Published in final edited form as:

Dev Biol. 2017 March 15; 423(2): 93–100. doi:10.1016/j.ydbio.2017.02.008.

Live-imaging analysis of germ cell proliferation in the *C. elegans* adult supports a stochastic model for stem cell proliferation

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Abstract

The *C. elegans* adult hermaphrodite contains a renewable pool of mitotically dividing germ cells that are contained within the progenitor zone (PZ), at the distal region of the germline. From the PZ, cells enter meiosis and differentiate, ensuring the continued production of oocytes. In this study, we investigated the proliferation strategy used to maintain the PZ pool by using a photoconvertible marker to follow the fate of selected germ cells and their descendants in live worms. We found that the most distal pool of 6–8 rows of cells in the PZ (the distal third) behave similarly, with a fold expansion corresponding to one cell division every 6 hours on average. Proximal to this region, proliferation decreases, and by the proximal third of the PZ, most cells have stopped dividing. In addition, we show that all the descendants of cells in rows 3 and above move proximally and leave the PZ over time. Combining our data with previous studies, we propose a stochastic model for the *C. elegans* PZ proliferation, where a pool of proliferating stem cells divide symmetrically within the distal most 6–8 rows of the germline and exit from this stem cell niche occurs by displacement due to competition for limited space.

Keywords

germline; stem cells; proliferation; lineage tracing

Introduction

The maintenance of many adult tissues depends on an adult stem cell system that contains a renewable, proliferative pool of cells that gives rise to differentiated cell types.

Understanding the patterns of proliferation and differentiation of stem cell systems is an essential part of elucidating mechanisms that regulate tissue maintenance and repair, as well as pathways that lead to misregulation of cell fate in disease progression and ageing.

Features of interest include the stem cell population size, cell cycle rate, and the strategy for control of cell fate to account for self-renewal and differentiation.

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The *C. elegans* germline is an important model for understanding adult stem cell systems. The hermaphrodite gonad contains a renewable population of ~200–250 germ cells that make up the progenitor zone (PZ), also known as the proliferative zone. The PZ cells are arranged in ~16–20 rows (cell diameters) at the distal end of the tube-shaped gonad (Fig 1a). A single somatic cell, called the distal tip cell (DTC, not shown), caps the distal end of the gonad and is required to maintain the PZ through GLP-1/Notch signaling (Austin and Kimble, 1987; Hansen and Schedl, 2013; Kimble and White, 1981). Cells proximal to the PZ display overt changes in chromatin appearance, indicating that they have already differentiated and entered meiosis. In the adult hermaphrodite, germ cells that enter meiosis either become oocytes or are eliminated via programmed cell death (Gumienny et al., 1999).

Previous studies have contributed to our understanding of various properties of the PZ, however the exact organization and patterns of proliferation within the PZ remain uncertain. One major approach has employed the incorporation of cytologically detectable nucleotides (BrdU or EdU) that label cells in both mitotic and meiotic S-phase. These studies suggested that germ cells in the PZ are not quiescent, but rather cycle continuously (Crittenden et al., 2006), that cells move through the meiotic region at an estimated rate of ~1 cell diameter per hour (Crittenden et al., 2006; Jaramillo-Lambert et al., 2007), and that meiotic S-phase cells are contained in the PZ (Crittenden et al., 2006; Fox et al., 2011). Also based on this approach, in combination with M-phase indices, estimates have been made of the cell cycle length, which have varied from 5–24 hours (Chiang et al., 2015; Crittenden et al., 2006; Fox et al., 2011; Seidel and Kimble, 2015). In these studies, properties of the PZ were inferred from population averages of fixed samples.

An important goal in the stem cell field is to elucidate the proliferation strategy used to ensure self-renewal and continued production of differentiated cells. Some stem cell systems, such as the *Drosophila* germline, have a hierarchical organization. In this system, a small population of stem cells is attached to the stem cell hub, and divides asymmetrically to produce a daughter that stays a stem cell, attached to the hub, and a daughter that detaches from the stem cell niche and subsequently goes through a specified number of cell divisions before differentiating (called transit-amplification) (Spradling et al., 2011). Asymmetric stem cell division (self-renewal) and transit-amplification are controlled by different pathways, and thus stem cells and transit-amplifying cells show different properties (Spradling et al., 2011; Xie and Spradling, 1998). More recently, other systems, such as the mammalian intestine, have been shown to have a stochastic organization, where stem cells divide symmetrically and compete for space in the stem cell niche. In this scenario, stem cells are displaced from the niche without undergoing asymmetric cell division, but due instead to divisions of other stem cells within the niche (Lopez-Garcia et al., 2010; Ritsma et al., 2014; Snippert et al., 2010).

In the *C. elegans* germline, it has been debated whether the mitotic cells in the PZ are developmentally equivalent or have a hierarchical organization. To date, genetic methods and cytology of fixed samples have been used to interrogate the response of PZ cells to either a block in cell-cycle progression (using a ts *emb-30* mutant) (Cinquin et al., 2010) or loss of GLP-1 signaling (using a ts *glp-1* mutant) (Fox and Schedl, 2015). Cinquin et al showed that germ cells in the distal 6–8 rows of the PZ responded differently than the rest of

the PZ to a cell-cycle block, and argued for a hierarchical organization, while Fox and Schedl showed that cells throughout the PZ responded similarly to loss of GLP-1, and argued for equivalency of proliferating cells.

In this study, we undertook a lineage-tracing approach to study the PZ dynamics in *C. elegans*, using photoconversion of Dendra2::H2B to mark and follow selected cells and their descendants over time in live animals. This approach complements previous studies, revealing new insights into the organization and operation of the PZ. We integrate our study with previous results to propose a model of the germline stem cell system.

Materials and methods

Strains and plasmids

C. elegans strains were maintained at 20°C on plates seeded with OP50, using standard methods. The following strains were used in this study: Bristol (N2) wild type, LX850 *lin-15(n765ts)* vsIs50 [HSNp::PTX; *lin-15(+)*] X (Tanis et al., 2008), OCF69 *ocfSi1[Pmex-5::Dendra2::HIS-58::tbb-2 3'UTR + unc-119(+)] I ; unc-119(ed3) III* (this study), OCF75 *ocfSi1[Pmex-5::Dendra2::H2B::tbb-2 3'UTR + unc-119(+)] I ; unc-119(ed3)? III; lin-15(n765ts)? vsIs50 [HSNp::PTX; lin-15(+)] X* (this study, made by crossing strains LX850 and OCF69).

The OCF69 strain was constructed as follows. Dendra2 (PCR amplified from pEG545, Addgene plasmid 40116, (Griffin et al., 2011)) was fused in frame to histone H2B gene *his-58* (PCR amplified from pCM1.151, Addgene plasmid 21386, (Merritt et al., 2008) by Gibson assembly (New England Biolabs) and inserted into Gateway pDONR221 by BP reaction. The resulting vector, pSR1, was combined with pJA252 (Addgene plasmid 21512, (Zeiser et al., 2011)) and pCM1.36 (Addgene plasmid 17249, gift from Geraldine Seydoux) in a 3-fragment Gateway LR reaction with destination vector pCFJ210 to create pSR3. pSR3 was used to perform MosSCI insertion into ttTi4348 site on chr. I of strain EG6701 using the published protocol ((Frokjaer-Jensen et al., 2012), <http://www.wormbuilder.org/>)

Viability, brood counts and egg-laying rate

For viability and brood counts, single L4 worms were picked to plates and transferred to new plates every 24 hours until egg-laying stopped. A day after removal of the parent, the number of live progeny and unhatched eggs were counted. For calculating average egg-laying rate, the number of eggs laid from 24 to 48 hours after L4 (a period where all worms had started laying eggs and none had stopped laying eggs) was divided by 24 hours to obtain a per hour average.

DAPI staining of gonads

To compare the strains of worms used in this study with and without Dendra2::H2B, dissection and DAPI staining of gonads was performed, as in (Rosu et al., 2013). Briefly, worms were dissected in egg buffer to release gonads and fixed in 1% paraformaldehyde solution for 5 minutes, followed by freeze cracking and methanol fixation for 1 minute.

Slides were washed with PBST and mounted in Vectashield with DAPI (Vector Laboratories). Microscopy was performed as below.

Mounting and recovery of worms for photoconversion and live imaging

Single worms that were 24 hours post L4 were immobilized on slides with 10% agarose pads in 4–6 μ L of 1:1 solution of M9 buffer and 0.1 μ m polystyrene beads (Polysciences, Inc, cat#00876). A coverslip was placed on top and sealed with melted petroleum jelly. Photoconversion was performed as described below. For recovery after imaging, the coverslip was lifted off with a razor blade, 4–6 μ L of M9 buffer was added to the worm, and the worm was gently sucked up with a pipet tip and returned to a plate. When recovery was successful, adult worms were able to move normally and lay eggs immediately upon return to plates. Worms that did not immediately recover were not studied further; this was usually due to physical damage to the worms when the coverslip was lifted, and amounted to less than 10% of worms.

For live imaging of embryos, adult worms were dissected in M9 buffer to release embryos, mounted on a slide with a 2% agarose pad and sealed with melted petroleum jelly.

Microscopy

Confocal images were taken on a Nikon Eclipse TE2000U spinning disk confocal microscope with a 60X/1.4 numerical aperture objective and a C9100-13 EMCCD camera by Hamamatsu. Metamorph software was used for image acquisition. Unconverted Dendra2 was imaged with a 491 nm laser at medium low intensity (20–40%; note that a higher intensity will result in unintended photoconversion), and converted Dendra2 was imaged with a 561 nm at high intensity (70–100%). Photoconversion was performed with a 405 nm laser at moderate power (30) for 3 sec using the Mosaic system for selective illumination. Z-stacks were taken at 1 μ m intervals. Images were analyzed using the ImageJ software. Images were prepared for display using Adobe Photoshop.

Cell cycle length determination

One cell division results in a population expansion ratio of 2, while two divisions result in a population expansion ratio of 4. Assuming all cells are dividing at the same rate, the observed ratio of 2.6 indicates a fraction of cells (x) are dividing once, while a fraction of cells (y) are dividing twice. Thus $2x+4y=2.6$, where $x+y=1$. Thus $x=0.7$ (70% of cells dividing once), and $y=0.3$ (30% of cells dividing twice) during the 8 hour period observed. The average number of divisions per hour is calculated as $(1*0.7+2*0.3)$ divisions/8 hrs=0.16 divisions/hour; this is equivalent to 1 division per 6.15 hours.

Statistical analyses

Prism 6 was used to perform ordinary one-way ANOVA, Tukey's multiple comparisons test.

Results and Discussion

Dendra2::H2B photoconversion enables lineage tracing in early embryonic divisions

We constructed a strain expressing histone H2B fused to Dendra2 (Dendra2::H2B) in the germline, under the control of the *mex-5* promoter and the *tbb-23* 3' UTR (a similar construct was recently described by (Bolkova and Lancot, 2016)). Dendra2 is a photoconvertible green-to-red fluorescent protein (Gurskaya et al., 2006). Our strain showed strong expression throughout the germline and in embryos (Fig. 1, Supplemental Fig. S1), and had similar viability and brood size to wild type (Supplemental Fig. S2a,b). To assess the use of Dendra2::H2B for lineage tracing, we performed photoconversion of a single cell in embryos, which undergo rapid cell divisions with a known lineage. When Dendra2::H2B in a single cell was photoconverted in the 2-cell embryo, all the descendants of the converted cell could be easily followed for at least 5 subsequent divisions (Fig. 1b). This indicates that in this context, the signal of the photoconverted Dendra2::H2B was sufficiently bright to carry out lineage tracing despite dilution of the converted population by half due to DNA replication in every cell division cycle.

Dendra2::H2B photoconversion enables tracing of a selected region of cells in the germline progenitor zone in live animals

We next assessed the use of Dendra2::H2B photoconversion for lineage tracing in the PZ. We found that due to the close proximity of cells in the distal germline, faint off-target photoconversion occurred in cells located in focal planes below the target cell when using laser intensity that was sufficient to track chromosomes during germline proliferation (data not shown). This made lineage determination ambiguous. To limit excitation in the Z-plane, we attempted to use a two-photon system. However, we observed that Dendra2 photoconversion did not occur under a two-photon regime, consistent with other reports (Dempsey et al., 2015).

We thus focused on photoconversion of a selected population of cells. The converting laser was aimed in the middle of the Z-stack of the tube-shaped gonad, thus targeting the entire cell population within a selected number of rows. As in the case of single cell photoconversion, cells at edges of the converted populations could be faintly converted, potentially resulting in either over or under estimating the proliferating pool (see more on this below). However, in general the number of cells at the edge of the population was small relative to the size of the population itself, minimizing the possible contribution of faintly converted cells. During photoconversion and imaging, individual worms were mounted on a slide and immobilized by friction, using a solution of polystyrene beads (Kim et al., 2013). Since germline progression stops in immobilized worms (data not shown and (McCarter et al., 1999)), after photoconversion the worms were recovered from the slides onto plates and then remounted and reimaged 8 hours later. Immediately after recovery, the vast majority of worms (>90%) moved and laid eggs normally; worms that did not move normally after recovery were discarded. For each gonad analyzed, we scored the number of converted cells and their position (rows from the distal tip) immediately after photoconversion, as well as the number and position of descendent cells 8 hours later. Our approach allows us to conduct

longitudinal studies of individual live worms, measuring proliferation, cell movement, and the fate of selected cells over time.

In wild type animals, the distal tip of the gonad in adult worms (24 hour post-L4) is often obscured by embryos or other tissue. To partially overcome this, we used a strain that clears embryos from the uterus faster due to the expression of a subunit of pertussis toxin, HSNp::PTX, in the HSN motor neurons that control egg-laying (Tanis et al., 2008). The HSNp::PTX strain had viability and brood size similar to wild type (Supplemental Fig. S2a,b). In addition, the average egg-laying rate did not increase, despite increased clearing of the uterus (Supplemental Fig. S2c). The Dendra2::H2B strain by itself had a slight decrease in average egg-laying rate, as well as a smaller average number of cells and rows in the PZ as compared to wild type (Supplemental Fig. S2c,d,e). Importantly, the double transgenic strain HSNp::PTX; Dendra2::H2B behaved similarly to the Dendra2::H2B strain (Fig. S2). Therefore we used this double transgenic strain for all subsequent analyses.

Pools of cells in the germline progenitor zone show distinct proliferation dynamics

As proof of principle, we started our initial analysis by dividing the PZ into roughly three parts of about 5–7 rows each (most distal, middle, and most proximal), depending on the PZ size in each worm. We then photoconverted each section of cells and determined their fate by imaging the gonads 8 hours later. Photoconverted cells originally in the most distal section of the PZ expanded an average of 2.6 fold (ratio of number of recovered cells: number of initial cells) in 8 hours, at which point they made up a large part of the PZ (Fig. 2a, Fig. 3a worms 1–3, and Supplemental Table S1 lines 1–3). The intensity of the Dendra2::H2B signal was qualitatively similar among the recovered cells, suggesting that all cells in the photoconverted population divided 1–2 times during this timeframe, rather than a few cells dividing many times. The converted Dendra2::H2B signal intensity was fainter than expected from dilution by DNA replication and cell division. As photoconversion leads to a covalent modification of the Dendra2 molecule that prevents it from reverting to green fluorescence (Chudakov et al., 2007), this observation suggests a substantial turnover of H2B molecules in the proliferating germ cell population. We did not observe intermixing of converted and unconverted cells in the recovered worms, showing that adjacent cells and their progeny move proximally together as more distal cells expand behind them. Moreover, the absence of intermixing allowed us to score the proliferation of this pool of distal germ cells in an additional manner, by following the pool of distal unconverted cells in worms where the middle section was photoconverted (Fig. 3a worms 4–6, Fig. 3b worms 26–28, and Supplemental Table S1 lines 4–9). These distal unconverted cells expanded an average of 2.6 fold, the same as the pool of converted cells in worms 1–3 described above.

In contrast to the distal pool, germ cells in the most proximal third of the PZ maintained a similar population size, expanding an average of 1.2 fold over 8 hours, by which time all have moved into the meiotic prophase zone (Fig. 2c, Fig. 3a worms 7–12, and Supplemental Table S1 lines 13–18). This indicates that most of the cells in this region of the PZ have stopped proliferating and have likely committed to meiosis.

The cells in the middle of the PZ region showed intermediate proliferation, with an average expansion of 1.6 fold (Fig. 2b, Fig. 3a worms 4–6, and Supplemental Table S1 lines 10–12).

This indicates that some cells have stopped proliferation, while others have undergone one division. This middle region likely represents the transition from the proliferative stage to the non-proliferative stage and commitment to meiosis. Taken together, these data show that it is possible to determine proliferation dynamics by live imaging and that different pools of cells within the PZ show distinct levels of proliferation.

In the worms analyzed above and in those that will be described below, the size of the PZ decreased in most gonads over the 8 hour time course (an average decrease of 13% in the number of cells and 11% in the number of rows). A similar decrease in cell number (11%) and a smaller decrease in row number (6%) were observed in control animals that were mounted on slides and imaged but not photoconverted ($n=8$). Thus, the decrease in PZ size is unlikely to be primarily due to photoconversion *per se*. This decrease may partly reflect a normal change in PZ size with age, also noted by other studies (Crittenden et al., 2006; Killian and Hubbard, 2005). It is also possible that the temporary stress from immobilization and imaging, and/or the genotype of our assay strain, may have contributed to the decrease in PZ size over this 8 hour window.

Subregions of the most distal pool of germline progenitor cells have similar proliferation dynamics

Whether cells in the first few rows of the distal tip proliferate differently than the rest of the proliferative cells has been an unresolved question. Studies analyzing the mitotic index in fixed gonads showed a slightly lower M-phase frequency in rows 1–2 (~2.9% of cells) compared to rows 3–9 (~4.3% of cells) ((Crittenden et al., 2006); a similar trend was reported in (Maciejowski et al., 2006)). Analysis of BrdU/EdU incorporation, on the other hand, suggested that cells throughout the PZ are cycling through S-phase (mitotic or meiotic) at a similar rate (Crittenden et al., 2006; Fox et al., 2011; Jaramillo-Lambert et al., 2007). These experiments were done with fixed samples, from which rates of proliferation can be difficult to extract. We thus used our direct proliferation assay to determine if subpopulations of the proliferative germ cell pool show different dynamics.

To this end, we photoconverted and analyzed small pools (2–4 rows) of cells in the distal and middle regions of the PZ. The germ cell pools from rows 1–4.5 expanded an average of 2.7 fold in 8 hours (Fig. 3b, worms 13–18 and 19–25, Fig 3c and Supplemental Table S2, lines 1–13). Note that the average expansion obtained from converted cells (in worms 13–18) and unconverted distal cells (in worms 19–25) differed (2.2 and 3.1 fold, respectively). This discrepancy is likely due to partially converted cells at the edge of the converted zone, which will lead to an underestimate of expansion when considering the converted pool (as the fluorescence in the daughter cells will be too faint to detect), and an overestimate of expansion when considering the unconverted distal pool (as daughters of partially converted cells at the edge will appear as if they were not previously converted). This effect is strongest for rows 1–4 because they contain the smallest number of cells (Table S2), whereas in other regions (e.g. the distal third, Supplemental Table S1 lines 1–9) the possible contribution of partially converted cells becomes negligible. Thus, to minimize the over- and underestimation problem, the fold expansion was calculated from both converted cells and unconverted distal cells.

The germ cell population from rows 3–8 similarly expanded an average of 2.7 fold in 8 hours (Fig 3b, worms 19–25, Fig 3c, Supplemental Table S2, lines 14–20). These values are not statistically different from the average fold expansion of rows 1–4.5 ($p=0.99$, one-way ANOVA, Tukey's multiple comparisons test). Taken together, our data suggest that subregions of the distal pool of germ cells have similar proliferation dynamics, which match the overall proliferation dynamics seen when considering the entire region of rows 1–7.5. This is significant because it indicates that the entire distal pool of cells up to rows 6–8 may have similar properties and thus may function as a stem cell pool. We note that due to the slightly smaller PZ size in the assay strain, the equivalently proliferating region in wild-type animals may be one row longer. In contrast, cells from rows 8–12.5 expanded significantly less, with a proliferation ratio of 1.5 (Fig 3b, worms 26–28, Fig 3C, and Supplemental Table S2 lines 21–23); this is consistent with a transition in this region from proliferative to non-proliferative state.

Proliferation dynamics indicate an average cell cycle length of about 6 hours

The average proliferation ratio of 2.6 in the distal region (up to rows 6–8) indicates that some cells underwent mitosis once in 8 hours, whereas the cells closest to entering M phase at the time of conversion likely divided twice. Assuming cells are dividing at an equal rate, this corresponds to an average cell cycle length of 6.15 hours, with ~30% of cells undergoing mitosis twice in 8 hours (see Materials and Methods). This represents a new method of calculating cell cycle length in this region of the germline, based on a direct proliferation assay. This result falls within the lower range of recent cell cycle estimates reported as 6–8 hours (Fox et al., 2011), 6–10 hours (Seidel and Kimble, 2015), and 5–7 hours (Chiang et al., 2015) for the adult hermaphrodite at 24 hours post-L4 stage at 20°C.

Pools of cells progress proximally along the PZ long axis at different rates

Our live cell analysis allows us not only to examine cell proliferation but to also determine the rate of cell progression through the PZ. To this end, the position of the proximal end of each converted zone (or distal unconverted zone) was measured at the initial time point and 8 hours later. We found that cells in the most proximal region, rows 10–23, where most cells have stopped proliferation, progress proximally an average of 1.3 ± 0.2 rows/hour (Fig. 4a). This rate is comparable to the 1 row/hour rate reported for cell movement out of PZ and throughout meiosis (Crittenden et al., 2006; Jaramillo-Lambert et al., 2007). This movement is likely the result of displacement by distal cell proliferation (input) as well as continued removal of oocytes proximal to this zone, either through fertilization or programmed apoptosis (output). In contrast, cells in rows 2–5, which are all proliferating, progress an average of 0.4 ± 0.1 rows/hour (Fig. 4a). This slower rate of movement is the result of both displacement by distally dividing cells and the axis of cell division, which is not just along the PZ long axis, but also laterally, consistent with previous observations (Crittenden et al., 2006). Lateral expansion is also reflected in the tapered geometry of the hermaphrodite gonad, where the number of cells per row increases from the distal tip to ~row 10, after which it remains constant (Morgan et al., 2010). Cells in between the distal and proximal pools (rows 6–8) have an intermediate progression rate, with an average of 1.0 ± 0.2 rows/hour (Fig. 4a).

Proliferation dynamics and other features of the germline progenitor zone favor a stochastic model of stem cell maintenance

An important question in the *C. elegans* germline field is which cells stay in the stem cell niche. We found that when cells in rows 3 and above were photoconverted, the entire population moved proximally as it expanded (Fig. 3b, worms 19–25, and Supplemental Table S2, lines 14–20). Conversely, when the population in the first 2–3 rows was photoconverted, their descendants filled an extended area of the distal tip, with no intermixing with unconverted cells that could have, potentially, stayed in place (Fig. 3b worms 13–18 and Supplemental Table S2, lines 1–6). While we cannot assay single cells or cells in just rows 1 or 2, we can conclude that descendants of cells in rows 3 and above move proximally and are not retained within the distal germline niche. If there is a population of cells that remains anchored at the tip, it would have to be small, ~11–13 cells or less (first two rows). However, based on the random orientation of cell divisions in this region (Crittenden et al., 2006) we favor the idea that there are no cells anchored at the distal tip, and that the reason why photoconverted cells in the first two rows remain at the tip is because there are no cells more distal to them that would expand and push them away from this region.

Our analysis of proliferation dynamics in the *C. elegans* adult hermaphrodite germline suggests that germ cells in the first 6–8 rows of the germline proliferate at a similar rate. This is the same region that showed a distinct response to cell cycle block in a *ts emb-30* mutant (Cinquin et al., 2010), prompting the authors to suggest these cells are in a stem cell-like state. Cinquin et al. went on to suggest that the stem-cell-like pool is organized in a hierarchical manner, and that proximal to this pool there is another pool of transit-amplifying cells. Instead, we propose that while the distal pool from row 1 up to rows 6–8 (~50–80 cells) is comprised of equivalent, proliferating stem cells, this pool is followed by a region where proliferation rate decreases, with no transient-amplifying cells (Fig. 4b). Recently, it was shown that transcription of 2 GLP-1/Notch targets is mainly restricted to the first 6–8 PZ rows (Lee et al., 2016). Thus, it appears that cells proximal to rows 6–8 have exited the stem cell niche, and are no longer responding to GLP-1 signal; once the GLP-1 signal is lost, cells undergo a final mitosis to finish the cell cycle before entering meiosis (Fox and Schedl, 2015). These cells are on their way to differentiation and are not undergoing transit-amplification divisions.

Taken together, our data support a stochastic model where proliferating stem cells divide symmetrically within the distal most 6–8 rows of the germline, and exit from this stem cell niche occurs by displacement. We show that (to the resolution permitted by our method), only descendants of cells in rows 1–2 remain at the distal tip, with the rest of the germ cells moving proximally over time. Thus competition for continued stem cell fate occurs in rows 1–2, where a dividing cell may displace a neighboring cell. Interestingly, this stochastic model predicts clonal drift over time (Snippert et al., 2010), where if mutations arise in the distal-most stem cells, some lineages may be lost and some may come to dominate (Fig. 4c).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Michael Krause, Eric Haag, and Alison Walters for comments on the manuscript, Christian Frøkjær-Jensen for advice regarding MosSCI, Michael Koelle for the LX850 strain, Hari Shroff, George Patterson, and Kevin O'Connell for microscopy advice, and members of the Cohen-Fix lab for helpful discussions. Work in the Cohen-Fix lab is supported by an intramural grant from NIDDK (#DK069012) and a Nancy Nossal Fellowship to SR. Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440)

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Highlights

- Photoconversion of histone H2B is used to determine germ cell proliferation rates and movement in live *C.elegans* adults
- The distal-most 6–8 rows of germ cells have similar proliferation rates and are likely stem cells
- Cells beyond rows 6–8 exit the proliferative state without transit-amplification
- All cells beyond row 2 will be displaced over time and exit the stem cell niche

Figure 1. Dendra2::H2B expression and photoconversion

Nuclei were visualized by the expression of histone H2B fused to the photoconvertible fluorescent protein Dendra2 (Dendra2::H2B). (a) Dendra2::H2B expression in one gonad arm (outlined in a dashed line) in a live immobilized young adult worm. The vertical line marks the cytologically visible transition from the PZ to meiotic prophase, based on chromatin appearance. Dendra2::H2B is also expressed in the sperm and embryos, as labeled. (b) Images of Dendra2::H2B in a live embryo undergoing mitotic divisions, where Dendra2::H2B in one cell was photoconverted from green to red at the 2-cell stage at time 0. Images were taken at the indicated time points. All daughter cells arising from the converted cell were marked and could be followed for at least 5 cell divisions. Top row: merged images of unconverted and converted Dendra2::H2B. Bottom row: converted Dendra2::H2B alone. Scale bars for both panels=15 μ m.

Figure 2. Tracing of germ cells in the PZ by photoconversion of Dendra2::H2B

The PZ was roughly divided into thirds and germ cells were photoconverted in the distal third (panel a), the middle third (panel b), or the proximal third (panel c). The distal part of the gonad arm is outlined in a dashed line. The yellow line marks the cytologically visible transition from the PZ to meiotic prophase, based on chromatin appearance. The left side of each panel (t=0) shows the gonad immediately after photoconversion, while the right side shows the same gonad 8 hours later. The top row in each panel shows a merged image of unconverted Dendra2::H2B (green) and photoconverted Dendra2::H2B (red). The bottom row shows photoconverted Dendra2::H2B alone. The arrowheads point to the edges of the photoconverted pool in the gonad. Scale bar=15 μm.

Figure 3. Analysis of cell movement and fold expansion of different pools of cells in the PZ (Panels a and b) Data for one gonad from each worm is displayed on the y-axis. The top bar represents the distribution of converted (orange) and unconverted (green) rows in the PZ immediately after photoconversion. The bottom bar represents the same gonad 8 hours later, where the orange sections mark cells retaining photoconverted Dendra2::H2B, and green sections mark cells without photoconverted Dendra2::H2B. The black vertical line marks the transition from PZ to meiotic prophase. The x-axis represents the row number within the gonad starting from the distal tip. (a) The converted regions were 5–7 rows in size, comprising about a third of each gonad as marked; fold expansion for each gonad was calculated as the ratio between the number of cells recovered containing converted Dendra2::H2B and the initial number of photoconverted cells. (b) The converted regions

were 2–4 rows in size, representing subregions in the distal and middle of the PZ. (c) The fold expansion was plotted for rows 1–4.5, 3–7.5 and 8–12.5 of worms shown in panel (b). Mean values are marked by a horizontal line, and are as follows: 2.7 ± 0.7 for rows 1–4.5, 2.7 ± 0.3 for rows 3–7.5, and 1.5 ± 0.03 for rows 8–12.5. * $p=0.02$, ** $p=0.01$, one-way ANOVA, Tukey's multiple comparisons test.

Figure 4. Analysis of progression along the PZ long axis and a model of germline PZ organization

(a) The x-axis represents the position of the proximal edge of each analyzed section at the time of photoconversion; the y-axis represents the rate of movement calculated from the difference in the proximal edge position of the analyzed section after 8 hours. (b) A proposed model for cell proliferation in the *C. elegans* adult PZ, where the distal pool up to rows 6–8 (~50–80 cells) is comprised of equivalent, proliferating stem cells, followed by exit from the stem cell niche. (c) Illustration of a stochastic system of stem cell maintenance, where exit from the stem cell pool occurs by displacement (each color represents a different lineage). Such system can give rise to clonal drift, where progeny of one cell (indicated in orange) can dominate the pool and others cells (indicated in blue) can be eliminated from the pool.