DIPLOID AND HAPLOID GENETICS AND RECOMBINATION MECHANISMS

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Contents

Summary

Most eukaryotic organisms, if not all, alternate between diploid and haploid generation through meiosis, reduction of chromosomes, and the fertilization/fusion of two sexually differentiated gametes. The period spent as diploid and haploid depends on the species and environmental conditions.

Lower organisms, like yeasts and molds, induce meiosis when their environment becomes unfavorable, e.g. from a lack of nutrition, and form spores. When conditions improve, the spores germinate and fuse together to form diploid cells (budding yeast) or divide as haploid cells (fission yeast). During meiosis, recombination takes palace at an extremely high frequency, several hundred times (organisms with smaller genome) to several thousands times (with large genome). Most meiotically induced recombinations are homologous, and cytological crossing-over is observed as chiasma at the end of the process. In higher organisms, pairing of homologous chromosomes follows recombination according to microscopic observations and genetic analysis, but in lower organisms, the pairing is induced by molecular recombination. Control of meiotic recombination without an effect on the other events of meiosis makes a great contribution to genetics. The application of the meiotic recombination process may result in the formation of new genetically modified plants.

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1. General Recombination

Whether Mendel used three genetic traits located on different chromosomes by chance or chose them from among several other traits is not mentioned in the literature. Nevertheless, Mendel's Law has been considered the basis of genetics, though hereditary phenomena that contradict his law have since been identified and are referred to as non-Mendelian genetics. First, hereditary events in mitochondria were observed in yeast. When a haploid containing a wild-type mitochondria mates with one containing a mutant mitochondria (for example, a chloramphenicol sensitive cell), all cells exhibit the wild-type phenotype (resistant). With successive round of mitosis, either the mutant or the wild-type mitochondria will gradually be lost from some daughters cells, with only one type of mitochondria in a particular cell, by a random process called mitotic segregation. During fertilization, only mitochondria of female cells are transmitted to the next generation: those of male cells are rejected.

The second event of Mendelian genetics is recombination. It was identified from linear linkage maps after the discovery of co-transmittance of certain pairs of genes in *Drosophila* and that their position corresponds to thread-like chromosomes. The connection between gene linkage maps and chromosomes was established by McClintock's experiments in maize. As gene mapping proceeded, the rearrangement of genes became clear and Whitehouse (1982) proposed a model for recombination. The chemical basis of chromosomes was established during the1950s but biological meaning came from the elucidation of the double helical structure of DNA by Watson and Crick (1953). The double stranded α-helix, with A-T and G-C pairs on opposite strands, explained how genetic information could be copied, and how mutations can arise and be transmitted to one of two off-spring. Naturally, mutation events deviate from Mendel's law. Using mutant and wild-type genes, the rearrangement of genes on chromosomes became evident, and was called recombination.

Recombination has been divided into two types based on the requirement of homology of paired double stranded DNAs. General recombination requires a certain amount of sequence homology between the strands and the double stranded cuts initiate the molecular exchange. Site-specific recombination does not require DNA-homology but exchange takes place at short, specific nucleotide sequences recognized by a variety of site-specific recombination enzymes. Nearly all early molecular and biochemical studies of recombination were carried out using bacteria (*E. coli*) and bacterial viruses. In recent yeast, however, the target of analysis has shifted to eukaryotic cells. Both general and site-specific recombination occur in somatic cells at low frequency, but the highest frequency is in the prophase of meiosis I, where a tremendous increase in general/ homologous recombination, but no increase in site-specific recombination, has been observed. The strand breaks, and repair may take place at various sites on a chromosome but fruitful recombination may result at only a limited number of spots which correspond to cross-over points. Carpenter (1979) showed a good relation between recombination sites or position and recombination nodules on a synaptonemal complex which appears in between paired homologues during pachytene. Exchange of DNA strand between homologues required several enzymes and gene products as summarized by Hays (1995).

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The next section focuses several events that occur during meiosis.

2. Recombination and Pairing of Homologous Chromosomes

Observations of chromosome behavior during meiotic prophase indicated an increase in the size of various heterochromatic regions inside the nuclear membrane and an increased number of nuclear pores in premeiotic interphase nuclei. As the cell approaches leptotene, the nuclear chromatin starts to form thin thread-like structures which increase in thickness with time. The pairing of homologous chromosomes begins during zygotene pachytene. The nuclear morphology changes from a round to a horse shoe-like form (see Figure 1A and B) with a synaptonema complex (SC) and recombination nodule (RN) present as shown in Figure 1D. There is no evidence of pre-existing pairing or alignment of homologous chromosomes before zygotene in most higher eukaryotes. Furthermore, in mutants lacking SCs or when the synthesis of SC is inhibited, the chiasma formation and recombination are suppressed. Thus, pairing is a pre-requisite to recombination in meiotic nuclei. However, in fission yeast, in which the central element of SC is absent and the nuclear membrane does not disappear, recombination is needed for the pairing of homologues. Fission yeast cells normally grow as haploid (+ or -) cells and + cells fuse to – cells just before meiosis, so the pairing occurs just before meiosis. In budding yeast in which recombination also precedes the pairing, SCs are normal while the nuclear membrane is retained throughout prophase. Although the order of homologous pairing and cross-over (recombination) differs between fungi and higher eukaryotes, the molecular mechanism is considered universal.

No matter which comes first, pairing or recombination, homologous chromosomes must move closer to each other. Some studies have suggested that they do this during the previous mitosis but organisms like fission yeast, in which plus and minus cells fuse just prior to meiosis, must find homologous chromosomes during the meiotic prophase. This is achieved by first gathering both ends of the chromosomes into a bouquet-like arrangement and letting them move from one end of the cell to the other as though being shaken while held at their ends (see Figure1 E). This kind of movement, observed by labeling the ends and centromeres with fluorescent dyes (Hiraoka, 1999), is probably helpful for sorting chromosomes by length, but cannot explain pairings with inversion, deletion and insertion. The bouquet shape is commonly observed in pachytene nuclei of higher organisms; nevertheless it is a very effective pair-inducing mechanism. Another interesting observation is that the nucleus in meiotic prophase is very irregular in shape, with many invaginations (Figure 1C, small insert) together with an increased number of nuclear pores, often covering the nuclear membrane just before its disappearance. Usually, the ends of chromosomes are attached to the nuclear membrane where pores do not exist. During the change in shape of the nucleus, the chromosome ends were pushed into the proper position, and the other ends were pushed together by the formation of pores in other spots. This may be caused by the meiosis-specific alteration of nuclear lamins, which are medium-sized filament proteins assumed to function in determining nuclear structure and considered part of the nuclear skeleton. The introduction of such a meiosis-specific lamin gene to somatic cells causes their nucleus to become meiotic (Furukawa, 1993, 1994).

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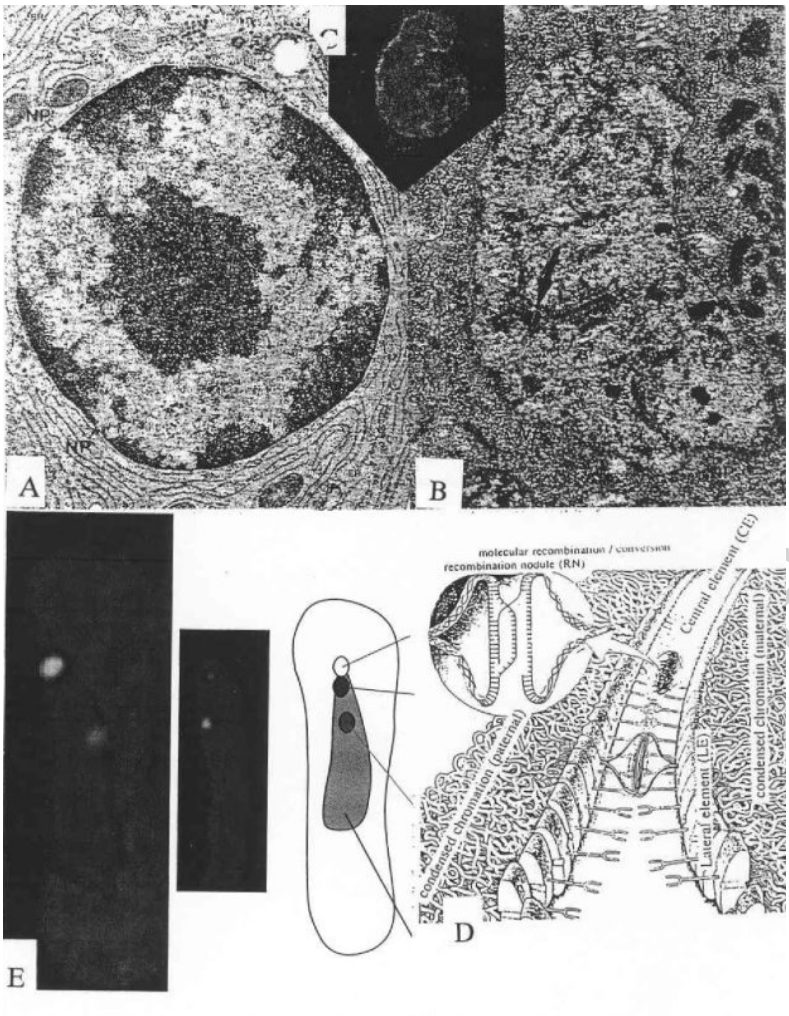

Figure 1. Nuclear morphology of meiotic nuclei and model of a synaptonema complex with a recombinational nodule (RN).

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According to gross analysis under an optical microscope, cross-over between homologues occurs nearly at random and with the same frequency along the chromosomes except in heterochromatic, centromeric and telomeric regions. Later, hot spots where recombination takes place at a much higher frequency, and cold spots where recombination scarcely occurs at all, were found on chromosomes and within genes of fungi and mammalian cells. Some of the structural features of hot spots are: a scattered telomere sequence, purine-pyrimidine repeats which can form transient left handed coils or Z-DNA forms, several repeats of recombination enzyme-binding sites, and a location between certain genes like the 5'end of the HIS gene and 3'end of the BIKI gene. At the molecular level, the hot spots are induced by double stranded breaks. These breaks are suppressed in mutants lacking the binding of transcriptional factors, Rap1p, Bas1p, and Bas2p, to the upstream region of HIS4 in yeast. There is a linear relation between the level of recombination and number of breaks (Fan, 1995). Rap1p (repressor activator protein 1) is an abundant nuclear protein that functions in the maintenance of telomeres, transactivation of genes and formation of inactive chromatin. The binding site of Rap 1 at the 5'end of HIS4 shows a very high frequency of recombination during meiosis by creating a negative supercoil after the binding and

rewinding of DNA (Gilson, 1994). Several studies found a telomere sequence near the centromere after detailed examination using drugs which induce a cross or Y shaped DNA at short repeats. A considerable number of DNA scission, repair and recombination events occur at distributed centromeres or close to them (Fernandez, 1995). A relationship was found between transcription and recombination. In yeast, there is a high correlation between transcription and mutation. Thus, we see a correlation between recombination and mutation. As a matter of fact, highly mutable regions are recombination hot spots. It is conceivable that recombination removes the undesirable mutated regions of chromosomes by inducing necessary enzymes and other protein activities, while gene conversion directly eliminates the mutation.

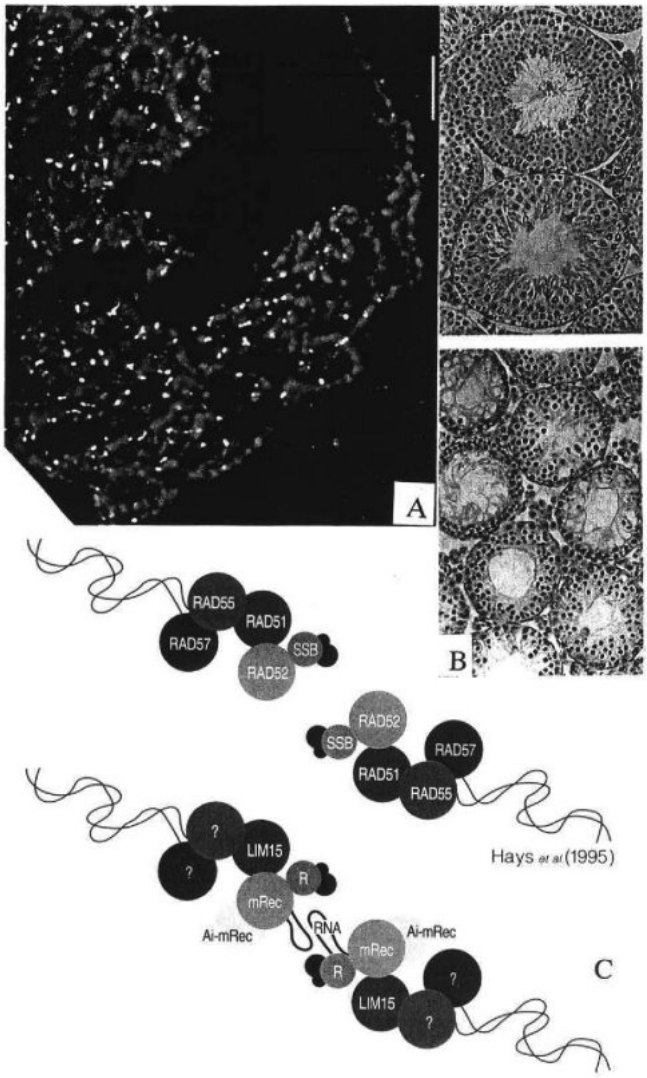

Figure 2. Homologous recombination.

The structure of recombination sites and the behavior of proteins together with other components, are of interest. An early study focused on the localization of a RecA-like protein and found two homologues, RAD52 and LIM15, that co-localizated on paired chromosomes at numerous spots (Terasawa, 1995) (see Figure 2A). Mutant mice

lacking such meiotic protein do not develop spermatids in feminiferous tubules (Fig. 2B). Later, Rad51 was identified at the sites of initiation and replication of chromatin during premeiotic replication of DNA. Also, its association with SC, and the R-banding region which are known as initiation sites of pairing and recombination were reported (Plug, 1996). The number of putative recombination spots (at least several hundred) identified by such proteins is far more than the number of chiasma (3-4 per bivalent), and their non-specific distribution can be compared to the incorporation of radioactive precursors into bivalents in pachytene which are at least several hundreds times more than anticipated from the number of chiasma, and distributed seemingly at random (Hotta, 1971). This may suggest that the initiation of recombination by the double stranded breaks occur at many points along a chromosome but most are repaired. However, some resulted in gene conversion and only at a small number of loci did recombination occur. In yeast, double stranded breaks during meiosis occur independent of the homologues, (as expected from wild-type cells) at normal frequency, at normal positions and with normal timing, and the repair of these breaks decides whether recombination proceeds. Since recombination is a useful tool for improvement of plants and for evolutionary studies, the induction or suppression of recombination is an interesting subject.

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