Drac1 and Crumbs participate in amnioserosa morphogenesis during dorsal closure in *Drosophila*

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Summary

Dorsal closure of the *Drosophila* embryo involves morphological changes in two epithelia, the epidermis and the amnioserosa, and is a popular system for studying the regulation of epithelial morphogenesis. We previously implicated the small GTPase Drac1 in the assembly of an actomyosin contractile apparatus, contributing to cell shape change in the epidermis during dorsal closure. We now present evidence that Drac1 and Crumbs, a determinant of epithelial polarity, are involved in setting up an actomyosin contractile apparatus that drives amnioserosa morphogenesis by inducing apical cell constriction. Expression of constitutively active Drac1 causes excessive constriction of amnioserosa cells and contraction of the tissue, whereas expression of dominant-negative Drac1 impairs amnioserosa morphogenesis. These Drac1 transgenes may be acting through their effects on the amnioserosa cytoskeleton, as constitutively active Drac1 causes increased staining for F-actin and myosin, whereas dominant-negative Drac1 reduces F-actin levels. Overexpression of Crumbs causes premature cell constriction in the amnioserosa, and dorsal closure defects are seen in embryos homozygous for hypomorphic *crumbs* alleles. The ability of constitutively active Drac1 to cause contraction of the amnioserosa is impaired in a *crumbs* mutant background. We propose that amnioserosa morphogenesis is a useful system for studying the regulation of epithelial morphogenesis by Drac1.

Key words: *Drosophila*, Rac, Small GTPase, Crumbs, Amnioserosa, Dorsal Closure, Cytoskeleton, Morphogenesis

Introduction

Dorsal closure of the *Drosophila* embryo has emerged in recent years as an excellent system for studying the regulation of epithelial morphogenesis (Noselli and Agnes, 1999). Following germband retraction, there is a hole present in the dorsal epidermis that is occupied by the amnioserosa, an epithelium of large flat cells. Dorsal closure proceeds through a dorsally directed migration of the lateral epidermis from both sides of the embryo, with the two flanks moving over the amnioserosa and meeting up along the dorsal midline. During dorsal closure, the cells of both the epidermis and the amnioserosa undergo dramatic changes in morphology. At the beginning of this process, the leading edge cells of the advancing epidermis elongate along the dorsoventral axis, and this is followed by a similar elongation of more ventrally located epidermal cells. Elongation of the leading edge cells appears to be achieved by an accumulation of filamentous actin (F-actin) and nonmuscle myosin (hereafter referred to as myosin) forming an actomyosin contractile apparatus present at their dorsal end (Young et al., 1993). Occurring simultaneously with the alteration of epidermal morphology is an extensive morphogenesis of the amnioserosa, which changes from an elliptically shaped squamous epithelium to a narrow, tubular structure. Transmission electron microscopy has shown that the cells of the amnioserosa shift from squamous to columnar in shape by elongating along the apical-basal axis (which runs dorsoventrally) and constricting apically (Rugendorff et al., 1994). This cell shape change is accompanied by an invagination of the tissue, leading to the final morphology. Laser ablation studies indicate that contractility of the amnioserosa contributes to dorsal closure and that cell shape change in this tissue is autonomous, and not simply a passive response to pushing by the advancing epidermis (Kiehart et al., 2000). How morphogenesis of the amnioserosa is regulated has not been characterized, although obvious candidate genes are those with known roles in dorsal closure.

Numerous genes have been identified that are required for correct dorsal closure, and these can be grouped into several categories including small GTPases (p21s), members of a Jun-amino-terminal kinase (JNK) cascade, members of a Decapentaplegic (Dpp) pathway, members of the Wingless pathway and various proteins associated with the cytoskeleton and/or cell junctions (Noselli and Agnes, 1999; McEwen et al., 2000). Disruption of the function of these genes leads to defects in dorsal closure that are frequently accompanied by failures of cell shape change in the epidermis and abnormalities in the distribution of F-actin and myosin at the leading edge. These results strongly support the idea that the leading edge cytoskeleton is driving

dorsal closure through effecting cell shape change in the epidermis. Studies in mammalian cells and model organisms have demonstrated the involvement of the Rho subfamily of Ras-related small GTPases in regulation of the actin cytoskeleton and cell shape, and we and others have characterized the involvement of Ras1 and three Rho subfamily members, Drac1, Dcdc42 and RhoA (Rho1), in dorsal closure (Harden et al., 1995; Harden et al., 1996; Harden et al., 1999; Magie et al., 1999; Ricos et al., 1999; Riesgo-Escovar et al., 1996; Strutt et al., 1997). Expression of dominant-negative versions of these p21s (and evaluation of loss-of-function Rho1 alleles) during embryogenesis demonstrates that only dominant-negative Drac1 is capable of preventing the accumulation of the leading edge actomyosin contractile apparatus. The other small GTPases have roles in the epidermis during dorsal closure, but impairment of their function does not completely disrupt the leading edge cytoskeleton.

We had previously seen a reduction in peripheral F-actin and spectrin staining in amnioserosa cells following heat shock induction of a dominant-negative *Drac1* transgene, *Drac1N17*, and we wondered if Drac1 might be regulating the cytoskeleton and morphology of the amnioserosa during dorsal closure (Harden et al., 1995). In this study, we address small GTPase function in the amnioserosa further by using the GAL4-UAS system (Brand and Perrimon, 1993) to drive expression of constitutively active and dominant-negative transgenes during dorsal closure with various GAL4 drivers. We show that, of the p21s tested, only Drac1 appears to be required for morphogenesis of the amnioserosa. A constitutively active version of Drac1 causes excessive contraction of the amnioserosa, whereas a dominant-negative version of Drac1 retards amnioserosa morphogenesis. We present evidence that Crumbs (Crb), a determinant of apical-basal polarity (Wodarz et al., 1995), is a component of Drac1-mediated amnioserosa morphogenesis.

Materials and Methods

Fly strains and GAL4 crosses

Transgenic flies bearing wild-type, activated and dominant-negative versions of human RhoA have been previously described (Harden et al., 1999). Flies bearing dominant-negative *Drosophila* RhoA in pUAST were from M. Mlodzik (Strutt et al., 1997), dominant-negative and activated forms of *Dcdc42* and *Drac1* in pUAST from L. Luo (Luo et al., 1994), dominant-negative Ras1 in pUAST from T. Lee (Lee et al., 1996) and activated Ras1 in pUAST (*UAS-Ras1Q13(II)B)* from B. Noll. Males from the pUAST transgenic lines were crossed to females from GAL4 lines and the progeny examined as embryos. The *69B-GAL4*, *GAL4*^{c381}, *UAS-crb*^{wt}, *crb*^{2} and *UAS-dpp* lines were obtained from the Bloomington Stock Center and the P-element insertions in *crb* were provided by the Szeged Stock Center. The *GAL4*$^{332.3}$ line was provided by B. Giebel and *Hs-GAL4*$^{M-4}$ and *UAS-lacZ*$^{1-71}$ flies were a gift of J. Roote.

Heat shock expression of Drac1V12

Embryos were collected and aged at 25°C until 8 to 12 hours after egg laying (AEL), placed in vials and heat-shocked for 10 minutes in a water bath set at 37°C. Following heat-shock, embryos were either aged for 7 hours at 21°C and fixed for immunohistochemistry or aged at 21°C for at least 48 hours and subjected to cuticle preparation.

Immunohistochemistry

Fixed embryos were staged independently of the degree of dorsal closure using the extent of head involution and/or central nervous system development instead (Campos-Ortega and Hartenstein, 1985). Unless otherwise stated, all staining procedures were carried out at room temperature. After dechorionation in 50% household bleach in 0.01% Triton X-100, embryos were washed in 0.01% Triton and fixed for 25 minutes in 4% paraformaldehyde in PBS (0.1 M NaCl, 10 mM phosphate buffer, pH 7.4)/heptane. Vitelline membranes were then removed by washing with methanol or 80% ethanol if embryos were to be phalloidin stained. Embryos were washed for 1 hour in three changes of PBT (PBS with 0.1% Triton X-100) and blocked for 1 hour in PBT containing 1% bovine serum albumin (BSA). Primary antibody incubations in PBT containing 1% BSA were done overnight at 4°C, and embryos were then washed for 1 hour in PBT. Fluorescent detection of primary antibodies was done using either secondary antibodies directly labeled with Texas Red or FITC, or biotinylated secondary antibodies and streptavidin labeled with Texas Red or FITC (all materials from Vector Laboratories). All secondary antibodies were diluted 1:200 in 1% BSA in PBT. Secondary antibody incubation was done for 2 hours, and embryos were then washed for 1 hour in three changes of PBT and incubated with a 1:1000 dilution of labeled streptavidin in PBS for 1 hour. If F-actin staining was required, FITC-labeled or TRITC-labeled phalloidin (Sigma) was added to a final concentration of 1 μg/ml 30 minutes into the streptavidin incubation. Embryos were washed for 20 minutes in several changes of PBS, mounted in Vectashield and examined using either a Bio-Rad MRC 600 or Zeiss LSM confocal laser scanning microscope. Confocal images were assembled from a series of Z-sections. Detection of primary antibodies for Nomarski microscopy was done using the glucose oxidase-DAB-nickel method (Hsu et al., 1988).

Cuticle preparations

Cuticles were prepared as described by Ashburner (Ashburner, 1989), except that the fixation step was omitted. At least 100 embryos were examined from each cross.

Plasmid rescue

Isolation of genomic sequences flanking the *crb*^{S010409} P-element insertion was done as described (O'Kane, 1998).

Results

Amnioserosa morphogenesis begins with apical constriction of cells at the anterior and posterior ends of the tissue accompanied by cytoskeletal changes

We initiated our study of the potential role of Drac1 in regulating amnioserosa morphogenesis by examining the effects of expressing a constitutively active *Drac1* transgene, *UAS-Drac1V12*, using a GAL4 driver, *GAL4*$^{332.3}$ (Wodarz et al., 1995), the expression of which is limited to the amnioserosa (Fig. 1F) and the brain during dorsal closure. The resulting embryos were examined with cuticle preparations, and the most prevalent defect seen was a failure to secrete cuticle, which occured in 41% of embryos. Of the embryos that did form a cuticle, 47% showed puckering of the dorsal surface, which suggests a mild defect in dorsal closure (data not shown). This result raised the possibility that Drac1V12 could affect dorsal closure through its action in the amnioserosa. Prior to evaluating the Drac1V12-induced phenotype, we did a careful evaluation of amnioserosa morphogenesis in wild-type embryos. We decided to stain

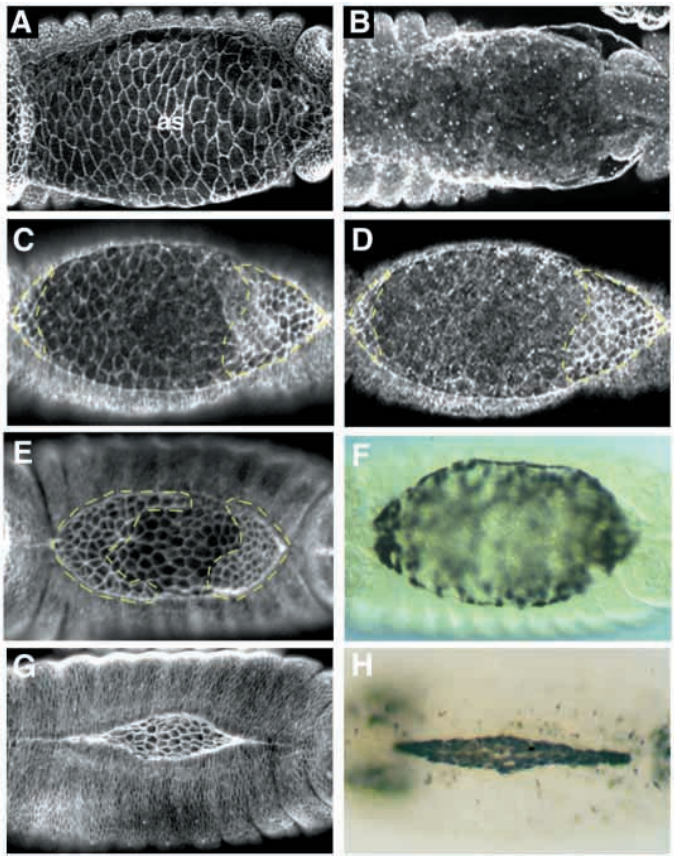

Fig. 1. Dorsal views of the amnioserosa showing morphogenesis of this tissue during dorsal closure. Anterior is to the left in this and subsequent figures. Dashed yellow lines demarcate clusters of apically constricted cells. (A,C,E,G) Embryos at progressively later stages of dorsal closure stained with anti-phosphotyrosine antibodies. (B,D) Embyros stained with anti-nonmuscle myosin heavy chain antibodies (Kiehart and Feghali, 1986). (F,H) Embryos stained with anti-β-galactosidase antibodies. (A) A stage 13 embryo prior to commencement of dorsal closure showing amnioserosa as a flat sheet with cells elongated perpendicular to the A-P axis of the embryo. (B) Embryo at similar developmental stage to that in A, showing no enrichment of myosin in the amnioserosa. (C,D) Early stage 14 embryo double-stained to show elevated levels of phosphotyrosine (C) and myosin (D) in cells at the anterior and posterior ends of the amnioserosa. These cells are apically constricted relative to cells in the middle of the amnioserosa (the apical ends of the amnioserosa cells are at the surface of the embryo). The hindgut can be seen as a stained structure under the posterior end of the amnioserosa. (E) Late stage 14 embryo midway through dorsal closure showing apically constricted cells at the ends of the amnioserosa and loss of original elongation of middle amnioserosa cells. (F) *UAS-lacZ*$^{1-71}$*;GAL4*$^{332.3}$embryo early in dorsal closure with anti-β-galactosidase staining revealing amnioserosa as an elliptical sheet of cells. Note the lack of expression of the *lacZ* reporter gene in the epidermis. That no epidermal cells were expressing the *lacZ* reporter gene was confirmed by double staining *UAS-lacZ*$^{1-71}$*;GAL4*$^{332.3}$ embryos with anti-phosphotyrosine antibodies to show cell boundaries (data not shown). (G) A stage 15 embryo late in dorsal closure showing that the middle amnioserosa cells are both elongated along the A-P axis and apically constricted. (H) A *UAS-lacZ*$^{1-71}$*;GAL4*$^{332.3}$embryo after the completion of dorsal closure with β-galactosidase staining revealing amnioserosa as a tubular structure. Note *lacZ* expression appearing in epidermal cells.

embryos for various components of the leading edge contractile apparatus in the belief that these might also be contributing to cell shape change in the amnioserosa. In addition to F-actin and myosin, phosphotyrosine-rich structures are found at the leading edge and may contribute to the assembly and/or function of the actomyosin contractile apparatus (Harden et al., 1996; Harden et al., 1999). Phosphotyrosine staining provides a clear view of the shape of the apical surface of amnioserosa cells, whereas myosin and F-actin staining can reveal the presence of any potential contractile apparatus. Following germband retraction but prior to the onset of dorsal closure, the amnioserosa cells have a large apical surface area and are elongated perpendicular to the anterior-posterior (A-P) axis of the embryo (Fig. 1A). There are no major accumulations of myosin (Fig. 1B) or F-actin (data not shown) in the amnioserosa at this time. As dorsal closure proceeds, cells at the anterior and posterior ends of the amnioserosa (the 'end cells') show a pronounced apical constriction and have elevated levels of peripheral phosphotyrosine, myosin and F-actin (Fig. 1C-E) (data not shown) relative to cells in the middle of the amnioserosa (the 'middle cells'). The middle cells gradually shift from being elongated laterally (Fig. 1A) to being elongated along the A-P axis late in dorsal closure (Fig. 1G). They also constrict apically, but lag behind the end cells. The overall change in amnioserosa morphology during dorsal closure can be followed by expressing a *UAS-lacZ* reporter gene with the *GAL4*$^{332.3}$ driver and staining embryos with anti-β-galactosidase antibodies. This staining allows easy visualization of even that portion of the amnioserosa that lies under the epidermis and reveals the narrowing of this tissue from its original elliptical shape (Fig. 1F,H).

Drac1V12 expression in the amnioserosa induced elevated levels of F-actin, myosin and phosphotyrosine in amnioserosa cells and contraction of this tissue

Embryos in which Drac1V12 had been induced with the *GAL4*$^{332.3}$ driver were stained for phosphotyrosine, myosin or F-actin and the amnioserosa compared to wild-type embryos of a similar age. As a result of Drac1V12 expression, the amnioserosa early in dorsal closure was transformed from a flat sheet of cells occupying the entire dorsal hole into a contracted tissue of greatly constricted cells occupying less than half the dorsal hole at its posterior end. In these embryos it appeared that all amnioserosa cells had constricted, rather than only the end cells as occurs in wild-type embryos (compare Fig. 2A with Fig. 1C). A similar, excessive contraction of the amnioserosa was induced through heat shock expression of Drac1V12 with the *Hs-GAL4*$^{M-4}$ driver (Fig. 2C). As the amnioserosa pulled away from the leading edge in Drac1V12-expressing embryos, it retained attachments to the epidermis, which were pulled taut (Fig. 2C, Fig. 3D). Optical sectioning of the amnioserosa in these embryos by confocal microscopy indicated that the tissue is no longer a monolayer of cells but rather a 'ball' of cells (data not shown), and we suspect that the amnioserosa cells that disappear from the anterior end of the dorsal hole are contained within this. The contraction of the amnioserosa was followed by 'bunched' closure of the epidermis around this tissue, as seen in embryos late in dorsal closure (Fig. 2B,D). The bunching of the epidermis causes

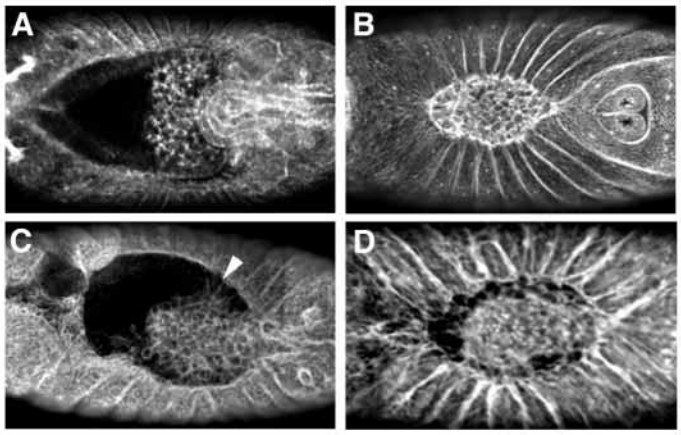

Fig. 2. Constitutively active *Drac1* transgene expression causes contraction of the amnioserosa. Embryos were stained with phalloidin to detect F-actin (A) or with the anti-phosphotyrosine antibody (B-D). (A) An early stage 14 *GAL4$^{332.3}$;UAS-Drac1V12* embryo showing dramatic contraction of amnioserosa into the rear half of dorsal hole. (B) A stage 15 *GAL4$^{332.3}$;UAS-Drac1V12* embryo late in dorsal closure showing bunched closure of the epidermis around contracted amnioserosa. Phosphotyrosine staining in the amnioserosa is very intense. (C) An early stage 14 *Hs-GAL4^{M-4}/UAS-Drac1V12* embryo showing an amnioserosa phenotype similar to that in A. Attachments of the amnioserosa to the epidermis can be seen (arrowhead). (D) Late stage 14 *Hs-GAL4^{M-4}/UAS-Drac1V12* embryo showing bunched closure of the epidermis around contracted amnioserosa.

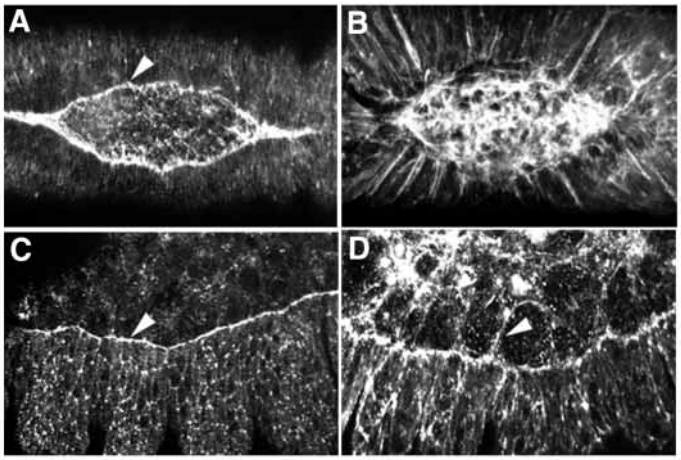

Fig. 3. Expression of constitutively active Drac1 causes an increase in F-actin and myosin staining in the amnioserosa. Embryos were stained with phalloidin to detect F-actin (A,B) or anti-nonmuscle myosin heavy chain antibody (C,D). (A) Dorsal view of the amnioserosa of a stage 15 wild-type embryo late in dorsal closure. There is heavy accumulation of F-actin along the leading edge (arrowhead). (B) Dorsal view of the amnioserosa of a stage 15 *Hs-GAL4^{M-4}/UAS-Drac1V12* embryo showing elevated F-actin staining as compared to the embryo in A. (C) Lateral view of a stage 14 wild-type embryo showing the boundary between the amnioserosa (top of micrograph) and the epidermis. There is accumulation of myosin along the leading edge (arrowhead). (D) Lateral view of a stage 14 *Hs-GAL4^{M-4}/UAS-Drac1V12* embryo showing the boundary between the amnioserosa (top of micrograph) and the epidermis. Compared with the embryo in C, there is increased myosin staining in the amnioserosa and parts of the epidermis. In this embryo the amnioserosa has begun to contract and pull away from the epidermis, although points of adhesion between the two tissues remain (arrowhead).

embryos to become bowed, with the head and tail pulled in towards each other.

The contraction of the amnioserosa following induction of Drac1V12 was caused by a striking change in cell shape. Cell shape change in the wild-type amnioserosa is accompanied by the accumulation of F-actin, myosin and phosphotyrosine. It is not unexpected, therefore, that the levels of these cytoskeletal components in the amnioserosa of Drac1V12-expressing embryos were often clearly higher than wild-type, even taking into account the fact that staining will look stronger in a compacted tissue (Fig. 2B, Fig. 3). As with our earlier studies on phosphotyrosine staining at the leading edge (Harden et al., 1996), we found through double-labeling studies of the amnioserosa in both Drac1V12-expressing and wild-type embryos that levels of phosphotyrosine staining correlated extremely well with the levels of F-actin and myosin (for example, compare Fig. 1C with 1D).

Expression of Drac1N17 in the amnioserosa impedes both amnioserosa morphogenesis and migration of the epidermis during dorsal closure

Having found that expression of Drac1V12 with the *GAL4$^{332.3}$* driver caused excessive contraction of the amnioserosa, we checked to see if amnioserosa-specific expression of a dominant-negative *Drac1* transgene, *UAS-Drac1N17*, would have any effect on this tissue. *GAL4$^{332.3}$* was used to drive Drac1N17 expression in embryos that were then subjected to cuticle preparation. 61% of such embryos failed to secrete cuticle; of the embryos that did secrete cuticle, 8% showed holes in the dorsal surface whereas the rest appeared to be wild-type (data not shown). We then expressed Drac1N17 with the *GAL4$^{332.3}$* driver in the presence of a *UAS-lacZ* reporter gene. These embryos were fixed and double-stained for phosphotyrosine and β-galactosidase in parallel with control embryos in which the *UAS-lacZ* reporter gene alone had been expressed with the *GAL4$^{332.3}$* driver. Examination of stained *GAL4$^{332.3}$;UAS-Drac1N17* embryos revealed impairment of both amnioserosa morphogenesis and migration of the epidermis. The amnioserosa of control embryos examined late in dorsal closure was in the process of narrowing from the original elliptical shape (Fig. 4A,C). The amnioserosa of similarly aged *GAL4$^{332.3}$;UAS-Drac1N17* embryos was still elliptical and was frequently ruptured by the hindgut (Fig. 4B,E). A lack of morphological change in the amnioserosa in *GAL4$^{332.3}$;UAS-Drac1N17* embryos appeared to impede the movement of the lateral epidermis, such that the dorsal hole was larger than in wild-type embryos of similar age (compare Fig. 4E with 4C and 4F with 4D). Occasionally, within a single embryo, there were patches of amnioserosa cells that had changed shape appropriately and patches of cells that had not (Fig. 5A). The amnioserosa cells towards the top of the dorsal hole in Fig. 5 have successfully elongated in the A-P direction and have a small apical surface area, whereas amnioserosa cells towards the bottom of the dorsal hole have retained a large surface area. Additionally, the epidermis has progressed further towards the dorsal midline on the side of the embryo, where

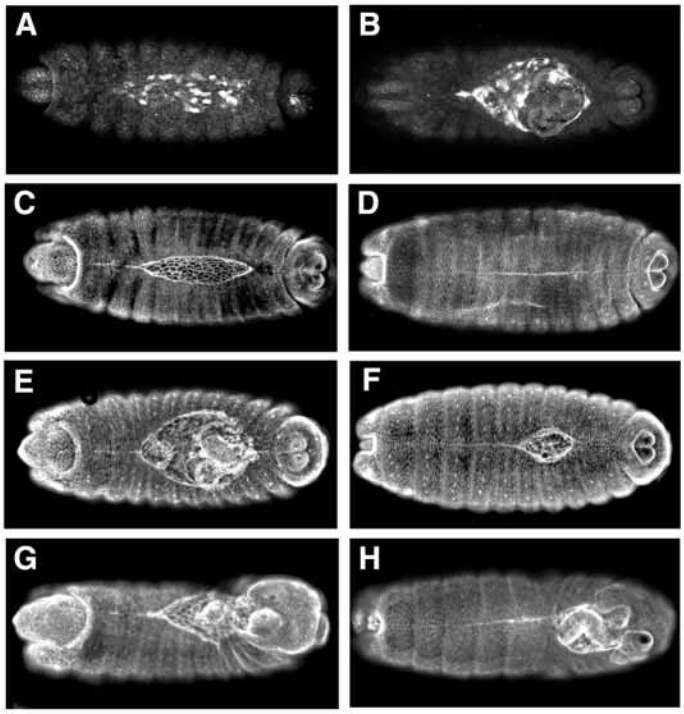

Fig. 4. Dorsal closure defects are seen following expression of dominant-negative Drac1 in the amnioserosa and in embryos homozygous for a hypomorphic allele of *crb*. Dorsal views of embryos stained with anti-β-galactosidase antibodies to label amnioserosa cells (A,B) or anti-phosphotyrosine antibodies to show morphology (C-H). (A,C) A double-stained stage 15 *UAS-lacZ*$^{1\text{-}71}$*;GAL4*$^{332.3}$ control embryo late in dorsal closure showing amnioserosa in transition from an elliptical to tubular morphology. (B,E) A double-stained *UAS-lacZ*$^{1\text{-}71}$*;GAL4*$^{332.3}$*;UAS-Drac1N17* embryo similar in age to that in A and C but in which the amnioserosa is still elliptical in shape. The hindgut has ruptured the amnioserosa in this embryo. (D) A wild-type embryo at the end of dorsal closure. (F) *UAS-lacZ*$^{1\text{-}71}$*;GAL4*$^{332.3}$*;UAS-Drac1N17* embryo similar in age to that in D showing persistence of a small dorsal hole. (G) A stage 15 embryo homozygous for *crb*^{S010409} showing germband retraction defect and impaired dorsal closure. There is robust phosphotyrosine staining along the leading edge. (H) An embryo homozygous for *crb*^{S010409}, similar in age to that in D, showing persistence of dorsal hole.

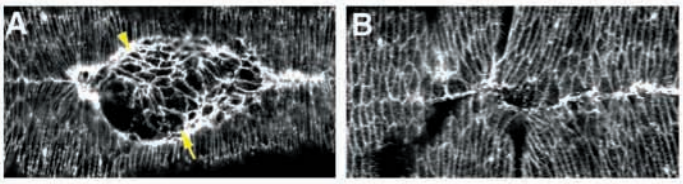

Fig. 5. (A) The dorsal hole of stage 15 *GAL4*$^{332.3}$*;UAS-Drac1N17* embryo late in dorsal closure showing strong leading edge phosphotyrosine staining. The amnioserosa cells near the top of the figure have changed shape properly (arrowhead), but the cells toward the bottom of the figure have not (arrow). The leading edge has progressed further toward the dorsal midline on the side of the embryo at the top of the figure. The amnioserosa cells and dorsal hole of this embryo can be compared with those of the wild-type embryo late in dorsal closure shown in Fig. 1G. (B) Dorsal surface of stage 16 *GAL4*$^{332.3}$*;UAS-Drac1N17* embryo showing successful but distorted dorsal closure.

the amnioserosa cells have changed shape effectively, than on the side where amnioserosa cell shape change is retarded. This finding supports the idea that lack of morphogenesis in the amnioserosa impedes movement of the epidermis. A consistent finding from the examination of many embryos was that there was an excellent correlation between the extent of amnioserosa morphogenesis and the degree of closure of the epidermis. The closure of the epidermis was clearly impaired in *GAL4*$^{332.3}$*;UAS-Drac1N17* individuals, but the leading edge cytoskeleton was not disrupted, and many embryos completed dorsal closure, although they frequently had mild defects in the dorsal surface (Fig. 5B).

Dominant-negative or constitutively active forms of the small GTPases Dcdc42, Ras1 or RhoA have little effect on amnioserosa morphogenesis

Many of the processes regulated by the Rac proteins, including dorsal closure, involve other small GTPases, and therefore we checked to see if other p21s had a role in amnioserosa morphogenesis. We expressed activated and dominant-negative versions of Dcdc42, Ras1 and human RhoA in the amnioserosa using the *GAL4*$^{332.3}$ driver and examined transgene-expressing embryos by cuticle preparation or staining for phosphotyrosine. None of these transgenes caused a significant frequency of dorsal cuticle defects, nor did any of them have substantial effects on amnioserosa morphology (data not shown). Expression of a dominant-negative *Drosophila* RhoA transgene with the *GAL4*$^{332.3}$ driver had no discernible effect on amnioserosa morphology (data not shown). These results suggest that Dcdc42, Ras1 and RhoA play little or no role in regulating amnioserosa morphogenesis.

Overexpression of Crumbs in the amnioserosa causes premature apical cell constriction

Are there any candidate participants in Drac1-mediated amnioserosa morphogenesis? To our knowledge only one gene has been previously described for which there is evidence for a role in this process. Crumbs (Crb) is a determinant of cell polarity expressed in epithelia, including the amnioserosa (Tepass et al., 1990). Overexpression of Crb in the amnioserosa using the *GAL4*$^{332.3}$ driver leads to contraction of the tissue into a dumbbell-shaped structure (Wodarz et al., 1995). We repeated this experiment using the transgene *UAS-crb*^{wt} and examined the resulting embryos with cuticle preparations or staining for phosphotyrosine and myosin. 34% of *GAL4*$^{332.3}$/*UAS-crb*^{wt} embryos failed to form cuticle, whereas 44% had dorsal holes (Fig. 6B) similar to those induced by Drac1N17 expression in the epidermis (Harden et al., 1995). In stage 13 *GAL4*$^{332.3}$/*UAS-crb*^{wt} embryos at the onset of dorsal closure, the end cells of the amnioserosa were apically constricted relative to the middle cells and showed elevated levels of phosphotyrosine and myosin staining (Fig. 7A,C). In wild-type embryos of this age, there is no such distinction between the end cells and the middle cells of the amnioserosa (Fig. 1A). The ends cells of the amnioserosa of stage 13 *GAL4*$^{332.3}$/*UAS-crb*^{wt} embryos looked similar to the end cells of late stage 14 wild-type embryos (compare Fig. 7C with Fig. 1E), suggesting that overexpressed Crb induced premature morphogenesis of this tissue. In older *GAL4*$^{332.3}$/*UAS-crb*^{wt}

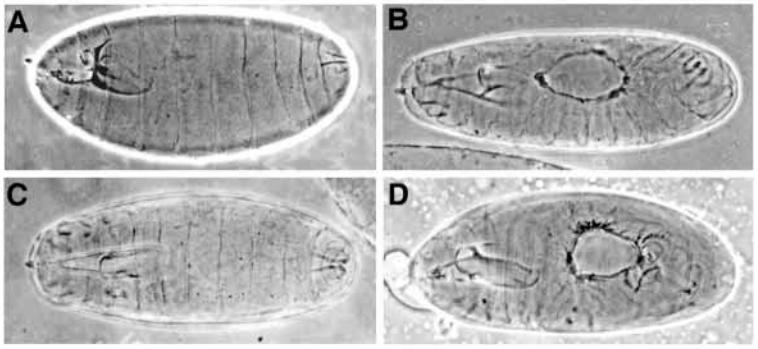

Fig. 6. Gains or losses of Crb function cause dorsal closure defects. (A) A dorsolateral view of wild-type embryonic cuticle. (B) A dorsolateral view of cuticle of *GAL4*$^{332.3}$/*UAS-crb*^{wt} embryo showing a dorsal hole resulting from overexpression of wild-type Crb in the amnioserosa. (C) Dorsal view of wild-type embryonic cuticle. (D) Dorsal view of cuticle of embryo homozygous for *crb*^{S010409}, showing dorsal hole and germband retraction defect.

embryos, a dumbbell-shaped amnioserosa was seen, as previously described (Fig. 7B). Distinct from Drac1V12-expressing embryos, the epidermis did not close up around the contracted amnioserosa in Crb-expressing embryos, and the gut and dorsal vessel were exposed in the large dorsal hole that remained. This failure of epidermal migration may have been caused by disruption of the leading edge cytoskeleton and a lack of epidermal cell elongation, as revealed by myosin/phosphotyrosine staining (Fig. 8A,B).

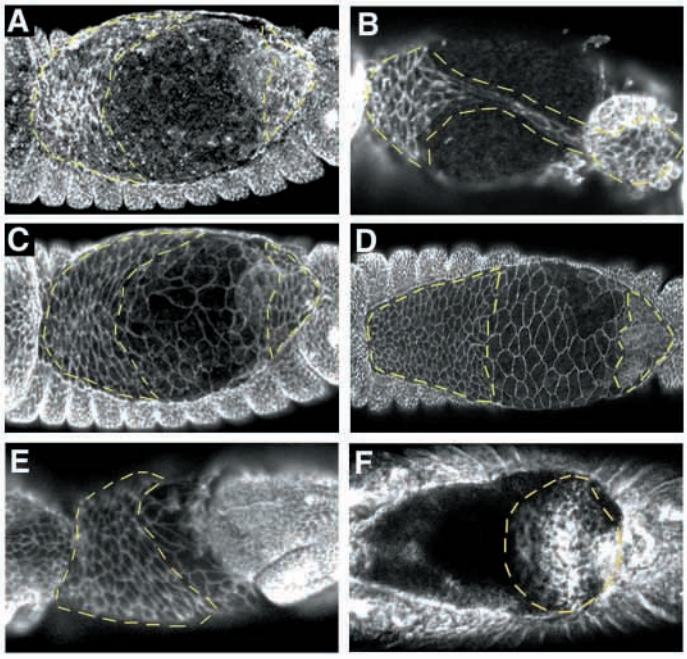

Fig. 7. Premature apical constriction of amnioserosa end cells is induced by overexpression of Crb or by expression of Drac1V12 in a *crb*^{S010409} mutant background. Dashed yellow lines demarcate clusters of apically constricted amnioserosa cells. Embryos were stained with anti-nonmuscle myosin heavy chain antibodies (A), anti-phosphotyrosine antibodies (B-E) or phalloidin (F). (A,C) A double-stained stage 13 *GAL4*$^{332.3}$/*UAS-crb*^{wt} embryo showing clusters of apically constricted cells with strong myosin staining at the anterior and posterior ends of the amnioserosa. (B) A stage 14 *GAL4*$^{332.3}$/*UAS-crb*^{wt} embryo showing dumbbell-shaped amnioserosa. (D) A stage 13 *GAL4*$^{332.3}$/*UAS-crb*^{wt}*;UAS-Drac1N17* embryo showing that impairment of Drac1 signaling does not block induction of premature cell constriction by overexpressed Crb. (E) A stage 13 *crb*^{S010409}, *UAS-Drac1V12*/*crb*^{S010409}, *Hs-GAL4*$^{M-4}$ embryo showing premature apical cell constriction at the anterior end of the amnioserosa. The posterior end of the amnioserosa in this embryo is obscured by the unretracted germband. (F) A stage 13 *Hs-GAL4*$^{M-4}$*/UAS-Drac1V12* embryo is included for comparison, showing contraction of amnioserosa into the posterior end of dorsal hole.

Characterization of a P-element-induced hypomorphic allele of *crb* reveals a requirement for Crb in dorsal closure and germband retraction

The fact that overexpression of Crb led to premature cell constriction in the amnioserosa prompted us to consider a role for Crb in amnioserosa morphogenesis and dorsal closure. It is not possible to evaluate dorsal closure in embryos homozygous for complete loss-of-function alleles of *crb*, as these embryos have disorganized epithelia and almost completely lack cuticle (Tepass and Knust, 1990). In a search of third chromosome P-element-induced lethals (Deak et al., 1997) for new participants in dorsal closure, we identified five insertions, *l(3)S010409*, *l(3)S025807*, *l(3)S025817*, *l(3)S025819*, and *l(3)S050920*, that all showed defects in dorsal closure and germband retraction and belonged to a single complementation group. Some of these insertions have been mapped by chromosome in situ hybridization to the vicinity of *crb* at 95F and are uncovered by the deficiency *Df(3R)crbS87-5* (Tepass and Knust, 1990). We confirmed that these five insertions are all alleles of *crb* through the finding that they all failed to complement the strong, amorphic allele *crb*^{2} (*crb*^{11A22}) (Tepass and Knust, 1990), and we have named these new *crb* alleles *crb*^{S010409} etc. We chose one of these alleles, *crb*^{S010409}, for

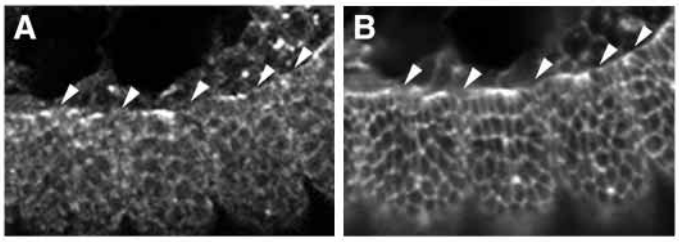

Fig. 8. Overexpression of Crb in the amnioserosa disrupts the leading edge cytoskeleton. Lateral view of leading edge of stage 14 *GAL4*$^{332.3}$/*UAS-crb*^{wt} embryo double-stained with anti-nonmuscle myosin heavy chain antibodies (A) and anti-phosphotyrosine antibodies (B). Phosphotyrosine and myosin are depleted in patches along the leading edge (arrowheads), and there is little elongation of epidermal cells. Compare the leading edge myosin staining in A with the wild-type leading edge myosin staining in Fig. 3C.

further analysis. Chromosome in situ hybridization on this line with a P-element probe shows an insertion at 95F3-10. To check that the lethality of this line is caused by P-element insertion, we mobilized the P-element by crossing in the transposase source P(Δ2-3) and established excision lines. Some of these excisions are homozygous viable, indicating that the lethality in the *crb*^{S010409} line is caused by the P-element insertion. We performed plasmid rescue on the P-element in the *crb*^{S010409} line and obtained a genomic sequence flanking the insertion. The P-element is inserted in an exon of the *crb* locus, 182 bp upstream of the predicted initiator methionine codon (Tepass et al., 1990). Cuticle preparations on the *crb*^{S010409} line revealed a high frequency of embryos with dorsal holes and germband retraction failures. 27% of embryos showed one or both of these defects, which was close to the expected frequency of 25% *crb*^{S010409} homozygotes. Most defective embryos showed both of these phenotypes together (Fig. 6D), although embryos could be found with either phenotype alone. Comparison of these results with cuticle data on other *crb* alleles indicated that *crb*^{S010409} is a hypomorphic allele, weaker than *crb*$^{S87\text{-}2}$ (Tepass and Knust, 1990). We stained homozygous *crb*^{S010409} embryos with anti-phosphotyrosine antibodies or with phalloidin to detect F-actin and examined them by confocal microscopy. *crb*^{S010409} embryos had an intact leading edge cytoskeleton that was comparable to wild-type but during dorsal closure consistently had a dorsal hole larger than wild-type embryos of similar age (Fig. 4G,H). The morphogenesis of the amnioserosa of *crb*^{S010409} embryos did not proceed correctly, and the sheet of amnioserosa cells was frequently ruptured by the hindgut. The epidermis was well organized in *crb*^{S010409} embryos and, except for the germband retraction defect, was wild-type in appearance. This is in contrast to embryos that are homozygous for amorphic alleles of *crb*, which show extensive disorganization and cell death in the epidermis (Tepass and Knust, 1990; Tepass et al., 1990). As with the cuticle preparations, we found stained *crb*^{S010409} embryos with defects in dorsal closure but without abnormalities in germband retraction (data not shown). This result indicated that the dorsal closure failures in *crb*^{S010409} embryos were not caused by germband retraction defects. Overall, the dorsal closure phenotypes of *crb*^{S010409} embryos and *GAL4*$^{332.3}$;*UAS-Drac1N17* embryos were similar in that both show impaired morphogenesis of the amnioserosa in the presence of an intact leading edge cytoskeleton. In comparison with *GAL4*$^{332.3}$;*UAS-Drac1N17* embryos, however, *crb*^{S010409} embryos tended to be less successful with their final degree of closure, and many late embryos were seen with a persistent dorsal hole and extrusion of the gut. This finding was consistent with the high frequency of dorsal holes seen in *crb*^{S010409} cuticle preparations.

Co-expression of Drac1N17 in the amnioserosa does not prevent induction of premature apical cell constriction by overexpressed Crb

The finding that both Drac1 and Crb participate in amnioserosa morphogenesis led us to look for interdependence of these proteins in regulating this process. To test for a requirement for Drac1 in induction of premature cell constriction by Crb, we created a line homozygous for both the *UAS-crb*^{wt} and *UAS-Drac1N17* transgenes and mated these flies to the *GAL4*$^{332.3}$ line. All the progeny from this cross will overexpress Crb in the amnioserosa and have impaired Drac1 signaling. Progeny from the cross were examined as embryos with anti-phosphotyrosine staining. As shown in Fig. 7D, impairment of Drac1 signaling did not prevent Crb from inducing premature apical cell constriction.

Contraction of the amnioserosa by Drac1V12 is weakened to a phenotype of premature apical cell constriction in a *crb*^{S010409} mutant background

To check for a requirement for Crb in Drac1V12-induced amnioserosa contraction, we expressed Drac1V12 by heat shock using the *Hs-GAL4*$^{M\text{-}4}$ driver in embryos homozygous for the *crb*^{S010409} allele and examined anti-phosphotyrosine-stained embryos by confocal microscopy. When Drac1V12 was expressed in the *crb*^{S010409} mutant background, it no longer generated contraction of the entire amnioserosa. Rather, embryos showed a premature apical constriction of cells at the anterior end of the tissue prior to the onset of dorsal closure in a manner very similar to that seen with Crb overexpression (Fig. 7E). The status of cells at the posterior end of the amnioserosa could not be assessed because of impaired germband retraction.

High frequencies of dorsal closure defects occur when *Drac1* and *crb* transgenes are expressed in the amnioserosa with the *GAL4*^{c381} driver

A problem with expression of the *Drac1* and *crb* transgenes with the *GAL4*$^{332.3}$ driver is that many embryos fail to form cuticle and their final degree of dorsal closure cannot be assessed. We believe that this failure to form cuticle is caused by expression of the driver in the epidermis following dorsal closure (Fig. 1H). This expression will lead to alterations in Drac1 or Crb function in the epidermis during cuticle secretion. Crb overexpression has previously been shown to have severe effects on the cuticle (Wodarz et al., 1995). During the course of this work we became aware of another amnioserosa driver, *GAL4*^{c381} (Manseau et al., 1997). We checked the expression of the *GAL4*^{c381} driver using a *UAS-LacZ* reporter gene and saw no β-galactosidase staining in the epidermis during or after dorsal closure (Fig. 9A-C). The *UAS-Drac1V12*, *UAS-Drac1N17* and *UAS-crb* transgenes were each crossed with the *GAL4*^{c381} driver and the progeny evaluated by cuticle preparation or as embryos stained with anti-phosphotyrosine. With the *GAL4*^{c381} driver, none of the transgenes caused failures of cuticle formation, and each transgene induced a very consistent phenotype, which occured in greater than 90% of embryos. Drac1V12-expressing embryos failed to undergo germband retraction but did complete dorsal closure, although their cuticles showed ‘puckers’ extending out from the dorsal surface (Fig. 9D). Drac1N17-expressing embryos did not complete dorsal closure and had a large dorsal hole in their cuticle extending form the middle of the dorsal surface to the rear of the embryo (Fig. 9E). Crb-overexpressing embryos also failed to close and had a cuticle phenotype very similar to that seen with Drac1N17. Examination of embryos stained for phosphotyrosine revealed that each transgene had similar effects on amnioserosa morphology to when expressed with the

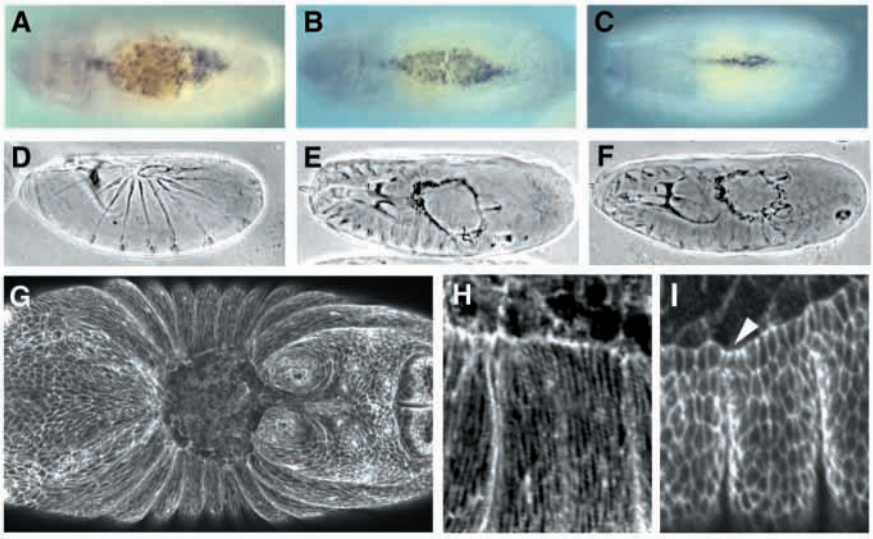

Fig. 9. Dorsal closure defects are seen following expression of *Drac1* and *crb* transgenes with the *GAL4^{c381}* driver. (A-C) Dorsal views of *UAS-lacZ$^{1-71}$;GAL4^{c381}* embryos stained with anti-β-galactosidase antibodies to show expression of the driver in the amnioserosa but not the epidermis during (A,B) and after dorsal closure (C). (D) Lateral view of cuticle of *UAS-Drac1V12;GAL4^{c381}* embryo showing germband retraction failure and puckers extending out from the dorsal surface. (E) Dorsolateral view of the cuticle of the *UAS-Drac1N17;GAL4^{c381}* embryo showing the large dorsal hole. (F) Dorsolateral view of cuticle of *UAS-crb^{wt};GAL4^{c381}* embryo showing large dorsal hole. (G) Dorsal view of stage 14 *UAS-Drac1V12;GAL4^{c381}* embryo stained with anti-phosphotyrosine antibodies to show contraction of the amnioserosa, germband retraction failure and bunching of the epidermis. (H,I) Lateral views of the leading edge in embryos stained with anti-phosphotyrosine antibodies. (H) Stage 14 *UAS-Drac1V12;GAL4^{c381}* embryo showing robust accumulation of phosphotyrosine at the leading edge and extensive epidermal cell elongation. (I) A stage 14 *UAS-crb^{wt};GAL4^{c381}* embryo showing loss of leading edge phosphotyrosine nodes and lack of epidermal cell elongation. The arrowhead marks a portion of the leading edge where phosphotyrosine nodes are intact.

GAL4$^{332.3}$ driver (Fig. 9G) (data not shown), but two major differences were noted in the final morphology of embryos. Consistent with cuticle preparations, *UAS-Drac1V12;GAL4^{c381}* embryos showed a high frequency of impaired germband retractions (Fig. 9G), and the epidermis failed to migrate over the amnioserosa in *UAS-Drac1N17;GAL4^{c381}* embryos (data not shown).

As described earlier, the behavior of the leading edge and dorsal epidermis following expression of Drac1V12 with the *GAL4$^{332.3}$* driver was distinct from that seen following overexpression of Crb in the same manner. These distinct epidermal phenotypes were also seen when these transgenes were expressed with the *GAL4^{c381}* driver. *UAS-Drac1V12;GAL4^{c381}* embryos showed bunched closure of the epidermis around the amnioserosa (Fig. 9G). This bunched closure made it difficult to evaluate the leading edge cytoskeleton, but those portions of the leading edge that could be assessed had an accumulation of phosphotyrosine comparable to wild-type embryos, and there was pronounced elongation of the epidermal cells along the dorsoventral axis (Fig. 9H). *UAS-crb^{wt};GAL4^{c381}* embryos showed disruption of leading edge phosphotyrosine, and there was little elongation of the epidermal cells (Fig. 9I).

Discussion

Drac1 regulates amnioserosa morphogenesis during dorsal closure

The cell shape changes that propel the epidermis over the amnioserosa to cause dorsal closure are believed to be mediated, at least in part, by an actomyosin contractile apparatus and associated phosphotyrosine-rich structures that are present along the leading edge of the advancing epidermis (Harden et al., 1996; Harden et al., 1999; Young et al., 1993). Drac1N17 can cause loss of these leading edge components during dorsal closure and may be regulating their accumulation at the leading edge (Harden et al., 1995; Harden et al., 1996; Harden et al., 1999). In this study, we have identified a role for Drac1 in another epithelial morphogenesis contributing to dorsal closure: the transformation of the amnioserosa from a squamous epithelium to a tubular structure of columnar cells. Dorsal closure is an excellent system for the study of the regulation of epithelial morphogenesis by small GTPases. Work in this area has largely focused on events at the leading edge and in the lateral epidermis, but our results indicate that active regulation of amnioserosa morphology is a component of normal dorsal closure. We previously showed that heat shock induction of Drac1N17 results in a decrease in F-actin staining in the amnioserosa (Harden et al., 1995), and we now demonstrate that expression of Drac1N17 in the amnioserosa slows morphogenesis of this tissue and it remains as a squamous epithelium for a longer period than in wild-type embryos. In Drac1N17-expressing embryos, where amnioserosa morphogenesis is lagging, the movement of the epidermis is also slowed, and the embryos have a larger dorsal hole than wild-type embryos of similar age. We believe that the impaired movement of the epidermis in such embryos is caused by lack of morphogenesis in the amnioserosa. These results are strong evidence that active cell shape changes in the amnioserosa are required for normal dorsal closure. Our examination of wild-type embryos has shown that this cell shape change in the amnioserosa begins with apical constriction of cells at the anterior and posterior ends of the amnioserosa. These cells have elevated levels of myosin, F-actin and phosphotyrosine, suggesting that an apically localized actomyosin contractile apparatus is driving their constriction. Early in dorsal closure, the middle cells in between the two clusters of apically constricted cells do not show elevated levels of F-actin or myosin but do change shape, losing their original elongation perpendicular to the A-P axis of the embryo. The middle cells may be stretching passively, in response to tension from the cell constrictions occurring at both ends of the amnioserosa. By the end of dorsal closure, the middle cells are both elongated along the A-P axis and apically

constricted, and it is conceivable that late in dorsal closure they undergo an active cell shape change as their neighbors did earlier.

Excessive Drac1 activity induces a dramatic contraction of the amnioserosa such that it shrinks to occupy less than half the dorsal hole, and this is accompanied by elevated levels of myosin, F-actin, and phosphotyrosine in this tissue. Our interpretation is that Drac1V12 is driving premature and excessive amnioserosa cell constriction through its effects on the cytoskeleton. We propose that Drac1 participates in amnioserosa morphogenesis by driving the assembly of an apical actomyosin contractile apparatus that constricts the amnioserosa cells, first at the ends of the tissue and possibly later in the middle. Contraction of an apical actomyosin belt has been implicated in diverse types of epithelial morphogenesis (Fristrom, 1988; von Kalm et al., 1995) including *Drosophila* gastrulation (Leptin, 1999), which shows some similarity to amnioserosa morphogenesis in that both processes involve apical construction of a monolayer of cells that then invaginates.

During the course of this work, Kiehart et al. (Kiehart et al., 2000) reported the results of a study that used cell ablation to address the contributions of the epidermis and amnioserosa to dorsal closure. They demonstrated that the amnioserosa is under tension, as ablation of cells in the amnioserosa causes the tissue to recoil away from the wound site, and the leading edge is pushed back away from the dorsal midline. They conclude, as we do, that there is active cell shape change in the amnioserosa that contributes to dorsal closure, rather than the tissue being simply compressed by the movement of the leading edge. Their finding that the recoiling of the amnioserosa after wounding pushes back the leading edge is consistent with our result that impairing amnioserosa morphogenesis through Drac1N17 expression hinders leading edge migration.

Crumbs, a determinant of epithelial apical-basal polarity, participates with Drac1 in establishing apical cell constriction in the amnioserosa

Overexpression of Crb in the amnioserosa leads to contraction of the tissue and failure of dorsal closure (Wodarz et al., 1995). We examined this phenotype in more detail and found that excessive Crb activity induces a premature constriction of cells at the ends of the amnioserosa. We have identified five P-element-induced *crb* alleles that are hypomorphic mutations, causing defects in dorsal closure and germband retraction. We have characterized one of these *crb* mutations, $crb^{S010409}$, in detail. Embryos homozygous for $crb^{S010409}$ show a dorsal closure defect similar to that seen with expression of Drac1N17 in the amnioserosa: amnioserosa morphogenesis is impaired, but the leading edge cytoskeleton is intact. In contrast to amorphic *crb* alleles, the epidermis is not disorganized in $crb^{S010409}$ mutants and it secretes cuticle. Amnioserosa morphogenesis and germband retraction may be particularly sensitive to the level of Crb activity. Our interpretation is that Crb activity in the amnioserosa is required for amnioserosa morphogenesis, although we cannot exclude the possibility that loss of Crb activity elsewhere in the embryo is affecting this process. Crb is a transmembrane domain protein with extracellular EGF-like and laminin A G-domain-like repeats that plays a key role in determining apical-basal polarity in epithelial cells (Tepass et al., 1990; Wodarz et al., 1995).

Drac1 may act through Crb in regulating the cytoskeleton, as the Drac1V12-induced phenotype of excessive contraction of the amnioserosa is weakened in a $crb^{S010409}$ mutant background. This weaker Drac1V12 phenotype of premature constriction of the end cells of the amnioserosa is very similar to that caused by Crb overexpression. There may be sufficient Crb in the $crb^{S010409}$ mutant embryos for Drac1V12 to be able to prematurely constrict cells at the ends of the amnioserosa but not to excessively contract the tissue. Crb overexpression does not appear to require Drac1 to cause premature constriction of amnioserosa cells, as it can achieve this in the presence of Drac1N17. The excessive contraction of the amnioserosa caused by Drac1V12 expression in embryos with wild-type Crb activity, and the dumbbell-shaped amnioserosa induced by Crb overexpression, could both result from excessive constriction of amnioserosa cells to produce a tissue that only occupies a fraction of the dorsal hole. Such excessive constriction may be driven by ectopic accumulation of a normally apically localized actomyosin contractile apparatus. A role for Crb in defining the location of the actomyosin contractile apparatus is consistent with the idea that Crb defines the range of the apical membrane cytoskeleton (Grawe et al., 1996; Wodarz et al., 1993; Wodarz et al., 1995). The actin-crosslinking protein $\beta_{Heavy}(\beta_H)$-spectrin normally has an apicolateral distribution, but upon overexpression of Crb is also found at the basolateral membrane, indicating a redistribution of the membrane cytoskeleton (Thomas and Kiehart, 1994; Wodarz et al., 1995). β_H-spectrin is required for apical constriction of follicle cells during *Drosophila* oogenesis and may participate in organization of an actomyosin contractile apparatus (Zarnescu and Thomas, 1999). It is conceivable that the ectopic localization of (β_H)-spectrin domain following Crb overexpression could be accompanied by an ectopic accumulation of F-actin and myosin. Future goals in studying Drac1-Crb function in amnioserosa morphogenesis will include addressing the nature of the interaction between the two proteins and defining which portion(s) of the Crb protein are required. The short cytoplasmic domain of Crb appears sufficient to execute all Crb functions studied to date (Klebes and Knust, 2000). No definitive role has been found for the large extracellular domain, although there is evidence that the *Drosophila* and human Crb proteins have non-cell-autonomous functions (Rashbass and Skaer, 2000).

Although Drac1 and Crb both generate premature contraction of the amnioserosa when their activity is experimentally upregulated in this tissue, their phenotypic effects are not identical. Drac1V12 expression drives constriction of all amnioserosa cells early in closure, whereas, at the same stage, Crb overexpression only promotes constriction of the end cells. A plausible explanation for this is that constriction of the middle cells requires Drac1 to activate Crb-independent processes and that Crb function is necessary but not sufficient for middle cell constriction. Crb overexpression in the amnioserosa causes disruption of the leading edge cytoskeleton and a failure of cell shape change in the epidermis, suggesting that a signal from the amnioserosa required for dorsal closure is disrupted. That communication between the amnioserosa and the epidermis is a component of

dorsal closure is demonstrated by two recent reports. Downregulation of JNK signaling in the amnioserosa is required for phosphotyrosine accumulation at the leading edge and dorsalward migration of the epidermis, and leading edge cells are specified wherever an interface of amnioserosa and dorsal epidermis occurs (Reed et al., 2001; Stronach and Perrimon, 2001). Drac1V12 expression in the amnioserosa does not disrupt the leading edge cytoskeleton or prevent closure of the epidermis, and this result suggests that Drac1V12 cannot activate a function of Crb that influences communication between the amnioserosa and the epidermis.

Amnioserosa morphogenesis as a system for studying small GTPase regulation of epithelial morphology

Evidence is emerging that Drac1 is a key regulator of epithelial cell morphology in *Drosophila*. *Drac1* transgene expression can affect head involution, germband retraction, border cell migration in the oocyte and, in the wing disc, adherens junction actin and planar polarity (Eaton et al., 1995; Eaton et al., 1996; Harden et al., 1995; Murphy and Montell, 1996). The present study and earlier work (Harden et al., 1995; Harden et al., 1999) indicate that Drac1 is essential for the morphogenesis of both the epidermis and the amnioserosa during dorsal closure. There is an enormous body of evidence showing that different small GTPases act in concert with each other to regulate diverse cellular events. However, none of the other small GTPases we have tested appear to have a major role in amnioserosa morphogenesis, as activated and dominant-negative versions of Dcdc42, RhoA and Ras1 do not substantially affect amnioserosa morphology. Thus, unlike the morphogenesis of the epidermis during dorsal closure, which is regulated by Drac1 and all these other small GTPases, only Drac1 may be required for amnioserosa morphogenesis. The finding that, of the p21s tested, only Drac1 has a substantial effect on amnioserosa morphology suggests that there may be a Drac1-specific control of amnioserosa morphogenesis and that this process may be a good system for identifying and characterizing Rac-specific effectors. Although Drac1, Dcdc42, RhoA and Ras1 all participate in dorsal closure (Harden et al., 1999), there is no evidence for the Cdc42→Rac→RhoA hierarchy of p21 activity demonstrated in some cultured mammalian cells (Allen et al., 1997; Nobes and Hall, 1995). Our results on p21 regulation of cell shape in the amnioserosa are a further indication that Rho subfamily hierarchies may often not be utilized in developmental cell shape change.

Our findings on Drac1-Crb regulation of amnioserosa morphology, taken together with other recent results indicating an active role for the amnioserosa in dorsal closure (Kiehart et al., 2000; Reed et al., 2001; Stronach and Perrimon, 2001), have important implications for the use of dorsal closure as a system for studying signal transduction and epithelial morphogenesis. It is now clear that one must consider the potential effects on the amnioserosa when interpreting the phenotype of any mutant with a dorsal closure defect. Genes acting solely in the amnioserosa may contribute to epidermal migration and, in this respect, we have identified a number of P[lacZ] insertion lines with dorsal closure defects that show high levels of β-galactosidase staining in the amnioserosa (N.H. and M.R., unpublished). The characterization of such genes should yield further insight into the control of amnioserosa morphogenesis and the role of the amnioserosa in dorsal closure.

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