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Chromosomally-retained RNA mediates homologous pairing

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Pairing and recombination of homologous chromosomes are essential for ensuring correct segregation of chromosomes in meiosis. In *S. pombe*, chromosomes are first bundled at the telomeres (forming a telomere bouquet) and then aligned by oscillatory movement of the elongated "horsetail" nucleus (Fig. 1).[1,2] Telomere clustering and subsequent chromosome alignment promote pairing of homologous chromosomes.[3-5] However, this telomere-bundled alignment of chromosomes cannot be responsible for the specificity of chromosome pairing. Thus, there must be some mechanism to facilitate recognition of homologous partners after telomere clustering. Recent studies in *S. pombe* have shown that RNA transcripts retained on the chromosome, or RNA bodies, may play a role in recognition of homologous chromosomes for pairing (Fig. 1).[6] Acting as fiducial markers of homologous loci they would abrogate the need for direct DNA sequence homology searching.

DSB-Independent Search for Homologous Chromosomes

It has been proposed that in the yeast *Saccharomyces cerevisiae*, interactions between homologous DNA sequences with DNA double-strand breaks (DSB) and the consequent recombination are involved in homology searching for pairing.[7,8] However, this issue remains debatable as it has been reported that pairing can be achieved through DSB-dependent or -independent pathways in other organisms.[7-9] We recently demonstrated that the *sme2* locus, which is located on chromosome II, shows robust pairing in the early stage of the horsetail movement and that the robust pairing occurs independently of DSB formation and, hence, independently of recombination.[6]

The *sme2* gene encodes a meiosis-specific polyadenylated non-coding RNA, named MeiRNA, that is essential for entry into meiosis in *S. pombe*.[10,11] We reported that the *sme2* RNA mediates robust pairing of homologous chromosomes in meiosis: deletion of the *sme2* sequence eliminates this robust pairing and transposition to other chromosomal sites confers the robust pairing at those sites (**Fig. 2**).[6] Remarkably, the *sme2* RNA transcripts accumulate at their respective gene loci and their retention at the homozygous *sme2* loci is essential for robust pairing at the *sme2* locus. Thus, the chromosomally-retained *sme2* RNA seems to act as an "identifier" of homologous chromosomes.

The 3'-Region of meiRNA-L is Crucial for Robust Pairing

MeiRNA was initially identified as a 0.5 kb *sme2* transcript that complements the defect of meiosis progression in the *sme2*Δ mutant.[10] On the other hand, we identified a longer transcript (1.5 kb), extended at the 3' end, as a major transcript during meiosis.[6] This discrepancy was explained by the finding that MeiRNA is a major target of Mmi1, which selectively eliminates meiosis-specific transcripts in vegetative cells.[11] In an *mmi1* temperature-sensitive mutant background, both the 1.5 kb and the 0.5 kb transcript were detected, suggesting that the short transcript is the product of Mmi1-mediated digestion.[11]

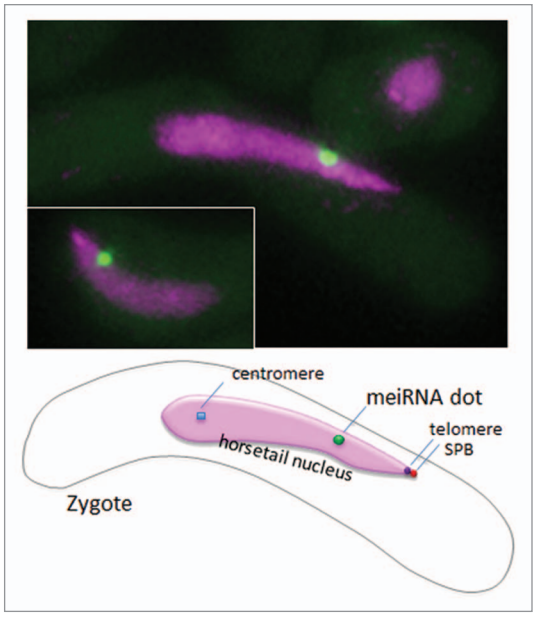

Figure 1. RNA retained on the chromosome. The sme2 RNA transcripts form a single dot (green) on the chromosome (magenta) in elongated horsetail nuclei (also see the schematic drawing). No dots are formed in the round nuclei of vegetative cells (upper right cell).

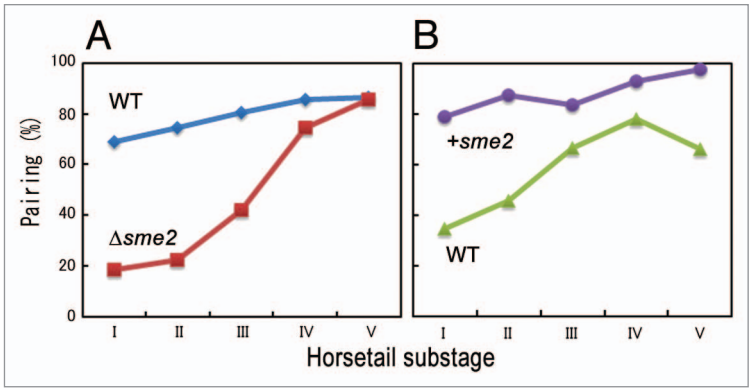

Figure 2. Robust pairing is mediated by the sme2 sequence. (**A**) Pairing frequency at the *sme2* locus during progression through the horsetail stage decreases with deletion of the *sme2* sequence (Δ*sme2*) compared with the wild-type (WT). (**B**) Pairing frequency at the *ade8* locus increases with insertion of the *sme2* sequence (+*sme2*) compared with the wild-type (WT).

Thus, the short (0.5 kb) and the long (1.5 kb) transcripts were named *MeiRNA-S* and *MeiRNA-L*, respectively.[6,11]

The sme2 transcript has two functional domains as shown in **Figure 3**. The Mei2 protein, a key regulator of meiosis in *S. pombe*, binds to the *sme2* transcript to form a distinct dot at the *sme2* locus in the horsetail nucleus.[12-14] The 5'-region of the *sme2* transcript, corresponding to meiRNA-S, is necessary for Mei2 binding, but unnecessary for robust pairing. In contrast, meiRNA-L is essential for robust pairing of the *sme2* loci.[6] Deletion of polyadenylation sites at the 3'-end of meiRNA-L results in the loss of meiRNA retention at the *sme2* locus and of robust pairing. The 3'-extended region specific to meiRNA-L (that is meiRNA-L with the meiRNA-S sequence deleted) is sufficient for its chromosomal retention and for robust pairing. Thus, we expect that chromosomal retention of meiRNA-L through its 3'-region has a role in robust pairing, as described below.

Roles for RNA in Homologous Pairing

Chromosomally-accumulated meiRNA-L at the *sme2* locus may play a role in recruiting specific RNA-binding proteins essential for pairing or recognition. On the other hand, even when the *sme2* locus was removed from chromosome II, no obvious defects in chromosome segregation or spore formation were detected. These observations raise the possibility that other as yet undiscovered pairing sites may also exist on chromosome II. An array of recognition complexes at multiple sites on each chromosome might act as chromosome-specific identifiers. Such recognition complexes could be arrays of RNA-protein complexes, transcription factories or specialized chromatin structures.

Although Mei2 forms a dot at the *sme2* locus in the horsetail stage, it has been demonstrated that Mei2 is unnecessary for robust pairing (**Fig. 3**).[6] Mmi1 also binds to MeiRNA-L and forms a dot at the *sme2* locus. However, in a hypomorphic *mmi1-48* mutant,[15] the robust pairing was enhanced, suggesting that Mmi1 negatively regulates robust pairing by degrading meiRNA-L.[6] Protein components that interact with the 3'-extended region specific to meiRNA-L may be involved in the robust pairing (**Fig. 3**), but such critical components are yet to be identified.

Alternatively, specific components may not be involved at all. Instead, RNA bodies formed at defined loci along the chromosome may act as a barcode for agglutination of homologous chromosomes. Recent reports demonstrate that RNA transcripts function as a scaffold in the assembly of many nuclear bodies at their respective gene loci.[16-18] The most striking example of RNA bodies is in the nucleolus formed around the rRNA genes. *S. pombe* rRNA gene clusters locate on both ends of chromosome III, and occupy a defined position within the nucleolus.[19,20] These findings provide an insight into the role of RNA nuclear bodies in the functional organization of chromosomes within the nucleus.

A previous model also proposed a role for transcription in homologous chromosome recognition and pairing:[21-23] this model depicts a chromosome as a linear array of transcription factories, providing

a possible mechanism for how transcription results in recognition and pairing of homologous chromosomes when chromosomes are pre-aligned in a chromosome bouquet. A similar model, in which meiosis-specific polyadenylated RNA transcripts initiate the pairing process, has been previously proposed in the lily.[24] Other studies have reported a similar link between pairing and transcription: in *Drosophila* embryos, somatic pairing is initiated at the histone gene cluster at the time when zygotic expression begins;[25] in *Drosophila* male meiosis, the pairing sites correspond to the highly transcribed rDNA loci and histone genes.[26] Another more direct link has been reported: in mammals non-coding RNA accumulated at their respective gene loci has been shown to mediate transient X-chromosome pairing and to mark the onset of X inactivation.[27,28] These findings imply that RNA transcripts retained at gene loci may act as chromosome identifiers for their homologous partner.

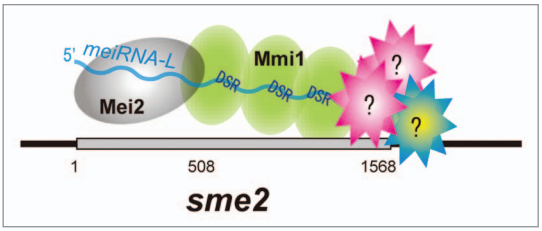

Figure 3. Distinct functional domains of the *sme2* transcript. The *sme2* RNA transcript can be divided into two distinct domains: the 5'-portion of meiRNA-L, corresponding to meiRNA-S, and the 3'-extended region specific to meiRNA-L. Mei2 protein binds to the 5'-portion of meiRNA-L (meiRNA-S). Mmi1protein binds to DSR motifs, which are concentrated in the 3'-extended region of meiRNA-L. We speculate that as yet unknown proteins may bind to meiRNA-L and play a role in pairing.

Precise molecular mechanisms for recognition between RNA bodies remain to be elucidated. Recognition can be achieved by an active mechanism based on DNA-RNA, RNA-RNA or protein-protein interactions, or by a passive mechanism based on agglutination of transcription factories, as discussed in recent literatures.[29,30] These questions can be answered once critical factors for recognition are identified in the *sme2* RNA body or similar pairing mechanisms are identified in other chromosomal loci of *S. pombe* or in other organisms.

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