© 2016. Published by The Company of Biologists Ltd | Development (2016) 143, 1160-1169 doi:10.1242/dev.134270

RESEARCH ARTICLE

G2 phase arrest prevents bristle progenitor self-renewal and synchronizes cell division with cell fate differentiation

Joseph O. Ayeni[1,‡], Agnès Audibert[2,‡], Pierre Fichelson[3,*], Martin Srayko[1], Michel Gho[3,¶] and Shelagh D. Campbell[1,¶]

ABSTRACT

Developmentally regulated cell cycle arrest is a fundamental feature of neurogenesis, whose significance is poorly understood. During *Drosophila* sensory organ (SO) development, primary progenitor (pI) cells arrest in G2 phase for precisely defined periods. Upon re-entering the cell cycle in response to developmental signals, these G2-arrested precursor cells divide and generate specialized neuronal and non-neuronal cells. To study how G2 phase arrest affects SO lineage specification, we forced pI cells to divide prematurely. This produced SOs with normal neuronal lineages but supernumerary non-neuronal cell types because prematurely dividing pI cells generate a secondary pI cell that produces a complete SO and an external precursor cell that undergoes amplification divisions. pI cells are therefore able to undergo self-renewal before transit to a terminal mode of division. Regulation of G2 phase arrest thus serves a dual role in SO development: preventing progenitor self-renewal and synchronizing cell division with developmental signals. Cell cycle arrest in G2 phase temporally coordinates the precursor cell proliferation potential with terminal cell fate determination to ensure formation of organs with a normal set of sensory cells.

KEY WORDS: Neural development, Sensory organs, Mitosis, G2 arrest, Cell differentiation

INTRODUCTION

The development of a functional nervous system requires precise coordination of self-renewal, proliferation and differentiation to ensure that the correct numbers of properly specified neuronal and non-neuronal cell types are produced. Developmental G2 phase arrest mediated by inhibitory phosphorylation of the Cdk1 mitotic regulator is an important mechanism for coordinating these processes in neural precursor cells (Edgar and O'Farrell, 1989, 1990). Precisely how G2 phase arrest contributes to neural development remains unclear (Tsuji et al., 2008).

Several features make *Drosophila* thoracic sensory organ (SO) development an excellent model system for studying this question. SO progenitor cells (referred to here as pI cells) are selected from clusters of G2-arrested proneural cells (Kimura et al., 1997; Usui and Kimura, 1992) and remain arrested for prolonged periods (Furman and Bukharina, 2008; Gho et al., 1999; Hartenstein and Posakony, 1989, 1990). Upon re-entering the cell cycle in response to developmental signals, they divide asymmetrically to produce two distinct daughter cells: a pIIa cell, which divides again to produce the so-called external or outer cells (the socket and shaft cells) and a pIIb cell, which generates the internal or inner cells (the glia, neuron and sheath cells) (Audibert et al., 2005; Bodmer et al., 1989; Fichelson and Gho, 2003; Gho et al., 1999; Hartenstein and Posakony, 1989).

Developmental mechanisms control the timing of pI cell division by regulating inhibitory phosphorylation of the master mitotic regulator Cyclin-dependent kinase 1 (Cdk1) (Fichelson and Gho, 2004; O'Farrell and Kylsten, 2008; Tio et al., 2001). Ectopic expression of Cdk1 inhibitors (Wee1, Myt1 or Tribbles) in the thoracic SO lineage uncouples pI cell division from cell fate determination, causing undivided pI cells to adopt a fate normally reserved for the pIIb daughter cell (Fichelson and Gho, 2004). Conversely, ectopic expression of $Cdc25^{stg}$, the Cdk-activating phosphatase String (Abdelilah-Seyfried et al., 2000; O'Farrell and Kylsten, 2008), or loss of the Cdk1 inhibitory kinase Myt1 (Jin et al., 2008) disrupts SO development. Several mechanisms have been proposed to account for how G2 phase arrest is used to coordinate *Drosophila* SO development. One hypothesis is that developmental G2 phase arrest affects the accumulation or segregation of cell-fate determinants that are asymmetrically distributed during pI cell division, suggested by the symmetric pI cell division phenotype of hypomorphic *cdc2* mutants (Tio et al., 2001). Another idea is that timing of Cdk1 activation could synchronize pI cell division with extrinsic developmental cues that specify certain cell fates, suggested by pIIb to pIIa cell fate transformations reported for ectopic $Cdc25^{stg}$ activity (O'Farrell and Kylsten, 2008). Regulation of Cdk1 by inhibitory phosphorylation could also influence choices between different modes of cell division (self-renewal, proliferative or terminal cell divisions) of precursor cells. No direct evidence for any of these alternatives exists that we are aware of.

In this study, we addressed these issues by manipulating inhibitory phosphorylation of Cdk1 to determine how forcing pI cells to divide prematurely would affect SO cell fate determination. Expression of either a transgenic Cdk1F inhibitory phosphorylation mutant to bypass G2 phase checkpoint arrest (Ayeni et al., 2014) or $Cdc25^{stg}$ to force pI cells to divide prematurely produced a secondary pI daughter cell instead of a neural precursor pIIb cell, as well as a precursor of external cells. Nonetheless, neural differentiation initiated at the normal time when the secondary pI cell divided generating an SO composed of single neuron and sheath cells, but supernumerary external cell types. These observations reveal new insights into mechanisms that couple cell cycle regulation with determination of terminal cell fate during *Drosophila* sensory development and a new role for G2 phase arrest in masking the self-renewal capability of neural precursor cells.

[1]Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2E9. [2]Sorbonne Universités, UPMC University of Paris 06, IBPS-UMR 7622, Laboratory of Developmental Biology, Paris 75005, France. [3]CNRS, IBPS-UMR 7622, Laboratory of Developmental Biology, Paris 75005, France.
*Present address: Health Interactions, Admiral House, London EC1V 9AZ, UK.
‡These authors contributed equally to this work

¶Authors for correspondence (michel.gho@upmc.fr; sc16@ualberta.ca)

Received 17 December 2015; Accepted 8 February 2016

Development (2016) 143, 1160-1169 doi:10.1242/dev.134270

RESULTS

Premature pI cell division results in SOs with supernumerary cells

Development of the *Drosophila* thoracic SO proceeds by a precisely timed program of asymmetric cell divisions that produces terminally differentiated cells that will comprise the adult organ (shaft, socket, neuron, sheath) and a glial cell that undergoes apoptosis, which are all derived from a single pI cell. Microchaete pI cells are specified in early pupal stages but then arrest in G2 phase for almost 10 h until the mitotic cycles begin (Hartenstein and Posakony, 1989; Huang et al., 1991; Usui and Kimura, 1992). To investigate how G2 phase arrest affected SO development, we used Gal4-driven expression of a transgenic Cdk1 phospho-acceptor mutant that efficiently bypasses developmental G2 phase checkpoint arrest (Ayeni et al., 2014). Genetic crosses with a *neur^{p72}-Gal4* strain were used to express Cdk1^{Y15F}-VFP (hereafter called Cdk1F) or wild-type Cdk1-VFP (as a control, hereafter called Cdk1WT) in arrested SO progenitor cells, several hours before they would normally divide (Bellaïche et al., 2001; Parks et al., 1997). When the adult progeny were examined for thoracic bristle phenotypes, 100% of flies expressing Cdk1WT had normal macrochaetae and microchaetae, each with a single socket and shaft (Fig. 1A, Tables S1 and S2). By contrast, Cdk1F-expressing lineages often produced aberrant bristles with multiple sockets and/or shafts (Fig. 1B). These defects were much more pronounced in macrochaetae (86% defective bristles, Table S1) than in microchaetae (45% defective bristles, Table S2).

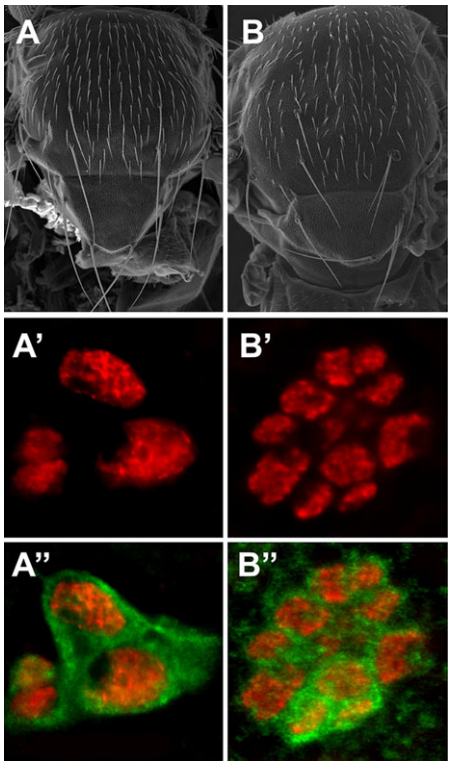

Fig. 1. Cdk1F produces SOs with supernumerary cells. SO phenotypes observed in progeny expressing Cdk1WT or Cdk1F under control of *neur^{p72}-Gal4*. (A) Scanning electron micrograph of normal bristles from an adult thorax expressing Cdk1WT. (A′,A″) Representative SO expressing Cdk1WT fixed at 24 h APF and labeled for Cut (red) and VFP (green) transgene expression. (B) Scanning electron micrograph of defective bristles in progeny expressing Cdk1F. (B′,B″) Representative SO expressing Cdk1F and fixed at 24 h APF showing supernumerary cells detected by labeling with Cut (red) and VFP (green).

Having established that Cdk1F expression perturbed SO development, we quantified the underlying cellular defects using anti-Cut antibodies to label SO cells (Blochlinger et al., 1990). Control macrochaetae expressing Cdk1WT always had four Cut$^+$ cells 24 h after puparium formation (APF), as shown in Fig. 1A′,A″. By contrast, macrochaetae in pupae expressing Cdk1F often had many more than four Cut$^+$ cells per cluster (up to 15 Cut$^+$ cells, n=12; Fig. 1B′,B″). Overexpression of Cdk1F therefore resulted in production of supernumerary sensory cells.

Supernumerary SO cells do not arise by pIIb-to-pIIa cell fate transformation

One possible explanation for SO with multiple bristles and with supernumerary cells in pupae expressing Cdk1F would be if aberrant SO were formed from multiple SOPs. Another possibility would be that multiple bristles resulted from a cell fate transformation of presumptive pIIb cells to a pIIa cell fate, similar to *numb* loss-of-function or *Notch* gain-of-function mutants (Frise et al., 1996; Guo et al., 1996; Justice et al., 2003; Rebay et al., 1993; Uemura et al., 1989). In the former case, we would expected the transformed SO to harbor multiple neurons. In the latter case, we would expect extra cells to arise at the expense of pIIb daughter cell types (neuron and sheath cells). To test these possibilities, we examined SO cell clusters expressing the *Cdk1* transgene at 24 h APF by labeling with ELAV and Su(H) antibodies to mark the neurons (Lin and Goodman, 1994) and socket cells, respectively (Gho et al., 1996). In SO lineages expressing Cdk1WT, each Su(H)$^+$ cell was associated with a single ELAV$^+$ neuron cell in 100% of the cell clusters examined (n=115; Fig. 2A-A‴). In microchaetae lineages expressing Cdk1F, we observed SO clusters with one Su(H)$^+$ cell (45%; n=120; Fig. 2B), two Su(H)$^+$ socket cells (31%, n=120; Fig. 2C) or three Su(H)$^+$ socket cells (24%; n=120; Fig. 2D). In each case, a single ELAV$^+$ cell was present (Fig. 2B′,C′,D′), even in the extreme example of a macrochaetae with seven or more Su(H)$^+$ socket cells shown in Fig. 2E. To determine whether each neuron was associated with a single sheath cell we co-labeled SO cells (at 24 h APF) with antibodies against both ELAV and Prospero (Spana and Doe, 1995). In lineages expressing either Cdk1WT or Cdk1F, each ELAV$^+$ neuron was associated with a single Pros$^+$ sheath cell (Fig. S1). Because we always observed SOs with normal numbers of terminally differentiated inner (neuron and sheath) cells, these results did not support either the possibility of extra SOPs merging or a pIIb-to-pIIa cell fate transformation as an explanation for supernumerary bristles in Cdk1F-expressing SOs.

Cdk1F expression specifically affects pI and pIIa daughter cell proliferation

We next addressed whether SOs with supernumerary bristles were caused by mis-regulation of Cdk activity within the pI cell, or in its descendant pIIa and/or pIIb lineages. To assess these alternatives, we used temperature-sensitive *neur^{p72}-Gal4, tubuline-Gal80^{ts}* to specifically express Cdk1F before or after pI division by shifting the temperature from 18°C to 30°C. Under control conditions at 24 h APF, SO clusters were composed of four cells: two pIIb-descendant inner cells and two pIIa-descendant external cells identified by Pdm1 immunoreactivity, one of which was a socket cell expressing high levels of Su(H) (Fig. 3A; Fichelson and Gho, 2004). When pupae were shifted from 18°C to 30°C at 0 h APF, we observed SO clusters with 3-6 pIIa-descendant external cells identified by Pdm1 immunoreactivity compared with two in control clusters (40 out of

Development (2016) 143, 1160-1169 doi:10.1242/dev.134270

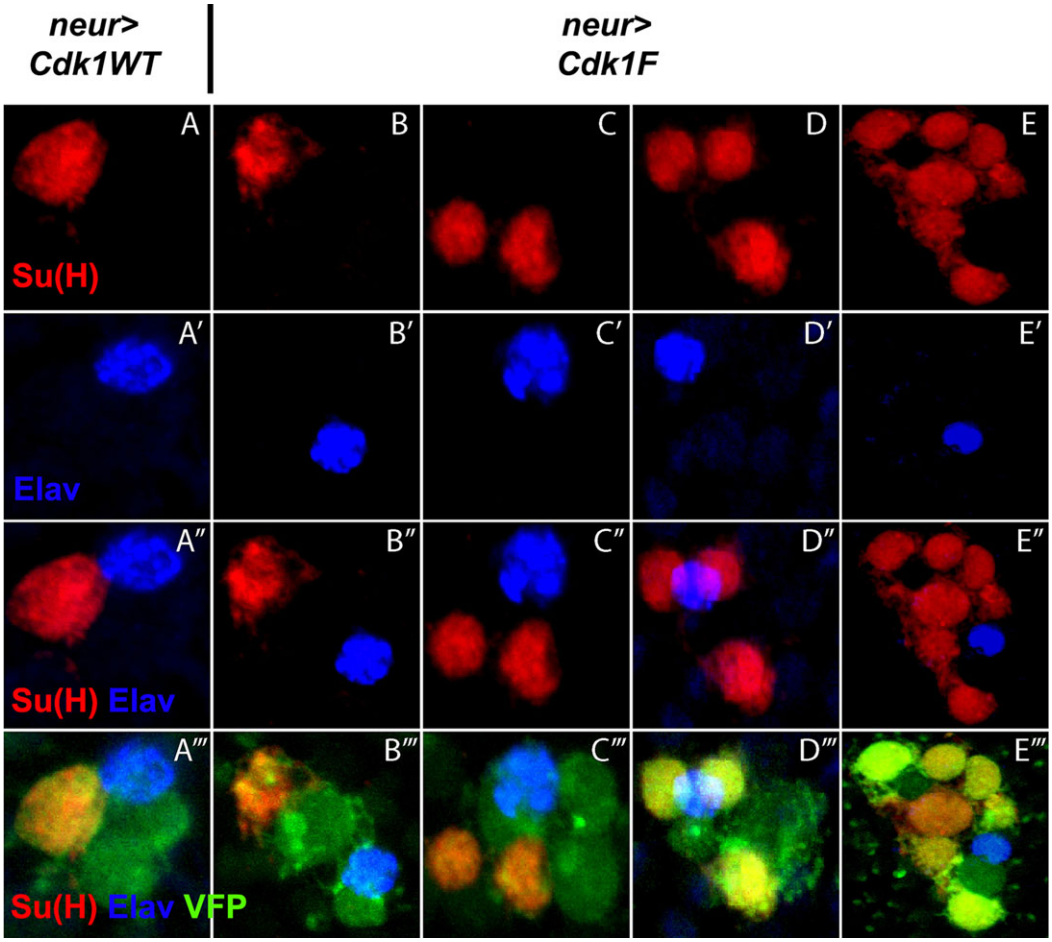

Fig. 2. Aberrant SOs with supernumerary socket cells have only a single neuron. Representative clusters of green (VFP)-labeled SO cells expressing Cdk1WT or Cdk1F at 24 h APF. Immunolabeling with antibodies against Su(H) in (red) and ELAV (blue) was used to detect socket cells and neurons, respectively. (A-A‴) Cdk1WT-expressing SO always had one socket cell and one neuron at 24 h APF. (B-D) Clusters representative of different Cdk1F-expressing microchaetae lineages, each of which have a single neuron at 24 h APF. (B-B‴) Normal SO with one socket and one neuron at 24 h APF. Mutant SO with two (C-C‴) or three (D-D‴) socket cells. (E-E‴) Example of a Cdk1F-expressing macrochaetae with at least seven socket cells, again only one ELAV^{+} neuron was observed.

160 SOs analyzed, Fig. 3B). Co-labeling of Pdm1^{+} pIIa cells with Su(H) revealed that 1-4 of these external cells were specified as socket cells (see Fig. 3D), consistent with data shown in Fig. 2. When *neur^{p72}-Gal4* was released later in development cells by shifting from 18°C to 30°C after the pI division (34 h APF at 18°C), clusters with three or four (Pdm1^{+}) external cells were observed, of which one or two were Su(H)$^{+}$ socket cells (40 out of 360 SOs analyzed, Fig. 3C,D). These data indicate that the supernumerary sensory cells induced by Cdk1F expression are produced, in part, by extra mitosis in secondary precursor cells. Next, we analyzed SO cell clusters after Cdk1F was expressed either in the pIIb sub-lineage using *pros-Gal4* (n=133) or specifically in neurons using *elav-Gal4* (n=144). In both cases, we observed no defects in the number of SO cells identified by Cut immunoreactivity (arrows in Fig. 3G,H compared with control in E,F), nor in the number of socket cells [identified by Su(H); red, Fig. 3G,H compared with control in E,F]. Expression of Cdk1F therefore induced overproliferation of pI- and/or pIIa-descendent external SO cells; however, the pIIb lineage was refractory to this effect.

Cdk1F expression induces self-renewal of pI cells and pIIa lineage amplification

To unambiguously define the origins of supernumerary cells induced by Cdk1F, we used a combination of live imaging and immunolabeling to follow the entire pattern of cell divisions in the microchaetae lineage. Genetic crosses with *neur^{p72}-Gal4* were used to co-express Cdk1WT or Cdk1F and Histone H2B-YFP as a DNA marker. At the end of each recording, the imaged notum was fixed and immunolabeled with anti-Pdm1 and anti-Su(H) to highlight external and socket cells, respectively (Fichelson and Gho, 2004). SOs that were previously analyzed *in vivo* could therefore be unambiguously recognized by their relative position in the fixed nota with respect to the midline, the position of the macrochaetae or the rows of microchaetae (Fichelson and Gho, 2004).

Beginning at 16 h APF, pupae co-expressing Cdk1WT and H2B-YFP were imaged for 8 h to capture the entire SO lineage (Fig. 4Aa-Ah). The pI cells divided at approximately 17 h APF and by 24 h, clusters consisting of four cells were observed (Fig. 4Ah; see also Movie 1). As shown in Fig. 4Ai (see also Fig. S2, co-expressing Cdk1WT with H2B::RFP and Pon::GFP), each cluster consisted of one socket cell [Pdm1^{+} and Su(H)$^{+}$, violet], one shaft cell (Pdm1^{+} in yellow) and two inner cells (YFP in green). Similar results were observed in 100% of the SO clusters analyzed (n=15). In lineages co-expressing Cdk1F and H2B-YFP, pI cells divided 2-3 h earlier than normal (between 12 and 14 h APF), so for this experiment, we started imaging at 12 h APF and continued for 10 h to capture the entire lineage, as shown in Fig. 4Ba-Bh (see also Fig. S6A). In the abnormal Cdk1F-expressing SO cluster shown in

Development (2016) 143, 1160-1169 doi:10.1242/dev.134270

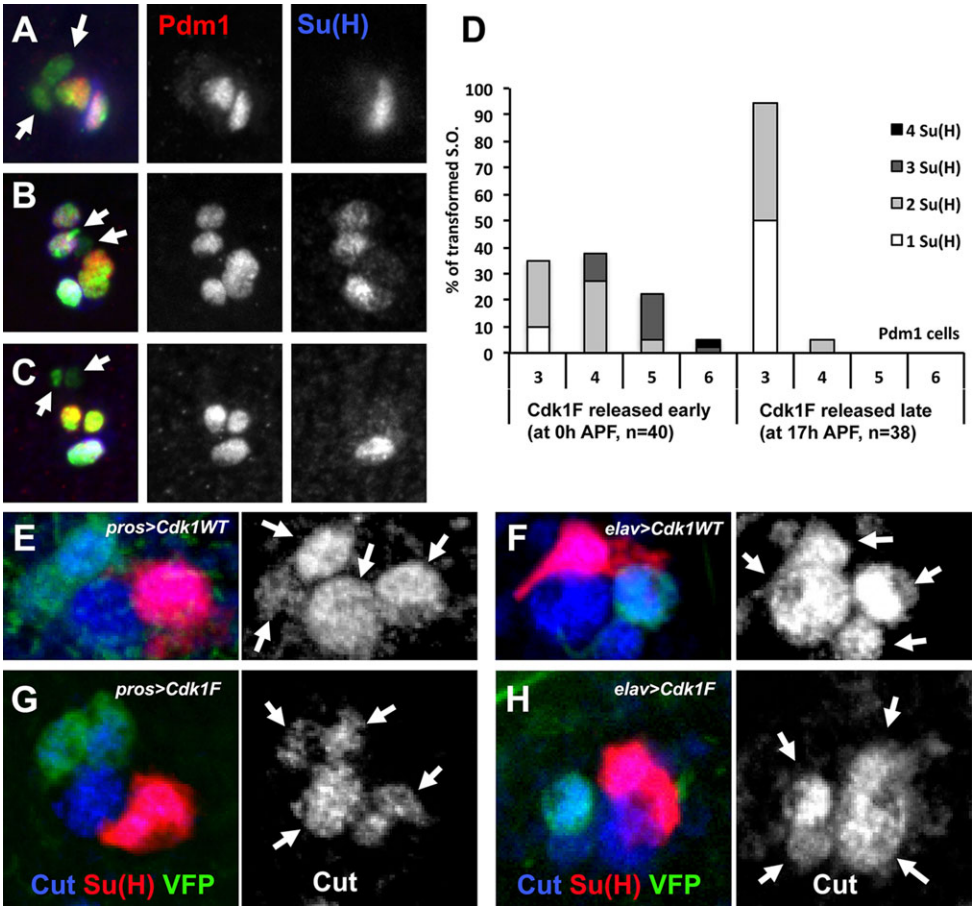

Fig. 3. Cdk1F expression in the pIIb lineage does not disrupt SO development. SOs from *neur^{p72}-Gal4 UAS-Cdk1F tub-Gal80^{ts}* pupae in (A) control conditions (no temperature shift) and after being shifted from 18°C to 30°C to induce Cdk1F expression at 0 h (B) and 17 h (C) APF, to induce Cdk1F in pI cells and after pI division, respectively, with dissection around 28 h APF. pIIa daughter cells are revealed by Pdm1 (red), socket cells by Su(H) (blue) and sensory cells by YFP immunoreactivity (green, UAS-H2B::YFP in all cases). Arrows indicate internal cells. Note that Cdk1F expression induced supplementary Pdm1$^+$ external cells exclusively. (D) Histogram showing the percentage of transformed SOs harboring 3-6 Pdm1$^+$ pIIa descendant cells after Cdk1F expression in pI and pII. The percentage of SOs with one socket cell (white) two socket cells (pale gray), three socket cells (gray) and four socket cells (black) is indicated. Note that late overexpression of Cdk1F induces extra mitoses in the pIIa-descendant cells. (E-H) Expression of Cdk1WT (E,F) or Cdk1F (G,H) under control of *pros-Gal4* (E,G) or *elav-Gal4* (F,H) in SOs fixed at 24 h APF. Labels show transgene expression (green, VFP), socket cells detected by immunolabeling of Su(H) in red and Cut$^+$ cells in blue. Note that Cdk1F expression in pIIb lineage or in the neuron does not induce modification in the number of SO cells (arrows).

Fig. 4Bi, the sensory cluster was composed of 8 cells, including three socket cells [Pdm1$^+$ and Su(H)$^+$, violet], three shaft cells (Pdm1$^+$, red) and two inner cells (YFP$^+$ only, green). Analysis of this recording showed that the anterior pI daughter cell displayed a similar pattern of cell divisions as a normal pI cell to produce five cells: two inner cells, a glial cell that underwent apoptosis, a shaft and a socket cell (Fig. 4Bj). This was observed in 6 out of 30 clusters analyzed (see also Fig. S3 and Movie 2). We also observed that the posterior pI daughter cell underwent further cell divisions producing supernumerary external cells (Movie 2). In the remaining clusters, the anterior pI daughter cell behaved normally, giving rise, after two rounds of divisions, to a glial cell, sheath cell and neuron. Proliferation defects were observed at lower frequency in this experiment than the one described in Fig. 2, where 45% of the SO clusters had more than one socket cell. This discrepancy might be due to subsequent ectopic divisions very late in the Cdk1F-expressing posterior lineage that would have been missed in these live-imaging experiments. In any case, we can conclude from these observations that forcing G2-arrested pI cells prematurely into mitosis often produced an anterior daughter cell that underwent a pattern of cell divisions similar to that observed for normal pI cells, as well as a posterior daughter cell, which underwent extra cell divisions that produced only external cells.

Cdk1F-expressing anterior pI daughter cells assume the identity of pI progenitor cells

To examine Cdk1F-expressing anterior pI daughter cell identity, we analyzed the expression of Prospero (Pros), a neural cell fate determinant that regulates self-renewal and neural cell fate determination (Choksi et al., 2006; Doe et al., 1991; Lai and Doe, 2014). In pupae expressing Cdk1WT or Cdk1F under *neur^{p72}-Gal4* control, SO cells were co-labeled with anti-phosphorylated Histone H3 to distinguish mitotic from interphase cells. Normally, Pros is absent during the pI cell division and first detected as a basal crescent when the anterior pI daughter cell (pIIb) divides (Gho et al., 1999). As expected, pI mother cells did not express Pros at any time during cell division in either genotype (not shown). Later in the SO lineages expressing Cdk1WT, a basal crescent of Pros$^+$ staining was observed in pIIb cells during mitotic entry in 100% of the clusters examined (appearing as a ring in horizontal optical sections, n=25, Fig. 5A). By contrast, in 25% of the SO lineages expressing Cdk1F, the anterior pI daughter cell did not express Pros during mitosis (n=25; arrows in Fig. 5B), just like a pI mother cell.

We examined the orientation of pI and the anterior pI daughter cell division in fixed nota, using Pon immunolabeling to mark the polarity of the asymmetric division (Lu et al., 1998). Normally, pI divides in an anterior-posterior axis within the plane of the epithelium (with the Pon crescent anteriorly located), whereas the anterior pIIb daughter cell divides in an apico-basal orientation (Pon crescent basally located). In Cdk1WT lineages, pI cells divided in the plane of the epithelium with an anteriorly oriented Pon crescent (Fig. 5C), then the anterior pI daughter cell divided along an apico-basal axis marked by a basal crescent of Pon (Fig. 5D). As a consequence, this division produced an apically located pIIIb cell and a basally located Pon$^+$ glia cell (Fig. 5E). In the Cdk1F-expressing lineage, the anterior pI daughter cell divided in the plane of the epithelium with an anteriorly oriented Pon crescent (Fig. 5G). As a consequence, the resulting daughter cells were all located within the plane of the epithelium (Fig. 5H), exactly like the pI cell

Development (2016) 143, 1160-1169 doi:10.1242/dev.134270

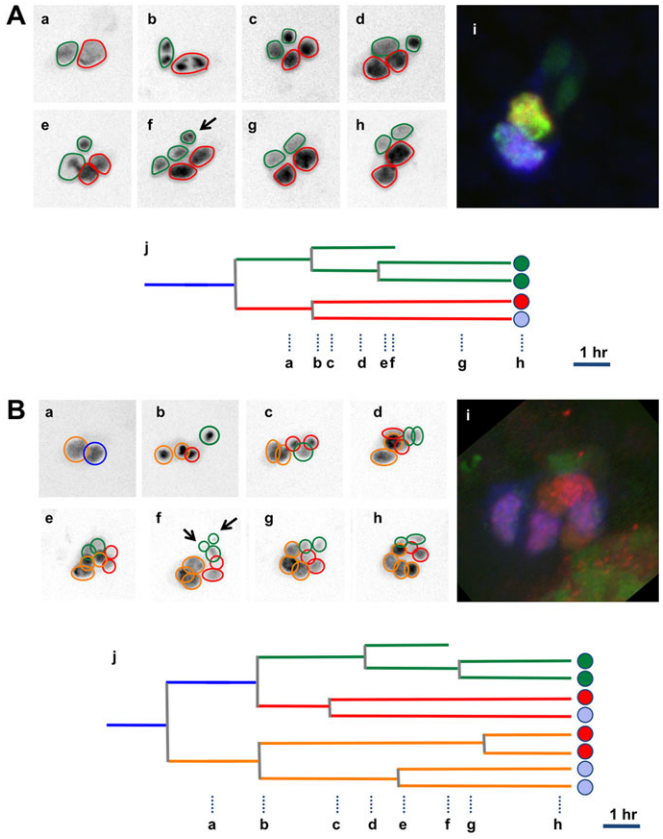

Fig. 4. Combined 4D live imaging and lineage analysis showing the origin of supernumerary cells. (A) Control conditions. Representative frames (Aa-Ah) from a time-lapse recording of one of the clusters expressing *neur p72*>Cdk1WT, H2B::YFP. Arrow in frame f shows fragmentation of the glial cell. pIIb cell and its progeny are outlined in green, pIIa cells and their progeny are outlined in red. (Ai) Immunostaining of the same cluster after the completion of time-lapse recording shown in frames Aa-Ah. SO cells are identified by YFP fluorescence (green). External cells are identified by Pdm1 immunostaining (red). Socket cell is marked by Su(H)-immunoreactivity (violet). (Aj) Schematic view of the lineage shown in a-h. Similar color coding to that in Aa-Ah; pI cell in blue. Temporal localization of each frame shown in Aa-Ah is indicated at the bottom of the lineage. On the right, terminal cells are colored as in Ai, except for the shaft cell shown in red (in Ai this cell appears yellowish because of the combination of red and green). (B) *neur>Cdk1F* expression conditions. Representative frames (Ba-Bh) from an SO expressing *neur p72>Cdk1F, H2B::YFP*. Note the apoptosis of the glial cell in frame Bf (arrows). pI and anterior pI cells are in blue, pIIb cell and its progeny in green, posterior pI cells and its progeny in ochre and pIIa cells and its progeny in red. (Bi) Immunolabeling of the same cluster fixed after the time-lapse recording. Similar immunolabeling was used to distinguish terminal cell types as previously. Note that in this case the cluster is composed of eight cells, which include three socket cells [Pdm^+ and $Su(H)^+$, violet]; three shaft cells ($Pdm1^+$ only, red) and two inner cells (marked by YFP in green). (Bj) Schematic view of the lineage shown in Ba-Bh; color coding is the same as in Ba-Bh. Temporal localization of each frame in Ba-Bh is again indicated in the bottom of the lineage. On the right, terminal cells are colored as shown in Bi.

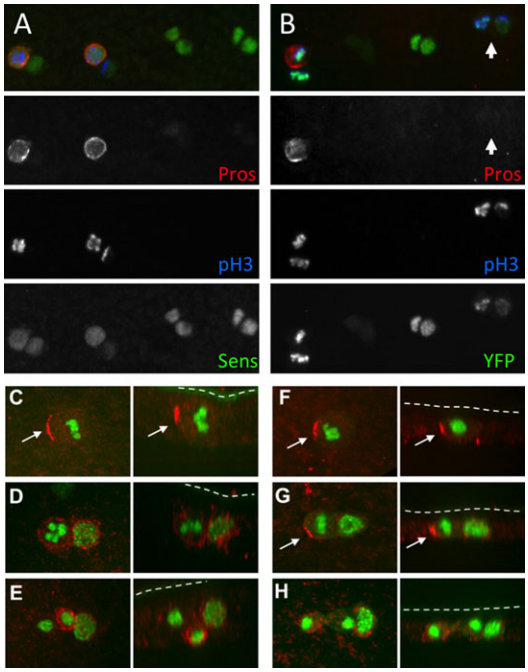

Fig. 5. The anterior pI daughter cell acquires a pI cell fate. (A,B) Expression of Prospero assessed in the anterior pI daughter cell. SOs expressing Cdk1WT or Cdk1F were labeled for Pros, Senseless, phospho-Histone H3 (pH3) or YFP, as shown. (A) Lineage expressing Cdk1WT showing anterior pI daughter cell expressing Pros (red) during mitosis (marked by PH3 labeling, in blue) in 100% of SOs analyzed (n=25). SO cells were identified by Sens immunostaining. (B) In organs expressing Cdk1F, roughly one quarter (n=25), showed anterior cell division that was not associated with Pros expression (arrow), reminiscent of a pI mother cell division. SO cells were identified by neur>H2B::YFP expression. (C-H) Cell division orientation and polarity of the pI cell and its anterior daughter cell examined in SO lineages expressing Cdk1WT (C-E) or Cdk1F (F-H). In each pair of confocal images, the left panel corresponds to a horizontal confocal plane (anterior towards the left) and the right panel to a vertical optical reconstruction (anterior towards the left, basal towards the bottom, dashed lines show the cuticle). The metaphase of pI cells is shown in C and F. The division of the anterior daughter cells is shown during metaphase (D,G) and telophase (E,H). SO cells were identified by the expression of Histone::YFP (Green). Pon (red) detected by immunoreactivity was used as cell polarity marker. The location of the Pon crescent formed in pI in SO cells expressing Cdk1WT and Cdk1F (arrows in C,F) shows that these cells are dividing anterior-posteriorly within the plane of the epithelium. The vertical reconstruction of the Cdk1WT-expressing control at metaphase shows that the anterior daughter cell has a crescent labeled for Pon located basally (D). As a consequence, this division produced an apical-located pIIIb and a basal-located Pon^+ and $Pros^+$ glia cell (E). In the Cdk1F-expressing SO, the anterior pI daughter cell shown in G has an anteriorly oriented Pon crescent (arrow), indicating that this cell is dividing within the plane of the epithelium (C,F). The vertical reconstruction of the Cdk1F-expressing SO (G) shows that the anterior Pon^+ cell will divide in the same plane as the pI cell (C,F), unlike the apical-basally oriented division shown in D. (H) The Pon^+ daughter divided in an anterior-posterior orientation to produce daughter cells in the same plane.

(arrows in Fig. 5C,F and G, respectively). Forcing pI cells to divide early therefore produced an anterior daughter cell that did not express Pros and divided in the plane of the epithelium, with the Pon crescent oriented toward the anterior pole. All of these cell behaviors are characteristic of pI cells. Because the Cdk1F-expressing anterior pI daughter cell also divided according to a pI cell pattern and generated a wild-type set of sensory cells, we concluded that this cell had acquired a pI cell identity. Consequently, we refer to it as a secondary pI cell.

Premature expression of $Cdc25^{stg}$ also induces self-renewal of the pI cell

During normal SO development, mitosis occurs when $Cdc25^{stg}$ phosphatase activates Cdk1 by removal of inhibitory phosphorylation (Edgar and O'Farrell, 1990; Huang et al., 1991; Kimura et al., 1997; O'Farrell and Kylsten, 2008; Usui and Kimura, 1992). To determine whether precocious activation of endogenous Cdk1 could also induce pI self-renewal, we used $neur^{p72}$-*Gal4* to co-express $Cdc25^{stg}$ with H2B::YFP (Fig. 6 and Movie 3) or $Cdc25^{stg}$ with H2B::YFP and

Development (2016) 143, 1160-1169 doi:10.1242/dev.134270

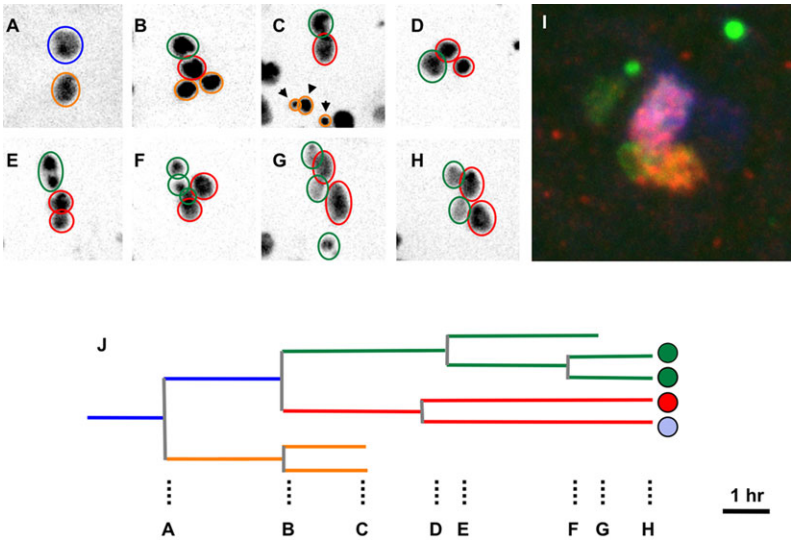

Fig. 6. Premature cell division induced by Cdc25^{stg} induces a self-renewal division in the microchaetae lineage. Combined 4D imaging in living pupae (A-H) and final immunostaining (I) of *neur>string*, H2B::YFP. Panels A-H show representative frames from a time-lapse recording of one cluster in this recording. pI and secondary pI cells in blue, pIIb and its progeny in green, pIIa and its progeny in ochre and secondary pIIa cells and its progeny in red. Note in C the fragmentation of the two posterior cells (arrowheads). (I) Immunostaining of the same cluster after the time-lapse recording. Shaft and socket cells are identified by anti-Pdm1 (red). The socket cell is also marked by Su(H) immunolabeling (purple). Bristle lineage cells are identified by YFP fluorescence (green). Note that the final cell composition of this cluster is identical to that of control *neurp72*>Cdk1WT, H2B::YFP cluster shown in Fig. 4. (J) Diagram of the cell lineage shown in A-H. Color code similar to that in A-H. On the right, terminal cells are colored as in I. The different frames depicted in A-H are temporally localized in the bottom of the cell lineage.

Pon::GFP (Fig. S4 and Movie 4). Live imaging was performed for 10 h to follow the entire microchaetae lineage and at the end of the recording the pupae were fixed and immunolabeled to determine the final differentiation state of the SO clusters. In lineages expressing Cdc25^{stg}, pI cells divided at 12-14 h APF, ~2-3 h earlier than normal, but similar to the timing of lineages expressing Cdk1F (see Fig. 4). In 75% of the clusters analyzed (n=32), the pI cell division produced an anterior-located daughter cell that behaved like a secondary pI cell, dividing in the plane of the epithelium with no detectable Pros (Fig. S5H and H′). More importantly, this cell generated an entire SO lineage with normal cell composition (Fig. 6A-I). In 90% of these clusters with a secondary pI cell (68% of the total of clusters analyzed), the posterior-located sister cell (or its descendants) underwent apoptosis (arrowheads in Fig. 6C, lineage depicted in Fig. 6J, see also Fig. S5A-F; n=32). In cases when this apoptosis occurred late in the lineage, as shown in Fig. S5A-F, the cell fragments were Ttk^{+} (Fig. S5G, asterisks). Because Ttk is expressed in external cells (Audibert et al., 2005), these observations indicate that apoptotic posterior cells adopted an external cell fate after overexpression of Cdc25^{stg}. In the remaining 10% of these clusters, descendants of the posterior cell do not die. This might explain the appearance of rare sensory organs with two shafts observed with expression of Cdc25^{stg} (not shown). Premature induction of mitosis by Cdc25^{stg} therefore produced a posterior cell that usually died and an anterior sibling that behaved like a pI progenitor cell, which was capable of recapitulating the entire SO lineage. The resulting adult SOs appeared normal in every respect. Collectively, these results show that pI cells can undergo self-renewal when forced to divide prematurely, with one daughter cells remaining competent for terminal differentiation and generating a full complement of inner and outer SO cells.

The ability of pI cells to perform self-renewal is temporally restricted

Finally, we determined when cells first assumed a neuronal fate during SO development under conditions of Cdk1F expression. At developmental times between 15 and 19 h APF, pupae expressing either Cdk1WT (Fig. 7A) or Cdk1F (Fig. 7B) were fixed and labeled with Pros antibodies (red). In 100% of the lineages expressing Cdk1WT, Pros^{+} cells were not observed at 15 h APF (n=39) or 17 h APF (n=51). Pros^{+} cells were detected only in late two-cell clusters at the time of pIIb cell division (19 h APF, 57 out of 98 SO analyzed, Fig. 7A and Fig. S6B). Similarly, there were no Pros^{+} cells in SOs expressing Cdk1F at 15 h APF, although these clusters were mainly composed of two cells (Fig. 7B and Fig. S6A; n=104). At 19 h APF, just as in control conditions, 60% of the

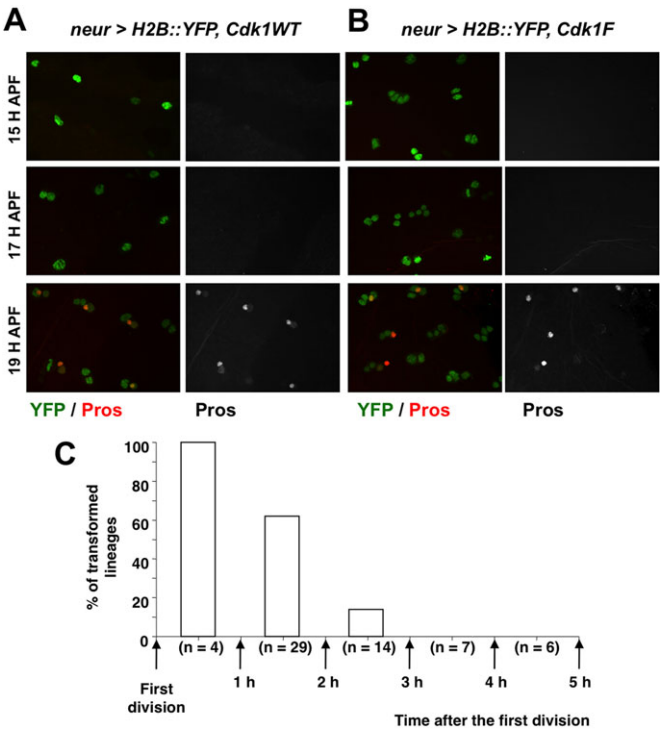

Fig. 7. The timing of neural differentiation as well as self-renewal ability is independent of the cell cycle. Nota expressing Cdk1WT (A) or Cdk1F (B) transgenes. Sensory organ cells at 15 h, 17 h and 19 h APF are identified by YFP immunoreactivity (green, *neur^{p72}-Gal4 UAS-H2B::YFP* in all cases). Neuronal fate was assessed by the accumulation of Pros (red). Note that Pros^{+} cells are detected at similar developmental times (19 h APF) in both cases, whereas the number of cells per sensory organ differs (mainly two cells in Cdk1WT-expressing sensory organs and four cells in Cdk1F-expressing sensory organs). (C) Histogram showing the percentage of pI cells undergoing self-renewal division as a function of pI division time. These cells divide in rows, and to adjust the different developmental time of each SO, the first pI division in a row is used as a frame of reference. Note that the probability of producing a self-renewal division is higher when the pI division occurs earlier.

Development (2016) 143, 1160-1169 doi:10.1242/dev.134270

clusters analyzed had one Pros^{+} cell (n=102; Fig. 7B and Fig. S6B). At this developmental time, the sensory clusters were composed mainly of four cells (Fig. S6A). These results show that forcing premature pI cell mitosis by Cdk1F expression does not accelerate the developmental timing of neuronal cell fate determination. Instead, they suggest the existence of a timer that is independent of cell division controlling the onset of neural differentiation.

The ability of pI cells to undergo self-renewal revealed by these experiments therefore appears to be transient, terminating with precise developmental timing once cells become committed to neuronal differentiation. To explore this idea, we studied the correlation between the time of pI division and the ability to perform pI self-renewal division. As pI cells do not divide synchronously but divide in rows (Usui and Kimura, 1993), we used the first pI cell division in a row as a frame of reference. The plot in Fig. 7C shows that pI cells that divided earlier in a given row had a higher probability of subsequently undergoing self-renewal. These observations are therefore in agreement with the idea that pI cell self-renewal capability is limited in time by the onset of a cell cycle-independent developmental signal that triggers terminal cell divisions and neural differentiation.

DISCUSSION

Cell cycle arrest in G2 phase has long been recognized as a mechanism for coordinating cell proliferation with terminal cell fate determination during *Drosophila* SO development (Hartenstein and Posakony, 1990; Huang et al., 1991; Kimura et al., 1997; Nègre et al., 2003). By forcing G2-arrested pI cells to undergo premature mitosis upon expression of Cdk1F, we discovered that pI cells can undergo asymmetric self-renewal division prior to terminal differentiation, producing a secondary pI and a sibling external daughter cell. The secondary pI daughter cell subsequently divided again at the normal developmental time to generate a full complement of differentiated SO cell types. The sibling daughter cell can also undergo additional cell divisions that produce only outer cells, explaining the observed supernumerary external structures. Precise cell cycle regulation of G2/M timing could therefore serve a dual purpose in the SO lineage: restraining pI self-renewal potential and synchronizing pI cell division with developmental signals that promote neuronal cell fate differentiation. Coordinating exit from G2 phase with reception of a neuronal cell fate signal would therefore provide an effective means of coupling these processes.

The major conclusion of our work is that pI cells are able to undergo self-renewal division before transitioning to a terminal mode of division. This self-renewal property was cryptic when these cells were arrested in G2 phase but revealed by forcing a precocious pI division. We propose two, non-exclusive mechanisms that could account for these results (Fig. 8). One possibility is that G2-arrested pI cells respond to differences in Cdk1 activity by adopting alternative modes of cell proliferation (Fig. 8A). Exposure of G2-arrested pI cells to elevated Cdk1 activity due to Cdk1F or Cdc25^{stg} expression can result in an asymmetric self-renewal division producing secondary pI daughter cells, whereas at intermediate (normal) levels of Cdk1 activity the pI cells divide asymmetrically to generate two differentiated cell types (pIIa and pIIb). At low levels of Cdk1 activity, pI cells either do not divide at all or undergo symmetric cell division to generate two post-mitotic terminal neurons, as reported for a hypomorphic *cdc2*^{E51Q} mutant (Tio et al., 2001). A second possibility is that a cell cycle-independent developmental signal triggers the cell fate transition from primary to secondary precursor identities (Fichelson and Gho, 2004). This idea is in agreement with the data of Fig. 7 showing that pI-like daughter cells initiated neuronal cell fate determination at the normal time of development, despite precocious pI cell divisions. We depict this developmental signal as a wave triggering changes in cell fate (Fig. 8B). At low levels of Cdk1 activity, this signal would trigger acquisition of pIIb cell identity in arrested pI cells (Fig. 8Ba) as reported earlier (Fichelson and Gho, 2004). At normal levels of Cdk1 activity, pI division and the development signal would be synchronous, resulting in a typical asymmetric pI division producing two distinct secondary precursor cells (Fig. 8Bb). Finally, at high levels of Cdk1 activity (Fig. 8Bc) division of the pI cell before the developmental signal that triggers cell fate transition produces a secondary pI cell by a classic self-renewal division.

There are two distinct processes accounting for the supernumerary external cells observed in SO lineages after Cdk1F expression: (1) premature pI division produces a secondary pI daughter cell, which can then undergo a normal pattern of cell divisions to produce a full complement of internal and external cell types and (2) a Ttk^{+} posterior cell that proliferates to produce a pIIa-like sub-lineage. Presumably, these pIIa-like external cells are already primed to undergo endoreplication and are therefore competent to reactivate their cell cycle machinery (Salle et al., 2012). It remains unclear why Cdk1F-induced overproliferation was

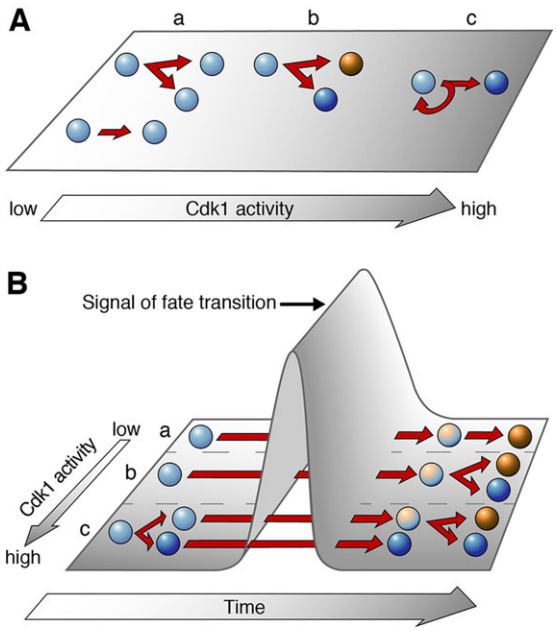

Fig. 8. Model depicting the relationship between precursor cell division and Cdk1 activity. (A) Sensory precursor cell division outcome reflects the level of Cdk1 activity. (Aa) At lower levels of Cdk1 activity, pI precursor cells divide symmetrically or not at all (Tio et al., 2001; Fichelson and Gho, 2004). (Ab) At intermediate (normal) levels of Cdk1 activity, precursor cells divide asymmetrically to generate two differentiated cells, pIIa and pIIb. (Ac) At high Cdk1 activity levels, the precursor cells undergo self-renewal asymmetric division. (B) The outcome of the pI precursor cell division depends on a temporal signal of cell fate transition that is independent of Cdk1 activity. (Ba) At lower levels of Cdk1 activity, the pI cell does not divide and a cell fate transition signal drives acquisition of a secondary cell identity depicted here with successive states from blue and ochre color. (Bb) At intermediate (normal) levels of Cdk1 activity, pI cells divide asymmetrically after receive the signal triggering cell fate transition. (Bc) At higher than normal Cdk1 levels, pI cells divide before receiving a cell fate transition signal and, as such, undergo a self-renewal division. The renewed pI-like cell receives the cell fate transition signal and behaves like a pI precursor cell.

Development (2016) 143, 1160-1169 doi:10.1242/dev.134270

so much more extreme in macrochaetae than microchaetae lineages (Fig. 2). Macrochaete precursor cells are determined well before microchaete SOP (Hartenstein and Posakony, 1989; Huang et al., 1991; Usui and Kimura, 1992). Our model presented in Fig. 8B predicts this would mean a large window of opportunity for multiple pI self-renewal divisions. Alternatively, macrochaetae might be more sensitive to Cdk1F-induced divisions later in the pIIa lineage. We were not able to resolve this issue by live imaging of macrochaetae development because this is not possible for technical reasons. Another unresolved issue concerns differences between outcomes observed after overexpression of Cdk1F and $Cdc25^{stg}$. $Cdc25^{stg}$ seems to be more effective at forcing premature cell divisions than Cdk1F (75% vs 20%). Moreover, unlike Cdk1F, overexpression of $Cdc25^{stg}$ usually induced apoptosis in the Ttk^{+} posterior pI daughter cell. In these respects, ectopic $Cdc25^{stg}$ behaves more like expression of a non-inhibitable $Cdk1^{T14A,Y15F}$ mutant, which causes gross chromosomal abnormalities, in addition to bypass of G2 phase checkpoint arrest (Ayeni et al., 2014). Alternatively, other factors affected by $Cdc25^{stg}$ might explain this discrepancy.

SO precursor cells are selected from groups of G2-arrested competent cells, called the proneural clusters (Ghysen and Dambly-Chaudiere, 1989). These G2-arrested cells seem to transit through three distinct states, revealing symmetric, self-renewal and terminal mode of division. Early in development, before the selection of sensory precursor cells, premature mitosis of proneural cells is associated with symmetric cell divisions and a loss of neural cell identity (Nègre et al., 2003). Once the G2-arrested SO precursor cells are specified, these cells undergo a self-renewal mode of proliferation when forced to divide precociously (this study). Thereafter, in response to a developmental signal, these sensory precursor cells divide terminally to produce differentiated cells that form each SO. Results presented here indicate that the transition from self-renewal to terminal mode of cell division involves a cell cycle-independent developmental signal.

What is the nature of this developmental signal? The probability of undergoing self-renewal seems to be correlated with when the SO precursor cell divided, indicating that this property is temporally limited. One possibility is that a temporally regulated cell-intrinsic factor caused cells to change their identity at a specific threshold of activity. For example, a temporal gradient for the Chinmo transcription factor confers temporal identity of neuroblast progeny during post-embryonic *Drosophila* brain development (Zhu et al., 2006). We examined this possibility but did not observe changes in SO cell fate within *chinmo*-null clones (not shown). Another intrinsic factor controlling cell fate and the choice between self-renewal and neural fate in *Drosophila* neuroblasts is the homeo-domain transcription factor Prospero (Berger et al., 2010; Choksi et al., 2006; Lai and Doe, 2014; Li and Vaessin, 2000; Reddy and Rodrigues, 1999). Temporal expression of Pros also correlates with neural determination in SO development (as shown in Figs 5 and 7); however, previous work showed that Pros is not involved in neural determination (Fichelson and Gho, 2003), but in neuronal differentiation (Manning and Doe, 1999).

Alternatively, the signal shown in Fig. 8B that terminates G2 phase arrest and promotes neural development might be extrinsic to the pI cell. An appealing candidate for an extrinsic signal would be the steroid hormone ecdysone, because pI cell division in the microchaetae lineage roughly coincides with a minor peak in ecdysone expression (Truman et al., 1994). Moreover, ecdysone has been shown to induce $Cdc25^{stg}$ expression in G2-arrested abdominal histoblasts and tracheal progenitor cells (Djabrayan et al., 2014; Ninov et al., 2009) and to induce metabolic changes linked to terminal cell cycle exit in *Drosophila* neuroblasts (Homem et al., 2014). Steroid hormone signaling could therefore serve as a global mechanism for synchronizing G2/M-regulated cell division with terminal cell differentiation during neural development. We tested this possibility by downregulation of the Ecdysone receptor using RNA interference and by overexpression of a dominant-negative form of this receptor, but did not observe any effect on SO development (data not shown). Identification and molecular characterization of this cell cycle-independent developmental signal remains an important goal for future analysis.

In conclusion, we have shown that G2 phase arrest serves a dual role in promoting formation of properly specified neural structures by: (1) preventing progenitor self-renewal and (2) synchronizing cell division with developmental signals that induce neural cell fates. As such, this study highlights the role of developmental cell cycle regulation in synchronizing different modes of cell division with signals promoting terminal cell differentiation during *Drosophila* sensory development.

MATERIALS AND METHODS

Fly strains

The construction and phenotypic characterization of *UAS-Cdk1WT* and *UAS-Cdk1F* transgenes expressed in imaginal wing discs and larval neuroblasts was previously described (Ayeni et al., 2014). To express transgenes in the SO lineage we used either $neur^{P72}$*-Gal4* (Bellaïche et al., 2001) or *w;* $neur^{P72}$*-Gal4,P UAS mRFP1-Pon[LD][1.2]/TM3,Sb* (a gift from J. Knoblich, Institute of Molecular Biotechnology, Vienna). To temporally control transgene expression, crosses were made with a $neur^{P72}$*-Gal4* strain bearing $Gal80^{ts}$ and the progeny were cultured at 18°C during embryonic and larval development. Pupae were then up-shifted at the indicated times to 29°C to allow the expression of $neur^{P72}$*-Gal4*. Crosses with *elav-Gal4* (Bloomington Stock Center) and *pros-Gal4* (a gift from C. Doe, Institute of Molecular Biology, University of Oregon) were used to express transgenes specifically in the pIIb lineage.

Immunohistology

Dissected larvae or pupae were fixed in 4% paraformaldehyde (PFA) for 25 min, then washed several times with 1× PBT (PBS with 0.3% Triton X-100) before incubation with primary antibodies. We used the following primary antibodies: mouse anti-Cut (DSHB, 1:250); rat anti-ELAV (DSHB, 1:100); mouse anti-ELAV (DSHB, 1:100); mouse anti-Prospero (DSHB, 1:500); rabbit anti-Su(H) [a gift from F. Schweisguth, Institut Pasteur, Paris (Schweisguth and Posakony, 1994), 1:500]; rabbit anti-phospho-Histone H3 (Upstate, 06-570, 1:4000); rabbit anti-PON [a gift from Y. N. Jan, University of California, San Francisco (Lu et al., 1998), 1:1000]; rabbit anti-Pdm1 (a gift from T. Préat, ESPCI, Paris, 1:200); guinea pig anti-Sens [a gift from H. Bellen, Baylor College of Medicine, Houston (Nolo et al., 2000), 1:1000], rabbit anti-Ttk (gift from A. Travers, Laboratory of Molecular Biology, Cambridge, UK, 1:500). Alexa Fluor 568- and 633-conjugated secondary antibodies were purchased from Molecular Probes and used at 1:1000.

Live imaging of SO development

We performed live imaging of the SO lineage in *neur-Gal4,UAS-Pon::RFP/UAS-Cdk1WT* or $neur^{p72}$*-Gal4,UAS-PON::RFP/UAS-Cdk1F* pupae, following protocols described previously (Audibert et al., 2005; Fichelson and Gho, 2004). White pupae were collected and aged for 15 h at 25°C before the pupae were dissected and mounted for imaging (Gho et al., 1999). Live imaging data shown in Figs 5 and 6 was collected using a spinning disk coupled to an Olympus BX-41 microscope (Roper Scientific, 40×, NA 0.75 objective, CoolSnapHQ^2 camera). The temperature of the recording chamber was carefully controlled (±0.1°C) using a Peltier device temperature controller fixed to the microscope stage. Data shown in Figs 4 and 6 were collected using an Olympus 1X81 microscope (60×, NA

Development (2016) 143, 1160-1169 doi:10.1242/dev.134270

1.42 oil objective) equipped with a spinning disk head (CSU10; Yokogawa) and an ORCA-R2 CCD camera (Hamamatsu). Both systems were driven by Metamorph software (Universal Imaging). Z-stacks of images were acquired every 3 min and assembled using ImageJ software (NIH).

Acknowledgements
We gratefully acknowledge Sophie Gournet (UPMC Illustration Services) as well as Jürgen Knoblich, Chris Doe, Yuh Nung Jan, Thomas Préat, Developmental Studies Hybridoma Bank (University of Iowa) and the Bloomington *Drosophila* Stock Center for providing fly stocks or antibodies.

Competing interests
The authors declare no competing or financial interests.

Author contributions
J.O.A., A.A., P.F., M.G. and S.D.C. designed and performed the experiments, and carried out data analysis. J.O.A., A.A., M.S., M.G. and S.D.C. prepared and edited the manuscript.

Funding
Funding was provided by the National Science and Engineering Research Council of Canada (J.O.A., S.D.C.), Canadian Institutes of Health Research (M.S.), the Centre National de la Recherche Scientifique (A.A., M.G.), the Université Paris VI Pierre et Marie Curie (A.A., M.G.). P.F. was supported by a fellowship Association pour la Recherche contre le Cancer.

Supplementary information
Supplementary information available online at
http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.134270/-/DC1

References
Abdelilah-Seyfried, S., Chan, Y. M., Zeng, C., Justice, N. J., Younger-Shepherd, S., Sharp, L. E., Barbel, S., Meadows, S. A., Jan, L. Y. and Jan, Y. N. (2000). A gain-of-function screen for genes that affect the development of the Drosophila adult external sensory organ. *Genetics* **155**, 733-752.

Audibert, A., Simon, F. and Gho, M. (2005). Cell cycle diversity involves differential regulation of Cyclin E activity in the Drosophila bristle cell lineage. *Development* **132**, 2287-2297.

Ayeni, J. O., Varadarajan, R., Mukherjee, O., Stuart, D. T., Sprenger, F., Srayko, M. and Campbell, S. D. (2014). Dual phosphorylation of cdk1 coordinates cell proliferation with key developmental processes in Drosophila. *Genetics* **196**, 197-210.

Bellaïche, Y., Gho, M., Kaltschmidt, J. A., Brand, A. H. and Schweisguth, F. (2001). Frizzled regulates localization of cell-fate determinants and mitotic spindle rotation during asymmetric cell division. *Nat. Cell Biol.* **3**, 50-57.

Berger, C., Kannan, R., Myneni, S., Renner, S., Shashidhara, L. S. and Technau, G. M. (2010). Cell cycle independent role of Cyclin E during neural cell fate specification in Drosophila is mediated by its regulation of Prospero function. *Dev. Biol.* **337**, 415-424.

Blochlinger, K., Bodmer, R., Jan, L. Y. and Jan, Y. N. (1990). Patterns of expression of cut, a protein required for external sensory organ development in wild-type and cut mutant Drosophila embryos. *Genes Dev.* **4**, 1322-1331.

Bodmer, R., Carretto, R. and Jan, Y. N. (1989). Neurogenesis of the peripheral nervous system in Drosophila embryos: DNA replication patterns and cell lineages. *Neuron* **3**, 21-32.

Choksi, S. P., Southall, T. D., Bossing, T., Edoff, K., de Wit, E., Fischer, B. E., van Steensel, B., Micklem, G. and Brand, A. H. (2006). Prospero acts as a binary switch between self-renewal and differentiation in *Drosophila* neural stem cells. *Dev. Cell* **11**, 775-789.

Djabrayan, N. J.-V., Cruz, J., de Miguel, C., Franch-Marro, X. and Casanova, J. (2014). Specification of differentiated adult progenitors via inhibition of endocycle entry in the Drosophila trachea. *Cell Rep.* **9**, 859-865.

Doe, C. Q., Chu-LaGraff, Q., Wright, D. M. and Scott, M. P. (1991). The prospero gene specifies cell fates in the Drosophila central nervous system. *Cell* **65**, 451-464.

Edgar, B. A. and O'Farrell, P. H. (1989). Genetic control of cell division patterns in the Drosophila embryo. *Cell* **57**, 177-187.

Edgar, B. A. and O'Farrell, P. H. (1990). The three postblastoderm cell cycles of Drosophila embryogenesis are regulated in G2 by string. *Cell* **62**, 469-480.

Fichelson, P. and Gho, M. (2003). The glial cell undergoes apoptosis in the microchaete lineage of Drosophila. *Development* **130**, 123-133.

Fichelson, P. and Gho, M. (2004). Mother-daughter precursor cell fate transformation after Cdc2 down-regulation in the Drosophila bristle lineage. *Dev. Biol.* **276**, 367-377.

Frise, E., Knoblich, J. A., Younger-Shepherd, S., Jan, L. Y. and Jan, Y. N. (1996). The Drosophila Numb protein inhibits signaling of the Notch receptor during cell-cell interaction in sensory organ lineage. *Proc. Natl. Acad. Sci. USA* **93**, 11925-11932.

Furman, D. P. and Bukharina, T. A. (2008). How *Drosophila melanogaster* forms its mechanoreceptors. *Curr. Genomics* **9**, 312-323.

Gho, M., Lecourtois, M., Géraud, G., Posakony, J. W. and Schweisguth, F. (1996). Subcellular localization of suppressor of hairless in Drosophila sense organ cells during Notch signalling. *Development* **122**, 1673-1682.

Gho, M., Bellaïche, Y. and Schweisguth, F. (1999). Revisiting the Drosophila microchaete lineage: a novel intrinsically asymmetric cell division generates a glial cell. *Development* **126**, 3573-3584.

Ghysen, A. and Dambly-Chaudiere, C. (1989). Genesis of the *Drosophila* peripheral nervous system. *Trends Genet.* **5**, 251-255.

Guo, M., Jan, L. Y. and Jan, Y. N. (1996). Control of daughter cell fates during asymmetric division: interaction of Numb and Notch. *Neuron* **17**, 27-41.

Hartenstein, V. and Posakony, J. W. (1989). Development of adult sensilla on the wing and notum of Drosophila melanogaster. *Development* **107**, 389-405.

Hartenstein, V. and Posakony, J. W. (1990). Sensillum development in the absence of cell division: the sensillum phenotype of the Drosophila mutant string. *Dev. Biol.* **138**, 147-158.

Homem, C. C. F., Steinmann, V., Burkard, T. R., Jais, A., Esterbauer, H. and Knoblich, J. A. (2014). Ecdysone and mediator change energy metabolism to terminate proliferation in Drosophila neural stem cells. *Cell* **158**, 874-888.

Huang, F., Dambly-Chaudière, C. and Ghysen, A. (1991). The emergence of sense organs in the wing disc of Drosophila. *Development* **111**, 1087-1095.

Jin, Z., Homola, E., Tiong, S. and Campbell, S. D. (2008). Drosophila myt1 is the major cdk1 inhibitory kinase for wing imaginal disc development. *Genetics* **180**, 2123-2133.

Justice, N., Roegiers, F., Jan, L. Y. and Jan, Y. N. (2003). Lethal giant larvae acts together with numb in notch inhibition and cell fate specification in the Drosophila adult sensory organ precursor lineage. *Curr. Biol.* **13**, 778-783.

Kimura, K., Usui-Ishihara, A. and Usui, K. (1997). G2 arrest of cell cycle ensures a determination process of sensory mother cell formation in Drosophila. *Dev. Genes Evol.* **207**, 199-202.

Lai, S.-L. and Doe, C. Q. (2014). Transient nuclear Prospero induces neural progenitor quiescence. *Elife* **3**, e03363.

Li, L. and Vaessin, H. (2000). Pan-neural Prospero terminates cell proliferation during Drosophila neurogenesis. *Genes Dev.* **14**, 147-151.

Lin, D. M. and Goodman, C. S. (1994). Ectopic and increased expression of Fasciclin II alters motoneuron growth cone guidance. *Neuron* **13**, 507-523.

Lu, B., Rothenberg, M., Jan, L. Y. and Jan, Y. N. (1998). Partner of Numb colocalizes with Numb during mitosis and directs Numb asymmetric localization in Drosophila neural and muscle progenitors. *Cell* **95**, 225-235.

Manning, L. and Doe, C. Q. (1999). Prospero distinguishes sibling cell fate without asymmetric localization in the Drosophila adult external sense organ lineage. *Development* **126**, 2063-2071.

Nègre, N., Ghysen, A. and Martinez, A.-M. (2003). Mitotic G2-arrest is required for neural cell fate determination in Drosophila. *Mech. Dev.* **120**, 253-265.

Ninov, N., Manjón, C. and Martín-Blanco, E. (2009). Dynamic control of cell cycle and growth coupling by ecdysone, EGFR, and PI3K signaling in Drosophila histoblasts. *PLoS Biol.* **7**, e1000079.

Nolo, R., Abbott, L. A. and Bellen, H. J. (2000). Senseless, a Zn finger transcription factor, is necessary and sufficient for sensory organ development in Drosophila. *Cell* **102**, 349-362.

O'Farrell, F. and Kylsten, P. (2008). A mis-expression study of factors affecting Drosophila PNS cell identity. *Biochem. Biophys. Res. Commun.* **370**, 657-662.

Parks, A. L., Huppert, S. S. and Muskavitch, M. A. T. (1997). The dynamics of neurogenic signalling underlying bristle development in Drosophila melanogaster. *Mech. Dev.* **63**, 61-74.

Rebay, I., Fehon, R. G. and Artavanis-Tsakonas, S. (1993). Specific truncations of Drosophila Notch define dominant activated and dominant negative forms of the receptor. *Cell* **74**, 319-329.

Reddy, G. V. and Rodrigues, V. (1999). Sibling cell fate in the Drosophila adult external sense organ lineage is specified by prospero function, which is regulated by Numb and Notch. *Development* **126**, 2083-2092.

Salle, F., Campbell, S. D., Gho, M. and Audibert, A. (2012). CycA is involved in the control of endoreplication dynamics in the *Drosophila* bristle lineage. *Development* **139**, 547-557.

Schweisguth, F. and Posakony, J. W. (1994). Antagonistic activities of Suppressor of Hairless and Hairless control alternative cell fates in the Drosophila adult epidermis. *Development* **120**, 1433-1441.

Spana, E. P. and Doe, C. Q. (1995). The prospero transcription factor is asymmetrically localized to the cell cortex during neuroblast mitosis in Drosophila. *Development* **121**, 3187-3195.

Tio, M., Udolph, G., Yang, X. and Chia, W. (2001). cdc2 links the Drosophila cell cycle and asymmetric division machineries. *Nature* **409**, 1063-1067.

Truman, J. W., Talbot, W. S., Fahrbach, S. E. and Hogness, D. S. (1994). Ecdysone receptor expression in the CNS correlates with stage-specific

Development (2016) 143, 1160-1169 doi:10.1242/dev.134270

responses to ecdysteroids during Drosophila and Manduca development. *Development* **120**, 219-234.

Tsuji, T., Hasegawa, E. and Isshiki, T. (2008). Neuroblast entry into quiescence is regulated intrinsically by the combined action of spatial Hox proteins and temporal identity factors. *Development* **135**, 3859-3869.

Uemura, T., Shepherd, S., Ackerman, L., Jan, L. Y. and Jan, Y. N. (1989). numb, a gene required in determination of cell fate during sensory organ formation in Drosophila embryos. *Cell* **58**, 349-360.

Usui, K. and Kimura, K. (1992). Sensory mother cells are selected from among mitotically quiescent cluster of cells in the wing disc of Drosophila. *Development* **116**, 601-610.

Usui, K. and Kimura, K. (1993). Sequential emergence of the evenly spaced microchaetes on the notum of Drosophila. *Development* **116**, 601-610.

Zhu, S., Lin, S., Kao, C.-F., Awasaki, T., Chiang, A.-S. and Lee, T. (2006). Gradients of the Drosophila Chinmo BTB-zinc finger protein govern neuronal temporal identity. *Cell* **127**, 409-422.