CHAPTER TEN

Small RNAs and their protein partners in animal meiosis

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Contents

Abstract

Meiosis is characterized by highly regulated transitions in gene expression that require diverse mechanisms of gene regulation. For example, in male mammals, transcription undergoes a global shut-down in early prophase I of meiosis, followed by increasing transcriptional activity into pachynema. Later, as spermiogenesis proceeds, the histones bound to DNA are replaced with transition proteins, which are themselves replaced with protamines, resulting in a highly condensed nucleus with repressed transcriptional activity. In addition, two specialized gene silencing events take place during prophase I: meiotic silencing of unsynapsed chromatin (MSUC), and the sex chromatin specific mechanism, meiotic sex chromosome inactivation (MSCI). Notably, conserved roles for the RNA binding protein (RBP) machinery that functions with small non-coding RNAs have been described as participating in these meiosis-specific mechanisms, suggesting that RNA-mediated gene regulation is critical for fertility in many species.

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Here, we review roles of small RNAs and their associated RBPs in meiosis-related processes such as centromere function, silencing of unpaired chromatin and meiotic recombination. We will discuss the emerging evidence of non-canonical functions of these components in meiosis.

Abbreviations

MSCI meiotic sex chromosome inactivation
RBP RNA binding proteins

1. Introduction

Meiosis is a specialized cell division that generates haploid gametes or spores in sexually reproducing organisms. Prophase I is the defining substage of meiosis, in which specific events take place to ensure accurate segregation of homologous (maternal and paternal) chromosomes and thus, reduction of the genetic content in daughter cells (Gray & Cohen, 2016). Chromatin structure is dynamic during prophase I, when homologous chromosomes must pair, synapse, and undergo meiotic recombination. Prior to meiosis, and progressing through prophase I, essential and stage-specific genes must be expressed within a tightly regulated timeline in order to co-ordinate these processes. As a consequence, meiosis is characterized by a stringently coordinated gene expression program. Moreover, transcriptional silencing shapes this expression program to be specific for each cell type and stage within the germline (Grive et al., 2019; Guo et al., 2018; Liu et al., 2021; Murat et al., 2021; Shi et al., 2020; Zhao et al., 2020). For example, in male mammals, transcriptional silencing of the X and Y chromosomes is a spatiotemporally-restricted event that contributes to the characteristic transcriptomic profile of pachytene spermatocytes (Turner, 2007, 2015). Even though many of the changes in transcriptome profiles during meiosis have been described, multiple aspects of the underlying regulatory mechanisms remain poorly understood.

A substantial fraction of the transcriptome is comprised of non-coding RNAs, a subset of which have roles in gene and genome regulation. In addition, cells possess different classes of RNA binding proteins (RBPs), some of which have important roles in gene regulation, often mediated by interactions with small RNAs (Hentze, Castello, Schwarzl, & Preiss, 2018). Certain RBPs and their small RNA cargoes exert their regulatory

influence in the nucleus by modulating chromatin function to control transcriptional and co-transcriptional gene expression, whereas others function in the cytoplasm to control transcript stability and translation and other aspects of mRNA fate. Many small RNAs are expressed in the germline and, more specifically, during prophase I of meiosis. Interestingly, knockout (KO) models for some of the RBPs associated with small RNA biogenesis have shown them to be critical for regulation of at multiple stages of meiosis (Giauque & Bickel, 2016; Girard et al., 2021; Nakamura et al., 2007; Yadav, Mäkelä, Hyssälä, Cisneros-Montalvo, & Kotaja, 2020). One of the most intensely studied examples is the RNA interference (RNAi) pathway, a conserved mechanism of post-transcriptional gene silencing mediated by sequence-specific small RNA molecules (Bhattacharjee, Roche, & Martienssen, 2019). However, the involvement of RNAi components in transcriptional regulation has also been reported, motivated by pioneering studies in the yeast *S. pombe*. This review summarizes the established and emerging roles of RBPs and associated small RNAs as facilitators of the gene regulatory environment unique to meiosis. We will focus on studies of meiosis in animals, while also incorporating studies in other organisms that implicate unexpected roles for small RNA pathways.

2. RNA binding proteins in non-coding RNA pathways

RBPs comprise a heterogeneous group of proteins defined by their ability to bind to RNA through RNA binding domains and form ribonucleoprotein complexes (Corley, Burns, & Yeo, 2020). A broad classification of RBPs is based on the specific RNA species with which they interact, including the following families: mRNA-binding, pre-rRNA-binding, tRNA-binding, small nuclear RNA-binding, small nucleolar RNA-binding, and non-coding RNA-binding proteins (Gerstberger, Hafner, & Tuschl, 2014). Knowledge of RBP structure has also facilitated a functional classification based on the type and number of RNA binding domains associated with their cellular functions (for a review see Corley et al., 2020). RBPs that bind non-coding RNA have deserved special attention, in particular, RBPs involved in biogenesis and processing of regulatory non-coding RNAs known to be key players of gene regulation. Regulatory non-coding RNAs can be categorized according to their transcript length into long non-coding RNAs (lncRNAs), which are longer than 200 nucleotides (nt) long, and small non-coding RNAs, which are shorter than 200 nt. LncRNAs represent the most diverse group in terms of both

their molecular mechanisms of action and the variety of regulatory functions they perform (Lee, Zhang, & Krause, 2019), a feature of lncRNAs that has greatly challenged our understanding of their functions. These diverse roles, across many cellular processes, have gained significant recognition over the past decade (Andric & Rougemaille, 2021) and have been reviewed extensively elsewhere (Quinn & Chang, 2016; Ransohoff, Wei, & Khavari, 2018; Taylor, Chu, Spektor, & Soloway, 2015). Small RNAs include microRNAs (miRNAs), endogenous small interfering RNAs (siRNAs) and PIWI-interacting RNAs (piRNAs), a classification based upon their biogenesis, mechanisms of action and functions. Recent advances in sequencing technologies have made classification of small RNAs more complex and they can now be divided further into multiple subclasses (Ghildiyal & Zamore, 2009). Here, we will focus on germline pathways that rely upon miRNAs and siRNAs, together with their associated RBPs (Table 1).

Table 1 Biogenesis of miRNA and siRNA, associated processes and proteins.

	miRNA	**Endo siRNA**
Organism	Animals, protists, plants, algae, viruses	Animals, fungi, protists, plants, algae
Genomic origin	– miRNA genes – Non-canonical miRNA (mirtrons)	– Repetitive sequences in transposons, centromere and telomere regions. – Convergent mRNA transcripts and sense-antisense pairs
Length	21–23 nt	21–22 nt
Nuclear processing	Microprocessor dependent for canonical miRNA	Microprocessor independent
Nuclear export	Exportin-5 dependent mechanism in mammals and flies.	*Blanks* dependent in *Drosophila*?
Cytoplasmic processing	Dicer dependent	Dicer dependent
Type of Argonaute	AGOs	AGOs (S)AGOs
Targeted genes	Coding genes	Coding genes Transposon and other repetitive sequences

2.1 Biogenesis of miRNA

Biogenesis of small RNAs starts from precursor RNA molecules and involves defined multi-step pathways both in the nucleus and cytoplasm (Fig. 1). Typically, miRNA genes are transcribed by RNA polymerase II, producing primary miRNA transcripts (pri-miRNA) (Hombach, Kretz, Hombach, & Kretz, 2016). In the canonical pathway, the pri-miRNA is cleaved in an RNA structure and sequence-dependent manner by Drosha (Starega-Roslan, Witkos, Galka-Marciniak, & Krzyzosiak, 2015), a member of the RNase III family, to generate a precursor-miRNA (pre-miRNA). Drosha requires a double stranded RNA-binding domain protein (dsRBD) partner, Dgcr8 (DiGeorge syndrome critical region 8) in vertebrates and Pasha in invertebrates, to facilitate efficient processing (Table 2). Together, Drosha and Dgcr8/Pasha form the nuclear-localized microprocessor complex, which is thought to have emerged early in metazoan evolution (Bråte et al., 2018; Grimson et al., 2008). The product of this first nuclear biogenesis step is a pre-miRNA with a 2-nucleotide overhang at the 3′ end (Lee, Han, Yeom, Jin, & Kim, 2006). The majority of miRNAs originate from this canonical pathway; however, a number of variations exist. For example, microprocessor-independent pre-miRNAs can originate from the action of the splicing machinery (Salim, Kumar, Kulshreshtha, & Vivekanandan, 2021); such miRNAs were first discovered in *Drosophila melanogaster* and *Caenorhabditis elegans* (Ruby, Jan, & Bartel, 2007) and termed mirtrons. Finally, transport of pre-miRNAs from the nucleus to the cytoplasm requires contributions from additional RBPs. In mammals, Exportin-5 recognizes the 2 nucleotide 3′ overhang in the pre-miRNAs and forms a complex with the co-factor Ran, a GTPase that releases the pre-miRNA into the cytoplasm upon GTP hydrolysis (Kim, 2004; Yi, Qin, Macara, & Cullen, 2003). Evidence suggests that Exportin-1 mediates pre-miRNA transport to the cytoplasm in flies and worms, the latter lacking an ortholog for Exportin-5 (Büssing, Yang, Lai, & Grohans, 2010). Thus, multiple factors are required for miRNA biogenesis, and inhibition or loss of any of these broadly impacts miRNAs. Therefore, each of these components represents an intervention point for drug targeting or KO studies to elucidate the roles of miRNA in a given cell type or pathway.

2.2 Biogenesis of siRNA

Endogenous siRNAs originate from double stranded RNA (dsRNA) precursors in the nucleus, and their biogenesis does not require microprocessor

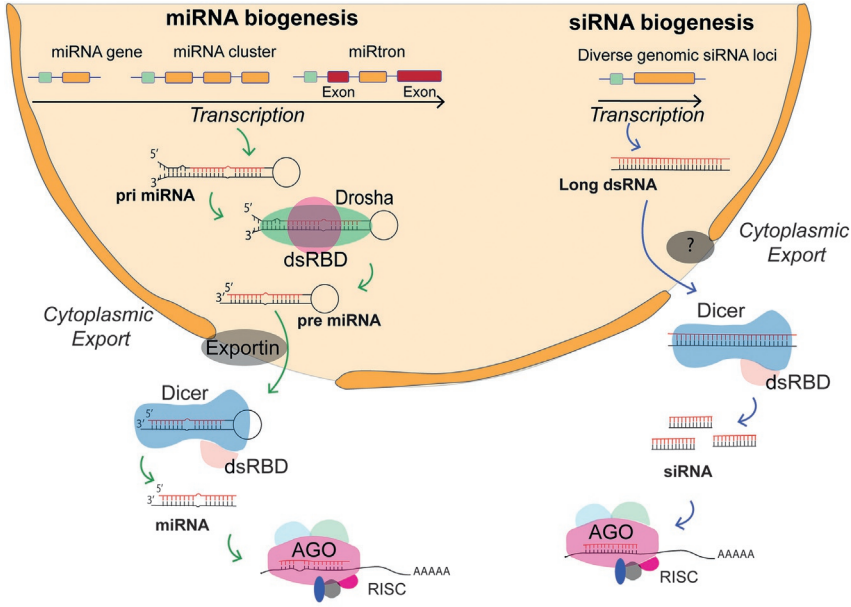

Fig. 1 Canonical pathway for the biogenesis of miRNA and siRNA. Biogenesis of the various classes of small RNAs starts from precursor RNA molecules and involves defined multi-step pathways both in the nucleus and in the cytoplasm. miRNA genes are transcribed by RNA polymerase II as primary miRNA (pri-miRNA) or alternatively, pri-miRNAs can originate from splicing of mRNA (mirtrons). In the nucleus, the microprocessor complex, consisting of Drosha and a dsRNA binding domain protein (dsRBD) Dgcr8, or Partner of Drosha, cleaves the flanking regions of the pri-miRNA to generate precursor-miRNA (pre-miRNA) with a 2-nucleotide overhang at the 3′ end. Endogenous siRNAs start as dsRNA precursors that arise from different genomic loci in the nucleus that do not require microprocessor activity such as repetitive sequences in transposons, centromere and telomere regions, convergent mRNA transcripts and sense-antisense pairs. Pre-miRNAs are transported from the nucleus to the cytoplasm by Exportin-5 in a GTP-bound Ran dependent mechanism in mammals, while the transport of siRNA precursors to the cytoplasm is not well known. Once they reach the nucleus, both miRNAs and siRNAs precursors are processed by Dicer enzymes. Small RNA duplexes are loaded into an Argonaute protein by Dicer in collaboration with other dsRBD proteins to mediate assembly of the RNA Induced Silencing Complex (RISC). Once interacting with the Argonaute protein, the miRNA/siRNA duplex is then unwound into single stranded small RNA. Argonaute proteins can either slice the target mRNA or cause destabilization or translation repression of the mRNA via recruitment of other associated effector proteins by the RISC complex.

Table 2 RBPs associated with miRNA and siRNA pathways in flies, worms and mice.

	Drosophila	*Caenorhabditis*	Mus
Microprocessor	Drosha Pasha	Drosha Pasha	Drosha DGCR8
Dicer	Dcr-1 (miRNA, siRNA) and Dcr-2 (siRNA)	Dcr-1	Dicer
Dicer associated dsRBPs	C3PO LoQs	RDE-4	TRBP
RdRP	Not present	EGO-1 (essential in the germline), Rrf-1, Rrf-2, and Rrf-3.	Not present
miRNA and siRNA interacting Argonautes	AGO clade (2)	AGO clade (6) WAGO clade (16)	AGO clade (4)

activity. These precursors derive from repetitive sequences found in transposons, centromeres and telomeres, convergent transcripts and other endogenous sense-antisense pairs (Carthew & Sontheimer, 2009; Golden, Gerbasi, & Sontheimer, 2008; Russo, Harrington, & Steiniger, 2016). Certain eukaryotes, such as worms and yeast (but not vertebrates or insects), possess RNA-dependent RNA polymerase (RdRP) enzymes that synthesize secondary siRNAs using primary siRNAs as templates, thus providing a system of small RNA signal amplification (Maida & Masutomi, 2011). The mechanism by which siRNA precursors are transported to the cytoplasm has not been fully characterized. Interestingly, recent work in *Drosophila* indicates that the dsRBD protein Blanks, previously shown to have a role in spermiogenesis, is involved in siRNA export (Gerbasi et al., 2011; Nitschko et al., 2020).

2.3 Processing of miRNA and siRNA in the cytoplasm

Within the cytoplasm, both miRNA and siRNA precursors undergo further processing by Dicer, a family of proteins conserved across eukaryotes (de Jong et al., 2009). Dicer recognizes pre-miRNAs and long dsRNAs (siRNA precursors) and cleaves them to produce miRNAs (~21–23 nt long) or siRNAs (~21–22 nt long) (Ha & Kim, 2014). Two Dicer paralogs exist in *Drosophila*, Dcr-1 and Dcr-2 (Lee et al., 2004); while Dcr-1 predominantly processes pre-miRNAs, Dcr-2 processes siRNA precursors

(Förstemann et al., 2005). However, Dcr-1 is also involved in siRNA biogenesis, as it may be required for loading of both miRNAs and certain siRNAs onto Argonaute proteins (Leuschner, Obernosterer, & Martinez, 2005). After processing by Dicer, the resulting miRNA or siRNA duplexes are loaded onto an Argonaute protein by Dicer and associated dsRBD proteins. During this process, the duplex is unwound into single stranded small RNA, discarding or degrading one strand, and resulting in the assembly of the RNA Induced Silencing Complex (RISC). The RISC is comprised of an Argonaute protein stably associated with a single stranded small RNA, referred to as the guide strand, reflecting the role of the small RNA in guiding RISC to a target RNA. The discarded strand is termed the passenger siRNA strand, or miRNA* (Ghildiyal & Zamore, 2009).

A number of dsRBD protein partners of Dicer have been described, with roles during or after initial processing. For example, in worms, Dicer interacts with RD-4, a dsRBD required for cleavage of dsRNA to siRNA, but which is not required later, during RISC assembly (Blanchard et al., 2011; Parker, Eckert, & Bass, 2006). In flies and mammals, duplex loading to Argonaute and RISC assembly by Dicer requires the protein loquacious (loQS; also known as R3D1) (Förstemann et al., 2005; Jiang et al., 2005; Saito, Ishizuka, Siomi, & Siomi, 2005) and trans-activation response RNA binding protein (TRBP) (Chendrimada et al., 2005; Wilson et al., 2015), respectively. Binding specificity and efficient passenger strand removal in *Drosophila*, is dependent on component-3 promoter of RISC (C3PO) (Li et al., 2012), which has also been found in mammalian cell lines and is critical for RISC activation (Ye et al., 2011).

2.4 Loading of small RNAs into Argonaute proteins and post-transcriptional gene silencing

Target recognition is based on the antisense complementarity between the guide strand of the small RNA bound by Argonaute and the target mRNA. The extend of pairing between the guide and target determine the mode of regulation and fate of the mRNA. In bilaterian animals, miRNAs are almost always partially complementary to their mRNA targets, with much of the pairing restricted to a limited and critical region of sequence at the 5′ end of the miRNA (nucleotides 2–7) called the seed region (Bartel, 2018; Lai, 2002). In contrast, siRNAs are usually fully complementary to the target mRNA. Generally, when a guide RNA is perfectly complementary to its RNA target, the Argonaute protein will catalyze endonucleolytic cleavage of the target (Liu et al., 2004; Zamore, Tuschl, Sharp, & Bartel, 2000). However, if there is imperfect complementarity between the small RNA

and the target mRNA region, as is usually the case for miRNAs, the mismatched bases disrupt the catalytic interactions at the Argonaute active site, reducing or eliminating cleavage efficiency (Hutvagner & Simard, 2008; Wee, Flores-Jasso, Salomon, & Zamore, 2012). In this case, silencing occurs via recruitment of other associated effector proteins by the RISC complex that set apart the transcript from the translational machinery and induce destabilization and/or translational repression (Filipowicz, Bhattacharyya, & Sonenberg, 2008), with accelerated mRNA decay being the predominant mode of regulation in mammals (Eichhorn et al., 2014).

Argonaute protein families have evolved across the prokaryote and eukaryote domains of life (Swarts et al., 2014). In animals, they can be classified into three major groups: AGO Argonautes, which interact with mi- and siRNAs, PIWI Argonautes, which bind piRNAs, and WAGOs, worm specific argonautes that interact with secondary small RNAs produced by an RdRP (Wynant, Santos, & vanden Broeck, J., 2017). *C. elegans* is notable due to the high number of Argonaute proteins, having 27, five of which belong to the AGO clade, and due to the production of diverse types of endogenous small RNAs (Wedeles, Wu, & Claycomb, 2013; Yigit et al., 2006). *Drosophila* has five Argonaute proteins, two of them (Ago1 and Ago2) belong to the AGO clade and they bind to miRNA and siRNA (Okamura, Ishizuka, Siomi, & Siomi, 2004), with the remaining three specific for piRNAs. Mammals possess multiple AGO and PIWI argonautes, for example, humans and mice possess four AGOs and 3 or 4 (respectively) PIWIs (Thomson & Lin, 2009).

Not all Argonaute proteins have slicer activity, for example, of the four mammalian AGO proteins, AGO2 alone can cleave mRNA targets effectively (Liu et al., 2004), although AGO3 may retain a degree of slicer activity (Park et al., 2017; Park, Sim, Kehling, & Nakanishi, 2020), while AGO1 and AGO4 lack this catalytic activity. Thus, the mechanism by which a miRNA regulates its mRNA target reflects not only the extent of complementarity between the miRNA and the mRNA, but also the specific Argonaute protein onto which the small RNA is loaded (Ghildiyal & Zamore, 2009). Beyond AGO2-specific slicer activity, whether additional functional specialization exists across the four AGO paralogs common to mammals remains an unanswered question.

In the course of evolution, Argonaute proteins acquired the ability to interact with pathway-specific proteins that enabled their participation in a wide range of cellular processes (Swarts et al., 2014). In animals, GW182 proteins (proteins containing multiple glycine-tryptophan repeats) are RISC binding partners known to mediate cytoplasmic mRNA decay.

GW182 family proteins can also promote aggregation of multiple RISC complexes into ribonucleoprotein granules (Bose, Barman, Goswami, & Bhattacharyya, 2017; Eulalio, Tritschler, & Izaurralde, 2009; Patel, Barbee, & Blankenship, 2016), and facilitate interactions between multiple RISCs on a single target mRNA (Briskin, Wang, & Bartel, 2020). Concentration of argonautes and other interacting RBPs and RNAs in cytoplasmic ribonucleoprotein granules of germ cells is a conserved feature of animal species (Mukherjee & Mukherjee, 2021). These structures have a variety of names depending on the organism and origin mechanism; examples include the P-body in *Drosophila* and *C. elegans* oocytes, Balbiani body in mouse oocytes and the chromatoid body in mouse spermatocytes (Mukherjee & Mukherjee, 2021; Voronina, Seydoux, Sassone-Corsi, & Nagamori, 2011). Intriguingly, these structures appear to be dynamic during gametogenesis; they can change position within the cell, exchange material with the cytoplasm and are even able to migrate between adjacent cells through intracytoplasmic bridges (Jaglarz, Kloc, Jankowska, Szymanska, & Bilinski, 2011; Onohara, Fujiwara, Yasukochi, Himeno, & Yokota, 2010; Parker & Sheth, 2007; Ventelä, Toppari, & Parvinen, 2003). In worms, at least four distinct germ granules have been identified and each of them seems to compartmentalize specific functions associated with small RNA pathways (Sundby, Molnar, & Claycomb, 2021). It is thought that these structures could act to compartmentalize/sequester the RNAi pathway machinery, as analysis of their components showed that they are rich in non-coding RNAs and RBPs such as Argonautes, GW182, VASA homologs, among others (Meikar & Kotaja, 2014; Onohara et al., 2010; Ouyang & Seydoux, 2022). Interestingly, mRNA-RBP complexes transported into P-bodies by the GW182 family may be degraded or isolated to suppress translation in a reversible way (Eulalio et al., 2009; Hubstenberger et al., 2017; Takimoto, Wakiyama, & Yokoyama, 2009). In vertebrates, the GW182 protein TNRC6 acts as scaffold protein, tethering associated effector proteins to destabilize and translationally repress target mRNAs by inducing their decapping and deadenylation (Jonas & Izaurralde, 2015). However, the roles of germ granules are not well understood and are likely not exclusive to small RNA-associated processes.

3. Roles for small RNAs and associated RBPs in the germline

The germ line represents a unique system in which specific small RNA pathways contribute to the differentiation and maintenance of germ

cells. Studies in animals using KO models point to diverse functions for both, miRNA and siRNA pathways during meiosis. For example, in *Drosophila* females, lack of Dcr-1, Pasha, Drosha or Ago1 causes impaired ability to sustain the meiotic cycle and loss of oocytes in the cyst (Azzam, Smibert, Lai, & Liu, 2012), indicating key roles for miRNAs in oogenesis. Furthermore, deletion of the siRNA-specific components Dcr-2 or Ago2 in *Drosophila simulans* males causes impaired meiosis and spermiogenesis; while KO males are unfertile, females exhibit only a mild fertility phenotype (Lin et al., 2018). Differences in phenotype between oogenesis and spermatogenesis in this species can be attributed to the role of siRNAs produced by Sex ratio suppressor genes involved in genomic conflict between the sex chromosomes. In this system, selfish genetic elements termed meiotic drivers that distort their transmission into gametes coexist with a suppressor element, in this case, small RNAs produced from an autosomal locus that interact with an X-linked driver (Lin et al., 2018; Muirhead & Presgraves, 2021; Vedanayagam, Lin, & Lai, 2021). Similar sexual dimorphic phenotypes are observed in the mouse in various mutants for genes encoding small RNA biogenesis associated RBPs. Loss of DGCR8 in female mice has no impact on oocyte development or fertility (Suh et al., 2010), while in testes, deletion of *Dgcr8* reduces the number of pachytene spermatocytes due to defective prophase I progression (Zimmermann et al., 2014). These, and other studies, have established that siRNAs, but not miRNAs are essential for meiosis in oocytes, with loss of Dicer associated siRNAs causing defects in spindle assembly during oocyte maturation and ovulation (Liu, Tang, He, & Rosenwaks, 2010; Ma et al., 2010; Mattiske, Han, & Mann, 2009; Murchison et al., 2007). However, miRNAs are expressed during mouse spermatogenesis (reviewed in Kotaja, 2014; Wang & Xu, 2015) where *Dicer* knockout causes loss of miRNAs and defective progression of Meiosis I (Greenlee et al., 2012; Lee et al., 2003; Modzelewski et al., 2015; Romero et al., 2011). Interestingly, Dicer KO in oocytes causes defects in maturation and ovulation, after prophase I is completed, while in spermatocytes, the meiotic defective phenotype seems to arise during prophase I, indicating differential small RNA requirements in meiosis I and II.

The detailed mechanisms giving rise to meiotic phenotypes in components of small RNA pathway mutant models is not fully understood. Given the basic cellular function of miRNAs and siRNAs in post-transcriptional gene regulation of all cell types, plus the fact that many genes encoding components of the meiosis machinery can be targeted by small RNAs, this type of gene silencing seems to be critical for meiosis. However, growing

evidence suggests that small RNA gene regulation in meiosis not only occurs via the canonical post-transcriptional silencing, but also through direct involvement of components of these pathways in chromatin reorganization. In the following section, we will discuss previous work showing how RBPs involved in small RNA biogenesis and RISC assembly localize to the meiotic nucleus and relate to key prophase I processes.

4. Meiotic silencing of unpaired chromatin

Pairing and synapsis between homologous chromosomes are highly regulated processes that define early prophase of meiosis I. Prophase I begins in pre-leptonema with the search for homology between maternal and paternal chromosomes, aided by the sequential formation of the synaptonemal complex (SC, in most organisms, exceptions include *S. pombe*). When an appropriate match is found, in zygonema, the physical tethering between homologous chromosomes is completed by assembly of the final component of the tripartite SC. By pachynema, homologous chromosomes are tethered along their entire length by the SC, with the notable exception of the sex chromosomes, as described below. The presence of any persistent unpaired DNA triggers an evolutionarily conserved response, first studied in *Neurospora crassa*, termed Meiotic Silencing of Unpaired DNA (MSUD) (Fig. 2) (Aramayo & Metzenberg, 1996). MSUD in *N. crassa* requires Dicer (DCL-1) and Argonaute family proteins and their associated small RNAs (for a thorough review see Hammond, 2017). During the pairing of homologous chromosomes, any unpaired regions are identified by an unknown molecular mechanism that involves the SAD-6 (suppressor of ascus dominance-6) homology search protein (Samarajeewa et al., 2014). Presence of unpaired DNA stimulates bidirectional generation of single-stranded aberrant RNAs (aRNAs) from the unpaired DNA, which are then exported to a meiotic silencing complex located in the perinuclear region (Decker et al., 2015). Here, the aRNAs are a template used by the RdRP, SAD-1, to generate dsRNA, which is a substrate for DCL-1, resulting in the production of MSUD-associated siRNAs (masiRNAs). The Argonaute SMS-2, loads the masiRNAs and targets any transcript from the unpaired locus for degradation, with targeting specified by pairing between the masiRNAs and their cognate loci. The mechanism of repression appears to be post-transcriptional and within the

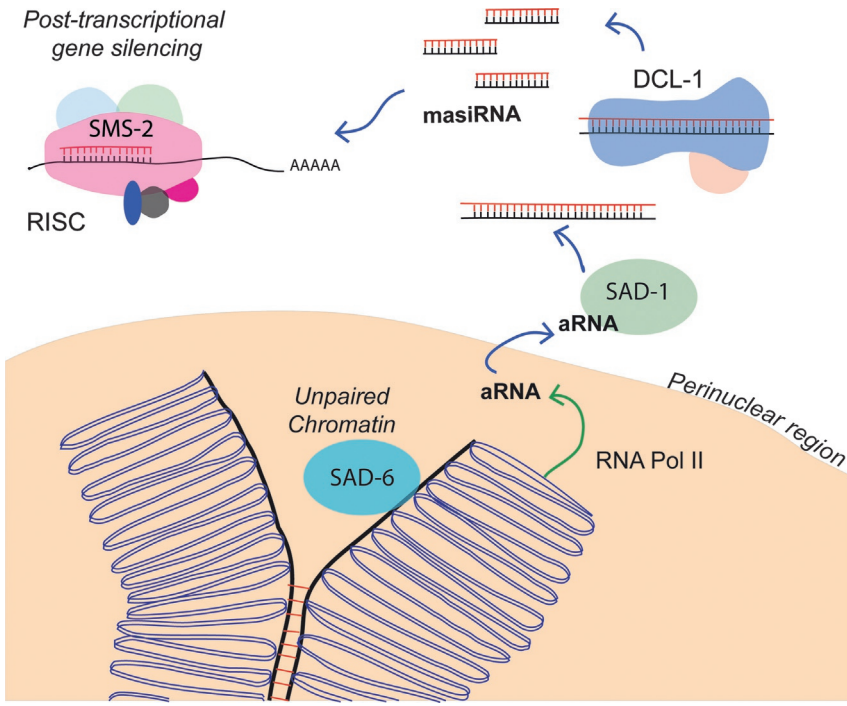

Fig. 2 Meiotic silencing of unpaired DNA (MSUD) in the meiotic nucleus of *Neurospora crassa*. During the pairing of homologous chromosomes, regions that are unpaired are identified by an unknown molecular mechanism that involves the homology search protein SAD-6. This signal stimulates bidirectional generation of single-stranded aberrant RNAs (aRNAs) from the unpaired DNA by RNA polymerase II that are exported to the perinuclear meiotic silencing complex. Here, the aRNAs are used as a template by the RdRP SAD-1 to generate dsRNA. Subsequently, a Dicer enzyme DCL-1 and other associated factors produce siRNAs from the dsRNAs termed MSUC-associated siRNAs (masiRNAs). The Argonaute SMS-2 loads the masiRNAs and targets any transcript from the unpaired locus for degradation.

cytoplasm, since SMS-2 has been shown to be exclusively perinuclear and cytoplasmic (Xiao et al., 2021). These results establish a fundamental role for small RNAs during meiosis in *Neurospora*.

Meiotic silencing likely evolved as a surveillance system to protect against aneuploidy, transposable elements or excessive gene dosage resulting from deleterious chromosomal rearrangements (Schimenti, 2005; Turner, 2015). In contrast to MSUD, a post-transcriptional process, the equivalent pathway in worms seems to occur via transcriptional silencing. In *C. elegans*, chromatin regions that fail to pair and synapse accumulate the repressive

histone modification H3K9me (Histone 3, trimethylated at lysine 9), as a result of a pathway called meiotic silencing of unpaired chromatin (Maine et al., 2005). Notably, in *C. elegans*, this meiotic silencing is dependent on the RdRP EGO1 and the Argonaute CSR-1 (Maine, 2010). Although the mechanistic details of this pathway are yet to be detailed, it has been proposed that EGO-1 and associated small RNAs are involved in recruitment of histone modifiers to the unpaired regions (Maine et al., 2005). Thus, although aspects of this pathway in *C. elegans* diverge from the *Neurospora* pathway, the fundamental role played by small RNAs is consistent.

In mammals, meiotic silencing is triggered by failure of homologous chromosomes to synapse, a process termed meiotic silencing of unsynapsed chromatin (MSUC). In mice, as autosomes become synapsed during pachynema, unsynapsed regions are marked by BRCA1, the kinase ATR and phosphorylated histone H2AX (phosphorylated by ATR at serine 139, termed γH2AX), which collectively trigger signaling cascades that result in transcriptional silencing (Baarends et al., 2005; Turner, Aprelikova, et al., 2004; Turner, Mahadevaiah, et al., 2004). Although mechanistic studies in mammals are limited, there is evidence for participation of small RNA pathways in MSUC. In mouse spermatocytes, AGO4 and AGO3 localize to autosomal regions that fail to pair, coincident with localization of BRCA1, ATR and γH2AX (Modzelewski, Holmes, Hilz, Grimson, & Cohen, 2012). Moreover, during pachynema, AGO4 may be involved in the localization of trimethylated H3K9me3, which contributes to the formation of transcriptionally silent heterochromatin. Loss of AGO4 leads to downregulation of multiple miRNA families in spermatocytes, however, the mechanism connecting these specific miRNAs and the establishment of silencing marks in unsynapsed chromosomes is unknown. Indeed, it is also possible that any role for AGO4 during MSUC involves a novel class of siRNAs, rather than miRNAs or does not require small RNAs, as Argonautes can have non-canonical functions in the nucleus of human cell lines and *Drosophila* independent of small RNAs (Acuña et al., 2020; Zaytseva et al., 2020). Nonetheless, the presence of AGO proteins at unpaired chromosomal regions during mammalian meiosis suggests additional links between MSUC and MSUD, raising the possibility that mammalian AGO proteins, likely in concert with unknown small RNAs, may be contributing to transcriptional (or co-transcriptional) silencing during MSUC, analogous to roles described for MSUD in *N. crassa*. More details on small RNA

participation on meiotic silencing pathways in animals come from the study of sex chromosome silencing, a naturally occurring process in many species, which we discuss below.

5. Meiotic silencing of sex chromatin

Components of small RNA pathways are also implicated in the silencing of sex chromosomes during meiosis, a specialized mechanism of unpaired homolog silencing. In species with morphologically distinct sex chromosomes that contain limited homology between maternal and paternal sex chromosomes, synapsis and recombination occurs only at regions that exhibit homology, referred to as pseudo-autosomal regions (PAR). During meiosis in male mice, the presence of unpaired sex chromatin triggers a feedforward mechanism that mediates the formation of a γH2AX domain on the sex chromosomes. This domain spreads to chromatin loops along with the accumulation of characteristic repressive chromatin marks, establishing a silencing domain known as the sex body (Abe et al., 2020) (Fig. 3). This specialized form of MSUC is termed meiotic sex chromosome inactivation (MSCI) (McKee & Handel, 1993; Turner, 2007), and occurs in pachynema, at which time transcriptional silencing of the XY pair stands in marked contrast to the active transcriptional state of the autosomes (Namekawa et al., 2006; Turner, 2015). Interestingly, the machinery that leads to both MSCI and MSUC appears to have common components, including proteins of the DNA Damage Response (DDR) pathway, whose accumulation seems to trigger silencing (ElInati et al., 2017). As in MSUC, BRCA1 accumulates on the unsynapsed sex chromosome axes and recruits the kinase ATR to phosphorylate H2AX (Turner, Aprelikova, et al., 2004). The mechanism by which γH2AX signal is spread along the chromatin loops is centered on MDC1, which binds γH2AX and enables the progressive activation of the ATR kinase through the activator protein TOPBP1 (Abe et al., 2020; Ichijima et al., 2011). TOPBP1-ATR then mediates adjacent γH2AX formation on chromatin loops to which MDC1 binds once again, and the mechanism continues progressively forwards (Ichijima et al., 2011).

Studies in mouse have shown that spermatocytes that lack *Dgcr8* or *Dicer*, thus with repression of miRNA biogenesis, or repression of both miRNA and siRNA biogenesis, respectively, show aberrant sex body establishment

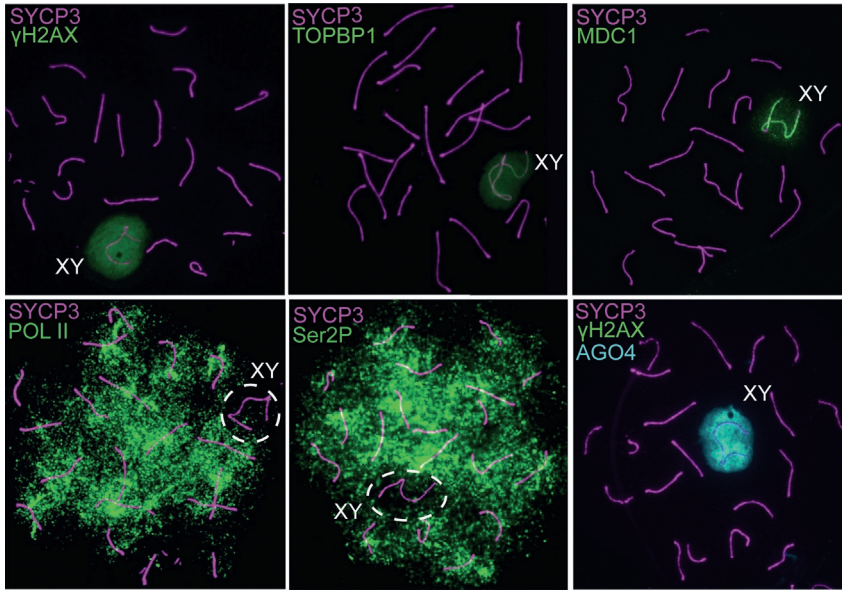

Fig. 3 Meiotic silencing of sex chromatin in male mouse. During pachynema, the sex body becomes apparent in spermatocytes, as shown by the markers γH2AX, TOPBP1 and MDC1 (upper panels), whose signal spreads from the Synaptonemal Complex (SC) axes to chromatin loops. Both total RNA Pol II (POL II) and phosphor-Pol II (phosphorylated at Ser2; Ser2P), indicative of active transcriptional elongation, are excluded from this silencing domain. AGO4 colocalizes to the sex body. MDC1, Ser2P and AGO4 images were kindly provided by Drs. Andrew Modzelewski, Tegan Horan and Adriana Alexander.

and mis-localization of DDR factors at pachynema (Modzelewski et al., 2015). In addition, loss of DICER or AGO4 in male mice causes overexpression of X chromosome associated genes that are known to be targeted for MSCI in pachynema (Greenlee et al., 2012; Modzelewski et al., 2012). Studies have shown that AGO3, AGO4 and associated small RNAs, localize to the sex body of pachytene spermatocytes and *Ago4*-deficient germ cells show deficient silencing of X and Y chromosomes and influx of RNA pol II to the sex body (Modzelewski et al., 2012). Moreover, AGO4 might be involved in γH2AX signal establishment, since deletion of AGO4 causes aberrant morphology of the sex body and mis localization of the DDR factors, γH2AX and ATR and TOPBP1 (Modzelewski et al., 2012). This study also showed that the X-linked miRNAs localize to the sex body along with AGO4 in wild type spermatocytes, suggesting that the mechanism behind sex body establishment might

involve miRNA binding. More studies are needed to establish the details of these silencing mechanisms, such as identifying the specific small RNAs that localize to the sex body and the possible target mRNAs or chromatin sites.

The existence of MSCI in *Drosophila* is controversial, and there seems to be no consensus on whether male flies exhibit sex chromosome transcriptional silencing; even less clear is whether small RNAs participate (Meiklejohn, Landeen, Cook, Kingan, & Presgraves, 2011; Vibranovski, 2014). MSCI in *C. elegans* is well documented, as X chromosomes in both males (X0) and hermaphrodites (XX) manifest reduced transcriptional activity up to diplotene (Kelly et al., 2002; Tzur et al., 2018). In hemizygous males, the single X chromosome is not able to pair during meiosis and is condensed, and interestingly, X chromosomes in hermaphrodites that are able to synapse are also silenced. Contrary to what is observed in mammals, similarities between silencing of X chromosomes during meiosis in *C. elegans* and MSUC is unclear, since both seem to respond to different pathways (Checchi & Engebrecht, 2011; Lamelza & Bhalla, 2012). Moreover, lack of synapsis might not be the trigger of X chromosome inactivation in *C. elegans* (Kelly et al., 2002; Rappaport et al., 2021). Nonetheless, loss of CSR-1 pathway factors results in mis-localization of the histone silencing mark H3K9me2 in both autosomes and X chromosomes, indicating that small RNA pathways are a common factor in sex chromosome silencing between worms and mammals (Maine et al., 2005; She, Xu, Fedotov, Kelly, & Maine, 2009).

6. Pericentromeric heterochromatin remodeling during meiosis

The meiotic nucleus undergoes progressive reorganization of heterochromatin as meiosis proceeds. Heterochromatin mediates proper segregation of chromosomes and facilitates long-range chromatin interactions between distant chromosomal regions. Increasing evidence suggests that certain histone modifications, which regulate the specialized architecture of meiotic chromosomes, play a fundamental role in centromere formation, pairing, synapsis, and recombination of homologs (Lam, Brick, Cheng, Pratto, & Camerini-Otero, 2019; Shi et al., 2021; Wang, Xu, Khawar, Liu, & Li, 2017). In most eukaryotes, centromeres are surrounded by densely packed pericentromeric heterochromatin (PCH) and this

characteristic organization is essential to build the kinetochore (Talbert & Henikoff, 2020). For this reason, PCH and centromeres have an important role as coordinators of sister kinetochore cohesion and homologous chromosome orientation at the Metaphase I spindle, thereby ensuring accurate chromosome segregation (Brar & Amon, 2008). A general mechanism of heterochromatin formation is deacetylation of histone H3 followed by methylation at lysine 9 (H3K9me3) (Weirich, Khella, & Jeltsch, 2021), which is catalyzed by a group of histone methyltransferases that contain a SET domain and a chromodomain, such as SUV39 in animals and Clr4 in yeast. Histone methylation provides a docking site for proteins that silence transcription, recruit chromatin modifiers, and maintain heterochromatin ultrastructure. Intriguingly, many reports suggest that small RNA pathways could contribute to the accumulation of proteins associated with heterochromatin formation at the PCH (Kanellopoulou et al., 2005; Miyata et al., 2021; Pal-Bhadra et al., 2004; Park et al., 2018; Chapman, van Nues, Theron, & Bayne, 2020).

S. pombe was the first system in which an RNA-induced transcriptional gene silencing complex (RITS) was shown to mediate PCH formation (Verdel et al., 2004). In this process, an RdRP produces dsRNAs from bidirectional transcription of repetitive sequences at the centromeres and serves as the initial source for siRNAs generated by Dcr1 (Sugiyama, Cam, Verdel, Moazed, & Grewal, 2005). These primary siRNAs are targeted back to the repetitive sequences by the RITS complex to recruit histone modifiers such as Clr4. In yeast Clr4 mediates H3K9 methylation, which is bound by the heterochromatin-associated factor Swi6. In *S. pombe*, deletion of Ago1, Dicer or RdRP results in impaired heterochromatin formation at the centromeres and at the mating type region during meiosis and mitosis (Hall, Noma, & Grewal, n.d.; Volpe et al., 2003). Thus, it is clear that the siRNA pathway plays a major role in establishment of the higher chromatin structure of chromosomes during meiosis in *S. pombe*.

Instead of having one centromere, as in *S. pombe*, *C. elegans* possess dispersed centromeres along the length of the chromosomes. Chromatin organization of these holocentric chromosomes in the germline is also maintained by a small RNA pathway. A specific germline nuclear WAGO, HRDE-1, was shown to regulate nuclear compaction by recruitment of siRNAs to nascent transcripts in the nucleus, resulting in co-transcriptional repression (Fields & Kennedy, 2019; Perales et al., 2018). Another member of the WAGO clade, CSR-1 was shown to localize to the diakinetic chromosomes in *C. elegans* oocytes. In contrast to *S. pombe*, CSR-1 also binds to

euchromatic regions in the nucleus of germ cells, where it targets nascent RNA for cleavage and also recruits chromatin-modifying factors to establish pericentromeric chromatin domains (Claycomb et al., 2009). This mechanism is dependent on small RNAs, as CSR-1 does not interact with genomic loci when small RNAs are depleted. Loss of CSR-1 and DRH-3 (Dicer related helicase) causes defects in chromosome segregation and aberrant accumulation of the repressive histone modification, H3K9me2 during meiosis (Claycomb et al., 2009; Davis et al., 2018; Duchaine et al., 2006; Nakamura et al., 2007; Yigit et al., 2006). Regardless of differences in genomic organization, recruitment of histone modifiers for chromatin compaction and PCH formation in *C. elegans* involves small RNA components acting in a non-canonical pathway reminiscent of that described in *S. pombe*.

Mouse meiotic spermatocytes and somatic cells also show PCH establishment pathway reminiscent of that in *S. pombe*, where RNA transcripts referred to as major satellite repeats are produced from the PCH and involved in heterochromatin formation (Fioriniello, Marano, Fiorillo, D'esposito, & Ragione, 2020; Fukagawa et al., 2004; Huo et al., 2020; Kanellopoulou et al., 2005; Khalil & Driscoll, 2010; Yadav et al., 2020). Using IF and *in situ* hybridization, DICER was shown to localize to heterochromatin areas of the spermatocyte nucleus, and colocalizing with major satellite transcripts by IF and RNA FISH (Yadav et al., 2020). The same authors showed that DICER interacts with major satellite repeat transcripts to produce mature small RNAs. Notably, *Dicer* and *Ago1*-null embryonic stem cells show upregulation of repetitive centromeric transcripts and defects in methylation of centromeric DNA and histone H3 (Kanellopoulou et al., 2005; Müller et al., 2022). In mouse, *Dicer* KO spermatocytes have reduced recruitment of SUV39H2 and H3K9me3 to the pericentric heterochromatin, along with a meiotic chromosome mis-segregation phenotype, suggesting a role for these small RNAs in promoting recruitment of histone modifiers to the PCH during meiosis (Yadav et al., 2020). Human somatic SUV39H1 localization to the PCH has also been shown to be regulated by small RNA binding (Johnson et al., 2017). In this regard, no evidence for an equivalent RITS complex has been detected in mammalian cells. It is important to note that *Dicer* mutants do not exhibit complete absence of heterochromatin formation, thus there are other mechanisms involved in this process. Moreover, mammals lack an RdRP to amplify small RNAs from their precursor transcripts, and therefore, the formation, spreading and maintenance of heterochromatin at the PCH likely occurs through mechanisms somewhat distinct from those in *S. pombe*. Nevertheless, key

factors for heterochromatin assembly such as involvement of Dicer are conserved in all eukaryotes (Talbert & Henikoff, 2020) suggesting participation of small RNA pathways in the mechanisms involved in PCH formation. Perhaps PCH formation in mammals occurs through modeling of the chromatin environment through direct interactions between Dicer and Argonautes, along with their small RNA cargoes, with the genome, reminiscent of the *C. elegans* system.

7. Regulation of meiotic recombination and double strand break repair

Meiotic recombination between homologs occurs during Pachynema generates a physical link between them, known as chiasmata, which is critical for accurate segregation during meiosis I (Gray & Cohen, 2016). This process starts early in leptonema with the formation of hundreds of double stranded DNA breaks (DSB) mediated by Spo11, which resolve as either crossovers (CO) or non-crossovers, depending on the proteins recruited to this DSB hot spots and the consequent pathways regulated by the corresponding protein complexes. Several reports using mammalian cell lines have shown that small RNAs are present at DSB sites, although the biogenesis mechanism for these small RNAs has not been elucidated (Burger et al., 2017; Francia et al., 2012; Lu et al., 2018; Ohle et al., 2016; Swahari et al., 2016). In these cells, the general model proposes that DSB serve as substrates for production of small RNA termed DSB induced RNAs (diRNAS), which can be used by RBPs such as Argonaute proteins to target these same regions for either histone modifications or direct recruitment of repair factors. Small RNAs from the sequences flanking the DSB can mediate recruitment of DDR factors such as TOPBP1 (Fragkos et al., 2019) and MDC1 (Burger et al., 2017) back to the DSB. Argonaute proteins may be involved in loading of DDR factors to the DSB, since knockdown of *AGO2* impairs homologous recombination efficiency in mammalian cell lines (Wei et al., 2012). In support of this possibility, the AGO2/diRNA complex interacts with RAD51, a DDR factor, facilitating its recruitment to DNA damage sites (Gao et al., 2014) and this interaction relies on AGO2 phosphorylation by a yet to be identified kinase (Hu et al., 2021). The mentioned studies were performed using *in vitro* somatic cell cultures and this mechanism has not been shown during DSB repair in meiosis. However, TOPBP1, MDC1 and RAD51 have also

been implicated in various repair processes in prophase I, and particularly in the events that result in MSCI as previously mentioned. *In vivo*, analysis of *Dicer* and *Dgcr8* KO models suggest that small RNA pathways could participate in DSB resolution and/or meiotic recombination in mouse, since KO spermatocytes show high levels of chromosomal instability (Modzelewski et al., 2015). Nonetheless, post transcriptional gene regulation through canonical miRNA pathways could be involved as well. For example, evidence shows that key crossover genes in the testes such as Separase-REC8, *MLH1* and *RAD1* are regulated by specific miRNAs (202-3p, 188-3p and 10a respectively) (Chen et al., 2022; Gao et al., 2014; Hilz, Modzelewski, Cohen, & Grimson, 2016; Song et al., 2017). Much research is needed to dissect a specific mechanism by which small RNA pathways could mediate recruitment of DDR factors and thus, influence crossover regulation and/or direct regulation of crossover machinery.

As described elsewhere within this volume, the multi-step process of crossover establishment must be tightly regulated, both temporally and spatially, to ensure correct chromosome segregation. It is well established, for example, that placement of crossovers in the vicinity of centromeres negatively influences meiotic chromosome segregation (Hassold & Hunt, 2001; Koehler, Hawley, Sherman, & Hassold, 1996). Interestingly, *S. pombe* KO mutants for Dcr1, Ago1 or the histone methyltransferase Clr4, show increased centromeric recombination (Ellermeier et al., 2010), suggesting participation of a small RNA pathway in crossover establishment through chromatin remodeling. New studies in the filamentous Ascomycete, *Sordaria macrospora*, have found that loss of Argonaute or Dicer results in increased chromosome length and altered chromatin loop/axis ratios along with increased crossover foci (Girard et al., 2021), also supporting a role of small RNAs in crossover formation through regulation of chromatin ultrastructure. Moreover, analysis of *Sordaria* KO model for Sms2 shows that proper localization of the E3 ligase Hei10 to crossovers sites relies upon presence of this Argonaute. Hei10 has been postulated as a candidate for signal integration by sensing state of SC structure formation and specific crossover events associated with recruitment of crossover factors (de Muyt et al., 2014). However, whether Hei10 interacts with Sms2 and both participate in SC formation, or recruitment of pro-crossover factors is not known. More studies in this model organism could provide mechanistic insights on whether small RNAs participate in meiotic recombination in *Sordaria*, or whether Sms2 behaves in a

non-canonical fashion by directly interacting with chromatin. Nonetheless, this leading study suggests that meiotic recombination, one of the hallmarks of meiosis might also be regulated by small RNA pathways.

8. Concluding remarks

Gene regulation through small RNA pathways has been implicated in a diverse repertoire of meiosis associated processes. The study of the structure, localization and mechanism of action of common RBP protein effectors such as Dicer and Argonaute can shed light on the roles for these pathways in animal meiosis. Molecular insights coming from model organisms such as yeast, have made tremendous contributions to the area. The finding of a RITS complex able to directly influence transcription during the assembly of heterochromatin in *S. pombe* expanded possible roles for small RNA pathways as chromatin remodelers during meiosis in multicellular organisms. It is clear from studies in *C. elegans*, where RNAi was discovered more than two decades ago, and with a demonstrated nuclear extensive small RNA regulatory network, that chromatin dynamics are regulated by small RNA pathways during meiosis. However, the study of these processes in mammalian meiosis is, largely, lacking. Studies coming from *in vitro* somatic cell culture systems are promising, since they allowed for mechanistic studies of the roles of small RNA pathways in shared processes between meiosis and mitosis such as DNA damage response, DSB and chromatin remodeling. The lack of a reproducible *in vitro* meiosis system limits our studies of meiosis associated processes to *in vivo* approaches. Nonetheless, work discussed here provides important clues such as DICER and AGO localization to the nucleus of mammalian meiocytes, participation of these proteins in key meiotic processes such as MSUC, MSCI, PCH formation and DSB resolution that are key starting points to unravel the non-canonical roles for small RNA pathways in mammal meiosis.

It is interesting to note that presence of unpaired chromatin during chromosome synapsis is detrimental for meiosis across multiple organisms, resulting in the evolution of silencing mechanisms that appear to involve common pathways. Chromatin remodeling during the establishment of MSUC in species with dimorphic sex chromosomes might respond to several different mechanisms; moreover, it is important to acknowledge that MSUC itself is a poorly understood process. It is known that MSUC and MSCI share common effectors, such as DDR proteins, and thus it seems plausible that roles for small RNA pathways could be similar between them.

Many studies using mouse KO models for *Dicer*, *Dgrc8 and Ago4* have shown that these genes are key to the localization of histone repressive marks to unsynapsed chromatin, indicating the small RNA pathways are a key mechanism involved in this meiotic silencing. In order to further our understanding of this process, we need to elucidate the molecular interactions between small RNA pathway components and chromatin repressive marks. This leads to several questions: Is there a RITS or RITS-like complex in mammals that recruits chromatin modifiers to the unsynapsed chromosomal regions? Or, are Argonaute proteins mediating chromatin silencing through recruitment or spreading of DDR signals to the unsynapsed chromatin? (Fig. 4). Since MSCI is a specialized form of MSUC and most of the, albeit limited, evidence comes from the study of sex chromosome silencing in mouse pachytene spermatocytes, a detailed proteomic and RNA sequencing of the components of the sex body would contribute to solving some of these questions. The study of Argonaute proteins during meiosis is also a useful tool that could provide a list of protein and small RNA binding partners and thus, start to reveal their role in chromatin condensation.

A current challenge in the field will be differentiate between classical post-transcriptional silencing involvement of small RNA pathways in meiosis from a potential role in transcriptional regulation, and to describe the molecular components behind such a mechanism. As gene regulation in animals is ensured by an integrated network of pathways, perturbations induced by gene mutations can often be compensated through alternative pathways. As a consequence of this complexity, KO models of RBPs or a particular small RNAs do not necessarily exhibit a phenotype that reflects their true function in this context. Thus, finding the components of the pathways is likely to be challenging. Sequencing efforts using model organisms allow to identify a subset of transcripts associated with a given small RNA component. However, identifying which of the differentially expressed transcripts are direct targets of small RNAs and which of them are being differentially expressed because of other disrupted cellular processes is very challenging. In addition, annotation of small RNAs is a growing process, making identification arduous. Future studies will take advantage of our growing ability to sequence and identify small RNAs, in particular those physically associated with specific Argonaute proteins, along with their subcellular localization.

The germ cell represents a fascinating model to study small RNA pathways that will not only contribute to our understanding of silencing in the

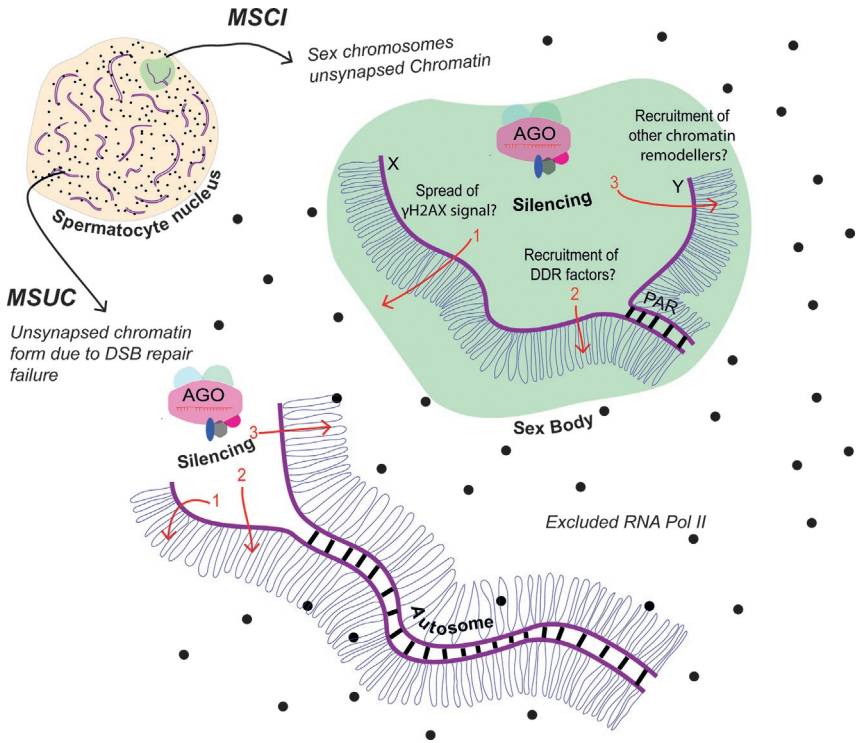

Fig. 4 Proposed model for a role of small RNAs and Argonaute proteins in Meiotic silencing of unsynapsed chromatin (MSUC) in male mammal meiosis. AGO proteins could mediate silencing of unsynapsed chromatin regions in autosomes formed due to double strand break (DSB) failure, and in the sex body during Pachynema by regulating the localization and spreading of γH2AX (1) or recruitment of DNA damage repair factors (DDR, 2) to the chromatin. On the other hand, it is possible that small RNA-AGO complexes interact directly with chromatin to regulate the accumulation of repressive histone marks (3) in a similar mechanism to PCH formation in *S. pombe*, where an RNA-induced transcriptional gene silencing complex (RITS) mediates recruitment of histone modifiers. MSCI: Meiotic sex chromosome inactivation. PAR: pseudo autosomal region

complex processes that underlie prophase I, but also, will sum to our knowledge on involvement of small RNA pathways as transcriptional regulators, besides their well-known role in posttranscriptional gene silencing.

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References

Abe, H., Alavattam, K. G., Hu, Y. C., Pang, Q., Andreassen, P. R., Hegde, R. S., et al. (2020). The initiation of meiotic sex chromosome inactivation sequesters DNA damage signaling from autosomes in mouse spermatogenesis. *Current Biology*, *30*(3), 408–420. e5. https://doi.org/10.1016/j.cub.2019.11.064.

Acuña, L. I. G., Nazer, E., Rodríguez-Seguí, S. A., Pozzi, B., Buggiano, V., Marasco, L. E., et al. (2020). Nuclear role for human Argonaute-1 as an estrogen-dependent transcription coactivator. *The Journal of Cell Biology*, *219*(9). https://doi.org/10.1083/JCB.201908097.

Andric, V., & Rougemaille, M. (2021). Long non-coding RNAs in the control of gametogenesis: Lessons from fission yeast. *Noncoding RNA*, 7(2), 34. MDPI AG. https://doi.org/10.3390/ncrna7020034.

Aramayo, R., & Metzenberg, R. L. (1996). Meiotic transvection in fungi. *Cell*, *86*(1), 103–113. https://doi.org/10.1016/S0092-8674(00)80081-1.

Azzam, G., Smibert, P., Lai, E. C., & Liu, J. L. (2012). Drosophila Argonaute 1 and its miRNA biogenesis partners are required for oocyte formation and germline cell division. *Developmental Biology*, *365*(2), 384–394. https://doi.org/10.1016/J.YDBIO.2012.03.005.

Baarends, W. M., Wassenaar, E., van der Laan, R., Hoogerbrugge, J., Sleddens-Linkels, E., Hoeijmakers, J. H. J., et al. (2005). Silencing of unpaired chromatin and histone H2A ubiquitination in mammalian meiosis. *Molecular and Cellular Biology*, *25*(3), 1041–1053. https://doi.org/10.1128/MCB.25.3.1041-1053.2005/FORMAT/EPUB.

Bartel, D. P. (2018). Metazoan MicroRNAs. *Cell*, *173*(1), 20–51. https://doi.org/10.1016/J.CELL.2018.03.006/ATTACHMENT/18013F94-01BD-4864-BD8E-C35285C19442/MMC1.XLSX.

Bhattacharjee, S., Roche, B., & Martienssen, R. A. (2019). RNA-induced initiation of transcriptional silencing (RITS) complex structure and function. *RNA Biology*, *16*(9), 1133–1146. Taylor and Francis Inc. https://doi.org/10.1080/15476286.2019.1621624.

Blanchard, D., Parameswaran, P., Lopez-Molina, J., Gent, J., Saynuk, J. F., & Fire, A. (2011). On the nature of in vivo requirements for rde-4 in RNAi and developmental pathways in C. elegans. *RNA Biology*, *8*(3), 458. https://doi.org/10.4161/RNA.8.3.14657.

Bose, M., Barman, B., Goswami, A., & Bhattacharyya, S. N. (2017). Spatiotemporal uncoupling of MicroRNA-mediated translational repression and target RNA degradation controls MicroRNP recycling in mammalian cells. *Molecular and Cellular Biology*, *37*(4). https://doi.org/10.1128/MCB.00464-16.

Brar, G. A., & Amon, A. (2008). Emerging roles for centromeres in meiosis I chromosome segregation. *Nature Reviews. Genetics*, *9*(12), 899–910. https://doi.org/10.1038/nrg2454.

Bråte, J., Neumann, R. S., Fromm, B., Haraldsen, A. A. B., Tarver, J. E., Suga, H., et al. (2018). Unicellular origin of the animal MicroRNA machinery. *Current Biology*, *28*(20). https://doi.org/10.1016/J.CUB.2018.08.018. 3288-3295.e5.

Briskin, D., Wang, P. Y., & Bartel, D. P. (2020). The biochemical basis for the cooperative action of microRNAs. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(30), 17764–17774. https://doi.org/10.1073/PNAS.1920404117/SUPPL_FILE/PNAS.1920404117.SAPP.PDF.

Burger, K., Schlackow, M., Potts, M., Hester, S., Mohammed, S., & Gullerova, M. (2017). Nuclear phosphorylated Dicer processes double-stranded RNA in response to DNA damage. *The Journal of Cell Biology*, *216*(8), 2373–2389. https://doi.org/10.1083/JCB.201612131.

Büssing, I., Yang, J. S., Lai, E. C., & Grohans, H. (2010). The nuclear export receptor XPO-1 supports primary miRNA processing in C. elegans and Drosophila. *The EMBO Journal*, *29*(11), 1830–1839. https://doi.org/10.1038/EMBOJ.2010.82.

Carthew, R. W., & Sontheimer, E. J. (2009). *Origins and mechanisms of miRNAs and siRNAs*. https://doi.org/10.1016/j.cell.2009.01.035.

Chapman, E., van Nues, R., Theron, E., & Bayne, E. H. (2020). Mkt1 is required for RNAi-mediated silencing and establishment of heterochromatin in fission yeast. *Nucleic Acids Research*, *48*(3), 1239–1253. https://doi.org/10.1093/NAR/GKZ1157.

Checchi, P. M., & Engebrecht, J. A. (2011). Caenorhabditis elegans histone methyltransferase MET-2 shields the male X chromosome from checkpoint machinery and mediates meiotic sex chromosome inactivation. *PLoS Genetics*, 7(9), e1002267. https://doi.org/10.1371/JOURNAL.PGEN.1002267.

Chen, J., Gao, C., Luo, M., Zheng, C., Lin, X, Nig, Y., et al. (2022). MicroRNA-202 safeguards meiotic progression by preventing premature SEPARASE-mediated REC8 cleavage. *EMBO Reports*, e54298. https://doi.org/10.15252/embr.202154298. Epub ahead of print.

Chendrimada, T. P., Gregory, R. I., Kumaraswamy, E., Norman, J., Cooch, N., Nishikura, K., et al. (2005). TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature*, *436*(7051), 740–744. https://doi.org/10.1038/NATURE03868.

Claycomb, J. M., Batista, P. J., Pang, K. M., Gu, W., Vasale, J. J., van Wolfswinkel, J. C., et al. (2009). The Argonaute CSR-1 and its 22G-RNA cofactors are required for holocentric chromosome segregation. *Cell*, *139*(1), 123–134. https://doi.org/10.1016/J.CELL.2009.09.014.

Corley, M., Burns, M. C., & Yeo, G. W. (2020). How RNA-binding proteins interact with RNA: Molecules and mechanisms. *Molecular Cell*, *78*(1), 9–29. Cell Press. https://doi.org/10.1016/j.molcel.2020.03.011.

Davis, G. M., Tu, S., Anderson, J. W. T., Colson, R. N., Gunzburg, M. J., Francisco, M. A., et al. (2018). The TRIM-NHL protein NHL-2 is a co-factor in the nuclear and somatic RNAi pathways in C. elegans. *eLife*, 7, e35478. https://doi.org/10.7554/ELIFE.35478.

de Jong, D., Eitel, M., Jakob, W., Osigus, H. J., Hadrys, H., DeSalle, R., et al. (2009). Multiple dicer genes in the early-diverging metazoa. *Molecular Biology and Evolution*, *26*(6), 1333–1340. https://doi.org/10.1093/MOLBEV/MSP042.

de Muyt, A., Zhang, L., Piolot, T., Kleckner, N., Espagne, E., & Zickler, D. (2014). E3 ligase Hei10: A multifaceted structure-based signaling molecule with roles within and beyond meiosis. *Genes & Development*, *28*(10), 1111–1123. https://doi.org/10.1101/GAD.240408.114.

Decker, L. M., Boone, E. C., Xiao, H., Shanker, B. S., Boone, S. F., Kingston, S. L., et al. (2015). Complex formation of RNA silencing proteins in the perinuclear region of Neurospora crassa. *Genetics*, *199*(4), 1017–1021. https://doi.org/10.1534/genetics.115.174623.

Duchaine, T. F., Wohlschlegel, J. A., Kennedy, S., Bei, Y., Conte, D., Pang, K. M., et al. (2006). Functional proteomics reveals the biochemical niche of C. elegans DCR-1 in multiple small-RNA-mediated pathways. *Cell*, *124*(2), 343–354. https://doi.org/10.1016/J.CELL.2005.11.036.

Eichhorn, S. W., Guo, H., McGeary, S. E., Rodriguez-Mias, R. A., Shin, C., Baek, D., et al. (2014). mRNA destabilization is the dominant effect of mammalian MicroRNAs by the time substantial repression ensues. *Molecular Cell*, *56*(1), 104–115. https://doi.org/10.1016/J.MOLCEL.2014.08.028.

ElInati, E., Russell, H. R., Ojarikre, O. A., Sangrithi, M., Hirota, T., de Rooij, D. G., et al. (2017). DNA damage response protein TOPBP1 regulates X chromosome silencing in the mammalian germ line. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(47), 12536–12541. https://doi.org/10.1073/pnas.1712530114.

Ellermeier, C., Higuchi, E. C., Phadnis, N., Holm, L., Geelhood, J. L., Thon, G., et al. (2010). RNAi and heterochromatin repress centromeric meiotic recombination. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(19), 8701–8705. https://doi.org/10.1073/pnas.0914160107.

Eulalio, A., Tritschler, F., & Izaurralde, E. (2009). The GW182 protein family in animal cells: new insights into domains required for miRNA-mediated gene silencing. *RNA*, *15*(8), 1433. https://doi.org/10.1261/RNA.1703809.

Fields, B. D., & Kennedy, S. (2019). Chromatin compaction by small RNAs and the nuclear RNAi machinery in C. elegans. *Scientific Reports*, *9*(1), 1–9. https://doi.org/10.1038/s41598-019-45052-y.

Filipowicz, W., Bhattacharyya, S. N., & Sonenberg, N. (2008). Mechanisms of post-transcriptional regulation by microRNAs: Are the answers in sight? *Nature Reviews. Genetics*, *9*(2), 102–114. https://doi.org/10.1038/NRG2290.

Fioriniello, S., Marano, D., Fiorillo, F., D'esposito, M., & Ragione, F. D. (2020). Epigenetic factors that control pericentric heterochromatin organization in mammals. *Gene*, *11*(6). MDPI AG. https://doi.org/10.3390/genes11060595.

Förstemann, K., Tomari, Y., Du, T., Vagin, V. V., Denli, A. M., Bratu, D. P., et al. (2005). Normal microRNA maturation and germ-line stem cell maintenance requires Loquacious, a double-stranded RNA-binding domain protein. *PLoS Biology*, *3*(7), 1187–1201. https://doi.org/10.1371/JOURNAL.PBIO.0030236.

Fragkos, M., Barra, V., Egger, T., Bordignon, B., Lemacon, D., Naim, V., et al. (2019). Dicer prevents genome instability in response to replication stress. *Oncotarget*, *10*(43), 4407–4423. https://doi.org/10.18632/ONCOTARGET.27034.

Francia, S., Michelini, F., Saxena, A., Tang, D., de Hoon, M., Anelli, V., et al. (2012). Site-specific DICER and DROSHA RNA products control the DNA-damage response. *Nature*, *488*(7410), 231–235. https://doi.org/10.1038/nature11179.

Fukagawa, T., Nogami, M., Yoshikawa, M., Ikeno, M., Okazaki, T., Takami, Y., et al. (2004). Dicer is essential for formation of the heterochromatin structure in vertebrate cells. *Nature Cell Biology*, *6*(8), 784–791. https://doi.org/10.1038/NCB1155.

Gao, M., Wei, W., Li, M. M., Wu, Y. S., Ba, Z., Jin, K. X., et al. (2014). Ago2 facilitates Rad51 recruitment and DNA double-strand break repair by homologous recombination. *Cell Research*, *24*(5), 532–541. https://doi.org/10.1038/cr.2014.36.

Gerbasi, V. R., Preall, J. B., Golden, D. E., Powell, D. W., Cummins, T. D., & Sontheimer, E. J. (2011). Blanks, a nuclear siRNA/dsRNA-binding complex component, is required for Drosophila spermiogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(8), 3204–3209. https://doi.org/10.1073/PNAS.1009781108.

Gerstberger, S., Hafner, M., & Tuschl, T. (2014). A census of human RNA-binding proteins. *Nature Reviews. Genetics*, *15*(12), 829–845. https://doi.org/10.1038/nrg3813.

Ghildiyal, M., & Zamore, P. D. (2009). Small silencing RNAs: An expanding universe. *Nature Reviews. Genetics*, *10*(2), 94–108. https://doi.org/10.1038/nrg2504.

Giauque, C. C., & Bickel, S. E. (2016). Heterochromatin-associated proteins hp1a and piwi collaborate to maintain the association of achiasmate homologs in drosophila oocytes. *Genetics*, *203*(1), 173–189. https://doi.org/10.1534/GENETICS.115.186460.

Girard, C., Budin, K., Boisnard, S., Zhang, L., Debuchy, R., Zickler, D., et al. (2021). RNAi-related dicer and argonaute proteins play critical roles for meiocyte formation, chromosome-axes lengths and crossover patterning in the fungus Sordaria macrospora. *Frontiers in Cell and Development Biology*, *28*(9), 684108. https://doi.org/10.3389/fcell.2021.684108.

Golden, D. E., Gerbasi, V. R., & Sontheimer, E. J. (2008). An inside job for siRNAs. *Molecular Cell*, *31*(3), 309. https://doi.org/10.1016/J.MOLCEL.2008.07.008.

Gray, S., & Cohen, P. E. (2016). Control of meiotic crossovers: From double-strand break formation to designation. *Annual Review of Genetics*, *50*, 175–210. Annual Reviews Inc. https://doi.org/10.1146/annurev-genet-120215-035111.

Greenlee, A. R., Shiao, M. S., Snyder, E., Buaas, F. W., Gu, T., Stearns, T. M., et al. (2012). Deregulated sex chromosome gene expression with male germ cell-specific loss of Dicer1. *PLoS One*, 7(10), e46359. https://doi.org/10.1371/journal.pone.0046359.

Grimson, A., Srivastava, M., Fahey, B., Woodcroft, B. I., Chiang, R. H., King, N., et al. (2008). Early origins and evolution of microRNAs and Piwi-interacting RNAs in animals. *Nature*, *455*(7217), 1193–1197. https://doi.org/10.1038/nature07415.

Grive, K. J., Hu, Y., Shu, E., Grimson, A., Elemento, O., Grenier, J. K., et al. (2019). Dynamic transcriptome profiles within spermatogonial and spermatocyte populations during postnatal testis maturation revealed by single-cell sequencing. *PLoS Genetics*, *15*(3), e1007810. https://doi.org/10.1371/journal.pgen.1007810.

Guo, J., Grow, E. J., Mlcochova, H., Maher, G. J., Lindskog, C., Nie, X., et al. (2018). The adult human testis transcriptional cell atlas. *Cell Research*, *28*(12), 1141–1157. https://doi.org/10.1038/S41422-018-0099-2.

Ha, M., & Kim, V. N. (2014). Regulation of microRNA biogenesis. *Nature Reviews. Molecular Cell Biology*, *15*(8), 509–524. Nature Publishing Group. https://doi.org/10.1038/nrm3838.

Hall, I. M., Noma, K.-I., & Grewal, S. I. S. (n.d.). RNA interference machinery regulates chromosome dynamics during mitosis and meiosis in fission yeast. https://doi.org/10.1073/pnas.232688099.

Hammond, T. M. (2017). Sixteen years of meiotic silencing by unpaired DNA. *Advances in Genetics*, *97*, 1–42. https://doi.org/10.1016/bs.adgen.2016.11.001.

Hassold, T., & Hunt, P. (2001). To err (meiotically) is human: The genesis of human aneuploidy. *Nature Reviews. Genetics*, *2*(4), 280–291. https://doi.org/10.1038/35066065.

Hentze, M. W., Castello, A., Schwarzl, T., & Preiss, T. (2018). A brave new world of RNA-binding proteins. *Nature Reviews. Molecular Cell Biology*, *19*(5), 327–341. Nature Publishing Group. https://doi.org/10.1038/nrm.2017.130.

Hilz, S., Modzelewski, A. J., Cohen, P. E., & Grimson, A. (2016). The roles of microRNAs and siRNAs in mammalian spermatogenesis. *Development*, *143*(17), 3061–3073. https://doi.org/10.1242/DEV.136721.

Hombach, S., Kretz, M., Hombach, S., & Kretz, M. (2016). Non-coding RNAs: Classification, biology and functioning. *Advances in Experimental Medicine and Biology*, *937*, 3–17. https://doi.org/10.1007/978-3-319-42059-2_1.

Hu, X., Li, Y., Zhang, T., Li, L., Chen, S., Wu, X., et al. (2021). Phosphorylation of Ago2 is required for its role in DNA double-strand break repair. *Journal of Genetics and Genomics*, *48*(4), 333–340. https://doi.org/10.1016/J.JGG.2021.03.011.

Hubstenberger, A., Courel, M., Bénard, M., Souquere, S., Ernoult-Lange, M., Chouaib, R., et al. (2017). P-body purification reveals the condensation of repressed mRNA regulons. *Molecular Cell*, *68*(1). https://doi.org/10.1016/J.MOLCEL.2017.09.003. 144-157.e5.

Huo, X., Ji, L., Zhang, Y., Lv, P., Cao, X., Wang, Q., et al. (2020). The nuclear matrix protein SAFB cooperates with major satellite RNAs to stabilize heterochromatin architecture partially through phase separation. *Molecular Cell*, 77(2). https://doi.org/10.1016/J.MOLCEL.2019.10.001. 368-383.e7.

Hutvagner, G., & Simard, M. J. (2008). Argonaute proteins: key players in RNA silencing. *Nature Reviews. Molecular Cell Biology*, *9*(1), 22–32. https://doi.org/10.1038/nrm2321.

Ichijima, Y., Ichijima, M., Lou, Z., Nussenzweig, A., Daniel Camerini-Otero, R., Chen, J., et al. (2011). MDC1 directs chromosome-wide silencing of the sex chromosomes in male germ cells. *Genes & Development*, *25*(9), 959–971. https://doi.org/10.1101/GAD.2030811.

Jaglarz, M. K., Kloc, M., Jankowska, W., Szymanska, B., & Bilinski, S. M. (2011). Nuage morphogenesis becomes more complex: Two translocation pathways and two forms of nuage coexist in Drosophila germline syncytia. *Cell and Tissue Research*, *344*(1), 169–181. https://doi.org/10.1007/S00441-011-1145-2.

Jiang, F., Ye, X., Liu, X., Fincher, L., McKearin, D., & Liu, Q. (2005). Dicer-1 and R3D1-L catalyze microRNA maturation in Drosophila. *Genes & Development*, *19*(14), 1674–1679. https://doi.org/10.1101/GAD.1334005.

Johnson, W. L., Yewdell, W. T., Bell, J. C., McNulty, S. M., Duda, Z., O'Neill, R. J., et al. (2017). RNA-dependent stabilization of SUV39H1 at constitutive heterochromatin. *eLife*, *6*. https://doi.org/10.7554/ELIFE.25299.

Jonas, S., & Izaurralde, E. (2015). Towards a molecular understanding of microRNA-mediated gene silencing. *Nature Reviews. Genetics*, *16*(7), 421–433. https://doi.org/10.1038/NRG3965.

Kanellopoulou, C., Muljo, S. A., Kung, A. L., Ganesan, S., Drapkin, R., Jenuwein, T., et al. (2005). Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. *Genes & Development*, *19*(4), 489–501. https://doi.org/10.1101/gad.1248505.

Kelly, W. G., Schaner, C. E., Dernburg, A. F., Lee, M. H., Kim, S. K., Villeneuve, A. M., et al. (2002). X-chromosome silencing in the germline of C. elegans. *Development*, *129*(2), 479–492. https://doi.org/10.1242/dev.129.2.479.

Khalil, A. M., & Driscoll, D. J. (2010). Epigenetic regulation of pericentromeric heterochromatin during mammalian meiosis. *Cytogenetic and Genome Research*, *129*(4), 280–289. https://doi.org/10.1159/000315903.

Kim, V. N. (2004). MicroRNA precursors in motion: exportin-5 mediates their nuclear export. *Trends in Cell Biology*, *14*(4), 156–159. https://doi.org/10.1016/J.TCB.2004.02.006.

Koehler, K. E., Hawley, R. S., Sherman, S., & Hassold, T. (1996). Recombination and nondisjunction in humans and flies. *Human Molecular Genetics*, 1495–1504. 5 Spec No(REVIEW). https://doi.org/10.1093/HMG/5.SUPPLEMENT_1.1495.

Kotaja, N. (2014). MicroRNAs and spermatogenesis. *Fertility and Sterility*, *101*(6), 1552–1562. https://doi.org/10.1016/J.FERTNSTERT.2014.04.025.

Lai, E. C. (2002). Micro RNAs are complementary to 3′ UTR sequence motifs that mediate negative post-transcriptional regulation. *Nature Genetics*, *30*(4), 363–364. https://doi.org/10.1038/ng865.

Lam, K. W. G., Brick, K., Cheng, G., Pratto, F., & Camerini-Otero, R. D. (2019). Cell-type-specific genomics reveals histone modification dynamics in mammalian meiosis. *Nature Communications*, *10*(1), 1–11. https://doi.org/10.1038/s41467-019-11820-7.

Lamelza, P., & Bhalla, N. (2012). Histone methyltransferases MES-4 and MET-1 promote meiotic checkpoint activation in Caenorhabditis elegans. *PLoS Genetics*, *8*(11), e1003089. https://doi.org/10.1371/JOURNAL.PGEN.1003089.

Lee, Y., Ahn, C., Han, J., Choi, H., Kim, J., Yim, J., et al. (2003). The nuclear RNase III Drosha initiates microRNA processing. *Nature*, *425*(6956), 415–419. https://doi.org/10.1038/NATURE01957.

Lee, Y., Han, J., Yeom, K. H., Jin, H., & Kim, V. N. (2006). Drosha in primary microRNA processing. *Cold Spring Harbor Symposia on Quantitative Biology*, *71*, 51–57. https://doi.org/10.1101/SQB.2006.71.041.

Lee, Y. S., Nakahara, K., Pham, J. W., Kim, K., He, Z., Sontheimer, E. J., et al. (2004). Distinct roles for Drosophila Dicer-1 and Dicer-2 in the siRNA/miRNA silencing pathways. *Cell*, *117*(1), 69–81. https://doi.org/10.1016/S0092-8674(04)00261-2.

Lee, H., Zhang, Z., & Krause, H. M. (2019). Long noncoding RNAs and repetitive elements: Junk or intimate evolutionary partners? *Trends in Genetics*, *35*(12), 892–902. https://doi.org/10.1016/J.TIG.2019.09.006.

Leuschner, P. J. F., Obernosterer, G., & Martinez, J. (2005). MicroRNAs: Loquacious speaks out. *Current Biology*, *15*(15), R603–R605. https://doi.org/10.1016/J.CUB.2005.07.044.

Li, L., Gu, W., Liang, C., Liu, Q., Mello, C. C., & Liu, Y. (2012). The translin-TRAX complex (C3PO) is a ribonuclease in tRNA processing. *Nature Structural & Molecular Biology*, *19*(8), 824–830. https://doi.org/10.1038/nsmb.2337.

Lin, C. J., Hu, F., Dubruille, R., Vedanayagam, J., Wen, J., Smibert, P., et al. (2018). The hpRNA/RNAi pathway is essential to resolve intragenomic conflict in the Drosophila male germline. *Developmental Cell*, *46*(3). https://doi.org/10.1016/J.DEVCEL.2018.07.004. 316-326.e5.

Liu, J., Carmell, M. A., Rivas, F. V., Marsden, C. G., Thomson, J. M., Song, J. J., et al. (2004). Argonaute2 is the catalytic engine of mammalian RNAi. *Science (New York, N.Y.)*, *305*(5689), 1437–1441. https://doi.org/10.1126/SCIENCE.1102513.

Liu, X., Li, W., Yang, Y., Chen, K., Li, Y., Zhu, X., et al. (2021). Transcriptome profiling of the ovarian cells at the single-cell resolution in adult Asian seabass. *Frontiers in Cell and Development Biology*, *9*, 560. https://doi.org/10.3389/FCELL.2021.647892/BIBTEX.

Liu, H. C., Tang, Y., He, Z., & Rosenwaks, Z. (2010). Dicer is a key player in oocyte maturation. *Journal of Assisted Reproduction and Genetics*, *27*(9–10), 571–580. https://doi.org/10.1007/S10815-010-9456-X.

Lu, W. T., Hawley, B. R., Skalka, G. L., Baldock, R. A., Smith, E. M., Bader, A. S., et al. (2018). Drosha drives the formation of DNA:RNA hybrids around DNA break sites to facilitate DNA repair. *Nature Communications*, *9*(1), 1–13. https://doi.org/10.1038/s41467-018-02893-x.

Ma, J., Flemr, M., Stein, P., Berninger, P., Malik, R., Zavolan, M., et al. (2010). MicroRNA activity is suppressed in mouse oocytes. *Current Biology*, *20*(3), 265–270. https://doi.org/10.1016/J.CUB.2009.12.042.

Maida, Y., & Masutomi, K. (2011). RNA-dependent RNA polymerases in RNA silencing. *Biological Chemistry*, *392*(4), 299–304. https://doi.org/10.1515/BC.2011.035.

Maine, E. M. (2010). Meiotic silencing in Caenorhabditis elegans. *International Review of Cell and Molecular Biology*, *282*(C), 91–134. https://doi.org/10.1016/S1937-6448(10)82002-7.

Maine, E. M., Hauth, J., Ratliff, T., Vought, V. E., She, X., & Kelly, W. G. (2005). EGO-1, a putative RNA-dependent RNA polymerase, is required for heterochromatin assembly on unpaired DNA during C. elegans meiosis. *Current Biology*, *15*(21), 1972–1978. https://doi.org/10.1016/J.CUB.2005.09.049.

Mattiske, D. M., Han, L., & Mann, J. R. (2009). Meiotic maturation failure induced by DICER1 deficiency is derived from primary oocyte ooplasm. *Reproduction (Cambridge, England)*, *137*(4), 625–632. https://doi.org/10.1530/REP-08-0475.

McKee, B. D., & Handel, M. A. (1993). Sex chromosomes, recombination, and chromatin conformation. *Chromosoma*, *102*(2), 71–80. https://doi.org/10.1007/BF00356023.

Meikar, O., & Kotaja, N. (2014). Isolation of chromatoid bodies from mouse testis as a rich source of short RNAs. *Methods in Molecular Biology*, *1173*, 11–25. https://doi.org/10.1007/978-1-4939-0931-5_2.

Meiklejohn, C. D., Landeen, E. L., Cook, J. M., Kingan, S. B., & Presgraves, D. C. (2011). Sex chromosome-specific regulation in the Drosophila male germline but little evidence for chromosomal dosage compensation or meiotic inactivation. *PLoS Biology*, *9*(8), e1001126. https://doi.org/10.1371/JOURNAL.PBIO.1001126.

Miyata, K., Imai, Y., Hori, S., Nishio, M., Loo, T. M., Okada, R., et al. (2021). Pericentromeric noncoding RNA changes DNA binding of CTCF and inflammatory gene expression in senescence and cancer. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(35), e2025647118. https://doi.org/10.1073/PNAS.2025647118.

Modzelewski, A. J., Hilz, S., Crate, E. A., Schweidenback, C. T. H., Fogarty, E. A., Grenier, J. K., et al. (2015). Dgcr8 and Dicer are essential for sex chromosome integrity during meiosis in males. *Journal of Cell Science*, *128*(12), 2314–2327. https://doi.org/10.1242/JCS.167148/260400/AM/DGCR8-AND-DICER-ARE-ESSENTIAL-FOR-SEX-CHROMOSOME.

Modzelewski, A. J., Holmes, R. J., Hilz, S., Grimson, A., & Cohen, P. E. (2012). AGO4 regulates entry into meiosis and influences silencing of sex chromosomes in the male mouse germline. *Developmental Cell*, *23*(2), 251–264. https://doi.org/10.1016/j.devcel.2012.07.003.

Muirhead, C. A., & Presgraves, D. C. (2021). Satellite DNA-mediated diversification of a sex-ratio meiotic drive gene family in Drosophila. *Nature Ecology & Evolution*, *5*(12), 1604–1612. https://doi.org/10.1038/s41559-021-01543-8.

Mukherjee, N., & Mukherjee, C. (2021). Germ cell ribonucleoprotein granules in different clades of life: From insects to mammals. *Wiley Interdisciplinary Reviews. RNA*, *12*(4), e1642. https://doi.org/10.1002/WRNA.1642.

Müller, M., Fäh, T., Schaefer, M., Hermes, V., Luitz, J., Stalder, P., et al. (2022). AGO1 regulates pericentromeric regions in mouse embryonic stem cells. *Life Science Alliance*, *5*(6), e202101277. https://doi.org/10.26508/LSA.202101277.

Murat, F., Mbengue, N., Winge, S. B., Trefzer, T., Leushkin, E., Sepp, M., et al. (2021). The molecular evolution of spermatogenesis across mammals. *BioRxiv*, *2021*(11), 08.467712. https://doi.org/10.1101/2021.11.08.467712.

Murchison, E. P., Stein, P., Xuan, Z., Pan, H., Zhang, M. Q., Schultz, R. M., et al. (2007). Critical roles for Dicer in the female germline. *Genes & Development*, *21*(6), 682–693. https://doi.org/10.1101/GAD.1521307.

Nakamura, M., Ando, R., Nakazawa, T., Yudazono, T., Tsutsumi, N., Hatanaka, N., et al. (2007). Dicer-related drh-3 gene functions in germ-line development by maintenance of chromosomal integrity in Caenorhabditis elegans. *Genes to Cells*, *12*(9), 997–1010. https://doi.org/10.1111/J.1365-2443.2007.01111.X.

Namekawa, S. H., Park, P. J., Zhang, L. F., Shima, J. E., McCarrey, J. R., Griswold, M. D., et al. (2006). Postmeiotic sex chromatin in the male germline of mice. *Current Biology*, *16*(7), 660–667. https://doi.org/10.1016/J.CUB.2006.01.066.

Nitschko, V., Kunzelmann, S., Fröhlich, T., Fröhlich, F., Arnold, G. J., Orstemann, K. F., et al. (2020). Trafficking of siRNA precursors by the dsRBD protein Blanks in Drosophila. *Nucleic Acids Research*, *48*(7), 3906–3921. https://doi.org/10.1093/nar/gkaa072.

Ohle, C., Tesorero, R., Schermann, G., Dobrev, N., Sinning, I., & Fischer, T. (2016). Transient RNA-DNA Hybrids are required for efficient double-strand break repair. *Cell*, *167*(4). https://doi.org/10.1016/J.CELL.2016.10.001. 1001-1013.e7.

Okamura, K., Ishizuka, A., Siomi, H., & Siomi, M. C. (2004). Distinct roles for Argonaute proteins in small RNA-directed RNA cleavage pathways. *Genes & Development*, *18*(14), 1655–1666. https://doi.org/10.1101/GAD.1210204.

Onohara, Y., Fujiwara, T., Yasukochi, T., Himeno, M., & Yokota, S. (2010). Localization of mouse vasa homolog protein in chromatoid body and related nuage structures of mammalian spermatogenic cells during spermatogenesis. *Histochemistry and Cell Biology*, *133*(6), 627–639. https://doi.org/10.1007/S00418-010-0699-5/FIGURES/8.

Ouyang, J. P. T., & Seydoux, G. (2022 Jan). Nuage condensates: Accelerators or circuit breakers for sRNA silencing pathways? *RNA (New York, N.Y.)*, *28*(1), 58–66. https://doi.org/10.1261/RNA.079003.121. rna.079003.121.

Pal-Bhadra, M., Leibovitch, B. A., Gandhi, S. G., Rao, M., Bhadra, U., Birchler, J. A., et al. (2004). Heterochromatic silencing and HP1 localization in Drosophila are dependent on the RNAi machinery. *Science (New York, N.Y.)*, *303*(5658), 669–672. https://doi.org/10.1126/SCIENCE.1092653.

Park, J., Lee, H., Han, N., Kwak, S., Lee, H. T., Kim, J. H., et al. (2018). Long non-coding RNA ChRO1 facilitates ATRX/DAXX-dependent H3.3 deposition for transcription-associated heterochromatin reorganization. *Nucleic Acids Research*, *46*(22), 11759–11775. https://doi.org/10.1093/NAR/GKY923.

Park, M. S., Phan, H. D., Busch, F., Hinckley, S. H., Brackbill, J. A., Wysocki, V. H., et al. (2017). Human Argonaute3 has slicer activity. *Nucleic Acids Research*, *45*(20), 11867–11877. https://doi.org/10.1093/nar/gkx916.

Park, M. S., Sim, G. Y., Kehling, A. C., & Nakanishi, K. (2020). Human Argonaute2 and Argonaute3 are catalytically activated by different lengths of guide RNA. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(46), 28576–28578. https://doi.org/10.1073/pnas.2015026117.

Parker, G. S., Eckert, D. M., & Bass, B. L. (2006). RDE-4 preferentially binds long dsRNA and its dimerization is necessary for cleavage of dsRNA to siRNA. *RNA (New York, N.Y.)*, *12*(5), 807–818. https://doi.org/10.1261/RNA.2338706.

Parker, R., & Sheth, U. (2007). P bodies and the control of mRNA translation and degradation. *Molecular Cell*, *25*(5), 635–646. https://doi.org/10.1016/J.MOLCEL.2007.02.011.

Patel, P. H., Barbee, S. A., & Blankenship, J. T. (2016). GW-bodies and P-bodies constitute two separate pools of sequestered non-translating RNAs. *PLoS One*, *11*(3), e0150291. https://doi.org/10.1371/JOURNAL.PONE.0150291.

Perales, R., Pagano, D., Wan, G., Fields, B. D., Saltzman, A. L., & Kennedy, S. G. (2018). Transgenerational epigenetic inheritance is negatively regulated by the HERI-1 chromodomain protein. *Genetics*, *210*(4), 1287–1299. https://doi.org/10.1534/GENETICS.118.301456.

Quinn, J. J., & Chang, H. Y. (2016). Unique features of long non-coding RNA biogenesis and function. *Nature Reviews. Genetics*, *17*(1), 47–62. Nature Publishing Group. https://doi.org/10.1038/nrg.2015.10.

Ransohoff, J. D., Wei, Y., & Khavari, P. A. (2018). The functions and unique features of long intergenic non-coding RNA. *Nature Reviews. Molecular Cell Biology*, *19*(3), 143–157. Nature Publishing Group. https://doi.org/10.1038/nrm.2017.104.

Rappaport, Y., Achache, H., Falk, R., Murik, O., Ram, O., & Tzur, Y. B. (2021). Bisection of the X chromosome disrupts the initiation of chromosome silencing during meiosis in Caenorhabditis elegans. *Nature Communications*, *12*(1), 4802. https://doi.org/10.1038/s41467-021-24815-0.

Romero, Y., Meikar, O., Papaioannou, M. D., Conne, B., Grey, C., Weier, M., et al. (2011). Dicer1 Depletion in male germ cells leads to infertility due to cumulative meiotic and spermiogenic defects. *PLoS One*, *6*(10), e25241. https://doi.org/10.1371/JOURNAL.PONE.0025241.

Ruby, J. G., Jan, C. H., & Bartel, D. P. (2007). Intronic microRNA precursors that bypass Drosha processing. *Nature*, *448*(7149), 83–86. https://doi.org/10.1038/nature05983.

Russo, J., Harrington, A. W., & Steiniger, M. (2016). Antisense transcription of retrotransposons in Drosophila: an origin of endogenous small interfering RNA precursors. *Genetics*, *202*(1), 107–121. https://doi.org/10.1534/GENETICS.115.177196.

Saito, K., Ishizuka, A., Siomi, H., & Siomi, M. C. (2005). Processing of pre-microRNAs by the Dicer-1-Loquacious complex in Drosophila cells. *PLoS Biology*, *3*(7), 1202–1212. https://doi.org/10.1371/JOURNAL.PBIO.0030235.

Salim, U., Kumar, A., Kulshreshtha, R., & Vivekanandan, P. (2021). Biogenesis, characterization, and functions of mirtrons. *Wiley Interdisciplinary Reviews. RNA*, *13*(1), e1680. https://doi.org/10.1002/WRNA.1680.

Samarajeewa, D. A., Sauls, P. A., Sharp, K. J., Smith, Z. J., Xiao, H., Groskreutz, K. M., et al. (2014). Efficient detection of unpaired DNA requires a member of the rad54-like family of homologous recombination proteins. *Genetics*, *198*(3), 895–904. https://doi.org/10.1534/genetics.114.168187.

Schimenti, J. (2005). Synapsis or silence. *Nature Genetics*, *37*(1), 11–13. https://doi.org/10.1038/ng0105-11.

She, X., Xu, X., Fedotov, A., Kelly, W. G., & Maine, E. M. (2009). Regulation of heterochromatin assembly on unpaired chromosomes during Caenorhabditis elegans meiosis by components of a small RNA-mediated pathway. *PLoS Genetics*, *5*(8), e1000624. https://doi.org/10.1371/journal.pgen.1000624.

Shi, Z., Lim, C., Tran, V., Cui, K., Zhao, K., & Chen, X. (2020). Single-cyst transcriptome analysis of Drosophila male germline stem cell lineage. *Development*, *147*(8), dev184259. https://doi.org/10.1242/DEV.184259.

Shi, J., Ma, Y., Hua, H., Liu, Y., Li, W., Yu, H., et al. (2021). Dynamic histone H3 modifications regulate meiosis initiation via respiration. *Frontiers in Cell and Development Biology*, *9*, 683. https://doi.org/10.3389/FCELL.2021.646214/BIBTEX.

Song, W. Y., Meng, H., Wang, X. G., Jin, H. X., Yao, G. D., Shi, S. L., et al. (2017). Reduced microRNA-188-3p expression contributes to apoptosis of spermatogenic cells in patients with azoospermia. *Cell Proliferation*, *50*(1), e12297. https://doi.org/10.1111/CPR.12297.

Starega-Roslan, J., Witkos, T. M., Galka-Marciniak, P., & Krzyzosiak, W. J. (2015). Sequence features of Drosha and Dicer cleavage sites affect the complexity of isomiRs. *International Journal of Molecular Sciences*, *16*(4), 8110–8127. https://doi.org/10.3390/IJMS16048110.

Sugiyama, T., Cam, H., Verdel, A., Moazed, D., & Grewal, S. I. S. (2005). RNA-dependent RNA polymerase is an essential component of a self-enforcing loop coupling heterochromatin assembly to siRNA production. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(1), 152–157. https://doi.org/10.1073/PNAS.0407641102.

Suh, N., Baehner, L., Moltzahn, F., Melton, C., Shenoy, A., Chen, J., et al. (2010). MicroRNA function is globally suppressed in mouse oocytes and early embryos. *Current Biology*, *20*(3), 271. https://doi.org/10.1016/J.CUB.2009.12.044.

Sundby, A. E., Molnar, R. I., & Claycomb, J. M. (2021). Connecting the dots: Linking Caenorhabditis elegans small RNA pathways and germ granules. *Trends in Cell Biology*, *31*(5), 387–401. https://doi.org/10.1016/J.TCB.2020.12.012.

Swahari, V., Nakamura, A., Baran-Gale, J., Garcia, I., Crowther, A. J., Sons, R., et al. (2016). Essential function of dicer in resolving DNA damage in the rapidly dividing cells of the developing and malignant cerebellum. *Cell Reports*, *14*(2), 216–224. https://doi.org/10.1016/J.CELREP.2015.12.037.

Swarts, D. C., Makarova, K., Wang, Y., Nakanishi, K., Ketting, R. F., Koonin, E. V., et al. (2014). The evolutionary journey of Argonaute proteins. *Nature Structural & Molecular Biology*, *21*(9), 743–753. Nature Publishing Group. https://doi.org/10.1038/nsmb.2879.

Takimoto, K., Wakiyama, M., & Yokoyama, S. (2009). Mammalian GW182 contains multiple Argonaute-binding sites and functions in microRNA-mediated translational repression. *RNA (New York, N.Y.)*, *15*(6), 1078–1089. https://doi.org/10.1261/RNA.1363109.

Talbert, P. B., & Henikoff, S. (2020). What makes a centromere? *Experimental Cell Research*, *389*(2), 111895. https://doi.org/10.1016/J.YEXCR.2020.111895.

Taylor, D. H., Chu, E. T. J., Spektor, R., & Soloway, P. D. (2015). Long non-coding RNA regulation of reproduction and development. *Molecular Reproduction and Development*, *82*(12), 932–956. https://doi.org/10.1002/mrd.22581.

Thomson, T., & Lin, H. (2009). The biogenesis and function of PIWI proteins and piRNAs: Progress and prospect. *Annual Review of Cell and Developmental Biology*, *25*, 355–376. https://doi.org/10.1146/annurev.cellbio.24.110707.175327.

Turner, J. M. A. (2007). Meiotic sex chromosome inactivation. *Development*, *134*(10), 1823–1831. https://doi.org/10.1242/dev.000018.

Turner, J. M. A. (2015). Meiotic silencing in mammals. *Annual Review of Genetics*, *49*, 395–412. https://doi.org/10.1146/ANNUREV-GENET-112414-055145.

Turner, J. M. A., Aprelikova, O., Xu, X., Wang, R., Kim, S., Chandramouli, G. V. R., et al. (2004). BRCA1, histone H2AX phosphorylation, and male meiotic sex chromosome inactivation. *Current Biology*, *14*(23), 2135–2142. https://doi.org/10.1016/J.CUB.2004.11.032.

Turner, J. M. A., Mahadevaiah, S. K., Fernandez-Capetillo, O., Nussenzweig, A., Xu, X., Deng, C. X., et al. (2004). Silencing of unsynapsed meiotic chromosomes in the mouse. *Nature Genetics*, *37*(1), 41–47. https://doi.org/10.1038/ng1484.

Tzur, Y. B., Winter, E., Gao, J., Hashimshony, T., Yanai, I., & Colaiácovo, M. P. (2018). Spatiotemporal gene expression analysis of the Caenorhabditis elegans germline uncovers a syncytial expression switch. *Genetics*, *210*(2), 587–605. https://doi.org/10.1534/GENETICS.118.301315.

Vedanayagam, J., Lin, C. J., & Lai, E. C. (2021). Rapid evolutionary dynamics of an expanding family of meiotic drive factors and their hpRNA suppressors. *Nature Ecology & Evolution*, *5*(12), 1613–1623. https://doi.org/10.1038/s41559-021-01592-z.

Ventelä, S., Toppari, J., & Parvinen, M. (2003). Intercellular organelle traffic through cytoplasmic bridges in early spermatids of the rat: mechanisms of haploid gene product sharing. *Molecular Biology of the Cell*, *14*(7), 2768. https://doi.org/10.1091/MBC.E02-10-0647.

Verdel, A., Jia, S., Gerber, S., Sugiyama, T., Gygi, S., Grewal, S. I. S., et al. (2004). RNAi-mediated targeting of heterochromatin by the RITS complex. *Science (New York, N.Y.)*, *303*(5658), 672–676. https://doi.org/10.1126/SCIENCE.1093686.

Vibranovski, M. D. (2014). Meiotic sex chromosome inactivation in Drosophila. *Journal of Genomics*, *2*, 104–117. https://doi.org/10.7150/jgen.8178.

Volpe, T., Schramke, V., Hamilton, G. L., White, S. A., Teng, G., Martienssen, R. A., et al. (2003). RNA interference is required for normal centromere function in fission yeast. *Chromosome Research*, *11*(2), 137–146. https://doi.org/10.1023/A:1022815931524.

Voronina, E., Seydoux, G., Sassone-Corsi, P., & Nagamori, I. (2011). RNA granules in germ cells. *Cold Spring Harbor Perspectives in Biology*, *3*(12), a002774. https://doi.org/10.1101/CSHPERSPECT.A002774.

Wang, L., & Xu, C. (2015). Role of microRNAs in mammalian spermatogenesis and testicular germ cell tumors. *Reproduction*, *149*(3), R127–R137. https://doi.org/10.1530/REP-14-0239.

Wang, L., Xu, Z., Khawar, M. B., Liu, C., & Li, W. (2017). The histone codes for meiosis. *Reproduction (Cambridge, England)*, *154*(3), R65–R79. https://doi.org/10.1530/REP-17-0153.

Wedeles, C. J., Wu, M. Z., & Claycomb, J. M. (2013). A multitasking Argonaute: Exploring the many facets of C. elegans CSR-1. *Chromosome Research*, *21*(6–7), 573–586. https://doi.org/10.1007/S10577-013-9383-7/TABLES/1.

Wee, L. M., Flores-Jasso, C. F., Salomon, W. E., & Zamore, P. D. (2012). Argonaute divides its RNA guide into domains with distinct functions and RNA-binding properties. *Cell*, *151*(5), 1055–1067. https://doi.org/10.1016/J.CELL.2012.10.036.

Wei, W., Ba, Z., Gao, M., Wu, Y., Ma, Y., Amiard, S., et al. (2012). A role for small RNAs in DNA double-strand break repair. *Cell*, *149*(1), 101–112. https://doi.org/10.1016/j.cell.2012.03.002.

Weirich, S., Khella, M. S., & Jeltsch, A. (2021). Structure, activity and function of the suv39h1 and suv39h2 protein lysine methyltransferases. *Life*, *11*(7), 703. MDPI AG. https://doi.org/10.3390/life11070703.

Wilson, R. C., Tambe, A., Kidwell, M. A., Noland, C. L., Schneider, C. P., & Doudna, J. A. (2015). Dicer-TRBP complex formation ensures accurate mammalian MicroRNA biogenesis. *Molecular Cell*, *57*(3), 397–407. https://doi.org/10.1016/J.MOLCEL.2014.11.030/ATTACHMENT/08369455-A78E-402D-99D5-A15CAF4E159D/MMC2.ZIP.

Wynant, N., Santos, D., & vanden Broeck, J. (2017). The evolution of animal Argonautes: Evidence for the absence of antiviral AGO Argonautes in vertebrates. *Scientific Reports*, *7*(1), 1–13. https://doi.org/10.1038/s41598-017-08043-5.

Xiao, H., Vierling, M. M., Kennedy, R. F., Boone, E. C., Decker, L. M., Sy, V. T., et al. (2021). Involvement of RNA granule proteins in meiotic silencing by unpaired DNA. *G3 (Bethesda, Md.)*, *11*(10), jkab179. https://doi.org/10.1093/G3JOURNAL/JKAB179.

Yadav, R. P., Mäkelä, J. A., Hyssälä, H., Cisneros-Montalvo, S., & Kotaja, N. (2020). DICER regulates the expression of major satellite repeat transcripts and meiotic chromosome segregation during spermatogenesis. *Nucleic Acids Research*, *48*(13), 7135–7153. https://doi.org/10.1093/nar/gkaa460.

Ye, X., Huang, N., Liu, Y., Paroo, Z., Huerta, C., Li, P., et al. (2011). Structure of C3PO and mechanism of human RISC activation. *Nature Structural & Molecular Biology*, *18*(6), 650–657. https://doi.org/10.1038/nsmb.2032.

Yi, R., Qin, Y., Macara, I. G., & Cullen, B. R. (2003). Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes & Development*, *17*(24), 3011–3016. https://doi.org/10.1101/GAD.1158803.

Yigit, E., Batista, P. J., Bei, Y., Pang, K. M., Chen, C. C. G., Tolia, N. H., et al. (2006). Analysis of the C. elegans Argonaute family reveals that distinct Argonautes act sequentially during RNAi. *Cell*, *127*(4), 747–757. https://doi.org/10.1016/J.CELL.2006.09.033.

Zamore, P. D., Tuschl, T., Sharp, P. A., & Bartel, D. P. (2000). RNAi: Double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. *Cell*, *101*(1), 25–33. https://doi.org/10.1016/S0092-8674(00)80620-0.

Zaytseva, O., Mitchell, N. C., Guo, L., Marshall, O. J., Parsons, L. M., Hannan, R. D., et al. (2020). Transcriptional repression of Myc underlies the tumour suppressor function of AGO1 in Drosophila. *Development*, *147*(11), dev190231. https://doi.org/10.1242/DEV.190231/225316.

Zhao, Z. H., Ma, J. Y., Meng, T. G., Wang, Z. B., Yue, W., Zhou, Q., et al. (2020). Single-cell RNA sequencing reveals the landscape of early female germ cell development. *The FASEB Journal*, *34*(9), 12634–12645. https://doi.org/10.1096/FJ.202001034RR.

Zimmermann, C., Romero, Y., Warnefors, M., Bilican, A., Borel, C., Smith, L. B., et al. (2014). Germ cell-specific targeting of DICER or DGCR8 reveals a novel role for endo-siRNAs in the progression of mammalian spermatogenesis and male fertility. *PLoS One*, *9*(9), e107023. https://doi.org/10.1371/JOURNAL.PONE.0107023.