Chapter 1
Role of Chromatin Modifications in *Drosophila* Germline Stem Cell Differentiation

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Abstract During *Drosophila* oogenesis, germline stem cells (GSCs) self-renew and differentiate to give rise to a mature egg. Self-renewal and differentiation of GSCs are regulated by both intrinsic mechanisms such as regulation of gene expression in the germ line and extrinsic signaling pathways from the surrounding somatic niche. Epigenetic mechanisms, including histone-modifying proteins, nucleosome remodeling complexes, and histone variants, play a critical role in regulating intrinsic gene expression and extrinsic signaling cues from the somatic niche. In the GSCs, intrinsic epigenetic modifiers are required to maintain a stem cell fate by promoting expression of self-renewal factors and repressing the differentiation program. Subsequently, in the GSC daughters, epigenetic regulators activate the differentiation program to promote GSC differentiation. During differentiation, the GSC daughter undergoes meiosis to give rise to the developing egg, containing a compacted chromatin architecture called the karyosome. Epigenetic modifiers control the attachment of chromosomes to the nuclear lamina to aid in meiotic recombination and the release from the lamina for karyosome formation. The germ line is in close contact with the soma for the entirety of this developmental process. This proximity facilitates signaling from the somatic niche to the developing germ line. Epigenetic modifiers play a critical role in the somatic niche, modulating signaling pathways in order to coordinate the transition of GSC to an egg. Together, intrinsic and extrinsic epigenetic mechanisms modulate this exquisitely balanced program.

Epigenetics is the study of how environmental factors influence gene expression. Historically, the term epigenetics has been used to describe events that could not be explained by classic genetics. C.H. Waddington's metaphor illustrates that during differentiation, a cell encounters a variable landscape that determines its fate (Goldberg et al. 2007). This definition of epigenetics has evolved to encompass

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both mitotically and meiotically heritable changes in gene expression, which are not consequences of changes in DNA sequence. The epigenome is the combination of chemical modifications in a cell that modulate gene expression. These chemical modifications include DNA methylation, histone posttranslational modifications, and histone variants, which can play pivotal roles in biological processes such as cellular reprogramming and DNA replication (Boros 2012).

The phenotypic effects of epigenetic modifications were first discovered in *Drosophila melanogaster*, paving the way for further studies using *Drosophila* as a model system (Muller 1930). One well-studied aspect of epigenetics is histone modifications (Yasuhara and Wakimoto 2008; Riddle et al. 2011). Histone modifications are deposited during early development, ensuring accurate cell fate determination of the next generation (Horard and Loppin 2015). As male *Drosophila* mostly lack histones, the female *Drosophila* germ line is the prime model for epigenetic studies (Rathke et al. 2014). *Drosophila* oogenesis consists of temporal stages that can be easily visualized and tracked (Fig. 1.1a) (Bastock and St Johnston 2008). During *Drosophila* egg production, there are several interactions between the soma and germ line that are essential for proper development, which is similar to mammalian

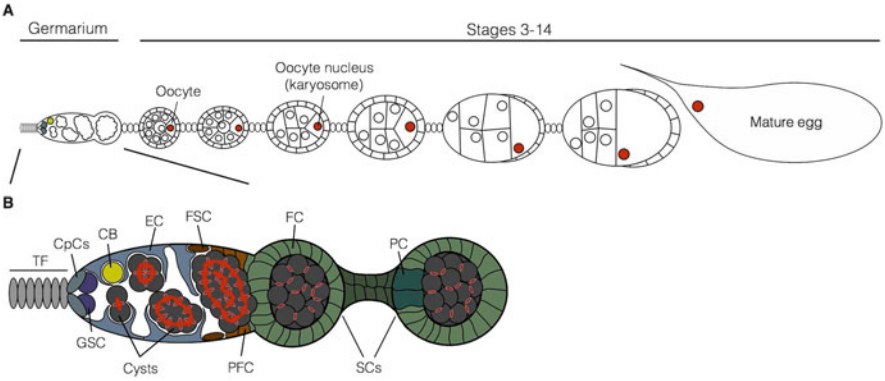

Fig. 1.1 (**a**) Schematic of *Drosophila* oogenesis. *Drosophila* females have two ovaries that consist of 16–20 ovarioles (assembly lines for mature eggs). Oogenesis can be split into 14 developmentally distinct stages, initiating with the division of GSCs that reside at the anterior region of the ovary in a structure, called the germarium. The GSC daughter then undergoes four incomplete rounds of mitosis, eventually giving rise to a 16-cell cyst. One of the 16 cells will become the egg, while the other 15 cells of the cyst will become polyploid nurse cells, which support the growth of the egg. The syncytium of nurse cell and oocyte is surrounded by a single layer of somatic follicle cells, constituting an egg chamber. The oocyte nucleus, also called the karyosome, assumes a characteristic structure in stage 3 of oogenesis. As development progresses, the nurse cells deposit mRNAs and proteins into the growing oocyte. This oocyte eventually becomes a mature egg (stage 14). (**b**) Inset germarium showing the somatic cell types that surround the germ line. TF and CpCs present at the anterior end of the germarium are intimately connected to the GSCs. Upon differentiation, the differentiating CB and 2- to 16-cell cysts make extensive contact with ECs. The 16-cell cyst then comes into contact with two FSCs. FSCs are progenitors of PFCs, FCs, SCs, and PCs. Of these, PFCs and FCs surround the developing egg chamber, SCs connect these separate egg chambers, and PCs present at both ends of the egg chambers specify polarity of the follicle epithelium

oogenesis (Gilboa and Lehmann 2006; Albertini and Bromfield 2010). Therefore, epigenetic studies in *Drosophila* can be extrapolated to higher organisms.

Drosophila oogenesis begins in the anterior-most part of the ovary, in a structure called the germarium (Fig. 1.1b) (Kirilly and Xie 2007; Spradling et al. 2011). The germarium consists of germ line stem cells (GSCs) and a specialized microenvironment called the somatic niche, which supports the GSCs. The somatic niche includes the terminal filament (TF), cap cells (CpCs), and escort cells (ECs) (Spradling et al. 2001, 2008; Lin 2002; Kirilly and Xie 2007). GSCs are attached to the niche via the CpCs and receive self-renewing signals from both the TF and CpCs. GSCs divide asymmetrically to both self-renew and give rise to a differentiated progeny, called the cystoblast (CB) (Chen 2003). After division, the CB comes into close proximity to ECs, which provide differentiation signals (Decotto and Spradling 2005). The CB expresses differentiation factor bag of marbles (Bam), which is necessary and sufficient for differentiation (McKearin and Spradling 1990; McKearin and Ohlstein 1995). Upon Bam expression, the CB undergoes four incomplete mitotic divisions to create a 16-cell cyst. One of the 16 cells becomes the oocyte, while the others become nurse cells that nourish the developing oocyte (Xie and Spradling 2000; Gilboa and Lehmann 2004) (Fig. 1.2).

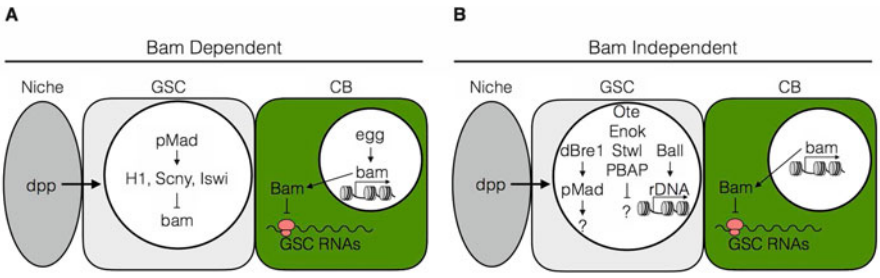

Fig. 1.2 Schematic of mode of function of epigenetic regulators in the GSC and CB during *Drosophila* oogenesis. The somatic niche provides Dpp signaling to the GSC which promotes pMad expression required for GSC self-renewal. pMad expression has shown to downregulate bam transcription in order to suppress a differentiation program. Upon GSC division, the CB turns on transcription of Bam which translationally suppresses GSC maintaining RNAs. However, several epigenetic factors can help mediate GSC self-renewal and differentiation in a Bam-dependent (A) or a Bam-independent (B) pathway. (A) Epigenetic factors H1, Scny, and Iswi act downstream of the incoming Dpp signaling to repress transcription of Bam, ensuring stem cell fate maintenance. On the other hand, dSETDB1/Egg expression in the CB is required to promote transcription of Bam to initiate a differentiation program. (B) dBRE1 expression in the GSC is required for proper pMad levels, although its downstream effector is not Bam. Ote, Enok, Stwl, and PBAP also repress a differentiation program by targeting expression of differentiating factors other than Bam. Ball has been implicated in promoting ribosomal biogenesis, which is crucial for GSC self-renewal and maintenance

1.1 GSC Self-renewal Is Associated with Epigenetic Silencing of Differentiation Program

GSC self-renewal and differentiation are controlled by several intrinsic and extrinsic regulatory factors. Extrinsic factors include extracellular molecules that trigger signal transduction pathways such as Decapentaplegic (Dpp) signaling, which promotes GSC self-renewal. Dpp signaling is initiated by the cytokine-like ligands, Unpaired (Upd) and Unpaired-2 (Upd-2), produced by the TF. Upd binds to Janus Kinase (JAK) surface receptors present on the CpCs (Lopez-Onieva et al. 2008; Wang et al. 2008), leading to the phosphorylation and activation of signal transducer and activator of transcription 92e (Stat92e). This activated Stat92e triggers the production of signaling molecules by the CpCs called Decapentaplegic (Dpp) or Glass bottom boat (Gbb), which are similar to Bone morphogenetic proteins (BMPs) in mammals (Xie and Spradling 1998; Kawase et al. 2004; Wang et al. 2008). Dpp binds to the Type I receptors Thick veins (Tkv) and Saxophone (Sax) on GSCs, leading to the phosphorylation of mothers against Dpp (pMad), a *Drosophila* Smad3 protein. pMad then complexes with Medea (Med), a *Drosophila* Smad4 protein, to transcriptionally silence the differentiation factor Bam (Brummel et al. 1994; Nguyen et al. 1998; Song 2004). The somatic niche also expresses Division abnormally delayed (Dally), a glypican protein which is responsible for distributing and stabilizing Dpp signaling (Guo and Wang 2009; Hayashi et al. 2009). However, Dpp signaling in the GSCs also results in a negative-feedback circuit that induces the expression of Daughters against Dpp (Dad), which dampens Dpp activity (Tsuneizumi et al. 1997). The interplay between Dpp signaling and the GSC's response to this incoming signaling controls self-renewal and differentiation.

Differentiation is initiated after GSC division, when the CB is displaced from the somatic niche. GSC differentiation requires downregulation of Dpp signaling in the CB through the turnover of TKV receptors by an E3 ubiquitin ligase, Smurf, to allow for *bam* transcription (Xia et al. 2010). Bam is a translational suppressor that downregulates GSC-maintaining RNAs, thereby initiating a differentiation program (Li et al. 2009b). The ECs express Epidermal Growth Factor (EGF) that transcriptionally represses *dally*, restricting the range of Dpp signaling to promote an efficient CB differentiation program (Schulz et al. 2002). Although, signaling pathways play an indispensable role in providing cues for maintaining GSCs and differentiation, epigenetic changes in the germ line and the surrounding somatic niche are also critical.

Gene expression is controlled by both epigenetic state and nuclear organization. The epigenome is a catalogue of chemical modifications that affect gene expression without directly altering the encoded genetic material (Goldberg et al. 2007). Several epigenetic modifications can occur directly on DNA or its associated proteins that constitute the chromatin (Cedar and Bergman 2009). The basic unit of chromatin is the nucleosome, consisting of five histones H2A, H2B, H3, H4, and a linker histone H1. The nucleosome octamer core contains one tetramer of H3 and

H4 and two heterodimers of H2A and H2B (Luger et al. 1997). Approximately, 150 base pairs of DNA wrap around each octomer, providing an epigenetic landscape that can be manipulated via different histone modifications (Jenuwein and Allis 2001). Each histone protein has a tail that can be modified to alter the local chromatin structure and regulate gene expression (Luger et al. 1997). Several modifications can be deposited onto histone tails such as methylation, acetylation, ribosylation, ubiquitination, and phosphorylation (Kouzarides 2007). These modifications together result in a specific readout of either gene activation or repression. For example, the trimethylation of lysine 9 and 27 residue of H3 (H3K9me3, H3K27me3) can result in gene repression, while trimethylation of lysine 4 and acetylation of lysine 9 of H3 (H3K4me3, H3K9ac) can result in gene activation (Bannister and Kouzarides 2011). H1 and its variants are essential for compaction of nucleosomes for chromatin remodeling (Bednar et al. 1998; Misteli et al. 2000; Bustin et al. 2005). Loss of H1 not only alters chromatin compaction but can also affect several histone modifications such as H3 lysine 4 (H3K4), lysine 9 (H3K9), and lysine 27 (H3K27), demonstrating its vital role in maintaining the epigenetic landscape for modulating gene expression (Zhang et al. 2012). Therefore, the combination of nuclear organization and chromatin modification is crucial for proper gene regulation. Direct DNA methylation can also contribute to the regulation of gene expression, but meaningful DNA methylation is not present in *Drosophila* (Raddatz et al. 2013), so it will not be addressed in this chapter.

1.1.1 Histones and Histone-Modifying Enzymes in the Germ Line that Regulate GSC Self-renewal and Differentiation

GSCs have a unique epigenetic state that is constantly altered to ensure proper differentiation (Buszczak and Spradling 2006; Li and Zhao 2008). H1 linker histone is intrinsically required for GSC maintenance, as depletion of H1 in the germ line results in loss of GSCs over time. Depletion of H1 in the GSC, however, does not alter response to Dpp signaling, as Mad is normally phosphorylated. However, in spite of normal pMad levels, Bam is prematurely expressed. Depletion of H1 also increases H4 lysine 16 acetylation (H4K16ac) in the GSCs, a mark associated with active chromatin. Additionally, overexpression of a specific H4K16 acetyl transferase, *male absent on the first* (*mof*), leads to GSC loss and premature expression of Bam (Sun et al. 2015). This suggests that H1 is responsible for silencing differentiation genes, while Mof activates differentiation genes to ensure remodeling of the chromatin to an active state for efficient GSC differentiation (Akhtar and Becker 2000). These studies indicate that histones modulate accessibility of differentiation genes via nucleosome compaction in the GSCs.

In addition to nucleosome compaction, GSC fate is also maintained at the chromatin level by histone modifications. H2B monoubiquitination (Ub-H2B)

plays an important role during chromatin regulation and is required for H3K4 methylation, which is an indicator of gene activation (Dover 2002; Sun and Allis 2002). In *Drosophila*, the gene *scrawny* (*scny*) encodes an H2B ubiquitin protease that deubiquitinates H2B to suppress methylation at lysine residues, resulting in gene repression (Gardner et al. 2005). GSCs express Scny at high levels, and loss of *scny* results in increased acetylation of H3 tails and early expression of Bam, causing a loss of GSCs (Buszczak et al. 2009). On the other hand, it has been shown that *dBre1* encodes an ubiquitin ligase required for the monoubiquitination of H2B and is expressed throughout the germarium (Bray et al. 2005). Loss of *dBre1* in the GSC results in GSCs with low levels of H3K4 trimethylation (H3K4me3). Although, GSCs lacking *dBre1* display low levels of pMad and Dad expression, *bam* transcription remains unchanged. This suggests that dBre1 modulates GSC maintenance by targeting differentiation factors other than Bam (Xuan et al. 2013). Collectively, these two studies have shown that an ubiquitin protease and an ubiquitin ligase maintain H3K4me3 levels in the GSCs to ensure the proper balance of gene expression.

A histone methyltransferase, *dSETDB1* or *eggless* (*egg*), has also been shown to regulate GSC fate during *Drosophila* oogenesis. dSETDB1 deposits the repressive H3K9me3 mark, which recruits heterochromatin protein 1 (Hp1) to form heterochromatin (Schotta 2002; Ebert 2004). H3K9me3 is present at low levels in the GSC, but increases in the CB once division is complete. This is concurrent with the finding that dSETDB1 in the GSCs is mostly cytoplasmic, but becomes increasingly nuclear after division (Rangan et al. 2011; Clough et al. 2014). Generation of *dSETDB1*-deficient GSCs leads to loss of self-renewal (Wang et al. 2011). And although the *dSETDB1*-deficient GSCs differentiate properly, they arrest mid-oogenesis. The targets of dSETDB1-mediated H3K9me3 marks were identified as Piwi-interacting small RNA (piRNA) clusters (Rangan et al. 2011). piRNA clusters produce small RNAs (piRNAs) that target transposable elements (TEs) for degradation (Gunawardane et al. 2007; Brennecke et al. 2007; Li et al. 2009a; Malone et al. 2009). Loss of *egg* in the germ line results in the loss of piRNAs and a failure to degrade TEs, leading to double-strand breaks (DSBs) in the genome (Rangan et al. 2011). A critical step prior to heterochromatin formation is the removal of methylations on H3K4 by *Lysine-specific demethylase 1* (*dLsd1*) (Rudolph et al. 2007; Di Stefano et al. 2007). Intriguingly, loss of *dLsd1* in the GSCs does not affect their maintenance (Eliazer et al. 2011). Germ line clones of *dLsd1* mutants also differentiate normally, with no observed block in the differentiation program, but result in sterile females (Eliazer et al. 2011). Together, these results indicate that methylation of histones mediated by intrinsic factors is imperative for GSC maintenance and fertility.

Although histone-modifying enzymes in the GSCs are responsible for promoting a repressive chromatin state, a recent study has shown that GSC self-renewal also depends on deposition of active marks (Xin et al. 2013). Acetylation of histones is associated with euchromatin formation, which makes the DNA more accessible to transcription factors (An 2007). A putative acetyl transferase, Enoki mushroom (Enok), has been shown to act intrinsically to maintain GSCs during *Drosophila*

oogenesis. Depletion of *enok* in the GSCs results in GSC loss over time. This loss of GSC phenotype does not result from inappropriate pMad levels or from premature Bam expression. This suggests that in the GSC, Enok acts intrinsically downstream of Dpp signaling and independent of the Bam pathway to control genes that may promote self-renewal (Xin et al. 2013). Cumulatively, these observations give insight into how histone-modifying enzymes mediate the balance of gene expression in the early stages of oogenesis to ensure the onset of differentiation.

1.1.2 Nucleosome Remodeling Complexes in the Germ Line that Regulate GSC Self-renewal and Differentiation

Various protein partners that interact with histone-modifying enzymes also contribute to the epigenetic state of a cell. Stonewall (Stwl), a DNA-binding protein that acts as a dominant Suppressor of variegation [Su(var)], is highly expressed in GSCs. Su(var)s usually modulate gene expression by depositing heterochromatin and promoting chromosomal rearrangement to inactivate the genes (Schotta 2002). Loss of *stwl* results in loss of GSCs, while overexpression results in a significant increase in GSC-like cells. These observations indicate that Stwl is an intrinsic GSC maintaining factor. However, loss of GSCs in a *stwl* mutant does not result from early expression of Bam, indicating that Stwl targets other differentiating factors for silencing in the GSC (Maines et al. 2007). Nanos (Nos) and Pumilio (Pum) are conserved RNA translational suppressors that suppress translation of differentiating mRNAs in the GSCs (Lin and Spradling 1997; Forbes and Lehmann 1998; Wang and Lin 2004). Interestingly, it was found that Stwl transcriptionally inhibits the genes that are translationally regulated by Nos and Pum (Maines et al. 2007). This shows that proteins such as Stwl interact with histone-modifying enzymes to maintain a repressive chromatin state that suppresses the transcription of differentiating mRNAs in the GSCs.

Chromatin remodelers, along with chromatin organization and epigenetic marks, are critical for GSC maintenance and differentiation. Chromatin remodeling proteins regulate gene expression by modulating nucleosome position, which allows transcription factors to access the DNA (Armstrong and Emerson 1998). In *Drosophila*, there are five chromatin remodeling factors: Brahma (Brm), Imitation SWI (Iswi), Domino (Dom), Kismet (Kis), and dMi-2 (Tamkun et al. 1992; Kehle 1998; Daubresse et al. 1999; Ruhf et al. 2001; Corona 2002). To remodel chromatin, Brm interacts with either Osa, a Trithorax group (TrxG) protein, to form the Brahma associated protein (BAP) complex or with Polybromo to form the Polybromo associated protein (PBAP) complex (Mohrmann et al. 2004; Moshkin et al. 2006). Brm is expressed throughout the germarium, in both the somatic niche and the germ cells, and loss of *brm* in the germ line results in GSC maintenance defects. Loss of Bap180, a PBAP complex member, in the germ line resulted in loss of GSCs over time. However, mutations in the BAP associated protein Osa did

not affect GSCs, suggesting that PBAP but not BAP is required for GSC maintenance. Although GSC loss is usually associated with misregulation of Dpp signaling and early expression of Bam, this phenomenon was not observed in the *bap180* mutants (He et al. 2014). These findings reveal that chromatin remodeling is critical for GSC maintenance, but their downstream targets in GSCs have yet to be identified.

Along with Brm, other chromatin remodelers have been shown to function in the GSCs: Iswi regulates transcription by compacting the chromatin, while Dom is responsible for exchanging histone variants to induce transcriptional repression (Deuring et al. 2000; Ruhf et al. 2001; Corona 2002; Badenhorst et al. 2002). Iswi protein levels are high in the nuclei of all germ cells, while Dom is present in high levels in the nuclei of GSCs and in low levels in the nuclei of somatic stem cells (SSCs). *iswi* and *dom* mutant oocytes arrest in mid-oogenesis, suggesting they are essential during *Drosophila* oogenesis. Intriguingly, it was reported that only loss of *iswi*, but not *dom*, resulted in the loss of GSCs. GSCs lacking *iswi* showed downregulation of Dad, but pMad levels were not altered concurrent with premature expression of Bam. This indicates that Iswi functions downstream of Dpp signaling to maintain GSCs by repressing Bam (Xi 2005). Although, Kismet's role during oogenesis has yet to be investigated, dMi-2 has been shown to play an important role in oocyte specification and karyosome formation, discussed in Sect. 1.2.3.

1.1.3 Nuclear Organization in the Germ Line that Regulates GSC Self-renewal and Differentiation

The cytoplasm and the nucleoplasm are separated by the nuclear envelope. Embedded beneath the nuclear envelope is a network of filamentous proteins, referred to as the nuclear lamina, which helps organize the chromatin during interphase (Gruenbaum et al. 2005). An important family of lamin-interacting proteins are the LEM domain (LEM-D) proteins, named after LAP2, Emerin, and MAN1 (Lin 2000; Mansharamani and Wilson 2005; Wagner and Krohne 2007). Protein domains in LEM-D proteins interact directly with transcriptional repressors as well as signaling cascade effectors, such as Smads, implying that they broadly contribute to gene regulation and chromatin organization (Holaska et al. 2003; Haraguchi et al. 2004; Lin et al. 2005; Mansharamani and Wilson 2005; Bakay 2006; Jiang et al. 2008). LEM-D proteins are constitutively expressed. However, they display tissue-specific pathological roles. For example, mutations in the LEM-D protein, Emerin, result in age enhanced, X-linked muscular dystrophy in humans (Manilal 1996, 1998). These observations suggest that diseases associated with LEM-D may result from dysregulation of adult stem cell maintenance. Otefin (Ote), the *Drosophila* homolog of the LEM-D protein Emerin, localizes to the nuclear envelope and physically interacts with nuclear lamin proteins B and YA

(Ashery-Padan et al. 1997; Goldberg et al. 1998). However, RNAi knockdown of Ote in *Drosophila* cell lines showed no defects in the organization of the nuclear envelope, suggesting a role beyond nuclear architecture (Wagner 2004). Jiang et al. (2008) showed that Ote is expressed in both the germ line and somatic cells of the *Drosophila* gonad. A mutation in the *ote* allele resulted in sterile females with rudimentary ovaries lacking GSCs with few differentiated germ cells and abnormal egg chambers. Germ line specific loss of *ote* by clone induction results in loss of GSC, suggesting that Ote is required intrinsically for GSC maintenance. And ectopic overexpression of Ote increased the number of GSCs in the ovary, suggesting a role in modulating differentiation. Concurrent Ote and Dpp antagonist Dad overexpression resulted in complete loss of GSCs, indicating that Ote acts as an agonist of the Dpp pathway. It was reported that loss of *ote* also resulted in Bam expression in the GSC, thereby promoting premature differentiation. Additionally, this group demonstrated that Ote physically interacts with Med and together associate with the silencer element in the *bam* gene to suppress its transcription, thereby conserving GSC fate (Jiang et al. 2008). In contrast to this work, studies conducted by Barton et al. reported that loss of *ote* during oogenesis results in differentiation defects with no upregulation of Bam expression. They also established that GSCs lacking *ote* failed to undergo proper differentiation despite ectopic expression of Bam, indicating that Ote does not target *bam* for GSC maintenance (Barton et al. 2013). Even though there is conflicting evidence on how Ote mediates GSC maintenance, it is evident that a LEM-D containing protein is indispensable for intrinsically maintaining GSC fate. Studies in vertebrates have shown that the LEM-D protein MAN1 binds to Smad2 and Smad3 to antagonize BMP signaling (Lin 2000; Ishimura 2006; Cohen et al. 2007). Altogether, these studies suggest that different LEM-D proteins may have opposing functions in maintaining homeostasis in various adult stem cell niches. Therefore, along with histone modifications, chromatin organization is required for GSC fate maintenance.

Studies have shown that the *Drosophila* gene *bällchen* (*ball*), also known as Nucleosomal Histone Kinase-1 (NHK-1), encodes a protein kinase orthologous to mammalian Vaccinia-related Kinase 1 (VRK1), which is required for both chromatin organization and appropriate histone modification patterns in the oocyte (Aihara et al. 2004). The Ball protein is expressed in the GSC and is required for long-term GSC self-renewal (Herzig et al. 2014). However, Ball neither affects the Dpp signaling pathway in the GSCs nor regulates Bam expression in the differentiating germ cells, suggesting that its function in GSC maintenance is independent of Bam regulation. It was found that Ball not only localized with the entire chromatin but was also highly enriched in the nucleolus. The nucleolus assembles around the chromatin region containing ribosomal DNA (rDNA) repeats. The GSCs have a large nucleolus as compared to those of the differentiating germ line cysts, which re-expand after the cysts begin to polyplodize (Neumüller et al. 2008). In the absence of Ball, half the GSCs exhibit fragmentation and disintegration of the nucleoli (Herzig et al. 2014). This indicates that Ball is not required for initiating but rather for maintaining nucleolar expansion of the GSCs. Overall, these studies

indicate that Ball is an intrinsic factor required to maintain nucleolar organization for increased ribosome biogenesis in the GSC. Studies conducted by Zhang et al. have shown that high levels of ribosomal RNA transcription is dependent on *underdeveloped* (*udd*) in the GSCs. Udd interacts with RNA polymerase I transcription-promoting factors to mediate GSC maintenance (Seifarth et al. 1991; Zomerdijk et al. 1994). Udd localization in the GSCs is highly nuclear with increased rRNA synthesis when compared to the CB. This increased rRNA synthesis leads to proper translation of GSC-fate maintaining RNAs, such as Mad, which allows the cell to promote its self-renewal properties (Zhang et al. 2014). Altogether, these studies show the importance of nucleolar assembly in the GSCs in order to promote rRNA transcription that ensures gene expression for self-renewal.

1.2 Epigenetic Mechanisms for Oocyte Specification and Maintenance

After GSC self-renewal and differentiation, the stem cell daughter undergoes four rounds of incomplete division. This produces an egg chamber cyst with a single cell that is a fully determined oocyte and 15 endocycling nurse cells. As the cyst migrates posteriorly, there is encapsulation by follicle cells (FCs). These encapsulation events lead to the formation of 14 distinct developmental stages or egg chambers (Spradling 1993).

Despite the oocyte being directly connected to the nurse cells, the developing oocyte has a unique cell cycle from that of the nurse cells. Nurse cells fluctuate expression of cyclin E (*CycE*) and *dacapo* (*dap*) to achieve an altered cell cycle where they increase their DNA content without dividing (Robinson and Cooley 1996; Su and O'Farrell 1997). The current hypothesis on how oocytes are specified proposes that fusomes and ring canals accumulate in cysts, facilitating RNA transport to promote this specification. Before the oocyte is specified, in meiotic prophase its chromosomes condense and align to form synaptonemal complexes (SCs) (Koch et al. 1970; Carpenter 1975; Schmekel and Daneholt 1995). Until recombination in prophase I is complete, the oocyte can be identified by the presence of SC members, such as crossover suppressor on 3 of Gowen (C(3)G) (Page 2001).

A growing body of research suggests that chromatin architecture and remodeling play a critical role in specifying the oocyte and modulating cell cycle and meiosis. Chromosomes store genetic information for propagation to subsequent generations. During meiosis, chromosomes have functions that demand opposing epigenetic states. Meiotic chromosomes are maintained in a haploid state for extended periods of time, so they must undergo dynamic structural changes to balance both transcriptional activation and silencing. Homologous meiotic chromosomes also undergo DSBs to allow recombination to take place. This process of homologous recombination (HR) is stabilized by the SC (Page and Hawley 2004), and the

assembly of the SC is mediated, in part, by epigenetic modifications (Ivanovska and Orr-Weaver 2014). Following recombination, a conserved, prolonged prophase I arrest occurs. In prophase I arrest, *Drosophila* chromosomes condense within the oocyte nucleus to form a structure called the karyosome, which is conserved in higher organisms. Although it has been postulated that karyosome formation serves to keep chromosomes close together in the growing oocyte nucleus, the reason for a chromosomal rearrangement in metazoan prophase I is, however, unclear (Gruzova and Parfenov 1993).

1.2.1 Histone-Modifying Enzymes and Histone Variants in Oocyte Specification and Maintenance

Histone-modifying complexes such as polycomb repressive complex 2 (PRC2) have been shown to be involved in oocyte specification and by extension karyosome formation. Enhancer of zeste [E(z)], the enzymatic subunit of PRC2, has previously been shown to deposit H3K27me3 marks to suppress gene expression (Czermin et al. 2001). Depletion of E(z) results in loss of oocyte markers, Orb and Bicaudal (BicD). By stage 3, when the meiotic chromosomes first condense to form the karyosome, the single C(3)G-positive cell in depleted ovaries lacked this karyosome structure. Chromatin immunoprecipitation (ChIP) assays using anti-E(z) and anti-H3K27me3 followed by qualitative real-time PCR (qPCR) assaying for *CycE* and *dap* loci showed that both loci are enriched for E(z) and H3K27me3 deposition. These data suggest that E(z) suppresses the nurse cell-like fate in the oocyte. This is mediated by the silencing of CycE and Dap expression. This in turn prevents improper endocycling before the oocyte becomes fully specified. And while PRC2 plays an important role in oocyte fate, loss of polycomb repressive complex 1 (PRC1) do not cause infertility or oocyte defects (Li et al. 2010; Gandille et al. 2010; Iovino et al. 2013) suggesting that PRC2 specifically has an epigenetic role in karyosome formation.

Gene regulation, modulated by histone modifiers, is critical for oocyte specification and karyosome formation. To characterize the oocyte epigenome, an antibody screen against 21 histone marks was carried out, and it was identified that the oocyte epigenome is unique, has both active and repressive marks, and is temporally dynamic (Navarro-Costa et al. 2016). One such dynamic mark that is deposited early during oogenesis and maintained is H3K4me3, which is found at transcription start sites of active genes (Santos-Rosa et al. 2002; Barski et al. 2007; Navarro-Costa et al. 2016). It was identified, through an RNAi screen, that *Drosophila* histone demethylase KDM5 (dKDM5) is responsible for regulating early H3K4me3 levels (Navarro-Costa et al. 2016). Tightly regulated H3K4me3 levels are crucial, as early H3K4me3 levels mediate karyosome remodeling and prophase I arrested-oocyte transcriptional reactivation (King 1970; Navarro-Costa et al. 2016). Thus, dKDM5 is required for timely transcriptional reactivation, proper karyosome remodeling, and fertility.

During meiotic recombination, there is an activation of a meiotic checkpoint due to the formation of DSBs (Su 2006). Ataxia telangiectasia-mutated (ATM) and ataxia telangiectasia-related (ATR/MEI-41) kinases are activated by these DSBs and phosphorylate several targets, including checkpoint-1 (Chk1), checkpoint-2 (Chk2/MNK), and histone H2A variant (H2Av) (Kurz and Lees-Miller 2004; Shiloh 2006; Joyce et al. 2011). It has been proposed that phosphorylation of H2Av recruits chromatin-remodeling complexes, such as the histone acetlytransferase Tip60, to DSB sites (Kusch et al. 2004). This suggests that DSBs recruit factors necessary for remodeling the local chromatin architecture for meiotic progression. When there is defective DSB repair, the meiotic checkpoint is activated for a prolonged period, which in turn results in inefficient accumulation of a TGFα-like protein called Gurken (Grk) causing alteration in the dorsal-ventral (DV) patterning in *Drosophila* oocytes (Ghabrial et al. 1998; Abdu et al. 2002).

Some members of the *spindle* group (which include *spindle* (*spn*)-*A*, *spn-B*, *spn-C*, *spn-D*, *spn-E*, *vasa*, *maelstrom*, *aubergine*, and *okra*) have been shown to have a role in meiotic checkpoint progression, oocyte specification, and chromatin organization. *spn-A*, *spn-B*, *spn-C*, *spn-D*, and *okra* were identified to be important in meiosis, as mutants for these genes had prolonged meiotic checkpoints, as demonstrated by H2Av accumulation, resulting in altered DV patterning phenotypes (Huynh and St Johnston 2004). Despite having similar phenotypes as the other *spindle*-group members, *spn-E*, *vasa*, *aubergine*, and *maelstrom* mutants did not show direct involvement during meiosis progression (Ghabrial et al. 1998; Abdu et al. 2002; Findley 2003; Tomancak et al. 2007). Thus, prolonged exposure to DSBs produces altered chromatin architecture that leads to defects in oocyte specification.

1.2.2 Nuclear Organization in Oocyte Specification and Maintenance

Chromosomes are anchored to the nuclear envelope during meiotic recombination (Su and O'Farrell 1997; Tange et al. 1998; Phillips and Dernburg 2006; Scherthan et al. 2007; Phillips et al. 2009). This association must be dynamic, given that after completion of meiotic recombination, chromosomes must detach from the nuclear envelope to form the karyosome (Cullen et al. 2005). SC formation takes place prior to recombination (McKim and Hayashi-Hagihara 1998). It was found that NHK-1, or Bällchen, a histone H2A kinase, is required for proper SC disassembly, histone H3 lysine 14 and histone H4 lysine 5 acetylation, and karyosome localization to the interior of the oocyte nucleus (Ivanovska 2005; Cullen et al. 2005). In *nhk-1* mutants, a recombination subunit of condensin, structural maintenance of chromosomes 4 (SMC4) does not localize to the karyosome (Chan et al. 2004; Ivanovska 2005). These findings suggest that NHK-1 plays a critical role in the formation and maintenance of the karyosome (Ivanovska 2005; Ivanovska and Orr-Weaver 2014).

Barrier to Autointegration Factor (BAF) binds to LEM-D proteins in the inner nuclear membrane and anchors DNA (Shumaker 2001; Furukawa 2003; Schmid and Nanda 2004; Mansharamani and Wilson 2005). Intriguingly, VRK1 in mammals has a high degree conservation of the kinase domain and structure to NHK-1 and has been shown to phosphorylate BAF (Nichols 2006; Gorjánácz et al. 2007). *Drosophila* karyosome and mouse oocytes exhibit similar chromatin architecture. *Vrk1* mutant oocytes exhibit nuclear organization defects, as compared to wild-type mouse oocytes. This suggests that VRK1 phosphorylation of BAF is critical for the release of DNA to form the karyosome (Schober et al. 2011). In *Drosophila*, mutation of the BAF phosphorylation site leads to continuous association of DNA with the inner nuclear membrane and loss of karyosome formation (Lancaster et al. 2007). Interestingly, *mnk, nhk-1* double mutants did not rescue the karyosome defect, suggesting that this defect is not a result of meiotic checkpoint activation. In *spn*-A, *spn*-B, and *spn*-D ovaries, the phosphorylation of histone H2A threonine 119, a mark deposited by NHK-1, was greatly reduced. This indicated that unrepaired DSBs suppress NHK-1 phosphorylation on histone H2A (Lancaster et al. 2010). Together, these findings support the model that NHK-1 kinase activity is suppressed during meiotic recombination while the oocyte chromosomes are anchored to the nuclear envelope. Once the oocyte has exited the meiotic checkpoint, NHK-1 phosphorylates BAF, leading to the release of chromosomes from the nuclear envelope (Ivanovska 2005; Lancaster et al. 2007, 2010; Ivanovska and Orr-Weaver 2014) (Fig. 1.3). Thus, NHK-1 is a critical factor mediating the dynamic architecture, which allows chromosomes to attach and detach from the nuclear envelope during meiosis.

1.2.3 Role of a Nucleosome Remodeling Complex in Oocyte Specification and Maintenance

The role of histone-modifying complexes in chromatin modification during oocyte specification and karyosome formation is still not fully understood (Kehle 1998; Brehm et al. 2000). dMi-2 is a member of multiple chromatin-remodeling complexes, including the NuRD complex (Kunert 2009). It been shown that loss of *dMi-2* causes chromosome decondensation and disrupts the association of cohesion with polytene chromosomes (Fasulo et al. 2012). While both dMi-2 and NHK-1 depleted oocytes had undercondensed chromosomes in mature oocytes, they most likely are involved in distinct pathways given that *dMi-2* oocytes show normal karyosome organization before nuclear envelope breakdown, unlike *nhk-1* mutants (Ivanovska 2005; Cullen et al. 2005; Nikalayevich and Ohkura 2015). Despite the fact that the mechanism of NuRD action in chromosome condensation has not yet been described, several suggestions have been offered. It has been proposed that perhaps nucleosome remodeling or even the histone deacetylase activity of NuRD is required for chromosome condensation; however, these proposals have yet to be tested (Nikalayevich and Ohkura 2015).

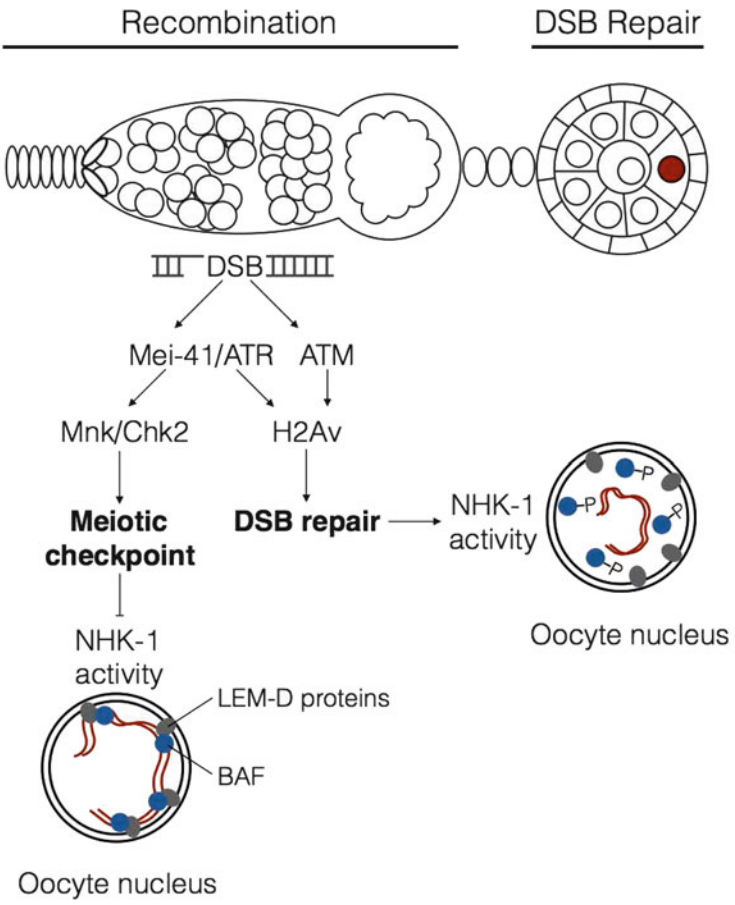

Fig. 1.3 Schematic of NHK-1 activity in the ovary. Upon double-strand break formation, meiotic recombination checkpoint is activated, NHK-1 kinase activity is suppressed, and BAF is unphosphorylated. This anchors chromosomes to the nuclear envelope via LEM-D proteins. Once recombination is complete, NHK-1 phosphorylates BAF and the chromosomes dissociate from the nuclear envelope, allowing karyosome formation. Adapted from Lancaster et al. (2007)

1.3 Chromatin Regulation in Somatic Niche Cells Regulates Oogenesis

Oogenesis requires proper GSC self-renewal and maintenance while also requiring close contact between the germ line and surrounding somatic niche. The soma–germ line contact is conserved from *C. elegans* to humans. In *C. elegans*, contact between the somatic sheath and germ cells is critical for oogenesis (Hall et al. 1999; Erickson 2008), and in mammals, somatic granulosa cells surround the oocytes to

promote maturation (Buccione et al. 1990; Canipari 2000; Matzuk et al. 2002; Gilchrist et al. 2004). A similar interaction between *Drosophila* germ cells and the somatic niche is critical for GSC self-renewal and differentiation (Morgan and Mahowald 1996; Xie and Spradling 2000).

In *Drosophila*, somatic niche cells such as the terminal filament (TF), cap cells (CpCs), and escort cells (ECs) surround the germ line and play an important role in GSC self-renewal and differentiation (Fig. 1.1b) (Song 2002). The TF consists of approximately seven to eight cells connecting the germarium with the sheath cells surrounding the ovariole (Lin and Spradling 1993; Sahut-Barnola et al. 1995). Attached to the TF is a cluster of CpCs (Forbes et al. 1996). GSC self-renewal requires adhesion to the CpCs, mediated by adherens junction molecules such as DE-Cadherin and Armadillo/β-catenin. Not only do CpCs provide somatic structural support but also are a source of Dpp signaling that is critical for GSC self-renewal (Song 2002).

Upon GSC division, the CB loses contact with the CpCs, turns on Bam, and undergoes four rounds of incomplete divisions to form a 16-cell cyst, as described earlier (Xie and Spradling 2000). The ECs form long processes that make extensive contact with the CB and all the differentiating cysts. These EC processes are required for proper differentiation (Schulz et al. 2002; Decotto and Spradling 2005). The 16-cell cyst containing the specified oocyte comes into contact with follicular stem cells (FSCs) (Margolis and Spradling 1995; Nystul and Spradling 2007). FSCs generate multiple somatic cell types, such as epithelial follicle cells, pre-follicular cells (PFCs), stalk cells (SCs), and polar cells (PCs) (Tworoger et al. 1999; Nystul and Spradling 2010). Epithelial follicle cells surround each maturing egg chamber and are required for proper follicle budding, oocyte development, and patterning (Huynh and St Johnston 2004; Nystul and Spradling 2010). The separate egg chambers are connected by an array of stalk cells and have specialized cells known as the polar cells at the anterior and posterior ends (Wu et al. 2008). All of these somatic cell types coordinate transition from GSC to the mature egg.

1.3.1 Chromatin Modifiers Regulate Signaling Pathways in the Somatic Niche

Numerous chromatin modifiers have been identified in the somatic niche cells that play a critical role in oogenesis. These molecules either act in a specific cell type or in multiple cell types to regulate different steps of this process. Female sterile (1) Yb (fs(1)Yb), also referred to as Yb, encodes a protein that has domains homologous to DEAD/DEAH box RNA helicase and Tudor domain (Szakmary et al. 2009). Fs(1)Yb interacts with other proteins of the piRNA pathway to form Yb bodies in somatic cells of the gonad (King and Lin 1999). These Yb bodies have

been identified as a major site for piRNA biogenesis (Qi et al. 2011). In the TF and CpCs, fs(1)Yb controls GSC maintenance by regulating two pathways: Piwi and Hedgehog (Hh) (King and Lin 1999; King et al. 2001).

Loss of Yb results in mislocalization of Piwi from Yb bodies in the somatic niche cells. Piwi, a nucleoplasmic factor that regulates chromatin state, is expressed in both the soma and germ line (Cox et al. 1998). It plays a pivotal role during oogenesis by promoting piRNA production (Thomson and Lin 2009). Loss of *piwi* in the germarium leads to pleotropic phenotypes, such as accumulation of GSC-like cells, loss of GSCs, and differentiation defects. *piwi* depletion in only the CpCs did not result in GSC tumors, but exhibited loss of GSCs suggesting that *piwi* is required in the CpCs for GSC maintenance (Cox et al. 1998, 2000; Ma et al. 2014). Corto, a chromodomain protein expressed in the TF and CpCs, binds to methylated lysine residues in histones. Corto has been shown to interact with both the Polycomb Group (PcG) genes that mediate gene silencing and the Trithorax Group (TrxG) genes that confer gene activation (Salvaing et al. 2003; Smulders-Srinivasan et al. 2010). It was shown that mutations in *corto* suppressed the GSC maintenance defects of *Yb* and *piwi* mutants. This suggests that an as yet unidentified regulatory mechanism acting downstream of Yb and Piwi in the CpCs is required for GSC maintenance (Smulders-Srinivasan et al. 2010).

piwi depletion in the ECs results in tumors containing GSC-like cells. This suggests that loss of *piwi* results in elevated Dpp signaling in the ECs (Jin et al. 2013). Further studies showed that reduction of *dpp* in these *piwi* depleted ECs did not drastically rescue the differentiation defects, indicating that Dpp upregulation is not the major cause of these defects. *piwi* is required in EC for survival and formation of its processes (Ma et al. 2014; Upadhyay et al. 2016). It has been shown that Piwi is also required for expression of Wingless-type mouse mammary tumor virus (MMTV) integration site family member 4 (dWnt4) in the ECs. dWnt4 signaling in the ECs is required for both proliferation and processes formation (Wang et al. 2015). Thus, in the ECs, Piwi is required for regulating Dpp and dWnt4 signaling to promote GSC differentiation.

Loss of *Yb* also phenocopies loss of *Hh* in the TF and CpCs, where FSCs underproliferate resulting in egg chamber defects (Forbes et al. 1996; King and Lin 1999). Loss of *piwi* does not affect Hh expression in the TF and CpCs, suggesting that piwi and Hh work in parallel, downstream of Yb (King et al. 2001). Studies have shown that Hh affects FSC proliferation and cell fate specification by coupling with the Hippo pathway (Huang and Kalderon 2014) or by regulating two transcription factors: Cubitus interruptus (Ci) and Castor (Cas) (Chang et al. 2013; Zhang and Kalderon 2001). Thus, Yb independent of *piwi* regulates Hh signaling to promote proper differentiation.

1.3.2 Histone-Modifying Enzymes Regulate Signaling Pathways in the Somatic Niche

Enok, a putative histone acetyltransferase, is required in both the GSCs and in the somatic niche for proper GSC maintenance. *Enok* depleted CpCs showed loss of GSC concomitant with reduced levels of pMAD, dally, and Notch reporters; however, Dpp levels were not altered. This suggests Enok acts downstream of Dpp signaling to control self-renewal. As Enok genetically interacts with Notch and Notch signaling has been shown to regulate CpC numbers (Ward et al. 2006; Song et al. 2007), it is thought that Enok contributes to GSC maintenance by maintaining CpC numbers. Thus, Enok not only controls the size of the niche by potentially activating the expression of Notch but also regulates the reach of Dpp signaling by modulating levels of Dally (Xin et al. 2013).

dBre1 and *dSet1* are required for regulating levels of H3K4me3, which is required for activating transcription (Ardehali et al. 2011; Hallson et al. 2012). Loss of *dBre1* or *dSet1* in the CpCs and ECs resulted in low levels of H3K4me3 in both of these cell types. *dBre1* depleted CpCs resulted in GSC loss over a period of time, indicating its role in GSC maintenance. Additionally, *dBre1* genetically interacts with *dSet1* to control GSC self-renewal. In TF and CpCs depleted of these enzymes, GSCs were found to have significantly reduced pMad expression, suggesting that Dpp signaling was compromised. However, *dally* mRNA levels were reduced, as opposed to *dpp* mRNA levels, suggesting that impaired dally expression is the cause for GSC loss. Furthermore, it was shown that *dBre1*/*dSet1* led to reduction in Adherens junctions (AJ) protein components, DE-Cadherin and Armadillo. This suggests that *dBre1*/*dSet1* regulates the accumulation of adhesion molecules at the CpC–GSC junction, thereby contributing to GSC maintenance. Thus, transcriptional activation mediated by *dBre1*/*dSet1* in the TF and CpCs is required both to modulate the reach of Dpp signaling by modulating levels of Dally and to regulate adhesion of CpCs to GSCs by modulating AJ components (Xuan et al. 2013).

dLsd1, a lysine demethylase, removes mono and dimethyl groups from H3K4, thus causing transcriptional silencing (Shi et al. 2004). dLsd1 is expressed in both the germ line and in somatic cells, but it is only required in the ECs for GSC differentiation. Loss of *dLsd1* in ECs leads to accumulation of pMad positive GSC-like cells. ChiP-seq assay revealed that dLsd1 binds to *engrailed* promoter in the ECs. Engrailed is normally only expressed in the TF and CpCs and encodes for a homeobox transcription factor (Morata and Lawrence 1975; Kornberg 1981; Desplan et al. 1985). Engrailed is known to promote Hh expression, which in turn drives Dpp expression (Lee et al. 1992; Tabata et al. 1992; Morata 2001; Alexandre and Vincent 2003). Loss of *dLsd1* resulted in ectopic expression of Engrailed in the ECs, and loss of *engrailed* in *dLsd1* ECs suppressed *dLsd1* phenotype. Additionally, *Hh* depletion in *dLsd1* mutants also suppressed *dLsd1* phenotype. This suggests that loss of GSC differentiation is due to the ectopic expression of Engrailed in the ECs, which promotes ectopic expression of Dpp (Fig. 1.4) (Eliazer et al. 2011,

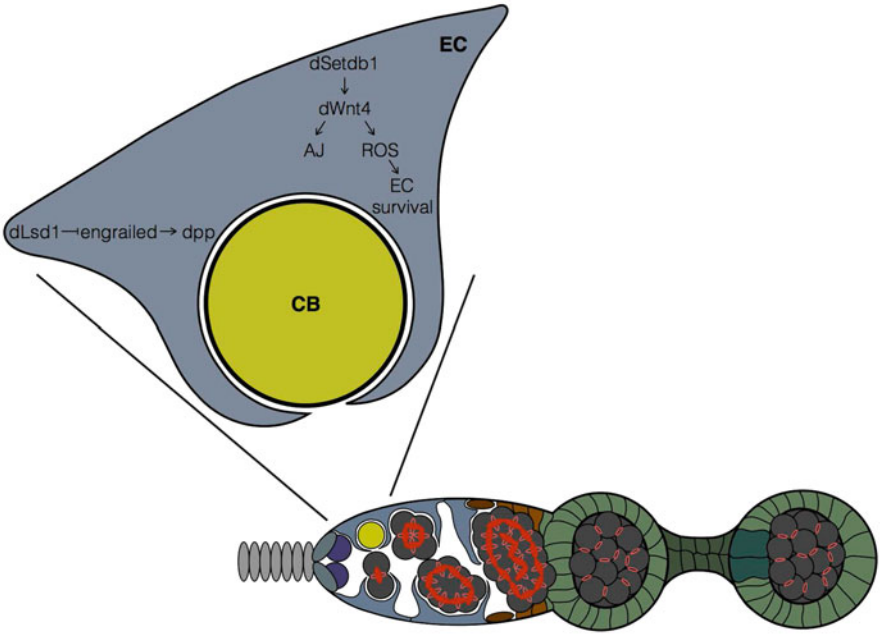

Fig. 1.4 Illustration of an escort cell (EC) enveloping the cystoblast (CB). Examples of two chromatin modifiers acting in the ECs are shown. dLsd1, a lysine demethylase represses engrailed and thereby dpp expression allowing proper differentiation. dSETDB1, a trimethylase regulates dWnt4 which promotes differentiation by regulating adherens junction proteins required for enveloping the CB and ROS required for EC survival in parallel

2014). Thus, silencing of self-renewal signaling in the ECs by dLsd1 is critical for promoting differentiation.

dSETDB1 or eggless (egg) transfers methyl groups on lysine 9 (K9) residues of Histone 3 and is also required for transcriptional silencing. Loss of *dSETDB1* in the ECs leads to loss of heterochromatin, upregulation of TEs, and Bam-dependent GSC differentiation defects (Rangan et al. 2011; Wang et al. 2011). Intriguingly, it was found that dWnt4 acts downstream of dSETDB1 and TEs to affect GSC differentiation. dWnt4 controls GSC differentiation by regulating AJ proteins and promoting the formation of cytoplasmic processes that envelope the differentiating daughter (Upadhyay et al. 2016). Furthermore, depletion of *disheveled* and *armadillo*, the downstream targets of Wnt signaling, leads to increased reactive oxygen species (ROS) levels in ECs, resulting in accumulation of CBs and EC loss. Overexpression of enzymes that help eliminate hydrogen peroxide, such as glutathione-S-transferase (Gst), catalase, or superoxide dismutase1, could rescue the differentiation defect and EC numbers. This suggests that Wnt signaling in the ECs maintains the reduced redox state and is responsible for germ cell differentiation and EC maintenance (Fig. 1.4) (Wang et al. 2015). Thus, silencing of TEs by

dSETDB1 is critical to promote dWnt4 signaling, which promotes EC proliferation and process formation.

1.3.3 Chromatin Remodeling Factors Regulate Signaling Pathways in the Somatic Niche

Chromatin remodeling factors that reposition nucleosomes are also required in the somatic niche for GSC maintenance and proper differentiation. As previously discussed, Polybromo interacts with Bap180 to form the PBAP complex in the germ line and is required for GSC maintenance. It was also shown that Polybromo associates with Bap170 in the TF and CpCs to control GSC differentiation. Loss of *Polybromo* and *Bap170* in the TF and CpCs resulted in lower pMAD levels leading to GSC loss over time (He et al. 2014). However, it is not known if it directly regulates Dpp signaling or indirectly by regulating dally levels.

PcG that can remodel chromatin is known to form two complexes, PRC1 and PRC2. Pc, Psc, Ph, Sce, Scm, and Su(z)2 together form the PRC1 complex (Shao et al. 1999; Saurin et al. 2001; Fritsch et al. 2003). The PRC2 complex is formed of Esc and E(z) (Ng et al. 2000; Tie et al. 2001). Of all these, only Ph, Sce, Scm, Psc, and Su(z)2 are critical for oocyte development. Ph is expressed both in the germ line and soma. Germ line clones for *ph* mutants revealed that Ph is not required in the germ line for oogenesis. Somatic clones of *ph* mutants showed that pre-follicular cells failed to form protrusions to encapsulate the 16-cell cyst and instead showed round morphology. These mutants also exhibited follicle stem cell, stalk and polar cell differentiation defects, and polar and stalk cell proliferation defects. Mutant *Sce* and *Scm* somatic mitotic clones showed multicyst follicles with several oocytes, disorganized encapsulation of the 16-cell cyst, and abnormal number of interfollicular stalk cells. However, no defect in polar cells was observed (Narbonne 2004). Psc functions with Su(z)2 to regulate FSC self-renewal. Although loss of *Psc* and *Su(z)2* shows germ line defects, these mutants are capable of differentiating into germ line cysts and follicular epithelia. This implies that these proteins are required for maintenance of GSCs and FSCs. Further studies showed that loss of these genes leads to sustained canonical and noncanonical Wnt signaling, resulting in loss of FSC self-renewal and causing basal epithelial extrusion, respectively (Li et al. 2010). All these studies suggest that PcG components also have a function independent of the canonical PRC1 complex in the somatic cells to regulate oogenesis.

1.4 Conclusion

The transition from GSC to a mature egg requires the fine balance of several opposing processes, which is inherently difficult. Transcription of differentiation factors must be silenced within the GSCs, only to be immediately upregulated in the

GSC daughter to counter the surrounding self-renewal signaling. While this chapter has outlined several repressive epigenetic mechanisms required for silencing in the GSCs, very little is known about what activates the GSC self-renewal program and the differentiation program in the daughter. However, it is known that a repressive epigenetic program is required in the GSC daughter to initiate the piRNA pathway that protects the genome against TEs. Upon differentiation, the specified oocyte enters meiosis. This transition is mediated by several epigenetic mechanisms to balance the chromosome's need to be anchored to the nuclear membrane to promote recombination and the chromosome's need to be released to proceed into prophase arrest for making an egg. The chromatin state of the oocyte nucleus is not known, nor is it known how this state changes during development to give rise to the karyosome. Lastly, the somatic niche plays a critical role in mediating these transitions. Key epigenetic regulators in the various somatic cells of the niche tune the signaling pathways to promote both self-renewal and differentiation. However, how these various signaling pathways are integrated to produce a specific epigenetic state in the egg is yet to be determined.

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