miRNA and piRNA localization in the male mammalian meiotic nucleus

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Abstract

During mammalian meiosis, transcriptional silencing of the XY bivalent is a necessary event where defects may lead to infertility in males. While not well understood, the mechanism of meiotic gene silencing is believed to be RNA-dependent. In this study, we investigated the types and localization of non-coding RNAs in the meiotic nucleus of the male mouse using a microarray screen with different cell isolates as well as FISH. We report that the dense body, a component of the murine spermatocyte sex body similar to that of a dense body in Chinese hamster spermatocytes, is DNA-negative but rich in proteins and RNA including miRNAs (micro RNAs) and piRNAs (PIWI associated small RNAs), or their precursors. Selective miRNAs and piRNAs localize to chromosome cores, telomeres and the sex body of spermatocytes. These RNAs do not have previously been detected in meiotic nuclei. These RNAs appear to associate with the nucleolus of the Sertoli cells as well as with the dense body. While in MIWI-null male mice the nucleolar signal from miRNA and piRNA probes in Sertoli cells is largely diminished, a differential regulation must exist in meiotic nuclei since the localization of these two components appears to be unaffected in the null animal.

Abbreviations

ncRNA non-coding RNA
FISH fluorescent in situ hybridization
db dense body
miRNA micro RNA
piRNA PIWI associated small RNA
SC synaptonemal complex
DSB double strand break
MSCI meiotic sex chromosome inactivation

laggard unsynapsed or partially synapsed autosome at pachytene stage
mRNA messenger RNA
snRNA small nuclear RNA
RNP ribonucleoprotein complexes
zygRNA zygotene RNA
RHA RNA helicase A
SYCP3 chromosome core component
PPIase peptidyl-prolyl cis-trans isomerase

Electronic supplementary material
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**Introduction**

During mammalian meiosis, homologous chromosomes align, physically associate via formation of the synaptonemal complex (SC), recombine their genetic material, and segregate to form two daughter cells that proceed through a second meiotic division to generate four haploid nuclei. Meiotic prophase can be classified into four sequential substages defined by the development of the synaptonemal complex. Chromosome cores appear in leptotene and synapse during zygotene to form the SC. This assembly is completed by pachytene. In diplotene, the two homologues pull apart until they are held together only at the sites of crossing-over/chiasmata (Figure 1).

Several processes are specific to meiosis. First, unlike somatic cells with their mostly accidental double-strand breaks (DSBs), the approximately 300 DSBs of meiosis are highly regulated and mandatory for meiotic progression (Romanienko & Camerini-Otero 1999, Baudat et al. 2000). Second, reciprocal recombination is a prerequisite for accurate chromosome segregation. Mutations of genes involved in the formation of stable chiasmata lead to infertility (Baker et al. 1996, Edelmann et al. 1996, 1999, Borts et al. 2000, Khazanehdari & Borts 2000, Lipkin et al. 2002). Third, while reciprocal recombination is an essential feature of mammalian gametogenesis, so too is interference—the reduced probability of multiple crossovers occurring on the chromosome in close proximity. Fourth, the processing of the XY bivalent is an essential feature of male meiosis but is poorly understood.

The XY bivalent is unique to the mammalian male meiotic nucleus. During meiotic prophase, the chromatin of the XY bivalent forms a distinct sex body that undergoes transcriptional silencing (meiotic sex chromosome inactivation: MSCI) that lasts until the spermatid stage (McCarrey et al. 1992, 2002, Hoyer-Fender 2003, Handel 2004, Khalil et al. 2004, Turner et al. 2005). While the molecular mechanisms of XY bivalent silencing are not well understood, several proteins associated with the XY chromosomes and/or the sex body such as ATR, γH2AX, TOPBP1, BRCA1, RAD51, DMC1, and RPA are also present on the unpaired autosomal axes during zygote. By mid-pachytene, ATR, γH2AX, BRCA1 and TOPBP1 are confined to the sex body and unsynapsed autosomes—the so-called ‘laggards’ (Moens et al. 1999, Perera et al. 2004, Marcon & Moens 2005, Turner et al. 2005). This distribution suggests that laggards, like X-Y chromatin, undergo silencing, possibly as a mechanism to prevent meiotic arrest caused by incomplete synapsis. Mutations in proteins unique to processing of heteromorphic sex chromosomes can lead to selective sterility in males but not in females (Celeste et al. 2002, Fernandez-Capetillo et al. 2003, Xu et al. 2003, Kolas et al. 2005).

The meiotic transcriptional repression pattern has already been established. In spermatogenic cells, DNA transcription rises during meiosis and decreases in post-meiotic nuclei until there is no detectable

![PROPHASE OF MEIOSIS](image)

*Figure 1.* Substages of meiotic prophase. Homologous chromosomes align in leptotene; synopsis starts in zygotene and is fully completed by pachytene. In diplotene, the homologues are pulled apart and are attached only at the sites of chiasmata. The sex chromosomes in late zygotene and early pachytene are synapsed at the pseudoautosomal region (PAR) and are clearly distinguishable from each other. As the cells progress through pachytene and into diplotene, the chromatin of the sex chromosomes condenses and the transcriptionally inactive sex body (SB) is formed. The dense body (DB) associated with the sex chromatin is observed in pachytene and in diplotene.