Yorkie regulates nutrient-independent proliferation of Mushroom Body neuroblasts (MB NBs) in *Drosophila*

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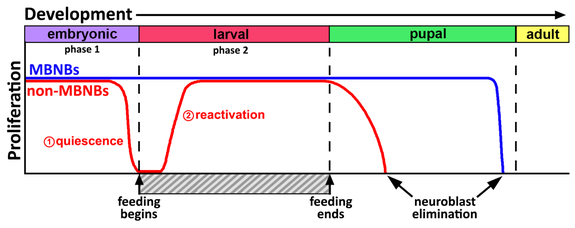
Introduction

The neural stem cell (NSC) population in the *Drosophila* central brain is ideal for investigating how systemic nutritional status affects stem cell proliferation. All neurons in the fly central brain are generated from asymmetric cell divisions of NSCs, known as neuroblasts (NBs). Most NBs enter a period of quiescence at the end of embyrogenesis coincident with declining maternal stores; upon larval feeding and reception of a nutrient-derived cue, these nutrient-sensitive NBs reenter the cell cycle and begin a second round of proliferation that continues until early pupal stages (**Fig. 1**). In contrast, mushroom body (MB) NBs, a subset of central brain NBs, never enter quiescence and divide continuously regardless of developmental stage or systemic nutritional state (Britton and Edgar, 1998). Both nutrient-sensitive NBs and nutrient-insensitive MBNBs reside in close proximity to one another, sharing a common environment, suggesting that quiescence versus proliferation decisions may be regulated in a cell-intrinsic manner. Here, we test this hypothesis by focusing on the role of the gene *Yorkie (Yki)*, a transcription coactivator that functions downstream of the evolutionarily conserved Hippo signaling pathway



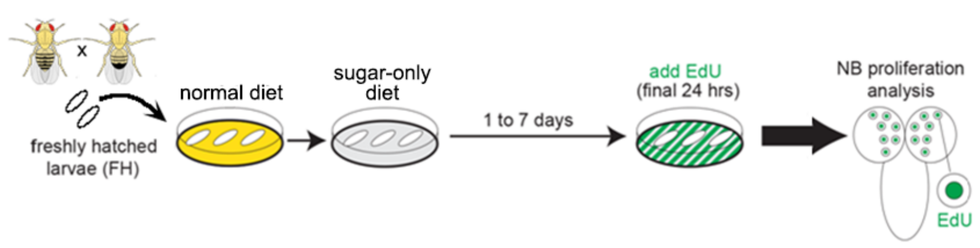


Figure 2. Experimental protocol to measure neuroblast proliferation during dietary nutrient restriction. Adapted from Britton and Edgar (1998). Freshly hatched larvae feed on a complete diet for 24 hours, reactivating non-MBNBs from developmental quiescence. Larvae are then transferred to a 20% sucrose solution and maintained for the specified number of days. Twenty-four hours before analysis, EdU its added to the sucrose solution to label proliferating cells. This figure was adapted from Sipe et. al. 2017

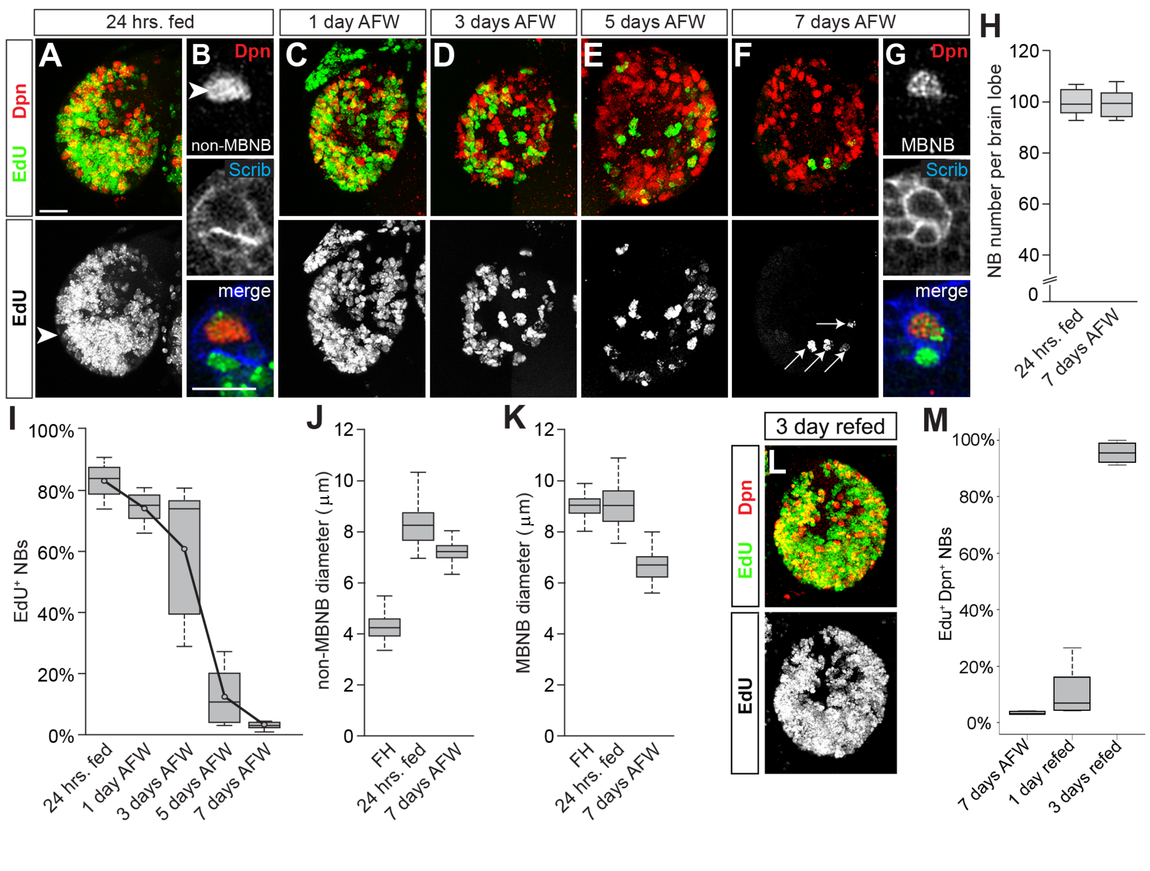


Figure 3. Two neuroblast subtypes in the central brain respond differently to dietary amino acid restriction. (A, C-F) Z-projections show cell proliferation in single brain lobes from larvae fed for 24 hours or at the indicated time points after food withdrawal (AFW). All neuroblasts are marked by Dpn (red). (B and G) Single Z-sections of a dividing non-MB NB (B) or MB NB (G). (H) The number of neuroblasts per brain lobe remains constant 7AFW. (I) Percent of proliferating neuroblasts per brain lobe at the indicated time points. (J,K) Non-MB NB (J) and MB NB (K) size at the indicated time points. (L) Z-projection of a brain lobe from a 7AFW larvae transferred back to complete diet and allowed to feed for 3 days. (M) Percent of proliferating neuroblasts per brain lobe after refeeding for the indicated number of days. This figure was taken from Sipe et. al. 2017

References

Britton, J and Edgar, B. Environmental control of the cell cycle in Drosophila: nutrition activates mitotic and endoreplicative cells by distinct mechanisms. *Development* **125,** 2149–2158 (1998). PMID: 9570778

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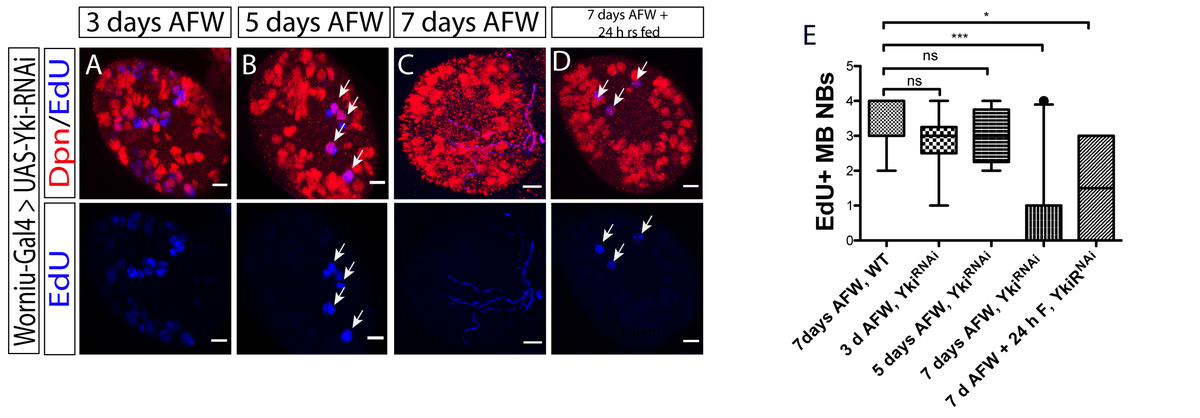

Figure 4: Yki is required for nutrient-independent MB NB proliferation
(A-D) Z-projections show cell proliferation in Yki knockdown single brain lobes at indicated time points. (C) Yki knockdown causes MB NBs stop proliferation in response to dietary nutrient withdrawal. (D) MB NBs resumed proliferation upon 24 hours of refeeding suggesting that Yki is necessary for nutrient-independent proliferation of the MB NBs. White arrows indicate proliferating MB NBs. Scale bar is 10 μm (E) quantification of the proliferating MB NB numbers at indicated time points. ***p≤0.001, *p≤0.033

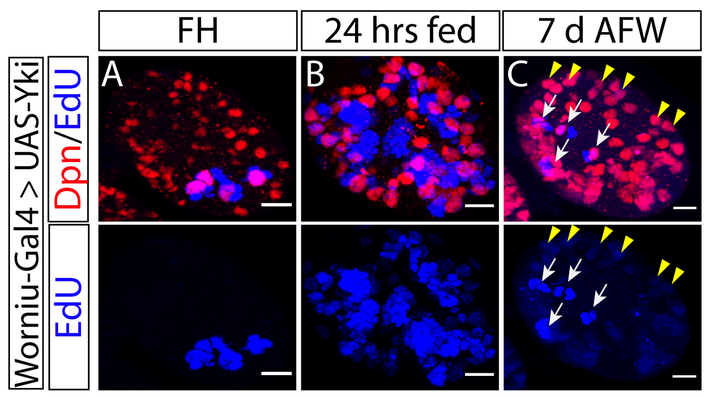

Figure 5: Yki is sufficient for driving NB proliferation in the absence of dietary nutrients
(A-C) Z-projections show cell proliferation in single brain lobes expressing a constitutively active form of Yki. Upon expression of a constitutively active form of Yki, NBs continue cell proliferation independent of dietary nutrient availability. This suggest that Yki is sufficient to drive NB proliferation in the absence of extrinsic dietary nutrient cues. White arrows mark four proliferating MB NBs similar to wild type brain (Figure 2F). Yellow arrow heads indicate some of the non-MB NBs that continue their proliferation in a nutrient-independent manner in response to Yki overexpression. Scale bar is 10 μm.

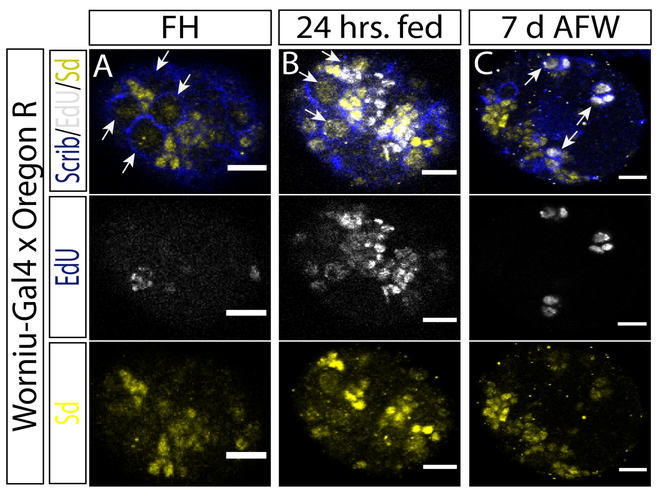

Figure 6: Sd is expressed in the NBs at different stages of larvae development
Yki requires a binding partner in order to induce its target gene expression. Scalloped (Sd) is the most well-known binding partner of Yki in different tissue types. However, the binding partner of Yki in the *Drosophila* brain remains unknown. (A-C) Z-projections show Sd expression at different stages of larvae development. Sd is expressed in NBs (both MB NBs and non-MB NBs) and their progeny cells. Sd expression level is higher in progeny cells compared to their progenitors (NBs). White arrows mark four MB NBs in a single brain lobe.

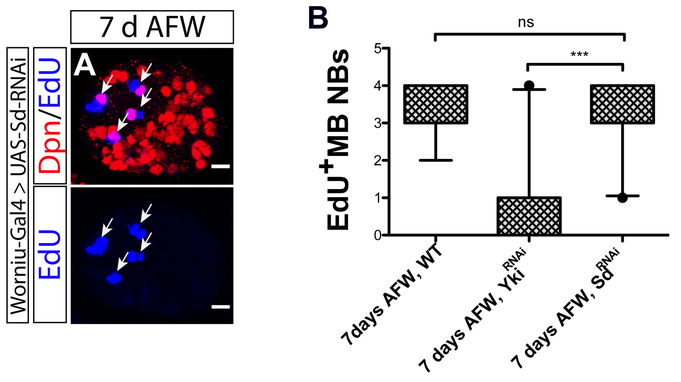

Figure 7: Sd knockdown does not affect dietary nutrient-independent proliferation of the MB NBs
We would expect Sd knockdown to phenocopy Yki knockdown in case of Sd being the binding partner of Yki in the NBs controlling proliferation. (A) Z-projection of a single brain lobe shows NB proliferation in Sd knockdown brain. Sd knockdown doesn't cause MB NBs to enter dietary nutrient restriction induced quiescence similar to Yki knockdown. This suggests that Sd is not the binding partner of Yki in the Drosophila brain in controlling NB proliferation. (B) quantification of the proliferating MB NB numbers at 7 days AFW. ***p≤0.001, *p≤0.033

Conclusions

1. MB NBs proliferate in a nutrient-independent manner whereas non-MB NBs require extrinsic nutrient cues in order to reactivate from quiescence and start their proliferation
2. Yki is necessary and sufficient to drive NB proliferation in the absence of extrinsic dietary nutrients cues
3. Sd seems to be not the binding partner of Yki in the NBs and our future work will determine the binding partner of Yki in the *Drosophila* brain in controlling NB proliferation