Opposing interactions between Drosophila Cut and the C/EBP encoded by Slow Border Cells direct apical constriction and epithelial invagination

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Stage 10 of Drosophila oogenesis can be subdivided into stages 10A and 10B based on a change in the morphology of the centripetal follicle cells (FC) from a columnar to an apically constricted shape. This coordinated cell shape change drives epithelial cell sheet involution between the oocyte and nurse cell complex which patterns the operculum structure of the mature eggshell. We have shown previously that proper centripetal FC migration requires transient expression of the C/EBP encoded by slow border cells (slbo) at 10A, due in part to Notch activation followed by slbo auto repression (Levine et al., 2007). Here we show that decreased slbo expression in the centripetal FC coincides with increased expression of the transcription factor Cut, a Cut/Cux/CDP family member, at 10B. The 10A/10B temporal switch from Slbo to Cut expression is refined by both cross repression between Slbo and Cut, Slbo auto repression and Cut auto activation. High Cut levels are necessary and sufficient to direct polarized, supracellular accumulation of Actin, DE-cadherin and Armadillo associated with apical constriction of the centripetal FC. Separately, Slbo in the border cell rosette and Cut in the pole cells have antagonistic interactions to restrict Fas2 accumulation to the pole cells, which is important for proper border cell migration. The opposing effects of Cut and Slbo in these two tissues reflect the opposing interactions between their respective mammalian homologs CAAT Displacement Protein (CDP; now CUX1) and CAAT Enhancer Binding Protein (C/EBP) in tissue culture.

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Introduction

Cell migration is fundamental to the construction of three-dimensional tissues from cellular units. Cells can move either as loosely organized streams, such as during vertebrate neural crest or neuronal precursor migration (reviewed in Sandell and Trainor, 2006), tightly adherent clusters, such as during border cell migration in the Drosophila ovary, or as coherent cell sheets, such as during Drosophila tracheal tube migration or convergent extension during vertebrate gastrulation (reviewed in Locascio and Nieto 2001; Berg, 2008; Caussinus et al., 2008; Friedl and Gilmour, 2009). The complex cytoskeletal rearrangements and changes in cell adhesion that occur as cells switch from stationary to invasive behaviors is coordinated by both intercellular signals and intrinsic cues, notably transcription factors. Examples include the C/EBP homolog encoded by the gene slow border cells (slbo; Montell et al., 1992; Rorth and Montell, 1992), which regulates levels of the homotypic adhesion molecule DE-cadherin necessary for migration of the border cell cluster, and the transcription factor Snail, which directs a switch from E- to N-cadherin expression required for mesoderm invagination (Cano et al., 2000), a molecular mechanism that is conserved with mass cell migration in vertebrates (reviewed in Come et al., 2004). In some cases, the opposing activities of two transcription factors control the timing and extent of tissue morphogenesis, illustrated by Drosophila knirps and spalt that promote the anterior and posterior tissue migratory behaviors, respectively, of the tracheal tube (Chen et al., 1998). Elucidating how transcription factors modulate the genetic circuits underlying the initiation, guidance and cessation of cell migration will be fundamental to understanding the molecular basis of tissue morphogenesis, wound repair and cancer invasion.

Drosophila oogenesis is an excellent model to probe the role of cell signaling during migration (reviewed in Horne-Badovinac and Bilder, 2005; Wu et al., 2008). The ovary is a paired structure composed of 15–20 ovarioles, each consisting of a series of attached egg chambers. Individual egg chambers are composed of a single oocyte connected to 15 nutritive nurse cells surrounded by a somatic follicle cell (FC) epithelium. Egg chamber production begins in the proximal germarium where a set of mesodermally-derived FC stem cells proliferate and encapsulate the oocyte-nurse cell syncytium in an epithelial monolayer. Like all epithelia, the FC are polarized cells that contact each other through specialized junction complexes connected to the internal cytoskeleton (Knust and Bossinger, 2002; Dow and Humbert, 2007). Adherens junctions (AJ) interconnect polarized FC, and apical and basal junctions connect the FC sheet to the oocyte and outer basement.
membrane, respectively (reviewed in Tanentzapf et al., 2000; Knust, 2002; Szafranski and Goode, 2007). Genetic and biochemical approaches show that the Aj, a complex of proteins including as E-Cadherin, Armadillo/β-catenin and α-catenin, is confined to the apicolateral domain by the combined action of (1) the apical Par6/Bazooka/PKC complex and (2) the marginal zone Crb complex, consisting of Crumbs, Stardust and Discs lost. These polarized epithelial junction complexes stably maintain epithelial sheet tension and tissue rigidity.

Genetic analysis reveals that junction complexes are required not only for the maintenance of the FC epithelial organization during early oogenesis, but also for cell migrations and rearrangements necessary to pattern the eggshell at later stages (Tepass, 2002; Carthew, 2005; Tepass and Harris, 2007; Niessen and Gottardi, 2008). At stage 9, Notch signaling in the anterior FC directs Aj remodeling during flattening of the nurse cell FC, a process coordinated with formation of the columnar main body FC, which pattern the main body features of the eggshell (Grammont, 2007). From stages 8–10, the anterior border cells (BC) delaminate from the FC epithelium, and migrate as a cluster (consisting of two polar cells surrounded by 10–12 rosette cells) through the nurse cell complex to arrive at the border between the nurse cell/oocyte syncytium (Montell, 2003). BC migration is associated with reorganization of the Aj components Armadillo and Cadherin to generate protrusive cell shape changes (Geisbrecht and Montell, 2002). At stage 14, coordinated cell shape changes in the dorsal anterior follicle cells drive the formation of the appendage tube (Berg, 2008).

At stage 10B, the centripetal migrating FC invaginate as an intact epithelium to cover the anterior of the oocyte (Figs. 5A,B). To accomplish this, the centripetal FC coordinately constrict apically at stage 10B and acquire adhesivity for the germ line. A set of leading edge FC derived from the nurse cell FC guides the cell sheet to migrate between the oocyte and nurse cells. These morphogenetic changes correlate with increased accumulation of non-muscle myosin and DE-cadherin in the centripetal FC, and mutations in either gene disrupt centripetal migration (Edwards and Kiehart, 1996; Niewiadowska et al., 1999). We have shown previously that Notch activates the transient expression of the C/EBP homolog encoded by slow border cells (slbo) in the centripetal FC at stage 10A prior to their involution (Levine et al., 2007). Here we show that as Slbo levels decrease in the centripetal FC at stage 10B, levels of the Cut gene increase and that high levels of Cut at stage 10B are necessary and sufficient for the apical constriction of these cells during centripetal migration. Our results from cross repression previously using the monoclonal antisera 2B10, Cut expression increases in the FC at stage 2 and Cut levels remain high until stage 6 (Fig. 1A; Blochlinger et al., 1993). At these early stages, Cut inhibits degradation of Cyclin E. Notch signaling represses Cut expression at stage 7, leading to a switch in DNA replication to the endocycle (Sun and Deng, 2005).
Following this switch to the endocycle, Cut expression remains low in most FC from stages 7–10A though high Cut levels persist in the pole cells, a subset of the border cells, throughout their migration (Figs. 1A–arrows and B–inset). At stage 10B Cut levels increase in the main body FC, with significantly higher levels in the centripetal FC (Figs. 1C–C″ and Blochlinger et al., 1993), marking these cells as they involute between the nurse cell/oocyte complex to cover the anterior of the oocyte and pattern the operculum.

To better define Cut expression in the centripetal FC, we compared Cut to the expression of the slbo reporter gene (slbo-lacZ hereafter), which is expressed in this tissue at stage 10A (Figs. 1A–C, and 1D; Montell et al., 1992; Rorth, 1996). When slbo-lacZ levels are high at stage 10A, Cut levels are low (nuclear lacZ shown in Figs. 1B–B″). Subsequently at stage 10B, slbo-lacZ levels decrease (Levine et al., 2007) concomitant with an increase in Cut levels, again highest in the centripetal FC and with lower Cut levels in the main body FC (Fig. 1C). As shown in Figs. 1D–G, by stage 11 slbo-lacZ expression persists only in the FC at the leading edge of centripetal migration, a set of cells that express Cut as well and are marked effectively by Eyes Absent expression (Eya, Figs. 1E–G; Bonini et al., 1993). In the border cell cluster, an overlapping pattern of slbo-lacZ and Cut can be seen as well, where Slbo is expressed in both the inner polar cells and the outer border cell rosette (latter revealed by slbo-lacZ, Fig. 1B, inset). In sum, expression of Cut and Slbo from stages 10–14 marks distinct groups of migrating cells that comprise the centripetal FC and border cells, respectively.

Slbo protein accumulation (detected by antisera to Slbo, Figs. 2A,B) parallels transient slbo-lacZ expression in the centripetal FC so that Slbo protein levels are high at stage 10A (Fig. 2A′) and subsequently undetectable by 10B (Fig. 2B′) at the onset of Cut expression (2B); Slbo protein expression in leading edge FC could not be detected by this rather weak antisera. The strict exclusivity of Slbo and Cut in these cells suggests that these genes may repress each other’s expression. To test this, we examined cut or slbo expression in genetic backgrounds that manipulate levels of the reciprocal gene. In FLP-out FC clones expressing Cut prior to 10B (Fig. 2C), we observed cell autonomous reduction of slbo-lacZ levels in both in the centripetal FC (inset from Fig. 2C shown in Figs. 2E–E″) and border cells (inset from Fig. 2C shown in Figs. 2D–D″), the effect of which we will describe in more detail, below. Cut misexpression also reduced Slbo protein levels in both tissues as well (Supplemental Fig. 3A,B). One concern was that misexpression of Cut simply generally reduces expression of all genes in the centripetal FC, so we examined the effect of Cut misexpression on the expression of the centripetal FC marker genes A359-lacZ (Fig. 2G, Dobens et al., 2000) and pPryVM32E(-112/-39)-lacZ (Fig. 2I, Cavaliere et al., 1997). As shown in Figs. 2J, Slbo2.6GAL4 driving misexpression of UAS-Cut in the centripetal FC lead to either increased levels of pPryVM32E(-112/-39)-lacZ or an increased number of cells expressing both markers, in stark contrast to slbo-lacZ which decreased under these conditions (not shown), indicating that Cut effects are gene-dependent. Complementary to Cut effects on slbo expression, misexpression of Slbo after 10A using the FLP-out driver resulted in reduced levels of Cut, both in the centripetal FC and in the main body FC where low levels of Cut occur (Figs. 2F–F″). FLP-out clones expressing Slbo recovered prior to stage 6 were sufficient to repress early Cut expression (Supplemental Fig. 1), indicating that at all stages of oogenesis misexpression of Slbo effectively can repress Cut expression.

To determine whether cut was necessary for slbo repression in the centripetal FC, we examined the effects of cut null allele clones on slbo expression. Consistent with the role of cut in promoting cell proliferation before stage 6, cut145 null clones were small at late...
Fig. 2. Cut and Slbo are sufficient to repress each other’s expression. (A,B). Accumulation of Slbo (red) and Cut (green) proteins detected by specific antiseria. Slbo levels are high in the centripetal FC at 10A (A’ when Cut levels are low (A)). Complementarily, at 10B Slbo levels are low (B’) while Cut levels are high (B) in the centripetal FC and low in the main body FC. (C). Slbo expression (slbo01310, red) at stage 10A is repressed by FLP-out misexpression of Cut (clones detected by GFP expression, green). Insets in D and E are boxed. Genotype in C–E: Actin5C–CD2–GAL4; slbo01310/UAS-Cut;UAS-GFP. Phenotype observed in 8/8 clones recovered in the CMFC and 6/9 clones recovered in the BC. (D). Inset from C shows close-up of a FLP-out Cut-expressing clone in the border cells (marked by GFP, green, D’) showing cell autonomous reduction of slbo-lacZ levels (arrowhead, red). (E). Inset from C of close-up of FLP-out Cut-expressing clones located in the centripetal FC (Marked by GFP, green, E’) showing cell autonomous reduction of slbo-lacZ (arrowhead, E’). (F). FLP-out Slbo-expressing clones (marked by GFP, green, F’) located in the centripetal FC (arrowheads) and main body FC (one clone indicated by arrow) result in cell autonomous repression of Cut (red, F”) in these cells at 10B. Genotype: Actin5C–CD2–GAL4;UAS-Slbo;UAS-GFP. Phenotype observed in 24/24 clones. (G). Wild type expression of centripetal FC marker gene A359 at 10B (surface view). Genotype: slbo2.6GAL4; A359-lacZ. (H). Increased expression of A359 occurs in centripetal FC expressing Cut under control of the Slbo2.6GAL4 promoter. Genotype: slbo2.6GAL4/UAS-Cut; A359-lacZ. (I). Wild type expression of centripetal FC marker gene A359 at 10B (cross-section view). Genotype: slbo2.6GAL4; pFpyVM32E(-112/-39)-lacZ. (J). Increased expression of pFpyVM32E(-112/-39)-lacZ occurs in centripetal FC expressing Cut under control of the Slbo2.6GAL4 promoter. Genotype: slbo2.6GAL4/UAS-Cut; pFpyVM32E(-112/-39)-lacZ.

stages, but these clones could be unambiguously detected using a positive marking system (Lee and Luo, 2001). cut145 clones examined before stage 10B had no effect on slbo-lacZ expression (data not shown), consistent with low levels of Cut seen at these stages (2A). cut mutant clones (positively marked in Figs. 3A–C) recovered in the centripetal FC at the onset of Cut expression at late stage 10A showed higher levels of slbo-lacZ (in cut145 clones, 3A) or Slbo protein (in cut287 clones, 3B) and in the posterior polar FC where Cut levels are low, cut145 clones led to higher levels of slbo-lacZ (Fig. 3C). In contrast, cut mutant clones recovered in the main body columnar FC at 10B showed no slbo-lacZ or Slbo misexpression (not shown). These results suggest that cut is necessary and sufficient during late oogenesis to repress Slbo expression in a subset of anterior and posterior follicle cells, presumably cells in which slbo can be activated. While Cut is necessary and sufficient to repress Slbo, and Cut is sufficient to repress cut, slbo null clones recovered in the anterior FC resulted in no Cut misexpression prior to 10B (data not shown) and no increased Cut expression after (Figs. 3D–D’). In weak slbo mutant allelic combinations (slbo21310/slabd22), low Slbo protein levels are seen in this genotype as shown in Fig. 7B) we noted that Cut and slbo-lacZ expression patterns do overlap (cf. WT in Figs. 3E–E’ to 3F–F‘), but this is due likely to misexpression of slbo-lacZ at 10B resulting from a loss of Slbo autorepression, as described previously (Levine et al., 2007). In sum, these data indicate that Slbo is sufficient to repress Cut, but reduced slbo activity is not sufficient to trigger precocious Cut expression before 10B.

High Cut levels in the centripetal FC is regulated by autoactivation

Previously, we have shown that Notch signaling tightly regulates Slbo expression in the centripetal FC: at 10A, Notch blocks Slbo autorepression so that high levels of Slbo expression can be detected (Fig. 2A); at 10B, decreased Notch signaling permits Slbo autorepression with reduced levels of Slbo as a consequence (Levine et al., 2007). Because Notch represses Cut at early stages of oogenesis (Sun and Deng, 2005), we tested whether Notch represses Cut at later stages by examining Cut levels in clones mutant for Notch (N55e11; Figs. 4A–E) or in clones expressing an activated version of the Notch receptor (Nintr; Fig. 4F).

Consistent with previous reports (Sun and Deng, 2005), N55e11 null clones resulted in increased Cut expression at stages 9 and 10A (Figs. 4A,B). At early 10B, N55e11 clones showed slightly reduced Cut accumulation compared to 10A clones stained in parallel (cf. Figs. 4B to 4C). This suggests that ectopic Cut expression in these 10B Notch
mutant clones may reflect perdurance of Cut from earlier stages. Consistent with this, at late 10B no increase in Cut expression above normal levels can be detected in Notch mutant clones, located either in the centripetal FC (Figs. 4D,E) or in the main body FC (Fig. 4E). As well, the onset of Cut expression at 10B appears normal in N⁰ ovaries shifted to the restricted temperature (data not shown; Xu et al., 1992; Dobens et al., 2005) and in clones of fringe or double mutant Delta and Serrate clones (data not shown). Misexpression of the intracellular portion of the Notch receptor, which activates Notch signaling, has been shown to repress Cut expression prior to stage 7 (Sun and Deng, 2005). We used the slbo2.6GAL4 driver to misexpress Nintra in the FC specifically at 10B and observed no repression of late Cut expression in the centripetal FC (Fig. 4F). Interestingly, scattered groups of GFP positive cells misexpressing Nintra that were located in the main body FC did repress effectively the low levels of Cut found there (Fig. 4F, arrow) indicating that Cut expression in the main body FC remains sensitive to ectopic Notch signaling, while in Cut regulation in the centripetal FC is not.

Because cut activates its own expression in some embryonic cell types (Blochlinger et al., 1991), we examined whether cut regulates its own expression in the FC. Cut protein levels were significantly reduced in positively marked mainbody FC clones of the protein null cutDB7 allele (Fig. 4G), indicating this allele is not a protein null and would be useful to examine Cut autoactivation. To further test this, we used hsGAL4 to transiently express UAS-Cut in this tissue. Following a 30 min heat shock induction of hsGAL4, UAS-Cut females (Brand and Perrimon, 1993), Cut levels increased from 3 to 6 h after recovery (data not shown). In contrast, a similar transient induction of hsGAL4,UAS-Slbo led to peak Slbo levels at 2 H and undetectable Slbo by 4 h (data not shown). By 24 h, very high levels of Cut could be detected throughout the FC layer in degrading egg chambers (Fig. 4I). Similar results were obtained following transient hsCut misexpression (data not shown). We conclude that Cut is sufficient to activate its own expression in all FC sub-types, but autoactivation has significance only for highest Cut levels in the centripetal FC.

cut and slbo have opposing effects on follicle cell polarity and apical constriction

High levels of Cut in the centripetal FC coincide with a their apical constriction at stage 10B (Figs. 5A,B) and is attendant with increased accumulation of several apical junction (AJ) components including DE-cadherin (Fig. 5B), β-catenin and F-actin (Tepass et al., 2000; Yagi and Takeichi, 2000; Gumbiner, 2005). Mutations in DE-cadherin and non-muscle myosin (NMM) have been shown to result in defective centripetal migration, indicating cell shape changes associated with this migration require cell–cell cohesion and coordinated cytoskeletal activity (Edwards and Kiehart, 1996; Tepass et al., 1996; Niewiadomska et al., 1999). To examine the significance of high Cut levels for FC shapes, we produced FLP-out Actin5C-GAL4 clones misexpressing UAS-Cut in the FC layer and noted the effect on both cell shapes and accumulation of AJ components. As can be seen in Fig. 5C, Cut-expressing clones detected by Cut antisera showed levels of Cut
comparable to the high Cut expression in the centripetal FC. When marked by UAS-lacZ and detected by histological staining, Cut-expressing clones showed two conspicuous phenotypes (Figs. 5D,E): in the columnar FC, clones were thickened compared to adjacent cells (Fig. 5D, arrow); and in the squamous FC, clones failed to flatten properly (cf. three closely arranged nuclei in Fig. 5D with WT arrangement in Fig. 5E; also see Supplemental Fig. 3C).

These phenotypes led us to examine the effect of Cut expression on epithelial polarity. As can be seen in Fig. 5F, FLP-out Cut-expressing clones in the main body at 10B showed increased DE-cadherin accumulation to levels comparable to centripetal FC (data not shown). Confocal cross-sections (Figs. 5F-I, panels from left to right, respectively, show sections of the FC epithelia from basal to apical sides) revealed that cells in Cut-expressing clones constrict apically...
and accumulate DE-cadherin (Fig. 5F), Armadillo (Fig. 5G) and F-actin (Fig. 5H) strongest at the apical vertices where cells in the clone make contact. As can be seen in Fig. 5F, right-most panel, the concentration of apically polarized DE-cadherin was greatest at areas of contact between the GFP positive, Cut-expressing cells, implying that these clones have a greater affinity for one another compared to the flanking WT cells. Cut-expressing cells can appear to pile up on adjacent cells in cross-section (Fig. 6B), but the epithelium remains intact. This collective change in cell shape and affinity in Cut-expressing clones lead to the loss of the normal FC hexagonal arrangements (numbered 0–6 in Fig. 5J) with the formation of aberrant FC contacts in small Cut-expressing clones of either five neighboring cells (numbered 1*–5*).
and outlined in Fig. 5) or four neighbors (not shown). In larger clones, the areas of contact between Cut-expressing cells and wild type cells resulted in a round clone shape (Fig. 5K), a cell arrangement that likely reflects differences in DE-cadherin levels and affinity (Carthew, 2005).

The striking effect of Cut misexpression on the apical face of the FC epithelium led us to examine the basolateral FC face of Cut clones. Basolateral junction marker Discs Large (Dlg) levels were increased overall in Cut misexpressing clones, but no change in either basal or apical accumulation of Dlg was evident (Fig. 5I). These data show that misexpression of Cut in the main body columnar FC is sufficient to remodel apical but not basal junctions, resulting in supracellular reorganization of the cytoskeleton that resemble the centripetally migrating FC at stage 10B (Fig. 5B).

Given the antagonistic effects of Cut and sblo in the FC, we examined more closely the effect of both on cell polarity in late stage egg chambers. Consistent with an opposing interaction between Cut

![Fig. 6. Cut and Slbo have opposing effects on FC cell shapes and polarity. (A–F). Cross-section of FC epithelium reveals effects of Cut and Slbo on cell polarity. Apical is to the top in all.

(A). DE-Cadherin (red) is apically localized in the FC (arrowhead) at stage 10. DNA is stained with Sytox-green. (B). FLP-out clones expressing high levels of Cut (GFP, green) accumulate high levels of apical DE-cadherin (arrowhead, red, cross-section shown). Genotype in B and C: Actin5C-CD2-GAL4;UAS-Cut;UAS-GFP. Phenotype observed in 12/12 egg chambers. (C). FLPOut clones expressing high levels of Cut (GFP, green) accumulate high levels of apical β-catenin (arrowhead, red, cross-section shown). Phenotype observed in 8/8 egg chambers. (D). Positively marked cut mutant clones (green) exhibit diffuse basolateral accumulation of DE-cadherin (arrowhead, red, cross-section shown). Genotype in D, E and H: FRT19A, tubP-GAL80 hsFLP1 w- / FRT19A cut145; UAS-mCD8::GFP.L; UAS-SrcGFP. Phenotype observed in 3/3 egg chambers. (E). Positively marked cut mutant clones (green) exhibit ectopic basolateral accumulation of β-catenin (arrowhead, red, cross-section shown). Phenotype observed in 5/5 egg chambers. (F). FLPOut clones expressing high levels of Slbo (GFP, green) exhibit ectopic basolateral accumulation of DE-cadherin (arrowhead, red, cross-section shown). Genotype: y1 w*; P[w-mc3=UAS-GAL4y2; P[w-mc=UAS-GFP.S65T];UAS-Slbo;UAS-GFP. Phenotype observed in 6/6 egg chambers. (G). Anterior slbo mutant clones (marked by absence of GFP) show increased accumulation DE-cadherin and convergent phenotype (arrow, red, surface-section shown). Genotype: hsFLP1; FRT42slboex2d / FRT42 UbiGFP. Phenotype observed in 3/5 anterior clones. (H). Anterior cut mutant clone (positively marked by GFP) show decreased and disorganized accumulation DE-cadherin (arrow, red, surface-section shown). Phenotype observed in 5/6 anterior clones.
and Slbo, UAS-Slbo FLP-out clones resulted in elevated levels of DE-Cadherin accumulating at basolateral positions (Fig. 6F), a phenotype similar to the expression seen in cut^{145} mutant clones (cf. Figs. 6D and 6F). FLP-out clones that co-expressed Slbo and Cut showed increased DE-Cadherin levels but the impact of Slbo co-expression on apical constriction phenotypes were difficult to interpret (data not shown). slbo^{026} null clones recovered in the main body FC prior to stage 10B did not have any effect on DE-cadherin levels nor did late slbo^{026} clones located posterior to the centripetal FC, where slbo levels are low (data not shown). In contrast, late stage slbo^{026} mutant clones that contact the centripetal FC resulted in increased DE-cadherin levels and convergence of cells to the clone’s center (Fig. 6G), a phenotype superficially similar to that seen in FLP-out Cut-expressing clones. Larger anterior slbo^{026} mutant clones led to a breakdown in FC epithelial integrity and egg chamber collapse and hence were difficult to document (data not shown). These data suggest that reduction of Slbo activity in anterior cells is necessary for apical constriction of cells in the sheet.

slbo and cut have opposing effects on Fas2 expression to regulate border cell migration

The opposing effects of Cut and Slbo on cell shapes and AJ accumulation during centripetal migration led us to examine their interactions during border cell migration. Cut protein accumulates in the pair of polar cells (Fig. 7A, arrowhead), while Slbo protein is expressed in both the polar cells and border cell rosette (Fig. 7A, arrow). The weak slbo mutant slbo^{01130}/slbo^{01336} resulted in reduced Slbo protein accumulation throughout the border cells (Fig. 7B') and ectopic, nuclear Cut accumulation in the rosette cells (Fig. 7B', arrow), indicating that slbo represses Cut in these cells. We have shown previously that slbo represses its own expression measured by sbo-lacZ in the centripetal FC (Levine et al., 2007) and here we observed that in the slbo transheterozygous mutant slbo^{01130}/slbo^{ex2}, (1) misexpression of Cut occurred in the rosette cells (Fig. 7D', weak misexpression in this genotype is marked by arrow) and (2) increased slbo-lacZ (Fig. 7D') expression occurred throughout the border cell cluster, including the polar cells that do not strongly express the slbo-lacZ reporter in the WT (cf. Fig. 7D' to WT in 7C' stained in parallel). Consistent with our observations in the centripetal FC, misexpression of Cut in the border cell rosette using either the FLP-out (Fig. 2C) or Slbo2.6Gal4 drivers (North, 1996) led to a reduction in Slbo levels (Figs. 7E,E') and a block in border cell migration (Fig. 7F). These data indicate that patterning of cut and sbo expression in the border cell cluster and centripetal FC relies on both cross repression and sbo autorepression (Fig. 2C and Levine et al., 2007).

In the course of these experiments we noted that Cut expression in wild type follicle cells parallels closely the reported expression of Fasciclin2 (Fas2; Hummel et al., 2000), a transmembrane cell adhesion molecule of the immunoglobulin superfamily that binds to the scaffolding protein Discs Large (Dlg; Szafranski and Goode, 2004). Prior to stage 7, Cut and Fas2, as detected by monoclonal antisera (1D4; Hummel et al., 2000), are co-expressed in all FC. At stage 7, accumulation of both proteins decreases in all cells except the polar cells (cf. Figs. 7A' and G'). In these cells, Fas2 accumulates in a polarized manner (1) at the membrane where the two polar cells contact and (2) apically at the leading edge of border cell migration (Szafranski and Goode, 2004). Szafranski and Goode (2004) showed that ectopic Fas2 expression in the border cell rosette disrupts border cell migration and concluded that Fas2 restricted to the polar cells is required to control the timing of border cell cluster delamination and the speed and direction of border cell migration.

Given both the similarities of both the expression patterns of Fas2 and cut in the pole cells and misexpression phenotypes on border cell migration, we tested if cut regulates Fas2 in this tissue. cut mutant clones in the outer rosette cells had no effect on border cell migration (not shown) consistent with low levels of Cut in these cells. Clones could not be recovered in the polar cells during their migration so we examined Fas2 levels following Slbo2.6Gal4 misexpression of Cut in the rosette border cells. Misexpression of Cut led to increased Fas2 in the rosette cells (Figs. 7H,H') resulting in reduced Fas2 at the interface of the polar FC and mis-accumulation of Fas2 at rosette cell/polar cell interfaces (Fig. 7H') in clusters that failed to migrate, a phenotype identical to the effect of Fas2 misexpression in the rosette cells (Szafranski and Goode, 2004).

In the main body FC, Fas2 and Cut have overlapping patterns of expression at several stages, indicating that Cut may regulate Fas2 in these cells. Prior to stage 6, positively marked cut mutant clones in the FC resulted in cell autonomous reduction of Fas2 levels and loss of basolateral Fas2 accumulation (Figs. 7I,I'). After stage 6 when Cut levels are low, FLP-out Cut-expressing clones recovered in the mainbody FC resulted in high levels of ectopic Fas2 (Fig. 7J'). At stage 10, Fas2 expression in the dorsal centripetal FC appears to overlap with Cut expression and consistent with activation by Cut at this stage, cut mutant clones led to reduced Fas2 in the dorsal centripetal FC (Figs. 7K,K') and FLP-out clones expressing Cut in the main body FC led to ectopic Fas2 expression (Figs. 7L'). We conclude that cut is necessary and sufficient for restricted Fas2 expression in the centripetal FC and border cell cluster and Fas2 regulation by Cut is important for cell migration of both cell types.

Discussion

cut directs apical constriction of anterior FC

The centripetal FC are patterned at stage 10A and then undergo a coordinated apical cell constriction at 10B leading to a collective involution of the FC epithelial sheet, which penetrates between the
germ line oocyte and nurse cells from stages 11–12. From a genetic screen for candidate targets of bunched repression that are expressed in the centripetal FC during stage 10, we identified Cut. Classic cut mutant phenotypes include wing margin notches and leg malformations and molecularly these map to a large and complex regulatory region that exerts tissue-specific control of cut expression; cut null mutations are embryonic lethal with defects in neural patterning (Johnson and Judd, 1979; Jack and DeLotto, 1995).

cut encodes a member of the Cux family of proteins found throughout the metazoan lineage (Alcalay and Vanden Heuvel, 2009); these homologs are expressed in diverse tissues and act generally to either prevent tissue differentiation or promote mitotic cycle entry in dividing cells or specify cell fate in postmitotic cells (reviewed in Nepveu, 2001). Cux family members are distinguished by a homeobox and multiple Cut repeats, which are conserved DNA binding motifs thought to act in a combinatorial manner to allow a single Cut-like protein to bind to diverse DNA sequences and regulate distinct promoters. To add to this complexity, distinct isoforms of human CDP have specific effects on cell differentiation and cancer (Goulet et al., 2002). Splice isoforms of the Drosophila cut gene have been detected
recently, but their specific functions and regulation are untested.

Here we show that high levels of Cut expression in the centripetal FC correlate with the apical constriction and coordinated involution of these cells during centripetal migration at stage 10B of oogenesis. This collective cell shape change can be recapitulated by misexpression of high levels of Cut in the columnar mainbody FC. Interestingly Cut misexpressing clones show supracellular cytoskeletal reorganization, remodeling cadherin-based interactions between expressing and non-expressing cells adjacent to the clone. Supracellular reorganization of cadherin–actin networks is associated with protrusion activities and retraction dynamics that involve many cells (reviewed in Friedl et al., 2004), but the mechanism underlying these shared and coordinated behaviors is unclear. cut mutant clones led to reduced DE-cadherin levels but only subtle effects on apical invagination. Because only small cut clones could be recovered due to the early requirement for cut in cell proliferation, it is possible that these small clones are effectively carried by their neighbors during the invagination process.

The correlation between high Cut expression levels in the apically constricted centripetal FC compared to lower Cut levels in the columnar-shaped main body FC recalls the distinct Cut levels in sensory neurons with distinct dendritic branching patterns: high Cut in neurons with extensive unbranched terminal protrusions, medium levels in neurons with complex arbor, and low Cut in neurons with simple dendrites (Gruener et al., 2003). Thus Cut levels may dictate cell shapes in some tissues. It is noteworthy that high Cut levels, either normally in the centripetal FC or in FC misexpressing Cut, result in no loss of epithelial character, quite unlike invasive phenotypes associated with mutations in Fas2, dgl, lethal giant larva (lgl), and Neuroglian (Nrg, Goode et al., 1992, 1996; Szafranski and Goode, 2004). The effect of Cut on the apical junction but not the basal junction may explain failure of these cells to lose the epithelium.

Cross repression between cut and slbo refines a genetic switch in gene expression

Cut and Slbo exhibit overlapping expression patterns in the leading edge FC and pole cells but complementary expression in the centripetal FC and border cell rosette (Figs. 1, 2 and 7). The opposing activities of their mammalian counterparts suggested to us that cut and slbo might repress each other’s expression in the latter cell types, a notion that is supported by our demonstration that (1) Slbo or Cut misexpression is sufficient to repress the expression of the reciprocal gene in the centripetal FC and border cell rosette and (2) cut mutant clones show increased slbo-lacZ and Slbo protein expression and slbo mutants show ectopic Cut in the border cells.

The switch from Slbo at 10A to high levels of Cut at 10B in the centripetal FC coincides with increased accumulation of key apical junction components and our genetic manipulations show that cut and slbo have opposing effects on the accumulation of DE-cadherin in this cell type. Cadherins are the dominant mediator of collective cell interactions in cells undergoing diverse motile processes such as gastrulation, primitive streak and neural crest migration, epiblast ingestion (Edelman et al., 1983; Hatta and Takeichi, 1986; Takeichi, 1988; Takeichi et al., 2000) as well as tumor invasiveness (reviewed in Peinado et al., 2004). Increased expression of cadherin isoforms and switching between cadherin types occurs during cell migration and positive and negative transcriptional regulation of E- and N-cadherin expression is exerted in part by a number of zinc-finger transcription factors including Snail, Slug and Twist (reviewed in Peinado et al., 2007). In Drosophila, Twist promotes motile phenotypes by directing E- to N-cadherin switching (Oda et al., 1998; Alexander et al., 2006). While no role for vertebrate Cux-like proteins in cadherin switching has been identified previously, the cut mammalian homologue CUTC mediates TGFβ1 enhancement of cancer cell motility and invasiveness by via transcriptional upregulation of N-cadherin (Maeda et al., 2005) and WNT5A (Michl et al., 2005; Michl and Downward, 2006).

Genetic and molecular approaches have characterized the large upstream regulatory region that controls Cut expression in diverse tissues (e.g. Bodmer et al., 1987; Liu and Jack, 1992) and several genes required for Cut expression in the embryonic nervous system have been identified (reviewed in Bellaiche and Schweiguth, 2001). Notch signaling has tissue-specific effects on Cut throughout development: during wing patterning, Notch activates Cut at the margin (de Celis and Bray, 1997; Michelli et al., 1997), while in the ovary Cut is repressed by Notch at stages 7–10A (Sun and Deng, 2005). Our data show that during late oogenesis, Cut levels in the centripetal FC at 10B increase as Notch-activated slbo expression decreases. Thus it was surprising that Notch mutant clones and Nintra misexpression had no effect on Cut levels in the centripetal FC, leading us to conclude that Cut activation in these cells occurs in a manner independent of Notch. In the mainbody FC by contrast, Nintra misexpression did effectively repress low Cut levels, indicating that distinct regulatory pathways regulate Cut expression in the centripetal FC compared to the main body FC. Consistent with this, cut145 clones in the centripetal FC show significant reduction in Cut levels, while mutant clones of cut145 recovered in the main body FC show no reduction in Cut expression. Evidence that Cut activates its own expression in embryonic cell types comes from experiments showing that transient, ubiquitous misexpression of a heat shock inducible Cut transgene during embryogenesis was sufficient for persistent Cut accumulation in transformed chordotonal cells, but not in other cell types (Blochlinger et al., 1991), and similarly, when we expressed heat shock Cut transiently in the FC, we observed a continuous increase in Cut levels so that by 24 h, Cut levels were very high in all FC subtypes in egg chambers that appeared degraded. We conclude that if expressed at high enough levels, Cut can activate its own expression throughout the FC, but Cut autoactivation normally occurs only in the centripetal FC to achieve the high Cut levels necessary to direct apical constriction of these migrating cells.

A working model summarized in Fig. 8 states that during border cell migration (8A), Cut is expressed in the polar cells and Slbo is expressed throughout the border cells, including both the polar cells and rosette cells and cross repression maintains this pattern of expression. In the centripetal FC (8B), transient Slbo expression at stage 10A represses both Cut expression (here) and its own expression (Levine et al., 2007). At 10B, Cut represses Slbo and activates high levels of its own expression in the centripetal FC as these constrict apically. We showed previously that in the centripetal FC, Notch signaling activates slbo-lacZ expression (Levine et al., 2007) while here we show that ectopic Nintra has no effect on late Cut expression in this tissue (Fig. 4F). This is difficult to reconcile with the ability of Slbo misexpression to effectively repress Cut (Fig. 2F). Based on work here, we note that slbo-lacZ levels can be high in the border cells when Slbo protein levels are low (cf. Fig. 7B* to 7D*). We speculate that Nintra blocks normal slbo autorepression to increase slbo-lacZ levels with slight to no affect on Slbo protein levels (unpublished observations) while Flip-out Slbo circumvents auto-repression entirely and effectively represses Cut. Epistasis experiments should elucidate further the significance of these proposed interactions for the Slbo/Cut expression switch.

Opposing activities of cut and slbo pattern border cell migration

Formation of the border cell cluster begins at stage 7 when the cytokine-like signal Unpaired, which is produced by the polar cells, activates the JAK/STAT pathway in neighboring cells recruiting these to the outer border cell fate. JAK/STAT signaling in turn activates a number of downstream border cell migration-associated genes, including slbo (Xi et al., 2003; Silver et al., 2005). Stage 7 coincides with the loss of Cut in all FC except the polar cells while Slbo protein is
expressed in both the inner polar and outer rosette cells of the border cell cluster (Fig. 7A*). The overlapping Slbo/Cut pattern is critical for proper border cell migration because in slbo mutants, ectopic Cut accumulates in the rosette cells of border cell clusters that fail to migrate. Complementarily, misexpression of Cut in the outer border cells leads to a block in border cell migration and Slbo repression. Cut activates Fas2 in both the pole cells and centripetal FC, the latter suggesting that polarized Fas2 coordinates centripetal FC invagination in addition to its role in polarizing the pole cell pair.

Superficially, the border cells and the centripetally migrating FC use different strategies to accomplish their respective migrations: the border cell cluster undergoes an epithelial to mesenchymal transition to delaminate from the FC layer and migrate as a cluster of cells while the centripetal FC retain the structure of an epithelial sheet throughout their involution. Slbo/Cut expression seems distinct as well: their patterns are complementary in distinct cell types but contemporaneous (Fig. 8A,C,D). Similarities in these tissues have been noted: the border cell cluster retains a quasi-epithelial organization throughout its migration; both migrating groups of cells presumably make similar changes in affinities to enter the germ cell environment and proceed following similar cues; and both migrating groups are marked by a leading edge cell with clear differences in cell morphology and gene expression (Borghese et al., 2006; Wang et al., 2006; Levine et al., 2007). Because diffuse, punctate DE-cadherin accumulation in the rosette cells of the migrating border cell cluster has been attributed to slbo-directed rapid endosomal-mediated turnover (Niewiadomska et al., 1999; Pacquelet and Rorth, 2005), it is possible that transient expression of slbo in the centripetal FC (Levine et al., 2007) at stage 10A initiates rapid endosomal-mediated turnover necessary for the launch of invagination. And as Cut regulates Fas2 to organize the border cell cluster, Cut at 10B directs polarized accumulation of DE-cadherin and Fas2 to coordinate apical constriction and cell sheet spreading. Thus it is likely that Cut/Slbo interactions during both border cell and centripetal migration regulate an underlying common molecular mechanism that works in both multicellular movements.

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Appendix A. Supplementary data


References


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