The chromatin remodeling factor Bap55 functions through the TIP60 complex to regulate olfactory projection neuron dendrite targeting

Tea and Luo
The chromatin remodeling factor Bap55 functions through the TIP60 complex to regulate olfactory projection neuron dendrite targeting

Joy S Tea, Liqun Luo*

Abstract

Background: The Drosophila olfactory system exhibits very precise and stereotyped wiring that is specified predominantly by genetic programming. Dendrites of olfactory projection neurons (PNs) pattern the developing antennal lobe before olfactory receptor neuron axon arrival, indicating an intrinsic wiring mechanism for PN dendrites. These wiring decisions are likely determined through a transcriptional program.

Results: We find that loss of Brahma associated protein 55 kD (Bap55) results in a highly specific PN mistargeting phenotype. In Bap55 mutants, PNs that normally target to the DL1 glomerulus mistarget to the DA4l glomerulus with 100% penetrance. Loss of Bap55 also causes derepression of a GAL4 whose expression is normally restricted to a small subset of PNs. Bap55 is a member of both the Brahma (BRM) and the Tat interactive protein 60 kD (TIP60) ATP-dependent chromatin remodeling complexes. The Bap55 mutant phenotype is partially recapitulated by Domino and Enhancer of Polycomb mutants, members of the TIP60 complex. However, distinct phenotypes are seen in Brahma and Snf5-related 1 mutants, members of the BRM complex. The Bap55 mutant phenotype can be rescued by postmitotic expression of Bap55, or its human homologs BAF53a and BAF53b.

Conclusions: Our results suggest that Bap55 functions through the TIP60 chromatin remodeling complex to regulate dendrite wiring specificity in PNs. The specificity of the mutant phenotypes suggests a position for the TIP60 complex at the top of a regulatory hierarchy that orchestrates dendrite targeting decisions.

Background

The stereotyped organization of the Drosophila olfactory system makes it an attractive model to study wiring specificity. The first olfactory processing center is the antennal lobe, a bilaterally symmetric structure at the anterior of the Drosophila brain. It is composed of approximately 50 glomeruli in a three-dimensional organization. Each olfactory projection neuron (PN) targets its dendrites to one of those glomeruli to make synaptic connections with a specific class of olfactory receptor neurons. Each PN sends its axon stereotypically to higher brain centers [1-3].

During development, the dendrites of PNs pattern the antennal lobe prior to axons of olfactory receptor neurons [4]. The specificity of PN dendrite targeting is largely genetically pre-determined by the cell-autonomous action of transcription factors, several of which have been previously described [5-8]. Furthermore, chromatin remodeling factors have been shown to play an important role in PN wiring [8], although very little is currently known about their specific functions. We report here a genetic screen for additional factors that regulate PN dendrite wiring specificity, and identify Brahma associated protein 55 kD (Bap55) as a regulator of PN dendrite wiring specificity as part of the TIP60 chromatin remodeling complex.

Bap55 is an actin-related protein, the majority of which physically associates with the Brahma (BRM) chromatin remodeling complex in Drosophila embryo extracts [9] (Figure 1A). There are two distinct BRM complexes: BAP (Brahma associated proteins; homologous to yeast SWI/SNF) and PBAP (Polybromo-associated BAP; homologous to yeast RSC), both of which contain Brahma, Bap55, and Snf5-Related 1 (Snr1) [10]. The human homologs of the BAP and

*Correspondence: lluo@stanford.edu
Howard Hughes Medical Institute, Department of Biology, Neurosciences Program, Stanford University, Stanford, CA 94305, USA

© 2011 Tea and Luo; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
PBAP complexes are called the BAF (Brg1 associated factors) and PBAF (Polybromo-associated BAF) complexes, respectively. The BRM/BAF complexes are members of the SWI/SNF family of ATP-dependent chromatin-remodeling complexes, and have been shown to both activate and repress gene transcription, in some cases, of the same gene [11-14].

In Drosophila, RNA interference knockdown of Bap55 in embryonic class I da neurons revealed dendrite misrouting phenotypes and reduced arborization [15]. The human homologs of Bap55 are BAF53a and BAF53b, with approximately equal homology. BAF53a is important for maintaining embryonic stem cell self-renewal and pluripotency as a part of the BAF complex found in embryonic stem cells (esBAF) [16]. BAF53b is important for dendritic outgrowth as a part of the BAF complex found in postmitotic neurons (nBAF) [17]. However, previous experiments have not clearly distinguished whether Bap55/BAF53b acts exclusively as a part of the BRM/BAF complex in regulating dendrite development.

In experiments purifying proteins in complex with tagged Drosophila Pontin in S2 cells, Bap55 was also copurified as a part of the TIP60 complex, as determined
by mass spectrometry [18] (Figure 1B). The TIP60 histone acetyltransferase complex has been shown to be involved in many processes, including both transcriptional activation and repression [19]. The complex contains many components, including Bap55, Domino (Dom), and Enhancer of Polycomb (E(Pc)) [18]. Dom, homologous to human p400, is the catalytic DNA-dependent ATPase; its ATPase domain is highly similar to *Drosophila* Brahma and human BRG1 ATPase domains [20]. E(Pc) is homologous to human EPC1 and EPC2 and is an unusual member of the Polycomb group; it does not exhibit homeotic transformations on its own, but rather enhances mutations in other Polycomb group genes [21].

We provide evidence that Bap55 functions as a part of the TIP60 complex rather than the BRM complex in postmitotic PNs to control their dendrite wiring specificity.

Results

Bap55 is required in projection neurons for dendrite targeting

To further our understanding of dendrite wiring specificity in *Drosophila* olfactory PNs, we performed a MARCM-based forward genetic screen using piggyBac insertional mutants [22]. MARCM allows visualization and genetic manipulation of single cell or neuroblast clones in an otherwise heterozygous background, permitting the study of essential genes in mosaic animals [23]. In this screen, we used GH146-GAL4 to label a single PN born soon after larval hatching [1], which in wild-type (WT) animals always projects its dendrites to the dorsolateral glomerulus DL1 in the posterior of the antennal lobe (Figure 2A). The DL1 PN also exhibits a stereotyped axon projection, forming an L-shaped pattern in the lateral horn, with additional branches in the mushroom body calyx (Figure 2B). We identified a

![Figure 2 Bap55 regulates projection neuron dendrite targeting](image-url)

**Figure 2** Bap55 regulates projection neuron dendrite targeting. (A) Wild-type (WT) DL1 PN dendrites target specifically to the posterior glomerulus DL1 (yellow dashed circle in A2) and never target to the anterior glomerulus DA4l (yellow dashed circle in A1). White arrowhead denotes cell body in all images. (B) WT DL1 axons show an L-shaped pattern in the lateral horn (LH), with branches in the mushroom body calyx (MBC; both outlined with white dashed circles). (C) Bap55^-/- PN dendrites fully mis-target to the anterior glomerulus DA4l (C2), abandoning the posterior glomerulus DL1 (C1). (D) Bap55^-/- PN axons show the stereotypical L-shaped pattern in the LH with branches in the MBC. (E) WT GH146 anterodorsal neuroblast clones are characterized by their cell body location dorsal to the antennal lobe (white arrowhead in all images). The dendrites target to stereotyped glomeruli in the antennal lobe. (F) WT GH146 lateral neuroblast clones are characterized by their cell body location lateral to the antennal lobe with dendrites targeting to stereotyped glomeruli. (G) WT GH146 ventral neuroblast clones are characterized by their cell body location ventral to the antennal lobe with dendrites targeting across the antennal lobe. (H, I) Bap55^-/- GH146-labeled anterodorsal (H) and lateral (I) neuroblast clones maintain their cell body location dorsal and lateral to the antennal lobe, respectively. Yet their dendrites do not target proper glomeruli. (J) Bap55^-/- GH146-labeled ventral neuroblast clones maintain their cell body location ventral to the antennal lobe, yet their dendrites target a small area of the antennal lobe. Green marks mCD8-GFP-labeled PNs generated by MARCM and labeled using GH146-GAL4. (A, C) show partial confocal stacks; (B, D, E-J) show full confocal stacks; magenta is the presynaptic marker nc82; extraneous magenta punctate staining outside of the antennal lobe in some panels is dsRed background from the piggyBac and/or GH146-GAL4 insertions. Scale bars: 20 μm in (A) (for A, C, E-J) and (B) (for B, D).
mutant, called LL05955, in which DL1 PNs mistargeted to the dorsolateral glomerulus DA4l in the anterior of the antennal lobe (Figure 2C). This phenotype is strikingly specific, with 100% penetrance (Table 1). Arborization of mutant axons, however, was not obviously altered (Figure 2D). We identified the piggyBac insertion site using inverse PCR [22] and Splinkerette PCR [24]. LL05955 is inserted into the coding sequence of Bap55 (Figure 1C), encoding a homolog of human BAF53a and BAF53b. Precise excision of the piggyBac insertion reverted the dendrite mistargeting phenotype, confirming that disruption of the Bap55 gene causes the dendrite mistargeting (Table 1).

In addition to causing DL1 mistargeting, Bap55 mutants also display neuroblast clone phenotypes. In WT, GH146-GAL4 can label three distinct types of PN neuroblast clones generated in newly hatched larvae. Two of these clones, the anterodorsal neuroblast clone (Figure 2E) and the lateral neuroblast clone (Figure 2F), possess cell bodies that lie dorsal or lateral to the antennal lobe, respectively. PNs from these two lineages project their dendrites to stereotyped and nonoverlapping subsets of glomeruli in the antennal lobe. The third type of clone, the ventral neuroblast clone, has cell bodies that lie ventral to the antennal lobe and dendrites that target throughout the antennal lobe due to the inclusion of at least one PN that elaborates its dendrites to all glomeruli (Figure 2G) [1,2].

In Bap55−/− PNs, anterodorsal neuroblast clones display a mild reduction in cell number, and their dendrites are abnormally clustered in the anterior dorsal region of the antennal lobe, including the DA4l glomerulus (Figure 2H). Lateral neuroblast clones display a severe reduction in cell number, and the remaining dendrites are unable to target to many glomeruli throughout the antennal lobe (Figure 2I). Ventral neuroblast clones display a mild reduction in cell number and a reduced dendrite mass throughout the antennal lobe (Figure 2J). During development, the lateral neuroblast first gives rise to local interneurons before switching to produce PNs [25]; in mutants affecting cell proliferation, this property of the lateral neuroblast displays as a severe reduction in GH146-labeled PNs. The severely reduced cell number in Bap55 mutants suggests that Bap55 is essential for neuroblast proliferation or neuronal survival. In the anterodorsal and ventral neuroblasts, PN numbers are not drastically changed; thus, the phenotypes indicate that Bap55 is important for dendrite targeting in multiple classes of PNs.

**Bap55 mutants cause derepression of a PN-GAL4**

In WT, Mz19-GAL4 labels a subset of the GH146-GAL4 labeling pattern. It labels a small number of PNs derived from two neuroblasts, which can be clearly identified in WT clones generated in newly hatched larvae. Anterodorsal neuroblast clones target their dendrites to the VA1d glomerulus (Figure 3A), as well as the DC3 glomerulus residing immediately posterior to VA1d (not easily visible in confocal stacks). Lateral neuroblast clones target all dendrites to the DA1 glomerulus (Figure 3B). Unlike GH146-GAL4, WT Mz19-GAL4 never labels ventral neuroblast clones because it is not normally expressed in those cells (Figure 3C).

In Bap55 mutant PN clones, however, Mz19-GAL4 labels additional PNs in anterodorsal, lateral, and ventral clones (Figures 3D-F) compared to their WT counterparts (Figures 3A-C). This phenotype suggests that some Mz19-negative PNs now express Mz19-GAL4. In anterodorsal clones, Mz19-GAL4 labels additional cells, although not as many as are labeled by GH146-GAL4. The PNs also mistarget their dendrites to the anterior antennal lobe (Figure 3D), similar to mutant phenotypes.

| Genotype         | Total n | %DL1 | %DA4l | %DL1 + DA4l | %DM6 | %DA4l + DM6 | %DA2 | %DA2 + DM6 |
|------------------|---------|------|-------|-------------|------|-------------|------|------------|
| WT               | 25      | 100  | 0     | 0           | 0    | 0           | 0    | 0          |
| Bap55−/−         | 28      | 0    | 100   | 0           | 0    | 0           | 0    | 0          |
| Bap55−/− precise excision | 19 | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| Bap55−/− + UAS-Bap55 | 32 | 90 | 0 | 0 | 10 | 0 | 0 | 0 |
| UAS-Bap55        | 16      | 100  | 0     | 0           | 0    | 0           | 0    | 0          |
| Bap55−/− + UAS-BAF53a | 19 | 68 | 0 | 0 | 32 | 0 | 0 | 0 |
| Bap55−/− + UAS-BAF53b | 21 | 94 | 0 | 0 | 6 | 0 | 0 | 0 |
| dom−             | 23      | 4    | 9     | 87          | 0    | 0           | 0    | 0          |
| dom− precise excision | 18 | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| dom− + UAS-Bap55 | 15      | 27   | 6     | 67          | 0    | 0           | 0    | 0          |
| E(Pc)−/−         | 22      | 5    | 45    | 0           | 14   | 0           | 36   | 0          |
| E(Pc)−/− + UAS-Bap55 | 20 | 0 | 0 | 0 | 70 | 5 | 25 | 0 |

Shown is the total number of analyzed PN single cell clones and percent targeting to listed glomeruli. In bold is the glomerulus with the highest percentage of targeting.
GH146-GAL4 anterodorsal neuroblast clones (Figure 2H). WT lateral neuroblast clones normally contain GH146-positive PNs and GH146-negative local interneurons [25]. In Bap55⁻/⁻ lateral neuroblast clones, Mz19-GAL4 predominantly labels local interneurons that send their processes to many glomeruli throughout the antennal lobe (Figure 3E) and do not send axon projections to higher brain centers. Lateral clones also show ectopic PN labeling with a lower frequency (data not shown). The Bap55 mutant appears to cause derepression of Mz19-GAL4, resulting in labeled local interneurons. Ventral clones are never labeled in WT Mz19-GAL4 (Figure 3C), yet are labeled in Bap55 mutants (Figure 3F). This further indicates a derepression of the Mz19-GAL4 labeling pattern.

Unlike GH146-GAL4, WT Mz19-GAL4 never labels single cell clones when clone induction is performed in newly hatched larvae (Figure 3G, H). This is because Mz19-GAL4 is not expressed in the DL1 PN, the only GH146-positive cell generated during this heat shock time of clone generation. However, in Bap55 mutant PN clones, Mz19-GAL4 ectopically labels single cell anterodorsal PN clones targeting to the DA4l glomerulus (Figure 3I), which show an L-shaped pattern in the lateral horn with branches in the mushroom body calyx (Figure 3J). The simplest interpretation is that this compound phenotype reflects first a derepression of Mz19-GAL4 in the DL1 PN, and second a DL1 to DA4l mistargeting phenotype in Bap55 mutants.

Bap55 is required in postmitotic PNs for dendrite targeting

To test whether Bap55 functions in the neuroblast or postmitotically in PNs, we used GH146-GAL4, which expresses only in postmitotic PNs [7], to express UAS-Bap55 in a Bap55⁻/⁻ single cell clone. We show that the dendrite mistargeting phenotype is rescued to the WT DL1 glomerulus (Figure 4A, Table 1) and conclude that Bap55 functions postmitotically to regulate PN dendrite targeting. The axon phenotype remains the stereotypical L-shaped pattern (Figure 4B).

However, in 2 out of 21 cases, expression of UAS-Bap55 in a Bap55⁻/⁻ single cell clone resulted in a de
novo phenotype. The PN dendrites targeted to neither the DA4l nor the DL1 glomeruli, but to the DM6 glomerulus in the anterior medial region of the antennal lobe (Figure 4C, Table 1). In addition, the axon showed a mistargeting phenotype, extending ventrally to the lateral horn (Figure 4D). The two cases showed correlated DM6 dendrite and ventral axon mistargeting; the remaining 19 out of 21 cases showed full DL1 rescue and an L-shaped axon pattern. Expression of UAS-Bap55 in a WT single cell clone, however, did not cause any phenotype (n = 16; Table 1).

Human BAF53a and b can rescue Bap55 mutant phenotypes

The Drosophila Bap55 protein is 70% similar and 54% identical to human BAF53a and 71% similar and 55% identical to human BAF53b. BAP53a and b are 91% similar and 84% identical to each other. Using GH146-GAL4
to express human BAF53a or b in a Bap55−/− single cell clone, we found that the human homologs can effectively rescue the Bap55−/− dendrite mistargeting phenotype (Figures 4E-H). Interestingly, both also cause the de novo DM6 dendrite and ventral axon mistargeting phenotypes in 6 out of 19 cases for BAF53a and 2 out of 32 cases for BAF53b. These phenotypes are quantified in Figure 4I and summarized in Table 1. Thus, human BAF53a and b can largely replace the function of Drosophila Bap55 in PNs.

Mutations in other BRM complex components have distinct PN dendrite targeting phenotypes

To address whether Bap55 functions as a part of the BRM complex in PN dendrite targeting, we tested two additional BRM complex mutants for their PN dendrite phenotypes. We first tested Brahma (brm), the catalytic ATPase subunit of the BRM complex, which is required for the activation of many homeotic genes in Drosophila [26] (Figure 1D). Null mutations have been shown to decrease cell viability and cause peripheral nervous system defects [27]. RNA interference knockdown of brm in embryonic class I da neurons revealed dendrite misrouting phenotypes, although not identical to the Bap55 phenotype [15]. The human homologs of brm, BRM and BRG1 (Brahma-related gene-1), both have DNA-dependent ATPase activity. Inactivation of BRM in mice does not yield obvious neural phenotypes, but inactivation of BRG1 in neural progenitors results in reduced proliferation. BRG1 is likely to be required for various aspects of neural development, including proper neural tube development [28].

In PNs, brm mutants displayed anterodorsal single cell clone mistargeting to non-stereotyped glomeruli throughout the antennal lobe, with each clone differing from the next (Figure 5A; n = 16). This is in contrast to the highly stereotyped DA4I mistargeting of Bap55 mutants. brm−/− neuroblast clones also displayed phenotypes where dendrites make small, meandering projections throughout the antennal lobe, which does not resemble the Bap55−/− phenotype (Figures 5B-D). They additionally exhibit a perturbed cell morphology phenotype, which is markedly more severe than the Bap55 mutant phenotype.

We next tested Snr1, a highly conserved subunit of the BRM complex (Figure 1E). It is required to restrict BRM complex activity during the development of wing vein and intervein cells [29] and functions as a growth regulator [30,31]. Its human homolog, SNF5, is strongly correlated with many cancers [32], yet little is known about its specific role in neurons.

In PNs, Snr1 mutants displayed similar phenotypes to brm mutants. The single cell clones displayed mistargeting to non-stereotyped glomeruli throughout the antennal lobe, with each clone demonstrating a unique phenotype (Figure 5E; n = 31). The neuroblast clones exhibited small meandering dendrites throughout the antennal lobe (Figures 5F-H), which also showed extremely perturbed cell morphology. These results, especially the non-stereotyped single cell clone phenotypes, indicate that the BRM complex functions differently from Bap55 in controlling PN dendrite targeting.

We further analyzed brm and Snr1 mutants with Mz19-GAL4 to determine whether their phenotypes resembled the Bap55 mutant phenotype of derepression. We were unable to generate any labeled clones,
indicating one of three possibilities: increased cell death, severe cell proliferation defects, or repression of the Mz19-GAL4 labeling pattern. In any of the three cases, the phenotype does not resemble the Bap55\textsuperscript{-/-} mutant phenotype of abnormal activation of Mz19-GAL4 in single cell or neuroblast clones, indicating that the BRM complex functions differently from Bap55 in PNs.

dom mutant PNs exhibit phenotypes similar to Bap55 mutants

In the same screen in which we identified the Bap55 mutation, we also independently identified LL05537, a mutation in dom that resulted in a qualitatively similar phenotype to Bap55 mutants. dom\textsuperscript{-/-} DL1 PNs split their dendrites between the posterior glomerulus DL1 and the anterior glomerulus DA4l (Figure 6A). Their axons exhibit a WT L-shaped pattern in the lateral horn (Figure 6B).

The LL05537 allele contains a piggyBac insertion in an intron just upstream of the translation start of dom (Figure 1F). Because the piggyBac insertion contains splice acceptor sites and stop codons in all three coding frames [22], this allele likely disrupts all isoforms of dom. Similarly to Bap55, we identified the piggyBac insertion site using inverse PCR [22] and Splinkerette PCR [24]. Precise excision of the piggyBac insertion reverted the dendrite targeting phenotype, confirming that disruption of the dom gene causes the dendrite mistargeting (Table 1). In addition, a BAC transgene that contains the entire dom transcriptional unit rescued the dom\textsuperscript{-/-} mutant phenotypes (Figure 6E).

Dom is the catalytic DNA-dependent ATPase of the TIP60 complex and has been shown to contribute to a repressive chromatin structure and silencing of homeotic genes. Dom is a member of the SWI/SNF family and its ATPase domain is highly similar to the Drosophila Brahma and human BRG1 ATPase domains [20]. The human homolog of Dom is p400, which is important for regulating nucleosome stability during repair of double-stranded DNA breaks [33] and an important regulator of embryonic stem cell identity [34].

To determine whether Bap55 and Dom genetically interact, we expressed UAS-Bap55 in a dom\textsuperscript{-/-} PN. This manipulation did not significantly alter the dendrite phenotype (Figures 6C, D, quantified in Figure 6E and Table 1; \(P > 0.05\) using two-way ANOVA with a Bonferroni posttest comparison across all columns). The axon branching pattern also was not altered.

E(Pc) mutant PNs also exhibit phenotypes similar to Bap55 mutants

We also examined another component of the TIP60 complex, E(Pc) (Figure 1G). In Drosophila, E(Pc) is...
suppressor of position-effect variegation [21,35] and heterozygous mutations in \( E(Pc) \) result in an increase in homologous recombination over nonhomologous end joining at double-stranded DNA breaks [36]. Following ionizing radiation, heterozygous animals also exhibit higher genome stability and lower incidence of apoptosis [36]. Yet little is known about its role in neurons.

In our study, we find that \( E(Pc)^{+/} \) DL1 PN dendrites also mistarget to the anterior glomerulus DA4l (Figure 7A) and exhibit the stereotyped L-shaped axon pattern in the lateral horn (Figure 7B). A BAC transgene that contains the entire \( E(Pc) \) transcription unit rescued the \( E(Pc) \) mutant phenotypes (Figure 7E). To determine whether Bap55 and \( E(Pc) \) genetically interact, we expressed \( UAS-Bap55 \) in an \( E(Pc)^{+/} \) DL1 PN. This manipulation caused the dendrites to split between the DA4l and DM6 glomeruli (Figure 7C), and resulted in axons targeting ventrally to the lateral horn (Figure 7D, E, Table 1).

dom and \( E(Pc) \) mutants derepress the expression of a PN-GAL4

Neuroblast clones mutant for dom also exhibit dendrite mistargeting phenotypes to inappropriate glomeruli throughout the antennal lobe. Anterodorsal and lateral neuroblast clones show a very mild reduction in cell number and their dendrites do not target to the full set of proper glomeruli (Figure 8A, B). Ventral neuroblast clones, when compared to WT, exhibit incomplete targeting throughout the antennal lobe (Figure 8C).

Further analysis of dom mutants by labeling with Mz19-GAL4 revealed the same derepression as in Bap55 mutants (Figure 3). dom mutant Mz19-GAL4 PN clones also label anterodorsal, lateral, and ventral neuroblast clones (Figures 8D-F) with phenotypes similar to GH146-GAL4 labeled neuroblast clones (Figures 8A-C). In anterodorsal and lateral neuroblast clones, Mz19-GAL4 labels a large number of PNs that target to many glomeruli throughout the antennal lobe, although the cell number is smaller than GH146-GAL4 labeling (Figures 8D, E). Ventral neuroblast clones are never labeled in WT Mz19-GAL4 (Figure 3C), yet are labeled in dom mutants (Figure 8F). Mz19-GAL4 also labels single cell clones that split their dendrites between the DA4l and DL1 glomeruli (Figure 8G) and form the stereotypical L-shaped axon pattern in the lateral horn (Figure 8H). As in Bap55 mutants, this compound phenotype likely results from ectopic labeling of a DL1 PN, which further mistargets to DA4l.

The \( E(Pc) \) phenotypes in GH146 and Mz19-GAL4 labeled neuroblast clones (Figure 8I-N), as well as Mz19-GAL4 labeled single cell clones (Figure 8O, P) displayed similar phenotypes to dom as described above.

![Figure 7](image-url)
The phenotypic similarities in single cell clone dendrite mistargeting and derepression of a PN-GAL4 in mutations that disrupt Bap55, dom and E(Pc) strongly suggest that these three proteins act together in the TIP60 complex to regulate PN development.

**Discussion**

In this study, we demonstrate a similar role for three members of the TIP60 complex in olfactory PN wiring. We find that the TIP60 complex plays a very specific role in controlling dendrite wiring specificity, with a
precise mistargeting of the dendrite mass in Bap55, dom, and E(Pc) mutants (Table 1). This specific DL1 to DA4l mistargeting phenotype has only been seen in these three mutants, out of approximately 4,000 other insertional and EMS mutants screened in our laboratory (unpublished data), supporting the conclusion that the TIP60 complex has a specific function in controlling PN dendrite targeting. We find that TIP60 complex mutants show discrete glomerular mistargeting, rather than randomly distributed dendrite spillover to different glomeruli. In contrast, perturbation of individual cell surface receptors often leads to variable mistargeted dendrites that do not necessarily obey glomerular borders [37,38], possibly reflecting the combinatorial use of many cell surface effector molecules. Even transcription factor mutants yield variable phenotypes [5,8]. Interestingly, BRM complex mutants yield non-stereotyped phenotypes in PNs. We do not see a stereotyped glomerular targeting for brm or Snr1 mutant dendrites; each PN spreads its dendrites across different glomeruli. Our data suggest that different chromatin remodeling complexes play distinct roles in regulating neuronal differentiation. The uni- or bi-glomerular remodeling to specific glomeruli implies that the TIP60 complex sits at the top of a regulatory hierarchy to orchestrate an entire transcriptional program of regulation.

Our study suggests a function for Bap55 in Drosophila olfactory PN development as a part of the TIP60 complex rather than the BRM complex. Another possibility could be that Bap55 also serves as the interface between the BRM and TIP60 complexes. While loss of core BRM complex components results in a more general defect, loss of Bap55 could specifically disrupt interactions with the TIP60 complex but maintain other BRM complex functions, causing a more specific targeting phenotype mimicking loss of TIP60 complex components.

Interestingly, both human BAF53a and b can significantly rescue the Bap55<sup>−/−</sup> phenotype. Though in mammals BAF53a is expressed in neural progenitors and BAF53b is expressed in postmitotic neurons [17], they can perform the same postmitotic function in Drosophila PNs. Further, both can function with the TIP60 complex in PNs to regulate wiring specificity. These data suggest that the functions for BAF53a and b (in neural precursors and postmitotic neurons, respectively) diverge after the evolutionary split between vertebrates and insects.

The discrete glomerular states of the mistargeting phenotypes may suggest a role for the TIP60 complex upstream of a regulatory hierarchy determining PN targeting decisions. It is possible that disrupting various components changes the composition of the complex. Additionally, overexpression of Bap55 in various mutant backgrounds might alter the sensitive stoichiometry of the TIP60 complex, resulting in targeting to different but still distinct glomeruli.

Studies in our laboratory have identified several mutants that cause DL1 PNs to mistarget to areas near the DM6 glomerulus [8] (Table 1 and unpublished results). Interestingly, WT DM6 PNs have the most similar lateral horn axon arborization pattern to DL1 PNs [2]. We hypothesize that the transcriptional code for DM6 is similar to that of DL1, which is at least partially regulated by the TIP60 complex. The genes described in this manuscript are the only mutants that have yielded specific DA4l mistargeting to date. It is possible that the targeting ‘code’ for DA4l, DL1, and DM6 may be most similar, such that perturbation of the TIP60 complex might result in reprogramming of dendrite targeting. PNs have previously been shown to be pre-specified by birth order [1]. Yet DA4l is born in early embryogenesis, DL1 is born in early larva, and DM6 is born in late larva [39]. This implies that the TIP60 transcriptional code does not correlate with PN birth order. The mechanisms by which the TIP60 complex specifies PN dendrite targeting remain to be determined.

**Conclusions**

In this study, we characterize PN phenotypes of mutants in the BRM and TIP60 complexes, with a focus on Bap55, which is shared by the two complexes. We find that the TIP60 complex plays a very specific role in regulating PN dendrite targeting; mutants mistarget from the DL1 to the DA4l glomerulus. This specific mistargeting phenotype suggests that TIP60 controls a transcriptional program important for making dendrite targeting decisions.

**Materials and methods**

**Fly stocks**

A MARCM-based mosaic screen was performed using piggyBac insertional mutants previously described and currently available at the Kyoto Drosophila Genetic Resource Center [22]. The insertion sites of piggyBac mutants in Bap55 (LL05955) and domino (LL05537) were confirmed by inverse PCR [22], precise excision, and Splinkertette PCR [24].

The brm<sup>2</sup>, Snr1<sup>01319</sup>, and E(Pc)<sup>1</sup> alleles were obtained from the Bloomington Drosophila Stock Center and Snr1<sup>6C</sup> was kindly provided by JA Simon [40]. We recombined the alleles onto FRT-containing chromosomes using standard techniques.

The GH146-GALA transgene has been previously described [41]. An insertion on the fourth chromosome was used in all MARCM experiments on 2R (Figures 2, 4, 6, 7, 8A-C, I-K).

**Immunostaining**

MARCM was performed as described and flies were raised at 25°C [42]. Fly brains of both genders were
dissected, fixed, and stained as described [43]. Antibody conditions: rat anti-mCD8a 1:100 (Invitrogen Caltag #RM2200 or #MCD0800, Carlsbad, CA, USA), mouse anti-nc82 1:40 (Developmental Studies Hybridoma Bank #nc82, contributed by E Buchner, Iowa City, IA, USA).

Construction of UAS-Bap55
To generate UAS-Bap55, a full length cDNA (LD29458, Berkeley Drosophila Genome Project Gold cDNA, Drosophila Genomics Resource Center, Bloomington, IN, USA) was amplified using the following primers (5’-3’): CACCCAAAATGAGTGGCGGCACCATATGCTATATG and TTACGGACACTTCCGCTCGACTTGG. The first primer amplifies from the 5’ end and adds a CACC overhang for the TOPO reaction and a Kozak sequence. The second primer amplifies to the stop codon. The PCR product was subcloned into pENTR-D/TOPO (Invitrogen) and recombined into the destination vector pLIAST-Gateway-attB [8] using LR Clonase II (Invitrogen).

The final construct was integrated into the 86Fb landing site [44] on the third chromosome.

Construction of UAS-BAF53a and UAS-BAF53b
To generate UAS-BAF53a and UAS-BAF53b, full length cDNAs (kind gifts of GR Crabtree [17]) were amplified using the following primers (5’-3’): for UAS-BAF53a, CACCCAAAATGAGGCGGCACCATGCTATATG and TCAAGGGCAGCTTTCTTTCTACACACTG; for UAS-BAF53b, CACCCAAAATGAGGCGGCACCATGCTATATG and TCAAGGGCAGCTTTCTTTCTACACACTG. The first primer amplifies from the 5’ end and adds a CACC overhang for the TOPO reaction and a Kozak sequence. The second primer amplifies to the stop codon. The PCR product was subcloned into pENTR-D/TOPO (Invitrogen) and recombined into the destination vector pLIAST-Gateway-attB [8] using LR Clonase II (Invitrogen).

The final construct was integrated into the 86Fb landing site [44] on the third chromosome.

Construction of BAC transgenic flies containing dom or E(Pc)
To generate BAC transgenic flies containing dom, BAC #CH321-01P07 in the attB-P[acman]-CmR vector from the BACPAC Resources Center (Oakland, CA, USA) was verified and sent to BestGene, Inc. (Chino Hills, CA, USA) for integration into the 86Fb landing site [44] on the third chromosome.

BAC transgenic flies containing E(Pc) were generated in the same manner, using BAC #CH322-140G22.

Enhancer of Polycomb; GFP- green fluorescent protein; PN- projection neuron, WT- wild type.

Acknowledgements
We thank the Developmental Studies Hybridoma Bank for antibodies; the Bloomington Drosophila Stock Center and JA Simon for fly stocks; the Drosophila Genomics Resource Center and GR Crabtree for DNA; O Schuldiner, D Berduk, and JM Levy for collaboration on the piggyBac screen; D Luginbuhl for technical assistance; YH Chou, GR Crabtree, L Liang, and TJ Mosca for comments on the manuscript. This work was supported by an NIH grant to LL (R01-DC005982) and a fellowship from the National Science Foundation (JST). LL is a Howard Hughes Medical Institute Investigator.

Authors’ contributions
JST carried out all the experiments. JST and LL designed the study and wrote the manuscript.

Competing interests
The authors declare that they have no competing interests.

Received: 30 October 2010 Accepted: 1 February 2011
Published: 1 February 2011

References
1. Jeffries G, Xie, Marin EC, Stocker RF, Luo L: Target neuron prespecification in the olfactory map of Drosophila. Nature 2001, 414:204-208.
2. Marin EC, Jeffries G, Korniyama T, Zhu H, Luo L: Representation of the glomerular olfactory map in the Drosophila brain. Cell 2002, 109:243-255.
3. Wong AM, Jiang, Axell R: Spatial representation of the glomerular map in the Drosophila protocerebrum. Cell 2002, 109:229-241.
4. Jeffries GS, Viau RM, Berduk D, Ramakers A, Stocker RF, Tanaka NK, Ito K, Luo L: Developmental origin of wiring specificity in the olfactory system of Drosophila. Development 2004, 131:117-130.
5. Korniyama T, Johnson WA, Luo L, Jeffries GS: From lineage to wiring specificity: POU domain transcription factors control precise connections of Drosophila olfactory projection neurons. Cell 2003, 112:157-167.
6. Korniyama T, Luo L: Intrinsic control of precise dendritic targeting by an ensemble of transcription factors. Curr Biol 2007, 17:278-285.
7. Spletter ML, Liu J, Liu J, Su H, Grueter C, Korniyama T, Quake S, Luo L: Lola regulates Drosophila olfactory projection neuron identity and targeting specificity. Neural Dev 2007, 2, 14.
8. Tea JS, Chiara T, Luo L: Histone deacetylase Rpd3 regulates olfactory projection neuron dendrite targeting via the transcription factor Prospero. J Neurosci 2010, 30:9993-9996.
9. Armstrong JA, Papoulos O, Daubreze G, Sperling AS, Lis JT, Scott MP, Tamkun JW: The Drosophila BRM complex facilitates global transcription by RNA polymerase II. EMBO J 2002, 21:5243-5254.
10. Mohrmann L, Langenberg K, Krijgsveld J, Kal AJ, Heck AJ, Venniger CP: Differential targeting of two distinct SWI/SNF-related Drosophila chromatin-remodeling complexes. Mol Cell Biol 2004, 24:3077-3088.
11. Ho L, Crabtree GR: Chromatin remodelling during development. Nature 2010, 463:474-484.
12. Tang L, Nogales E, Ciferri C: Structure and function of SWI/SNF chromatin-remodeling complexes and mechanistic implications for transcription. Prog Biophys Mol Biol 2010, 100:122-128.
13. Becker PB, Hor W: ATP-dependent nucleosome remodeling. Annu Rev Biochem 2002, 71:247-273.
14. Chi TH, Wan M, Lee PP, Akashi K, Metzger D, Chambon P, Wilson CB, Crabtree GR: Sequential roles of Brg, the ATPase subunit of BAF chromatin remodeling complexes, in thymocyte development. Immunity 2003, 19:169-182.
15. Parrish JZ, Kim MD, Jan LY, Jan YN: Genome-wide analyses identify transcription factors required for proper morphogenesis of Drosophila sensory neuron dendrites. Genes Dev 2006, 20:820-835.
16. Ho L, Ronan JU, Wu J, Staahl BT, Chen L, Kuo A, Lessard J, Nesvizhskii AI, Ranish J, Crabtree GR: An embryonic stem cell chromatin remodeling complex, esBAF, is essential for embryonic stem cell self-renewal and pluripotency. Proc Natl Acad Sci USA 2009, 106:5181-5186.
17. Wu J, Lessard J, Olave IA, Qiu Z, Ghash G, Graef IA, Crabtree GR: Regulation of dendritic development by neuron-specific chromatin remodeling complexes. Neuron 2007, 56:94-108.
