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Drosophila puckered regulates Fos/Jun levels during follicle cell morphogenesis

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SUMMARY

***puckered* (*puc*) encodes a VH1-like phosphatase that down-regulates Jun kinase (JNK) activity during dorsal closure of the *Drosophila* embryo. We report a role for *puc* in follicle cell morphogenesis during oogenesis. *puc* mRNA accumulates preferentially in the centripetally migrating follicle cells and cells of the elongating dorsal appendages. Proper levels of Puc activity in the follicle cells are critical for the production of a normal egg: either reduced or increased Puc activity result in incomplete nurse cell dumping and aberrant dorsal appendages. Phenotypes associated with *puc* mutant follicle cells include altered DE-cadherin expression in the follicle cells and a failure of nurse cell dumping to coordinate with dorsal appendage elongation, leading to the formation of cup-shaped egg chambers. The JNK pathway target *A251-lacZ* showed cell-type-specific differences in its regulation by *puc* and by the small GTPase DRac1. *puc* mutant cells displayed region-specific ectopic expression of the *A251-lacZ* enhancer trap whereas overexpression of a transgene encoding Puc was sufficient to suppress *lacZ* expression in a cell autonomous fashion. Strikingly, decreased or increased *puc* function leads to a corresponding increase or decrease, respectively, of Fos and Jun protein levels. Taken together, these data indicate that *puc* modulates gene expression responses by antagonizing a Rho GTPase signal transduction pathway that stabilizes the AP-1 transcription factor. Consistent with this, overexpression of a dominant negative DRac1 resulted in lower levels of Fos/Jun.**

Key words: *Drosophila* oogenesis, epithelial migrations, JNK signaling, dual specificity phosphatase, small GTPase

INTRODUCTION

During organogenesis, tightly connected sheets of cells, or epithelia, undergo coordinated cell shape changes and cell migrations to create the three-dimensional architecture of a tissue. A striking example of this process occurs during oogenesis in *Drosophila*, when domains of cells within the somatic follicular epithelium undergo distinctive migrations and changes in shape. Here we show that the *puckered* (*puc*) gene is required to fine-tune follicle cell rearrangements that create the structures of the anterior eggshell. *puc* encodes a dual specificity phosphatase that down-regulates Jun kinase (JNK) signaling during epithelial morphogenesis (Martín-Blanco et al., 1998; Ring and Martinez-Arias, 1993; Glise and Noselli, 1997; Agnes et al., 1999; Martín-Blanco et al., 2000). In addition to its roles in epithelial rearrangements during embryogenesis and pupariation, our data on oogenesis indicate that *puc* has a general role in modulating signal transduction during epithelial sheet movements.

The *Drosophila* egg is formed from coordinated development of somatic follicle cells (FC) and germ cells (Spradling, 1993). The follicle cells form an epithelial sheet around a cyst of germ cells, the oocyte and 15 nurse cells, which then develops as an egg chamber unit. Late in oogenesis, specific groups of follicle cells become distinct. During stage 9, most follicle cells migrate posteriorly to cover the oocyte surface in a columnar epithelium (Fig. 1A,B). Approximately 50 cells – the nurse cell FC (NCFC; Fig. 1C) – flatten to form a squamous domain over the nurse cells. Subsequently, subgroups of the columnar FC change shape and migrate (Fig. 1D-I). Beginning in stage 10B, the centripetally migrating FC (CMFC; Fig. 1C) move inward between the oocyte and nurse cells (Fig. 1C-I). These cells will create anterior eggshell structures such as the operculum (Fig. 1I). The main body FC (MBFC; Fig. 1C) stretch during stage 11 (Fig. 1E and not shown), as the oocyte rapidly enlarges with the transfer of the nurse cell contents, a process called nurse cell dumping (1D,E; reviewed by Robinson and Cooley, 1997). During stages 12 through 14, two dorsal anterior groups of FC migrate anteriorly to create the dorsal appendages (Fig. 1F-I). The posterior pole

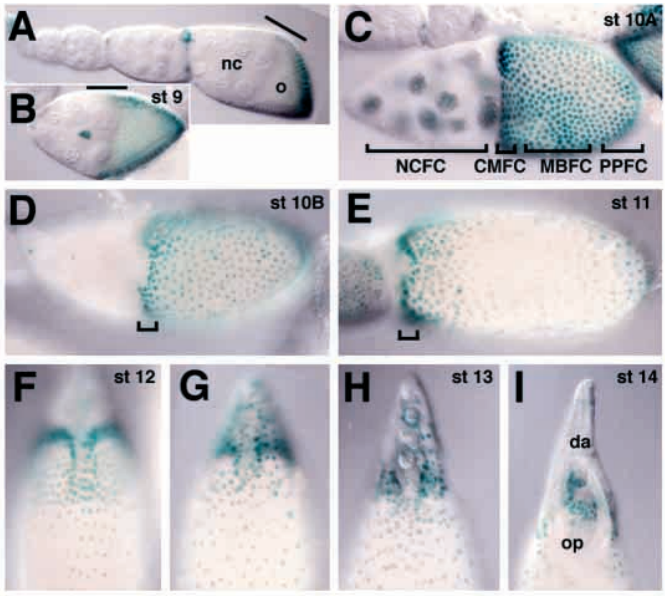

Fig. 1. Enhancer trap B42.1 marks follicle cell migrations during late oogenesis (see text for discussion): nc, nurse cells; o, oocyte; NCFC, nurse cell follicle cells; CMFC, centripetal migrating FC; MBFC, main body FC; PPFC, posterior pole FC; da, dorsal appendages; op, operculum. Anterior is left for A-E; up, for F-I.

FC (PPFC; Fig. 1C) include cells that produce the aeropyle of the eggshell. The FC that will create a specific eggshell structure are fated earlier in oogenesis, as revealed by specific patterns of gene expression (reviewed by Dobens and Raftery, 2000).

The Rho family of small GTPases plays a central role in cell morphology and actin cytoskeletal organization in tissue culture cells (Hall, 1998), but little is known about their role in FC morphogenesis. Transcriptional responses to Rac and Cdc42 activation can be mediated by the Jun kinase (JNK) pathway in mammalian systems (reviewed by Canman and Kastan, 1996; van Aelst and D'Souza-Schorey, 1997). In *Drosophila*, migration of an embryonic epithelium during dorsal closure requires JNK pathway signaling (Glise and Noselli, 1997; Hou et al., 1997; Riesgo-Escovar and Hafen, 1997a; Riesgo-Escovar and Hafen, 1997b; Zeitlinger et al., 1997; reviewed by Noselli and Agnes, 1999). Components of the pathway include *basket* (*bsk*), a Jun kinase, and *hemipterous* (*hep*), a Jun kinase kinase (Glise et al., 1995). Key mediators of the pathway are DFos and DJun (the AP-1 transcription factor; Peverali et al., 1996; Zeitlinger et al., 1997). Target genes include *decapentaplegic* (*dpp*) and *A251-lacZ*, an intron II enhancer trap in *puc*. Dpp, a member of the TGFβ family, is expressed at the leading edge of the dorsal epithelium. Dpp signaling is required for the trailing cells to elongate (reviewed by Martín-Blanco, 1997; Noselli, 1998). *puc* plays a critical role in restricting JNK activity in time and space to coordinate cell shape changes that occur during dorsal closure (Martín-Blanco et al., 1998).

We demonstrate that *puc* is required to coordinate follicle cell morphogenesis during nurse cell dumping. *puc* mRNA is expressed throughout the FC, but is strongest in the centripetally migrating FC and extending dorsal appendages. Levels of Puc activity are critical: anterior FC morphogenesis loses coordination with nurse cell dumping either when Puc levels are decreased or when they are increased. Similar to regulatory pathways in embryonic dorsal closure, *puc* functions in the FC to down-regulate gene expression activated by small GTPases. At late stages, FC showed regional differences in responsiveness to Puc and to small GTPases. In all FC domains, the levels of the pathway mediators DFos and DJun are sensitive to activity of the pathway. These results indicate that Puc is a key modulator of signal transduction in FC morphogenesis, and suggest AP-1 levels mediate the region-specific effects of *puc* activity.

MATERIALS AND METHODS

Drosophila strains

puckered alleles used were puc^{A251}, puc^{B48}, puc^{E69} and puc^{0694} (Martín-Blanco et al., 1998; The FlyBase Consortium, 1999). Transgenes have been described previously: B42.1 (Grossniklaus et al., 1989), *Actin>CD2>Gal4* (Pignoni and Zipursky, 1997), UAS-GFP (Duffy et al., 1998), hs-GAL4 (from Norbert Perrimon), UAS-Puc (Martín-Blanco et al., 1998); UAS-DRac1V12, UAS-DRac1N17 (Luo et al., 1994).

Generation of mitotic clones

Female genotypes were: (1) *w^{1118};[w+mC=Ubi-GFP],P[ry^+; hs-neo; FRT]82B P[ry^+; w^+]90E, (2) w^{1118} hsFLP; P[ry^+; hs-neo; FRT]82B P[mini-w^+;hsNM]88C (82-NM)*, and (3) *w; P[ry+t7.2=neoFRT]82B P[w+mC=ovoD1-18]3R1 P[w+mC=ovoD1-18]3R2/st1 betaTub85DD ss1 es/TM3, Sb^1* (Chou et al., 1993; Xu and Rubin 1993). FC clones were generated using *hs-FLP1; FRT82B puc^{A251} /FRT82B hs-NMYC or hs-FLP1; FRT82B puc^{A251}/FRT82B,Ubi-GFP* to generate recombination as previously described (Xu and Rubin, 1993). Females were subjected to 60 minutes at 37°C, 3-4 days prior to dissection. *puc* mutant germline clones were induced in *hs-FLP1; FRT82B puc^{A251}/FRT82B ovo^{D1}* females during the first or second larval instar as described previously (Chou et al., 1993).

Generation of flip-out clones

Flip-out FC clones were created using the flip-out GAL4 transgene with female genotypes: (1) Fig. 7C and 7F, *hsFLP1/actin5C>CD2>GAL4; UAS-DRac1V12/UAS-GFP puc^{A251}*, (2) Figs 7B and 8I, *hsFLP1/actin5C>CD2>GAL4; UAS-DRac1N17/UAS-GFP puc^{A251}*; (3) Fig. 7D, *hsFLP1/actin5C>CD2>GAL4; UAS-DCdc42V12/UAS-GFP puc^{A251}*, (4) Figs 7E, *hsFLP1/actin5C>CD2>GAL4; UAS-DCdc42N17/UAS-GFP puc^{A251}*; (5) Fig. 4I, *hsFLP1/actin5C>CD2>GAL4; UAS-Puc*; (6) Figs 6E and 8H, *hsFLP1/actin5C>CD2>GAL4; UAS-Puc/UAS-GFP puc^{A251}*. 2 days before dissection, clones were induced with a 30- to 60-minute heat shock at 37°C. Flies carrying UAS-DRac1V12 were subjected to only 20-30 minutes at 37°C; longer heat shock treatments were lethal. Clones were detected with a sensitive UAS-green fluorescent protein (GFP) reporter (Duffy et al., 1998).

In situ hybridization, histochemical and immunofluorescence staining

Full-length sense or antisense *puc* digoxigenin-labelled RNA probes were used for whole-mount in situ hybridization (Wasserman and Freeman, 1998).

Ovaries were dissected, fixed and stained for β-galactosidase activity or immunostained as previously described (Dobens et al., 1997). We used mouse anti-Broad Complex (1:200; Emery et al., 1994), mouse anti-Myc (1:100; Oncogene Science; Uniondale, NY), rabbit anti-α-spectrin (1:1000; #354 from Daniel Branton; Pesacreta et al., 1989), rat anti-DE-cadherin (Oda et al., 1994), rabbit anti-β-

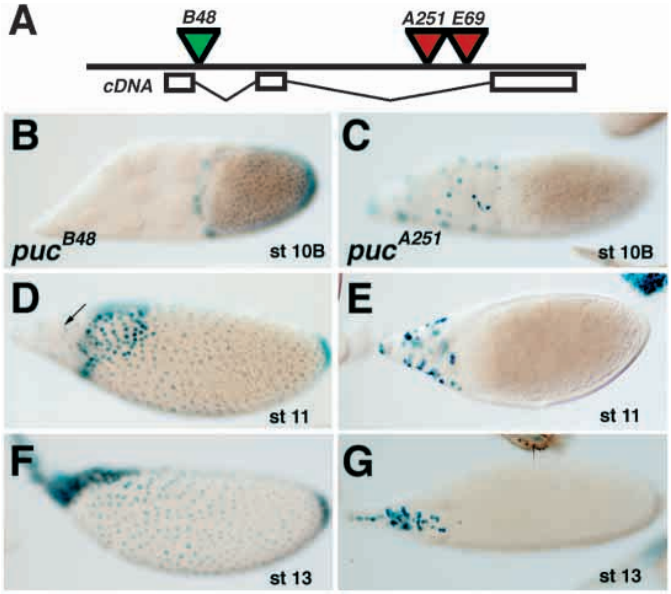

Fig. 2. Follicle cell expression of *puc* enhancer traps. (A) Schematic of the *puc* gene with enhancer trap insertions indicated (Martín-Blanco et al., 1998). (B,D,F) Expression of puc^{B48} from stage 10B to stage 13 occurs throughout the follicle cells: higher levels are detected in the centripetal migrating FC, elongating dorsal appendages (F and Fig. 3G) and in the posterior pole FC. Lower levels occur in the main body FC with very low levels in the nurse cell FC (arrow, D). (C,E,G) Expression of puc^{A251} is restricted to the nurse cell FC, stretched thin over the nurse cell complex, from stages 10-13. Note that nurse cell FC remain associated with nurse cell remnants at the extreme anterior (F, dorsal view). Late expression in the posterior pole FC is shown in Fig. 4D.

galactosidase (1:10,000 following preadsorption; Cappell, West Chester, PA), rabbit anti-DFos (1:200; Zeitlinger et al., 1997) and rabbit anti-DJun (1:2000; Peverali et al., 1996). Secondary antibodies were FITC-conjugated goat anti-mouse, goat anti-rabbit Alexa 580 (Molecular Probes) and Cy5-conjugated goat anti-rabbit and rat (1:200; Jackson ImmunoResearch; West Grove, PA). Confocal images were collected on a Leica TCS-NT microscope and figures prepared using Photoshop 4.0.

RESULTS

puckered mRNA accumulates in the migrating follicle cell subgroups

In our studies of anterior FC patterning, we identified an enhancer trap insertion at 84E, *l(3)A251.1F3* (Grossniklaus et al., 1989), with *lacZ* expression specifically in the nurse cell FC. Recombination mapping, complementation analysis, and reversion by P element excision indicated that the associated P element caused a mutation in the *puc* gene (data not shown). Molecular analysis indicated that the P element was inserted in the second intron of the *puc* transcription unit (Martín-Blanco et al., 1998). We generated a recombinant chromosome bearing this mutation, puc^{A251}, and the nearby 82B FRT insertion.

To further characterize *puc* expression in the follicle cells, we examined the expression of other enhancer traps in the gene. Surprisingly, two classes of *lacZ* expression patterns were revealed by β-galactosidase activity staining. Like puc^{A251}, enhancer traps puc^{E69} and puc^{0694} showed *lacZ* expression specifically in the nurse cell FC beginning at stage 10B. Staining persisted in the nurse cell FC throughout nurse cell dumping (Fig. 2C,E,G and data not shown). At stage 14 new expression was seen in the posterior pole FC (shown in Fig. 4D). Members of this class of *puc* enhancer traps also express *lacZ* in the leading edge cells during embryonic dorsal closure (Martín-Blanco et al., 1998; and data not shown)

A distinct pattern of β-galactosidase activity was observed for the enhancer trap allele puc^{B48} (Fig. 2B,D,F). Expression of *lacZ* was only weakly detected in the nurse cell FC (Fig. 2D, arrow), as well as in the mainbody FC. Strong *lacZ* expression was observed in the centripetally migrating FC and posterior pole FC at stages 10-11 (Fig. 2B,D); at stages 12-14, expression was restricted to elongating dorsal appendage cells (Figs 2F and 3G). This enhancer trap also showed a distinct expression pattern during embryonic dorsal closure in the amnioserosa (Martín-Blanco et al., 1998; E.M.-B. and A.M.A., unpublished data). The different *lacZ* expression patterns for the two classes of *puc* enhancer traps correlated with their distinct P element integration sites. puc^{A251} and puc^{E69} are each inserted in intron 2 (Fig. 2A, red); puc^{B48} is inserted in intron 1 (Fig. 2A, green; Martín-Blanco et al., 1998).

To determine the sites of *puc* mRNA accumulation, we performed in situ hybridization using antisense *puc* probe (Fig.

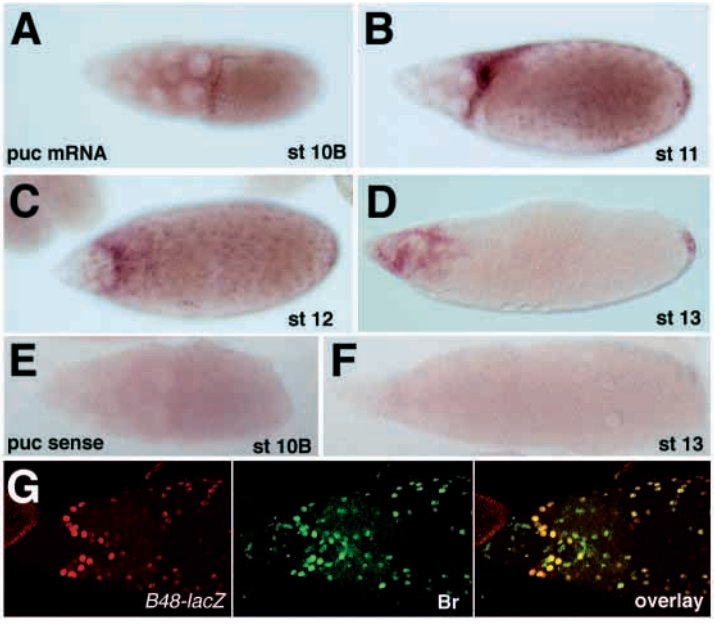

Fig. 3. *puc* RNA expression parallels expression of puc^{B48}. (A,B) *puc* mRNA accumulates throughout the follicle cells and nurse cells from stage 10 to 11 with higher levels in the centripetal migrating FC and at the posterior pole FC (B). (C,D) At stages 12 and 13, *puc* mRNA accumulates in cells at the tip of the extending dorsal appendages and at the posterior pole (D). (E,F) No strong staining is seen at any stage using a labeled probe specific to the *puc* sense strand. (G) Late expression of puc^{B48} (red) at leading edge of migrations. These cells express BR-C (green; Emery et al., 1994).

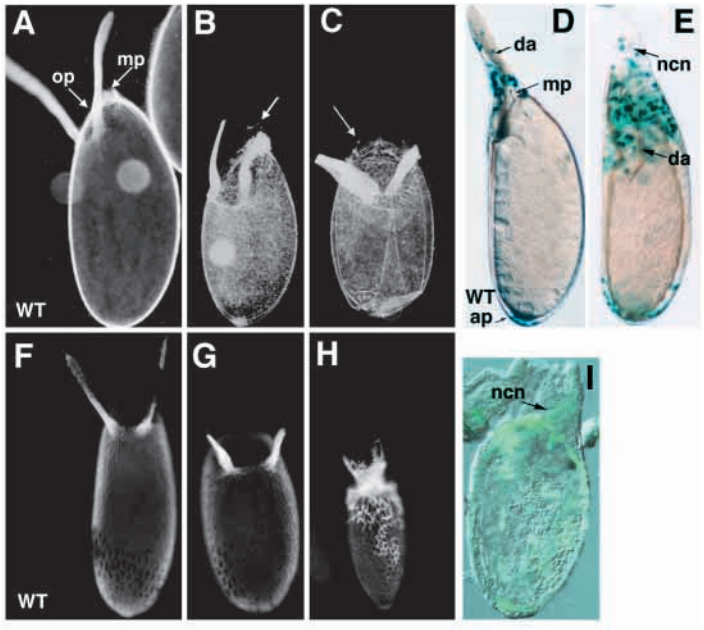

Fig. 4. *puc* levels are critical for proper eggshell patterning. (A,F) Wild type eggshell. Anterior is up in all panels. Anterior specializations include the micropyle (mp) and operculum (op). (B,C) 3 days following induction of FLP-recombinase in *FRT-puc*^{A251} females, 5% of all eggs (*n*=530) were small with shortened dorsal appendages. Two examples shown; operculum is open (arrows in B,C). (D) Expression of *A251-lacZ* in *puc*^{A251}/+ at stage 14 occurs in the nurse cell FC located between the extending dorsal appendages (compare Fig. 2G) and in the posterior-most follicle cells overlying the aeropyle (ap). (E) Egg chamber from *FRT-puc*^{A251} females 3 days after induction of FLP recombinase. Presence of a *puc* clone is inferred from the strength of *A251-lacZ* expression (compare Fig. 6E,F and see text). Given the presence of dorsal appendages and the extent of chorion formation, this egg chamber is nearly mature, yet nurse cell dumping was not complete. The egg is small and nurse cell nuclei (ncn) are still present beneath the nurse cell FC and dorsal appendages (da) are short. Ectopic *A251-lacZ* expression is present in FC at the anterior and posterior of the egg chamber. (G,H) Heat shock overexpression of Puc (genotype hs-GAL4; UAS-Puc) results in small eggs with shortened dorsal appendages. (I) Ectopic UAS-Puc expression in large flip-out FC clones expressing UAS-GFP,UAS-Puc (green) is associated with incomplete nurse cell dumping.

3). *puc* mRNA was detected in egg chambers beginning at stage 10, with higher expression in the centripetally migrating FC (Fig. 3A,B). Lower levels of *puc* mRNA were detected throughout the FC, including the nurse cells; no signal was seen using a *puc* sense probe (Fig. 3E,F). From stages 11-13, *puc* mRNA levels remained elevated in the centripetally migrating FC (Fig. 3C), with additional high expression in cells at the anterior tip of the extending dorsal appendages and at the posterior pole FC (Fig. 3D). *puc* mRNA levels were low by stage 14 (not shown). Thus the *lacZ* expression pattern exhibited by *puc*^{A251} and *puc*^{E69} does not represent the pattern of *puc* mRNA accumulation in the follicle cells. Instead, *puc* mRNA accumulates in a pattern that parallels *lacZ* expression from the enhancer trap *puc*^{B48}, highest in follicle cell subgroups undergoing morphogenetic cell shape changes, including the centripetally migrating FC and dorsal appendage FC (compare Fig. 3D with 3G).

puckered levels are critical to anterior follicle cell morphogenesis

To investigate the role of *puc* in oogenesis, we used the FRT82B *puc*^{A251} chromosome for FLP/FRT-mediated clonal analysis (Xu and Rubin, 1993). The *puc*^{A251} allele is among the strongest alleles; homozygous embryos die with abnormal suturing and patterning defects at the dorsal midline (Ring and Martinez, 1993; McEwen et al., 2000). 48 hours after induction of *puc* mutant clones, females laid small, unfertilized eggs with aberrant eggshells (Fig. 4B,C; cf. wild type in 4A). The eggshells enclosed the anterior ends and had micropyles, indicating that FC migrated centripetally. However, eggshells were open in the anterior, with inadequate sealing between the operculum and the collar, and the dorsal appendages were shorter and variable in width. These phenotypes indicate that morphogenesis is defective in the anterior egg chamber, but do not indicate whether *puc* is required in the germline or in the somatic follicle cells. To distinguish between the two tissues, we selected for eggs derived from *puc* mutant germ cells using the FLP/FRT-dominant female sterile system (Chou et al., 1993). In contrast to the previous experiment, all eggs derived from mutant germ cells had normal eggshells and were fertilized (data not shown).

These data indicate that the defective eggs result from reduced *puc* activity in the somatic follicle cells. Small eggs may arise from incomplete nurse cell dumping, and dorsal appendage defects are often associated with defects in nurse cell dumping (Schüpbach and Wieschaus, 1989; Cooley et al., 1992). To examine nurse cell phenotypes in *puc* mutant egg chambers, ovaries were hand-dissected 72 hours after clone induction. Egg chambers that had chorion-encased small eggs also bore a mass of nurse cells similar in size to stage 11 nurse cells (Fig. 4E, cf wild type in 4D). Thus, nurse cell dumping was not completed.

To further examine the phenotypes of *puc* mutant FC, we detected the location of *puc* clones using ubiquitin-driven GFP to mark the *puc*$^{+}$ chromosome, and co-stained with DE-cadherin, to outline FC shapes. In wild-type egg chambers at stage 10 of oogenesis, high levels of DE-cadherin accumulate in the centripetally migrating FC (Fig. 5A) with lower levels in the main body FC. Strong staining accumulates in follicle cells that produce the dorsal appendages and operculum (Fig. 5C,E,F,I). Anterior *puc* clones exhibited high levels of DE-cadherin staining beginning at stage 10 (Fig. 5B). At later stages, clones were associated with defects in dorsal appendage formation (Fig. 5D,G,H). Because nurse cell dumping is incomplete and dorsal appendages are defective, such aberrant egg chambers cannot be staged. At late stages, we also observed aberrant cell shapes and defects in epithelial continuity in main body FC mutant clones (Fig. 5J). This class of eggs includes flaccid cup-shaped eggs that are likely resorbed and not laid, because they are not detected in collections of laid eggs (an example of this phenotype is shown in Fig. 8G). Thus *puc* is required to coordinate epithelial organization during FC morphogenesis.

During embryogenesis, the level of Puc activity is critical; dorsal closure defects arise both from reduced and elevated Puc activity (Martín-Blanco et al., 1998). We examined the effects

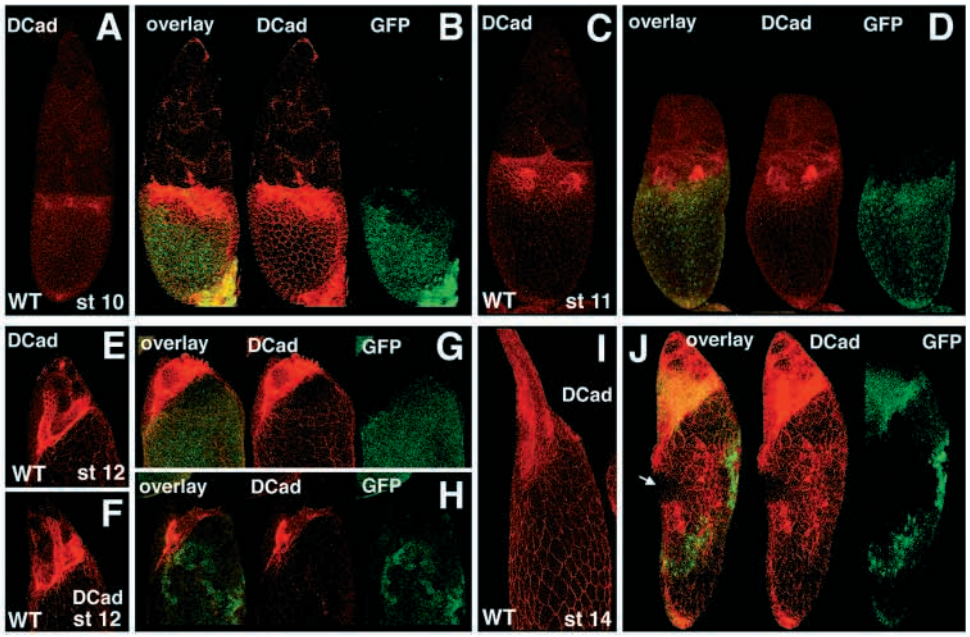

Fig. 5. *puc* is required to modulate DE-cadherin levels and to direct late FC migrations. Marked clones of *puc* are GFP^{-}; DE-cadherin is red in all panels. (A) Wild-type DE-cadherin accumulates throughout the follicle cells at stage 10, with the highest accumulation in the prospective CMFC. (B) Egg chamber with anterior *puc*$^{-}$ clone showing misexpression of DE-cadherin (red) in more anterior cells at approximately stage 10. (C,E,F) At stages 11-13, strong accumulation of DE-cadherin occurs in the extending dorsal appendages, and outlines these cells. (D,G,H) In anterior *puc*$^{-}$ clones, elevated levels of DE-cadherin accumulation are associated with shortened dorsal appendages. Proper staging of *puc*$^{-}$ mutant egg chambers is difficult because nurse cell dumping is incomplete. (I) DE-cadherin is stronger in extending dorsal appendage FC at stage 14 and marks the basolateral periphery of every FC, giving a honeycomb-like appearance. (J) Late stage *puc* mutant clones show aberrant accumulation of DE-cadherin and discontinuities in the main body FC. These disruptions are found in flaccid egg chambers, which are likely resorbed because they are not observed among laid eggs.

of overexpressing Puc during oogenesis using hsGAL4 to transiently drive high levels of UAS-Puc expression. Within 24 hours, eggs laid were variably short, with dorsal appendage defects (Fig. 4G,H; wild type in 4F). Strikingly, small eggs with abnormal appendages arise both from reduced and from elevated Puc activity (cf. Fig. 4B,C). Clones of follicle cells expressing UAS-Puc were generated by flip-out-induced expression of yeast GAL4 from the constitutive actin 5C promoter (Pignoni and Zipursky, 1997). Widespread overexpression of Puc in GFP-marked FC clones resulted in defective egg chambers composed of a significant mass of nurse cell material connected to a small chorion-encased egg (Fig. 4I), similar to egg chambers with reduced Puc function (Fig. 4E). These observations indicate that a proper balance of Puc levels in the follicle cells is required to coordinate morphogenesis with nurse cell dumping.

puckered regulates expression of *A251-lacZ*

puc encodes a phosphatase that modulates the strength and duration of MAP kinase signaling in mammalian cultured cells (Martín-Blanco et al., 1998). During embryonic dorsal closure, *puc*^{E69}*-lacZ* is expressed in the dorsal-most migrating epithelial cells of wild-type embryos. Expression of this enhancer trap depends on Jun kinase signaling during dorsal closure (Glise and Noselli, 1997). Furthermore, *puc*^{E69}*-lacZ* is regulated by Puc itself; expression is elevated and expanded in some tissues in a *puc* mutant background (Ring and Martinez-Arias, 1993). Conversely, widespread Puc overexpression is sufficient to inhibit *puc*^{E69}*-lacZ* expression (Martín-Blanco et al., 1998). Thus during embryogenesis, *puc* down-regulates expression of JNK pathway target genes.

To take advantage of *puc*^{A251}*-lacZ* as a reporter during FC morphogenesis, we first confirmed that *puc*^{A251}*-lacZ* (hereafter referred to as *A251-lacZ*) is negatively regulated by *puc* during embryogenesis (data not shown). We then tested for regulation by *puc* in the follicle cells. Strong ectopic *A251-lacZ* expression was observed in egg chambers from females bearing *puc*^{A251} mutant clones (Fig. 6A-D). In the absence of clone production, *A251-lacZ* expression was not detected before stage 10B. In contrast, precocious expression of *A251-lacZ* was observed in nurse cell FC of stage 9 egg chambers, 3 days after clones were induced (Fig. 6A). In stage 10B egg chambers, the nurse cell FC had elevated levels of β-galactosidase activity (Fig. 6B,C). The late stage small egg *puc* phenotype was strongly associated with elevated levels of *lacZ* expression (Fig. 4E, cf. WT in 4D). This reinforces the link between ectopic *A251-lacZ* and the presence of *puc* mutant cells, although it is important to point out that these cells have two copies of the *lacZ* insertion.

To directly detect *puc* mutant cells, we used the N-Myc epitope (Xu and Rubin, 1993) to mark the *puc*$^{+}$ chromosome in double immunofluorescence staining for β-galactosidase (Figs 6C,D and 7A). At early stages, marked *puc* mutant clones that occur anywhere in the epithelium result in precocious expression of *A251-lacZ* (Fig. 6C). At later stages, misexpression of *A251-lacZ* occurred only in specific subsets of follicle cells. In the nurse cell FC, the cytoplasmic N-Myc epitope was detected by the presence of a Myc halo surrounding the nucleus (Fig. 6D inset); the absence of this Myc halo indicated a *puc* mutant cell. Nurse cell FC that lacked the Myc halo had substantially elevated levels of β-galactosidase (Fig. 6D). These data indicate that *A251-lacZ* is negatively regulated by *puc* in the nurse cell FC during stage 9 and even at 10B when *A251-lacZ* in normally expressed.

We next examined the effects of increased Puc activity on *A251-lacZ* expression. Flip-out-induced clones of cells expressing UAS-Puc marked with UAS-GFP prevented expression of *A251-lacZ* in the nurse cell FC in a cell autonomous manner (arrowheads, Fig. 6E). In regions that normally lacked *A251-lacZ* expression, flip-out expression of

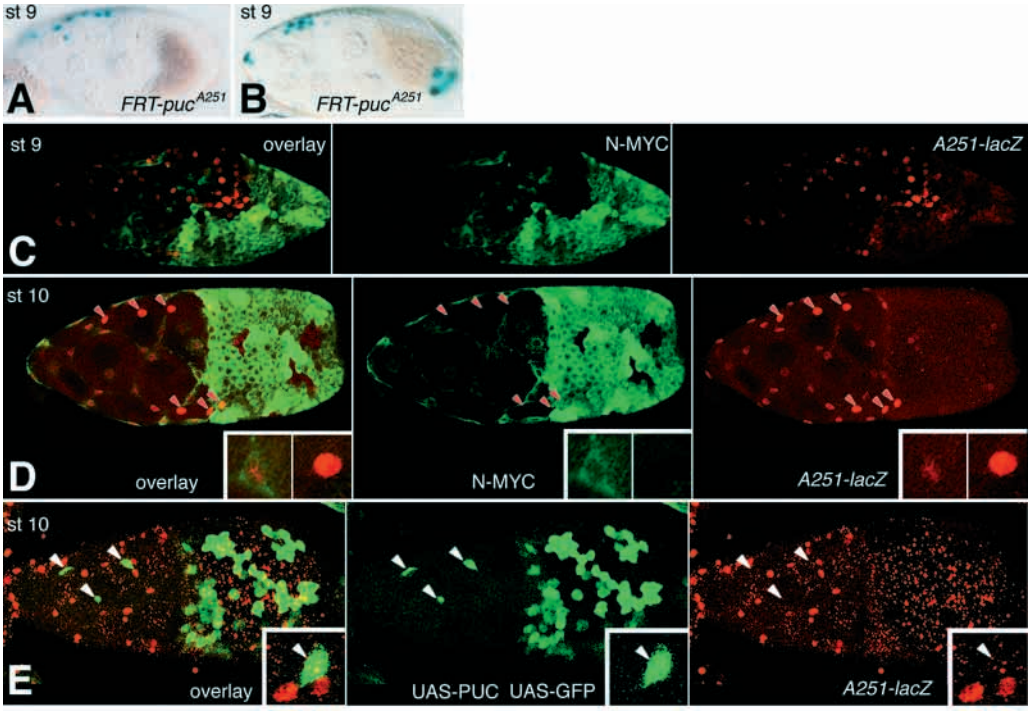

Fig. 6. *puc* regulates *A251-lacZ* expression in nurse cell FC. (A-C) Ectopic expression of *A251-lacZ* is detected by staining for β-galactosidase following clone induction. (A) Stage 9 egg chamber with strong precocious *A251-lacZ* expression in the nurse cell FC. (B) Stage 9 egg chamber shows ectopic staining in clones located in the nurse cell FC and the posterior pole FC. (C,D) Marked *puc* mutant clones designated by lack of Myc marker (green) show cell autonomous ectopic expression of *A251-lacZ*. (C) At stage 9, the expression of *A251-lacZ* (red) is limited to the *Myc*$^{-}$ cells in both the nurse cell FC and columnar FC. (D) At stage 10, mutant nurse cell FC nuclei lacking Myc marker antigen (visible around the nuclei of heterozygous wild-type cells) exhibit very high levels of *A251-lacZ* expression (red, arrowheads) compared to adjacent heterozygous nuclei that have a halo of Myc (see inset). The increase in β-gal levels appears greater than the 2× increase expected for two copies of *lacZ*. (E) Overexpression of UAS-Puc in nurse cell FC flip-out clones at stage 10 (UAS-GFP, green; see Materials and Methods for genotypes) is sufficient to block expression of *A251-lacZ* cell autonomously (white arrowheads marking three cells). Insets show higher magnification views of three nuclei, one of which overexpresses UAS-Puc (green) and lacks *A251-lacZ* expression.

Puc had no effect. These data indicate that *puc* is both necessary and sufficient to negatively regulate *A251-lacZ* expression in the nurse cell FC and *A251-lacZ*, the *puc* intron II enhancer trap, apparently marks FC domains of low *puc* activity.

A251-lacZ regulation is domain specific

Late stage egg chambers bearing unmarked *puc* mutant clones showed expanded *A251-lacZ* staining at the anterior and posterior, but not in the main body FC (not shown). This staining pattern could reflect an underlying competence of the cells to express *A251-lacZ*, or differential survival of *puc* mutant cells in different regions of the egg chamber. To distinguish between these possibilities, we sought marked *puc* mutant clones in the main body FC of late egg chambers (Fig. 7A). Unlike stage 9 clones, mutant clones in the main body FC at stage 10B did not show strong ectopic *A251-lacZ* expression (Fig. 7A). In contrast, we observed ectopic *A251-lacZ* expression in anterior columnar FC, which will become the centripetally migrating FC, and in the posterior pole FC (Fig. 7A). Indeed, mutant clones that fall across the boundary between the main body FC and the posterior pole FC showed strong ectopic *A251-lacZ* expression only in posterior cells (Fig. 7A inset; one posterior cell expresses *A251-lacZ*). No morphological abnormalities occur in the follicular epithelium clones at these stages.

These data suggest that *puc* regulates *A251-lacZ* expression differently in distinct regions of columnar FC. In the centripetally migrating and posterior pole FC, *A251-lacZ* expression is normally repressed by Puc activity. Consistent with this, the centripetally migrating and posterior pole FC normally accumulate high levels of *puc* mRNA (Fig. 3A-F). Thus, Puc may be most active in these domains. The main body FC repress *A251-lacZ* expression by a Puc-independent mechanism. By these criteria, the nurse cell FC appear to have moderate Puc activity. Paradoxically, it is this intermediate level of Puc activity that leads to the nurse cell FC-specific staining pattern of *A251-lacZ* that first attracted our attention (Fig. 1B,D,F). Given its functions as a MAP kinase phosphatase, Puc likely antagonizes one or more MAP kinase signal transduction pathways in the different FC domains, and the activity of these signals is critical for proper coordination of the distinct morphological changes of FC during late oogenesis.

Rho family GTPases regulation of *A251-lacZ* is domain specific

The location and timing of *puc* mutant defects suggest that Puc coordinates morphogenesis of the anterior egg chamber. Inward migration of the centripetally migrating FC, where Puc appears to be most active, must be coordinated with stretching of the dorsal appendage FC, and with nurse cell dumping. Changes in cell shape and cell motility are often mediated by the Rho family of small GTPases (van Aelst and D'Souza-Schorey, 1997). Activated forms of two members of this family, DRac1 and DCdc42, have been shown to increase expression of *A251-lacZ* during dorsal closure (Glise and Noselli, 1997). Thus, we examined the effects of activated forms of DRac1 and DCdc42 (DRac1V12=Act-DRac and DCdc42V12=Act-DCdc42, respectively) and a dominant negative form of each (DRac1N17=DN-DRac and DCdc42N17=DN-DCdc42; Luo et al., 1994) on expression of *A251-lacZ* in the follicle cells.

Overexpression of DN-DRac1 in clones located in the nurse cell FC, induced with the flip-out technique, was sufficient to suppress *A251-lacZ* (white arrows; Fig. 7B). In contrast, cells expressing Act-DRac1 were not recovered in the nurse cell FC. Act-DRac1 expression in clones elsewhere in the FC showed strong ectopic expression of *A251-lacZ*, but only in portions of the clone that include the posterior FC and never in main body FC clones (Fig. 7C). Notably, ectopic *A251-lacZ* occurs both in the Act-DRac1 clone and in cells adjacent to the clone (arrowheads, Fig. 7C). These results suggest that DRac1 is required for *A251-lacZ* expression in the nurse cell FC but that ectopic elevation of DRac1 activity may be deleterious in this domain. They further suggest that downregulation of DRac1 activity in the posterior pole FC is required to silence *A251-lacZ* expression there. Alteration of DRac1 activity had no detectable effect in the anterior columnar FC, the region that includes the centripetally migrating FC. Thus, different FC domains show distinct reponses to altered DRac1 activity.

Surprisingly, DCdc42, while showing domain-specific responses, had the opposite effect on *A251-lacZ* expression. Again, we over-expressed activated and dominant negative versions of DCdc42. In the rare Act-DCdc42 clones that occurred in the nurse cell FC, we observed reduction in *A251-lacZ* expression (Fig. 7D); conversely, overexpression of DN-DCdc42 led to misexpression of *A251-lacZ* in posterior pole FC clones at stage 10 (Fig. 7E), before its normal expression in these cells at stage 14 (data not shown).

Altered levels of DRac1 and DCdc42 activity also had profound effects on follicle cell morphology. Expression of either Act-DRac1 (Fig. 7C) or DN-DCdc42 (Fig. 7E, inset) in the posterior pole FC resulted in loss of monolayer organization. This is shown for Act-DRac1 by immunostaining for α-spectrin, which localizes to the apicolateral follicle cell contacts (Fig. 7F; Lee et al., 1997). In contrast, DN-DRac1 overexpression led to aberrant formation of the nurse cell FC (Fig. 7B). Morphology of the mainbody FC was not perturbed by expression of any of these mutant proteins, further emphasizing the distinct nature of this cell population.

Cell-type variations in Fos and Jun levels in the follicle cells

The cell-type-specific effects of Puc and Rho GTPase family members suggest that the main body FC differ in their capability to respond to these signaling molecules. Regulation

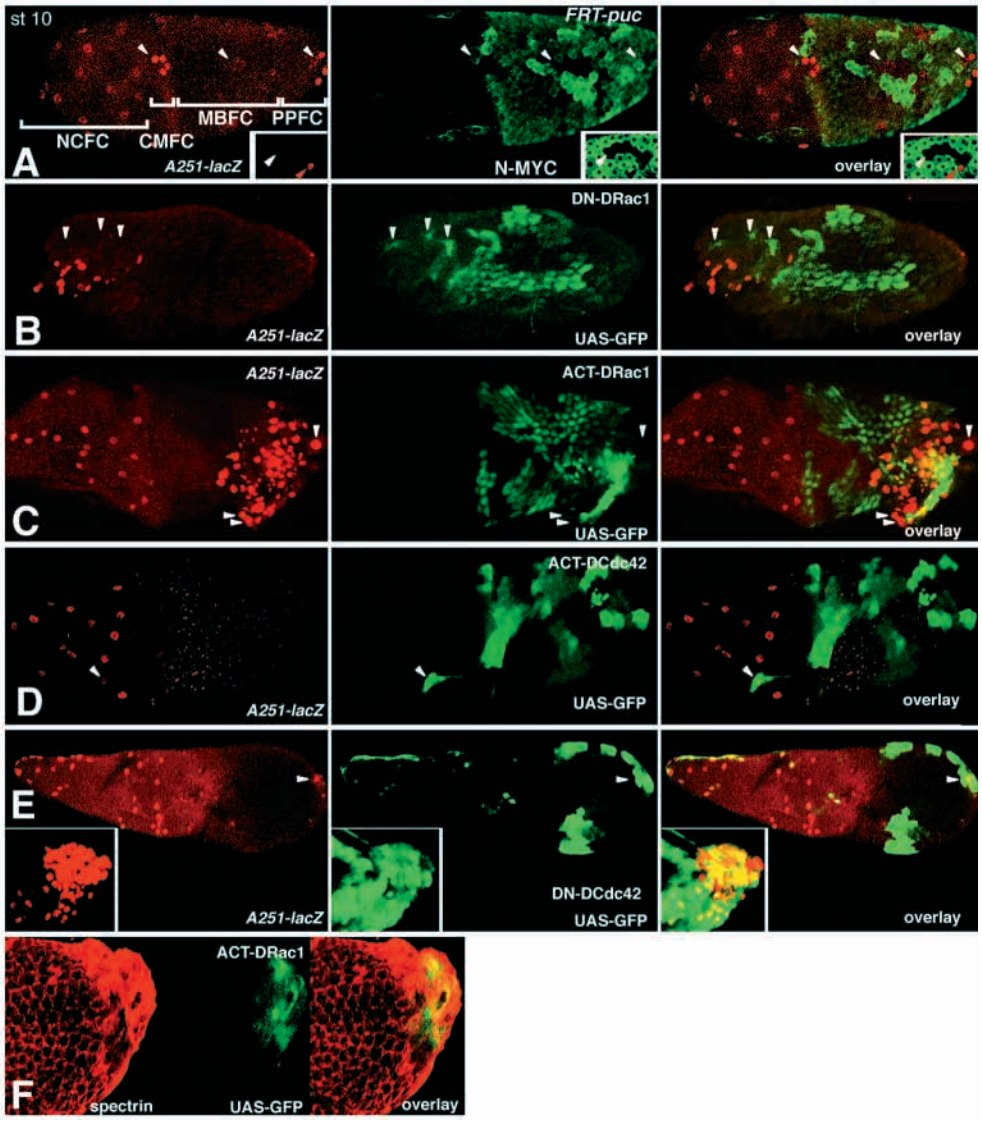

Fig. 7. Regional variations in *A251-lacZ* responses. (A) By stage 10, marked *puc* mutant clones show regional variations in altered *A251-lacZ* expression. Three mutant clones (arrowheads) marked by absence of MYC staining in the centripetally migrating FC (at nurse cell/oocyte border) and posterior pole FC show strong expression of *A251-lacZ* (red). At the same time, several main body FC *puc* mutant clones (arrowheads) show only weak expression of *A251-lacZ*. Inset: in a clone that straddles the boundary between the main body FC and the posterior pole FC, shows *A251-lacZ* expression only in the posteriormost cell (red arrow). (B) Flip-out clones expressing DN-DRac1 visualized using a UAS-GFP marker (green in B-F; white arrowheads) suppress *A251-lacZ* in the nurse cell FC in a cell autonomous manner and cause aberrant morphology. (C) Act-DRac1-expressing cells elevate *A251-lacZ* (red) in the posterior but not in the main body portion. Ectopic *A251-lacZ* occurs both in cells overexpressing Act-DRac1 and in adjacent GFP^{-} cells (white arrowheads). Posterior cells in and adjacent to the clone delaminate and leave the continuous epithelium. (D) Act-DCdc42-expressing cells suppress *puc-lacZ* expression in the nurse cell FC (white arrow). (E) DN-DCdc42 can induce ectopic *puc-lacZ* in the posterior FC. Posterior cells in large clones delaminate and leave the continuous epithelium (inset). (F) Cells in posterior clone overexpressing Act-DRac1 (green) outlined by α-spectrin staining show loss of monolayer organization in a posterior clone.

of gene expression by members of the Rho GTPase family often involves Jun kinase signaling to activate the AP-1 transcription factor. Consistent with this, the AP-1 components Dfos (also known as Kay; Kayak) and DJun (also known as Jra; Jun-related antigen) are required for dorsal closure (reviewed by Noselli and Agnes, 1999). We speculated that one of these proteins might be absent from the main body FC.

Expression of DFos and DJun is dynamic during late oogenesis (summarized in Fig. 8A-C). At stage 10A, DFos and DJun antisera (Zeitlinger et al., 1997; Peverali et al., 1996) each detect a nuclear protein in the follicular epithelium with noticeably higher levels in the nurse cell FC. At later stages, accumulation of these proteins changes rapidly. As shown in Fig. 8C, DJun levels increase in the anterior FC, including the centripetally migrating FC and nurse cell FC, with a corresponding decrease in the mainbody FC and in the posterior pole FC (Fig. 5E). Levels then decrease in the dorsal anterior FC (not shown).

It has been suggested that levels of MAPK-like signaling can affect the stability of DFos and DJun (Okazaki and Sagata, 1995; Chen et al., 1996; Musti et al., 1997). The nurse cell FC expression of DFos and DJun parallels the expression of Jun kinase target gene *A251-lacZ* and suggests that levels of these putative mediators of JNK signaling may be regulated by *puc*. To test this, we examined DFos and DJun levels in FC mutant for *puc*. *puc* mutant clones showed strong elevation of DFos and DJun protein levels in all FC subpopulations (Fig. 8D-F). Elevated DFos and DJun were detected in both the nucleus and cytoplasm of mutant clones and correlated with high levels of DE-cadherin (Fig. 8F) in such clones. High Fos/Jun levels positively marked degenerating egg chambers, which provided a means of readily identifying a strong *puc* egg chamber phenotype (Fig. 8G). Conversely, Fos and Jun levels were reduced in FLP-out clones expressing Puc in the nurse cell FC (Fig. 8H). Furthermore, nurse cell FC overexpressing DN-DRac1 exhibited lower levels of DJun (Fig. 8I), supporting the notion that the phosphorylation state of Fos/Jun affects their cellular levels. While increased levels of Fos and Jun vary within cells of a *puc*$^{-}$ clone, we observed effects in all regions of the egg chamber.

DISCUSSION

Coordinated epithelial cell movements play a role in a number of developmental events, such as wound healing and epiboly during gastrulation. In *Drosophila*, the spreading of the lateral epithelia during dorsal closure to cover the amnioserosa (Martinez-Arias, 1993) and the spreading of anterior follicle cells during nurse cell dumping have several similarities (Young et al., 1993; Edwards and Kiehart, 1996). Both involve actin-myosin driven changes in cell shape coordinated with a spreading of epithelia to enclose, respectively, the dorsal side of the embryo and the anterior of the oocyte. Here we describe an additional parallel: the involvement of the phosphatase Puc.

puc was first identified because it is required for dorsal closure during embryogenesis (Ring and Martinez-Arias, 1993). Some *puc* enhancer traps are expressed in the edge cells

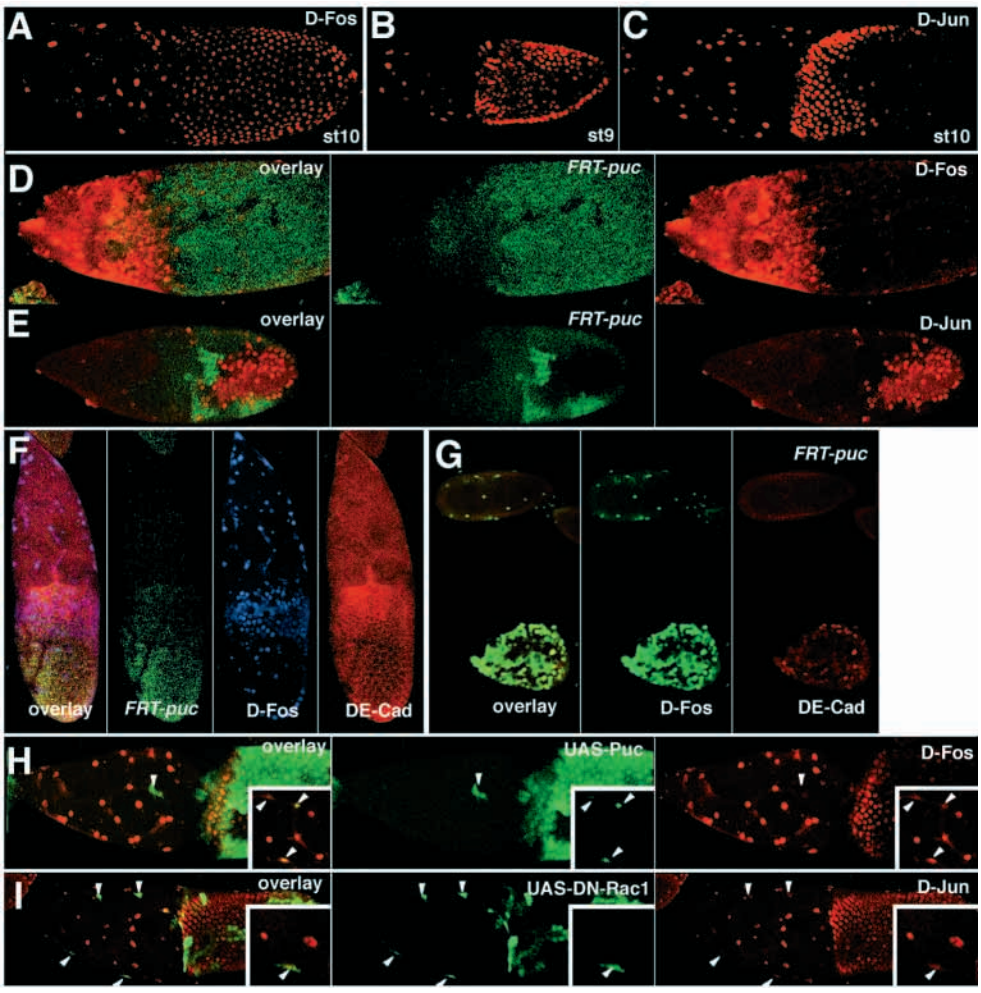

Fig. 8. *puc* and DN-DRac1 modulate levels of DFos and DJun in the FC. (A,B) At stage 10, nuclear DFos and DJun can be detected throughout the FC with higher levels in the nurse cell FC. (C) At stage 10B, DJun levels change dynamically: a rapid decrease in the dorsal anterior main body FC and posterior pole FC occurs. (D-G) In *puc* clones (clones marked by GFP^{-}), high levels of both DJun (E) and DFos (D,F,G) accumulate in both the nucleus and cytoplasm (red). Overexpression of DFos and DJun in these clones correlate with high levels of DE-cadherin (F), with aberrant accumulation permitting detection of small, cup-shaped egg chambers (G). (H,I) Overexpression of DN-DRac1 (H) and Puc (I; clones marked by GFP) results in subtle but consistent down-regulation of DJun levels in the nurse cell FC.

that lead the spreading ectoderm. In *puc* mutants, dorsal closure apparently initiates normally. However, the leading edge cells fail to suture properly when the two sides meet at the dorsal midline (Ring and Martinez-Arias, 1993). *puc* encodes a member of the VH-1 family of dual specificity phosphatases that have been implicated in the downregulation of MAP kinase activity (Martín-Blanco et al., 1998; reviewed by Keyse, 2000). Some enhancer traps in *puc* are regulated by the Jun kinase signaling pathway during dorsal closure (Glise and Noselli, 1997; Hou et al., 1997; Riesgo-Escovar and Hafen, 1997b; Zeitlinger et al., 1997). These *lacZ* reporters have been used to demonstrate that Puc down-regulates Jun kinase activity during dorsal closure. We report here a new role for *puc* in coordinating follicle cell movements during oogenesis.

puckered levels are critical for anterior follicle cell morphogenesis

puc mRNA levels become elevated in the centripetal migrating FC and dorsal appendage FC and remain high during morphogenesis of these structures. Production of *puc* mutant clones in the ovary led to the formation of small eggs with anterior eggshell defects. The junction between the operculum and ventral collar was aberrant, so that the eggshells were open in the anterior, and the dorsal appendages were short and broad. The presence of opercula and micropyles indicated that centripetal migration occurred, but the specific defects suggested that it did not proceed normally. Late-staged egg chambers from these females retained significant nurse cell material at the anterior after the eggshell was deposited, indicating that nurse cell dumping was not coordinated with follicle cell morphogenesis. Germline clonal analysis indicated that these *puc* mutant phenotypes derive solely from a requirement for *puc* in the soma. Overexpression of Puc in the FC also leads to production of small eggs with dorsal appendage defects. Because either increased or decreased *puc* function disrupts the coordination of follicle cell morphogenesis and nurse cell dumping, we conclude that Puc activity must be poised at the proper level to coordinate anterior morphogenesis. These data parallel the subtly different defects in dorsal closure that result from either reduced or increased Puc activity, and point to a general role for Puc in coordinating epithelial sheet movements.

We further demonstrate that loss of *puc* in the follicle cells leads to aberrant cell shapes. Puc mutant dorsal appendage FC accumulate elevated levels of DE-cadherin and fail to extend properly. Late in oogenesis the mainbody FC are irregular in shape when mutant for *puc*. We conclude that the level of Puc activity finely modulates the morphogenesis of the follicular epithelium. This requirement is most evident in the dorsal appendage FC, but is also important for morphogenesis of the operculum boundary.

Domain-specific regulation of the JNK signaling target gene *A251-lacZ*

Enhancer traps *A251-lacZ* and *E69-lacZ* are inserted into the same region of the *puc* second intron. Both exhibit leading edge cell expression during embryonic dorsal closure and nurse cell FC-specific expression during nurse cell dumping. In contrast, *puc* mRNA expression is more widespread in both tissues. In the egg chamber, it accumulates at low levels throughout the FC and at high levels in the centripetally migrating FC, dorsal appendage FC and posterior pole FC. This pattern is recapitulated by the *B48-lacZ* enhancer trap insertion located in *puc* intron 1. Consistent with this, the *puc* gene is required in more FC than the limited expression of *A251-lacZ* would predict. Given the widespread expression of *puc* RNA in the embryo, *A251-lacZ* reveals only a subset of the complete *puc* expression pattern in this tissue as well (Martín-Blanco et al., 1998; McEwen et al., 2000).

These *lacZ* reporters have been useful to dissect the regulation of epithelial cell morphogenesis during dorsal closure in embryos. Reporter expression is positively regulated by components of the JNK pathway, and negatively regulated by Puc itself. It has not been reported whether *puc* RNA levels are similarly regulated. We found that *A251-lacZ* expression is also negatively regulated by Puc in the FC. First, when *puc* mRNA levels are low, before stage 10, there is a requirement for Puc activity to prevent *A251-lacZ* expression throughout the FC. Second, after stage 10, when clear FC domains are established, we observe striking regional differences in the requirement for Puc to regulate *A251-lacZ* expression.

In the nurse cell FC, *A251-lacZ* expression is increased by reduced Puc function and repressed by overexpression of UAS-Puc. Because *puc* mRNA levels are low in these cells at this stage, we infer that Puc activity is at an intermediate level so that Jun kinase-like signaling is only partially repressed.

In the centripetally migrating FC, low *A251-lacZ* levels are Puc dependent because reduction of Puc function results in ectopic *A251-lacZ* expression. We conclude that Jun kinase-like signaling is active in these cells, but strongly suppressed by the high levels of Puc activity normally there. Consistent with this, we see high levels of *puc* mRNA in these cells. High *puc* mRNA levels correlate with strong repression of *A251-lacZ* at stage 10 in the posterior pole FC, as well. The strong requirement for Puc activity in anterior FC correlates well with the *puc* mutant defects seen in anterior FC morphogenesis.

Low levels of *A251-lacZ* in the main body FC are not dependent on *puc*: mutant clones show only a slight derepression of *A251-lacZ* at very late stages (data not shown). Consistent with this, these cells express only low levels of *puc* mRNA, and overexpression of Puc has no visible effect on the morphology of these cells. However, Puc is active in the main body FC, for these cells accumulate Fos and Jun when mutant.

Activated Rho GTPases have opposing, domain-specific effects on *A251-lacZ* expression

The Rho family of small GTPases are important modulators of cell shape (reviewed by Hall, 1998) and epithelial cell reorganization (reviewed by Eaton, 1997). Specific Rho proteins can manifest specialized cellular functions, including unique effects on transcriptional control, cell growth control and membrane trafficking (reviewed by van Aelst and D'Souza-Schorey, 1997). Moreover, individual Rho family members can exhibit cell type-specific effects. When first identified in Swiss 3T3 cells, microinjection of either Cdc42 or Rac were shown to induce the formation of filopodia or lamellopodia, respectively (reviewed by Hall, 1998). The cell-type specific effects extend to their abilities to stimulate the JNK pathway. For example, both Rac and Cdc42 stimulate the MAP kinase-related JNK and p38 activity in HeLa, Cos and NIH-3T3 cells; in contrast, Cdc42 and Rho but not Rac can stimulate JNK activity in human kidney 293 T cells (Coso et

al., 1995; Minden et al., 1995; Teramoto et al., 1996). In *Drosophila*, differing requirements for Rac, Rho and Cdc42 have been observed during embryonic dorsal closure and imaginal disk development (Glise and Noselli, 1997; Harden et al., 1999; Agnes et al., 1999; Lu and Settleman, 1999; Ricos et al., 1999; Martín-Blanco et al., 2000). The activities of Rho family members in *Drosophila* oogenesis were tested previously, but the focus was on their phenotypes in the nurse cells and the border cells (Murphy and Montell, 1996). The differing requirements for Puc in different regions of the follicular epithelium suggested that these regions would show distinct sensitivities to the actions of Rho family GTPases.

Because of evidence that DRac1 and DCdc42 regulate expression of *A251-lacZ* during embryogenesis (Luo et al., 1994), we tested the ability of these small GTPases to regulate *A251-lacZ* expression in the FC. We found two opposing effects: activated DRac1 and dominant negative DCdc42 activate *A251-lacZ* expression in posterior cells; dominant negative DRac1 and activated DCdc42 repress *A251-lacZ* expression in anterior cells. Clones for activated DRac1 were not recovered in the anterior nurse cell FC, suggesting that these cells are highly sensitive to increased Rac activity. These data suggest that a balance between these two small GTPases may define the level of *A251-lacZ* expression in the nurse cell FC and the posterior pole FC. This may reflect the shared origins of these distinct cell groups as terminal follicle cells (González-Reyes and St Johnston, 1998).

Neither DRac1 nor DCdc42 appeared to regulate *A251-lacZ* expression in the main body FC or the centripetally migrating FC. This latter observation is surprising, given the strong requirement for Puc activity in these cells. Perhaps a distinct Rho family GTPase is active in these cells, such as Rho1. Consistent with this, mutants in PAK kinase, which acts in parallel to JNK signaling during dorsal closure, have a variable defect in centripetal migration (Lu and Settleman, 1999; J. Settleman, personal communication).

Activated GTPase expression in the follicle cells resulted in additional phenotypes. First, activated DRac1 had non-autonomous phenotypes in the posterior pole FC. Similarly, activated DRac1 had non-autonomous effects on gene expression in the dorsal ectoderm of the embryo (Glise and Noselli, 1997). This suggests that in both tissues, a short-range signal dependent on DRac1 signaling can upregulate expression of these intron II enhancer traps in adjacent cells. Dpp is a local signal to the dorsal ectoderm during dorsal closure; however, our data suggest that Dpp is not the key signal to induce *A251-lacZ* in the posterior FC (L. L. D. and L. A. R., unpublished data). We note that loss of *puc* in our marked clones never results in upregulation of *A251-lacZ* expression in adjacent cells, indicating that whatever its identity, the short range signal is not regulated by *puc*.

Expression of activated DRac1 in the posterior pole FC caused domain-specific disorganization of the epithelium. Dominant negative DCdc42 also caused this phenotype, similar to genetic loss of DCdc42 activity (Genova et al., 2000). Murphy and Montell (Murphy and Montell, 1996) reported a similar phenotype for activated RhoL. Thus the balance of Rho GTPases in the posterior follicular epithelium may be important to maintain epithelial structure, similar to roles ascribed to Rac in metastasis (Khosravi et al., 1995; Qiu et al., 1995a; Qiu et al., 1995b; Qiu et al., 1997).

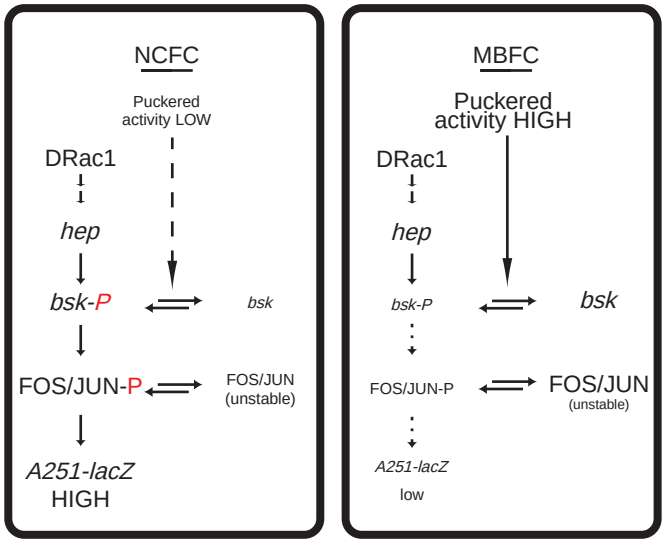

Fig. 9. Model for *puc* regulation of Jun/Fos levels and consequent *A251-lacZ* activation in anterior FC.

puckered is a rheostat that modulates Fos/Jun levels to coordinate follicle cell migrations

Based on data presented here, we propose that *puc* functions as a rheostat to modulate gene expression responses to Rho family GTPases (Fig. 9). This modulation is critical to coordinate morphogenesis in distinct FC domains. We have discussed evidence that opposing activities of DRac1 and DCdc42 sets the level of *A251-lacZ* expression in the terminal domains of the FC. Because Puc can act as a Jun kinase phosphatase in the embryo, we speculate that Puc modulates the activity of similar kinases that act downstream of the small GTPases at the FC termini. It is likely that *puc* modulates Jun kinase signal in the anterior FC, because the Jun kinase kinase encoded by *hemipterous* (*hep*; Glise et al., 1995) is reported to be required for anterior *A251-lacZ* expression (Maglie, Perrimon and Noselli, personal communication). However, *puc* is also required at the posterior, whereas late *A251-lacZ* expression is not dependent on *hep* (Maglie, Perrimon and Noselli, personal communication). Components of the posterior pathway remain to be identified.

The insensitivity of main body FC to stimulation by Rac or Cdc42 might suggest that these cells are incompetent to respond to small GTPase activation during the late stages of oogenesis. However, our data suggest that responses are suppressed by an independent mechanism. The dynamic pattern of DJun and DFos (Fig. 5C; Dequier, Lepesant and Yanicostas, personal communication) and *puc* itself, suggests that FC may vary greatly, both in time and in space, in their competence to respond to the JNK pathway. To complicate this picture, our data indicates that *puc* regulates levels of Fos and Jun, even in the main body FC. Loss of *puc* leads to elevation of Fos/Jun protein levels. Conversely overexpression of either Puc or DN-DRac1 lowers Fos/Jun levels. Currently we do not know the direct mechanism for *puc* regulation of Fos/Jun levels, but these results recall observations that stability of Fos and Jun depends on phosphorylation. In cell culture, site-specific mutations in Fos or Jun that block phosphorylation confer instability; conversely, phosphate-mimetic mutations confer

greater stability (Okazaki and Sagata, 1995; Chen et al., 1996, Musti et al., 1997). We conclude that proper levels of Puc phosphatase modulates Rho family GTPase signal output to coordinate follicle cell morphogenesis. These results are similar to the effect of both loss- and gain-of-function Puc in blocking dorsal closure (Martín-Blanco et al., 1998) and a role for *puc* in disc epithelial morphogenesis (Agnes et al., 1999; Martín-Blanco et al., 2000). The precise regulation of Puc activity levels is critical to coordinate epithelial cell sheet spreading in at least three tissues.

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