**Introduction**

The development of a multicellular organism requires regulation of cellular behavior that is highly orchestrated temporally and spatially. The coordination of cellular behavior at the molecular level is made possible by mechanisms of cell-cell signaling, which permit cells to influence each other’s fate and behavior. One of the most important mechanisms of cell signaling is mediated by Notch, a transmembrane receptor that coordinates a signaling system known as the Notch pathway, which is evolutionarily conserved across metazoans and has a key role in cell-fate determination and pattern formation (Artavanis-Tsakonas et al., 1999; Bray, 1998; Greenwald, 1998; Schweisguth, 2004). Notch signals through a conserved and regulated intramembrane proteolysis (Mumm and Kopan, 2000; Selkoe and Kopan, 2003). Binding between the extracellular domain of the receptor and the ligand activates the proteolysis and the release of the intracellular domain of Notch (NICD), which is then shuttled into the nucleus and converts the transcriptional repressor Su(H) into an activator.

The Notch pathway has a wide range of biological roles in Drosophila development. Notably, it is implicated in a lateral inhibition process during neurogenesis that restricts sensory-organ formation and boundary formation in the wing imaginal disc (Beatus and Lendahl, 1998; Bray, 1998). In oogenesis, Notch signaling has been shown to be implicated in the specification of the polar follicle cells and regulation of cell-cycle programs in the follicle-cell epithelium (Althauser et al., 2005; Deng et al., 2001; Grammont and Irvine, 2001; Lopez-Schier and St Johnston, 2001; Ruohola et al., 1991; Torres et al., 2003).

**Summary**

During Drosophila mid-oogenesis, follicular epithelial cells switch from the mitotic cycle to the specialized endocycle in which the M phase is skipped. The switch, along with cell differentiation in follicle cells, is induced by Notch signaling. We show that the homeodomain gene cut functions as a linker between Notch and genes that are involved in cell-cycle progression. Cut was expressed in proliferating follicle cells but not in cells in the endocycle. Downregulation of Cut expression was controlled by the Notch pathway and was essential for follicle cells to differentiate and to enter the endocycle properly. cut-mutant follicle cells entered the endocycle and differentiated prematurely in a cell-autonomous manner.

By contrast, prolonged expression of Cut caused defects in the mitotic cycle/endocycle switch. These cells continued to express an essential mitotic cyclin, Cyclin A, which is normally degraded by the Fizzy-related-APC/C ubiquitin proteosome system during the endocycle. Cut promoted Cyclin A expression by negatively regulating Fizzy-related. Our data suggest that Cut functions in regulating both cell differentiation and the cell cycle, and that downregulation of Cut by Notch contributes to the mitotic cycle/endocycle switch and cell differentiation in follicle cells.

**Key words:** cut, Endocycle, Cell cycle transition, Notch signaling, Drosophila melanogaster
CCAAT displacement protein (CDP) (Nepveu, 2001). Unlike homologues have been identified in vertebrates, e.g. the Brewster et al., 2001). In addition, Cut expression has been shown in vertebrates (e.g. to be required (Blochlinger et al., 1991; Lopez-Schier and St Johnston, 2001). At stage 10B, main-body follicle cells exit the mitotic cycle and undergo amplification of specific genomic regions (e.g. the chorion gene region; this stage could therefore be referred to as the chorion-gene amplification or amplification stage; Fig. 1A) (Calvi et al., 1998; Caiyirlioglu et al., 2001).

The switch from the mitotic cycle to the endocycle is induced by Notch signaling (Deng et al., 2001; Lopez-Schier and St Johnston, 2001). At around stage 6 of oogenesis, DI expression is elevated in the germline cells, coincident with the transition from the mitotic cycle to the endocycle and downregulation of FasIII. Indeed, removal of DI function in the germline cells or Notch function in the follicle cells keeps follicle cells in the mitotic cycle during mid-oogenesis (stages 7 to 10A). In addition, the immature follicle-cell-fate marker, FasIII, remains expressed at a high level in these cells. Notch-dependent cell-cycle transition and cell differentiation require protein cleavage, as γ-secretase components Presenilin (Psn) and Nicastrin are involved in this process (Lopez-Schier and St Johnston, 2001; Lopez-Schier and St Johnston, 2002). In addition, the nuclear effector of Notch signaling, Su(H), is needed for the switch (Deng et al., 2001; Lopez-Schier and St Johnston, 2001). Notch signaling negatively regulates String (Stg)/cdc25 phosphatase and G2 cyclins and positively regulates Fizzy-related/Hec1/Cdh1 (Fzr) (Deng et al., 2001; Schaeffer et al., 2004; Shcherbata et al., 2004). Fzr, also known as Retina aberrant in pattern, is a conserved WD domain protein. Previous studies have shown that Fzr is required for degeneration of G2 cyclins in an anaphase promoting complex/cyclosome (APC/C) E3 ligase-dependent manner. Loss of fzr function in Drosophila follicle cells causes defects in the mitotic-to-endocycle transition and results in the maintenance of CyclA expression after stage 6 during oogenesis (Schaeffer et al., 2004).

Cut is a DNA binding protein that contains a unique homeodomain and three Cut repeats (Nepveu, 2001). Its expression and function have been characterized in several developmental processes in Drosophila. In wing imaginal discs, it is induced by Notch signaling in the dorsal-ventral (DV) border to maintain the DV boundary (de Celis and Bray, 1997; de Celis et al., 1996). During neurogenesis, Cut is involved in sensory organ differentiation, a process for which Notch function is also required (Blochlinger et al., 1991; Brewster et al., 2001). In addition, Cut expression has been found in follicle cells. It is required for egg-chamber encapsulation and coordination of the germline and follicle-cell differentiation (Jackson and Blochlinger, 1997). Cut homologues have been identified in vertebrates, e.g. the CCAAT displacement protein (CDP) (Nepveu, 2001). Unlike its Drosophila homologue, vertebrate Cut is known to be involved in cell-cycle progression in some cell types (Coqueret et al., 1998). Here we show that, in Drosophila follicle cells, Cut also regulates the cell cycle, a function that may have been conserved during evolution.

Our data show that Cut expression is downregulated by the Notch pathway in follicle cells during mid-oogenesis and that the downregulation is necessary for proper entry of follicle cells into the endocycle and for their differentiation. In our experiments, loss of cut function resulted in premature entry into the endocycle and cell-autonomous differentiation. This promoted CyclA expression by negatively regulating Fzr. Our results suggest that Cut functions as a linker between Notch and genes that are involved in cell-cycle progression and cell differentiation.

Materials and methods

Fly stocks

The following fly stocks were used: N55e11/FRT101 [N55e11 is an amorphic allele of Notch (N); a gift from Y. N. Jan]; FRT 82B DI (Drosophila) is an amorphic allele of DI that is produced by excision of the promoter region, transcription start site, and first exon) (Haltiwanger and Simpson, 1991; Zeng et al., 1998); Su(H) and FRT40A (Su(H) is a strong loss-of-function allele of Su(H), a gift from S. Blair); psnC1/FRT2A (psnC1 is a null mutant of psn (Struhl and Greenwald, 1999)); ctC145 FRT18A (Jackson and Blochlinger, 1997); ctC145 and ctC145 mutant clones, we used yw Ub-GFP[FRT101];MKRS P[y=hsFLP]86E/TM6BTb, hsFLP;FRT2B Ub-GFP/TM3, hsFLP;GFP FRT40A/Cyo, GFP FRT2A, GFP FRT 18A; MKRS P[y=hsFLP]86E/TM6B, and hsFLP FRT18D;MKRS P[y=hsFLP]86E/TM6Bh Tub, respectively. For analysis of the overexpression phenotype, the following stocks were used: UAS-NICD (an active form of Notch (Rauskolb et al., 1999)), UAS-cut (a gift from R. Bodmer), UAS-stg (Reis and Edgar, 2004) and hsFLP;act<CD2<Gal4, UAS-GFP/TM3. The fzd-lacZ line (fzd1032a, a P[lac-W] element inserted in the first intron of the Fizzy-related gene) (Peter et al., 2002) and stg-lacZ line [psztbetaR6.4 (Deng et al., 2001; Lehman et al., 1999)] were used to detect the fzd and stg expression in the Cut-overexpressing follicle-cell clones, respectively. A101/TM3 (neutralized-lacZ) and PZ80/Cyo were also used as the polar-cell-fate marker (Larkin et al., 1996; Adam and Montell, 2004). w1118 was used as a wild-type control.

Generation of follicle cell and germline clones

Fly culture and crosses were performed according to standard procedures. Mutant clones were generated by mitotic recombination with the FLP/FRT system (Xu and Rubin, 1993) by either X-chromosome or third-chromosome heat-shock-inducible flippase (hsFLP). After heat shock, all flies were put in fresh food vials with wet yeast for 1-2 days before dissection. Clones were marked with ubi-GFP or histone-GFP (hGFP) (Maines et al., 2004). To obtain germline clones, we heat shocked flies as second- and third-instar larvae for 2 hours at 37°C (Deng and Ruohola-Baker, 2000). For follicle cell clones, 1- to 5-day-old flies were heat shocked as adults for 1 hour at 37°C and put into fresh food vials. For generation of Cut mutant clones in early-stage egg chambers, flies were dissected 1-2 days after heat shock. For generation of flip-out Gal4 overexpression clones, adult flies were heat shocked for 45 minutes and put into fresh food vials for 2-3 days before dissection.
Immunocytochemistry and BrdU labeling

Immunocytochemistry was carried out as described previously (Deng et al., 2001). The following antibodies were used: mouse anti-FasIII 1:15, mouse anti-CycB 1:5, mouse anti-Cut 1:15, mouse anti-Eya 1:10 (Developmental Studies Hybridoma Bank; DSHB), mouse anti-BrdU 1:50 (BD Bioscience), rabbit anti-ORC2 1:3000 (a gift from S. P. Bell) (Royzman et al., 1999), rabbit anti-Bib 1:2000 (a gift from Y. Jan), rabbit anti-PH3 1:200 (Upstate Biotechnology, NY, USA), rabbit anti-CycA 1:500 (a gift from C. Lehner), rabbit anti-β-galactosidase 1:5000 (Sigma), guinea pig anti-CycE 1:500 (a gift from T. Orr-Weaver), Alexa Fluor 546 goat anti-guinea pig, Alexa Fluor 546 or 633 goat anti-mouse (1:500), and Alexa Fluor 488, 546 or 633 goat anti-rabbit (1:500) (Molecular Probes). Images were acquired with a Zeiss LSM 510 confocal microscope and assembled in Adobe Photoshop.

BrdU labeling was carried out as described by Calvi et al. (Calvi et al., 1998), with the following modifications. Dissected ovaries were incubated in 0.5 mg/ml BrdU (Sigma) in Grace’s medium (Mediatech-Cellgro, VA) for 1 hour at room temperature, followed by fixation in EM-grade formaldehyde/buffer B/dH2O (1:1:4) for 15 minutes. Ovaries were then washed twice (15 minutes each rinse) in PBS + 0.4% Triton X-100, twice (15 minutes each rinse) in DNase I reaction buffer (66 mM Tris, pH 7.5, 5 mM MgCl2, 1 mM fresh 2-mercaptoethanol), and incubated in 12 μl DNase I (25 mg/ml)/1 ml DNase reaction buffer at 37°C for 45 minutes, washed three times (1 minute each wash) then once more (15 minutes) in PBT, and blocked in PBT with 5% normal goat serum overnight at 4°C. BrdU was detected with the mouse anti-BrdU antibody described above.

Results

Homeodomain gene cut is downregulated by Notch signaling in follicle cells undergoing endoreplication

To determine how Notch regulates the switch of cell-cycle programs, we looked for genes that could act between the Notch pathway and the intrinsic cell-cycle machinery. Cut was a candidate because it has been shown to be expressed in follicle cells from germarium region 2B to stage 6 of oogenesis (Jackson and Blochlinger, 1997), during which time the follicle cells are in the mitotic cycle. Its expression is downregulated in main-body follicle cells from stage 7 to stage 10A and reappears afterwards (Fig. 1B). To determine whether downregulation of Cut during mid-oogenesis is coupled with the exit from the mitotic cycle in follicle cells, we double-labeled the wild-type egg chamber with antibodies against Cut and the mitotic marker PH3. Indeed, downregulation of Cut and PH3 were almost concurrent. During stage 6, we could detect Cut in all follicle cells, whereas PH3 was detected in cells in late G2 or M phases. At stage 7, staining with both antibodies was diminished in main-body follicle cells (Fig. 1B).

Because downregulation of Cut expression in main-body follicle cells was coupled with the switch from the mitotic cycle to the endocycle, a process induced by Notch signaling, we hypothesized that the Notch pathway negatively regulates Cut during the cell-cycle switch. To test this hypothesis, we first generated Dl germline clones using the FLP/FRT-induced mitotic recombination technique (Xu and Rubin, 1993). In follicle cells that surrounded the Dl germline clone, Cut was continuously expressed after stage 6 (Fig. 2A,A’). Next, we examined Cut expression in Notch follicle-cell clones, as Notch is required cell-autonomously in follicle cells for the mitotic cycle/endocycle switch (Deng et al., 2001; Lopez-Schier and St Johnston, 2001). As expected, in egg chambers older than stage 6, Notch mutant follicle cells upregulated Cut expression in a cell-autonomous fashion, whereas their wild-type

Fig. 1. Switch of cell-cycle programs and the Cut expression pattern in follicle cells. (A) During Drosophila oogenesis, somatically derived follicle cells undergo two cell-cycle switches: (1) the mitotic cycle to endocycle switch and (2) the endocycle to gene-amplification switch. From the gerarium (G) to stage (S) 6, follicle cells undergo unsynchronized mitotic cycles. During stages 7 to 10A, these cells go through three rounds of endoreplication and thereafter switch to a localized amplification pattern characteristic of chorion gene amplification. (B) Cut expression in follicle cells. Expression (shown in red) began in region 2B of the gerarium. It persisted in all follicle cells, including the polar cells and the interfollicular stalk cells, until about stage 6 and diminished afterwards, concurrent with the first cell-cycle switch. PH3 (shown in green) was used to mark the M phase of the mitotic cycle. Between stages 7 and 10A of oogenesis, Cut expression ceased in all follicle cells except the polar cells. At about stage 10B, Cut expression resumed in the columnar follicle cells that surround the oocyte. DAPI (in blue) was used to reveal the nuclei.
neighbors showed no Cut expression (Fig. 2B,B’). In addition to Notch, psn and Su(H) clones resulted in upregulated Cut expression in egg chambers beyond stage 6 (Fig. 2C,C’,D,D’), but Su(H) clones had a less severe effect in regulating Cut expression. Cut expression was rarely detected in Su(H) clones in stage 9 or 10A egg chambers, but its expression was very common in Notch and psn clones (data not shown). Nonetheless, the findings that DI germline clones and Notch, psn and Su(H) follicle-cell clones all induced Cut expression during mid-oogenesis suggest that the canonical Notch pathway is required for downregulation of Cut expression at the mitotic cycle/endocycle switch.

Loss of Cut function in follicle cells results in precocious entry into the endocycle in a cell-autonomous manner

Because Cut is expressed in proliferating follicle cells and is downregulated when follicle cells exit the mitotic cycle, we set out to determine whether Cut function is required for follicle-cell proliferation. A null allele of cut, ctΔb7 (Brewster and Bodmer, 1995), was recombined into the FRT18D chromosome to generate mitotic clones in follicle cells. We found that ctΔb7 follicle-cell clones had larger nuclei than their wild-type neighbors (Fig. 3A,B), a phenotype similar to that previously reported in ctΔ145 mosaics (Jackson and Blochlinger, 1997). Although ctΔ145 is also a null allele of cut, it produces an extremely low frequency of clones (Jackson and Blochlinger, 1997) (data not shown), so it is not suitable for mosaic studies. To determine whether the ctΔb7 follicle-cell clones prematurely exit the mitotic cycle, we focused on follicle-cell clones that were in young egg chambers (prior to the mitotic cycle/endocycle switch). We generated these clones 24-48 hours before fixation to avoid analyzing clones that were produced when follicle cells were still in the germline, where cut is involved in egg-chamber encapsulation (Jackson and Blochlinger, 1997). During stages 4-6, the cut-mutant follicle cells already had larger nuclei than their sister clones (Fig. 3B,B’). To determine whether these cells had defects in the follicle-cell mitotic cycle, we used a series of mitotic markers to stain the cut mosaic egg chambers. CycA, the only essential mitotic cyclin in Drosophila (Jacobs et al., 1998), was detected either in the nuclei or in the cytoplasm in about 50% of the cells that were in the mitotic cycle during stages 4-6 (our results) (Shcherbata et al., 2004). In contrast, in ctΔb7 follicle cell clones during these stages, CycA staining was not detected (Fig. 3B,B’). Furthermore, two other mitotic markers, CycB and PH3, were also not detected in ctΔb7 clones during these stages (data not shown). Lack of expression of mitotic markers in cut mutant clones indicates that the cells cease mitotic cycle prior to the developmentally programmed stage. Consistent with this observation, the number of cells in the mutant clone was significantly lower than that in the sister clone. Typically, 2-8 cells were found in each of the ctΔb7 mutant cell clones, whereas a 2- to 5-fold greater number of cells was seen in the sister clone (Fig. 3C,C’). The lower number of cells present in the cut clone and the lack of mitotic marker expression indicate that cut is required for follicle-cell proliferation and maintenance of the mitotic cycle.

Next, we investigated whether ctΔb7 mutant cells undergo the endocycle after exiting the mitotic cycle. CyclE expression and BrdU incorporation oscillate in endocyte cells (Edgar and Orr-Weaver, 2001). In ctΔb7 follicle-cell clones from stages 4-6, CyclE staining was detected in a sporadic pattern (Fig. 3D,D’). In addition, we observed BrdU incorporation in some of these mutant cells (data not shown). Oscillation of CyclE and BrdU incorporation and lack of CycA, CycB and PH3 staining in cut clones suggest that the mutant cells precociously entered the endocycle.

Next, we set out to determine whether the cut mutant cells switched from the endocycle to gene amplification correctly. These cells appeared to incorporate BrdU in a foci-like pattern in endocyte cells already had larger nuclei than their sister clones (Fig. 3C,C’). This result suggests that the switch from the endocycle to gene amplification was not affected, which also indicates that late Cut expression, starting at stage 10B (Fig. 1B), in follicle cells is not essential for chorion gene amplification.

Prolonged Cut expression during mid-oogenesis causes defects in the mitotic cycle/endocycle switch

Because Cut was required for follicle-cell proliferation and its expression was downregulated by Notch signaling upon entry into the endocycle, we set out to determine whether extending Cut expression beyond stage 6 could maintain the follicle cells in a mitotic cycle. Ectopic Cut expression in follicle cells after stage 6 was achieved by means of the flip-out Gal4/UAS technique. DAPI staining showed that Cut-misexpressing cells had slightly smaller nuclei than their wild-type neighbors (Fig. 4A,A’). These cells also appeared to be more densely distributed, a phenotype resembling that of the Notch clones.
(Deng et al., 2001). To determine whether these cells exhibited features of a mitotic cycle, we applied a series of cell-cycle markers to stain the mosaic egg chambers. Interestingly, we found that 62% of egg chambers with Cut-misexpressing clones showed CycA expression during the middle stages, we used a reporter to stain the mosaic egg chambers. Interestingly, we found that 62% of egg chambers with Cut-misexpressing clones (arrow). The sister clones (outlined and marked by higher levels of GFP) had higher levels of Cut expression than the heterozygous cut07 follicle cells. (B,B',B'') cut07 mutant follicle cells had larger nuclei and less CycA expression. In a stage 6 egg chamber, DAPI staining showed that cut07 mutant follicle cells (outlined) had larger nuclei than the wild-type cells. In the clone, CycA expression is absent (red in B and B’), but in the neighboring wild-type cells about half of the cells were CycA positive. (C) Number of nuclei in cut07 mutant clones (blue bars) compared with that in their associated sister clones (purple bars) in stage 10 egg chambers. The x-axis represents the clone number, and the y-axis represents the number of cells per clone or corresponding sister clone. (C’) On average, the number of nuclei in the clonal area was one third of that in the associated sister clone. (D,D’,D’’) CycE (red in D and white in D’) had normal oscillating expression pattern in the clone cells (outlined). (E,E’,E’’) cut07 mutant follicle cells properly switched to the gene-amplification stage. A foci-like pattern of BrdU incorporation (red in E and white in E’) was found in the cut-mutant follicle cells in a stage 10B egg chamber, (e,e’) The circled areas in E and E’ show four dots of BrdU incorporation foci (red in e and white in e’) in each nucleus.

is probably the reason why ectopic CycA was present in Cut-misexpression clones.

To induce the mitotic cycle/endocycle switch, Notch signaling not only promotes M phase exit by upregulating Fzr, it also suppresses Stg, a CDC25 homolog required for G2/M progression (Edgar et al., 1994; Kumagai and Dunphy, 1991). Although misexpression of Cut downregulated Fzr, it did not seem to be able to regulate stg expression. A stg-lacZ reporter gene showed no change of expression in Cut-misexpressing cells (Fig. 4D,D’), but misexpression of both Stg and Cut in follicle cells at stages 7-8 could drive an extra round of cell cycle, as shown by Ph3 staining in some GFP-positive cells (Fig. 4E,E’; 60% of egg chambers counted had PH3-positive cells; n=70). In contrast, misexpression of either Cut or Stg alone was not able to drive an extra round of cell division (data not shown).

To determine whether cells with prolonged Cut expression would proceed to gene amplification, we examined BrdU incorporation and ORC2 and CycE staining in mosaic egg chambers. In wild-type cells, ORC2, a component of the origin recognition complex, is colocalized at the BrdU incorporation foci in cells undergoing chorion gene amplification (Fig. 4F,F’). In Cut-
Downregulation of Cut is required for cell differentiation in follicle cells

During stages 6-7, Notch signaling also induces the follicle cells to differentiate, a step that can be identified by downregulation of FasIII expression (Lopez-Schier and St Johnston, 2001). To determine whether Notch-dependent downregulation of Cut is necessary for cell differentiation, we studied FasIII expression in follicle cells with extended Cut expression between stages 7 and 10. Invariably, a high level of FasIII expression was maintained in cells ectopically expressing Cut (Fig. 5A,A'). During oogenesis, strong FasIII expression is present not only in immature main-body follicle cells but also in polar cells, interfollicular stalk cells, and their precursors. To determine which cell fate Cut-misexpressing cells took, we further stained the egg chamber with an antibody against Big brain (Bib), which is expressed in mature stalk cells and the precursor cells of the polar cell/stalk cell lineage (Larkin et al., 1996). In main-body follicle cells that ectopically expressed Cut, Bib staining was not detected, indicating that the stalk-cell fate or polar/stalk precursor fate was not taken (Fig. 5B,B').

Persistent Cut misexpression/overexpression during mid-oogenesis caused the follicle cells to be more densely distributed and their nuclei to be slightly smaller than those of the wild type. (B,B') Cyclin A (shown in red in B and white in B') was found to be expressed in some of the follicle cells misexpressing Cut in a stage 9 egg chamber, whereas CycA was no longer present in wild-type cells. (C,C') β-gal expression (red in C and white in C') from a fzr enhancer trap line fzr-G0326 was downregulated in follicle-cell clones with overexpression of Cut during mid-oogenesis. (D,D') β-gal expression (red in D and white in D') from stg-lacZ showed no change in cut misexpressing clones during mid-oogenesis. (E,E') PH3 (red in D and white in D') staining was found occasionally during mid-oogenesis in follicle cells misexpressing both Cut and Stg. (F,F',F'') Follicle-cell clones overexpressing Cut in stage 10B egg chambers did not switch to the amplification stage. BrdU incorporation (red in F and white in F') and Orc2 staining (blue in F and white in F'') were not present in the clonal cells (outlined). (G,G') Overexpression of Cut in a stage 10B egg chamber affected the uniform Cyclin E expression pattern. No Cyclin E expression (red in G and white in G') was detected in the clone misexpressing Cut (outlined).
To determine whether Cut is required in main-body follicle cells to maintain an undifferentiated fate, we examined the expression of FasIII and Eya in transient cut^{PZ80} clones at stages 4-6. In most cases, FasIII expression was downregulated at the basal-lateral surface of the follicle cells (Fig. 6A,A',B,B'), but at the apical-lateral region, FasIII expression was still detected (Fig. 6B,B', arrowhead). To determine whether this type of FasIII staining is normally present, we re-examined the expression pattern of FasIII in wild-type follicle cells. Surprisingly, an apical-lateral staining of FasIII was detected in wild-type follicle cells during stages 7-9 (Fig. 6C,C'), in contrast to its location along the entire lateral membrane during earlier stages (Fig. 6D,D'). We therefore concluded that the restriction of FasIII to the apical-lateral membrane reflected follicle-cell differentiation and that cut mutation caused follicle cells to differentiate prematurely. This conclusion was supported by staining for Eya in cut follicle-cell clones, which showed cell-autonomous loss of Eya expression (Fig. 6E,E',F,F'). Lack of Eya is shown in differentiated columnar cells and the polar cells during oogenesis (Bai and Montell, 2002). A polar cell fate marker, PZ80, was not expressed in the clone (Fig. 6F), revealing that these cells did not take the polar-cell fate. Together, these results suggest that cut mutant follicle cells show a differentiated fate and that cut is required to maintain the follicle cells in an immature stage in early oogenesis.

### Discussion

The switch of cell-cycle programs in Drosophila follicle cells provides an excellent opportunity to study how developmental signals control the intrinsic cell-cycle machinery. A switch from the mitotic cycle to the endocycle in follicle cells is induced by the Notch pathway. Cell-cycle regulators such as CycA, CycB, Stg and Fzr are regulated by Notch during this process. In our experiments, the homeodomain gene cut acted between the Notch pathway and some of these cell cycle regulators. Its expression was downregulated by the Notch pathway in main-body follicle cells during the mitotic cycle/endocycle switch. Cut function was required cell-autonomously for maintenance of the mitotic cycle and an immature cell fate in main-body follicle cells. Cut downregulation by Notch signaling was a key step allowing proper entry into the endocycle and cell differentiation. Fzr, an adaptor of the APC/C that promotes endocycle, was negatively regulated by Cut, but Stg was not regulated by Cut (Fig. 7).

### Interaction between the Notch pathway and Cut

Several roles for cut during Drosophila oogenesis have been described (Jackson and Blochlinger, 1997). First, cut negatively interacts with Notch for partitioning of individual germline cysts into egg chambers. Second, cut defines a signaling pathway from the follicle cells to the oocyte to maintain the germline integrity. In addition, the rarely produced cut^{214} mutant follicle-cell clones have fewer, but larger, cells. This last phenotype agrees with our findings that...
cut is required to maintain the follicle cells in mitotic cycle and that cut\textsuperscript{re} mutation results in premature entry into the endocycle. Study of the involvement of cut in this process required generation of clones after the egg chamber exited the gerarium, so that defects caused by gerarium requirements of cut would not interfere. Interestingly, during both egg-chamber encapsulation and cell-cycle switch, cut function was related to the Notch pathway. Cut expression was downregulated by Notch signaling during the cell-cycle transition at stages 6-7. In the gerarium, cut negatively interacts with Notch, as heterozygous cut mutation suppresses the Notch phenotype (Jackson and Blochlinger, 1997). Whether Cut acts as a target of Notch signaling at this stage is unclear. We have studied gain- or loss-of-function clones of Notch prior to stage 6 and detected no obvious change of Cut expression (data not shown), which argues that Cut is not a downstream target of Notch during early oogenesis.

The interaction between Notch and cut is not restricted to the follicle cells during Drosophila development. In wing imaginal discs, cut also interacts with Notch (de Celis and Bray, 1997; de Celis et al., 1996), but the Notch pathway positively regulates Cut expression in DV boundary formation. Clones of Notch eliminate Cut expression cell-autonomously at the DV border, a pattern opposite to that of the follicle-cell-cycle switch. Ectopic expression of constitutively active Notch causes Cut to be ectopically activated in the disc. Notch downstream genes, such as strawberry notch and Enhancer of split (E(spl)), also positively regulate Cut expression in the disc, suggesting Notch-regulated Cut expression is indirect (Majumdar et al., 1997). In follicle cells, downregulation of Cut expression by Notch signaling seems to be indirect as well, because NICD/Su(H) usually functions as a transcriptional activator (Mumm and Kopan, 2000). Downregulation of Cut is probably achieved by a transcriptional repressor activated by NICD/Su(H). E(spl) is unlikely to be the mediator in this process, because loss of E(spl) has no effect on follicle-cell
cycle transition and follicle-cell differentiation (Deng et al., 2001; Lopez-Schier and St Johnston, 2001). Two other nuclear proteins, bHLH transcription factor dMyc (Dm, encoded by diminutive – FlyBase) and bHLH protein Emc (encoded by extra macrochaetae), are both required in follicle cells for proper entry into the endocycle (Adam and Montell, 2004; Maines et al., 2004; Shcherbata et al., 2004). Removal of the function of dMyc results in lack of endocycle in follicle cells, but whether dMyc expression is regulated by Notch is uncertain, because the expression of dMyc is uniform throughout oogenesis (Maines et al., 2004). Emc expression is detected in main-body follicle cells from the germarium to stage 8 of oogenesis; its expression after stage 6 depends on Notch signaling (Adam and Montell, 2004). Loss of emc function results in upregulated CycB and FasIII in follicle cells after stage 6, a phenotype similar to that of loss of Notch (Adam and Montell, 2004). Emc could function as a transcriptional repressor (Campuzano, 2001), and it is known to be involved in promoting differentiation (Cubas et al., 1994), so it is a good candidate for mediating Notch-dependent downregulation of Cut during the mitotic cycle/endocycle switch. We did not, however, find any change of Cut expression in emc loss-of-function clones generated by a null allele, emc<sup>AP5</sup> (data not shown), thus excluding the possibility that cut is repressed by emc during the mitotic/endocycle switch. Another transcription factor may therefore be involved.

The role of Cut in cell differentiation and cell cycle progression

The known role of Cut in Drosophila is mostly related to cell differentiation. During neurogenesis, Cut is involved in cell-fate determination in sensory-organ cells. Loss of cut function causes transformation of the external sensory organ into the chordotonal sensory organ, whereas overexpression of Cut has the opposite effect (Merritt, 1997; Blochlinger et al., 1991). In main-body follicle cells, we have shown that overexpression of Cut maintained expression of immature cell-fate markers FasIII and Eya, whereas loss of cut function in early stages repressed their expression. Although Cut is involved in cell differentiation in these two developmental processes, its roles in the two are significantly different. In sensory organs, the role of Cut is post-mitotic, whereas in main-body follicle-cell differentiation, it appears to be correlated with Cut function in the mitotic cycle. The requirement for Cut in main-body follicle-cell differentiation may be related to its function in cell-cycle regulation.

The role of cut in polar-cell differentiation is intriguing. Cut expression is normally retained in these specialized cells while its expression in main-body cells is decreased. We have shown that consistent expression of Cut led follicle cells to take the immature main-body cell fate, but these cells eventually took the polar-cell fate (Fig. 5G). Main-body cell fate and polar/stalk-cell fate are separated in the germarium, which requires Notch activity. Continuous Cut activity seemed able to reverse this differentiation process.

In contrast to the role of Drosophila Cut in cell differentiation, mammalian Cut has mainly been shown to be involved in regulating cell-cycle progression in some cell types (Coqueret et al., 1998; Gupta et al., 2003; Trusscott et al., 2003; Wu and Lee, 2002). CDP, the mammalian homolog of Cut, has been shown to be physically associated with the complex regulating the G1/S progression (Coqueret et al., 1998). Cut could functionally replace E2F in forming a complex with RB in regulating cell-cycle progression (Gupta et al., 2003). The requirement for Cut in maintaining the mitotic cell cycle in Drosophila follicle cells echoes its role in mammalian systems. Whether Drosophila E2F has a function in follicle cell proliferation is not known; weak alleles of E2F1 and E2F2 affect gene amplification, whereas no defect appears in the mitotic cycle (Cayirlioglu et al., 2001; Cayirlioglu et al., 2003; Royzman et al., 1999). Cut may functionally replace E2F for cell-cycle progression in proliferating follicle cells, but it is not an essential regulator of the cell cycle machinery because the mitotic cycle did not seem to be affected in cut germline clones (data not shown). In addition, cut function has been extensively studied during embryogenesis and in imaginal discs, but no reported function is related to cell-cycle regulation in these developmental stages. The requirement for Cut in cell-cycle regulation is therefore probably specific to follicle cells in Drosophila.

We would like to thank A. B. Thistle, J. Horabin, L. Epstein and J. Poulton for critical reading of the manuscript. We thank S. Qian, C. Green, G. Barrio, A. Wolfson and other members of the Deng lab for technical help and enthusiastic discussions throughout the research project. We are also thankful to S. Bell, S. Blair, R. Bodmar, N. Dyson, B. Edgar, E. Giniger, S. Jackson, Y. Jan, C. Lehner, D. Montell, T. Orr-Weaver, H. Ruohola-Baker, D. Stein and G. Struhl and Developmental Studies Hyridomab Bank, Bloomington Stock Center, for sending us fly stocks and antibodies. K. Riddle and the Biological Science Imaging Facility at FSU helped with confocal microscopy. W.M.D. is supported by a Scientist Development Grant from the American Heart Association, Florida/Puerto Rico affiliate, a Planning Grant from CRC FSU, and the FSU College of Arts and Sciences set-up fund.

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