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Transcriptional regulation during Drosophila spermatogenesis

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Drosophila spermatogenesis has become a paradigmatic system for the study of mechanisms that regulate adult stem cell maintenance, proliferation and differentiation. The dramatic cellular differentiation process from germline stem cell (GSC) to mature sperm is accompanied by dynamic changes in gene expression, which are regulated at transcriptional, post-transcriptional (including translational) and post-translational levels. Post-transcriptional regulation has been proposed as a unique feature of germ cells. However, recent studies have provided new insights into transcriptional regulation during Drosophila spermatogenesis. Both signaling pathways and epigenetic mechanisms act to orchestrate the transcriptional regulation of distinct genes at different germ cell differentiation stages. Many of the regulatory pathways that control male gamete differentiation in Drosophila are conserved in mammals. Therefore, studies using Drosophila spermatogenesis will provide insight into the molecular mechanisms that regulate mammalian germ cell differentiation pathways.

Overview of Drosophila Spermatogenesis

The Drosophila testis is a long tubular structure with a stem cell niche at the apical tip and a linear distribution of germ cells that are progressively differentiated toward the basal end of the tube. A cluster of post-mitotic cells, termed the hub, anchors both germline stem cells (GSCs) and cyst stem cells (CySCs) in the testis niche. The GSCs divide asymmetrically to self-renew and produce gonialblasts (GBs), which are displaced from the niche to start the cellular differentiation program. Each GB undergoes four rounds of mitosis as transit-amplifying spermatogonial cells that remain interconnected by incomplete cytokinesis. These 16 spermatogonia then undergo a pre-meiotic S phase before switching to an elongated G_2 phase as spermatocytes. A robust transcription program is turned on in spermatocytes to actively express genes required for meiotic division and terminal differentiation.[1]

Along with GSC division, the CySCs, two of which encapsulate each GSC, also divide asymmetrically.[2] While one daughter cell retains its stem cell identity, the other becomes a cyst cell, which never divides again. These two cyst cells continue to enclose the synchronously dividing and differentiating germ cells to form a distinct cyst until the individualization stage when the spermatids are separated, followed by release into the seminal vesicle as mature sperm.

Because of the physical association between cyst cells and germ cells, these two cell types act cooperatively throughout spermatogenesis. Here we will review transcriptional regulation of Drosophila spermatogenesis in a stepwise manner and in the context of the intimate soma-germline interaction.

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Transcriptional Regulation in the Stem Cell Niche

Adult stem cells normally reside in a microenvironment called the niche. The Drosophila male GSC niche is one of the best characterized niches. In this niche, GSCs associate with two types of somatic cells: hub cells located at the tip of the testis and CySCs. The niche provides a polarized extrinsic environment where GSCs are maintained through cell-cell adhesion and niche-to-GSC signaling.[3] At the cellular level, GSCs undergo stereotypical asymmetric cell division,[4,5] and at the molecular level, GSCs probably maintain a unique chromatin structure and gene expression profile.[6,7] Because both signaling pathways and epigenetic mechanisms change the transcriptional profile of cells, we will discuss both of them here. Although highly anticipated, a direct link between these two mechanisms has not been reported in the male GSC niche.

Two major signaling pathways play important roles in the male GSC niche: Bone Morphogenetic Protein (BMP) pathway and Janus Kinase-Signal Transducer and Activator of Transcription (JAK-STAT) pathway. In both pathways, ligands [Glass bottom boat (Gbb) and Decapentaplegic (Dpp) for BMP; Unpaired (Upd) for JAK-STAT] emanating from the niche act upon their corresponding receptors at stem cells [Saxophone (Sax), Thick veins (Tkv) and Punt for BMP; Domeless for JAK-STAT] to promote phosphorylation and translocation of their downstream transcription factors [Mothers against Dpp (Mad) for BMP; Stat92E for JAK-STAT].[8-13] A recent study revealed that a regulator of BMP signaling called Magu is specifically expressed in hub cells and required for GSC maintenance.[14] Activated transcription factors subsequently initiate a cascade of gene expression in GSCs and CySCs. Although direct target genes of both pMad and pStat92E have been identified in other cell types in Drosophila,[15-18] their

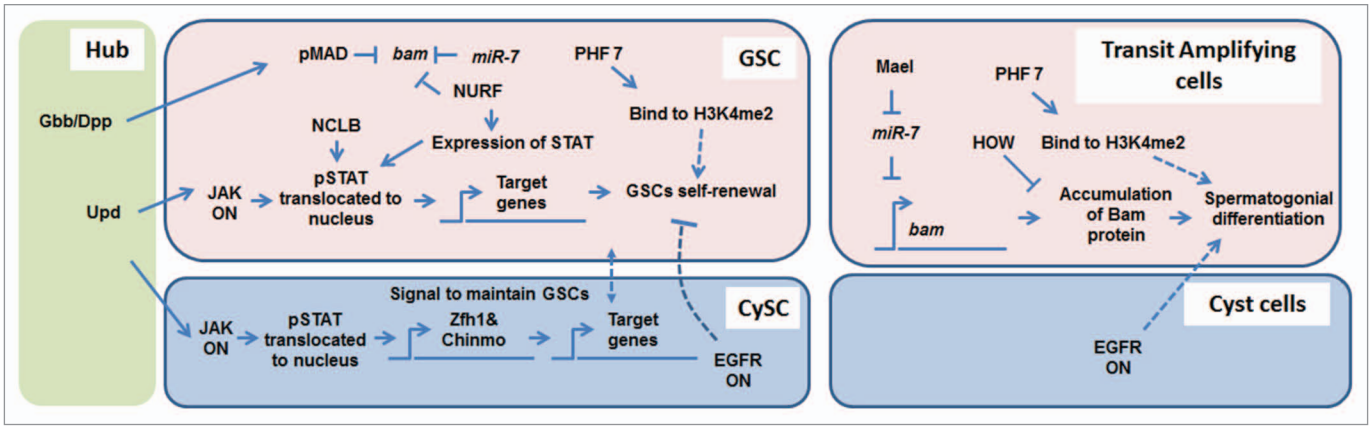

Figure 1. Summary of transcriptional regulation in stem cell niche and mitotic germ cells. Hub cells are in green, GSC and transit-amplifying cells are in pink, CySC and cyst cells are in blue. Solid lines denote direct regulation, dashed lines denote indirect regulation or lack of evidence for direct regulation. See text for detailed discussion.

direct targets in GSCs or CySCs remain unidentified. However, it has been shown that GSCs unable to respond to the BMP pathway have ectopic transcription of a differentiation gene called *bag of marbles* (*bam*), which leads these GSCs to undergo premature differentiation and leave the niche.[12,13] Therefore, it is possible that normal BMP activity represses *bam* transcription in male GSCs, just as it does in female GSCs.[15] Recent studies demonstrate that the major role of JAK-STAT in GSCs is to increase GSC-hub adhesion,[11] suggesting that cell-cell adhesion molecules, such as Drosophila E-cadherin homolog (DE-cadherin, DE-cad), are potential downstream targets of Stat92E. To search for Stat targets at a genome-wide level, microarray analysis was performed to identify genes whose expression dramatically changes in response to hyperactivated Stat.[19] Interestingly, validation of the Stat-responsive genes revealed that most of them are expressed in CySCs instead of GSCs, suggesting that active Stat signaling in somatic cells predominates and is required for maintaining GSCs. Consistent with this finding, ectopic expression of the Stat92E target genes *Zinc-finger homeodomain protein 1* (*Zfh-1*) or *Chronologically inappropriate morphogenesis* (*Chinmo*), both encoding transcription factors, in cyst cells is sufficient for GSC self-renewal outside of the niche.[10,20] Zfh-1 has been implicated in guiding GSC self-renewal, probably by activating BMP signaling in CySCs,[10,11] as well as providing crosstalk between the BMP and JAK-STAT pathways. Another particularly interesting Stat target gene in somatic cells is *Suppressor of cytokine signaling 36E (Socs36E)*, which encodes an antagonist of the JAK-STAT pathway and acts to maintain a balanced ratio of CySCs and GSCs in the niche (**Fig. 1 and Table 1**).[21] Because the microarray analysis was performed using the entire tissue (i.e., testes), it does not provide a cell type-specific transcriptional profile. Furthermore, some of the identified target genes are transcription factors that regulate other genes. Thus, many of the genes with changed expression may not be direct target genes. Further studies with purified cells, in combination with chromatin immunoprecipitation (ChIP) using specific antibodies against pMad or pStat92E, will reveal genes that are direct targets of BMP and JAK-STAT in the testis niche.

In addition to signaling pathways, epigenetic mechanisms also play important roles in regulating GSC activity.[7] Epigenetic regulation changes chromatin structure and gene expression without changing DNA sequences. Two major classes of chromatin regulators have been identified for their functional roles in the male GSC niche: ATP-dependent chromatin remodelers and histone modifying enzymes. For example, one chromatin remodeler, the Nucleosome Remodeling Factor (NURF) complex, has been shown to positively regulate JAK-STAT signaling.[22] Because chromatin remodelers act in both transcriptional activation and repression, it is possible that NURF either promotes transcription of JAK-STAT activators or represses transcription of JAK-STAT inhibitors. A deubiquitinating enzyme of mono-ubiquitinated H2B encoded by *scrawny* (*scny*) serves as an example of the histone-modifying enzymes. Since Scny normally functions in gene silencing, it was postulated to maintain GSCs by repressing the transcription of differentiation genes.[23] Both NURF[24,25] and Scny[23] also regulate female GSC function. By contrast, a novel chromatin factor encoded by *no child left behind* (*nclb*) specifically regulates male, but not female, GSC maintenance.[26] Nclb is enriched at chromatin regions with active transcription. In *nclb* mutant GSCs, Stat92E has decreased expression or accumulation,[26] suggesting that Nclb may act via signaling pathways to determine GSC fate in the niche (**Fig. 1 and Table 1**). However, no direct connection between epigenetic mechanisms and signaling pathways has been reported. This partly results from the difficulty in precisely mapping their direct target genes in different cell types from the niche. Finally, RNA-binding proteins, such as Musashi (Msi),[27] Held-out-wings (HOW)[28] and IGF-II mRNA binding protein (Imp),[29] are all required for GSC maintenance, suggesting an important role of post-transcriptional regulation in the testis niche.

Transcriptional Regulation in the Transit-Amplifying Cells

After GSCs exit the niche, they enter a transit amplification stage consisting of mitotically dividing GBs and spermatogonial cells.

Table 1. Factors required in transcriptional regulation of spermatogenesis

Cell types	Stage	Gene product(s)	Function(s)	Reference(s)
Somatic cells	Hub cells	Gbb/Dpp	Ligand of BMP signaling pathway	12–13
		Upd	Ligand of JAK-STAT signaling pathway	8–10
		Magu	Regulates BMP signaling and GSC maintenance	14
	Cyst stem cells	Rac and Rho	Small GTPases, downstream of Egf pathway. Rac promotes Egf signaling while Rho inhibits Egf signaling. Egf pathway inhibits GSC self-renewal and promotes GSC to GB transition.	38, 41
		pSTAT	Downstream transcription factor of JAK-STAT pathway, sufficient for GSC self-renewal	10
		Chinmo	Transcription factor, a target gene of JAK-STAT signaling, sufficient for GSC self-renewal	20
		Zfh1	Transcription factor, a target gene of JAK-STAT signaling, sufficient for GSC self-renewal	10
		Socs36E	An inhibitor and also a target gene of JAK-STAT signaling, maintains a balanced ratio of CySCs and GSCs in the niche	21
	Cyst cells	Rac and Rho	Small GTPases, downstream of Egf pathway. Egf pathway inhibits spermatogonial division and promotes spermatogonia-to-spermatocyte transition.	38, 41
Germ cells	GSCs	Nucleoporin98–86	Nuclear envelope components, regulate proper GSC-to-GB transition, act upstream of BMP, JAK-STAT and Egfr pathways	40
		pMAD	Downstream transcription factor of BMP pathway, important for GSC self-renewal	12–14
		pSTAT	Downstream transcription factor of JAK-STAT pathway, required for GSC-Hub cell adhesion	11
		Scny	A deubiquitinating enzyme targeting mono-ubiquitinated H2B, represses differentiation gene expression in GSCs	23
		NCLB	A chromatin factor that has male-specific roles for GSC maintenance through regulating JAK-STAT pathway	26
		NURF	A chromatin remodeler that positively regulates JAK-STAT signaling	22
		PHF7	An epigenetic reader that recognizes H3K4me2 and regulates GSC self-renewal	48
		miR-7	A microRNA that binds to *bam* mRNA 3'UTR and downregulates *bam* expression	34
		Msi	A RNA binding protein required for GSC maintenance	27
		HOW	A RNA binding protein required for GSC maintenance	28
		Imp	A RNA binding protein that stabilizes *upd* mRNA and maintains GSC	29
	Spermatogonia	Bam	A differentiation factor required for spermatogonial differentiation	30–32
		Mael	A RNA binding protein that represses *miR-7* and upregulates *bam* expression	34
		PHF7	An epigenetic reader that recognizes H3K4me2 and regulates spermatogonia differentiation	48
		HOW	A RNA binding protein required for spermatogonial proliferation	28
		Nucleoporin98–86	A nuclear envelope component, regulates proper spermatogonia-to-spermatocyte transition and acts upstream of BMP, JAK-STAT and Egfr pathways	40

Table 1. Factors required in transcriptional regulation of spermatogenesis

Cell types	Stage	Gene product(s)	Function(s)	Reference(s)
	Spermatocyte	tMAC (Aly,Achi/ VisComr, Topi, Tomb,Mip40)	Regulate tTAF proper nuclear localization and binding to target genes, transcription of meiotic cell cycle and spermatid differentiation genes	61–66, 71–73
		tTAFs) (Can, Sa, Mia, Nht, Rye)	Transcriptional activation of spermatid differentiation genes, antagonizes PcG-mediated gene silencing, accumulation of Boule protein	69–70
		WUC	Maturation of spermatocyte and meiotic divisions	64, 82
		THO-complex	Maturation of spermatocyte and meiotic divisions	83
		NURF	Maturation of spermatocyte and meiotic divisions	81
	Spermatid	Unknown	Post-meiotic transcription of cup and comet genes, required for individualization of sperm	84, 90–93

The *bam* gene encodes a differentiation factor that is detected in 4- to 16-cell spermatogonia with a peak level in 8-cell spermatogonia,[30] but not in GSCs.[31,32] Ectopic expression of Bam in GSCs causes their premature differentiation or cell death.[31,33] The HOW RNA-binding protein[28] and microRNA-7 (*miR-7*) have both been implicated in binding to *bam* mRNA and down-regulating *bam* expression[34] post-transcriptionally. Another RNA binding protein, Maelstrom (Mael), is required in spermatogonia to repress *miR-7* and upregulate *bam* expression so that GB can enter the normal transit-amplification stage and divide as spermatogonia (**Fig. 1 and Table 1**).[34] The Bam protein subsequently accumulates to a threshold level that is required for spermatogonia to become spermatocytes.[32] As this threshold is never reached in the absence of Bam, *bam* mutant testes are filled with continuously dividing spermatogonial cells.[30,35] Although regulation of *bam* expression has been elucidated at both transcriptional and post-transcriptional levels, the exact mechanism Bam utilizes to regulate transit-amplifying cell differentiation is not yet clear. Another differentiation gene, *benign gonial cell neoplasm* (*bgcn*), has been shown to have mutant phenotypes similar to *bam* in both male and female germline.[30] Although the mechanism that Bam utilizes to regulate male germ cell differentiation is unknown, studies in female germ cells demonstrate that Bam and Bgcn form a protein complex to antagonize factors for GSC self-renewal and promote differentiation gene expression in transit-amplifying cells.[36] Because Bgcn is predicted to be an RNA-binding protein, further characterization of proteins and RNAs with which the Bam-Bgcn complex interacts will illuminate their functions in transit-amplifying cells.

In spermatogenesis, the switch from mitosis to meiosis is critical. Too early transition to meiosis may lead to fewer germ cells and decreased fertility, while failure in this transition may lead to germline tumors. The Epidermal growth factor (Egf) signaling pathway plays an important role in the regulation of this switch. The Egf receptor (Egfr) ligand Spitz is processed by Stet, a transmembrane protease, in germ cells.[37] Activated Spitz then acts on Egfr expressed in somatic cells.[38] Egf signaling acts through the guanine nucleotide exchange factor (GEF) Vav to activate Rac-type small GTPases, which are antagonized by the Rho-type small GTPases.[39] Egfr signaling acts in cyst cells to restrict GSC self-renewal and spermatogonial proliferation, while promoting GSC-to-GB and spermatogonia-to-spermatocyte transitions.[38] Recent studies also demonstrate that Egfr signaling decreases the frequency of GSC division in adult, but not larval, testes, suggesting a dynamic mode of Egfr regulation.[40] In addition, mutations in a serine/threonine kinase signal transducer encoded by *raf* result in a phenotype similar to the *Egfr* mutant, suggesting that the receptor tyrosine kinase (RTK) pathway is, in general, required in cyst cells for proper transit-amplification.[41] The direct target genes for the Egfr/Raf pathway have not been identified; however, because compromised Egf signaling leads to defects in germline-soma interaction and overproliferation of spermatogonial cells, it is possible that the target genes regulate proper encapsulation of germ cells by cyst cells.[37,39] Furthermore, a recent study reported that a nuclear envelope component, Nucleoporin98–86, regulates proper GSC-to-GB and spermatogonia-to-spermatocyte transitions and that its function is upstream of the BMP, JAK-STAT and Egfr signaling pathways.[42] These results highlight the importance of nuclear structure in regulating cellular differentiation during spermatogenesis.

In order to study the transcriptional profile and chromatin state in transit-amplifying cells, *bam* mutant testes were used because they are enriched with over-proliferative spermatogonial cells, as described previously. In addition, wild-type (wt) testes were used as a comparison because they contain germ cells at all stages. High-throughput mRNA sequencing (RNA-seq) studies reveal that both chromatin remodeling factors and histone-modifying enzymes have more abundant transcripts in *bam* testes compared with wt testes.[43] Furthermore, ChIP followed by high-throughput sequencing (ChIP-seq) reveals a different chromatin structure in *bam* testes compared with other stem cell lineages, such as embryonic stem cells (ESCs).[44] In ESCs, it has been shown that differentiation genes have both the repressive H3K27me3 and the active H3K4me3 modifications (i.e., "bivalent" chromatin signature), as well as stalled RNA polymerase II (Pol II, i.e., "poised" genes), at their promoter regions.[45-47] By contrast, differentiation genes required for spermatocyte maturation

and spermiogenesis are either enriched with H3K27me3 only, or deprived of H3K4me3 and H3K27me3, in *bam* testes, and they are not associated with stalled Pol II.[44] This distinct chromatin structure in *bam* testes may prevent ectopic transcription of the differentiation genes in transit-amplifying cells. On the other hand, the chromatin structure in spermatogonia-enriched *bam* testes suggests that dramatic changes at the promoter regions of differentiation genes are needed to turn on their robust transcription in spermatocytes. In addition to these genome-wide studies, it was recently reported that an epigenetic reader-encoding *Plant Homeodomain Finger* 7 (*PHF7*) gene is specifically expressed in GSCs and transit-amplifying cells. PHF7 recognizes the active H3K4me2 histone modification and is required for GSC maintenance and proper spermatogonial differentiation.[48] Further studies to identity the target genes of PHF7, which should be enriched with H3K4me2 or H3K4me3, will shed light on its roles in maintaining GSCs and regulating differentiation of transit-amplifying cells.

Recent studies also revealed that spermatogonia can dedifferentiate to reoccupy the niche and become GSC-like cells.[33,49-52] Although dedifferentiated spermatogonia have cellular features that are distinct from bona fide GSCs,[50] it is less clear whether they have a different transcriptional profile and chromatin structure. Nevertheless, the dedifferentiated spermatogonia can undergo asymmetric cell division just like GSCs,[50] suggesting that they properly respond to signaling from the niche, possibly because of a permissive chromatin landscape in spermatogonia. By contrast, spermatocytes are unable to dedifferentiate to become GSCs,[33] suggesting that germ cells commit to an irreversible program at the spermatocyte stage. Similar irreversible commitment may also apply to female meiotic germ cells because only 4- to 8-cell transit-amplifying cells have been reported to undergo dedifferentiation to become GSC-like cells in the ovary.[53]

Transcriptional Regulation in Meiotic Spermatocytes

The transition from spermatogonia to spermatocytes is accompanied by a series of transcriptional, epigenetic and morphological changes. After transit-amplification, germ cells undergo the last S phase followed by an extended G_2 phase that initiates the spermatocyte stage. Spermatocytes grow 25 times in volume and turn on a robust transcription program to activate genes required for spermatocyte maturation, as well as genes needed for meiotic divisions and terminal differentiation.[54] Most genes required for meiotic divisions, as well as terminal differentiation, are under translational repression until a later time when their encoded proteins are required.[55]

The G_2/M transition in meiosis I requires Cyclin B, Boule (a RNA-binding protein) and Twine (Cdc25 homolog), all transcribed in spermatocytes.[54,56,57] Boule translocates from the nucleus to the cytoplasm to trigger the G_2/M transition in meiosis I by allowing translation of Twine.[58] At this point in time, Cyclin B protein also escapes from translational repression and accumulates in the cytoplasm of spermatocytes.[54] In both *boule* and *twine* mutant testes, spermatid differentiation occurs independently of meiotic cell cycle progression, suggesting that these two processes can be uncoupled.[56,59] However, the discovery of two classes of genes expressed in early spermatocytes reveals a high degree of coordination between meiotic divisions and spermatid differentiation.[60] Mutations in any of these genes arrest meiosis and block spermatid differentiation, leading to testes filled with immature spermatocytes. These genes are named "meiotic arrest" genes, which are classified into "*aly*-class" and "*can*-class" based on morphological differences in the chromosomal structure of the mutant spermatocytes[54,60] and the distinct target genes they regulate.[54,60-69] For example, transcription of meiotic cell cycle genes, such as *Cyclin B*, *boule* and *twine*, rely on *aly*-class, but not *can*-class, genes.[54] However, Boule protein accumulation requires the *can*-class genes.[70] Because meiotic arrest genes regulate transcription or translation of meiotic cell cycle genes, their functions ensure that the meiotic cell cycle does not proceed until terminal differentiation genes are robustly transcribed.[54,60]

The five known *aly*-class genes are *always early (aly), cookie monster (comr), matotopetli (topi), tombola (tomb)* and *achintya/vismay (achi/vis)*. All of the *aly*-class genes, except *achi/vis*, are expressed exclusively in primary spermatocytes.[62-65,71,72] Four of the five *aly*-class proteins have putative DNA binding domains, including Comr, which contains a winged helix; Topi, which contains multiple Zn-finger motifs; Tomb, which has a CXC domain; and Achi/Vis, products from a gene duplication, which have homeodomains. Thus, it is thought that these proteins regulate transcription of target genes by directly binding to DNA sequences, although their direct target genes have not been identified. Immunoaffinity purification studies have revealed that Aly and Tomb proteins are copurified with Mip40 (Myb interacting protein, 40kDa) to form the testis meiotic arrest complex tMAC, which also contains Topi, Comr and CAF1.[66] A second form of tMAC contains Aly, Comr and Achi/Vis.[71] The tMAC resembles the MIP/dREAM complex in mammals and the SynMuv complexes in *C. elegans*.[54,61-66] While the mode of action of tMAC is not fully elucidated, it is thought to have an active, rather than repressive, transcriptional role. This is based on results demonstrating that expression of Achi/Vis fused with a strong transactivation domain, VP16, rescued the *achi/vis* mutant phenotype, while the fusion of Achi/Vis with a repression domain, EnR, failed to rescue this phenotype.[73] Consistent with these findings, all tMAC subunits have been found to co-localize with euchromatin in primary spermatocytes.[61,63,64,71]

The *can*-class genes encode testis-specific homologs of the ubiquitously expressed subunits of the general transcription factor II D ($TF_{II}D$). $TF_{II}D$ is one of the general transcription factors that constitute the RNA Pol II preinitiation complex composed of TATA-binding protein (TBP) and 13–14 TBP-associated factors (TAFs).[74-76] $TF_{II}D$ coordinates the interaction between RNA Pol II and gene promoter regions. The characterized *can*-class genes include *cannonball (can, TAF5L), meiosis I arrest (mia, TAF6L), no hitter (nht, TAF4L), ryan express (rye, TAF12L)* and *spermatocyte arrest (sa, TAF8L)*. Among these five TAF homologs, four, including Mia, Nht, Rye and Sa, share similar structural domains called histone folding motifs for protein-protein interaction, and Can is a WD40-repeat-containing protein.[68]

Indeed, Nht and Rye form a heterodimer in vitro.[67] These testis-specific TAFs (tTAFs) are thought to form a testis-specific complex required for the transcriptional activation of terminal differentiation genes.[67,68] Such predicted functions of tTAFs suggest that they localize at the euchromatin in spermatocyte nuclei. However, while a proportion of the total protein of each tTAF associates with chromosomes in spermatocytes, most tTAF protein is localized to a subcompartment within the nucleolus.[70,77] Interestingly, Polycomb (Pc) and other components of the Polycomb Repressive Complex 1 (PRC1) are co-localized to the same nucleolar subcompartment with tTAFs in spermatocytes. Furthermore, localization of PRC1 components to the spermatocyte nucleolus is coincident with tTAF expression and dependent on wild-type tTAF function.[70] These results suggest that tTAFs act as derepressors by sequestering PRC1 to the spermatocyte nucleolus to counteract Polycomb Group protein (PcG)-induced repression. However, removing PcG activity is not sufficient to turn on terminal differentiation genes in the absence of tTAFs,[69] suggesting that the chromatin-associated tTAFs are required for activating terminal differentiation genes. Consistent with this observation, tTAFs were reported to turn on the transcription of more than 1,000 genes, and many of them are required for spermatid differentiation.[54,69] Among the tTAF-dependent genes, three are shown to be tTAF direct target genes by ChIP assay: *fuzzy onions* (*fzo*), which encodes a protein required for mitochondrial fusion in early spermatids;[78] *mst87F*, which encodes a component of the sperm tail[79] and *don juan* (*dj*), which encodes a sperm-specific DNA-binding protein that also localizes to mitochondria.[80] ChIP analysis at the promoter regions of these three tTAF direct target genes showed that levels of the repressive H3K27me3 mark and paused Pol II are high, while levels of the active H3K4me3 mark are low in *can* and *aly* mutant testes.[69] These data suggest that tTAFs and tMAC might recruit the Trithorax group complex (TrxG), whose activities antagonize PcG, to methylate H3K4 at promoters of terminal differentiation genes (**Fig. 2 and Table 1**).[70]

Although the mode of interaction between tMAC components (*aly*-class) and tTAFs (*can*-class) is not fully understood, it was reported that the function of *aly* is required for the binding of TAF8L to target gene promoters. Aly is also required for the proper nucleolar localization of several tTAFs and Pc in spermatocytes, suggesting that tMAC acts upstream of tTAFs.[69] This is consistent with assays using northern blot, in situ hybridization and microarray analysis.[54,68,69] In addition, while Mip40 is co-immunoprecipitated with tMAC components, loss of *mip40* results in spermatocytes with condensed chromosomes, a phenotype similar to mutants of *can*-class genes,[66] suggesting that Mip40 might mediate the interaction between tMAC and tTAFs. Both tMAC and tTAFs have their canonical counterparts that act generally in other tissues. Similarly, the canonical chromatin remodeler NURF has a germline-specific function in regulating meiotic divisions and spermatocyte differentiation,[81] most likely through using an alternatively spliced isoform.

Recently, two other meiotic arrest genes were identified, which cannot be classified as either the *aly*-class or the *can*-class.[82,83] Wake-up-call (Wuc) was identified by its physical interaction with Aly in a yeast-two-hybrid screen.[64] In spermatocytes, the Wuc protein is highly expressed and associated with chromatin, similar to other tMAC components.[82] However, unlike tMAC or tTAF mutants, loss of *wuc* does not abolish expression of either meiotic cell cycle genes or spermatid differentiation genes.[82] Another study showed that disruption of a component of the THO complex, THOC5, led to the meiotic arrest phenotype.[83] The THO complex is known to export mRNAs from nucleus to cytoplasm. However, no mRNA export defects were detectable in the *thoc5* mutant. Moreover, neither meiotic cell cycle genes nor spermatid differentiation genes have decreased transcription in the *thoc5* mutant.[83] Finally, THOC5 is localized to a perinucleolar region, and loss-of-function in *thoc5* leads to disrupted nucleolar structure and tTAF localization.[83] Identification of *wuc* and *thoc5* mutants and detailed characterization of their phenotypes demonstrate the existence of meiotic arrest genes other than *aly*-class and *can*-class. Understanding their molecular mechanisms will lead to new information about spermatocyte maturation.

Transcriptional Regulation in Post-Meiotic Cells

After germ cells exit the extended G2 phase, they undergo two meiotic divisions followed by spermatid differentiation. One of the major epigenetic events in post-meiotic germ cells is the displacement of histones by transition nuclear proteins (Tnps), followed by protamines (Prms).[84] The replacement of histones with protamines allows for DNA condensation and packaging in the sperm nuclei. To prepare for this replacement, histones undergo a series of post-translational modifications that open up the chromatin, including hyperacetylation of H3/H4 tails,[84,85] mono-ubiquitylation of H2A,[84] and phosphorylation of H4S1 (**Fig. 2**).[86]

Replacement of histones with protamines causes chromatin condensation, which may block transcription in spermatids.[84] Autoradiography studies of nucleic acid synthesis demonstrate a lack of transcription in post-meiotic cells.[87,88] Thus, consensus has held that spermatid differentiation genes are mostly transcribed in spermatocytes, and remain under translational repression. For example, *dj* is transcribed in spermatocytes, but remains translationally repressed until the Dj protein is required for sperm tail formation in elongated spermatids.[89] However, immunostaining showed that phosphorylated and active Pol II reappears in canoe-stage spermatids, indicating reinitiation of active transcription at this late stage during spermatogenesis.[84] Using a sensitive single-cyst quantitative RT-PCR method, another study identified 24 genes, termed "comets and cups" based on their localization patterns, which showed active transcription in Drosophila spermatids.[90] For example, *scotti* is a post-meiotically transcribed comet gene required for normal actin cone progression during spermatid individualization.[91] Consistent with its function, *scotti* mutant males are sterile.[90] More recently, microarray analysis[92] and 5-bromouridine (BrU) incorporation assay[93] were used to profile and visualize de novo transcription in spermatid bundles. These new results demonstrate that transcription is reactivated in 20–30% of testis genes prior to the histone-to-protamine change.[90,92]

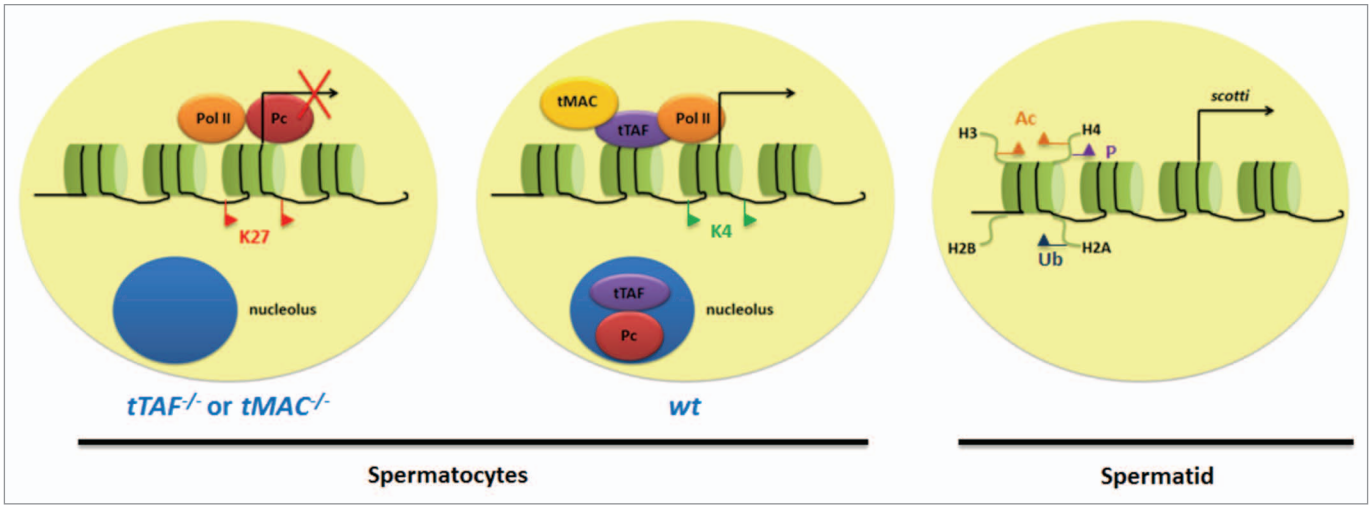

Figure 2. Summary of transcriptional regulation in meiotic and post-meiotic germ cells. A schematic diagram outlines potential chromatin state in spermatocyte mutants for tMAC or tTAF (left, analyzed with *can* or *aly* mutant testes) compared with mature wt spermatocytes (middle, analyzed with wt testes). K27: H3K27me3, K4: H3K4me3. Adapted from Chen et al.[69] On the right, a schematic diagram outlines potential chromatin state in spermatids prior to the histone-to-protamine transition. The *scotti* gene is transcribed in elongating spermatids. Ac, acetylation; P, phosphorylation; Ub, ubiquitylation.

Active transcription in spermatids has been described in mammals.[94,95] These new discoveries of actively transcribed genes in Drosophila spermatids provide a striking similarity between fly and mammalian spermatogenesis. However, although genes transcribed in mammalian spermatids almost always encode components of the mature sperm, most genes transcribed in Drosophila spermatids (22 of the 24) do not encode sperm proteins.[90] Further investigation of these genes is warranted to better understand their functions during spermatogenesis.

Conclusions and Perspectives

In conclusion, Drosophila spermatogenesis has provided an ideal model system to study transcriptional regulation of the cellular differentiation pathway in an endogenous stem cell linage. Recently, new technologies, including RNA-seq and ChIP-seq, have greatly improved our understanding of transcriptional regulation. In the future, the combination of highly sensitive genomic techniques with purified cell types at distinct stages of spermatogenesis will reveal transcriptional profiles with much higher spatiotemporal resolution. Furthermore, ongoing efforts to identify direct target genes downstream of key signaling pathways will illuminate regulatory networks that control transcriptional changes during spermatogenesis.

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