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Activated Armadillo/β-Catenin Does Not Play a General Role in Cell Migration and Process Extension in *Drosophila*

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Human β-catenin and its fly homolog Armadillo are best known for their roles in cadherin-based cell–cell adhesion and in transduction of Wingless/Wnt signals. It has been hypothesized that β-catenin may also regulate cell migration and cell shape changes, possibly by regulating the microtubule cytoskeleton via interactions with APC. This hypothesis was based on experiments in which a hyperstable mutant form of β-catenin was expressed in MDCK cells, where it altered their migratory properties and their ability to send out long cellular processes. We tested the generality of this hypothesis *in vivo* in *Drosophila*. We utilized three model systems in which cell migration and/or process extension are known to play key roles during development: the migration of the border cells during oogenesis, the extension of axons in the nervous system, and the migration and cell process extension of tracheal cells. In all cases, cells expressing activated Armadillo were able to migrate and extend cell processes essentially normally. The one alteration from normal involved an apparent cell fate change in certain tracheal cells. These results suggest that only certain cells are affected by activation of Armadillo/β-catenin, and that Armadillo/β-catenin does not play a general role in inhibiting cell migration or process extension.

INTRODUCTION

Cell migration and cell shape changes play key roles in morphogenesis. For example, migrating neural crest cells shape the mammalian peripheral nervous system. Likewise, dramatic cell shape changes, such as axon extension by neurons or process extension by insect tracheal cells, produce highly asymmetric cells whose asymmetry is critical to their function. Underlying both cell migration and cell shape changes are highly organized rearrangements of the actin and microtubule cytoskeletons. Recently, attention has focused on how these cytoskeletal events are regulated. Many mechanisms have been postulated and tested in cultured cells. Genetic technologies in model organisms such as *Drosophila melanogaster*, *Caenorhabditis elegans*, and the mouse now allow us to begin to test these proposed mechanisms *in vivo*. The results of *in vivo* tests sometimes differ from those in cultured cells. For example, several roles for αv-integrins postulated from *in vitro* studies were not observed in mice lacking this integrin subfamily (reviewed in Sheppard, 2000). Thus, *in vivo* tests are critical for evaluating the roles of different cytoskeletal regulators in the complex environment of the intact animal.

One potential cytoskeletal regulator during cell migration and process extension is β-catenin, a multifunctional protein adapter involved in the assembly of several multiprotein complexes with distinct biological activities. For example, β-catenin is a core component of the cadherin–catenin complex, at the heart of the cell–cell adherens junction. Transmembrane cadherins mediate intercellular adhesion, while catenin proteins bind directly or indirectly to the cadherin tail and link the adherens junction to the actin cytoskeleton. The cadherin–catenin complex plays a critical role in establishing and maintaining tissue architec-

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ture in epithelial cells. Reduced cadherin levels have been traditionally viewed as required to allow epithelial cells to lose adhesion for neighboring cells and become migratory, both in normal situations such as neural crest migration and in pathological situations such as tumor cell metastasis. However, more recent data suggest that the picture is more complex: DE-cadherin promotes border cell migration during *Drosophila* oogenesis (Niewiadomska *et al.*, 1999), while N-cadherin promotes motile behavior in cultured mammalian tumor cells (Nieman *et al.*, 1999).

In most epithelial cells, any β-catenin that is not assembled into adherens junctions forms a complex with the tumor suppressors adenomatous polyposis coli (APC) and Axin. This interaction promotes assembly of a multiprotein complex that targets β-catenin for phosphorylation by glycogen synthase kinase-3β (GSK3β), and subsequent proteasomal destruction (reviewed in Peifer and Polakis, 2000). β-Catenin destruction can, however, be countered if the cell is exposed to Wnt family intercellular signals, which inactivate the "destruction complex," stabilize β-catenin, and allow it to enter the nucleus. There, β-catenin again acts as an adapter, in this case mediating interactions between DNA-binding proteins of the TCF/LEF family and the basal transcriptional machinery. This drives gene activation and can influence cell fate in many different ways.

A series of experiments in cultured mammalian cells suggested that β-catenin modulates cell migration and process extension (Barth *et al.*, 1997; Pollack *et al.*, 1997). Mutant forms of β-catenin that could not be targeted for proteasomal destruction, as their GSK3β phosphorylation sites had been deleted (referred to below as activated β-catenin), were expressed in cultured MDCK cells. This led to the accumulation of nonjunctional β-catenin and had dramatic effects on cell behavior. The morphology and colony-forming properties of subconfluent MDCK cells were altered, and the ability of these cells to extend cell processes or migrate in response to Hepatocyte Growth Factor (HGF)/Scatter Factor was nearly abolished. These results suggest that β-catenin can negatively regulate both cell migration and process extension in MDCK cells.

It was hypothesized that these effects of mutant β-catenin might be via its effects on APC (Barth *et al.*, 1997; Pollack *et al.*, 1997). This hypothesis was based on a variety of data suggesting that APC may be a cytoskeletal modulator, both in response to Wnt signals and possibly independent of them (reviewed in McCartney and Peifer, 2000). APC can bind and bundle microtubules *in vitro* (Munemitsu *et al.*, 1994; Smith *et al.*, 1994). Endogenous APC localizes to membrane puncta which are often associated with the ends of microtubule bundles (Näthke *et al.*, 1996), APC-GFP fusion proteins bind to and traffic along microtubules (Mimori-Kiyosue *et al.*, 2000), and *Drosophila* APC2 localizes to the cortex at the ends of the mitotic spindle of asymmetrically dividing neural stem cells (McCartney *et al.*, 1999). Further, APC binds to the protein EB1(Su *et al.*, 1995), which associates with the mitotic spindle in yeast and human cells and affects spindle function in yeast (reviewed in Bloom, 2000). Other data suggest that APC may also interact with the actin cytoskeleton. *Drosophila* APC2 colocalizes with actin in a variety of contexts (McCartney *et al.*, 1999; Yu and Bienz, 1999; Townsley and Bienz, 2000), and human APC was recently shown to associate with the Rho-GEF ASEF (Kawasaki *et al.*, 2000). When stabilized β-catenin was expressed in MDCK cells, it formed stable complexes with APC at the cell cortex (Barth *et al.*, 1997; Pollack *et al.*, 1997). These data together prompted the hypothesis that APC might promote cell migration and process extension by stabilizing microtubule bundles in cell processes, and that this role might be negatively regulated by its binding partner β-catenin. Of course, β-catenin could also affect cell migration in other ways. It might alter migration via its role as a catenin, or could indirectly affect cell behavior via effects on gene expression.

We thus set out to test the generality of the proposed role of β-catenin in cell migration and process extension in the more complex situation found in the living animal. *Drosophila* development provides a number of quite well-characterized situations in which cells migrate and/or extend processes. We chose three of the best studied as models: the migration of the border cell population of follicle cells in the ovary, the extension of axons in the central nervous system, and the combination of process extension and cell migration that occurs during the development of the tracheal system. In each case, we examined whether expression of a hyper-stable mutant form of Armadillo (Pai *et al.*, 1997; referred to below as activated Armadillo), the β-catenin homolog, perturbed any of these processes *in vivo*.

MATERIALS AND METHODS

Stocks

Canton S was used as the wild-type stock. UAS-Arm^{S10} and UAS-Arm$^{\Delta N}$ were previously described in Pai *et al.* (1997). The transgene in those stocks was expressed in specific tissues with the following GAL4 drivers: two different Elav-GAL4 drivers (Lin and Goodman, 1994; Luo *et al.*, 1994) were used for expression in embryonic postmitotic neurons, with the Elav-GAL4 line C155 of Lin and Goodman (1994) used for most experiments, C306-GAL4 and slbo-GAL4 were used for expression in oocyte follicle cells (Manseau *et al.*, 1997; Rorth *et al.*, 1998), and N722 btl-GAL4 (Shiga *et al.*, 1996) was used for expression in the embryonic tracheal system. UAS-lacZ was used to confirm expression patterns of GAL4 drivers and as a comparison with the expression of UAS-Arm^{S10}.

Antibodies and Immunodetection

Embryos were prepared for immunofluorescence as in Peifer *et al.* (1993). Samples were imaged on a Zeiss laser-scanning confocal microscope (LSM 310). Antibodies 2A12, anti-FasIII, and BP102 were purchased from the Developmental Studies Hybridoma Bank and were diluted 1:50, 1:50, and 1:100, respectively. Anti-FasII was

a gift from Corey Goodman and was used at 1:100. Preabsorbed anti-lacZ (Cappel) was used at 1:1000, and preabsorbed anti-c-myc (9E10) was used at 1:10.

Quantitation of Border Cell Migration

The extent of border cell migration was determined at stages 9, 10a, and 10b of oogenesis. The stage of each egg chamber was determined by estimating the extent of oocyte enlargement relative to the size of the entire egg chamber (20% at stage 9, 30–40% at stage 10a, and 50% at stage 10b). The extent of border cell migration was visually estimated by microscopic analysis and recorded as a fraction of the total distance from the origin of migration to the anterior end of the oocyte, and this was plotted as a function of developmental stage.

Immunoblotting

Nine- to eighteen-hour-old wild-type embryos or equivalently aged embryos carrying both a particular GAL4 driver and UAS-Arm^{S10} were collected, bleach dechorionated, and ground in an equal volume of 2× SDS–PAGE sample buffer and boiled for 5 min. Ovaries were dissected from adult females carrying both C306-GAL4 and UAS-Arm^{S10} and treated as above. Samples were analyzed by 6% SDS–PAGE and immunoblotting with preabsorbed antibody to the myc-epitope which tags Arm^{S10} (DSHB; 1:1). Equal amounts of total protein were loaded in each lane, as assessed by staining the blot with Ponceau S. HRP-conjugated secondary was used for chemiluminscent detection (Amersham). NIH Image was used for signal quantitation.

RESULTS

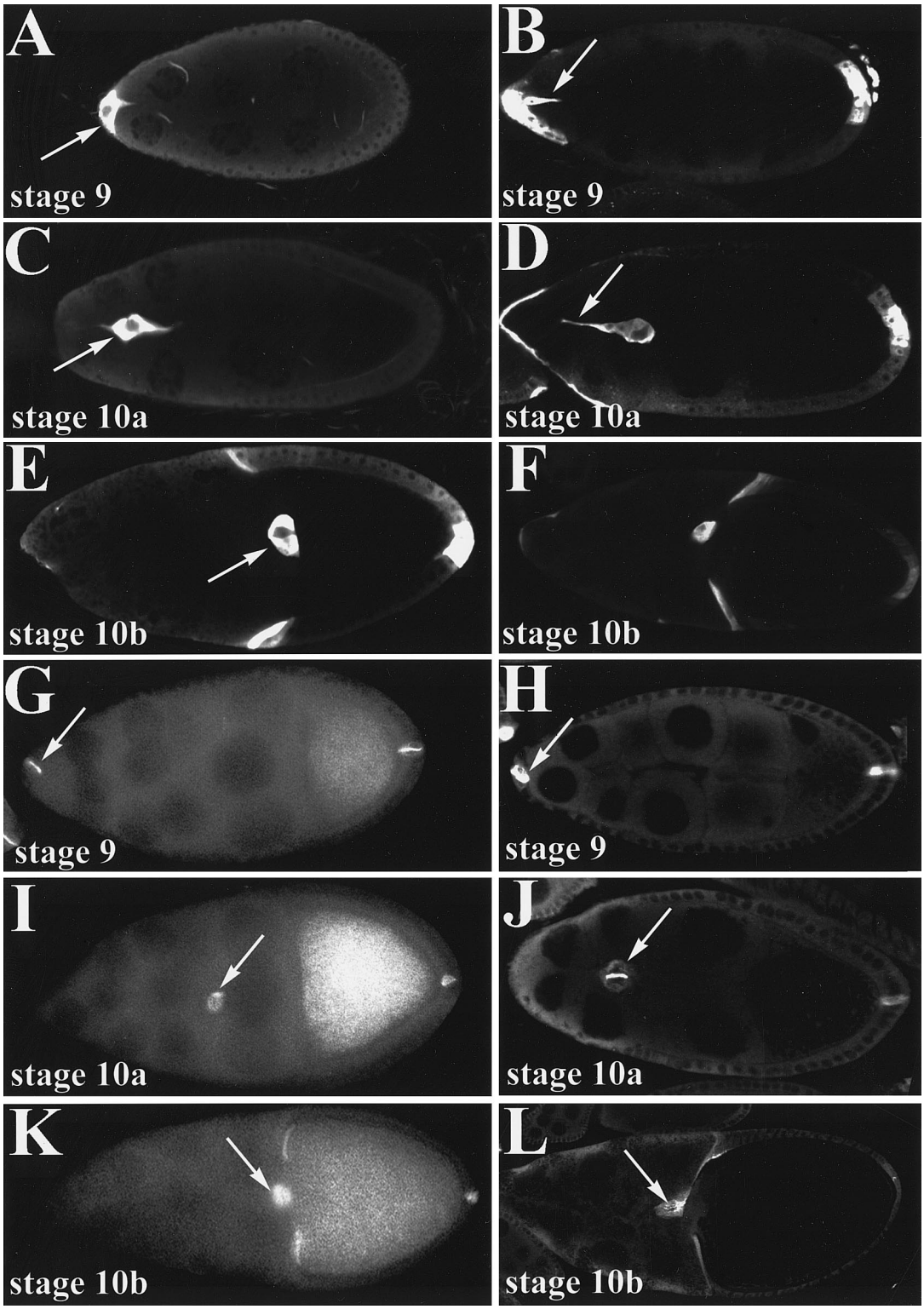
Activated Arm Has No Effect on Border Cell Migration

In cultured MDCK cells, expression of activated β-catenin can alter cell migration, process extension, and tubulogenesis (Barth *et al.*, 1997; Pollack *et al.*, 1997). We set out to test whether similar effects would be observed *in vivo*, using *Drosophila* as a model. To test effects on cell migration, we first examined the migration of the border cells of the fly ovary, a subset of the somatic follicle cells that surround the oocyte and nurse cells. These cells are a superb model for cell migration (reviewed in Montell, 1999). At the midpoint of oogenesis, the border cells separate from the follicular epithelium and migrate as a group between the nurse cells to the presumptive anterior end of the oocyte. The process of border cell migration is known to require DE-cadherin (Niewiadomska *et al.*, 1999). Border cells accumulate elevated levels of both Arm (Peifer *et al.*, 1993) and DE-cadherin (Niewiadomska *et al.*, 1999), consistent with them working together in adhesion in this cell type; however, if Arm function in the developing follicular epithelium is severely compromised, egg chambers degenerate prior to border cell migration (Tantenzapf *et al.*, 2000) so this cannot be tested directly. No role has been reported for Wg signaling in border cells.

To express activated Armadillo (Arm^{S10}; Pai *et al.*, 1997) specifically in the border cells, we made use of the GAL4-UAS system. We utilized two different drivers, C306-GAL4 and *slbo*-GAL4, each of which directs expression of the GAL4 transcription factor in the border cells and not in most of the other somatic cells of the ovary (Figs. 1A, 1C, and 1E). We created females carrying one of these drivers together with UAS-Arm^{S10}, in which activated Arm is driven by a promotor containing GAL4-binding sites, thus driving expression of Arm^{S10} in border cells. We confirmed Arm^{S10} expression in the border cells by staining ovaries with anti-myc antibody, which specifically recognizes the myc-epitope-tagged Arm^{S10} protein (Figs. 1B, 1D, and 1F). This also revealed that Arm^{S10} accumulated throughout the border cells, including in long cell processes. Border cell expression of activated Arm using C306-GAL4 had no significant effect on endogenous Arm, which continued to accumulate in border cells at normal levels and with unaltered subcellular localization (Figs. 2C–2G). This lack of effect of activated Arm on ubiquitous Arm accumulation seemed to be generally true, as ubiquitous expression of activated Arm in embryos using the e22c-GAL4 driver also did not significantly alter levels of endogenous wild-type Arm (Fig. 2B).

We then compared border cell migration in wild-type and activated Arm-expressing border cells. We labeled border cells with antibody to Fasciclin III (Fas III), which is specifically expressed in these cells (Brower *et al.*, 1981; Figs. 1G, 1I, and 1K) and calculated the rate at which they migrated, expressed as the ratio of border cell position from the anterior egg chamber pole to the anterior end of the oocyte, and used the retraction of the follicle cells from the

FIG. 1. Expression of activated Arm does not alter border cell migration. All panels depict egg chambers, with anterior to the left. In each set, three stages of oogenesis are displayed: (A), (B), (G), and (H) are in Stage 9. (C), (D), (I), and (J) are in Stage 10a. (E), (F), (K), and (L) are in Stage 10b. (A, C, E) Expression pattern of slbo-GAL4 revealed by crossing to UAS-lacZ and immunostaining for β-galactosidase. This driver directs expression in the border cells (indicated by arrows), the posterior polar follicle cells and some of their immediate neighbors, and in the centripetal cells which migrate between the oocyte and the nurse cells. (B, D, F) Accumulation pattern of Arm^{S10} driven by either C306-GAL4 (B, D) or slbo-GAL4 (F), and detected with antibody to the myc-epitope. Arm^{S10} accumulates throughout the migrating border cells, including in long cellular processes (arrows in B and D). (G–L) Border cell migration in wild-type (G, I, K) or Arm^{S10}-expressing (H, J, L) egg chambers, as assessed by immunofluorescence for the border cell marker anti-Fas III. Border cells are indicated by arrows. Border cell migration was qualitatively normal as assessed by this procedure.



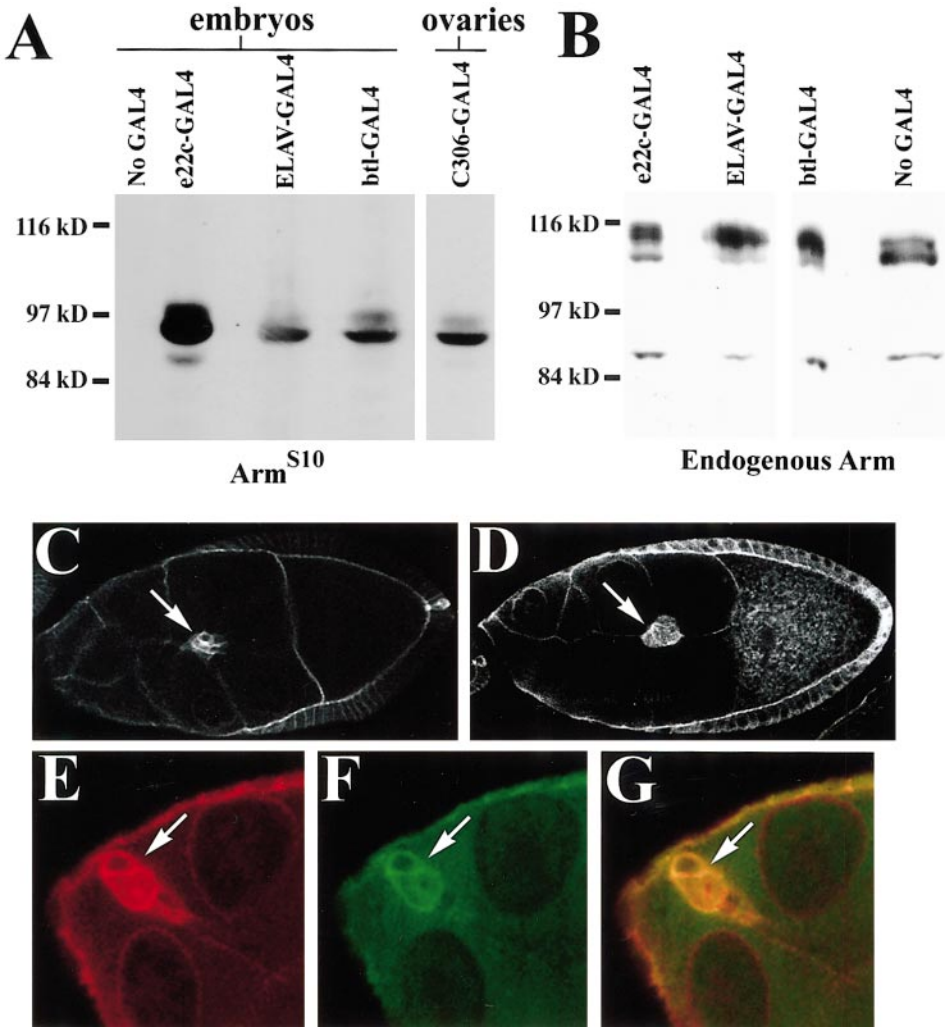


FIG. 2. Levels of expression of activated Arm driven by various GAL4 drivers, and effect of activated Arm expression on levels of endogenous Arm. (A) Equal amounts of total protein from 9 to 18-h-old wild-type embryos (leftmost lane), equivalently aged embryos carrying both the indicated GAL4 driver and UAS-Arm^{S10} (middle three lanes), or ovaries dissected from adult females carrying both C306- GAL4 and UAS-Arm^{S10} (rightmost lane) were analyzed by SDS–PAGE and immunoblotted with antibody to the myc-epitope which tags Arm^{S10}. The position of the MW markers are shown at the left and the position of Arm^{S10} is shown at the right. Note that, while e22c-GAL4 drives expression in most if not all embryonic cells, the other GAL4 drivers only drive expression in a small subset of the cells in the embryo or ovary. (B) Expression of activated Arm does not significantly reduce total levels of endogenous Arm. Equal amounts of total protein from 9 to 18-h-old wild-type embryos (rightmost lane) or equivalently aged embryos carrying both the indicated GAL4 driver and UAS-Arm^{S10} (left three lanes) were analyzed by SDS–PAGE and immunoblotted with anti-Arm monoclonal antibody 7A1, which recognizes wild-type endogenous Arm but does not recognize Arm^{S10} due to deletion of the epitope. The upper set of bands represents canonical wild-type Arm, while the lower band is the alternatively spliced neural isoform. (C–G) Expression of activated Arm in border cells using the C306-GAL4 driver does not significantly reduce levels of endogenous Arm in those cells. (C, D) Ovaries from wild-type animals (C) or from those expressing Arm^{S10} in border cells (D) were stained with anti-Arm monoclonal antibody 7A1, which recognizes wild-type endogenous Arm but does not recognize Arm^{S10}. Note that levels of endogenous Arm in border cells (arrows) remain high even after expression of activated Arm. (E–G) Endogenous wild-type Arm continues to accumulate at high levels in border cells of animals expressing activated Arm. Ovaries from animals expressing Arm^{S10} in border cells were stained with anti-Arm monoclonal antibody 7A1 (E), which recognizes wild-type endogenous Arm but does not recognize Arm^{S10}, and with anti-Arm C-terminal polyclonal antibody (F), which recognizes both endogenous Arm and activated Arm. (G) The merged images.

nurse cells and the size of the oocyte to calculate stage. Border cell migration was completely unperturbed by expression of Arm^{S10}, both in qualitative terms (Figs. 1G, 1I, and 1K vs. Figs. 1H, 1J, and 1L) and in overall rate (Figs. 3A and 3C). We also expressed in border cells a second activated form of Arm, Arm$^{\Delta N}$, which lacks both the GSK3β

phosphorylation sites and also the α-catenin-binding site (Pai *et al.*, 1996, 1997). $Arm^{\Delta N}$ expression also had no discernable effect on border cell migration (Fig. 3B). Consistent with this, females expressing C306-GAL4 and UAS-Arm^{S10} are fertile at 25°C (data not shown).

These results are unlikely to be due to low levels of transgene expression. We examined the level of expression of activated Arm driven by the C306-GAL4 driver by immunoblotting (Fig. 2), comparing expression driven by this GAL4 driver to that of the e22c-GAL4 driver in embryos, which we previously characterized. e22c-GAL4 drives ubiquitous expression of Arm at levels comparable to those of endogenous wild-type Arm (Cox *et al.*, 1999; Pai *et al.*, 1997). In comparison, when normalized to equivalent amounts of total protein analyzed, C306-GAL4 drove expression of activated Arm to levels about fivefold less than those driven by e22c-GAL4. However, on a cell-by-cell basis, expression driven by C306-GAL4 is likely considerably higher, as e22c-GAL4 drives ubiquitous expression, while, in ovaries carrying the C306-GAL4 driver and UAS-Arm^{S10}, only a small fraction of the cells (the border cells) express activated Arm. We thus expect we are driving expression to levels similar to or exceeding those of endogenous Arm. This is similar to the relative levels of activated and endogenous β-catenin seen in the previous experiments in MDCK cells where alterations in cell migration and process extension were noted (Barth *et al.*, 1997; Pollack *et al.*, 1997).

Activated Arm Does Not Significantly Alter Axon Outgrowth

To examine cell process extension, we turned to a second cell type in which process extension plays an critical role. Cells of the central nervous system (CNS) send out long processes, forming the axonal scaffold of the CNS and extending axons into the periphery. Axon outgrowth depends on a variety of different cues. Among these is information from the cadherin/catenin complex—embryos lacking cadherin or catenin function have defects in the outgrowth of certain axon tracts (Iwai *et al.*, 1997; Loureiro and Peifer, 1998). Consistent with this role, Arm accumulates heavily in the axons of the developing CNS (Loureiro and Peifer, 1998). Arm also plays a role in CNS development via its role in Wg signaling—this role is early in the process, in assigning neuroblasts specific identities (Chu-Lagraff and Doe, 1993; Loureiro and Peifer, 1998; Richter *et al.*, 1998). There is no known role for Wg signaling in axon outgrowth in the embryonic CNS, though it does play a role in axon outgrowth in the visual centers of the larval brain (Kaphingst and Kunes, 1994).

Mammalian APC localizes to the termini of neurites (Näthke *et al.*, 1996), and overexpression of activated β-catenin blocked HGF-stimulated process extension by MDCK cells (Pollack *et al.*, 1997). We thus tested whether expression of activated Arm affected axon outgrowth in *Drosophila*. We once again used the GAL4-UAS system, making use of Elav-GAL4, which specifically drives expression in the postmitotic CNS and PNS (Lin and Goodman, 1994; Luo *et al.*, 1994; Fig. 4A). We targeted expression to postmitotic cells both because this is when axons are extended, and because earlier work revealed that Wg signaling acts through Arm to pattern neural precursors (Chu-Lagraff and Doe, 1993;

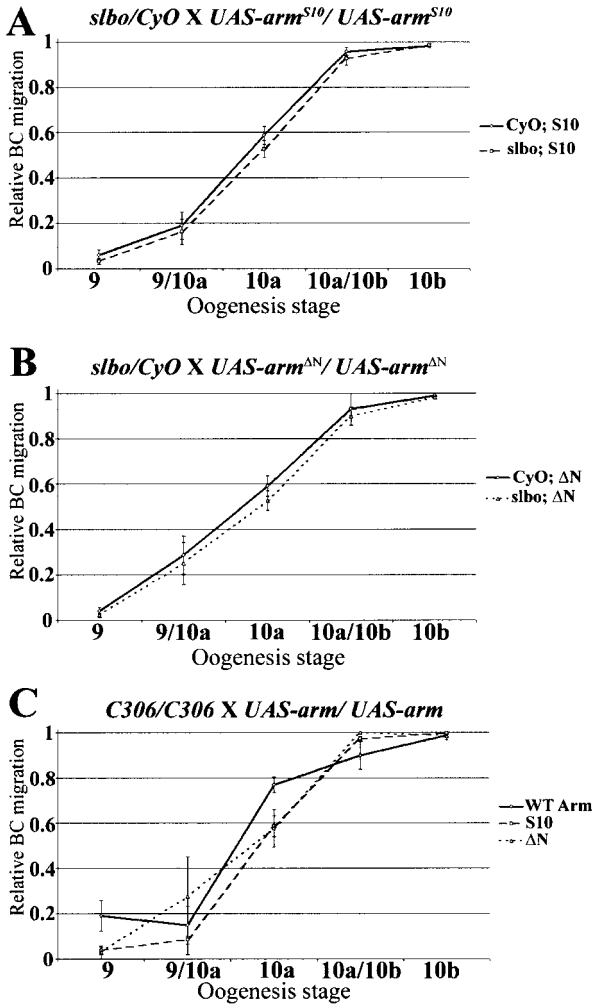


FIG. 3. The rate of migration of border cells expressing various forms of activated Armadillo. (A, B) Ovaries dissected from slbo-GAL4; UAS-Arm^{S10} or slbo-GAL4; UAS-$Arm^{\Delta N}$ females were compared to females from the same crosses that did not express activated Armadillo. (A) Egg chambers accumulating Arm^{S10} (slbo; S10; $n = 367$) have a comparable border cell migration pattern as egg chambers that do not express Arm^{S10} (CyO; S10; $n = 277$). (B) Border cell migration in egg chambers accumulating $Arm^{\Delta N}$ (slbo; ΔN; $n = 258$) was indistinguishable from that in control egg chambers that do not express $Arm^{\Delta N}$ (CyO; ΔN; $n = 126$). (C) Similar results were seen using the GAL4 driver C306. Overexpression of wild-type Arm^{S2} (WT Arm; $n = 149$), Arm^{S10} (S10; $n = 194$), or $Arm^{\Delta N}$ (ΔN; $n = 61$) in egg chambers did not grossly alter the progression of border cells through the nurse cells. In all graphs, standard error bars are shown for each time point.

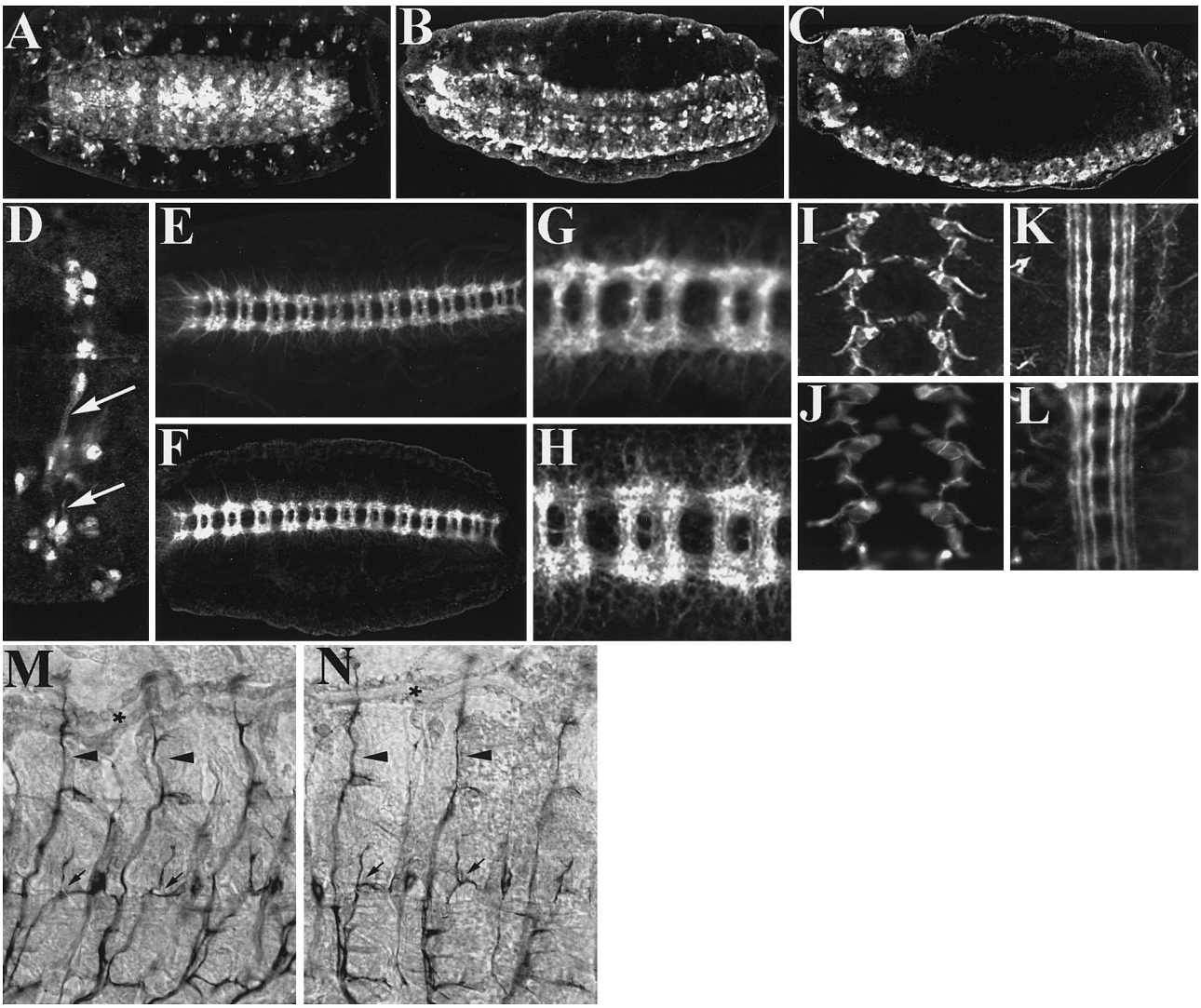


FIG. 4. Expression of activated Arm does not block axon outgrowth during development of the embryonic nervous system. (A) Ventral view of a stage 16 embryo, showing the expression pattern of Elav-GAL4 as revealed by crossing to UAS-lacZ and immunostaining for β-galactosidase. (B–D) Accumulation pattern of UAS-Arm^{S10} driven by Elav-GAL4, in the CNS (B; ventral view; C, lateral cross-section) and PNS (D, lateral view) of stage 16 embryos, as detected with antibody to the myc-epitope. Arm^{S10} protein appears to accumulate primarily in neuronal nuclei and cell bodies, though some axonal accumulation can be seen in the CNS (data not shown) and the PNS (arrows) (D). (E–H) The development of the CNS appeared normal as assessed by staining with the pan-axonal marker BP102. We should note that we cannot rule out subtle changes in the trajectory of individual axons in this analysis. (E–H) Ventral views of stage 16 embryos; (G) and (H) are close-ups. (E, G) Wild-type embryos. (F, H) Embryos expressing UAS-Arm^{S10}. (I–L) The pattern of axons positive for Fas II also appears normal both early (I, J) and later (K, L) in development, as assessed by immunofluorescence with anti-Fas II. (I–L) Close-ups of ventral views of stage 16 embryos. (I, K) Wild-type; (J, L) Embryos expressing UAS-Arm^{S10}. (M, N) Photomicrographs of stage 17 wild-type embryos (M) and embryos expressing Arm^{S10} in all neurons (N) stained with mAb 1D4 (anti-Fas II). Axons from the segmental nerve extend and branch at the appropriate choice point (arrows). Axons from the intersegmental nerve (marked with arrowheads) extend past the trachea (*) to their dorsal muscle targets.

Loureiro and Peifer, 1998), and we wanted to avoid indirect effects via mispatterning. Elav-GAL4 drove strong and specific expression of Arm^{S10} protein in the cells of the CNS and PNS (Figs. 4B–4D). Interestingly, unlike wild-type Arm, which accumulates predominantly in axons (Loureiro and Peifer, 1998), Arm^{S10} accumulated predominantly in cell bodies, in a punctate pattern which may represent nuclear accumulation (Figs. 4B and 4C). Arm^{S10} could also be observed at lower levels in the axons of the PNS (Fig. 4D). We examined levels of expression of activated Arm by immunoblotting, using the

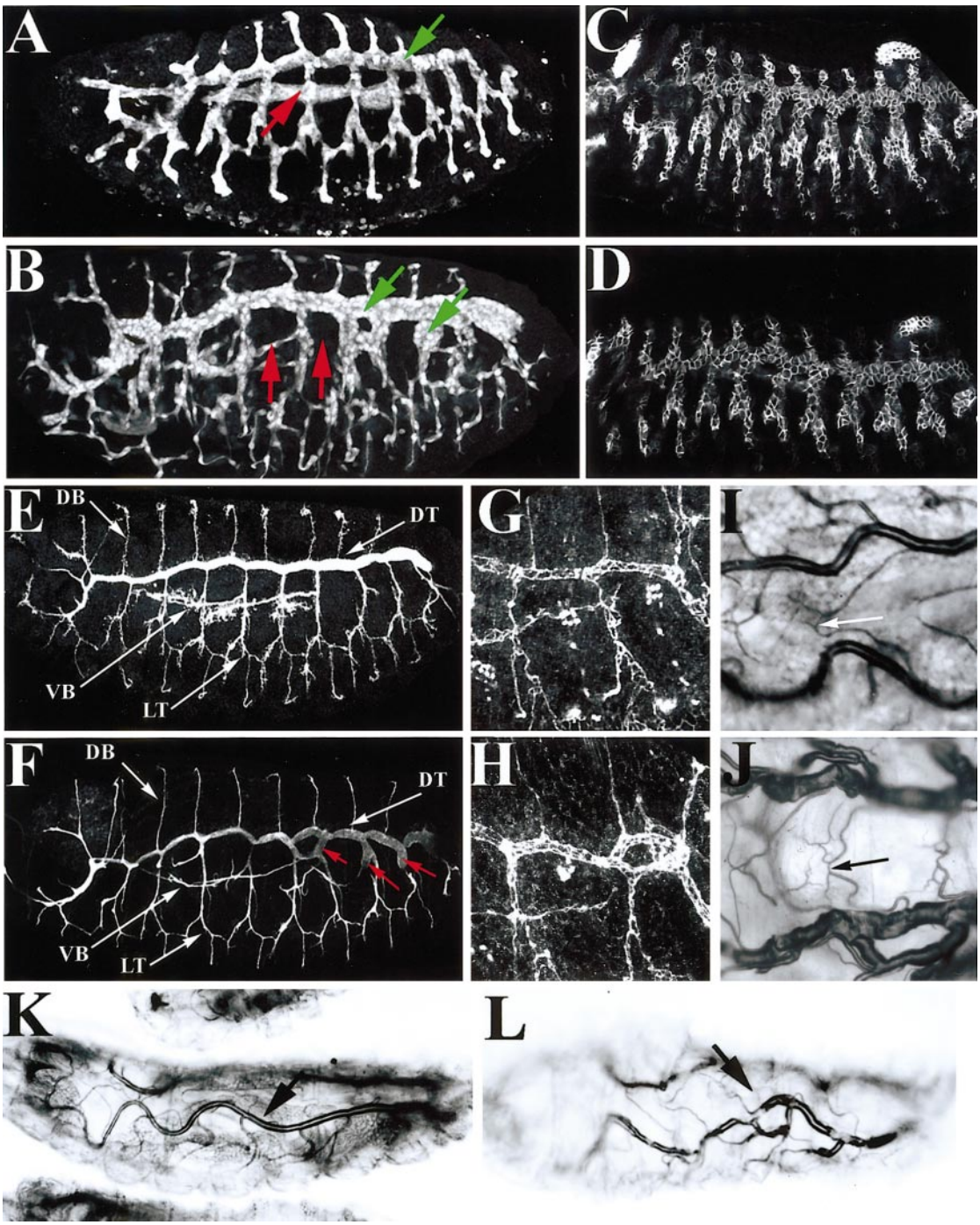


FIG. 5. Expression of activated Armadillo does not prevent tracheal cell migration or process extension, but does alter tracheal cell fates. In all pictures anterior is to the left, and all are lateral views, except (I) and (J) which are viewed from the dorsal side. (A) The expression pattern of btl-GAL4 in a stage 15 wild-type embryo as assessed using UAS-lacZ and immunofluorescence with anti-β-galactosidase antibodies. The dorsal trunk and visceral branches are indicated with green and red arrows, respectively. (B) The accumulation pattern of Arm^{S10} driven by btl-GAL4 in a stage 15 embryo, detected by immunofluorescence with antibodies directed against the myc-epitope. Note that Arm^{S10} protein accumulates throughout tracheal cells, but is concentrated in nuclei. At this stage, the dorsal trunk is enlarged and has abnormal ventrally directed loops or outgrowths (green arrows), while the visceral branches are sometimes reduced (red arrows). (C, D) Fas II expression in the developing trachea of stage 13 wild-type (C) or Arm^{S10}-expressing embryos (D). The Fas II expression pattern is quite normal at this stage. (E, F) The tracheal system of stage 15 wild-type (E) and Arm^{S10}-expressing embryos (F) revealed with antibodies against

ubiquitously expressed e22c-GAL4 as a positive control. Levels of Arm^{S10} driven by Elav-GAL4 are within three- to fivefold of those driven by e22c-GAL4 (Fig. 2). Taking into account the fraction of cells which make up the CNS, we thus estimate that Elav-GAL4 drives Arm^{S10} expression at or near the level of endogenous wild-type Arm within the cells of the CNS. Expression of activated Arm in the CNS did not substantially reduce levels of either the canonical Arm isoforms or the alternately spliced neural isoform (Fig. 2B).

We then examined the effect of Arm^{S10} expression on the development of the axon pattern. We utilized antibodies to both a general neuronal marker, BP102 (Figs. 4E and 4G), and to a marker of a subset of axons, the cell adhesion molecule Fasciclin II (Fas II; Figs. 4I and 4K). We chose this latter marker as the Fas II-positive neurons are among those whose axon pattern requires the function of the N-cadherin/catenin system (Iwai *et al.*, 1997; Loureiro and Peifer, 1998). The CNS of an embryo expressing Arm^{S10} under Elav-GAL4 control appeared wild-type in its axon patterns, as visualized both using BP102 (Figs. 4E and 4G vs. Figs. 4F and 4H) or anti-Fas II (Figs. 4I and 4K vs. Figs. 4J and 4L). We also used anti-FasII to examine the outgrowth of peripheral motoneuron axons. We found that axons of the segmental and intersegmental nerves appeared essentially normal when expressing Arm^{S10} (Fig. 4M vs. 4N). It should be noted that we cannot, of course, assess every axon and thus cannot rule out subtle differences. Consistent with a normal axon pattern, most if not all animals expressing Arm^{S10} under Elav-GAL4 control lived to adulthood (data not shown; Ahmed *et al.*, 1998).

Activated Arm Does Not Prevent Tracheal Cell Migration or Cell Rearrangements but Does Alter Tracheal Cell Fates

The third cell type we utilized were the tracheal cells, which undergo a dramatic, postmitotic program of cell migrations, cell rearrangements, and process extension to produce the larval tracheal system (reviewed in Manning and Krasnow, 1993). Several of these morphogenetic events closely parallel the events observed in the migration of MDCK cells stimulated by HGF (Barth *et al.*, 1997; Pollack *et al.*, 1997). For example, in forming the tracheal vessels, tracheal cells migrate out as a line of cells, some of which later rearrange to form multicellular tubes with a lumen. Tracheal cells also extend terminal branches that resemble the cell extensions produced by the MDCK cells. Arm and DE-cadherin are expressed at high levels in the developing tracheae, and tracheal development is known to require cadherin–catenin function (Uemura *et al.*, 1996).

We tested a number of GAL4 drivers for expression of Arm^{S10} in the tracheal system. Several drivers (e.g., E132 or P127) activated Wg signaling in the epidermis, as assessed by the production of excess naked cuticle (data not shown). We thus did not examine these further, as the interpretation of the results would thus have been complicated by alterations in the landscape across which tracheal cells were migrating. We instead focused on a tracheal driver that did not affect epidermal cell fate choices (data not shown), btl-GAL4. This driver directs expression throughout the tracheal system from the time at which tracheal cells invaginate (Fig. 5A).

btl-GAL4 drove strong expression of Arm^{S10} throughout the tracheal system (Fig. 5B), as assessed using an antibody directed against the myc-epitope. Arm^{S10} protein accumulated throughout tracheal cells, including in cell processes, although it was enriched in tracheal nuclei (Fig. 5B). The level of activated Arm expression, as measured by immunoblotting, was higher than those driven by Elav-GAL4 (Fig. 2). By comparison with e22c-GAL4 (Cox *et al.*, 1999) and taking into account the relatively small proportion of the embryo which gives rise to the trachea, it appears that levels of Arm^{S10} accumulation driven by btl-GAL4 likely approximate or exceed the levels of endogenous Arm.

Many aspects of tracheal development appeared unperturbed by Arm^{S10} expression driven by btl-GAL4. The proper pattern and approximately correct number of tracheal cells were present soon after invagination, as revealed by Fas II staining (Fig. 5C vs. 5D). These cells were able to migrate to distant locations, as do wild-type tracheal cells, and to form many normal tracheal structures. For example, tracheal cells organized dorsal and lateral longitudinal trunks, as well as dorsal tracheal branches, transverse connectives, and visceral tracheal branches (Fig. 5E vs. 5F). In fact, many segments of individual embryos looked nearly normal. Normal-looking terminal branches were also observed (Fig. 5I vs. 5J). One striking and consistent defect was noted, however. In many segments, we noted a pronounced hypertrophy of the presumptive dorsal longitudinal trunk, often associated with a corresponding reduction in the

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the tracheal lumenal marker 2A12. The dorsal branches (DB) and lateral trunk (LT) appear quite normal. Ectopic loops and ventral outgrowths of the dorsal trunk (DT) are observed in the embryo expressing Arm^{S10} (red arrows). The segment with a loop lacks a normal visceral branch (VB). (G, J) Close-ups showing the cellular morphology of the normal dorsal trunk (G) and of an ectopic loop in an embryo expressing Arm^{S10} (H), revealed by antibodies to DE-cadherin. The loop has a cellular morphology similar to that of the normal dorsal trunk. (I–L) Transmitted light micrographs of first instar larvae. (I, J) Dorsal view of wild-type (I) or Arm^{S10}-expressing (J) first instar larva, showing that fine tracheal branches (arrows) can be formed even in segments with large loops in the dorsal trunk. (K, L) Lateral view of wild-type (K) or Arm^{S10}-expressing (L) first instar larva. The arrow indicates the dorsal trunk, which in the wild-type larva has filled with air, as indicated by the clear outline of the tracheal lumen. In the mutant larva, normal gas-filling has not occurred (note gaps in outline of tracheal lumen).

presumptive visceral branch (Fig. 5B). The resulting dorsal trunk was misshapen, often forming loops ventral to and reconnecting with the dorsal longitudinal trunk, or ventral outgrowths (Figs. 5B, 5F, 5H, 5J, and 5L). These loops had lumens of a diameter more similar to that of the dorsal longitudinal trunk than of the transverse connective (Fig. 5H). These defects were associated with gas-filling problems in larvae after hatching, as assessed by Nomarski optics (Fig. 5L). None of these defects was ever observed in wild-type controls.

This phenotype resembled that caused by the overexpression of the zinc-finger transcription factor Spalt (Kuhnlein and Schuh, 1996). At this point in our analysis, we learned that two other groups were independently investigating the role of the Wingless signaling pathway during tracheal development (Chihara and Hayashi, 2000; Llimargas, 2000). These groups observed similar tracheal defects resulting from activation of the Wg pathway by several means, including misexpression of Wg itself and of activated Arm. They further found that this phenotype resulted from inappropriate transcriptional activation of the *spalt* gene. This suggests that the phenotype we and they observed was not due to a direct effect of activated Arm on the cytoskeleton, but rather was mediated via its role as a transcriptional coactivator.

DISCUSSION

A novel role of β-catenin has been suggested by mammalian tissue culture studies. Activated β-catenin has an inhibitory role in HGF-induced process outgrowth and cell migration of MDCK epithelial cells (Barth *et al.*, 1997; Pollack *et al.*, 1997). Expression of activated β-catenin dramatically modulated cell behavior: the ability of cells to form colonies was altered, and their ability to send out cell processes, migrate, and form tubular processes in response to HGF was substantially reduced. Interestingly, activated forms of β-catenin had the same effects regardless of whether they were able to bind α-catenin. Although both forms of activated β-catenin bind E-cadherin, neither appeared to disrupt the function of endogenous wild-type β-catenin at the adherens junction. This suggests that activated β-catenin may disrupt other processes. The authors thus hypothesized that β-catenin acts as a negative regulator of cell migration, perhaps via its interaction with APC. This hypothesis was supported by experiments in the murine colon epithelium (Wong *et al.*, 1998). Here, epithelial migration along the crypt-villus axis was slowed by expression of activated β-catenin; here, however, the orderliness of migration was not affected, and the morphology of the epithelial cells was normal.

We tested the generality of this hypothesis by examining the effect of expressing activated Arm in several cell types in *Drosophila*, selected because cell migration and process extension are part of their normal developmental or physiological program. The experiments above led to the hypothesis that Arm/β-catenin might inhibit cell migration via effects on APC. Previous work also revealed that activated Arm is a powerful modulator of cell fate in certain tissues, via its transcriptional role. For example, it mimics activation of Wg signaling and thus shapes cell fate in the embryonic epidermis, the wing, or the eye (Pai *et al.*, 1997; Zecca *et al.*, 1996; Ahmed *et al.*, 1998). Given Arm's ability to modulate morphogenesis via transcriptional and potentially nontranscriptional effects, we anticipated that misexpression of activated Arm would have drastic consequences in the three model tissues selected.

To our surprise, the effects of activated Arm were quite modest. We first tested the effects on border cell migration or axon outgrowth. Both processes seemed good candidates to be affected, as both involve cell migration or process extension over a cellular substrate and both require cadherin–catenin function (Iwai *et al.*, 1997; Loureiro and Peifer, 1998; Niewiadomska *et al.*, 1999). However, in both cases, no significant defects were observed. We also tested whether activated Arm would alter the cell migratory events and cell shape changes that occur during morphogenesis of the tracheal system. Tracheal cells resemble the MDCK cells whose migration is affected by activated β-catenin in several ways—the two divergent cell types share the ability to migrate in columns from an epithelial progenitor, to form multicellular tubes, and also to send out long cell processes. We thus were surprised at how little perturbation of tracheal development was caused by expression of activated Arm. The cell biological abilities of tracheal cells were essentially unimpaired—cells retained the ability to migrate in columns, form tubes, and extend processes. The one striking effect that was observed appeared to be a misallocation of cells into different cell types, with more cells choosing the dorsal tube fate and fewer cells choosing the visceral branch fate. This change did result in a dramatic change in the morphogenesis of a subset of the tracheal system, as these two cell types differ significantly in their cellular behaviors.

From these data, we can draw two general conclusions. First, these data point out very clearly that the response of a cell to activated Arm/β-catenin varies substantially between different tissues. While some cells, such as the epithelial cells of the embryonic epidermis or the imaginal discs, respond to activated Arm with dramatic shifts in cell fate, other cell types, such as the border cells or postmitotic neurons, seem to be refractory to its effects. This was particularly striking as activated Arm was observed to accumulate in or even become concentrated in the nuclei of the cells in which it was expressed. This conclusion is further substantiated by a study by Schüpbach and Wieschaus (1998) in which they found that expression of activated Arm in various follicle cell subsets had little or no effect on eggshell morphology or fertility. Several possible explanations may explain this differential sensitivity. First, cells may vary in their expression of Arm's transcriptional partner dTCF—we view this as less likely, as dTCF is expressed at apparently uniform levels in different cells in

the embryo (van de Wetering *et al.,* 1997). Second, the Arm/dTCF complex may require other cofactors to regulate gene expression, which are not uniformly expressed. We feel the most likely explanation, however, is that most Wingless-target genes may only be activated by the combinatorial action of different transcription factors which deliver inputs from a variety of different signal transduction pathways (e.g., Halfon *et al.,* 2000). This allows activation of different subsets of target genes in different tissues, and also ensures that inappropriate activation of a given pathway will often not have serious consequences, unless the tissue is already programmed to respond to that pathway. Thus, for example, in the tracheae, the only cells affected by expression of activated Arm are those which normally are programmed to respond to Wg signal (Chihara and Hayashi, 2000; Llimargas, 2000; our data).

Our second conclusion is that expression of activated Arm/β-catenin does not have a general inhibitory effect on cell migration or process extension. These processes were unaffected by expression of activated Arm in the border cells or neurons, and were only affected in a small proportion of cells in the developing trachea. These observations provide a striking contrast to the dramatic effects of activated β-catenin expression in MDCK cells (Barth *et al.,* 1997; Pollack *et al.,* 1997) and the significant, though less dramatic, effects seen in the colon epithelium of mice (Wong *et al.,* 1998). It is possible that these differences could be due to differences in level of expression of activated Arm/β-catenin. However, we think this is less likely, as in our experiments (Fig. 2) and those of Barth *et al.* (1997) and Pollack *et al.* (1997), the level of expression of activated Arm/β-catenin was approximately equal to that of endogenous Arm/β-catenin, while in the experiments of Wong *et al.* (1998), the activated β-catenin accumulated to levels only three- to fivefold higher than that of the endogenous protein. There are several alternate explanations for the differences between our results and those in MDCK cells. First, as discussed above with respect to Arm/β-catenin's transcriptional effects, there may be differences in expression of partners or cofactors required for effects of β-catenin on migration—e.g., APC family members. Second, it may be that the effects observed in the MDCK and colon cells were mediated by the transcriptional role of β-catenin—in the colon, at least, it is already clear that these cells respond to activation of the Wnt pathway by altering their transcriptional profile. We and others have found that expression of activated Arm in the dorsal trunk cells of the trachea leads to dramatic alterations in the morphogenesis of this cell type; this effect is clearly due to effects on the Wg/Wnt regulated transcriptional program, with the downstream transcription factor Spalt a key target (Chihara and Hayashi, 2000; Llimargas, 2000). Thus β-catenin's role in cell migration may be primarily indirect, via alterations in cell fate mediated by its role in regulating gene expression. This can now be tested directly in mammalian cells by examining whether downstream gene expression via the TCF/LEF pathway is essential for β-catenin's effects.

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