Notch signaling regulates the timing of neural stem cell entry into quiescence in *Drosophila*

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Abstract:

Most somatic stem cells are maintained in a quiescent state during development and in adult tissues. They switch between periods of proliferation and quiescence in response to nutrients, to maintain tissue homeostasis or to repair damaged tissue. Using *Drosophila* neural stem cells (known as neuroblasts, NB), we are interested in understanding how quiescence versus proliferation decisions are regulated during development. We use *Drosophila* as a model system because of the availability of genetic tools and because the population of neuroblasts is relatively simple and defined. Most neuroblasts in the central brain enter and exit quiescence in a nutrient-dependent and PI3-kinase regulated manner (Fig 1). To better understand how neural stem cell proliferation decisions are regulated we carried out a large-scale RNAi screen. From this screen, we identified components of the Notch signaling pathway. Notch is an evolutionarily conserved juxtacrine cell signaling pathway that in the *Drosophila* central brain allows for cross talk between both neuroblasts and their daughters and between neuroblasts and their glial niche. To activate Notch signaling, Notch receptor binds to its ligand, Delta. Here we investigate how Notch pathway regulates neuroblast entry and exit from quiescence during the embryonic to larval transition. We found that Notch pathway is active in proliferating neuroblasts but not in quiescent neuroblasts. When Notch pathway was knocked down in neuroblasts, we found that neuroblast proliferation continued for longer compared to control during embryo-to-larval transition. Next, we assayed expression of Notch signaling components as neuroblasts entered into quiescence. We found that Delta expression and Notch pathway activity was downregulated as the neuroblasts entered quiescence. Additionally, we found that Notch pathway downregulates expression of Delta in neuroblasts and that ectopic expression of Delta is sufficient to delay neuroblast entry into quiescence most likely through cis-inhibition of Notch pathway. Altogether, this suggests that Notch pathway promotes neuroblast entry into quiescence by downregulating expression of Delta which in turn forms a self-regualtory feedback loop of Notch pathway.

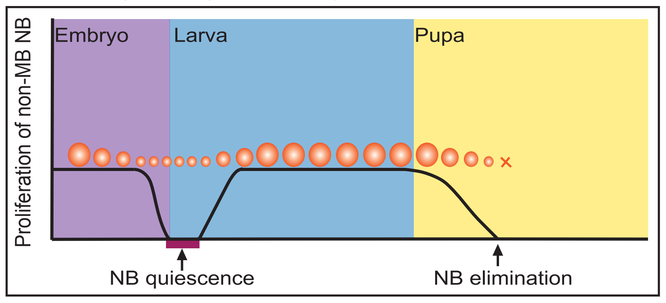

Figure 1: Proliferation of neuroblasts during development.

Results:

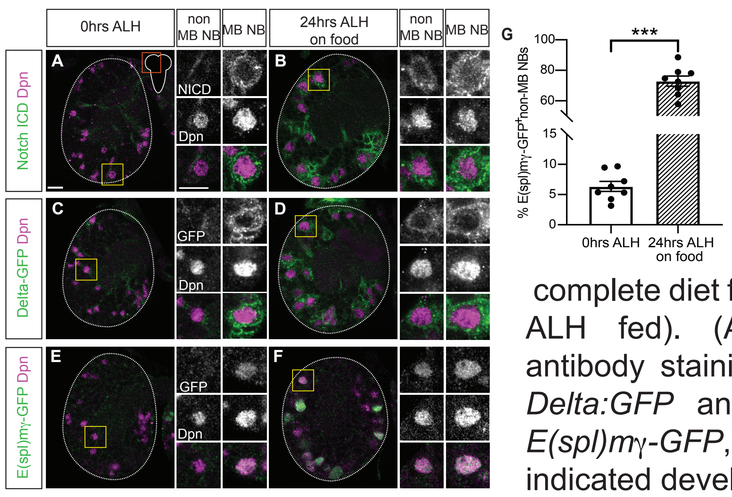

Figure 2: Notch pathway is active in proliferating but not quiescent neuroblasts.
A-I) Single plane images of a brain hemisphere from freshly hatched larvae (0hrs after larval hatching (ALH)) and larvae fed on a complete diet for 24hrs after larval hatching (24hrs ALH fed). (A-B) Notch intracellular domain antibody staining, (C-D) GFP antibody detecting *Delta:GFP* and (E-F) GFP antibody detecting *E(spl)mγ-GFP*, reporter for Notch signaling at the indicated developmental time points. Panels on the right show the mushroom body (MB) NBs and the non-MB NB (marked by yellow box) from the same brain hemisphere at a higher magnification. In this and subsequent figures, neuroblasts are marked with Dpn (magenta). Scale bar-10μm. (J) Graph shows percentage of non-MB NBs expressing the Notch pathway reporter *E(spl)mγ-GFP* at the indicated time points. Data represents mean ± SEM. ***p≤0.001.

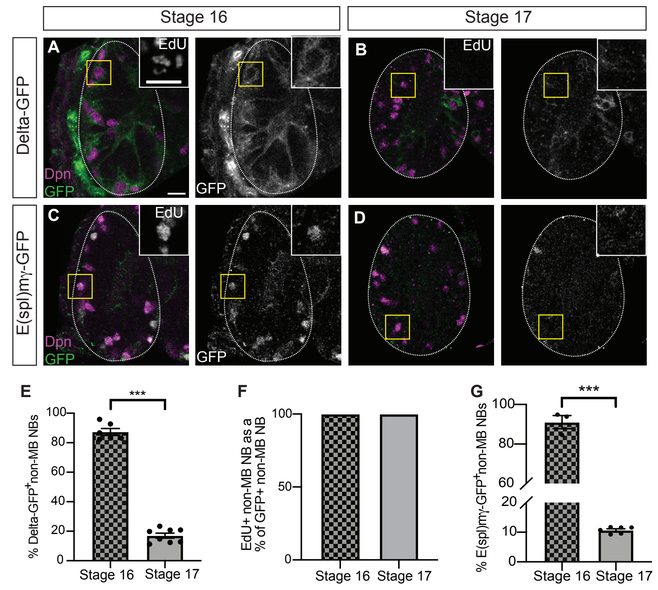

Figure 4: Loss of Delta expression and Notch pathway activity is correlated with neuroblast entry into quiescence.
(A-B) Single plane images of a brain hemisphere from larvae expressing *Delta:GFP* at the indicated embryonic time points. Panels show a non-MB NB (marked by yellow box) at a higher magnification. (E) Graph of percentage of non-MB NBs expressing *Delta:GFP* at the indicated time points. (F) Graph representing the percentage of EdU incorporating non-MB NBs that are also expressing *Delta:GFP* at the indicated time points. (C-D) Single plane images of a brain hemisphere from larvae expressing *E(spl)mγ-GFP* at the indicated embryonic time points. Panels show higher show a non-MB NB (marked by yellow box) at a higher magnification. (G) Graph of percentage of non-MB NBs expressing *E(spl)mγ-GFP* at the indicated time points. Data represents mean ± SEM. ***p≤0.001.

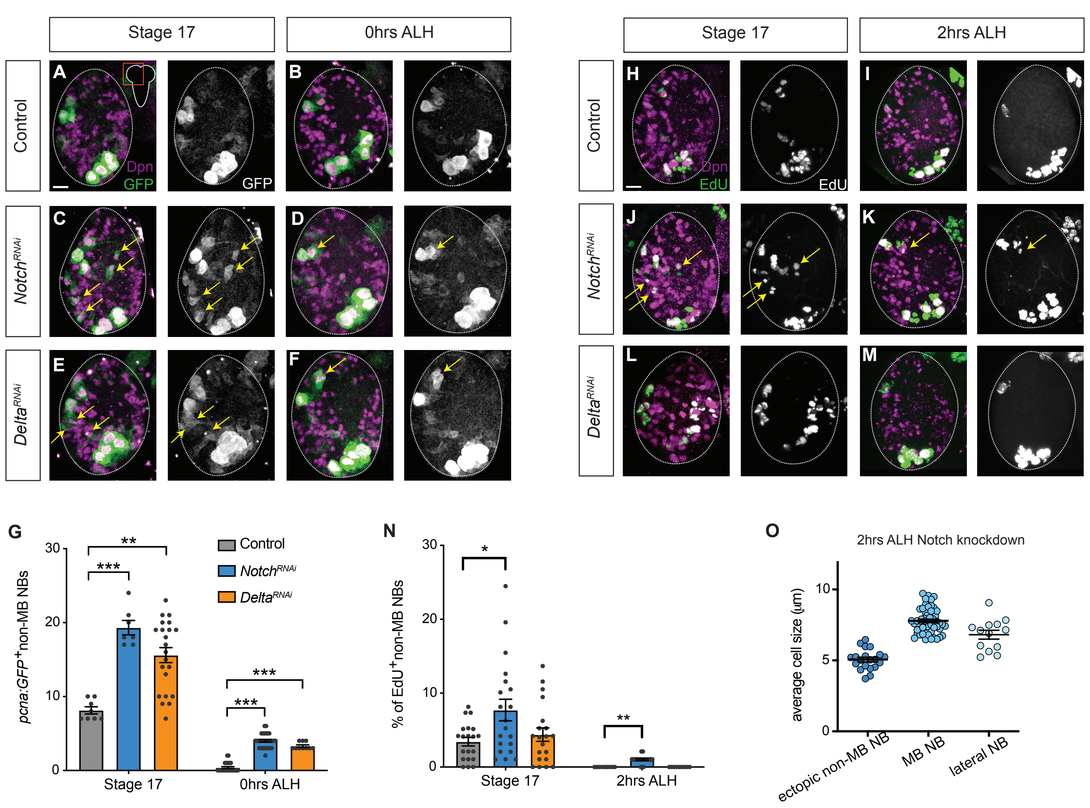

Figure 3: Notch signaling regulates the timing of neuroblast entry into quiescence.
(A-F) Maximum intensity projections from single brain hemispheres show *pcna:eGFP* expression in (A-B) Control larvae, (C-D) Notch knockdown larvae and (E-F) Delta knockdown larvae at the indicated developmental time points. All transgenes are driven by *Worniu-Gal4*. In this and subsequent figures, arrows indicate the ectopically proliferating NBs. (G) Graph shows number of non-MB NBs expressing *pcna:eGFP* at the indicated developmental time points. Numbers in each bar indicates the number of brain hemispheres analyzed. (H-M) Maximum intensity projections from single brain hemispheres show EdU incorporation in (I-J) Control larvae, (K-L) Notch knockdown larvae and (M-N) Delta knockdown larvae at the indicated developmental time points. (N) Graph representing the percentage of non-MB NBs incorporating EdU at the indicated developmental time points. (O) Scatter plot of the average cell size of the EdU incorporating neuroblasts at 2hrs ALH in Notch knockdown larvae. Data represents mean ± SEM.***p≤0.001, **p≤0.002,* p≤0.033.

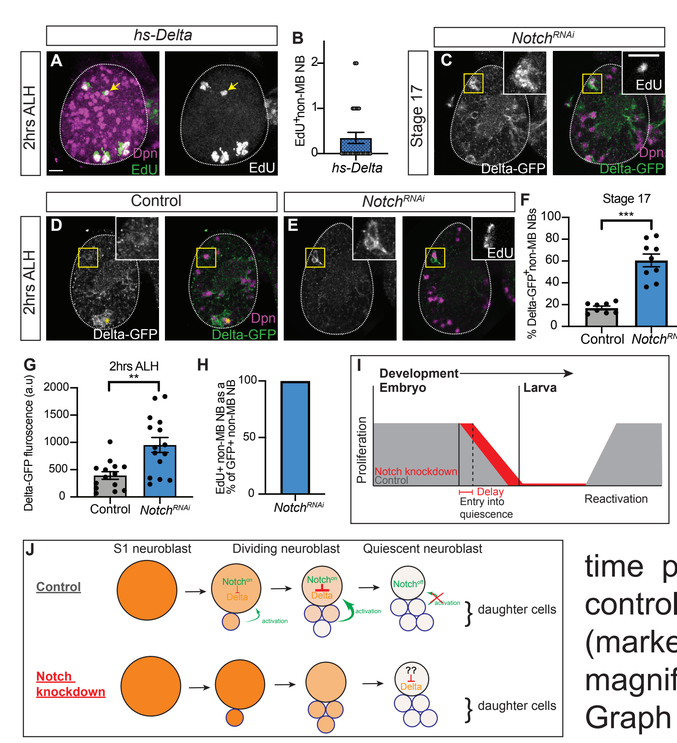

Figure 5: Notch pathway downregulates Delta expression in neuroblasts to drive neuroblast entry into quiescence
(A) Maximum intensity projections from single brain hemispheres show EdU incorporation in *hs-Delta* larvae. The animals were heat-shocked at stage 16 for 30mins. (B) Graph representing the number of non-MB NBs incorporating EdU (C-E) Single plane images of a brain hemisphere from larvae expressing *Delta:GFP* at the indicated embryonic time points in (C, E) Notch knockdown (D) control larvae. Panels show a non-MB NB (marked by yellow box) at a higher magnification. Asterisk (*) marks the MB NB. (F) Graph of percentage of non-MB NBs expressing *Delta:GFP* at stage 17 in control and Notch knockdown larvae. (G) Graph quantifying the fluroscence intensity of *Delta:GFP* in the ectopically proliferating neuroblast in Notch knockdown larve and its respective counterpart neuroblast in the control larvae. (H) Graph representing the percentage of ectopically proliferating non-MB NBs in Notch knockdown larvae that are also expressing *Delta:GFP* at the indicated time points.Data represents mean ± SEM. ***p≤0.001, **p≤0.002. (I-J) Model and mechanism of how Notch pathway regulates the timing of neuroblast entry into quiescence.

Conclusions:

1. Notch pathway is only active in proliferating neuroblasts.
2. Notch pathway is necessary for proper timing of neuroblast entry into quiescence.
3. Expression of Delta is downregulated in neuroblasts as they enter quiescence.
4. Notch pathway forms a feedback loop by inhibiting expression of Delta in neuroblasts.

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