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Nuclear architecture as an intrinsic regulator of Drosophila female germline stem cell maintenance

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Homeostasis of Drosophila germline stem cells (GSC) depends upon the integration of intrinsic and extrinsic signals. This review highlights emerging data that support nuclear architecture as an intrinsic regulator of GSC maintenance and germ cell differentiation. Here, we focus on the nuclear lamina (NL) and the nucleolus, two compartments that undergo alterations in composition upon germ cell differentiation. Loss of NL or nucleolar components leads to GSC loss, resulting from activation of GSC quality control checkpoint pathways. We suggest that the NL and nucleolus integrate signals needed for the switch between GSC maintenance and germ cell differentiation, and propose regulation of nuclear actin pools as one mechanism that connects these compartments.

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Introduction

Germline stem cells (GSCs) are a unique adult stem cell population responsible for transmitting information across generations [1]. These stem cells divide asymmetrically to generate one daughter that retains its stem cell identity and a second daughter that differentiates into an egg or sperm. Maintaining the balance between GSC self-renewal and germ cell differentiation is critical for sustained gametogenesis, with failures leading to infertility.

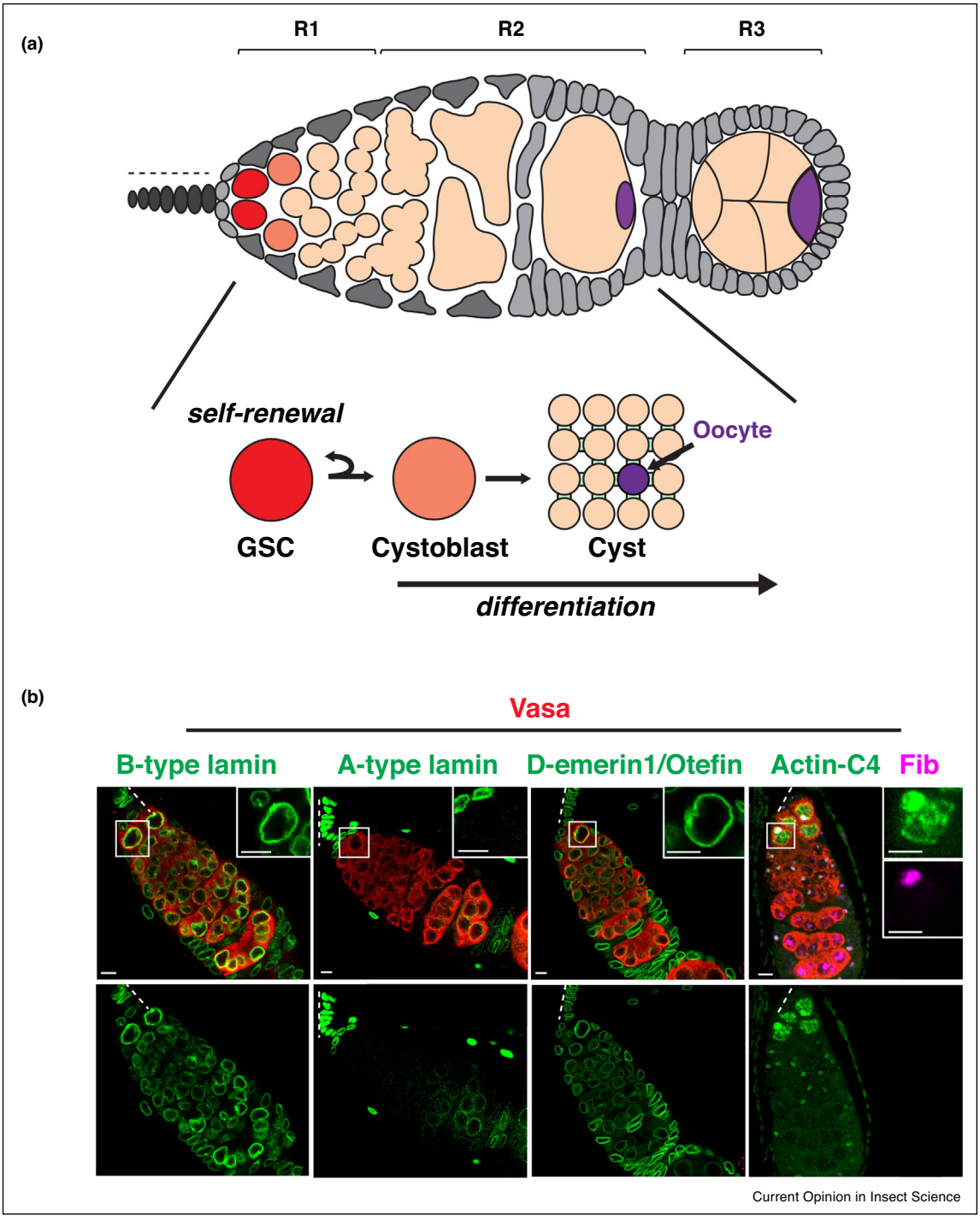
The Drosophila ovary is one of the pioneer systems that has advanced our understanding of GSC health and maintenance [2,3]. Oogenesis begins in a specialized structure called the germarium (Figure 1a). Within each germarium, somatic niche cells anchor two or three GSCs. Asymmetric GSC divisions produce one germ cell that self-renews and one cystoblast (CB) that commits to differentiation. CBs enter into four incomplete mitotic divisions to generate an interconnected 16-cell cyst. Once this cyst is formed, germ cells enter a sex-specific gametogenesis program, with one cell becoming the oocyte and the remaining cells becoming nurse cells that support the developing oocyte.

Extrinsic and intrinsic mechanisms regulate the decision between stem cell self-renewal and differentiation [4]. Extrinsic mechanisms include somatic niche signals that inhibit differentiation, such as BMP signaling that directs transcriptional repression of the key differentiation gene, *bag of marbles* (*bam*) in GSCs [2]. GSCs also produce signals important for maintenance of the niche [5••,6]. Intrinsic mechanisms are varied and center on the regulation of gene expression through effects on chromatin structure, transcription, RNA processing and translation [7,8••]. Here, we discuss the role of nuclear architecture as an intrinsic regulator of GSC maintenance and commitment to differentiation in Drosophila. Notably, distinct cell fates commonly accompany global differences in the three dimensional arrangement of genomes [9–11]. For this reason, we focus on two nuclear compartments critical to nuclear architecture, the nuclear lamina (NL) and nucleolus [9,12], as these compartments anchor and organize genomic domains to establish the spatial arrangement of chromosomes [13]. We summarize recent data that highlight how the NL and nucleolus contribute to germ cell homeostasis, with a focus on regulation in the Drosophila ovary.

The NL contributes to chromosome positioning in germ cells

The NL is an extensive protein network that lies beneath the inner nuclear envelope [14]. This dense meshwork is comprised of lamins, including B-type lamins that localize to the inner nuclear membrane through post-translational carboxy-terminal farnesylation and A-type lamins that localize to the inner nuclear membrane and nuclear interior. Lamins scaffold hundreds of interacting proteins [15,16], including proteins in the LEM-Domain (LEM-D) family. LEM-D proteins share an ability to interact with Barrier-to-autointegration Factor (BAF), a chromatin and DNA binding protein [17–19]. LEM-D proteins have a prominent role in nuclear architecture, acting as

Figure 1



Nuclear architectural components are dynamic in the germarium.
(a) Schematic representation of the structure of a germarium. Germ cells include germline stem cells (GSCs, red), cystoblasts (CBs, orange), and differentiating germ cells (peach) including the future oocyte (purple). Somatic cells include cells of the niche (dashed line) and elsewhere in the germarium (gray). Each germarium is divided into three regions (R1–R3). In R1, GSC divisions produce a self-renewed daughter and a CB. The CB undergoes four additional mitotic divisions to produce a 16-cell cyst. In R2, 16-cell cysts differentiate, including identification of the oocyte (purple). In R3, somatic cells surround the 16-cell cyst to form an egg chamber that forms the unit of oocyte differentiation during oogenesis. **(b)** Confocal images of germaria stained for Vasa (red), nuclear architectural components (green) and Fibrillarin (magenta), which marks the nucleolus. Inserts are magnifications of boxed regions. Both the B-type lamin (Lamin Dm0) and D-emerin/Otefin are enriched in GSCs and become downregulated as germ cells differentiate. The A-type lamin (Lamin C) is absent in GSCs and is upregulated in 16-cell cysts. The Actin-C4 antibody recognizes polymeric actin at the nuclear periphery and monomeric actin in the nucleolus of GSCs and CBs [54••]. Nucleolar C4 actin

bridging proteins that tether genomic regions to the NL. The NL establishes nuclear structures that are important for stem cell function and differentiation [9,20–23], as illustrated by the age-dependent progression of human diseases associated with NL dysfunction [5••,24].

The composition of the NL is cell-type specific and changes during development [16,25,26]. Commonly, little or low levels of the A-type lamin are found in stem cells, with levels of this lamin increasing upon differentiation [23,27,28]. Such changes in NL composition contribute to cell-type specific nuclear mechanics that are important for transcription, replication and genome stability [19,29,30]. Similarly, the NL composition changes during development of Drosophila female and male germ cells. GSCs express only the B-type lamin (Lamin Dm0). This lamin declines during germ cell differentiation, as expression of the A-type lamin (Lamin C) begins [Figures 1, 2; [31]]. These changes accompany adjustments in lamin-interacting proteins, exemplified by the Drosophila orthologue of the LEM-D protein emerin (D-emerin/Otefin). GSCs express high levels of D-emerin/Otefin, which decline during differentiation (Figures 1b, 2 a). Notably, D-emerin/Otefin is essential for GSC survival [32,33], indicating that GSCs might use a changing composition of the NL to regulate nuclear events required for germ cell development.

Chromosome positioning and pairing in Drosophila GSCs depend upon proteins in the NL. Notably, chromosome organization in GSCs differs from other Drosophila cell types. GSCs are the only adult cells that carry unpaired chromosomes [34,35], an unexpected arrangement considering that progeny of these stem cells are destined to enter meiosis. In GSCs, unpaired chromosomes align with the nuclear periphery along their length [34]. D-emerin/Otefin contributes to the separation of these chromosomes, evidenced by heterochromatin coalescence in *d-emerin/otefin* mutant GSCs [36••]. Further, the NL SUN and KASH domain proteins, Klaroid and Klarsicht, respectively, play a central role in reorganization of nuclear architecture that is needed for meiosis [37]. These proteins bridge the nuclear interior and the cytoskeleton, structurally supporting the microtubule-driven nuclear rotation that promotes homologue pairing [37]. Together, these observations highlight that NL proteins have many roles in chromosome organization in early germ cells.

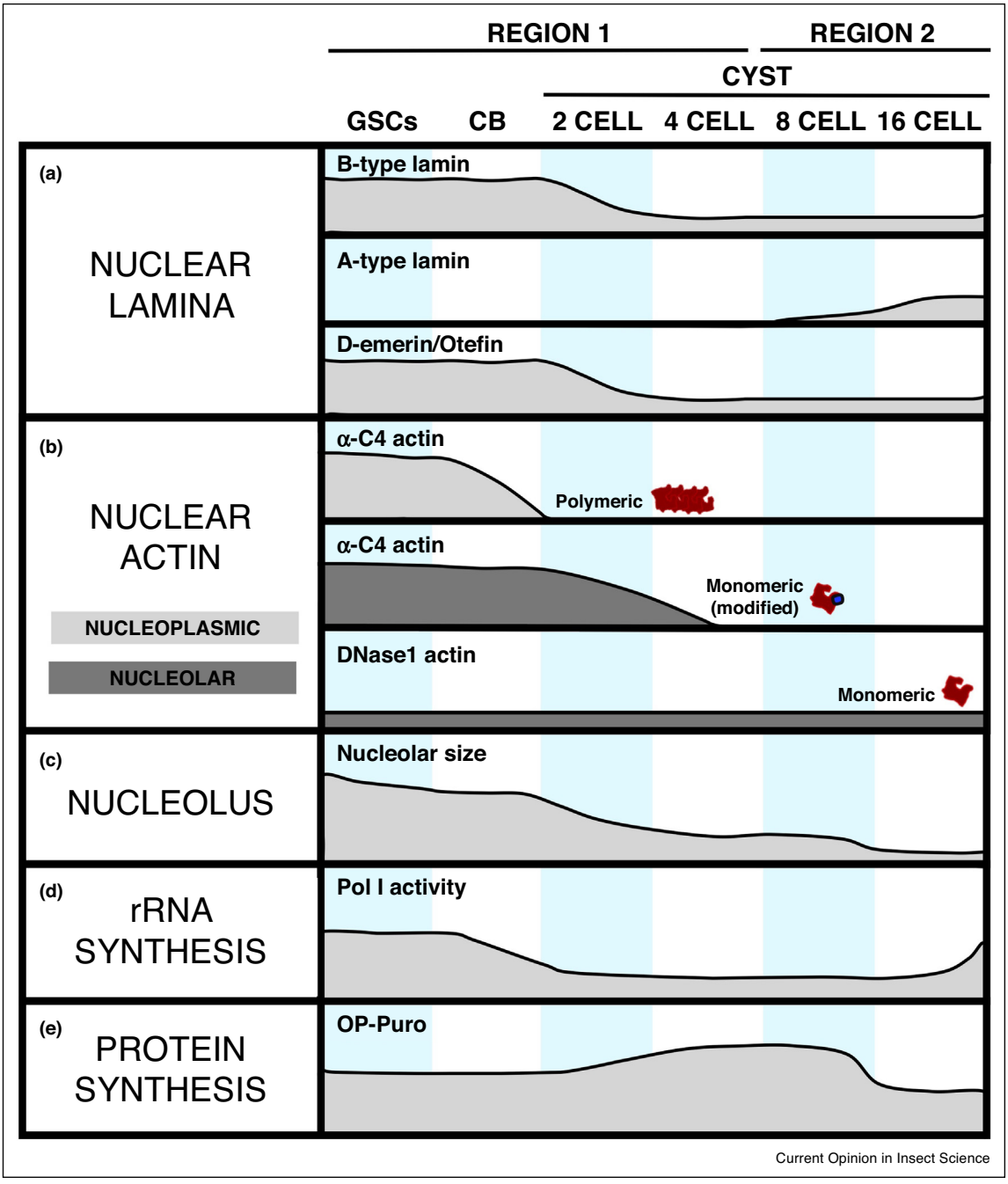
Contributions of the NL to transcriptional repression in germ cells

GSC divisions produce one self-renewing and one differentiating daughter. Differentiating CBs shift their transcriptional program to promote adoption of a developmental fate. Notably, these changes are accompanied with movement of chromosomes away from the NL (Figure 3; [34]), a nuclear compartment that is associated with transcriptional repression and depletion of active histone marks, such as acetylation [38,39]. Low levels of acetylation of chromatin at the NL are maintained in part by sequestration of histone deacetylases by NL proteins [40,41]. Indeed, the LEM-D protein emerin interacts with histone deacetylase HDAC3, with this interaction stimulating deacetylase activity [40]. Strikingly, Drosophila GSC maintenance requires low levels of acetylation. For example, GSC numbers decline in *scrawny* mutant females and males, because loss of this histone H2B ubiquitin protease increases H3K4me3 and H3 acetylation [42]. Additionally, the loss of H1 causes premature differentiation of germ cells. H1 loss is coupled with elevation of H4K16 acetylation due to the absence of H1-dependent antagonism of the histone acetyltransferase Males Absent First (MOF) [43]. In both cases, increased acetylation upregulates transcription of differentiation genes, such as *bam*, emphasizing the importance of maintaining levels of acetylation. Furthermore, the intrinsic requirement for H1 is intriguing, as H1 is a component of a distinct type of repressive chromatin called BLACK chromatin [44]. This chromatin type is also enriched for the B-type lamin and the satellite binding protein D1, but devoid of classic heterochromatin marks of H3K9me3 and H3K27me3 [44]. These observations suggest that GSCs capitalize upon the close proximity of chromosomes to the NL for gene silencing, conferring repression that is independent of additional chromatin marks. This type of chromatin state might facilitate adoption of the distinct transcriptional program needed for CB differentiation.

NL association represents only one mechanism of transcriptional repression in GSCs. Canonical H3K9me3 and H3K27me3 repression pathways are also used, but these pathways display unique features. For example, the histone methyltransferase SETDB1 (Eggless) controls local accumulation of H3K9me3 over testis-specific genes, depositing a restricted mark that does not spread into neighboring loci [45••]. Formation of such localized heterochromatin is essential for maintenance of the female cell fate [45••]. Although untested, NL association might also contribute to repression of testis-specific genes, as many testis genes are organized in NL-associated gene clusters that translocate away from the NL upon transcriptional activation in the testis [46]. In a second example, the H3K27me3 writer, Polycomb Repressive Complex 2 (PRC2), is sequestered in the nucleoplasm by Piwi, causing decreased H3K27me3 deposition [47•]. This

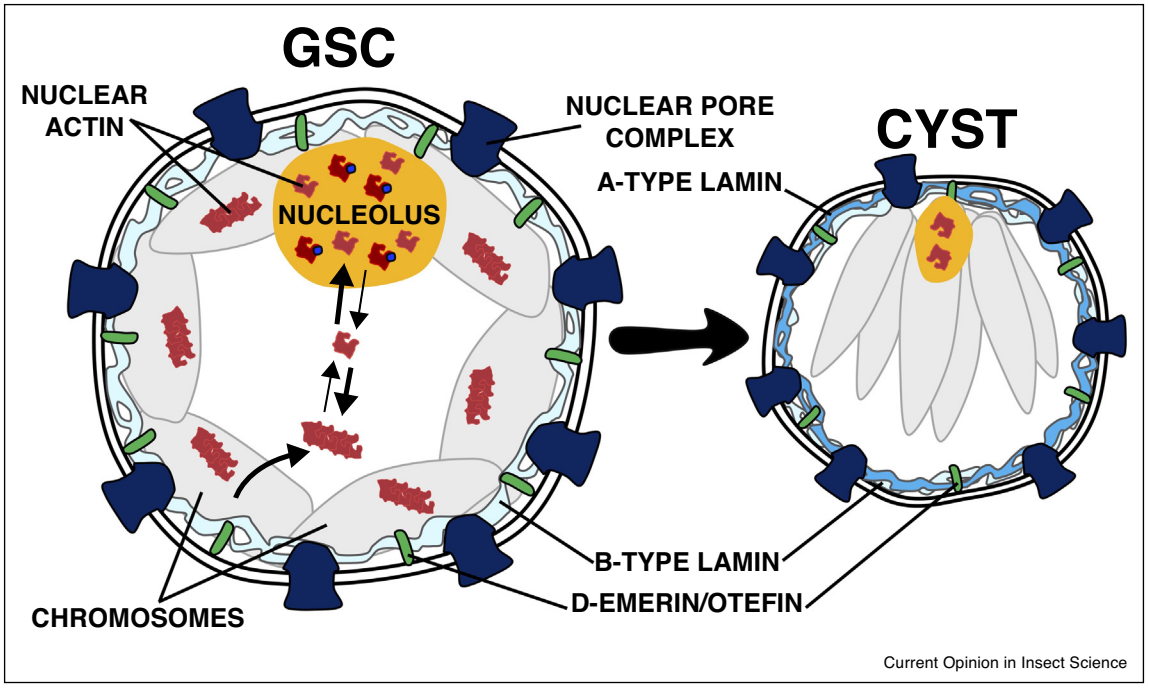
(Figure 1 Legend Continued) decreases as germ cells differentiate. Anterior is top left, with the position of the GSC niche shown as a dashed white line. Scale bars represent 5 μm.

Figure 2



Compositional and functional changes in nuclear compartments in the germarium.
(a–e) Schematic of the trends of protein levels and activity of molecular processes indicated across regions 1 (R1) and 2 (R2) of the germarium. Trend lines are relative and should not be compared across categories. The 16-cell stage refers to the lens stage and does not represent changes that occur in region 3 (R3), 16-cell cysts. (a) NL composition changes across the germarium. The B-type lamin (Lamin Dm0) and D-emerin/Otefin are high in the GSCs and decrease to low levels by 4-cell cysts. The A-type lamin (Lamin C) is undetectable until the 8-cell cyst. See Figure 1b for confocal images of NL components. (b) Multiple nuclear actin pools are found in the nucleoplasm (gray) and nucleolus (dark gray) across the germarium. The Actin C4 antibody (a-C4 actin) recognizes a polymeric nucleoplasmic pool of actin that is specific to GSCs and CB [54••]. A second pool of C4-positive monomeric actin persists in the nucleoli from GSCs through the 4-cell cyst. In contrast, DNaseI detects a stable monomeric pool in nucleoli throughout all of oogenesis. (c) GSCs have the largest nucleoli, with nucleolar size decreasing steadily throughout R1 and R2 as germ cells differentiate [8••]. (d) rRNA synthesis is high in GSCs and CBs and plateaus as cysts differentiate [69]. (e) Global protein synthesis ramps up in 2 to 8-cell cysts and decreases as 16-cell cysts move into R3 [8••].

Figure 3



Model summarizing nuclear architecture as an intrinsic regulator of GSC maintenance and germ cell differentiation.
A schematic model for how nuclear architecture influences the transcriptional state of euchromatic and rDNA genes required for GSC maintenance and germ cell differentiation. In GSCs (left), the nuclear lamina (NL; B-type lamin, light blue and A-type lamin, dark blue), LEM-domain proteins such as D-emerin/Otefin (green), and nuclear pores (navy) maintain nuclear integrity and tether chromatin (light gray) to the periphery. Autosomes (gray) are unpaired in GSCs, whereas *X* chromosomes are paired at the nucleolus (yellow). Proximity to the NL contributes to transcriptional repression. We propose that activities of the NL and nucleolus are integrated through regulation of nuclear actin (red) dynamics. In GSCs, nuclear actin is found in three populations, one at the NL (a C4-positive, polymeric pool) and two in the nucleolus (a DNaseI-positive [red monomer] pool and a C4-positive [red monomer with blue modifier] pool). These pools dynamically exchange between compartments. Components of the NL, including D-emerin/Otefin and lamins, can facilitate the polymerization of actin (black arrow) to regulate actin structures in each compartment (weighted arrows). Nuclear actin, in turn, can influence transcription in both compartments. Upon germ cell differentiation, the NL composition changes (B-type lamin and D-emerin/Otefin decline and A-type lamin increases), and the nuclear and nucleolar size decrease (right: 8-cell cyst nucleus). These latter changes correlate with decreased rRNA synthesis and increased protein synthesis. Notably, in Regions 1 and 2 of the germarium, differentiating cysts contain only the DNase I monomeric actin pool. This shift towards monomeric actin is likely a consequence of decreased NL components which facilitate actin polymerization, such as D-emerin/Otefin and the B-type lamin. Further, chromosomes adopt a Rabl conformation, positioning domains away from the NL. We propose that these changes in nuclear architecture are required for germ cells to differentiate. Differences in overall nuclear size are to scale, however sizes of individual proteins and complexes are not to scale.

Piwi-dependent PRC2 sequestration appears to be germ cell-specific, as somatic over expression of Piwi has no discernable defects. Finally, transient transcriptional silencing occurs during the GSC to CB transition [48•], due to brief expression of Polar Granule Component (Pgc), a small peptide inhibitor of RNA Polymerase II [49]. This mechanism enhances progression from a stem cell to a differentiated state within one cell division. Taken together, these data indicate that transcriptional repression mechanisms are tailored to the needs of GSCs.

The NL might make indirect contributions to transcriptional regulation through an impact on nuclear actin pools. Nuclear actin regulates transcription in multiple ways, including acting as a component of chromatin remodeling complexes and all three RNA polymerases [50,51•]. Recently, lamin has been identified as a candidate regulator of nuclear actin polymerization [52] and the LEM-D protein emerin, is an actin capping protein [53]. Strikingly, a polymeric actin pool, recognized by the C4 actin antibody, localizes to the nuclear periphery of GSCs and CBs, but is absent in differentiating cysts (Figures 1b, 2 b; [54••]), mirroring the downregulation of the B-type lamin and D-emerin/Otefin (Figures 1b, 2 a). Taken together, these observations suggest that changes in NL components might have the capacity to alter nuclear actin pools and transcriptional output during germ cell development.

Loss of NL integrity triggers a GSC quality control checkpoint

D-emerin/Otefin is a NL component that is required for survival of female and male GSCs [5••,55]. Without this LEM-D protein, GSCs fail to differentiate and are lost.

Strikingly, GSC survival and germ cell differentiation are rescued by inactivation of the DNA damage response (DDR) kinases, ATR and Chk2. Even though this germline checkpoint uses components of the DDR pathway, genetic and cytological data failed to support a role for DNA damage as a checkpoint trigger [56,57]. Instead, checkpoint activation is linked to structural deformation of the NL. Multiple mechanisms might connect nuclear architecture changes to ATR/Chk2 activation. These include changes in genomic contacts needed for appropriate transcriptional regulation, with resulting gene expression changes prompting activation of the checkpoint. Alternatively, disruptions in the NL might affect trafficking of products between the nucleus and cytoplasm, altering pools of key factors, such as nuclear actin. Finally, structural alteration in the NL might itself trigger ATR/Chk2 activation [58]. Indeed, emerging evidence implicates ATR as a general sensor of the structural integrity of cellular components [59].

Progression from stem cell to differentiation requires nucleolar functions

The nucleolus is an RNA and protein dense non-membrane bound organelle whose formation depends upon transcription of the 35S ribosomal DNA (rDNA) genes [60]. In Drosophila, ~600 rDNA genes are organized into repeat arrays found on the *X* and *Y* chromosomes [61]. Only some rDNA genes are actively transcribed, with the rest imbedded in heterochromatin that encases the nucleolus [60,62,63]. Reduction of heterochromatin disperses the rDNA and causes nucleolar fragmentation [64]. Strikingly, deletion of rDNA genes compromises heterochromatin-induced gene silencing elsewhere in the genome [65]. These data suggest that the nucleolar function is likely integrated into global chromatin regulation of GSCs.

GSCs have a large nucleolus that decreases in size as germ cells differentiate (Figure 2c; [8••,66]). The size of the nucleolus reflects the rate of rDNA transcription [8••,67,68]. Indeed, regulated levels of rDNA transcription in GSCs are critical for stem cell maintenance and germ cell differentiation (Figure 2d). Ribosomal DNA genes are transcribed by RNA Polymerase I (RNAPI). Transcriptional activation of these genes depends upon Under developed (Udd), a subunit of the Drosophila general RNAPI regulatory complex that is analogous to human Selectivity Factor 1 [69]. Udd is enriched in GSC nucleoli. GSC divisions generate CBs that inherit lower levels of Udd, causing downregulation of rDNA transcription and germ cell differentiation. Notably, loss of Udd is associated with small ovaries due to the failure to maintain GSCs, whereas Udd over expression causes accumulation of undifferentiated germ cells [69]. Similarly, the RNAPI transcriptional regulator Facilitates Chromatin Transcription (FACT) is required for GSC maintenance [67], demonstrated by GSC loss upon RNAi knockdown of the FACT subunit SPT16. Nuclear actin might also regulate rDNA transcription, based on findings that nuclear actin is required for RNAPI activity [70,71,72•] and is a component of multiple chromatin remodeling complexes, including one involved in rDNA regulation [72•,73]. In the Drosophila germline, the nucleolus contains two pools of monomeric nuclear actin (Figures 1b, 2b), including a constant pool recognized by DNase I and a dynamic pool recognized by the C4 actin antibody [54••]. Notably,C4 nuclear actin is progressively lost during germ cell differentiation, consistent with the downregulation of rDNA transcription (Figure 2b, d). These data imply that the C4 actin pool might be involved in GSC maintenance. Maturation of rRNA also appears to impact nucleolar function in GSCs, evidenced by the requirement for the pre-rRNA processing U3 snoRNP, Wicked, in GSC maintenance [74]. Notably, Wicked is asymmetrically inherited by CBs. Further, loss of Wicked results in premature differentiation and GSC depletion. Taken together, these data indicate that multiple pathways are utilized to establish balanced rRNA levels in GSCs.

Despite having large nucleoli, protein synthesis is low in GSCs (Figure 2e; [8••,75]). Instead, high levels of ribosome biogenesis and protein synthesis occur upon germ cell differentiation. These observations indicate that GSCs and differentiating germ cells have different ribosome needs. Indeed, GSCs express distinct ribosomal proteins and regulators of ribosomal biogenesis [76]. Several lines of evidence suggest that blocking increased protein synthesis prevents differentiation. For example, downregulation of protein synthesis by reducing signaling of the nutrient responsive Target of Rapamycin (Tor) pathway causes accumulation of GSCs [8••]. Additionally, knockdown of ribosomal assembly factors affects stem cell cytokinesis, resulting in the formation of stem cysts [8••], structures comprised of GSC-like cells that fail to complete abscission and remain connected. Formation of stem cysts also results upon loss of the histone chaperone, Chromatin Assembly Factor 1 (CAF1), which causes nucleolar fragmentation and GSC loss through activation of Chk2 [77•]. These findings correlate nucleolar structure and regulation of protein synthesis with the transition from stem cell self-renewal to differentiation.

Disruption of the nucleolus might trigger a GSC quality control checkpoint

The nucleolus is multi-functional. In addition to rDNA transcription and ribosome biogenesis [78,79], the nucleolus contributes to cell cycle progression, proliferation, and cell death [78–80]. These processes are integrated through the nucleolar stress response pathway [NSP; [78,81]]. Activation of NSP decreases rDNA transcription, ribosome biogenesis, and protein production, which inhibits proliferation and potentially causes cell death. Similar phenotypes are observed when GSC nucleolar activity is disrupted [8••,66,67,69,74]. These observations

suggest that GSCs might use the NSP as a means of quality control. One factor that might contribute to a GSC nucleolar checkpoint is nuclear actin. Nuclear actin polymerizes into thick, filamentous rods in response to stresses that induce NSP [50]. As such, alteration of nuclear actin levels or structure might disrupt GSC nucleolar function and activate NSP. Strikingly, the downstream effectors of the NSP are ATR and Chk2 [82,83], components of the NL quality control checkpoint [36••]. Further studies are needed to identify whether and how nucleolar dysfunction triggers a GSC-specific checkpoint.

Conclusions and future perspectives

GSCs demonstrate specialized structures of two nuclear compartments, the NL and the nucleolus (Figures 1, 2). Disruption of these compartments activates GSC quality control checkpoints that lead to germ cell loss [5••,8••,36••,55,66,67,69,74]. We present evidence that these compartments integrate signals needed for the switch between GSC maintenance and germ cell differentiation, proposing a model that highlights connections between transcription and nuclear actin (Figure 3). In GSCs, chromatin is held in a reversible transcriptional state by the NL and nucleolus. Interestingly, both compartments are enriched for nuclear actin, with polymeric actin at the NL and monomeric actin within the nucleolus [54••]. Strikingly, NL proteins both increase nuclear actin levels and promote its polymerization [52,53]. Further, the nucleolus appears to sequester actin monomers to influence the balance between monomeric and polymeric states [78]. By controlling nuclear actin dynamics, the NL and nucleolus might facilitate the switch between GSC self-renewal and germ cell differentiation. We speculate that disruption of either compartment drives formation of stress-induced nuclear actin rods that activate GSC quality control checkpoints involving ATR and Chk2 [36••,82,83]. Further studies are needed to fully define the impact of nuclear actin on the roles of the GSC NL and nucleolus. Whereas we focus our discussion on female GSCs, the NL and nucleolar compartments in male germ cells also have critical roles in self-renewal and differentiation [5••,28,69,84].

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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