Laser Microdissection of Sensory Organ Precursor Cells of *Drosophila* Microchaetes

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**Abstract**

**Background:** In *Drosophila*, each external sensory organ originates from the division of a unique precursor cell (the sensory organ precursor cell or SOP). Each SOP is specified from a cluster of equivalent cells, called a proneural cluster, all of them competent to become SOP. Although, it is well known how SOP cells are selected from proneural clusters, little is known about the downstream genes that are regulated during SOP fate specification.

**Methodology/Principal Findings:** In order to better understand the mechanism involved in the specification of these precursor cells, we combined laser microdissection, to isolate SOP cells, with transcriptome analysis, to study their RNA profile. Using this procedure, we found that genes that exhibit a 2-fold or greater expression in SOPs versus epithelial cells were mainly associated with Gene Ontology (GO) terms related with cell fate determination and sensory organ specification. Furthermore, we found that several genes such as pebbled/hindsight, scabrous, miranda, senseless, or cut, known to be expressed in SOP cells by independent procedures, are particularly detected in laser microdissected SOP cells rather than in epithelial cells.

**Conclusions/Significance:** These results confirm the feasibility and the specificity of our laser microdissection based procedure. We anticipate that this analysis will give new insight into the selection and specification of neural precursor cells.

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**Introduction**

In *Drosophila*, the small external sensory organs (microchaetes) located on the dorsal part of the thorax have become an excellent system to analyse mechanisms involved in the acquisition and maintenance of neural precursor cell identity from a non-differentiated state \([1,2]\). In this system, each sensory organ develops from a single SOP that arises from a cluster of equivalent cells called proneural cluster. Cells of a proneural cluster are defined by the expression of the proneural genes *achaete* and *scute* (*ac/sc*) that provide them with the competence to become SOP \([3,4]\). In each cluster, the proneural competence is progressively restricted to only one cell that accumulates the highest level of proneural proteins and that will become the SOP whereas the others cells remain epithelial cells. This process of SOP selection depends on both the auto and cross regulation of proneural gene expression \([4]\) and the activation of the Notch signalling pathway. This later involves cell-cell interactions mediated by the Notch receptor and its ligand Delta in such a way that in each cluster, one cell (the future SOP) will express higher level of the ligand Delta and will activate Notch receptors in neighbouring (future epithelial) cells \([3,6]\). Notch signalling promotes the transcription of *Enhancer of split complex* genes that repress proneural gene expression and prevents the acquisition of neural fate \([4,7,8]\).

Despite considerable progress in our knowledge of the mechanisms underlying SOP selection, relatively few downstream target genes regulated by this proneural regulatory network are known. The gene *senseless* (*sens*), which encodes a zinc finger transcription factor, is one of the known downstream genes. Sens is expressed in SOPs and has been shown to act as a binary switch in the proneural cluster. High levels of Sens up regulate *ac/sc* expression and, conversely, low levels repress *ac/sc* expression \([8,9]\). It is tempting to speculate that other Sens-like factors remain to be discovered.

In order to determine the genome-wide response associated with SOP fate acquisition, we propose an original protocol that combines laser microdissection, to isolate individually SOPs from epithelial cells, and transcriptome analysis, to compare the RNA profiles of SOPs cells from that of their sibling epithelial cells. Our analysis revealed that genes exhibiting a two-fold or greater expression in SOPs were mainly associated with gene ontology (GO) term related to sensory organ specification and neurogenesis. Moreover, from this set of genes, almost twenty genes were previously found to be expressed in SOPs. These data show the feasibility and the specificity of the laser microdissection technique in isolating identified cells from this type of system. We anticipate that this approach will give new insights into the selection and specification of neural precursor cells. Furthermore, we believe...
that this technique can easily be extended to different epithelia and as such will be useful in investigating specific cell transcriptomes.

**Materials and Methods**

**Fly Stocks**

The *neuralized*Gal4 driver was used to express in pl cells the construction mCD8::GFP using the UAS/Gal4 system [10]. *neu>mCD8::GFP* flies were reared on a standard *Drosophila* diet. White pupae were selected and maintained at 25°C prior dissection.

**Fixation and Mounting**

The notum from *neu>mCD8::GFP* pupae were dissected out in PBS and fixed in cold absolute ethanol for 10 minutes. A fixation longer than 15 min, hardes the tissue and makes the microdissection difficult. Three to five fixed nota were transferred directly from ethanol with a Pasteur pipette and then carefully flattened and dried with the epithelium facing down on a thermobable membrane slides for laser microdissection (See Fig S1).

**Laser Microdissection**

Laser microdissection was realized on a MMI cellcut microdissection system coupled to an Eclipse TE-2000 inverted fluorescent microscope (Nikon Instrument). The parameters used were: focus 40, speed 1, power 74 at objective 60X and 4 to 8 laser rounds were required to cut through a notum. Selected areas were cut from the tissue by an UV laser beam. To keep the SOP integrity and preserve RNA from the heat of the laser, we took care to leave a space between the laser circle and the cell limit (around 5 μm).

**RNA Extraction and Amplification**

Total RNA was extracted from microdissected cells by using the picopure RNA isolation kit (Molecular devices - Arcturus) following manufactures instructions with minor modifications as described below. We incubated the tubes containing microdissected cells with 20 μl of extraction buffer at 42°C upside down for 30 min. Then, after centrifugation, the extracts pooled were passed through a single RNA purification column. During purification, we treated the column with DNAse I (Qiagen) for 30 min at room temperature to avoid genomic DNA contamination. We obtained 0.1–0.5 μg of total RNA from a sample of 1000 microdissected cells.

After extraction, RNA was amplified by using the MessageAmp II aRNA Amplification Kit (Ambion). We proceeded with two rounds (9 h each) of *in vitro* transcription. After each round, the RNA purification column was treated by DNAse I (Qiagen) for 10 min at room temperature before aRNA elution. For better RNA integrity, we carried out all the amplification processes in one step directly after RNA extraction to avoid freezing the sample. Indeed, in addition to the usual recommendations about manipulating RNA, we avoided, as much as possible, freezing both the tissue before microdissection and the RNA samples between extraction and amplification. After two rounds of amplification, we obtained 20–50 μg of aRNA from a sample of 1000 microdissected cells.

For microarray hybridizations, UTP- amino allys were integrated during the second round of *in vitro* transcription, for subsequent labelling with dyes Cy3 or Cy5 (Amino Allyl MessageAmp II kit - Ambion).

**qRT-PCR**

We performed reverse transcription on 1 μg of aRNA using random primers from Roche and the SuperscriptII reverse transcriptase from Invitrogen. The same quantity of cDNA (50–100 ng) from SOPs or epithelial cells was then used to perform semi quantitative PCR (30 cycles) or qRT-PCR for several genes.

**Microarray**

Amplified and differently labelled aRNA from 1000 microdissected SOPs and an equivalent surface of epithelial cells were hybridized to INDAC Drosophila GeneChips (platform Montpellier GenomiX, Institut de Génomique Fonttonnelle, UMR 3203 CNRS – U661 INSERM, Montpellier, France). Normalization of raw data was performed by LIMMA. The flagged spots and controls were removed from the analysis. No background correction was performed before normalization. Lowess normalization was used to normalize the M values for each array separately (within-array normalization). Genes exhibiting a signal ratio SOPs/epithelial cells superior than two were considered as SOPs-overexpressed genes for subsequent analysis. Gene Ontology analysis was performed with Flymine [11] that provides enriched GO terms ranked by significance. P values were calculated following a hypergeometric distribution (with Bonferroni correction).

**Data Deposition**

The raw data associated with this manuscript are available on the Gene Expression Omnibus (GEO) according to MIAME standards under the following accession number: GSE18615.

**Results**

**Purification of SOPs by Laser Microdissection**

In order to identify SOP cells, we specifically expressed the construction mCD8::GFP to label SOP membranes and their progeny by using the Gal4/UAS expression system and the specific driver line neuralizedGal4 (neur>[11]). The dorsal epithelium (or notum) of neu>mCD8::GFP pupae at 16h after puparium formation (APF) was dissected and fixed in ethanol. At this developmental time, most of the SOPs have not yet divided [12,13].

After mounting on a membrane slide, SOPs expressing GFP were identified by fluorescence and circled manually with a circle radius of 9 μm (Fig. 1A). Microdissected cells were then collected on an adhesive lid of a microtub placed onto the area (Fig. 1G–J). The success of the cell capture was visually confirmed by the gaps in the tissue after lid removal (Fig. 1G–F). We collected around 20 cells per notum, 20–50 cells on a cap and pooled around 20–50 tubes to proceed to the RNA extraction. Altogether, we collected around 1000 SOPs from 50 nota. In parallel, we captured tissue free of SOP fluorescent cells corresponding to epithelial cells (Fig. 1B). A similar integrated surface (around 250 000 μm²) was collected in order to standardize both samples.

Once the required number of cells was been collected, total RNA was extracted and amplified for analysis.
Figure 1. Laser Microdissection. Laser Microdissection of SOP cells (left column) and epithelial cells (right column). Fixed nota from
mCD8::GFP flies (16 h APF) that express GFP specifically in SOP
cells. SOP cells were laser-cut following a circle pathway centered on
each SOP (A). After cut, gaps corresponding to each SOP encircled
remained on the nota (transmitted light in C and fluorescent light in E).
In contrast, the captured SOP cells stuck to the lid of a microtube
(transmitted light in G and fluorescent light in H). A similar procedure
is shown for epithelial cell capture. These cells were isolated from areas
without fluorescent SOP cells (B). Note that sometimes for SOP (not shown)
as well as epithelial cell microdissection (asterisks in D) some areas
were not captured and remain on the nota. Note also that, the
fluorescence level was strongly reduced after laser beam application
(I and J).
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Quantitative Real Time PCR and Microarray Data Confirm
Differential Expression of Known Genes

We carried out reverse transcription following by PCR on some
SOPs specific (ac, sens, [4,9]) and non-specific (rp49, taf11) genes to
verify the aRNA extracted and amplified from microdissected cells.
Semi quantitative PCR (30 cycles) performed on the same quantity
of cDNA from SOPs and epithelial cells showed that Rp49, taf11 and
ac seem to be expressed at similar levels in SOPs and epithelial cells.
In contrast, as expected, gfp (that was ectopically expressed in SOPs)
and sens were more highly expressed in SOPs than in epithelial cells
(not shown). To verify these results, we performed quantitative real
time PCR (qRT-PCR). We calculated the ratio of SOP/epithelial
mRNA levels for each gene (Fig. 2A). Using this procedure, we
confirmed that at expression was not significantly different in SOPs
and epithelial cells (ratio = 0,9), whereas the expression of gfp and
sens was higher in SOP than in epithelial cells (11,2 and 11,4 times
respectively) (Fig. 2A and Fig. S2). The significant enrichment of
transcripts corresponding to sens and gfp in SOPs confirms the
usefulness and the specificity of aRNA material collected using the
laser microdissection technique on fixed Drosophila nota.

Concomitantly to qRT-PCR analysis, we used DNA microarrays
to identify genes differently expressed between microdissected
SOPs and epithelial cells. This analysis revealed 127 genes
whose expression was increased 2-fold or greater between SOPs
and epithelial cells (Table 1). To analyse whether a particular
biological process could be overrepresented in this data set, we
regrouped the genes of this set according to their function that has
been ascribed using Gene Ontology (GO) terms (www.geneontology.org).
This analysis showed that 58% of these genes were associated with a specific function. Interestingly, 27% of this subset
of genes were related to the nervous system. This category showed
more than a three fold enrichment in the SOP-gene data set
(Fig. 2B). More precisely, a hypergeometric test applied on this set
of 127 genes, revealed a significant enrichment in GO terms
related to nervous system development, sensory organ development
and cell fate specification. Moreover, several eye photore-
ceptor cell development associated GO terms were also enriched
in our SOP-gene data set (Fig. 2B). Conversely, among GO terms
that are significantly underrepresented and, as a consequence,
enriched in their sibling epithelial cells, we found cuticle
development and epithelium morphogenesis (data not shown).
Furthermore, neither genes already known to belong to SOP-
enriched genes nor genes associated with GO terms related with
cell fate determination and sensory organ specification were found
in this SOP non-enriched set of genes.

Our data set of SOP-specific genes includes 19 known genes
that have already been shown to be expressed in SOPs or involved
in sensory organ development related mechanisms (Table 2).
Among them, we found sens, confirming our qRT-PCR analysis,
and other SOP-specific genes such as cut (ct), neuralized (neur)
and phyllopod (phyl) [14–16]. We can also note pebbled/hindsight (peb)
and seven up (svp) that are involved in photoreceptor development
[17,18], scabrous (sca) that plays a role in lateral inhibition processes
via the regulation of Notch activity [19], and miranda (mira)
involved in neuroblast and SOP asymmetric divisions [20,21].
Moreover, among these genes, eleven have already emerged from
microarray analysis performed on proneural clusters by Reeves
and Posakony [22]. In addition to the well characterised genes
such as mira, peb, neur or phyl, we can cite as an example of new
SOP genes, mutant and insensitive (Table 2). In contrast, some genes in
our data set didn’t appear in Reeves and Posakony microarray
results. The most relevant examples are sens and ct, two well known
SOP-specific genes [8,14]. We can also cite shaven, sea or ncery, all
three being involved in sensory organ development [19,23,24].

Discussion

In this study, we used laser microdissection to isolate SOPs from
the dorsal epithelium of Drosophila in order to subsequently analyse
the mRNA expression profile. Laser microdissection permits the
isolation of single cells from a heterogeneous tissue [25]. The high
level of cell homogeneity obtained with this technique permits one
to obtain reliable microarray data. In this regard, microdissection,
although cumbersome, has certain advantages over other methods of isolating populations of cells such as FACS. In our study, microdissection was applied to tissue freshly dissected and simply fixed in absolute ethanol. This was made possible because the tissue of interest is an epithelium that we are able to dissect from the animal and than flatten. As such, the protocol described here may be adapted to other thin tissues similar to epithelia.

The principal challenge with this technique was to obtain a significant quantity of RNA from SOPs and to ensure that the integrity of the RNA after laser microdissection was sufficient for subsequent gene expression analysis such as quantitative real time PCR and microarrays. Here, we verify the utility and the specificity of the RNA extracted from microdissected SOPs and epithelial cells by performing qRT-PCR on particular genes and undertaking microarray analysis. As expected, we observed by qRT-PCR that sens, known to be up regulated in SOPs by proneural protein activity and repressed in non-SOP cells by Notch signaling activation [8,9], was indeed significantly more expressed in SOPs than in epithelial cells. This result was confirmed by microarray analysis where sens was found among the genes exhibiting a two-fold or greater overexpression in SOPs.

Figure 2. Microdissected SOP cells show specific gene expression and are enriched with genes associated with cell fate determination. (A) Fold changes represent the ratio between SOP and epithelial cell mRNA levels measured by qRT-PCR. Values obtained in epithelial cells were normalized to 1. Transcripts for gfp (ectopically expressed in SOP cells) as well achaete (ac) and senseless (sens) are shown. The mean and standard deviation of at least 3 independent experiments for each gene are represented. The difference between SOP and epithelial cell expression levels was considered significant when student test P value was inferior to 0.05 (indicated with asterisk). Note that, achaete (ac) expression was not different in SOPs and epithelial cells (ratio = 0.8), whereas gfp and senseless (sens) were expressed 11.2 and 11.4 more times in SOPs than in epithelial cells respectively. (B) Genes overexpressed in SOPs (ratio of SOP/epithelial cell transcripts $\geq 2$) were grouped according to their function on the basis of their ascribed GO terms. The 16 categories having lowest $P$-values with enrichment $\geq 3$ are shown ranked. $P$-values (on the right) were calculated following a hypergeometric distribution (with Bonferroni correction). Fold enrichment was calculated as the ratio between the percentage of genes associated with a given GO term among SOP-over expressed genes and the percentage of genes associated with the same GO term throughout the entire genome. Note that many of the significant categories concern fate determination and nervous system (underlined).

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### Table 1. Genes whose expression was increased 2-fold or greater between SOPs and epithelial cells.

| Flybase ID  | Gene symbol | Gene name                      | SOPs/Epithelial cells signal ratio |
|-------------|-------------|--------------------------------|------------------------------------|
| FBgn0005561 | sv          | shaven                         | 14,121155                          |
| FBgn0003053 | peb         | pebbled                        | 13,55055                           |
| FBgn0019830 | colt        | congested-like trachea         | 10,6780015                         |
| FBgn0030396 | CG2556      |                                 | 9,546415                           |
| FBgn0030589 | CG9519      |                                 | 9,1127835                          |
| FBgn0052023 | CG32023     |                                 | 7,569719                           |
| FBgn0037844 | CG4570      |                                 | 7,20862                            |
| FBgn0052150 | CG22150     |                                 | 6,548325                           |
| FBgn0002891 | mus205      | mutagen-sensitive 205          | 6,133705                           |
| FBgn003326  | sca         | scabrous                       | 5,26793                            |
| FBgn0005636 | nvy         | nervous                        | 6,05058                            |
| FBgn0040842 | CG15212     |                                 | 5,903055                           |
| FBgn0052392 | CG23392     |                                 | 5,86501                            |
| FBgn0033995 | vvl         | ventral veins lacking          | 5,2886                             |
| FBgn0021776 | mira        | miranda                        | 5,26211                            |
| FBgn0028536 | CG15281     |                                 | 4,9381                             |
| FBgn0002573 | sens        | senseless                      | 4,56789                            |
| FBgn0033772 | CG12488     |                                 | 4,427495                           |
| FBgn0030432 | CG4404      |                                 | 4,36984                            |
| FBgn003996  | w           | white                          | 3,8498                             |
| FBgn0034692 | CG13502     |                                 | 3,582385                           |
| FBgn0033739 | Dyb         | Dystrobrevin-like              | 3,511521                           |
| FBgn0028537 | CG31775     |                                 | 3,443735                           |
| FBgn0029839 | CG4660      |                                 | 3,220815                           |
| FBgn0015393 | hoip        | hoi-polloi                     | 3,114765                           |
| FBgn0053200 | ventrally-expressed-protein-D |     | 3,071915                           |
| FBgn0033507 | CG12909     |                                 | 3,051625                           |
| FBgn0004779 | Ccp84Ae     |                                 | 3,036835                           |
| FBgn0050118 | CG30118     |                                 | 3,034455                           |
| FBgn0015393 | hoip        | hoi-polloi                     | 2,982565                           |
| FBgn0036124 | CG7839      |                                 | 2,97755                            |
| FBgn0036839 | CG18136     |                                 | 2,9365975                          |
| FBgn0030027 | CG1632      |                                 | 2,89352                            |
| FBgn0036137 | CG7628      |                                 | 2,86596                            |
| FBgn0036369 | CG10089     |                                 | 2,83541                            |
| FBgn0003187 | qua         | quail                          | 2,827855                           |
| FBgn0030833 | CG8915      |                                 | 2,8224225                          |
| FBgn0001090 | bnb         | bangles and beads              | 2,7679                             |
| FBgn0039154 | CG6164      |                                 | 2,745795                           |
| FBgn0051523 | CG31523     |                                 | 2,727035                           |
| FBgn0032871 | CG2611      |                                 | 2,7265                             |
| FBgn0039118 | CG10208     |                                 | 2,719125                           |
| FBgn0004511 | dy          | dusty                          | 2,7177                             |
| FBgn0051800 | CG31800     |                                 | 2,69935                            |
| FBgn0010383 | Cyp1Ba1     | Cytochrome P450-18a1            | 2,688865                           |
| FBgn0013765 | cnn         | centrosomin                    | 2,67734                            |
| FBgn0058454 | CR40454     |                                 | 2,6720405                          |
| FBgn0038318 | CG6236      |                                 | 2,6341                             |
| FBgn0035878 | CG7182      |                                 | 2,619305                           |
| Flybase ID | Gene symbol | Gene name | SOPs/Epithelial cells signal ratio |
|-----------|-------------|-----------|-----------------------------------|
| FBgn0033275 | CG14756 | 2,57977 |
| FBgn0037723 | SpdS | Spermidine Synthase | 2,57505 |
| FBgn0031273 | CG2839 | 2,56041 |
| FBgn0051352 | CG31352 | 2,559855 |
| FBgn0030001 | CG15335 | 2,546375 |
| FBgn0037240 | Cont | Contactin | 2,5257925 |
| FBgn0039152 | CG6129 | 2,52151 |
| FBgn0002952 | neur | neuralized | 2,515405 |
| FBgn0052827 | CG32827 | 2,47839 |
| FBgn0031764 | CG9107 | 2,451235 |
| FBgn0037137 | Nopp140 | 2,450615 |
| FBgn0019938 | Rpl1 | RNA polymerase I subunit | 2,44853 |
| FBgn0003651 | syp | seven up | 2,439685 |
| FBgn0036656 | CG17922 | 2,43033 |
| FBgn0038916 | CG6560 | 2,4265 |
| FBgn0039169 | CG5669 | 2,42484 |
| FBgn0039630 | CG11843 | 2,386245 |
| FBgn002778 | mnd | minidiscs | 2,37934 |
| FBgn0038120 | CG10148 | 2,3619 |
| FBgn0050349 | CG30349 | 2,34567 |
| FBgn0039335 | CG5127 | 2,337975 |
| FBgn0029568 | CG11381 | 2,3251455 |
| FBgn0004198 | ct | cut | 2,319 |
| FBgn0010105 | comm | commissureless | 2,310285 |
| FBgn0035521 | CG1268 | 2,299415 |
| FBgn0050007 | CG30007 | 2,299075 |
| FBgn0034224 | CG6520 | 2,29819 |
| FBgn0031706 | nmr2 | neuromancer2 | 2,27797 |
| FBgn0037314 | CG12000 | 2,271605 |
| FBgn0000409 | Cyt-c-p | Cytochrome c proximal | 2,267055 |
| FBgn0031824 | CG15433 | 2,26653 |
| FBgn0000707 | CG14543 | 2,261375 |
| FBgn0027903 | CG12018 | 2,25671 |
| FBgn0028855 | CG15282 | 2,23759 |
| FBgn0035532 | CG15014 | 2,222355 |
| FBgn0034528 | CG11800 | 2,21393 |
| FBgn0033802 | CG17724 | 2,20619 |
| FBgn0030958 | CG6900 | 2,20591 |
| FBgn00036017 | CG4115 | 2,194645 |
| FBgn0026378 | Rep | Rab escort protein | 2,173765 |
| FBgn0028510 | CG15261 | 2,173175 |
| FBgn0052344 | CG32344 | 2,163085 |
| FBgn0031434 | insv | insensitive | 2,159285 |
| FBgn0039563 | CG4951 | 2,15345 |
| FBgn0015907 | bl | bancal | 2,152305 |
| FBgn0011638 | La | La autoantigen-like | 2,150125 |
| FBgn0032297 | CG17124 | 2,142305 |
| FBgn0039271 | CG11839 | 2,13788 |
| FBgn0036043 | CG8177 | 2,136985 |
| FBgn0000340 | cno | canoe | 2,136715 |
Similarly, gfp, whose expression was driven specifically in SOPs and their progeny by neur-GAL4, was more expressed in microdissected SOPs. However, gfp transcripts were still detected in the epithelial sample. This was unexpected since epithelial cells were collected from non-fluorescent areas. It might be possible that a few SOPs, not fluorescent enough to be detectable, were included in epithelial cell selected areas. It might also be possible that there is a weak leak of the neur-GAL4 driver onto epithelial cells insufficient to induce a detectable fluorescence.

Unexpectedly, we observed by qRT-PCR and confirmed by microarray, a relatively constant level of ac (ratio SOP/epithelial cells = 0.8 by qRT-PCR and 0.95 by microarray). Indeed, ac is a proneural gene whose expression has been shown to be specifically upregulated in proneural clusters and restricted to one cell during SOP specification [3,4]. However, the expression of ac in SOPs has been shown to decrease before cell division [3]. Since we use pupae at 16 h APF, at the moment of SOP first division, we suggest that the relatively similar level of ac transcripts observed in SOP and epithelial cells was due to this downregulation phase.

The SOP-enriched genes of the data set obtained in this analysis were classified using Gene Ontology associated terms. This analysis confirmed the specificity of the microdissected SOP samples. Indeed, microdissected SOPs samples were enriched in genes involved specifically in sensory organ development and cell fate related GO terms. Interestingly, eye photoreceptor cell development related GO terms were also enriched in our data. This is not surprising since photoreceptor cells share similar mechanisms of selection with the SOPs including the isolation of one cell among equivalent cluster cells by lateral inhibition mediated by Notch signalling [26]. In this regard, it is interesting that peb was highly expressed in SOPs compared to epithelial cells. It has been recently shown that one role of peb is to modulate Delta expression during cone cell induction during ommatidial formation [18]. It remains to be known whether peb plays a similar role during SOP selection, which is characterised by an elevated level of Delta.

In accordance with previous studies, many genes (19 out of 127) belonging to the SOP enriched genes identified in our study have been already recognized to be SOP specific. In particular, 11 out of 127 of these known SOP enriched genes are in common with a whole-genome microarray analysis performed with cells belonging to proneural cell clusters [22]. In contrast, some known SOP-specific genes as sens and ct, were identified in our analysis but not in Reeves and Posakony’s study. In their study, proneural cells were sorted by FACS (Fluorescence-Activated Cell Sorting) by using E(spl)m4-GFP as proneural cluster-specific marker. As such,

| Flybase ID | Gene symbol | Gene name | SOPs/Epithelial cells signal ratio |
|------------|-------------|-----------|-----------------------------------|
| 101        | FBgn0039829 | CG15561   | 2.13596                           |
| 102        | FBgn0042092 | CG13773   | 2.123165                          |
| 103        | FBgn0036096 | CG8003    | 2.120365                          |
| 104        | FBgn0052645 | CG32645   | 2.11955                           |
| 105        | FBgn0041004 | CG17715   | 2.112665                          |
| 106        | FBgn0002563 | Lsp1β     | 2.10427                           |
| 107        | FBgn0029761 | SK        | 2.09956                           |
| 108        | FBgn0052677 | CG32677   | 2.071425                          |
| 109        | FBgn0005630 | lola      | 2.068285                          |
| 110        | FBgn0037248 | CG9809    | 2.064895                          |
| 111        | FBgn0004551 | Ca-P60A   | 2.06413                           |
| 112        | FBgn0030501 | BthD      | 2.063545                          |
| 113        | FBgn0023214 | edl       | 2.05935                           |
| 114        | FBgn0015558 | tty       | 2.05836                           |
| 115        | FBgn0003890 | βTub97EF  | 2.05672                           |
| 116        | FBgn0050080 | CG30080   | 2.054815                          |
| 117        | FBgn0038640 | CG7706    | 2.05049                           |
| 118        | FBgn0030345 | CG1847    | 2.041705                          |
| 119        | FBgn0046704 | Liprin-α  | 2.03972                           |
| 120        | FBgn0039685 | Obp99b    | 2.03933                           |
| 121        | FBgn0029704 | CG2982    | 2.03666                           |
| 122        | FBgn0036460 | CG5114    | 2.03641                           |
| 123        | FBgn0026015 | Top3β     | 2.032305                          |
| 124        | FBgn0036133 | CG7638    | 2.022935                          |
| 125        | FBgn0033942 | CG10112   | 2.01533                           |
| 126        | FBgn0036569 | CG5414    | 2.014675                          |
| 127        | FBgn0024734 | PRL-1     | 2.013035                          |
the analysis was performed with all cells of proneural clusters including the future SOP. Thus, we expect that some subset of SOP-specific genes also belongs to the genetic profile triggered during proneural cell determination and that another subset is specific for the acquisition of the SOP identity. It is interesting to note that target genes involved in the Notch-mediated lateral inhibition as the *E(spl)* or *bearded* (*brd*) gene family, which are activated in the future epithelial cells during SOP selection, were either similarly expressed in SOP and epithelial cells or underrepresented in SOPs (for instance, the ratio SOP/epithelial cells for *brd* was 0.35).

Overall, our result confirm the SOP specificity of the gene set identified and we are confident that the approach combining laser microdissected cells and transcriptome analysis will produce exploitable data. Finally, we would like to highlight that a successful characterisation of the transcriptional profile of well-identified precursor cells at a precise moment of development opens multiple possibilities concerning the analysis of the mechanisms underlying precursor cell determination. Thus, the development of a procedure combining laser microdissection and transcriptome analysis represents an undeniably important technical advance for the analysis of biological processes such as fate determination of defined precursor cells.

### Supporting Information

Figure S1 Schematic representation of the procedure. The notum from pupae was manually dissected in PBS, fixed and transferred to a thermolabile membrane slide. The epithelium was facing down membrane. Once dry, the notum, stuck to the membrane, was covered with a slide to maintain the mechanical stability during microdissection. During microdissection the adhesive lid was pressed against the membrane and microdissected cells remained stuck to the lid when the microtube was removed.

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Figure S2 qRT-PCR analysis. *taf11, ac, gfp,* and *sens* mRNA transcripts from microdissected SOPs and epithelial cells were analysed by qRT-PCR. For each gene, (on the left) PCR amplification curves as function of the number of PCR cycles and (on the right) standard curves, Ct (Cycle threshold) were plotted against serially diluted cDNA samples obtained from aRNA extracted and amplified from 20 whole nota. Note that PCR amplification curves corresponding to SOPs and epithelial cells for *taf11* and *ac* are super-imposed. Ct for SOPs and epithelial cells are similar and data points corresponding to SOPs and epithelial cells cluster together in standard curves (red points). In contrast, PCR amplification curves corresponding to *gfp* and *sens* transcripts are shifted to the left in SOP compared to epithelial cells, showing a stronger expression in SOPs than in epithelial cells. Accordingly two separate groups of data points were observed on the standard curves.

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### Author Contributions

Conceived and designed the experiments: EB MG. Performed the experiments: EB. Analyzed the data: EB. Wrote the paper: EB MG.

### References

1. Pi H, Chien CT (2007) Getting the edge: neural precursor selection. J Biomed Sci 14: 467–473.
2. Bertrand N, Castro DS, Guillemot F (2002) Proneural genes and the specification of neural cell types. Nat Rev Neurosci 3: 517–530.
3. Cubas P, de Celis JF, Campuzano S, Modolell J (1991) Proneural clusters of achaete-scute expression and the generation of sensory organs in the Drosophila imaginal wing disc. Genes Dev 5: 996–1008.
4. Gull J, Modolell J (1996) Proneural gene self-stimulation in neural precursors: an essential mechanism for sense organ development that is regulated by Notch signaling. Genes Dev 12: 2036–2047.
5. Schweisguth F (2004) Regulation of Notch signaling activity. Curr Biol 14: 129–138.
6. Bray SJ (2006) Notch signalling: a simple pathway becomes complex. Nat Rev Mol Cell Biol 7: 678–689.
7. Bailey AM, Posakony JZ (1995) Suppressor of hairless directly activates transcription of enhancer of split complex genes in response to Notch receptor activity. Genes Dev 9: 2069–2076.
8. Jafar-Nejad H, Acar M, Nolo R, Lacin H, Pan H, et al. (2003) Senseless acts as a binary switch during sensory organ precursor selection. Genes Dev 17: 2966–2978.
9. Nolo R, Abbott LA, Bellen HJ (2000) Senseless, a Zn finger transcription factor, is necessary and sufficient for sensory organ development in Drosophila. Cell 102: 349–362.
10. Lyne R, Smith R, Rutherford K, Wakeling M, Varley A, et al. (2007) FlyMine: an integrated database for Drosophila and Anopheles genomics. Genome Biol 8: R129.
11. Brand AH, Perrimon N (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development 118: 401–415.

### Table 2. Microarray data confirm differential expression of known SOP genes.

| Known SOP genes | Molecular function | SOP/epithelial signal | References |
|-----------------|-------------------|-----------------------|------------|
| shaven          | Transcription factor 14.1 | 23) |
| pebbled*        | Transcription factor 13.5 | 18) |
| CG32150*        | Protein binding 6.5 | 22) |
| scabrous        | Signal transduction 6.1 | 19) |
| nery            | Transcription factor 6 | 24) |
| CG2392*         | Microtubule binding 5.9 | 22) |
| ventral vein lacking* | Transcription factor 5.3 | 28) |
| miranda*        | Actin binding 5.3 | 20) |
| senseless       | Transcription factor 4.6 | 9) |
| phyllopod*      | Ras/MAPK signaling 3.1 | 16) |
| quax*           | Actin binding 2.8 | 22) |
| Cytochrome P450-18a* | Cytochrome P450 2.7 | 22) |
| neuronalised*   | E3 ubiquitin ligase 2.5 | 15) |
| seven up        | Transcription factor 2.4 | 17) |
| cut             | Transcription factor 2.3 | 14) |
| insensitive*    | Unknown 2.1 | 22) |
| bancal          | RNA binding 2.1 | 27) |
| ETS-domain lacking* | Ras/MAPK signalling 2 | 30) |
| scratch*        | Transcription factor 2 | 29) |

Genes previously found to be expressed in SOPs and included in the 127 candidate genes whose expression exhibits a 2.0-fold or greater elevation in SOPs versus epithelial cells.

*Genes also found expressed in proneural clusters according to microarray data obtained by Reeves and Posakony (2005) [22].

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12. Gho M, Bellaiche Y, Schweisguth F (1999) Revisiting the Drosophila microchaete lineage: a novel intrinsically asymmetric cell division generates a glial cell. Development 126: 3573–3584.
13. Fichelson P, Gho M (2004) Mother-daughter precursor cell fate transformation after Cdc2 down-regulation in the Drosophila bristle lineage. Dev Biol 276: 367–377.
14. Blochlinger K, Jan LY, Jan YN (1993) Transformation of sensory organ identity by ectopic expression of Cut in Drosophila. Genes Dev 5: 1124–1135.
15. Yeh E, Zhou L, Rudzik N, Boulianne GL (2000) Neuralized functions cell autonomously to regulate Drosophila sense organ development. Embo J 19: 4827–4837.
16. Pi H, Wu HJ, Chien CT (2001) A dual function of phyllopod in Drosophila external sensory organ development: cell fate specification of sensory organ precursor and its progeny. Development 128: 2699–2710.
17. Miller AC, Seymour H, King C, Herman TG (2008) Loss of seven-up from Drosophila R1/R6 photoreceptors reveals a stochastic fate choice that is normally biased by Notch. Development 135: 767–775.
18. Pickup AT, Ming L, Lipshitz HD (2009) Hindsight modulates Delta expression during Drosophila cone cell induction. Development 136: 973–982.
19. Li Y, Futschik M, Lai ZC, Baker NE (2003) Scabrous and Gp150 are endosomal proteins that regulate Notch activity. Development 130: 2819–2827.
20. Roegiers F, Younger-Shepherd S, Jan LY, Jan YN (2001) Two types of asymmetric divisions in the Drosophila sensory organ precursor cell lineage. Nat Cell Biol 3: 56–67.
21. Mollinari C, Lange B, Gonzalez C (2002) Miranda, a protein involved in neuroblast asymmetric division, is associated with embryonic centrosomes of Drosophila melanogaster. Biol Cell 94: 1–13.
22. Reeves N, Posakony JW (2005) Genetic programs activated by proneural proteins in the developing Drosophila PNS. Dev Cell 8: 413–425.
23. Miller SW, Avidor-Reiss T, Polyakovovsky A, Posakony JW (2009) Complex interplay of three transcription factors in controlling the tormogen differentiation program of Drosophila mechanoreceptors. Dev Biol 329: 386–399.
24. Wildonger J, Mann RS (2005) Evidence that nervy, the Drosophila homolog of ETO/MTG8, promotes mechanosensory organ development by enhancing Notch signaling. Dev Biol 286: 507–520.
25. Erickson HS, Gillespie JV, Emmert-Buck MR (2008) Tissue microdissection. Methods Mol Biol 424: 433–448.
26. Sawamoto K, Okano H (1996) Cell-cell interactions during neural development: multiple types of lateral inhibitions involved in Drosophila eye development. Neurosci Res 26: 205–214.
27. Abdelilah-Seyfried S, Chan YM, Zeng C, Justice NJ, Younger-Shepherd S, et al. (2000) A gain-of-function screen for genes that affect the development of the Drosophila adult external sensory organ. Genetics 155: 733–752.
28. Inbal A, Levanon D, Salzberg A (2003) Multiple roles for u-turn/ventral veinless in the development of Drosophila PNS. Development 130: 2467–2478.
29. Rouk M, Sturtevant MA, Emery J, Vaessin H, Grell E, Bier E (1995) scratch, a pan-neural gene encoding a zinc finger protein related to snail, promotes neuronal development. Genes Dev 9: 2384–2396.
30. Yamada T, Okabe M, Hirumi Y (2003) EDL/MAE regulates EGF-mediated induction by antagonizing Ets transcription factor Pointed. Development 130: 4085–4096.