

# Expression of constitutively active Notch arrests follicle cells at a precursor stage during *Drosophila* oogenesis and disrupts the anterior-posterior axis of the oocyte

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## SUMMARY

During early development, there are numerous instances where a bipotent progenitor divides to give rise to two progeny cells with different fates. The *Notch* gene of *Drosophila* and its homologues in other metazoans have been implicated in many of these cell fate decisions. It has been argued that the role of Notch in such instances may be to maintain cells in a precursor state susceptible to specific differentiating signals. This has been difficult to prove, however, due to a lack of definitive markers for precursor identity. We here perform molecular and morphological analyses of the roles of Notch in ovarian follicle cells during *Drosophila* oogenesis. These studies show

directly that constitutively active Notch arrests cells at a precursor stage, while the loss of Notch function eliminates this stage. Expression of moderate levels of activated Notch leads to partial transformation of cell fates, as found in other systems, and we show that this milder phenotype correlates with a prolonged, but still transient, precursor stage. We also find that expression of constitutively active Notch in follicle cells at later stages leads to a defect in the anterior-posterior axis of the oocyte.

Key words: constitutively active Notch, precursor, cell fate, oogenesis, axis, *Drosophila*

## INTRODUCTION

In *Drosophila*, the establishment of both the anterior-posterior (A-P) and dorsal-ventral (D-V) body axes takes place during oogenesis. The establishment of this polarity requires signaling between the oocyte and the somatic follicle cell layer that surrounds it. In particular, the asymmetric localization of morphogens in the oocyte depends on interactions between the oocyte and subpopulations of specialized follicle cells. The discrimination of cell types among the somatic follicle cells is thus a committed event leading to the specification of embryonic polarity.

The follicle cell subpopulation that is necessary for establishment of the A-P axis is the posterior follicle cell group (Ruohola et al., 1991; Gonzalez-Reyes et al., 1995; Roth et al., 1995; reviewed in Rongo and Lehman, 1996). Recent experiments have shown that signaling from the posterior follicle cells is required for polarization of the oocyte microtubule cytoskeleton (Clark et al., 1994, Lane and Kalderon, 1994). Polarization of this cytoskeleton is necessary for proper localization of anterior and posterior morphogens (Pocrywka and Stephenson, 1991; Clark et al., 1994; Lane and Kalderon, 1994). It is therefore important to determine how the posterior follicle cells are defined.

Three signaling pathways are known to be required for differentiation of subgroups of follicle cells: the EGF receptor pathway, the Notch pathway and the Hedgehog pathway (Gonzalez-Reyes et al., 1995, Roth et al., 1995; Ruohola et al., 1991; reviewed in Rongo and Lehman, 1996; Forbes et al., 1996). The EGF receptor pathway transmits inductive information from the oocyte to the follicle cells, while the Hedgehog and Notch pathways act in communication among the follicle cells. Notch functions in determining two follicle cell populations: the posterior follicle cells and the stalk cells which separate the egg chambers from one another and from the germarium, the structure in which the germ-line divisions take place. In Notch loss-of-function mutants, both expansion of the posterior follicle cell field and loss of stalk cells are detected. The expansion of the posterior follicle cell field correlates with a defect in the establishment of the A-P polarity of the oocyte.

Despite the wealth of information about the loss-of-function phenotype of Notch in oogenesis, the precise role of Notch activity remains obscure. Notch is a transmembrane receptor which functions in a wide variety of cell fate specification events in multiple species. The mechanism by which Notch signaling mediates these cell fate choices is under investigation. We have addressed this question by studying the effects

produced by activation of Notch function in follicle cells during oogenesis. For this purpose, we have taken advantage of Notch derivatives that uncouple intracellular signal transduction from ligand binding. Specifically, recent studies have shown that a fragment of Notch lacking the extracellular domain is constitutively active, regardless of the presence or absence of ligand. Persistent or transient ubiquitous expression of activated Notch in *Drosophila* causes an overproduction of epidermis at the expense of neural structures (Lieber et al., 1993; Rebay et al., 1993; Struhl et al., 1993), a phenotype opposite to that seen with the loss-of-function allele of *Notch* (Poulson, 1940; Lehmann et al., 1983; Hartenstein and Posakony, 1990; Heitzler and Simpson, 1991). Activated Notch causes defects in developmental processes in *C. elegans* (Roehl and Kimble, 1993), *Xenopus* (Coffman et al., 1993), chicken (Austin et al., 1995) and humans (Ellisen et al., 1991), as well as *Drosophila* eye development (Fortini et al., 1993). Absence or delay of normal differentiation in some of these cases has led to the proposal that ongoing Notch activity prevents competent cells from undergoing differentiation *in vivo*. However, this has been hard to prove due to the lack of definitive molecular markers for the precursor stage.

Here we show directly by molecular and morphological markers that, in early oogenesis, constitutively active Notch arrests follicle cells in a specific precursor stage blocking the development of both stalk cells and polar cells. Loss of Notch function eliminates this precursor stage. In a later stage of oogenesis, activation of Notch in follicle cells leads to aberrant anterior-posterior axis formation.

## MATERIALS AND METHODS

### Stocks

*Drosophila melanogaster* stocks were raised on standard cornmeal-yeast-agar medium at 25°C or at 18°C. The following fly strains were used: *Oregon R*, *w<sup>-</sup>*, *GAL4(112A1)/FM6*, *GAL4 109(3)9* (generated in the Jan laboratory), *HS-GAL4/CyO*, *UASLacZ/CyO*, *UASLacZ/FM6* (Brand and Perrimon, 1993), *UASStau-GFP(5.8)* on the second chromosome (Brand, 1995), *93F/TM3* (Ruohola et al., 1991), *KL503/TM3* (Clark et al., 1994), *A101/TM3* (Bier et al., 1989), *UAS-ΔEN* lines: *B33c-3/TM3*, *B2a-2/CyO* and *B2a-3/TM3* (for details of the construct see Fuerstenberg and Giniger, unpublished data), *Act5C>y<sup>+</sup>>Notch(intra)#2 and#3 (=N(intra))* and a line carrying *P[ry<sup>+</sup> hsp70-flp]* on the X-chromosome (generous gifts from Gary Struhl; Struhl et al., 1993).

To construct *UASΔEN* lines, *ΔEN* was placed immediately downstream of the *GAL4* upstream activating sequence (*UAS*, Brand and Perrimon, 1993) and introduced into flies using P-element-mediated transformation. To verify that *ΔEN* acts similarly to other published extracellular deletion constructs, we investigated the effect of *ΔEN* expression in neurogenesis. *UASΔEN* was crossed to a line that expresses *GAL4* protein in embryonic epidermis (*GAL4(112)*). The embryos from this cross were stained with mAb22C10 antibody to visualize the neurons (Zipursky et al., 1984). Fewer neurons were detected in *ΔEN* embryos than in control embryos (compare Fig. 1B to 1A). Since the phenotype of loss-of-function *N* mutations is hyperplasia of neurons, *ΔEN* behaves as expected for a gain-of-function mutation in neurogenesis. Western blotting analysis showed that this construct produced a truncated form of Notch in ovaries (data not shown).

### Heat-shock treatment

To induce constitutively active Notch expression from the *UASΔEN* construct, *UASΔEN(33c-3)* was crossed to *HS-GAL4*, progeny were

raised at 18°C and heat shocked to induce expression of *GAL4* by the *hsp70*-promoter. For adult heat shocks, glass vials containing 3-4 day old *HS-GAL4/+;UASΔEN(33c-3)/+* animals were incubated in a 39°C water bath for 15 minutes (or 37°C for 30 minutes). After heat shocks, the animals were returned to 18°C for the appropriate times.

To induce expression of the intracellular form of *N* (*N(intra)*), a *FRT>y<sup>+</sup>>FRT* cassette was excised between an actin promoter and the *N(intra)* construct by heat-shock-induced expression of the *flipase* gene from the *hsp70* promoter. To achieve this, *Ni<sup>#2</sup>/Ni<sup>#2</sup>;Ni<sup>#3</sup>/Ni<sup>#3</sup>* was crossed to the *hsp70flp/hsp70flp* animals and the progeny were raised at 25°C. The progeny were heat shocked at 37°C for 30 minutes (adults) or at 39°C for 1 hour (late pupae). After heat shocks the animals were returned to 25°C for the appropriate times. Both adult and pupal heat-shock paradigms will result in a *caN-intermediate* phenotype at early time points (1-2 days) and a *caN-strong* phenotype at late time points (6-7 days).

### Immunocytochemistry, in situ hybridization and other staining procedures

Immunocytochemistry, in situ hybridization and X-Gal-staining procedures were performed as described earlier (Ruohola et al., 1991), with the following minor modifications. For immunocytochemistry and in situ hybridizations, the ovaries were dissected in PBS, fixed for 1 hour in 4% paraformaldehyde/PBS/0.1% DMSO, dehydrated and stored at -20°C. For X-Gal staining, the ovaries were dissected in PBS, fixed in 2% glutaraldehyde/PBS for 5-7 minutes, rinsed with PBS and incubated in 0.2% X-Gal solution in Fe/NaP buffer at 37°C overnight. Samples were washed in PBS, fixed for 20 minutes in 4% paraformaldehyde/PBS and mounted in glycerol.

The antibodies used in this study were mAb22C10 (Zipursky et al., 1984), anti-Fasciclin III (1:15 dilution, Ruohola et al., 1991), anti-65F (1:4000 dilution), anti-Big Brain (1:1500; D. Doherty and Y. N. Jan, personal communication), anti-Notch (intracellular domain, 1:1000) and anti-Staufen (1:3000; St Johnston et al., 1991; a gift from Daniel St Johnston). The DNA plasmids used for in situ hybridization probes were pGEM3bcd (Driever et al., 1990) and BSKS-osk (Ephrussi et al., 1991).

### Western blot analysis

The ovary samples were prepared as described earlier (Markussen et al., 1995) and run on 7% SDS-PAGE gels with 3.5% stacking gels. Proteins were transferred to a 0.45 μm Protran nitrocellulose membrane (Schleicher and Schuell) and probed with polyclonal antibody to the intracellular domain of Notch (1:3000 dilution). Antibodies were visualized by chemiluminescence using a goat anti-rabbit Ig(H+L) secondary antibody conjugated to alkaline phosphatase and the chemiluminescent substrate, Lumigen PPD (Boehringer Mannheim).

### Microscopy

Microscopy was performed on a Leitz DMRB with Nomarski differential interference contrast. Confocal images were collected using the MRC 600 laser scanning confocal microscope system (Bio-Rad Microsciences Division) using the 488 and 568 wavelengths of a krypton argon laser.

### Cytoplasmic streaming assays

Bulk cytoplasmic movements within living oocytes were assayed as follows: adult females of genotype *B2A3/109(3)9* were transferred to a slide and covered with halocarbon oil (700). Ovaries were removed and dissected into separate ovarioles under oil. The slide was then transferred to a confocal microscope and yolk granules were imaged using the fluorescein filter set. Single frame images were collected in 10 second intervals for 100 seconds (10 images total). Time-lapse images were created by projecting the time series into a single image. For the time-lapse videomicroscopy, the egg chambers were viewed on a Leitz DMRB microscope with Nomarski optics and recorded

with a AIMS CV-252 CCD camera, a Boeckeler IMG-100 image processor and a Mitsubishi HS-S5600U time lapse video-recorder at the rate of one frame every 8 seconds. To analyze the streaming movement, the images were projected on a screen (Sony PVM-135) at 30 frames per second (240 $\times$ ).

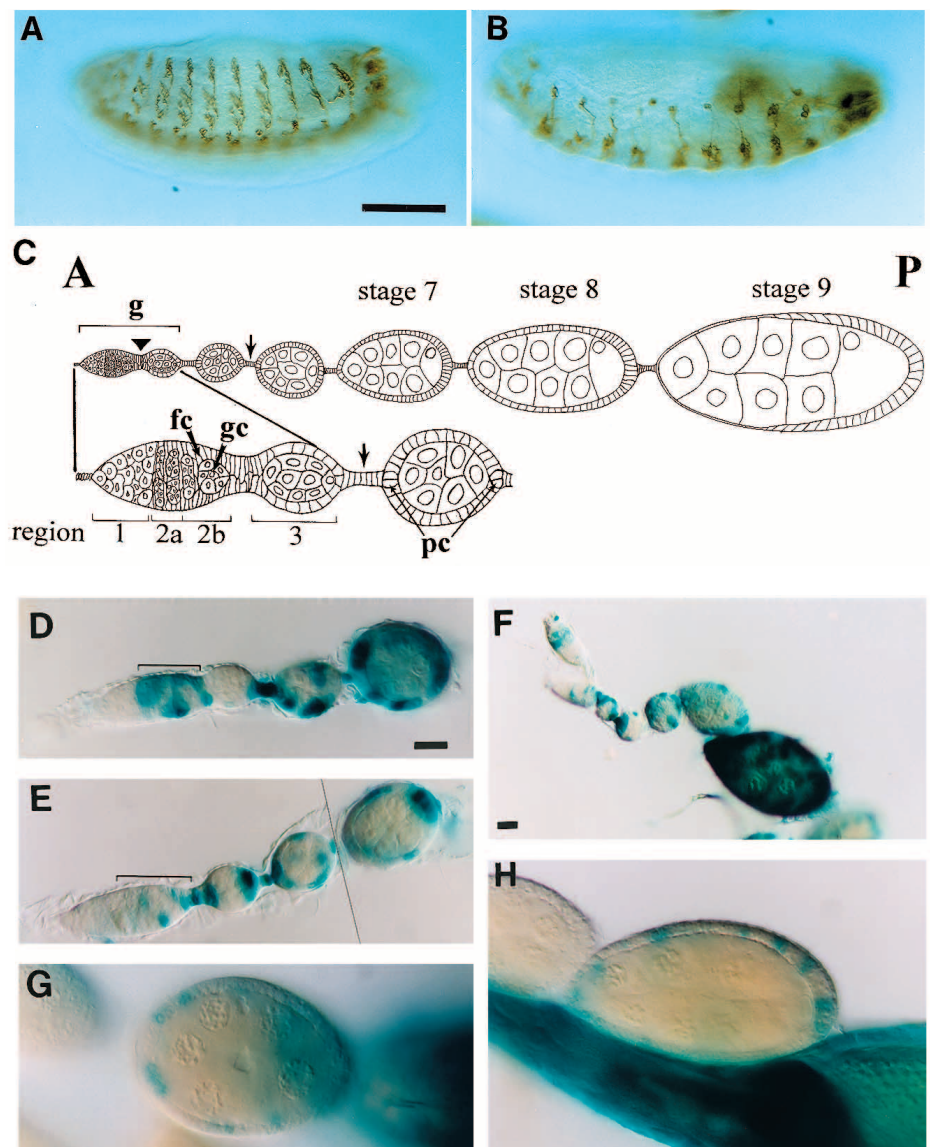
## RESULTS

### Constitutively active Notch constructs and experimental design

Two constructs of *Notch* (*N*) were used in these studies in order to express constitutively active *N* either persistently or transiently: *N(intra)* and  $\Delta EN$ . *N(intra)* consists of the intracellular domain of *N*, separated from an actin promoter by a cassette which is surrounded by two Flipase recognition target (FRT) sites and contains a stop codon. Flies carrying this construct were crossed to flies carrying flipase (*flp*) downstream of an *hsp70* promoter. Progeny were heat shocked, resulting in a pulse of *flp* expression which excises the cassette and allows *N(intra)* to be expressed under the control of the actin promoter (for details see Experimental Procedure and Struhl et al., 1993). This construct has been shown to act like a gain-of-function mutation of *N* (Struhl et al., 1993).

$\Delta EN$  expression under the control of GAL4 upstream activating sequence (UAS, Brand and Perrimon, 1994) leads to production of a protein fragment comprising the last four amino acids of the extracellular domain of Notch, the transmembrane domain, and the amino-terminal half of the intracellular domain, including the Su(H) interacting domain and the CDC10/ankyrin repeats. This construct is expected to behave as a constitutively active form of Notch based on earlier studies (Lyman and Young, 1993; Coffman et al., 1993; Fortini et al., 1993; Lieber et al., 1993; Rebay et al., 1993; Struhl et al., 1993; Roehl and Kimble, 1993; Kopan et al., 1996), and this has been verified by analyzing its phenotype in embryonic neurogenesis (Fig. 1A,B).

Functionally, the significant difference between  $\Delta EN$  and *N(intra)* is that excision of the FRT cassette from



**Fig. 1.** (A,B) *UASΔENotch* construct behaves as a gain-of-function mutation in neurogenesis. The embryonic neurons are visualized by mAb22C10 antibody staining in (A) control *GAL4(112)/(+ or y)* and (B) mutant *GAL4(112)/(+ or y); UASΔEN(33c-3)/+* embryos. Fewer neurons are detected in the embryos expressing  $\Delta EN$  due to GAL4 expression in embryonic epidermis by the GAL4-line *112A* than in the control embryos indicative of an anti-neurogenic phenotype. (C) Developmental sequence of oogenesis: diagram of an adult wild-type ovariole. *Drosophila* oogenesis is divided into 14 stages (stages 7, 8 and 9 indicated). The anterior-most structure, the germarium (g), contains the stem cells for germ line and the somatic follicle cells. The germarium is divided into four regions: in region 1 germ-line cells divide four times to produce a cyst of 16 germ cells (gc), 15 nurse cells and one oocyte. When the cyst has reached region 2, follicle cells (fc) migrate between two successive egg chambers and begin to intercalate, causing the region 3 egg chamber to 'pinch off' from the germarium (arrowhead). These cells then take the fate of either stalk cells (arrows) or follicle cells that surround the egg chamber, including the polar cells (pc). (D-F) *HS-GAL4* and (G-H) *GAL4-109(3)9* induce the expression of the target gene in follicle cells. One day after heat-shock induction (30 minutes, 37°C)  $\beta$ -gal expression was detected by X-Gal staining starting in region 2 in germaria of *HS-GAL4/UASLacZ* flies (D,E, brackets). Some variability in the staining pattern is detected which might account for variability in the penetrance of the phenotype. From the stage 7-8 on the expression of  $\beta$ -gal increased considerably (F). Patchy GAL4 expression detected in ovaries of *GAL4-109(3)9/UAS-LacZ* flies is first detected around stage 6 increasing by stage 9 in all of the follicle cells (G,H). Scale bars are equivalent to 20  $\mu$ m. A and B are of the same magnification; D,E,G and H are of the same magnification.

between *N(intra)* and the actin promoter results in persistent expression of constitutively active N, while  $\Delta EN$  is only transiently expressed as long as GAL4 is present.

To induce  $\Delta EN$  expression in oogenesis, the flies carrying the *UAS $\Delta EN$*  construct were crossed either to flies expressing GAL4 under heat-shock control (*HS-GAL4*) or to the enhancer trap GAL4 line, *109(3)9*. The GAL4 expression patterns in ovaries for both of these lines were examined by crossing them to a line carrying *UAS-lacZ* and staining the progeny with X-Gal, or by crossing them to *UAS-tau-GFP* and analyzing the GFP pattern by fluorescence microscopy. *HS-GAL4* induced *UAS-LacZ* expression in follicle cells beginning in region two of the germarium (Fig. 1D,E), whereas *109(3)9* induced follicle cell expression is not detected prior to stage 6 in oogenesis (Fig. 1G,H). No germ-line expression of GAL4 was detected (Fig. 1G-H; data not shown).

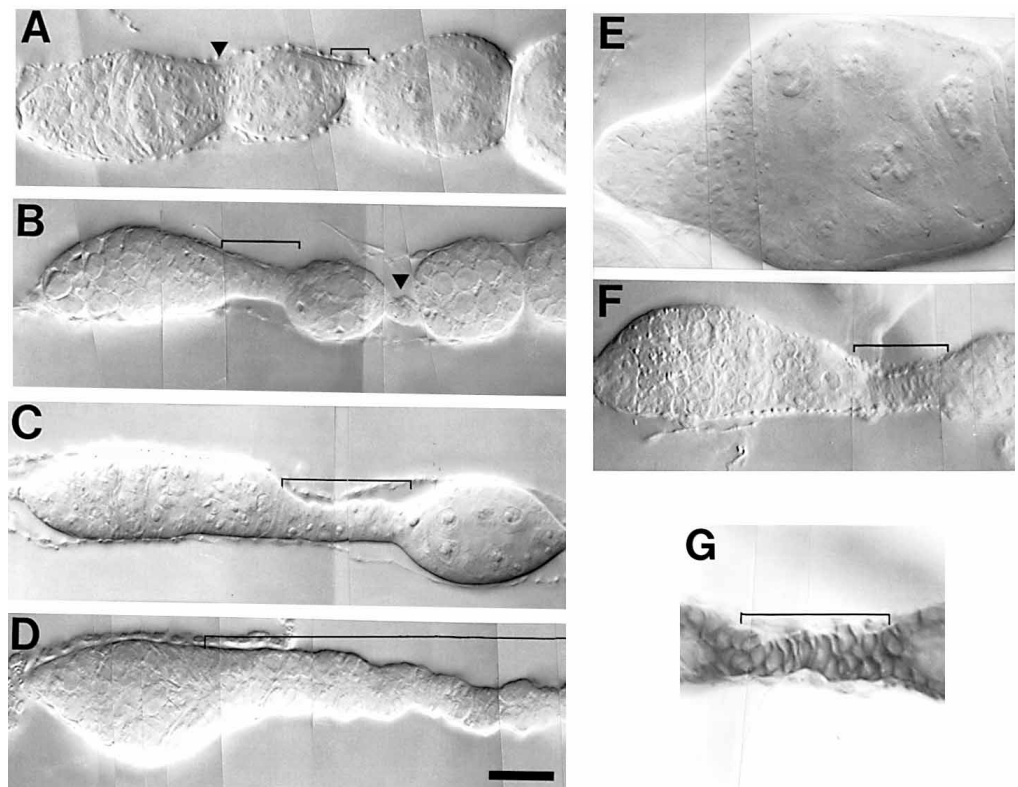
### Expression of constitutively active Notch in the germarium follicle cells generates long stalk structures

Ovaries contain egg chambers in a developmental order. In the anterior-most region of the germarium, region 1, the germ-line stem cells produce cystocytes, which divide through incomplete cytokinesis to form 16 cell cysts. The cysts are surrounded by a follicle cell layer and released from the germarium by 'pinching-off' (Fig. 1C, arrowhead). This process results in the formation of two kinds of specialized follicle cells: stalk cells, which intercalate between each other to form the stalk that separates two successive egg chambers (Fig. 1C, arrows), and polar cells, which form a cap at the anterior end of the older egg chamber and the posterior end of the younger egg chamber (Fig. 1C, pc). In loss-of-function *N<sup>ts</sup>* mutant ovaries, no stalk cells are detected at the restrictive temperature and the egg chambers never leave the germarium (Fig. 2E; Ruohola et al., 1991; Xu et al., 1992).

To address whether expression of constitutively active N could induce long stalks between the egg chambers, a phenotype opposite to the loss of stalks observed with *N<sup>ts</sup>*, we

expressed constitutively active N transiently in the germarial follicle cells using *HS-GAL4* induced expression of  $\Delta EN$ . This leads to a dramatic phenotype in the early stages of oogenesis. Instead of a wild-type stalk of 6-8 cells (Fig. 2A, bracket), long stalk structures consisting of an average of 15 cells were detected (Fig. 2B, bracket).

The sequential nature of oogenesis allows us to study whether ovarioles that include abnormally long stalks continue to develop, and at what stage of development the length of both wild-type and long stalks is determined. If expression of  $\Delta EN$  is induced by a 15 minute heat shock at 39°C and the flies are then allowed to develop for 24 hours at 18°C, long stalks are first detected between stages one and two (Fig. 2B, bracket). At this timepoint, long stalks are not observed between the later stages (Fig. 2B, arrowhead), indicating that the length of fully formed stalks is not altered. Previous studies have indicated



**Fig. 2.** Expression of constitutively active Notch generates long stalk-like structures; phenotypic series. (A) In wild-type ovary, a stalk forms in the germarium (arrowhead). A fully formed stalk consisting of 6-7 cells is detected between each egg chamber (bracket). (B) Transient expression of constitutively active N (*caN-mild*, 39°C, 15 min) caused long stalk formations two days (at 18°C) after induction (15.3 cells/stalk, bracket). A fully formed stalk was not affected (arrowhead). (C) Persistent expression of constitutively active N lead to even longer stalks when analyzed 24 hours (at 25°C) after the induction of expression (*caN-intermediate*, 22 cells, bracket). (D) Extreme stalks were detected after induction of constitutively active N when analyzed 6 days after heat shock (*caN-strong*, 101 cells, bracket). (E) No stalks are formed in *N<sup>ts</sup>* ovaries allowed to develop at the restrictive temperature (one day at 32°C). (F) *N<sup>ts</sup>/N<sup>ts</sup>; UAS $\Delta EN$ /HS-GAL4*. The *N<sup>ts</sup>* phenotype is suppressed by the expression of constitutively active N (*caN-mild*), suggesting that constitutively active N acts without endogenous N and that both mutations affect the same pinching-off event. (G) Constitutively active N (*UAS $\Delta EN$* ) was induced by *HS-GAL4* expression due to two heat-shock pulses (39°C, 15 minutes and 39°C, 20 minutes) in a one day interval. The ovaries were dissected 2 days after the last induction and stained with Fasciclin III (Fas III). Fas III marks all the follicle cells during the pinching-off event and becomes restricted to the polar cells around stage 3 in oogenesis. The long stalk consists of 22 cells, indicating that longer stalks are detected with the *UAS $\Delta EN$*  construct after multiple heat-shock inductions. Scale bar is equivalent to 20  $\mu$ m.

**Table 1. Stalk is determined in the germarium**

caN-mild	Position of long stalk	Total cells in stalk	% long stalks with 93F <sup>-</sup> cells	% long stalks with A101 <sup>+</sup> cells	% A101-positive cells
39°C 15 minutes, 2 days	stages 2-3	15.3 (n=17)	77 (10/13)	79 (34/43)	18.3
39°C 15 minutes, 3 days	stages 3-6	16.5 (n=20)	79 (19/24)	73 (22/30)	15.5
39°C 15 minutes, 4 days	stages 6-8	16.5 (n=10)	82 (14/17)	79 (49/62)	11.5
37°C 30 minutes, 2 days	stages 2-3	11.8 (n=4)	NA	64 (7/11)	8.5
37°C 30 minutes, 3 days	stages 3-6	12.3 (n=10)	NA	64 (16/25)	11.4

NA= not analyzed.

that a new egg chamber pinches off every 24 hours at this temperature (10-12 hours at 25°C; King, 1970; Margolis and Spradling, 1995). Since long stalks are detected between stages 1 and 2 only 24 hours after induction of  $\Delta EN$ , this suggests that constitutively active N acts during or just before the beginning of the pinching-off event and has no effect on fully formed stalks. The expression pattern of *HS-GAL4* under these conditions indicates that it induces target gene expression in the follicle cells at regions 2 and 3 of the germarium (Fig. 1D-F), the region where pinching-off occurs. 48 hours after the induction of constitutively active N, the long stalks have progressed and are present between stage 2 and 3 egg chambers (Fig. 3B) and, after three days, the long stalk has moved from the germarium region to the region between stages 3 and 6 in oogenesis (Fig. 4B). The long stalks detected in the later stages are of the same average length as those seen earlier, indicating that the final number of cells in these long stalks is also determined in the germarium (Table 1).

To address whether constitutively active N affects the same developmental process as the loss-of-function mutation, we determined the phenotype produced when activated Notch is expressed in the background of *N<sup>ts</sup>*. Only the long stalk phenotype of constitutively active N was detected in these ovaries (Fig. 2F, bracket). This suggests that, in stalk formation, activated Notch affects the same process as the wild-type protein and, further, that constitutively active N can act without endogenous N. Similar results have been seen in other systems, such as the nervous system (Struhl et al., 1993).

Long stalk structures were also observed when the flip-FRT system was used to drive expression of constitutively active N (Struhl et al., 1993). 24 hours after heat-shock induction of flipase, long stalk structures were detected (Fig. 2C, bracket). The long stalks observed one day after heat-shock induction resembled the long stalks detected after *GAL4*-induced expression. However, the penetrance of the phenotype was higher and the stalks were longer (Table 2), indicating a

stronger phenotype either due to the different *N* construct or the experimental procedure. Multiple heat-shock inductions of  $\Delta EN$ , in order to avoid the transient nature of *GAL4* expression, result in longer stalks, which resemble those generated by *N(intra)* (Fig. 2G, bracket). This indicates that longer stalks can be generated by longer expression of  $\Delta EN$  and the stronger phenotype of *N(intra)* is most likely due to the fact that it is expressed persistently.

To examine whether the long stalks generated by *N(intra)* also progressed through oogenesis, we looked at ovaries at later time points. 2 days after heat shock, the long stalk had progressed to between stages 3 and 6, and was usually followed by a second long stalk. 6 to 7 days after heat shock, extremely long stalk structures were detected (Table 2; Fig. 2D, bracket), which have presumably continued to grow for 3 days since the heat shock. Since they are detected at a later time point, these extremely long stalks could be due to a stem cell carrying *N(intra)* under the control of the actin promoter, leading to progeny that all express constitutively active N. The lengths of stalks generated in (1)  $\Delta EN$  (37°C, 30 min or 39°C, 15 min), 2 days after induction, (2) *N(intra)*, 1 day after induction and (3) *N(intra)*, 6-7 days after induction suggest that the three treatments constitute a phenotypic series of increasing severity. These three treatments are referred to as *caN* (for constitutively active Notch)-*mild*, *caN-intermediate* and *caN-strong*, respectively, throughout the remainder of the paper.

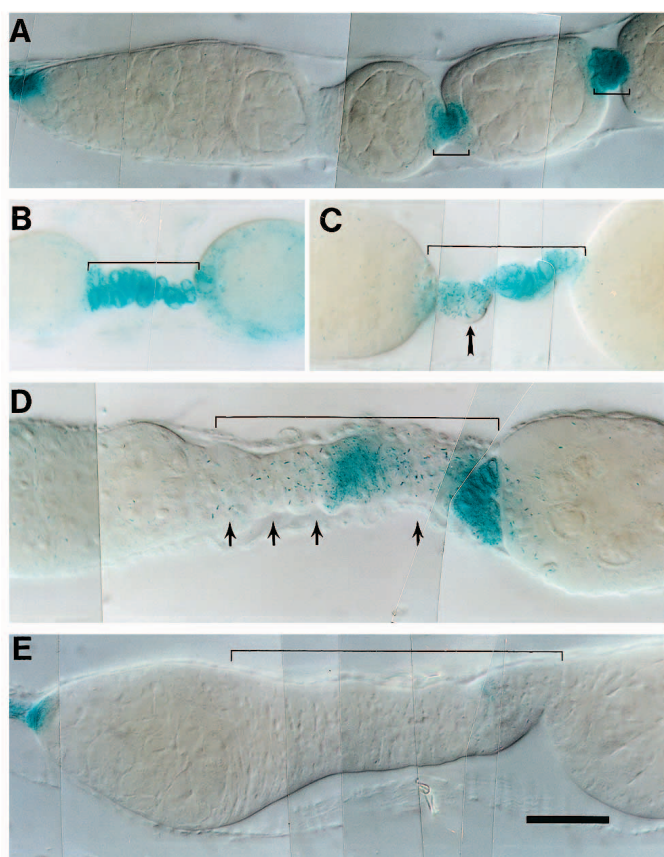
In addition to varying lengths of stalk structures, the experimental procedures generated varying cellular morphology within the long stalks. The cellular morphology of *caN-mild* stalks resembles that of a wild-type stalk: a single row of aligned, disc-shaped cells (Fig. 2B, bracket; and Fig. 1C, arrow). Cells in the *caN-intermediate* and *caN-strong* stalks are not aligned in a single row, the stalks appear much thicker than wild-type stalks and the cells are organized as several aligned columns of cells (Fig. 2C,D). This morphology is reminiscent of the cellular organization observed earlier in the germarium

**Table 2. Expression of constitutively active Notch: phenotypic series**

Constit. active N construct	Heat-shock conditions and survival time	Title	Penetrance (% long stalks)	Average length of stalk	Percent of stalks with A101 <sup>+</sup> cells	Percent of A101 <sup>+</sup> cells in stalk
–	*	Control	0	6 cells	0	0
$\Delta EN$	37°C 30 minutes, 2 days at 18°C	<i>caN-mild</i>	69 (11/16)	12 cells (4)	64 (7/11)	8.5
$\Delta EN$	39°C 15 minutes, 2 days at 18°C	<i>caN-mild</i>	81 (43/55)	15 cells (17)	79 (34/43)	18
<i>N(intra)</i>	37°C 30 minutes, 1 day at 25°C	<i>caN-intermediate</i>	94 (44/47)	22 cells (17)	98 (43/44)	45
<i>N(intra)</i>	39°C 1 hour, 6 days at 25°C	<i>caN-strong</i>	100 (48/48)	101 cells (25)** 38 cells (25)	– 0	– 0

\*The controls were heat shocked under the same conditions as the constitutively active Notch constructs.

\*\*The full length of these stalks is an average of 101 cells. A101-positive cells are found at the posterior end of some stalks, so the average length of stalk which does not contain any A101-positive cells is 38. In the most severe cases, none of the cells are A101 positive.



**Fig. 3.** A stalk cell fate marker is expressed in *caN-mild* and *caN-intermediate* stalks, but not in *caN-strong* stalks. (A) The *93F* enhancer trap line stains the terminal filament cells and stalks (brackets). (B,C) *caN-mild*: three days (at 18°C) after the heat shock (39°C, 15 minutes), two groups of long stalks were detected (brackets). 21% of the long stalks consisted entirely of *93F*-positive cells (B, bracket), indicating that all of the cells in long stalks had taken the stalk cell fate. However, 79% of the long stalks consisted of *93F*-positive (C, bracket) and *93F*-negative cells (C, arrow). (D) *caN-intermediate*: extra *93F*-positive and *93F*-negative (arrows) cells are detected in long stalks (bracket). (E) *caN-strong*: the extremely long stalks (bracket) do not show *93F* staining. In some cases, a few *93F*-positive cells are seen at the very posterior end of the long stalk in the cells adjacent to the egg chamber (data not shown). Scale bar is equivalent to 20 µm.

at the time of the pinching-off event, before the cells have begun to intercalate (Fig. 2A, arrowhead; Fig. 1C, arrowhead).

### A stalk cell fate marker is expressed in *caN-mild* and *caN-intermediate* stalks, but not in *caN-strong* stalks

To determine whether more cells had actually taken the fate of stalk cells, we examined the identity of the cells in the long stalks with *93F*, an enhancer trap line that is expressed in stalk cells (Fig. 3A; Ruohola et al., 1991). *93F* expression was analyzed in *caN-mild*, *caN-intermediate* and *caN-strong* backgrounds. In control ovaries, all stalk cells were *93F* positive (Fig. 3A, brackets). In the case of *caN-mild*, some long stalks were detected that consisted entirely of cells expressing the stalk cell marker (Fig. 3B), consistent with the prediction that more cells become stalk cells with expression of *caN*. However, the majority of the long stalks (mean value 43/54,

80%, Table 1) contained a group of cells that did not express the stalk cell marker, despite being located in the stalk structures (Fig. 3C).

In *caN-intermediate*, the long stalks also contained more *93F*-positive stalk cells than are contained in normal stalks, indicating that additional cells had taken the fate of stalk cells. However, many *93F*-negative cells were also detected in these long stalks (Fig. 3D). The number of cells that did not stain with the stalk cell marker also increased in number from *caN-mild* to *caN-intermediate*. Strikingly, no *93F*-positive stalk cells were detected in the extreme long stalks induced by *caN-strong* (Fig. 3E, bracket), indicating that none of the cells in these 'stalks' had taken the fate of stalk cells. Therefore, three different kinds of long stalk-like structures can be generated by the expression of constitutively active N: those that consist entirely of *93F*-positive stalk cells, those that consist of *93F*-positive and *93F*-negative cells and those that contain only *93F*-negative cells.

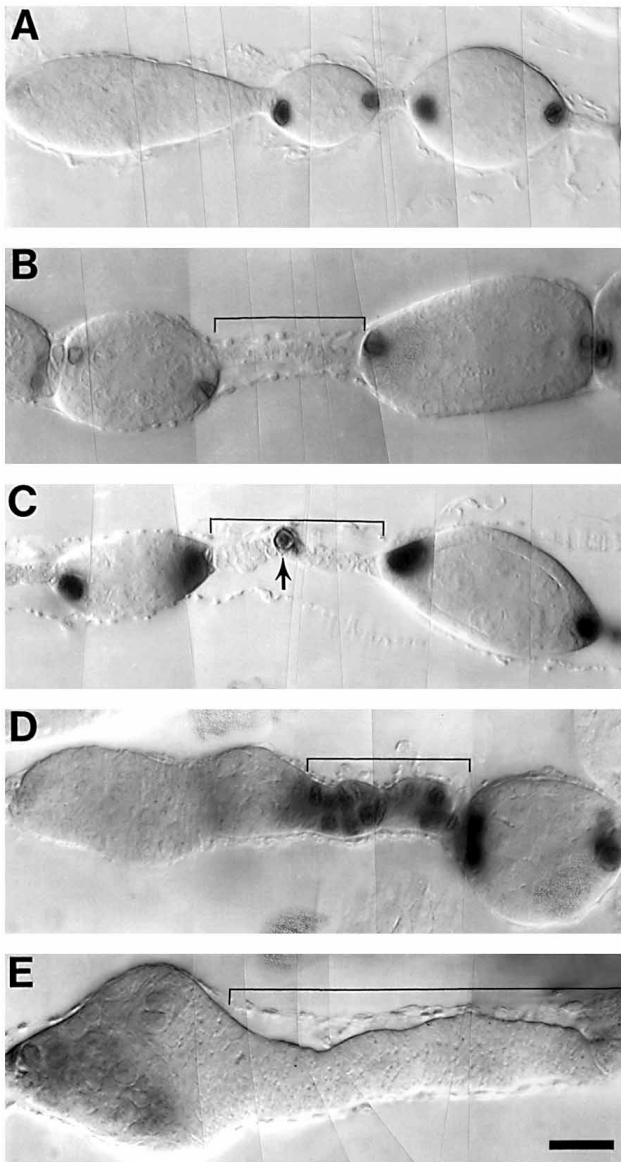
### A polar cell marker is expressed in *caN-mild* and *caN-intermediate* stalks but not in *caN-strong* stalks

To assess the identity of the cells in long stalks that did not stain with the stalk cell marker, we used a marker for a subpopulation of follicle cells that differentiates at the time of, or just prior to, stalk formation: the *A101* enhancer trap line. This mutation is a P-element insertion in the *neuralized* gene and shows a  $\beta$ -galactosidase-staining pattern similar to that seen in an *in situ* hybridization using a *neuralized* cDNA probe.  $\beta$ -galactosidase activity is first detected in some of the polar cell precursors at the time of stalk formation. By stage four of oogenesis, its expression is restricted to two anterior and two posterior polar follicle cells (Fig. 4A; Ruohola et al., 1991).

In the case of *caN-mild*, some long stalks were detected that contained no *A101*-positive cells (Fig. 4B). However, a large group of long stalks were detected that did have some *A101*-positive cells located within the stalk structures (Fig. 4C; Tables 1, 2). Based on the penetrance of the phenotype and the number of cells that express *A101*, it appears that the cells that express the polar cell marker likely correspond to those cells in the long stalks that did not express the stalk cell marker. There was no trend in the position of the *A101*-positive cells in the long stalk. In most cases, the normal number of polar cells was still found in the egg chambers surrounding the long stalks. However, in a few instances only one of the usual two polar cells was detected. *caN-intermediate* stalks had more *A101*-positive cells than *caN-mild* mutant stalks (Fig. 4D, bracket indicates stalk). However, long stalks in *caN-strong* ovaries had no *A101*-positive cells (Fig. 4D, bracket; Table 2). Based on the expression of *93F* and *A101*, three different kinds of long stalk-like structures can be generated by the expression of constitutively active N: those that consist entirely of stalk cells, those that consist of stalk cells and polar cells and those that do not contain either of these two cell types.

One possibility for the polar cells located in long stalks is that N function is required both for the initiation of stalk cell fate as well as the maintenance of this fate. According to this model, with transient expression of constitutively active N, the initiation of the stalk cell fate was induced in a group that normally should become polar cells. However, when the transiently expressed N was degraded, the cells return to their normal fate: polar cells. This is unlikely since polar cells are

detected in *caN-intermediate* stalks where N(intra) is expressed persistently. This model also predicts, in the case of transient expression of constitutively active N, that the longer the time period after heat shock, the more long stalks that have



**Fig. 4.** A polar cell marker is expressed in *caN-mild* and *caN-intermediate* stalks but not in *caN-strong* stalks. (A) The *A101* enhancer trap line is a P-element insertion in the *neuralized* gene and induces  $\beta$ -gal expression in the follicle cells at the anterior tip of germarium, in polar cell precursors (5-6 cells at the anterior and posterior pole of the stage 1-3 egg chambers) and polar cells (two cells at the anterior and posterior pole of egg chambers). (B,C) Two groups of long stalks were detected in the *caN-mild* mutant ovaries. 4 days after the heat-shock induction (39°C, 15 minutes) 21% of the long stalks did not stain with a polar cell marker *A101* (B, bracket). However, 79% of long stalks had 1-5 *A101*-positive cells in the stalk structures (C, arrow). (D) *caN-intermed.* 98% of long stalks contain cells that are *A101* positive. (E) *caN-strong*. The most severe long stalks do not contain any *A101*-positive cells. The stalk shown in E consists of 151 cells which do not express the marker *A101* (bracket). Scale bar is equivalent to 20  $\mu$ m.

*A101*-positive cells would be detected. In addition, each long stalk should have more *A101*-positive cells the further they progress through oogenesis. As neither prediction is upheld (Table 1), it seems unlikely that Notch is required for the maintenance of stalk cell fate.

#### The number of cells expressing a precursor marker increases in the long stalks as the severity of the phenotype increases

As mentioned previously, we noted a morphology reminiscent of precursor cells in some of the long stalks. In addition, expression of two markers for more differentiated cells, *93F* for stalk cells and *A101* for polar cells, was not detected in the long stalk-like structures generated by *caN-strong* (Figs 3E, 4E). To address whether N may inhibit the differentiation of stalk cells, we examined the stalks with a marker that reflects the differentiation state of stalk cells: Big Brain (Bib) protein. In wild-type ovaries, Bib is expressed in the follicle cells that are going to intercalate to form a stalk (Fig. 5B, arrowhead) as well as fully developed stalk cells (Fig. 5B, arrow). Importantly, the subcellular localization of Bib differs in these two cell populations. In the precursors, Bib is detected predominantly at the apical tip of the cells as they constrict (Fig. 5B, arrowhead and inset 1). In the stalk cells that have aligned into a single row, the apical pattern is lost and Bib is detected along the lateral surface (Fig. 5B, arrowhead and inset 2). Another marker, Fasciclin III (Fas III) protein is first detected in all follicle cells in region 2 of the germarium (Fig. 5A, bracket). It begins to restrict to the polar cell precursors by region 3 of the germarium, and is fully restricted once the chamber is completely pinched from the germarium (Fig. 5A, arrows).

In the long stalks generated by *caN-mild* and *caN-intermediate*, more cells were detected that stained with the Bib precursor pattern and early Fas III pattern than in wild-type ovaries, indicating that more precursors are present in these ovarioles (Fig. 5C-F). However, if these stalks were allowed to develop, the cells matured (or partially matured) to stalk cells or polar cells (Fig. 5E,F). In *caN-strong*, more precursors were also detected; however, these cells did not mature into stalk cells or polar cells, but stayed in the precursor stage, based on Bib and FasIII staining (Fig. 5G,H). Therefore, the strongest phenotype observed due to constitutively active N expression is persistence of the Bib-positive precursor stage.

Therefore, three different classes of long stalk-like structures can be generated by the expression of constitutively active N: those that consist of too many stalk cells (I), stalk cells and polar cells ('confusion', II), or precursor cells (III) (Fig. 7). A transient excess of precursors was also detected in classes I and II suggesting that the severity of the phenotype correlates with the length of the precursor stage, culminating in a complete block at the precursor stage (class III).

In order to address whether the precursor stage was also affected by the loss of N function, we stained *N<sup>ts</sup>* ovaries with Bib and Fas III antibodies. After 25 hours at the restrictive temperature, no Bib-positive cells were detected in *N<sup>ts</sup>* germaria (Fig. 5J). This indicated that no cells were in the precursor stage, either because they never develop to this stage or because this stage is transient and therefore not detected.

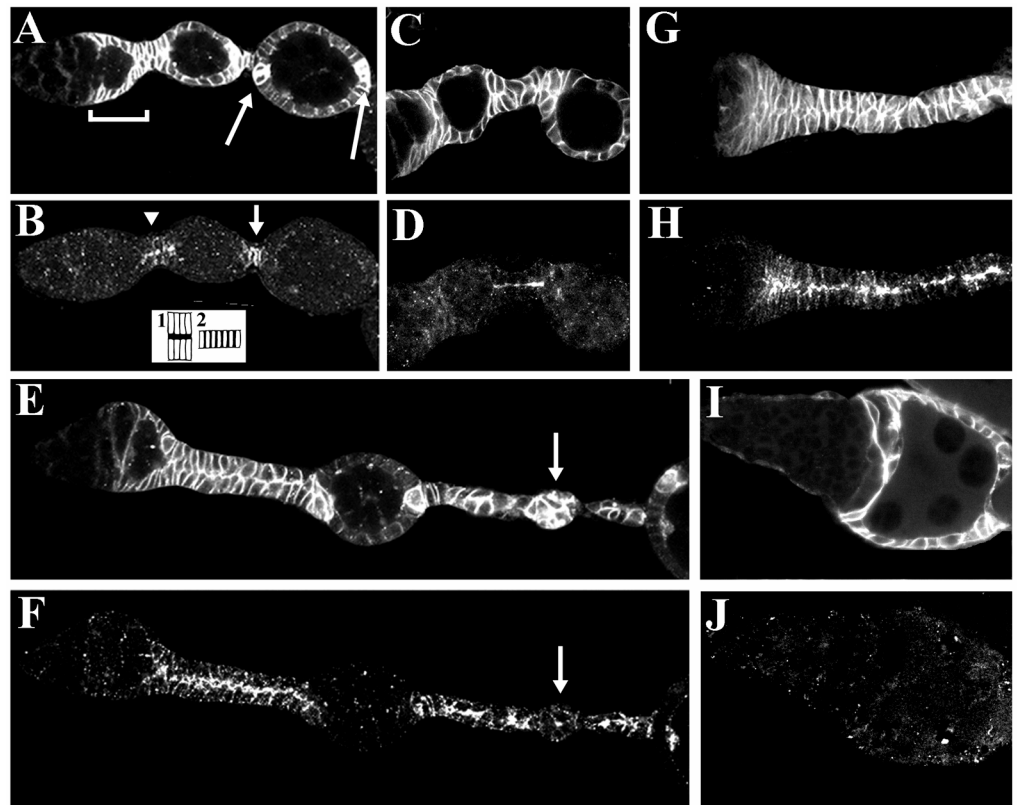
### Expression of constitutively active Notch in follicle cells results in a defect in the oocyte anterior-posterior axis

Based on the loss-of-function mutant phenotypes of *Notch* and *Delta*, it has previously been proposed that a signal from the posterior follicle cells is required for setting the proper A-P axis of the developing oocyte (Ruohola et al., 1991; Clark et al., 1994; Ruohola-Baker et al., 1994). However, the nature of the signal and the posterior follicle cell type which may send the signal are not known. To further investigate this, we have analyzed how expression of constitutively active N in follicle cells affects the posterior follicle cells and the anterior-posterior axis of the underlying oocyte.

In wild-type egg chambers after stage 4, two Fas III-positive cells are detected at the anterior and posterior poles of each egg chamber (Fig. 6A). After two heat-shock inductions of  $\Delta EN$  in a one day interval, egg chambers were observed in which no Fas III-positive cells are detected (Fig. 6B-D). These egg chambers were not separated by a long stalk, indicating that, in this case, constitutively active N acts in a cell fate decision separate from stalk formation. The egg chambers that lack Fas III-positive cells did not develop further but instead became necrotic. Therefore, in order to address the effects that expression of constitutively active N in the follicle cells has on the underlying oocyte during the later stages of oogenesis, we expressed constitutively active N either under *HS-GAL4* with milder heat-shock conditions [ $\Delta EN(HS)$ ] or under *GAL4-109(3)9* [ $\Delta EN(109)$ ]. Neither of these results in detectable follicle cell fate defects; two Fas III-positive cells are detected at each pole of the egg chambers, and there is no change in the domain of *pointed*, a posterior follicle cell marker (data not shown; Morimoto et al., 1996). However, we cannot rule out the possibility that further differentiation of the posterior follicle cells is defective. The A-P polarity of the oocyte was examined in these egg

chambers using markers used in analysis of *N<sup>ts</sup>* (Ruohola et al., 1991). The effects of constitutively active Notch on embryonic polarity could not be addressed because development was blocked prior to embryogenesis.






An early marker for anterior-posterior polarity, *oskar* (*osk*) mRNA, is localized to the posterior pole of wild-type oocytes at stage 8 (Ephrussi et al., 1991; Kim-Ha et al., 1991; Fig. 6D) and is essential for development of the abdomen and formation of the germ-line precursor cells at the posterior pole of the embryo (Lehmann and Nüsslein-Volhard, 1986; Ephrussi and Lehmann, 1994). Another marker for A-P polarity, Staufen (*Stau*) protein is localized to the posterior pole of the oocyte at



**Fig. 5.** The number of cells expressing a precursor marker increases in the long stalks as the severity of the constitutively active Notch long stalk phenotype increases. Wild-type (A,B) and mutant (C-J) ovaries are double stained with Fas III and Bib antibodies and visualized by confocal microscopy. (A) Fas III is first detected in all follicle cells in region 2 of the germarium (bracket). It becomes restricted to the polar cell precursors by region 3 of the germarium and is fully restricted once the chamber is completely pinched from the germarium (arrows). (B) Stalk cell precursors (arrowhead) and mature stalk cells (arrow) are stained with Big brain (Bib) antibody. Bib is first expressed in the apical tip of stalk precursors as they meet each other (arrowhead; inset 1). As the stalk matures and the cells align in a single row, this staining pattern ceases and Bib is detected as a line along the lateral surface of the cells (arrow; inset 2). (C,D) *caN-mild*: 2 days after the heat shock, more precursors are detected in the stalk between the germarium and the first egg chamber than in the wild type, based on precursor Bib (D) and Fas III (C) staining. However, these cells developed to stalk cells or polar cells later in oogenesis since older stalks express *93F* and *A101* markers (Figs 4B,C, 3B,C). (E,F) *caN-intermediate*: 48 hours after the heat-shock induction, the long stalks between the germarium and the most anterior egg chamber (Fas III staining, E) consists of precursor cells (Bib precursor staining, F). Stalks removed from the germarium begin to include a small subset of cells which show more intense Fas III staining (E, arrow) and less intense Bib staining (F, arrow) or begin to show the apical staining pattern and morphology of a mature stalk. (G,H) *caN-strong*: The extremely long stalk-like structures (Fas III staining, G) consist of precursor cells (Bib precursor pattern, H). (I,J) *N<sup>ts</sup>*: after 24 hours at the restrictive temperature, Fas III (I) but no Bib expression (J) is detected in the follicle cells which have failed to form a pinch.



**Table 3. Posterior components are mislocalized in constitutively active Notch mutant egg chambers**

					
<i>ΔEN(109); oskar</i>	42% (27/65)	54% (35/65)	5% (3/65)	0%	0%
Staufen	38% (47/125)	54% (68/125)	8% (10/125)	0%	0%
K-βgal	19% (17/91)	46% (42/91)	35% (32/91)	0%	0%
<i>ΔEN(HS); oskar</i>	17% (18/108)	63% (68/108)	8% (9/108)	0%	12% (13/108)
Staufen	23% (11/47)	38% (18/47)	21% (10/47)	0%	17% (8/47)
K-βgal	11% (3/28)	54% (15/28)	0%	36% (10/28)	0%

stage 8 (Fig. 6G; St Johnston et al., 1991). Whole-mount in situ hybridization with an *osk* cDNA probe, as well as immunocytochemistry with Stau antibody revealed that expression of constitutively active N caused mislocalization of these posterior components: they are mislocalized in ~80% of  $\Delta EN(HS)$  egg chambers and in ~60% of  $\Delta EN(109)$  egg chambers (Table 3). Mislocalization was either to the center of the oocyte (Fig. 6F,I; Table 3) or to a streak from the posterior (Fig. 6E,H; Table 3). In addition, expression of activated N under the control of *HS-GAL4* also generated mislocalization of both *osk* and Stau in granules throughout the oocyte (Table 3 and data not shown).

*bcd* mRNA is localized to the anterior pole of the oocyte in wild-type egg chambers (Fig. 6M) and is also crucial in the establishment of anterior-posterior polarity (Nüsslein-Volhard et al. 1987; St Johnston et al., 1989; Berleth et al., 1988). In situ hybridization indicated that *bcd* mRNA was localized properly to the anterior end of the oocyte of  $\Delta EN(HS)$  ovaries (data not shown). However, in 15% (11/73) of the  $\Delta EN(109)$  stage 8 egg chambers, *bicoid* was detected at both the anterior and posterior poles of the oocyte (Fig. 6N). This phenotype has been detected previously in *N*, *Delta*, *Protein Kinase A*, *gurken*, *torpedo* and *cornichon* mutants, and reflects a defect in microtubule reorganization that takes place between stages 4 and 7 of oogenesis (Ruohola et al., 1991; Lane and Kalderon, 1994; Gonzales-Reyes et al., 1995; Roth et al., 1995).

To analyze whether the microtubular structures of the oocyte are oriented properly, a strain that expresses a Kinesin-βgal fusion protein (Clark et al., 1994) was crossed to both  $\Delta EN(HS)$  and  $\Delta EN(109)$ . This fusion protein has been used previously as a reliable marker for microtubule organization (Clark et al., 1994; Lane and Kalderon, 1994; Gonzales-Reyes et al., 1995; Roth et al., 1995; Gillespie and Berg, 1995). In control egg chambers, Kinesin-βgal localizes to the posterior of the oocyte (Fig. 6J), suggesting that at least a subset of microtubules are oriented with their plus ends directed towards the posterior. Kinesin-βgal was mislocalized in 89% of  $\Delta EN(HS)$  and 81% of  $\Delta EN(109)$  egg chambers (Table 3). These mislocalizations were either to the center of the oocyte (Fig. 6L; Table 3), to a posterior streak (Fig. 6K; Table 3), or throughout the oocyte (Table 3), as described above for other posterior components.

### Constitutively active Notch in the follicle cells induces premature ooplasmic streaming

The defect in localization of Kinesin-βgal indicated a defect in the organization of the microtubules. Another indirect method for analyzing the organization of the microtubules is to assay whether ooplasmic streaming commences at the

correct time. Ooplasmic streaming is a microtubule-dependent process (Gutzeit and Koppa, 1982). In wild-type egg chambers at stage 8, an anterior-to-posterior gradient of microtubules is present in the oocyte. At this stage, no ooplasmic streaming is detected (Fig. 6O). A rearrangement of microtubules is observed at stage 10b, which results in arrays of parallel microtubules adjacent to the oocyte cortex that are associated with ooplasmic streaming (Theurkauf et al., 1992). Premature ooplasmic streaming was detected at stage 8 in 65% (13/20) of  $\Delta EN(109)$  egg chambers (Fig. 6P), suggesting that the normal gradient of microtubules is replaced by subcortical microtubule bundles. In controls (*109(3)9/TM3*, 15 stage 8 egg chambers analyzed) random flushing movements were detected, but no continuous circular swirling movements.

## DISCUSSION

To better define the mechanism of Notch action, we have investigated the effects of expressing constitutively active N in oogenesis. Specifically, we have tested the hypothesis that N functions in follicle cell fate determination and that signaling from the posterior follicle cells is required for the establishment of the oocyte A-P axis (Ruohola et al., 1991; Xu et al., 1992).

We have shown that, at two stages of oogenesis, constitutively active Notch induces follicle cell fate defects that are opposite to those generated by a loss-of-function allele of *N*: hyperplasia of stalk cells early in oogenesis and later, a loss of polar cells. The most extreme phenotype obtained with constitutively active Notch was the persistence of a precursor stage phenotype in the follicle cells required for pinching-off the egg chamber. This observation suggests a novel function for N in oogenesis in differentiation of these follicle cells: holding them in a precursor stage of development.

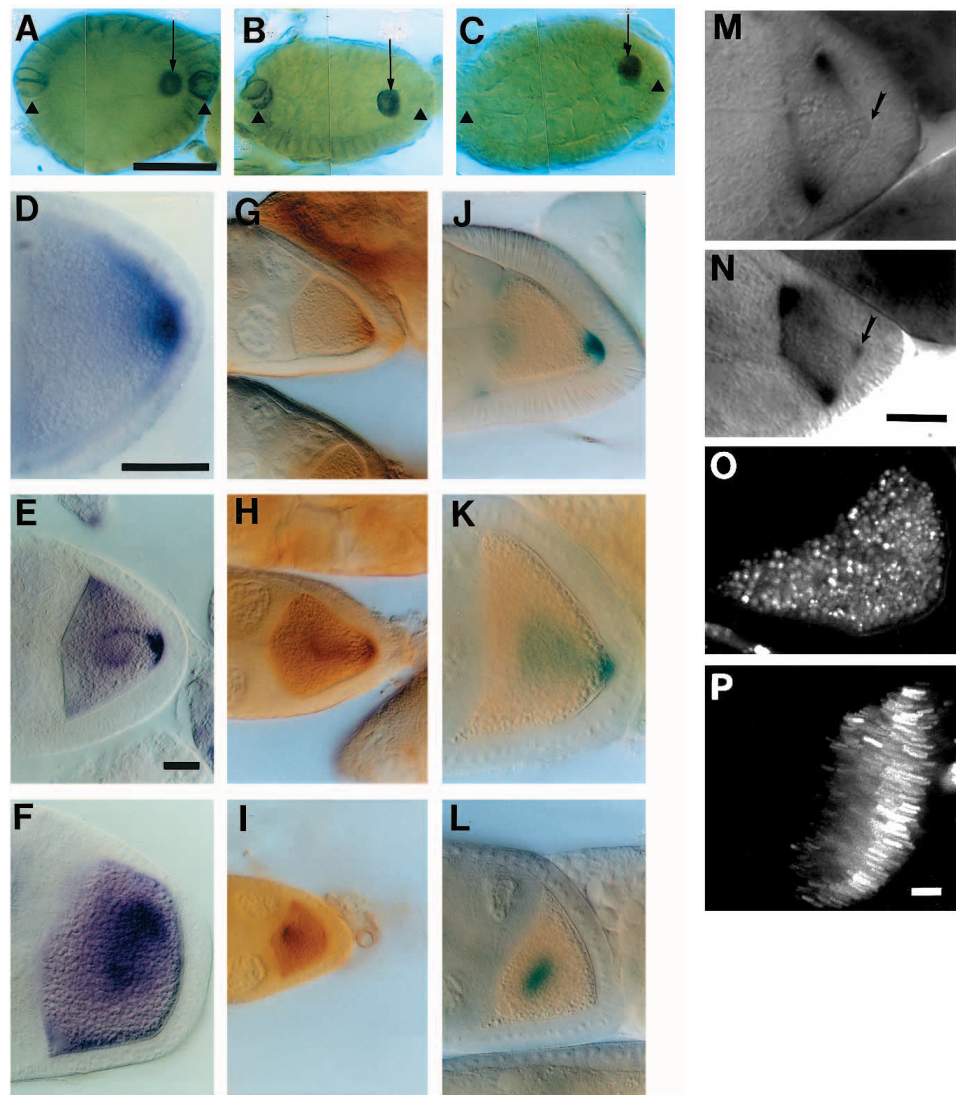
We have also shown that expression of constitutively active Notch in the follicle cells affects localization of the posterior components *oskar* and *Staufen*. In addition, mislocalization of a Kinesin-βgal fusion protein and premature onset of cytoplasmic streaming was seen, as well as mislocalization of the anterior component, *bicoid*. These phenotypes reflect a defect in the organization of the microtubules within the oocyte and implicates information from the follicle cells in regulation of this organization.

### Models for Notch action in oogenesis

Three models for Notch action have been proposed previously: instructive, permissive and prohibitive (reviewed in:

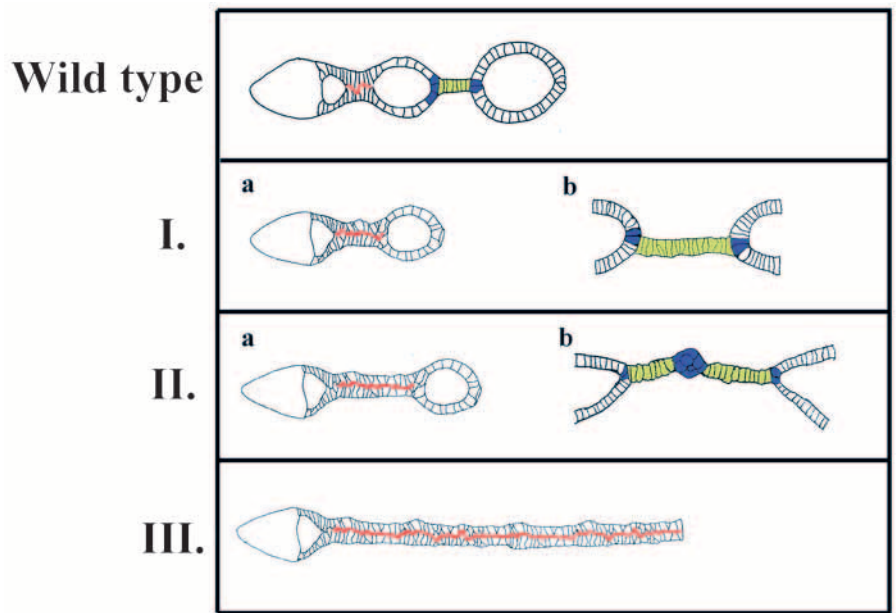
Greenwald, 1994; Muskavitch, 1994; Artavanis-Tsakonas, 1995). In the instructive model, Notch directs cells to adopt one of two alternative fates. This binary cell fate decision can be a choice between two differentiated cell fates or between remaining a precursor and achieving a more differentiated fate (Struhl et al., 1993; Greenwald, 1994). In the permissive model, Notch makes the cells competent to respond to inductive signals (Muskavitch, 1994). In the prohibitive model, Notch maintains cells in an uncommitted state preventing them from responding to external differentiation signals (Fortini et al., 1993; Coffman et al., 1993; Muskavitch, 1994; Artavanis-Tsakonas, 1995). Therefore, in both the permissive and prohibitive models, Notch modulates the cells' ability to accept additional signals.

Our data suggest that Notch is sufficient to keep cells in a specific precursor stage. This is consistent with either the prohibitive model or the instructive model, but is difficult to reconcile with the permissive model. The prohibitive model proposes that Notch holds cells in an uncommitted state, which could correspond to the *Bib*-positive precursor stage retained by expression of constitutively active Notch. However, the prohibitive model invokes additional differentiation signals for which there is no evidence thus far. Recent results have shown that Hedgehog acts as an inductive signal affecting follicle cell fate around region 2B of the germarium; however, it appears to affect a decision prior to that controlled by Notch (Forbes et al., 1996). Our data are also consistent with the binary cell fate decision model if a



**Fig. 6.** Effects of *caN* expression in the follicle cells later in oogenesis. Expression of constitutively active Notch induces the loss of polar cells (A-C). Wild-type and mutant egg chambers are stained with antibodies against Fas III and 65F proteins. Normal locations of polar cells are indicated by arrowheads; the 65F-positive oocyte nucleus by an arrow. (A) In wild-type egg chamber, two Fas III-positive polar cells are detected at the anterior and posterior ends (arrowheads). (B,C) Constitutively active N was induced by *HS-GAL4* expression due to two heat-shock pulses (39°C, 15 minutes and 39°C, 20 minutes) in a one day interval. In mutant ovaries dissected two days after the last induction, egg chambers are detected which lack either posterior polar cells (B) or both anterior and posterior polar cells (C). Expression of constitutively active Notch ( $\Delta EN$ ) in follicle cells results in a defect in oocyte anterior-posterior axis. (D-L) Posterior components and a Kinesin- $\beta gal$  fusion protein are mislocalized. (D-F) Localization of *oskar* mRNA in wild-type and  $\Delta EN$  egg chambers. (G-I) Localization of Staufin (Stau) protein in wild-type and  $\Delta EN$  egg chambers. (J-L) Localization of Kinesin- $\beta gal$  in wild-type and  $\Delta EN$  egg chambers. In wild-type egg chambers, the posterior components, *osk* and Stau, are localized tightly to the posterior pole of the oocyte (D,G). A Kinesin- $\beta gal$  fusion protein is also detected at the posterior pole (J). After expression of constitutively active N in the follicle cells by *GAL4-109(3)9*, posterior components and Kinesin- $\beta gal$  are detected either diffusing from the posterior (E,H,K), or in the center of the oocyte (F,I,L). (M,N) The anterior morphogen, *bicoid*, is mislocalized. (M) In wild-type egg chambers, *bicoid* mRNA is localized as a ring at the anterior margin of the oocyte. (N) In *GAL4-109(3)9* egg chambers, *bicoid* is detected at both the anterior and posterior poles in 15% (11/73) of stage 8 mutant egg chambers (arrows indicate posterior pole of oocyte). (O,P) Cytoplasmic streaming commences early. Yolk granules within the oocyte are detected by autofluorescence. (O) In wild-type egg chambers at stage 8, no cytoplasmic movement is detected within the oocyte. Ooplasmic streaming does not begin until stage 10b of oogenesis. (P) In *GAL4-109(3)9*, premature ooplasmic streaming is detected in 55% (10/18) of the stage 8 oocytes (O). (A-D, M-N) 100 $\times$ , (E-L, O-P) 40 $\times$ , (E-F, J-L, O-P; not dehydrated). All scale bars are equivalent to 20  $\mu m$ .

**Fig. 7.** Summary: three different types of long stalks are generated by expression of constitutively active Notch. Big brain (Bib) expression is shown in red, *A101* expression in blue and *93F* expression in green. (Wild type) In wild-type ovarioles, Bib is expressed in the precursor pattern in the cells which are going to constrict to form a stalk, *93F* is expressed in a in the fully formed stalks and *A101* is expressed in the polar cells at both poles of the egg chambers. Expression of constitutively active N generates three types of long stalks: (I) In the germarium, too many precursors are formed (a) and these further differentiate to form a long stalk which consists entirely of stalk cells (b). (II) Too many precursors are formed in the germarium (a), but these further differentiate into a long stalk which consists of stalk cells and polar cells (b). (III) A long stalk-like structure is formed in which all of the cells are in the precursor stage and they remain in this stage throughout development.



binary switch is assumed between precursor fate and a more differentiated cell fate (Greenwald, 1994).

### The anterior-posterior axis of the oocyte

The mislocalization of *bicoid* both to the anterior and posterior ends of the oocyte (Fig. 6N) and the localization of posterior components to the middle of the oocyte (Fig. 6E-F, H-I, K-L) are phenotypes reminiscent of those seen with ovaries from mutants in *N*, *Delta*, *Protein Kinase A*, *torpedo*, *gurken* and *cornichon* (reviewed in Rongo and Lehmann, 1996). During stages 1-6 of oogenesis, a microtubule-organizing center (MTOC) is located at the posterior pole of the oocyte and extends a microtubule network from the oocyte into the nurse cells. During midoogenesis, this cytoskeleton is reorganized so that an anterior-to-posterior gradient of microtubules is present in the oocyte, suggesting that the MTOC at the posterior degenerates and the microtubules are nucleated at the anterior of the oocyte. It has been postulated that, in these mutants (*N*, *Di*, *PKA*, *top*, *grk*, *cni*), the posterior MTOCs are not properly destroyed at stage 4-7 of oogenesis, resulting in oocytes with both anterior and posterior microtubule concentrations (Lane and Kalderon, 1994; Ruohola-Baker et al., 1994). A mirror-image axis duplication results (Ruohola et al., 1991; Lane and Kalderon, 1994; Gonzalez-Reyes et al., 1995; Roth et al., 1995). One possibility is that mislocalization of the posterior components as a streak from the posterior is a milder phenotype resulting from this type of defect in microtubule organization. In egg chambers that express constitutively active Notch in the follicle cells, ooplasmic streaming in the oocyte commences early, beginning around stage 8 of oogenesis. This is presumably also a secondary consequence of defects in the microtubule reorganizations.

One surprising result is that expression of constitutively active N either under the control of *GAL4-109(3)9* or *HS-GAL4* (mild heat shock) can lead to defects in the A-P axis of the oocyte without generating any obvious defects in the fate of the posterior follicle cells. By analogy to *caN* action in the germarium, it is possible, however, that the timing of

posterior follicle cell differentiation is altered in the mutant situation. Isolation of markers specific for different stages of posterior follicle cell differentiation should shed light on this question. It is also possible that the Notch pathway is closely linked to the signaling pathway from the follicle cells to the oocyte and expression of constitutively active Notch has affected the signaling without affecting the fates of these cells. Further analysis of this subject requires a better understanding of the signaling pathway from the follicle cells to the oocyte.

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