Functions of homologous DNA recombination

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Homologous DNA (or genetic) recombination plays important roles in heredity and evolution. A recent study has shown that a physical linkage established by recombination between two or more homologous DNA regions or molecules is essential for the precise disjunction of homologous chromosomes in meiosis and the recovery of collapsed DNA replication forks. These physical linkages in recombination may also be involved in the establishment of mitochondrial homoplasmy. A recent study has also challenged some notions that were regarded as dogmas, such as the role of Holliday intermediates in gene conversion and crossing over. The concept that homologous recombination is generally promoted by proteins of the RecA/Rad51 family has been challenged by the observations that homologous pairing is promoted *in vitro* by ATP-independent proteins encoded by recombination genes.

Introduction

Homologous recombination is the exchange ("crossing over") or replacement ("gene conversion") of a DNA region by its homologous DNA sequence from the homologous chromosome or the sister chromatid (Fig. 1). Homologous recombination also occasionally occurs between similar DNA sequences on nonhomologous chromosomes or within a chromosome. A single gene conversion event changes one of the pair of homologous sequences without changing any other parts of the genome.

In this article, the recent progress made in understanding homologous recombination as well as in answering the remaining questions that need to be solved is reviewed.

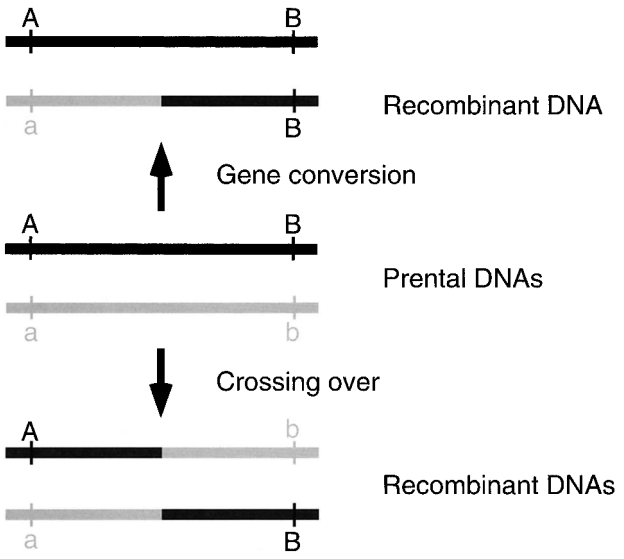

Fig. 1. Two classes of homologous recombination: gene conversion and crossing over.

The role that Holliday intermediates play in homologous recombination is being challenged

In meiosis, one-half of a gene conversion event is associated with the crossing over of flanking genetic markers. This consistently documented correlation suggests that both gene conversion and crossing over, which are classes of homologous recombination, have a common mechanism. Early physicochemical studies revealed that homologous recombination occurs through the breaking and rejoining of parental DNA molecules.[1)] Initially, it was not clear how this mechanism could lead to gene conversion. Later, however, Holliday explained well how both classes of homologous recombination were mediated by common intermediates.[2)] The intermediates called Holliday intermediates consist of pairs of parental double-stranded DNA molecules aligned at a homologous sequence by a pair of heteroduplex joints and have been shown to be present in prokaryotic and eukaryotic cells undergoing DNA recombination. The joints are formed by the exchange of a pair of strands of the same orientation (Fig. 2). Holliday suggested that gene conversion can occur through mismatch repair within the heteroduplex, while crossing over can occur if the Holliday intermediate is resolved in one of the two ways. Recently, however, the original proposal of Holliday regarding the mechanisms underlying gene conversion and crossing over is being challenged.[3)]

Gene conversion contributes to both the maintenance of genetic information and creation of genetic diversity

The accurate repair of double-stranded DNA breaks is performed through gene conversion, in which DNA regions flanking each double-stranded break are replaced by an intact copy from the sister chromatid or homologous chromosome. This cellular mechanism serves to protect the genetic information in the cell from damage. Homologous recombination is actively induced in bacteria and simple eukaryotes when cells are subjected to conditions unfavorable for their survival, such as nutritional starvation and a high cell density. While meiotic crossing over is supposed to create genetic diversity by producing new combinations of the alleles derived from

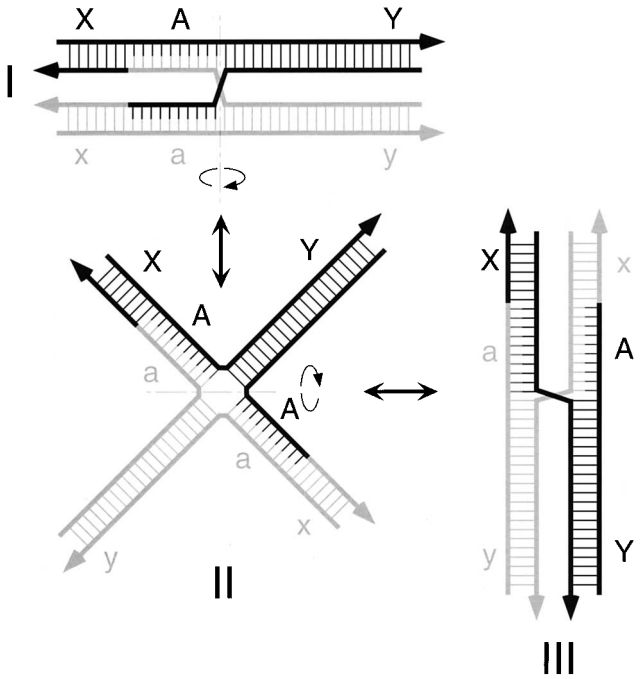

Fig. 2. Holliday intermediate. A heteroduplex is formed around heterozygous locus A/a. The locus is flanked by X/x and Y/y loci. The Holliday intermediate at conformer I with parallel parental DNAs is converted to conformer III with parallel crossed-over DNAs through extended conformer II. The heteroduplex has a mismatch base pair(s) at A/a locus. A mismatch repair system corrects the mismatch base pair(s) using black strands as templates, and gene conversion from a to A occurs. If gray strands are used as templates, gene conversion from A to a occurs. Since conformer II has a twofold rotational symmetry (considering the base sequence), the intermediate is resolved into X-A/a-Y and x-A/a-y (parental configuration with respect to X and Y loci) or X-A/a-y and x-A/a-Y (crossing over between X and Y loci) by Holliday-junction-resolving endonuclease at the same frequency.

parents and the genetic diversity may help cells to adapt to such unfavorable conditions, the significance of meiotic gene conversion has not been well understood.

Mitotic gene conversion that results in the creation of genetically altered cells has been documented. For example, the mating-type switching observed in homothallic mitotic haploid cells of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* is due to the gene conversion that occurs at the active mating-type locus. Through gene conversion, the active locus is replaced with the information stored in either of the two silent mating-type loci. These three loci have a common sequence in addition to the specific sequence that defines each mating type and this enables switching of the locus through homologous recombination.

Gene conversion between nonallelic genes can also potentially generate a gene with novel functions by shuffling parts of the parental open reading frame (ORF) with aligned coding frames. This type of gene conversion is the mechanism used to create immunoglobulin diversity in chickens and some mammals. In these organisms, a unique copy of a rearranged immunoglobulin v gene undergoes repeated cycles of gene conversion with one of several variable pseudogenes, promoting antibody diversity.[4] A possible role of meiotic gene conversion in evolution has been discussed recently with respect to the creation of novel genes.[5]

Formation of physical connections between DNA molecules is required for meiosis and mitosis

Many mutations defective in meiotic homologous recombination cause the non-disjunction of homologous chromosomes during meiosis, which generates aneuploids.[6] Some of the mutants are defective in crossing over but not in gene conversion. These mutants still exhibit meiotic non-disjunction, which indicates that the mechanism that induces gene conversion is not sufficient to establish precise disjunction. Extensive studies have revealed that precise meiotic disjunction requires that the homologous chromosome pair must be physically connected by chiasmata, which are formed by the crossing over between homologous chromosomes.[7, 8]

Defective gene mutations that are essential to homologous recombination in microorganisms result in the death of numerous cells, and in higher eukaryotes, such mutations often arrest embryonic development and are lethal during culture. These mutations abrogate the ability of homologous recombination to resume the movement of replication forks that collapsed at sites of DNA damage.[9]

Homologous recombination may participate in establishing or maintaining mitochondrial homoplasmy

Each eukaryotic cell has 10^2–10^3 copies of mitochondrial DNA (mtDNA). Mitochondrial homoplasmy is the state in which all mtDNA copies within each cell or individual are genetically indistinguishable. Homoplasmy of the mitochondrial genome is the normal situation for all organisms from yeast to higher eukaryotes. In humans, mitochondrial heteroplasmy is closely associated with serious diseases of nerve and muscle systems, as well as with aging. While homoplasmy is a basic non-Mendelian genetic phenomenon, the molecular mechanisms underlying its establishment and maintenance are unknown. A study that examined the functions of *MHR1*, a gene in the yeast *Saccharomyces* that is required for mtDNA recombination, has beeb revealing that this gene participates in establishing homoplasmy in heteroplasmic cells (F. Ling, unpublished observations). This probably occurs by forming physical connections among each clone of mtDNA through homologous recombination.

That homologous recombination is always promoted by ATP-dependent homologous pairing proteins is being questioned

In bacteria to higher eukaryotes, homologous recombination in nuclear DNA involves the formation of heteroduplexes from homologous single-stranded DNA and double-stranded DNA by the RecA/Rad51 family proteins through ATP-dependent reactions.[10–12] Heteroduplex formation by the RecA/Rad51 family proteins involves homologous pairing and strand exchange. Homologous pairing is the formation of core heteroduplex joints by searching for homology between double-stranded DNA and single-stranded DNA, and strand exchange is the extension of the region of the heteroduplex. Although the ATP-binding motifs of proteins of this family are conserved, some human Rad51 paralogues have been shown to promote *in vitro* homologous pairing

in an ATP-independent manner.[13] Furthermore, the human Rad52 protein, which has no homology to Rad51 and is required for various homologous recombination, promotes an ATP-independent homologous pairing.[14] In bacteria, the ATP-independent homologous pairing proteins were identified as RecT[15] and RecO[16] and it was shown that the RecT-dependent homologous recombination of plasmid DNA in bacteria does not require RecA. *E. coli* phage lambda also has its own recombination machinery that functions independently of the RecA/Rad51 family proteins. Thus, it can be postulated that homologous recombination in small genomes does not require ATP-dependent homologous pairing proteins such as RecA. Although the ATP-hydrolysis-dependent reaction of RecA stabilizes heteroduplexes through strand exchange when the homologous region is sufficiently large, the same reaction results in the dissociation of previously formed heteroduplexes if the homologous region is small.[17] This suggests that the ATP-dependent reaction prevents heteroduplex formation between nonallelic sequences. This corrective system may not be required for the recombination of small genomes such as those of viruses and plasmids, or may be harmful to such recombination that uses a small homologous region in eukaryotes.

Perspectives

While homologous recombination plays important roles in heredity and evolution, it is also a means by which physical connections between two homologous DNA molecules are formed. It has recently been discovered that the establishment of such physical connections between homologous DNA molecules is required for the recovery of collapsed replication forks. These physical connections probably play additional, but as yet unidentified, roles in other systems. In addition, a recent study on homologous recombination, particularly at the molecular level, challenges notions that were once regarded as dogmas. These include the function of Holliday intermediates and the general roles played by the RecA/Rad51 family proteins in homologous recombination. Such advances provide us with a greater understanding of the functions and molecular mechanisms of homologous recombination, as well as enhance our knowledge of the regulatory mechanisms that control it. This knowledge about the natural processes by which genetic information evolves will enable us to develop new technology for breeding useful agricultural or industrial organisms and gene therapy.

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