *et al.*, 1998) nor *APC2* (McCartney *et al.*, 1999) single mutants have defects in patterning of the imaginal discs, precursors of the adult epidermis. To test this, we induced mitotic recombination to produce clones of double mutant cells in animals heterozygous for a wild-type and a double mutant chromosome. In animals in which we induced clones, we found alterations of imaginal disc patterning consistent with activation of Wg signaling (Fig. 5). In the wing, one function of Wg is to specify the wing margin. Activation of Wg signaling by removing *zw3* function was previously observed to transform cells from a wing blade to a wing margin fate, leading to patches of margin bristles in the blade (Blair, 1992). We observed similar patches of bristles in the wing blades of animals in which *APC2*^{d40} *APC1*^{Q8} double mutant clones were induced (Figs. 5B, 5C, 5E, and 5F); they varied in size from tens to hundreds of cells. We also saw regions of the notum where too many cells adopted bristle fates (Fig. 5H, arrow); this was also previously observed in clones mutant for *zw3* (Simpson and Cateret, 1989).

These data suggest that loss of both APC family members activates Wg signaling in imaginal discs, presumably via Arm stabilization. To test this hypothesis, we examined the effect of clonal loss of both *APC* genes on Arm levels. We generated clones of cells that were homozygous *APC2*^{d40} *APC1*^{Q8} double mutant in an *APC2*^{d40} *APC1*^{Q8}/+ heterozygous background. In doing so, one generates adjacent "twin spots"—clones of homozygous wild-type cells. We detected mutant clones by the absence of APC2 staining; both mutant clones (Fig. 5J, arrows) and their homozygous wild-type twin spots (with elevated APC2; Fig. 5J, arrowheads) could be easily distinguished from their heterozygous neighbors. In *APC2*^{d40} *APC1*^{Q8} double mutant clones, we saw elevated Arm accumulation. Thus, loss-of-function of both APC family members is sufficient to deregulate Arm accumulation in the imaginal discs.

DISCUSSION

APC is a tumor suppressor that negatively regulates Wnt signaling, functioning in a multiprotein complex that targets the Wnt effector Arm/β-cat for proteolytic destruction (reviewed in Polakis, 2000). This model of APC function was strongly supported by genetic analysis of the roles of APC proteins during *Drosophila* development (Ahmed *et al.*, 1998; McCartney *et al.*, 1999). However, this relatively simple picture recently became more complex. There are two APC family members in both flies and mammals, with both conserved and divergent structural elements, raising questions about their overlapping or divergent functions in both cell biological and biological events.

A Complex Functional Relationship between APC1 and APC2

Our data reveal a complex pattern of overlapping functions between APC1 and APC2. When we began, we knew that, in certain tissues, individual APCs play critical roles: APC1 is essential in photoreceptors (Ahmed *et al.*, 1998), while APC2 plays critical roles in syncytial embryos and the embryonic epidermis (McCartney *et al.*, 1999, 2001). This functional partition is paralleled by distinct embryonic expression patterns, with APC1 on at high levels in axons (Hayashi *et al.*, 1997) and APC2 on at high levels in the ectoderm (McCartney *et al.*, 1999). In other tissues, such as imaginal discs, neither APC family member is essential, even though both Wg signaling and regulation of Arm stability are critical there. This raised the question of whether the two proteins are redundant in these tissues or whether APC-independent means of regulating Arm stability exist.

Our data suggest a complex picture in which some tissues depend exclusively on one APC family member (photoreceptors), others depend primarily on one family member (the embryonic ectoderm), while in some, either family member provides sufficient function (the larval brain or imaginal discs). The embryonic ectoderm provides a striking example of this complexity. APC2 plays an important role there, with the Wg pathway activated in *APC2* mutants (McCartney *et al.*, 1999). However, certain aspects of the *APC2* phenotype were puzzling; embryos null for another destruction complex component, *zw3*, have a more severe phenotype and more highly elevated Arm levels (Peifer *et al.*, 1994), suggesting that residual destruction complex activity remains in APC2's absence.

We considered several explanations for this. First, the *APC2* allele we used is not a protein null allele. Second, residual destruction complex function might remain in the absence of all APC family members. However, our data support a third possibility: in the absence of APC2, APC1 provides function in the embryonic epidermis that is not sufficient for wild-type pattern, but does allow residual activity of the destruction complex. This is somewhat surprising in view of the expression pattern of APC1 in embryos, which was initially thought to be restricted to axons and primordial germ cells. However, the uniform maternal mRNA (Hayashi *et al.*, 1997) and low levels of APC1 protein (Fig. 3) appear to provide a low level of APC1 function in epidermal cells. While this paper was under review, a similar study of the redundancy of the two fly APC proteins was published (Ahmed *et al.*, 2002). These authors also document redundancy between the *APC1* and *APC2* in both the embryo and the imaginal discs. They further demonstrate that raising the level of expression of *APC1* can rescue the embryonic defects of *APC2*, and that elevated expression of *APC2* can rescue the eye phenotype of *APC1* mutants. These data further underscore the functional overlap between the two proteins.

Together, these two studies raise questions about the possible overlap in function of the mammalian APC proteins in development and oncogenesis and also raise interesting questions about the evolution of small multigene families. The two APC proteins in flies and in mammals appear to have been derived from independent gene duplication events in each lineage. After duplication, the two genes diverged in their patterns of expression and their domain structures (APC2 appears to have lost certain domains, such as the putative microtubule-binding domain). It thus remains to be determined whether there is selective pressure to retain low levels of APC1 in the embryonic ectoderm, or whether residual expression is simply a relict of a time when flies may have had only a single APC. APC family proteins also have Wnt-independent cytoskeletal roles, and may have overlapping roles in these as well; the accompanying paper describes an apparent example of this during brain development.

Overlapping Functions and Divergent Localization

The functional overlap of the two APC family members is even more striking given their distinct structures and intracellular localizations. All APC family proteins share a core structure: a block of highly conserved Arm repeats, which in APC2 is essential for cortical localization and function in the destruction complex, and a set of short repeated sequences that bind Arm/β-cat or Axin, the number and arrangement of which are variable. APC2 contains only this core, which is thus sufficient for both regulating Arm levels as well as for APC2's Wnt-independent role in spindle anchoring. APC1 is longer at its N and C termini, and shares with mammalian APC (at least by sequence similarity) a domain known to bind microtubules. These structural differences confer strikingly different cell biological properties upon the two fly APCs. When overexpressed, APC2 localized almost exclusively to the cell cortex, resembling its endogenous localization. In contrast, overexpressed APC1 localized to the region of the centrosome and to cytoplasmic microtubules, a localization potentially mediated by its putative microtubule-binding domain.

This striking difference in localization raises several interesting issues. We suspect that the localization of APC proteins depends on the availability of and the affinity for different binding partners, as we previously observed for Arm. We hypothesize that the core APC domains are sufficient for cortical localization, but that the additional domain(s) in APC1 redirect it to microtubules. We further hypothesize that if APC1 were detached from microtubules, it would exhibit a default localization to the cortex. This hypothesis is supported by Bienz and colleagues, who showed that one could shift mammalian APC from association with microtubules to actin by disrupting microtubules (Rosin-Arbesfeld *et al.*, 2001).

These data also have implications for the destruction complex. Two mutations that disrupt the localization of APC2 to the cell cortex also disrupt its function in Arm

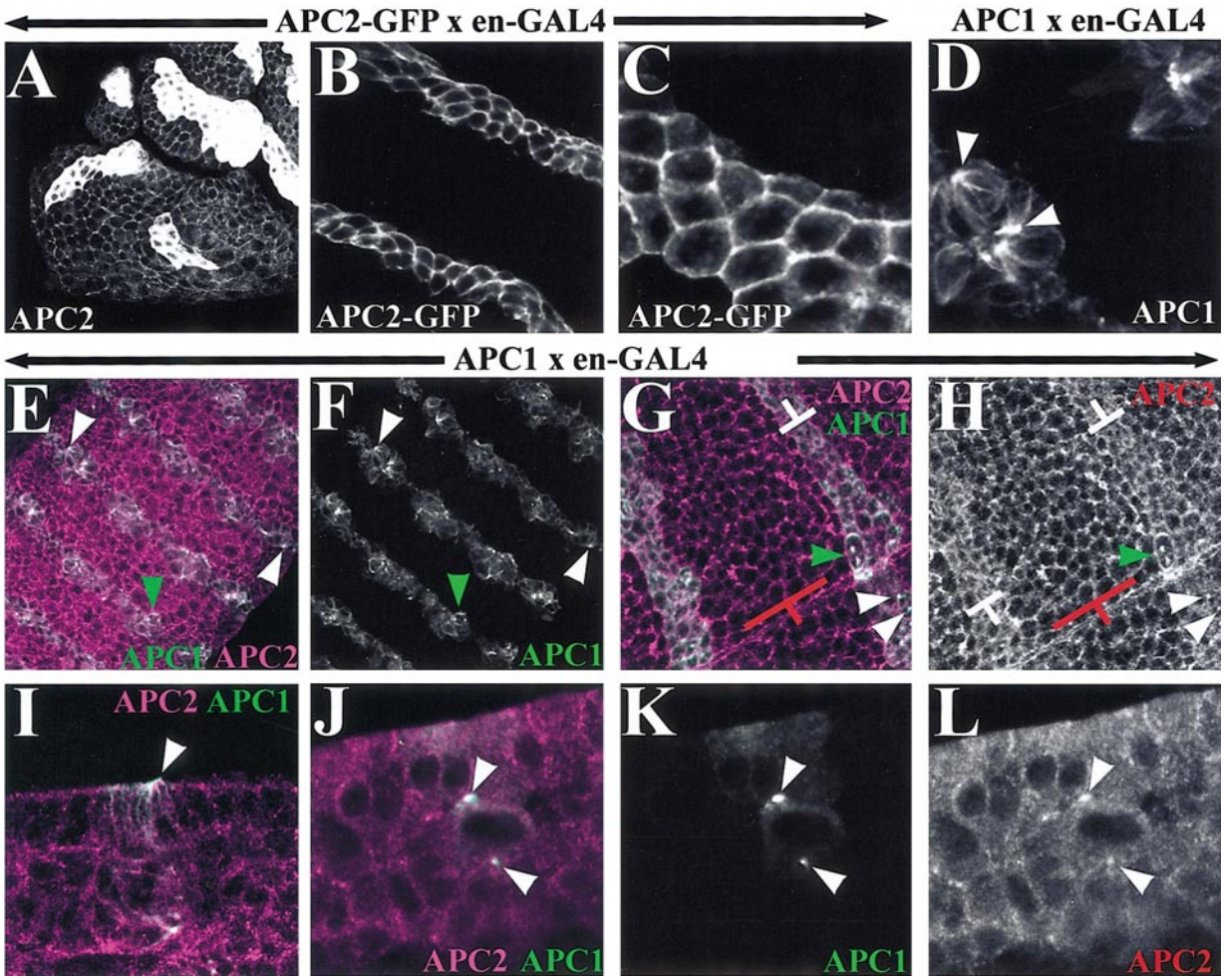

FIG. 4. APC1 and APC2 localize differently when overexpressed in embryos. (A–H) Surface views of extended germ-band embryos. (I–L) Optical cross-sections. (A–C) Embryos expressing APC2-GFP under the control of *en*-GAL4. (A) Anti-APC2 antibody. (B, C) GFP visualized directly. (A) *en*-GAL4-driven APC2-GFP accumulates in segmental stripes, at levels much higher than that of endogenous APC2. (B, C) APC2-GFP localizes to the cell cortex, similar to endogenous APC2. (D–L) Embryos expressing APC1 under the control of *en*-GAL4. (D) Close-up. APC1 localizes to centrosomes and associated microtubules (arrowheads). (E–L) Double-labeled embryos: APC1 (E, G, I, J green, F, K), endogenous APC2 (E, G, I, J purple, H, L). Overexpressed APC1 does not accumulate at the cortex—instead, it accumulates diffusely in the cytoplasm with stronger localization to structures that appear to be centrosomes and microtubules (white arrowheads). (E, F) Green arrowhead, cell with separated centrosomes. (G, H) Green arrowhead, cell in the late stages of mitosis. Endogenous APC2 is normally cortical (G, H, red bracket). In cells expressing APC1, endogenous APC2 becomes diffusely cytoplasmic (G, H, white bracket) and is recruited to presumptive centrosomes with APC1 (white arrowheads). (I) Ectodermal cell, cross-section. APC1 and APC2 colocalize to the apical centrosome and the microtubules radiating from it (white arrowhead). (J–L) Mitotic neuroblast, cross-section. APC1 and APC2 accumulate at the separated centrosomes (white arrowheads).

regulation (McCartney *et al.*, 1999, 2001), suggesting that cortical localization of the destruction complex is important for function. However, the distinctive localization of APC1 raises questions about this conclusion. The simplest interpretation of our data are that APC1 and APC2 localize the destruction complex to distinct places, but that the complex functions at either location. There are several caveats to this interpretation, not least of which is that the predominant localization of APC1 upon overexpression may mask a small amount localized to the cortex; our genetic experiments suggest that nearly undetectable levels of APC1 can confer Arm regulatory activity. It is also possible that both APC2 and APC1 bind to similar partners but with different affinities. Thus, in the absence of APC2 (in *APC2* mutants or in tissues where it is not normally expressed), APC1 may localize to places where APC2 is normally found.

Finally, our data suggest that APC1 and APC2 may

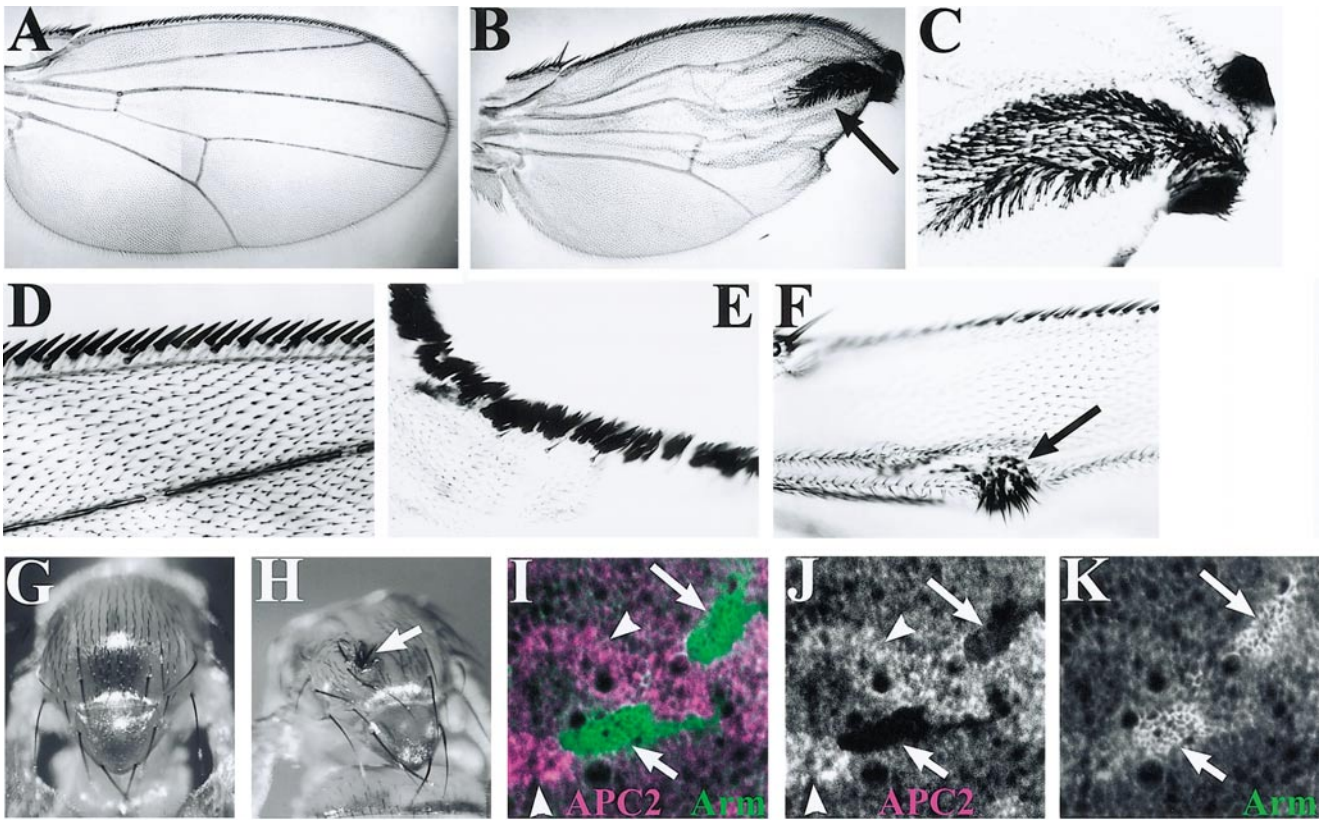

FIG. 5. *APC1* and *APC2* play redundant roles in imaginal disc development. (A–F) Wings from wild-type adults (A, D), or adults in which we induced clones of cells double mutant for $APC2^{d40}$ $APC1^{Q8}$ (B, E, F; C, close up of region in B indicated by arrow). Variable size patches of ectopic wing margin bristles occur in wing blades of animals in which clones were induced (arrows). (D) Wild-type wing margin. (E) Excess cells adopting the wing margin bristle fate. (G, H) Notums. Wild-type (G) and adult in which we induced clones of cells double mutant for $APC2^{d40}$ $APC1^{Q8}$ (H). Note ectopic notal bristles (arrow). (I–K) Wing imaginal disc with clones of cells double mutant for $APC2^{d40}$ $APC1^{Q8}$. Double labeled: APC2 (I purple, J), Arm (I green, K). Double-mutant cells can be identified by reduced APC2 levels (J, arrow); homozygous wild-type twin spots have increased APC2 (J, arrowheads). Double-mutant cells have elevated Arm levels (K, arrows) relative to heterozygous mutant or wild-type cells; this was most evident when one focused below the apical-most plane.

interact. Overexpression of APC1 triggered relocalization of endogenous APC2 from the cortex to centrosomes and microtubules. When both APCs were overexpressed, APC2 recruited APC1 to the cortex (accompanying paper). The mechanism by which this occurs is not clear. Mammalian APC homodimerizes via an N-terminal coiled-coil (Day and Alber, 2000). A clear match to this region is found in mammalian APC2, raising the possibility that mammalian APC and APC2 heterodimerize. However, there is only a weak, partial similarity to this region in fly APC1, and fly APC2 lacks this region entirely. Other mechanisms of oligimerization may exist, or a linker protein or proteins could mediate this putative interaction.

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