Mechanisms of Inter-chromosomes Communications

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Genetic information is coded by DNA sequence and the long DNA filament is folded and organized into a chromosome. There are numbers of chromosomes in a cell nucleus of eukaryote. These chromosomes are not packed into the nucleus randomly but in a highly organized way. To coordination of complex life events, inter-chromosomal communication is required. We have recently found a meiotic specific non-coding RNA molecule that plays a role in inter-chromosomal communication.

1 Introduction

The reason eukaryotes have survived on the planet and thrived is because they adapted to changes in the environment and acquired almost infinite diversity through a never-ending battle with pathogenic bacteria to prevent penetration and infection by them. Sexual reproduction is one of the mechanisms by which organisms evolved and acquired this kind of diversity. Basically, sexual reproduction is the exchange of genetic information between individuals of different sexes, or to be more specific, it is communication between the chromosomes from the father and those from the mother.

All information on organisms is coded into the chromosomal DNA as genetic information, and organisms with good information, or in other words organisms with more genetic information that is useful for its survival, ultimately gain the upper hand in the race for survival. Cell division leading up to sexual reproduction is known as "meiosis", and in animals it is cell division that produces eggs and sperm. In meiosis, parts of chromosomal DNA from individuals of different sex are mixed and exchanged, resulting in the genetic diversity of organisms, and this is what has ensured the survival and thriving of species.

Our cells each have two sets of chromosomes (23 chromosomes × 2), one inherited from the father, and the other inherited from the mother. Chromosomes of approximately the same gene sequences are homologous chromosomes. In meiosis, there is genetic exchange (homologous recombination) between these homologous pairs. Before genetic exchange takes place, the homologous chromosomes first locate one another and align themselves side-by-side. This is believed to be the first step in chromosomal communication. We discovered that the formation of the telomere bouquet plays a central role in the alignment of homologous chromosomes. Observing the behavior of chromosomes in live fission yeast cells reveals that the ends of chromosomes (telomeres) form a cluster. This telomere clustered chromosome arrangement is known as a telomere bouquet (Fig. 1A). Then the nucleus oscillates back and forth led by the telomere, and the homologous chromosomes quickly begin to form pairs while swinging as if they were jump ropes with both ends held in one hand and swung back and forth (Fig. 1B)[1]. Our research group investigated the molecular mechanism

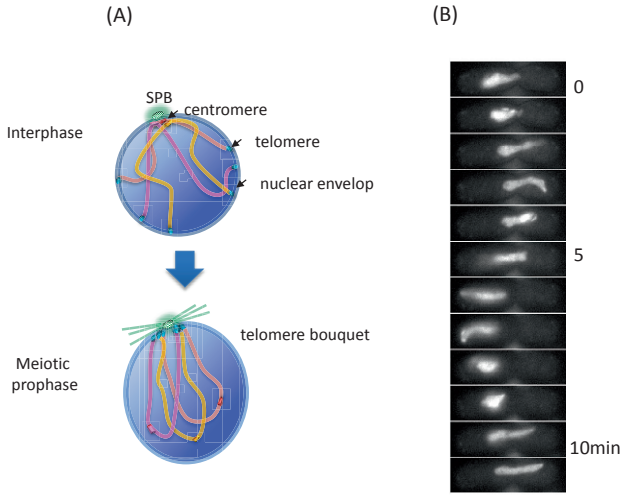

Fig. 1 Spatial chromosome rearrangement and nuclear movement during meiotic prophase

(A) Upper panel: Chromosome arrangement in interphase cell during vegetative growth. The centromeres cluster near spindle pole body (SPB), and telomeres locate on nuclear envelop. Down panel: Chromosome arrangement during meiotic prophase. The telomeres cluster near SPB and centromeres detach from SPB, forming a telomere bouquet.

(B) Time-lapse images of nuclear movement in meiotic prophase.

of the formation of this telomere bouquet[2]–[5]. We found that the formation of this telomere bouquet and the bundling and alignment of the chromosomes promotes the pairing of homologous chromosomes[6]. However, it was not known how chromosomes found their corresponding partners from among the numerous other chromosomes (46 chromosomes in the case of human beings) to form homologous pairs. We recently discovered that the key to solving this mystery lies in non-coding RNA.

2 One shining dot within the nucleus leads to discovery

Model organisms play major roles in biological research based on the fact that the fundamental molecular reactions supporting life are the same in a wide variety of organisms ranging from unicellular organisms such as yeast to higher order organisms such as human beings. In spite of the fact that meiosis is a universally vital process to all eukaryotes, live observation of this process in the cells of higher order animals is extremely difficult. On the other hand, meiosis occurs in fission yeast cells within a short time (approximately 8 hours) making it relatively easy to observe, and detailed analyses have been carried out on the behavior of chromosomes during the meiotic phase. For these reasons, we used fission yeast as our model organism to study meiosis.

Fission yeast has a protein known as Mei2, which controls meiosis. This protein gathers in a particular location on the chromosome, known as the *sme2* gene locus, during the meiotic prophase to form what is called a Mei2 dot[7] (Fig. 2). Despite the fact that cells have two homologous *sme2* gene loci, only one Mei2 dot was seen in most cells observed (Fig. 2). These observations suggest that the homologous *sme2* gene locus are always co-localized with each other, or easy pairing.

To observe the behavior of homologous chromosomes, we first prepared fission yeast cells with a specific part of their chromosomes fluorescently tagged. This was done by inserting repeated lactose operon (lacO) arrays to a specific part of the chromosome and creating a fusion protein (GFP-LacI) with lactose repressor protein (LacI), which bonds specifically to the arrays, and a green fluorescent protein (GFP) to make it visible. Observing the pairing of a variety of chromosomes in living cells revealed that the frequency of pairing increases gradually as meiosis progresses[6]. In order to examine the frequency of pairing at the *sme2* gene locus we inserted a lacO array that bonds

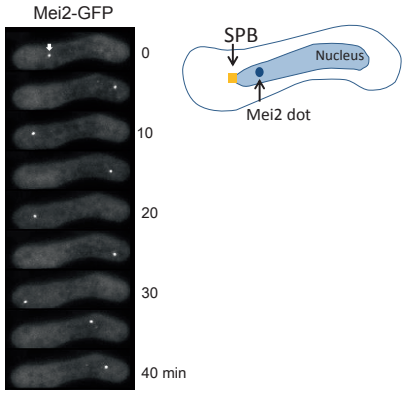

Fig. 2 A single Mei2 dot was observed in meiotic prophase
Time-lapse images of a Mei2 dot (arrow) moving in a cell, accompanying with the nuclear movement.

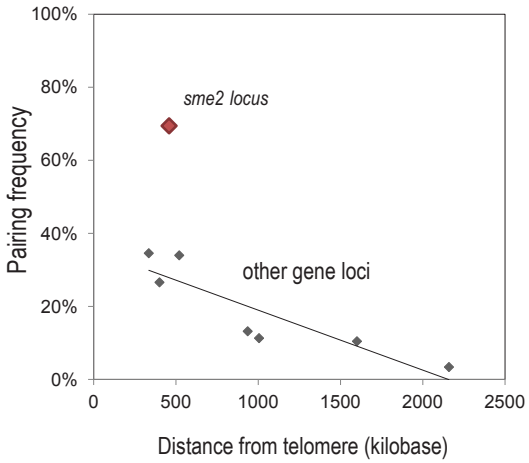

Fig. 3 sme2 locus show a much higher pairing frequency comparing with other gene loci

with GFP-LacI near the *sme2* gene locus, and investigated the pairing that occurs when the living cell undergoes meiosis. As a result, we found that pairing frequency is highest at the *sme2* gene locus in all chromosomes loci we examined (Fig. 3)[8]. Furthermore, deletion of the *sme2* gene (*Δsme2*) decreases the pairing frequency, while insertion of the *sme2* gene at another (*ade8*) gene locus (*ade8* + *sme2*) boosts the pairing frequency at the new site. This showed that the *sme2* gene locus strongly promotes the pairing of homologous chromosomes (Fig. 4). It was also found that the promotion of homologous chromosome pairing at the *sme2* gene locus does not occur in cell strains that have had their telomere bouquet forming or nuclear movement impaired. This indicates that the close alignment of homologous chromosomes by the formation of the telomere bouquet, and nuclear movement, are the required preconditions for the promotion of pairing at the *sme2* gene locus[8].

From these results, we concluded that homologous

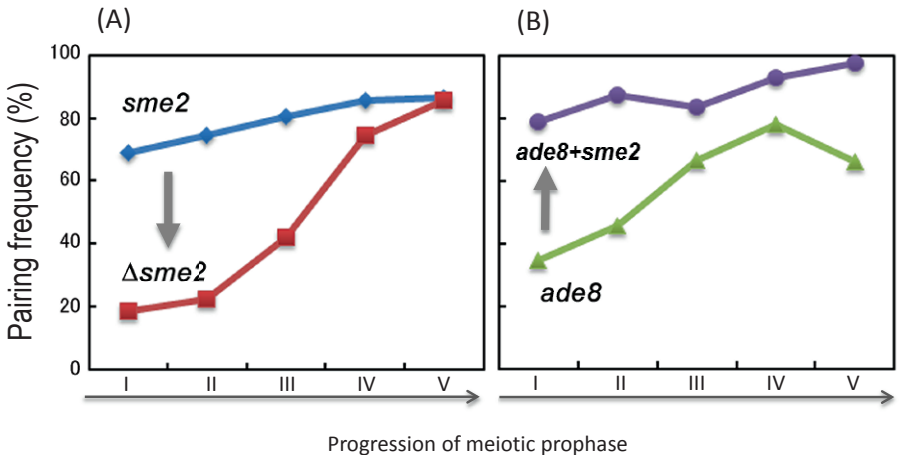

Fig. 4 Pairing frequency is dependent on the existence of *sme2* locus
In the deletion strain of *sme2* (*Δsme2*), the pairing frequency decreased (A). When *sme2* was inserted at *ade8* locus (*ade8+sme2*), the pairing frequency increased (B).

chromosomes become closely aligned by the formation of the telomere bouquet and nuclear movement, they next recognize one another to form pairs due to the function of the *sme2* gene locus.

3 Non-coding RNA gathered around the gene locus promotes recognition of homologous chromosomes

We asked why pairing frequency is boosted around the *sme2* gene locus. The *sme2* gene codes for a meiotic phase-specific RNA of around 1.5 kb in length, known as meiRNA. Unlike normal messenger RNA, meiRNA is known to be non-coding RNA, which does not carry a genetic code for proteins[9]. When the transcription of meiRNA (the synthesis of RNA, which uses DNA as its template) is inhibited by mutating the DNA array controlling the *sme2* gene, the pairing frequency decreases. Thus, meiRNA made by the *sme2* gene is necessary for promoting pairing[8]. Moreover, from the fact that the promotion of pairing is not seen when transcription only occurs in one of a pair of homologous chromosomes indicates that meiRNA transcription is necessary in both chromosomes of a homologous pair for pairing to take place[8].

To investigate the localization of meiRNA within the cell, a U1Atag was inserted into the 5' end of the *sme2* gene, and the U1Atag-meiRNA transcription product was visualized by a U1Atag binding protein (U1Ap) which fused with GFP (U1Ap-GFP). Visualization of the meiRNA localization revealed that it accumulates at the same *sme2* gene locus on the chromosome as Mei2 protein (Fig. 5).

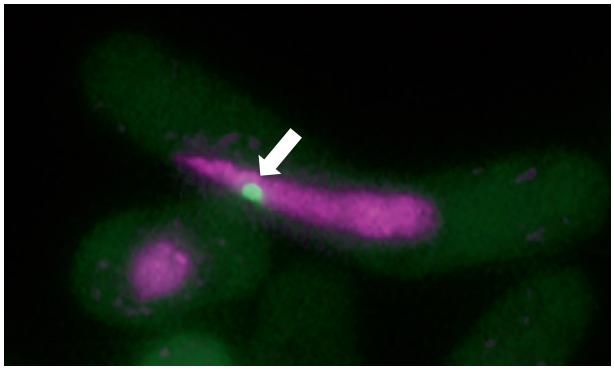

Fig. 5 Visualization of meiRNA dot (the green dot pointed by an arrow) by GFP
The chromosome DNA was visualized by a fluorescent dye (magenta).

Mei2 protein actually accumulates because it can bind with meiRNA. In addition, removing the end of the *sme2* gene disables the meiRNA's ability to accumulate at the *sme2* gene locus and inhibits pairing, indicating that the meiRNA which accumulates at the *sme2* gene locus is responsible for identifying homologous chromosomes and facilitating pairing[8].

4 Conclusions

Homologous recombination involving the double-strand DNA break (DSB) is necessary for pairing in mammals, plants and true fungi. On the other hand, organisms such as the fruit fly are able to carry out pairing without homologous recombination, suggesting that there is a mechanism of chromosomal recognition that does not depend on DSB formation. The formation of telomere bouquets and chromosome movement bring chromosomes together and align them, but they are not the mechanisms

directly involved in the recognition of individual homologous chromosomes. Our discovery that the non-coding RNA, which accumulates on chromosomes, is responsible for homologous chromosomal recognition, explained this mechanism for the first time. Copies of RNA can be synthesized many times, and it is extremely practical that RNA should be used to identify homologous chromosome pairs, with DNA as its template, instead of using the genomic DNA itself with only two copies, which if damaged may lead to lethal errors. The accumulation of non-coding RNA at several locations on a chromosome will enable the allocation of easily recognizable properties to chromosomes, much like bar codes. In the future we hope to discover pairing sites other than the *sme2* gene locus, which will enable the identification of the common characteristics of chromosomal DNA and protein factors, and we plan to further clarify the molecular mechanism behind the accumulation of RNA.

Organisms no doubt have a diverse range of chromosomal communication strategies that have evolved in the process of adapting to environmental changes and specialization. Expectations are held for the elucidation of each and every one of these strategic mechanisms to not only allow the analysis of the various issues pertaining to the phenomenon of life, but it will also bestow indispensable wisdom upon us for application in agriculture and medicine. We have only just begun to shed light on these issues, but we hope that our discovery that non-coding RNA plays a central role in homologous chromosomal communication during meiosis is a milestone in the research of chromosomal communication.

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