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Supplemental Information

Cell-Type-Specific Profiling of Gene Expression and Chromatin Binding without Cell Isolation: Assaying RNA Pol II Occupancy in Neural Stem Cells

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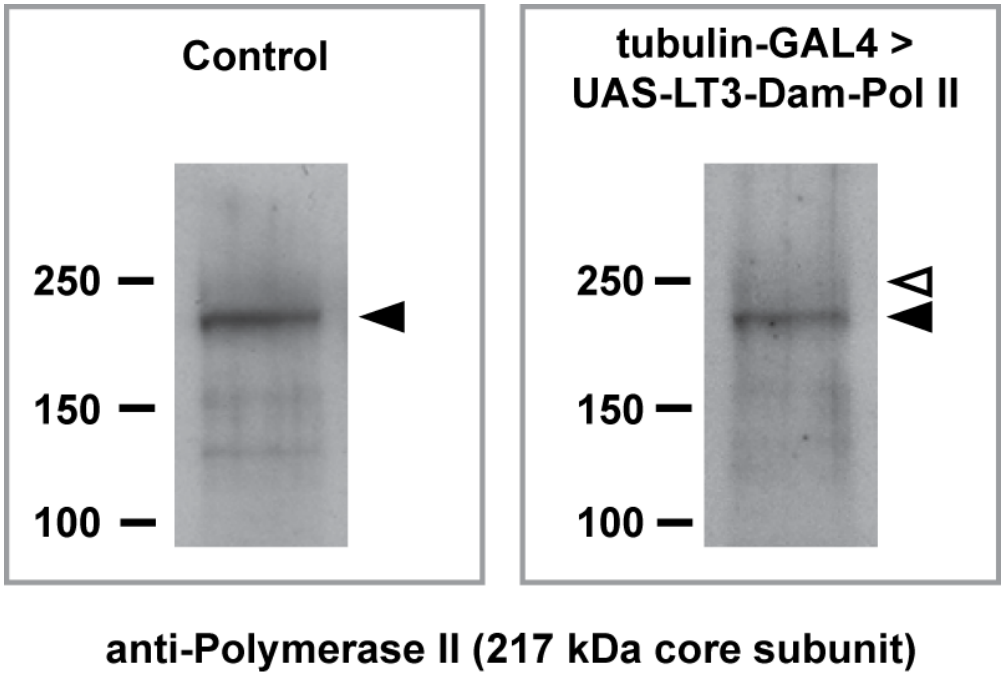

Figure S1, related to Figure 1. Dam-Pol II protein is undetectable relative to endogenous Pol II. Western blot showing endogenous Pol II levels (217 kDa, black arrowheads) in wildtype third instar larvae and third instar larvae expressing Dam-Pol II in all cells. Intensity levels were normalised to total protein loaded. No Dam-Pol II protein is detectable in the tubulin-GAL4>UAS-LT3-Dam-Pol II lane (predicted size 245 kDa; empty arrowhead). We compared the intensity of the band at 217 kDa with the region of the gel at predicted size 245 kDa for both samples, following background subtraction. The ratio of intensity is very similar: control = 2.4, larvae expressing Dam-Pol II = 2.3.

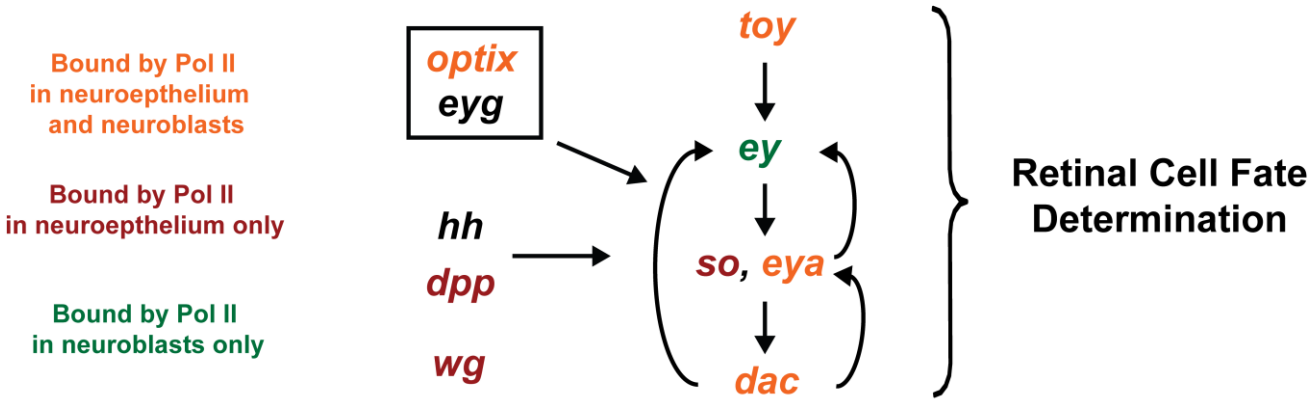

Figure S2, related to Figure 5. Pol II occupancy at genes involved in the retinal determination network. The majority of the retinal determination network (adapted from (Chen and Mardon, 2005) genes are bound by Pol II in the neuroepithelium, in neuroblasts or in both.

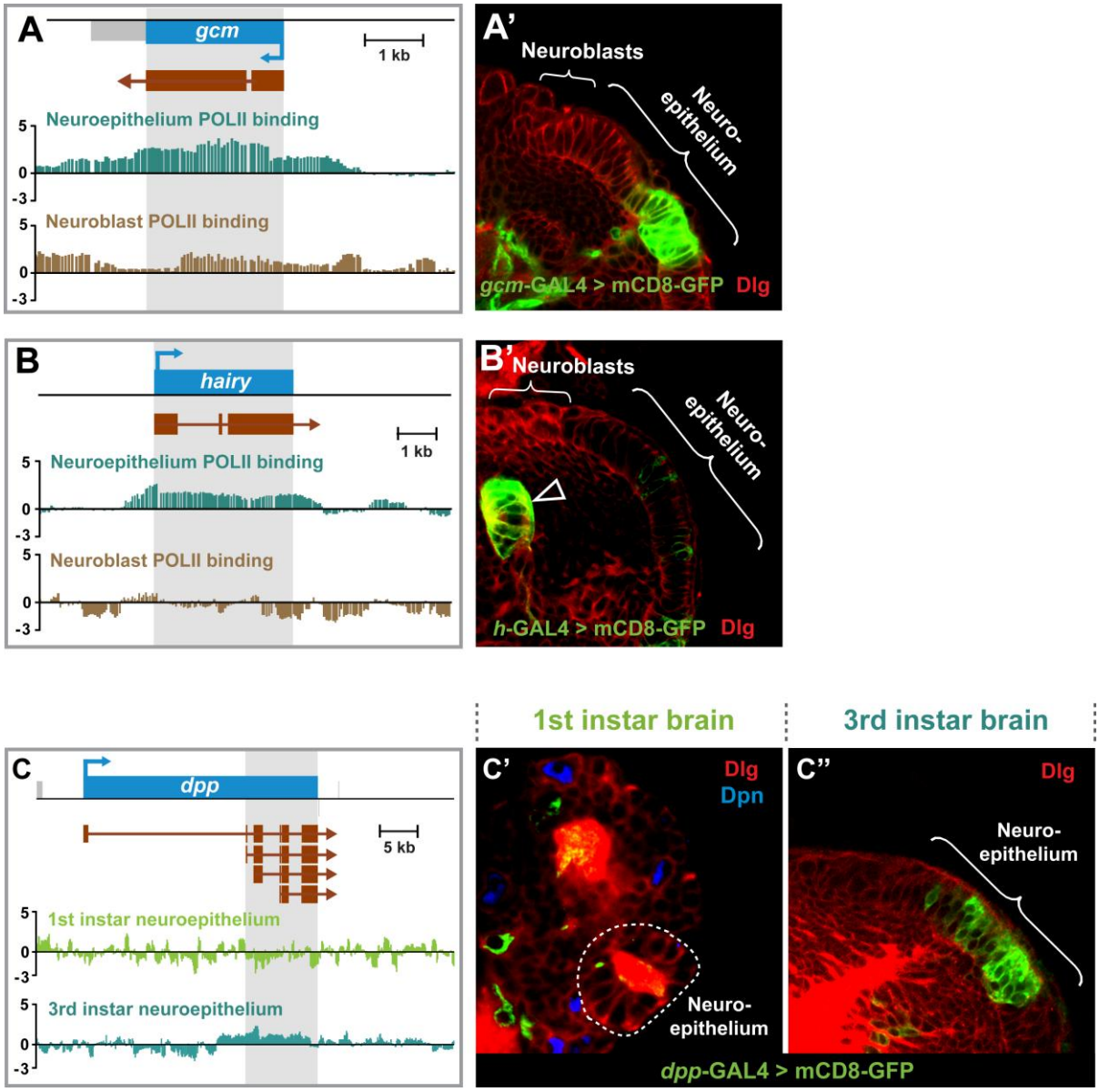

Figure S3, related to Figures 6 and 7. Cell type-specific and temporal differences in RNA Pol II occupancy reflect differences in gene expression. (A) and (B) Differential Pol II occupancy in the neuroepithelium and neuroblasts. (A') and (B') Expression pattern of the respective genes in the larval brain. (B') *hairy* is expressed in the neuroepithelium of the inner proliferation centre (IPC; open arrowhead). (C) Differential Pol II occupancy between the early and late larval neuroepithelium. (C') Expression of *dpp* in the first instar larval brain. (C'') Expression of *dpp* in the third instar larval brain. Scale bars represent log2 ratio change between Dam-Pol II and Dam (reference) samples.

Supplemental Tables

Gene	Significant Pol II occupancy	Reference
Tom	yes	(Egger et al., 2010)
Cad99C	yes	(Fung et al., 2008)
fat	yes	(Reddy et al., 2010)
sca	yes	(Mlodzik et al., 1990)
sc	yes	(Egger et al., 2007)
l(3)mbt	yes	(Richter et al., 2011)
tll	yes	(Yasugi et al., 2008)
HLHm5	yes	(Egger et al., 2010)
upd	yes	(Yasugi et al., 2008)
pnt	yes	(Yasugi et al., 2010)
rho	yes	(Yasugi et al., 2010)
HLHmγ	yes	(Yasugi et al., 2010)
N	yes	(Egger et al., 2010)
yan/aop	yes	(Yasugi et al., 2010)
Vsx1	yes	(Erclik et al., 2008)
ex	yes	(Reddy et al., 2010)
ds	yes	(Reddy et al., 2010)
fj	yes	(Reddy et al., 2010)
Dll	yes	(Kaphingst and Kunes, 1994)
wg	yes	(Kaphingst and Kunes, 1994)
dpp	yes	(Kaphingst and Kunes, 1994)
arm	yes	(Hayden et al., 2007)
Dl	yes	(Egger et al., 2010)
shg	yes	(Orihara-Ono et al., 2011)
Mcm2	yes	(Orihara-Ono et al., 2011)
neur	yes	(Boulianne et al., 1991)
CycA	yes	(Zhu et al., 2008)
mnb	yes	(Tejedor et al., 1995)
l'sc	yes	(Yasugi et al., 2008)
th/Diap1	yes	(Richter et al., 2011)
dpp	yes	(Kaphingst and Kunes, 1994)
bi/omb	yes	(Li and Padgett, 2012)
ft	yes	(Kawamori et al.)
Vdup1	yes	(Chang et al., 2010)
hth	yes	(Hasegawa et al., 2011)
lft	yes	(Mao et al., 2009)
yki	yes	(Reddy et al., 2010)
patj	no	(Egger et al., 2007)
mer	no	(Reddy et al., 2010)
aPKC	no	(Orihara-Ono et al., 2011)
dome	no	(Wang et al., 2010)
STAT92E	no	(Wang et al., 2010)

Table S1, related to Figure 4. Comparison of Dam-Pol II occupancy with published expression data. Literature search for genes reported to be expressed in the optic lobe neuroepithelium (determined by antibody staining, RNA in situ hybridisation or enhancer trap expression).

Table S2, related to Figures 4, 5, 6 and 7. Genes with significant Pol II occupancy in 3rd instar neuroepithelial cells, 3rd instar neuroblasts and 1st instar neuroepithelial cells (separate excel file).

Supplemental Experimental Procedures

Primer name	Sequence (5' to 3')
LT1-Ndam FW	GGAATTCATGGGAGGATCAGCTGGATAATAAGGCATGAAGAAAAATCGCGCTTTTTTG
LT1-Ndam RV	GGGAGATCTCGCAGATCCTCTTCAGAGATGAGTTTC
mGFP6 FW	GGAATTCATGAGTAAAGGAGAAGAACTTTTC
ΔmGFP6 RV	CAAAAAAGCGCGATTTTTCTTCATCTTATTACCGCTTCATATGATCAGGGTAAC
LT2-Ndam FW	GTTACCCTGATCATATGAAGCGGTAATAAGATGAAGAAAAATCGCGCTTTTTTG
Ndam RV	GGGAGATCTGCGCCGGCCAGATCCTC
mCherry FW	GGAATTCATGGCAACTAGCGGCATGGTTAG
mCherry RV	CAAAAAAGCGCGATTTTTCTTCATGTTATTATGCGGTACCAGAACCTTTG
LT3-Ndam FW	CAAAGGTTCTGGTACCGCATAATAACATGAAGAAAAATCGCGCTTTTTTG
mGFP6 RV	CAGATCTCCTTTGTATAGTTCATCCATGCCATG
RpII215 FW	GTACGCGGCCGCTCATGAGCACCCCCACGGACTCGAAG
RpII215 RV	GTCTAGATCAGTCTTCGCTCTCCTCGAACGTGG

Primer sequences for generating constructs. LT1-Ndam FW and LT1-Ndam RV were used to generate pUAST-LT1-NDam. mGFP6 FW, ΔmGFP6 RV, LT2-Ndam FW and Ndam RV were used to generate pUAST-LT2-NDam. mCherry FW, mCherry RV, LT3-NDam FW and NDam RV were used to generate pUASTattB-LT3-NDam. mGFP FW and mGFP RV were used to generate pUAST-NGFP. RpII215 FW and RpII215 RV were used to amplify RpII215 for cloning into pUAST-NGFP and pUASTattB-LT3-NDam.

Supplemental References

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