RECOMBINATION-TRANSFORMATION, TRANSDUCTION, AND CONJUGATION

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Summary

Evolution and the enormous diversity among terrestrial organisms are caused by mutation, transduction, conjugation, and recombination. Recombination is the major and most basic factor that increases and decreases chromosomal and genetic components. I would like to review studies on homologous and non-homologous recombination, meiotic and somatic recombination, and the applications of basic mechanisms of recombination today, such as genetically modified plants and foods.

There are increasing numbers of publications in these areas and not all results and hypotheses can be covered in this article. Although conjugation and transduction have been observed in higher plants, they are fundamental to the alteration of the genome. All of these processes contribute to the transformation of plants.

1. Introduction

The events mentioned in the title involve a common process, namely, the alteration of genomic structure by the addition or loss of DNA through DNA strand-breaks and repairs. In plant science, transformation has led to genetically modified food. Although these events were first found in bacteria and later described in eukaryotes, only limited information is available in plants, partly, because transduction and conjugation seldom occur or simply have not been studied. Here, I will describe studies concerning these events in plants, but I also intend to encourage further study of them by mentioning some advanced research in microorganisms and animals.

2. Recombination

As genome projects proceed in human and other organisms, especially those of experimental and economic importance, the alteration of genes on chromosomes is becoming clear and the events of recombination are shown to play a major role. For example, the mammalian Y-chromosome, originally homologous to the X-chromosome, has changed in structure over 300 million years and continues to do so. Originally, X and Y were homologous, like autosome pairs but early in the development of mammals, one chromosome was mutated to Y. If one compares X and Y, one finds homologous regions between them where chiasma form (homologous recombination at chromosomal level). Also, genes that are localized on Y but do not recombine with X are found on the X-chromosome. Some 95% of Y is not committed to homologous recombination with X. These findings support the hypothesis that Y evolved from X. Where homologous recombination is missing, mutations, even devastating ones, accumulate to cause loss of function.

The suppression of homologous recombination started outside of the *SRY* (Sex Reversal of Y) gene and spread to other regions in a rather spontaneous fashion, leading to the loss of genes and chromatin. It has been speculated that the cause of suppression is inversion on the Y-chromosome. When the homologous recombination, a kind of DNA repair method, is induced, the nucleotide sequences of two homologous genes will probably diverge in a random fashion. The higher the homology between the two, the more recent the diversification is. If the homology is low, the recombination events are suppressed, and such a reduction may have caused the diversification of genes many years ago. The measurement of sequences in various regions of Y indicates that the greatest difference was near *SRY*. Thus, both *SRY* and suppression of recombination around *SRY* are thought to have occurred 300 million years ago. The inversion that caused the first suppression of homologous recombination took place between 320 and 240 million years ago, and the second suppression, 170 to 120 million years ago when mammalians separated from marsupials. The third suppression occurred between 130 and 80 million years ago and the fourth 5 to 3 million years ago, after primates appeared but before monkeys separated from other mammals. All these events are results of recombination or lack of recombination and some cases induced a shortening of chromosomes. This may be related to the excision of gene(s) introduced into the genome due to the fragile nature of the transformed gene(s).

Although most plants do not have cytologically recognizable sex chromosomes, about 70 species do. The first report of sex chromosomes in plants was on *Sphaerpcarpus* in 1917, followed by *Rumex, Elodea, Dorstenia*, and others in 1923. Later, the presence of sex chromosomes was reported in many other species, including some water plants. Apart from a wild strawberry with the Japanese name of “Takaichigo,” which has a Z-W sex chromosome (homologous in the male and heterologous in the female), all cases studied so far are of the X-Y type (homologous in the female and heterologous in the male). In plants, it is not necessary for X to be larger than Y. Further, there are two Ys in Rumex. How these sex chromosomes evolved is unknown. However, the sex determining genes are hidden in autosomes, and their endogenous or exogenous condition activates proper gene(s) to determine individual sex or differentiation of sex organs, as in reptiles. In plants without detectable sex chromosomes, the autosomes pair

completely and show chiasma between the homologs.

The shortening or loss and lengthening of chromosomes that can be observed under an optical microscope are based on DNA strand-breaks and repairs at the molecular level, including the jumping of certain portions of genomic DNA and/or chromosome fragments. Thus the first chromosome alteration is a DNA break. Currently, recombination events are considered to start with double stranded breaks (DSBs) (Figure 1).

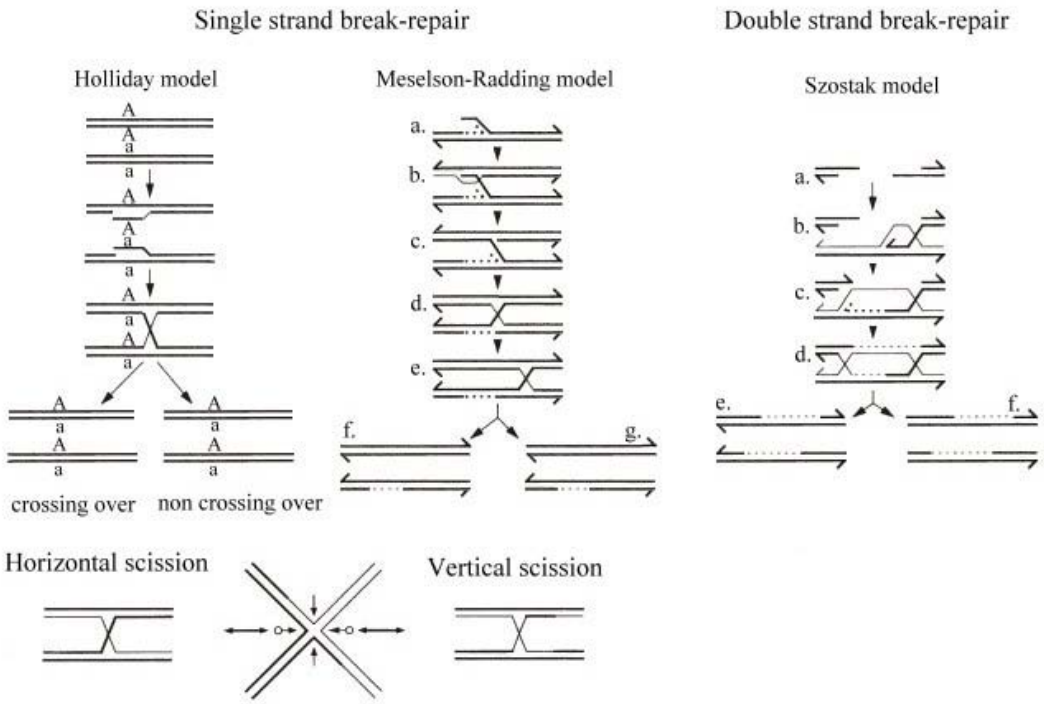

Figure 1. Proposed model of recombination

Two models with a single-stranded nick, gap formation and repair were proposed on the basis of cytological studies (Holliday model and Meselson-Radding model). The double-strand break and repair model was proposed on the basis of molecular studies in yeast. The model has been established in yeast but did not eliminate the occurrence of break repair events.

In higher organisms, single-stranded breaks occur during meiosis but the relation to recombination events remains unclear. Holliday junction recombination will be established by horizontal scission and gene conversion by vertical scission.

DSBs can be caused by exogenous factors like radiation and chemical agents, and because some of them are lethal, they must be repaired. The most economical form of repair is ligation with available DNA, as observed in many higher organisms, but this can be accompanied by loss of genetic material and may lead to gross chromosomal rearrangements. An alternative is recombination using several proteins like RecA, RecBCD (Rec stands for recombination), and Rad 52 (radiation sensitive) in

combination with exonuclease V and helicase. DSBs also result from the breakage of arrested replication forks, which are also repaired by recombinational proteins. All of these DSBs are potential sites of recombination but it is difficult to control such sites on chromosomes.

Particularly in plant biotechnology, the directed recombination of specific sequences can bring about profound changes in gene expression and the genome organization. Reciprocal exchange between two short identical DNA sequences is carried out by three systems, *Cre-lox*, FLP-*FRT*, and *R-RS*, where Cre, FLP, and R are recombinases and lox, RT, and RS are the recombination sites. Many variations of site-specific deletion/inversions of transgenes to activate or deactivate gene expression have been tested using Cre-*lox*, FLP-*FRT*, and the R-*RS* system. In haploid organisms, a large chromosomal aberration induced by R-*RS* site-specific recombination may be lethal, but plants with polyploidy accept such large chromosomal rearrangements. Recombinase-mediated inter-chromosomal translocations have been produced in tobacco using Agrobacterium-mediated transformation. Cre-*lox*-mediated reciprocal chromosomal translocations have been observed in mouse embryonic cells. An advantage of animal over plant model systems is the availability of homologous recombination, which can direct the placement of recombination sites and cause predetermined genome aberrations.

For intra-chromosomal recombination, the intervening DNA must be flanked by a pair of recombination sites, a process difficult to achieve through the random placement of transgenes. Unlike fungal and animal systems, the insertion of transgenes through homologous recombination in plants is not yet practical. To examine intra-chromosomal recombination, the dissociation element (Ds) and the activator element (Ac), transposons, a specific system was developed. In this system, depending on the relative orientation of the two recombination sites, deletion or inversion of the intervening DNA segment will occur when the recombinase is introduced through sexual hybridization. These studies are supported by experiments in an *in vitro* system using Cre-catalyzed and FLP-catalyzed reactions (Figure 2). The recombination-rearrangement of the genome in blocks in nature shows that the same set of basic linkage blocks stays together but in different arrangements. This suggests the shuffling of genomic blocks through site-specific recombination and may enable us to create diversity in plants and different species.

The BLM gene, if muted, causes Bloom's syndrome; the WRN gene, if muted, causes Werner's syndrome; and the RecQ4 gene, if muted, causes Rothmud-Thompson syndrome. All of these genes belong to the RecQ family of DNA helicase genes and the defect induces sensitivity to sunlight, reduced fertility, and a high incidence of cancer. It has been shown that cells with a defective helicase have an unstable genome due to low repair activity.

Foreign DNA or a fragment which is homologous to bacterial DNA or carrying a homologous region at two sites can be integrated using the homologous recombination mechanism. An example of the integration of circular DNA of F-plasmid is shown in (B).

A gene homologous to *BML* has been identified in *Drosophila melanoguster* and named *Dmblm*. At the end of a double-stranded break, Ku70 protein binds and facilitates repair by blocking genetic inactivation of *Dmblm. Dmblm* mutant flies are sterile, probably due to a defect in meiosis. Their homologous chromosomes become interlocked, causing non-segregation.

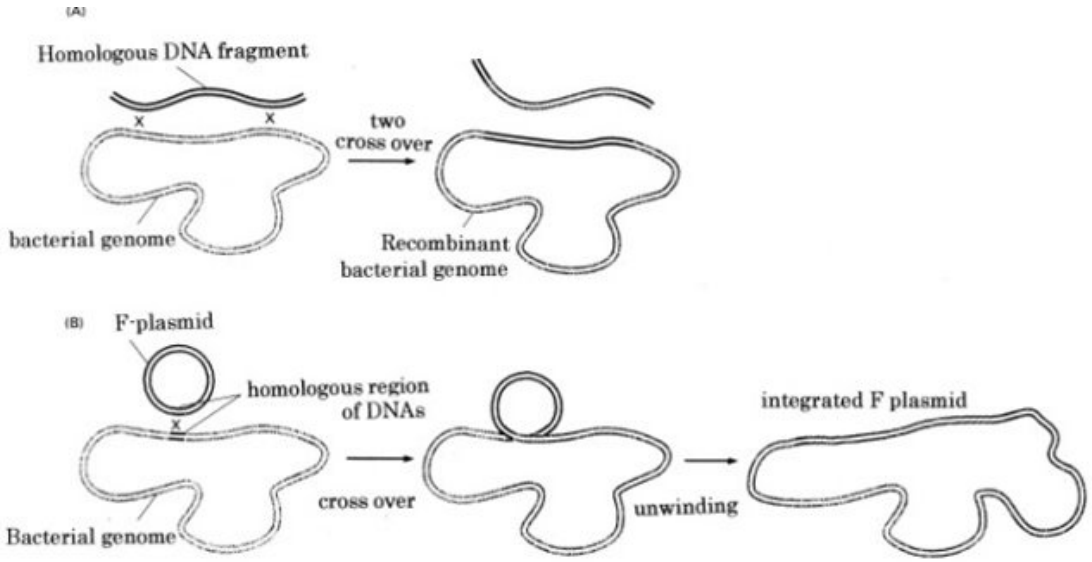

Figure 2. Integration of DNA fragment (A) and a circular DNA by two homologous recombination events.

The insertion of a wild-type copy of the *Dmblm* gene into the fly genome partially restores fertility. Furthermore, insertion of Ku70 of either fly or human also partially ameliorates the sterile condition. Together with the structurally related Ku80, Ku70 is crucial for the repair of double-strand breaks in DNA. Such breaks are repaired principally by error-free homologous recombination and error-prone non-homologous end-joining. The former requires genes of the *RAD52* epistasis group in yeast. RecQ helicases are thought to be involved in homologous recombination in a non-essential fashion because RecQ helicase mutants appear proficient in performing general recombination with break-repair. In contrast, the mutants of RecQ helicase show hyper-recombination between both homologs and sister chromatids. The suppression of *Dmblm* mutations by a component of the end-joining pathway (that is, overproduction of Ku70) suggests the presence of an unidentified molecular link between end-joining and homologous recombination, which until now have been viewed as two different processes. The maintenance of genomic stability requires not only the repair of damaged DNA, but also the coordination of DNA repair with processes such as DNA replication and chromosome segregation. The relative sensitivity of RecQ helicase mutants to agents that induce DSBs suggests that RecQ helicases are likely to act in a specialized pathway to repair double-strand breaks, perhaps during the S phase. The involvement of helicase has been suggested in DNA replication in plants cells, but this concept is new and thus the mechanisms of recombination in plant cells require further study.

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Biographical Sketch

Professor Yasuo Hotta graduated from the Faculty of Science, Nagoya University in 1959 with a Dr. Sci., and served as a postdoctoral fellow of the Canada National Research Council at the Plant research institute in Ottawa till 1960. He was Research Associate at the Department of Botany, University of Illinois, Urbana, from 1960 to 1965. He worked at the Department of Biology, University of California, San Diego, as Associate Research Biologist until 1971, and then as Research Biologist until 1986. He was appointed professor at the School of Science, Nagoya University in 1985, and was professor at the Graduate School of Bioscience, Nara Institute of Science and Technology from 1994 till 1999. He served as professor at the Department of Library and Information Science, Aichi Shukutoku University, and Division Head at the Gifu International Institute of Biotechnology from 1999 till 2001. He is currently, professor at the Department of Health and nutrition, School of Medical Technology, Niigata University of Health and Welfare, and Head of the University Library. He is Professor Emeritus of the University of California (1986), the Nagoya University (1994) and the Nara Institute of Science and Technology (1999).