Genetic Recombination and Meiosis

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Mini-review:

Modern genetics started from Mendel who luckily chose three genetic traits of beans which are located on different chromosomes, or picked out those three, out of several other traits whose occurence he could not explain. Mendel's law has been considered the basis of genetics for many years, however, many hereditary phenomena that contradict his law are found. Firstly, hereditary events in mitochondria observed in yeast contradict Mendel's law. When haploid cells containing the wild type mitochondria and cells containing a mutant type mitochondria, for example chloramphenicol sensitive mate, all cells become resistant to the drug. With successive mitosis, either the mutant or the wild type mitochondria will gradually be diluted out to daughter cells, forming only one type of mitochondria in any particular cell by the random process called mitotic segregation. In fertilization, only mitochondria of female cells are transmitted to the next generation while the mitochondria from male cells degrade in oocytes by a yet unknown mechanism.

A second event contrary to Mendelian genetics is recombination. The discovery of linear linkage of genes in *Drosophila* chromosomes, where a group of genes co-transmit together to the next generation, was important. The connection between linkage maps and chromosome structure was established by McClintock, using maize. After many studies of gene mapping, the rearrangements of genes became clear as Whitehouse proposed a model for recombination (1982). The chemical and physical structure of chromosomes were established through the discovery of the double strand model of Watson and Crick (1953) and the genetic basis of DNA was made evident by Meselson and Stahl (1958) and Taylor (1963) who showed a semi-conservative replication of DNA and chromosomal distribution. Following this, the molecular basis of mutation, transformation and recombination, all of which create non-Mendelian phenomenon, came to light. Today, only the last of laws, concerning dominant-recessive segregation and independence is acceptable.

Recombination is divided into two categories, based on the requirement of homology for pairing. One category is general recombination which requires a certain length of sequence homology between two double stranded DNAs and the scission of strand(s) to initiate the exchange of the strands. The other is site-specific recombination, which an excision and insertion of DNA, occurring in the presence of a short, specific nucleotide sequence recognized by a variety of recombination enzymes. Nearly all of the molecular and biochemical studies of recombination have been carried out by using bacteria (ex. *E. coli*) and bacterial viruses (phages), but recently, yeast has been a favorite target for analysis among various eukaryotic cells. Both types of recombination occur in somatic cells with low frequency, but the rate of homologous recombination increases tremendously in meiotic prophase cells with rates 1000 to 10,000 times grater, depending

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on organism, sex and environmental condition, while no increase of site-specific recombination is observed. In meiotic recombination, the breakage and repair of nuclear DNA takes place at various sites, although there are hot spots and cold spots, and some of the former may be lead to recombination. Carpenter (1979) showed a good correlation between recombination sites and the position of recombination nodules (RN) in synaptonema complex(SC), which appears in between paired homologs during pachytene. Exchange of DNA strands between homologs requires several enzymes and other gene products as summarized by Hays (1995) using microscopic detection on the chromosomes. This mini-review intends to explain several events occuring in meiosis.

Chromosomal behavior and meiotic recombination: Cytologists' observations of meiotic prophase chromosomes indicated an increase of hetero-chromatic regions inside the nuclear membrane and an increase in the number of nuclear pores that eventually replace most of the membrane. Towards leptotene, nuclear chromatin condensation starts, forming thin thread-like structures and these increase in thickness through time. Chromosomes move around inside the nuclear membrane as though one homolog is trying to look for a partner. All the ends of chromosomes attach to the nuclear membrane and these pieces of membrane become the seed of membrane formation during anaphase. During zygotene/pachytene when chromosomal pairing and crossing-over take place, the nuclear shape changes from ball-like to horse shoe-like, as a bouquet-type arrangement of chromosomes can be observed in some organisms. Under the microscope, evidence of pre-existing pairing has not been detected, but recombination between homologous DNA preceeds pairing in yeast, and it leads to pairing, while in *Drosophila* pairing must be present before crossing-over, and a disturbance of SC formation or mutants lacking pairing do not recombine. Such discrepancies have not been resolved. However, one can see that fission yeast normally grow in a haploid condition(+ or -) and that + cells fuses to - cells just before meiosis, meaning the fusion of cells and chromosomal meetings takes place prior to meiosis. In budding yeast, the nuclear membrane does not disappear during nuclear and cell division. Although the sequence of pairing and recombination is different between fungi and higher eukaryotes, the molecular mechanism is considered to be universal.

The bouquet-like arrangement of all chromosome ends and that chromosomes move around inside the cell or nucleus is very helpful in finding homologs to each other. If inversion or deletion exists in the chrosomes, they can arrange themselves to maximize pairing to each other. Alteration of nuclear morphology may be induced by nuclear lamins specific for meiotic prophase nuclei. Introduction of such lamin-genes to somatic cell has induced the nucleus to becomes horse shoe-like (Furukawa, 1993,1994). If the nuclear shape is related to chromosomal recombination, induction of meiotic lamin gene(s) may stimulate recombination.

The presence of recombination hot spots and cold spots is well documented and their possible structures include these; scattered telomere sequences, purine-pyrimidine repeats which can form transient left handed coil or Z-DNA form, several repeats of recombination enzyme binding sites and the structure between certain genes like the 5'end of the HIS gene and the 3'end of the BIKI gene. The hot spots are induced by double strand breaks of DNA molecules. This break is suppressed in mutants lacking the binding of transcriptional factors, Rap1p (repressor activator protein), Bas1p, Bas2p on the upstream of HIS4 in yeast. Also, there is a parallel relation between recombination frequency and the number of DNA breaks (Fan,Q., 1995). Rap1p is an abundant

nuclear protein working for the maintenance of telomere structure, trans-activation of genes and inactive chromatin. The binding site of Rap1 at the 5'end of the HIS gene shows very high recombination during meiosis (Gilson,E., 1994). Treatment with a drug that induces the formation of short X-shape or Y-shape DNA at the telomere-like sequence near the centromere also induces a considerable amount of DNA scission, repair and recombination (Fernandez,L.L., 1995).

There is no doubt that highly mutable regions are recombination hot spots and it is conceivable that recombination removes undesirably mutated regions of chromosomes using enzymes and structure proteins while gene conversion directly eliminates the mutation. An early study focused on the location of RecA-like proteins and RAD52 and LIM15 revealed their co-localization on numerous spots of homologs going to and completing pairing (Terasawa,M., 1995). Mutant mice that lack such meiotic proteins fail to complete their meiosis and to form spermatids, and end up infertile.

Rad 51 protein was detected on replicating points of pre-meiotic chromatin and near SC of the R-banding region, namely, the known initiation sites of recombination (Plug,A.W., 1996). Such putative sites of recombination exist in several hundreds on lily meiotic chromosomes but the number of actual sites, crossing-over and chiasma are far less than this (3 to 4 chiasma per bivalent). The amount of repair synthesis of chromosomal DNA also exceeds the expected DNA synthesis due to recombination (Hotta,Y., 1971). All these suggest that initiation of recombination occurs in many spots and double strand breaks may be repaired as normal or through gene-conversion at breaking points, however, only some spots can exchange their strands for recombination.

Induction of recombination: In budding yeast, at least ten genes (Spo11, Mre11, Rad50, Xrs2, Mer2, Mek1/Mre4, Mei4, Rec104, Rec114 and Rec102) are indispensable for double strand breaks. Such breaks needed for recombination induction are not necessary on both stands but are needed in either one of two and should occur at the near location on the other strand. They can be nicks or gaps in DNA to form a 3 stranded shape (Shan, 1998). Among these, Rad50 and Mre11 are required for both introduction and repair of double strand breaks. Mre11s (mutant gene of Mre11) leaves a break for a longer period leading to local non-homologous recombination. This period also maintains the pairing of homologs and increases homologous recombination (Nairz and Klein, 1997). During late zygotene and pachytene, a considerable number of breaks are bound with recombination protein having 3'-P and 5'-OH endings created by meiosis-specific DNase (Stern, 1974). It is conceivable that this end structure contributes to a longer period of opening, which in turn helps to increase recombination, because this must be conversion to 3'OH and 5'-P termini in order for repair by DNA polymerase and ligase.

Double strand breaks occur at the QAGR promoter site and the region between YCR47c and YCR48w gene which are hypersensitive regions to nuclease and appear as multiple scissions on DNA. All of them first show 5'-overhangings instead of the usual 3'-ones, indicating the involvment of a meiosis-specific DNase. These 5'-overhangs should be removed before recombination. A Rad50S mutant lacks the digestionability with an identified protein tightly bound at the 5'-overhang end and this protein can not be removed from the end of DNA by deproteinization. A similar structure has been found in fission yeast during meiosis. Namely, the structure is the binding of SPO11 protein to Rad50S DNA. The promoter region of the AGR locus is a recombination hot spot having 14 adenine chains, and, if this region is removed, the rate of gene conversion will be reduced to 25-30

percent of the wild type. Since Rad51 mutant has suppressed recombination and do not have such overhanging structure, such single strand structure must be important for recombination.

To complete molecular recombination during meiosis, the genes to check for damage of DNA in mitosis, RAD9, RAD17, RAD24, MEC1, must work and the mutants of these genes also suppress meiosis. On the other hand, a mutant of XRS (X-ray and chemical mutagen resistant) gene of *Arabidopis* reduces somatic recombination but not meiotic recombination. These facts suggest the presence of a relationship between the two types of recombination.

Pairing and recombination: Cyto-genetic studies in higher organisms showed that pairing of homologs should occur ahead of the crossing-over events, however, studies in yeast demonstrated that homologous recombination is a requirement of pairing. Later, studies using *Drosophila* supported the idea that pairing is pre-requisite for homologous recombination. In any case, a stable pairing of homologs is indispensable for chromosome segregation.

In vitro assays of homologous recombination have been hampered by difficulties in purifying all the necessary components and the removal of DNase(s) from cell extract. However, several trials were conducted in the early 1980s, and the necessity of RecA-like protein was universally demonstrated. Two such proteins were isolated from mouse spermatocytes and lily microspores and somatic cells and named m-rec and s-rec, respectively. M-rec consists of ATP-dependent and independent unwinding proteins. An assay mixture which contained two mutant plasmids which can form a wild type (drug resistant) after homologous recombination; by addition of m-rec into the cell extract reaction mixture, recombination activity increased 100 fold but did not do so with s-rec. A comparable fraction with m-rec was found in yeast meiotic cells and it also increased in vitro recombination activity while the fraction isolated from somatic cells did not. The S-rec did not interfare with m-rec activity. It should be noted that initiation of meiosis needs a suppression of somatic transcription and translation The maximum amount of m-re activity coincides with pachytene, when chromosomal crossing-over occurs and is not found in achiasmatic cells (Hotta,1985) Such an assay system has been improved and many modifications are recorded (Hino, 1991).

Establishment of recombination: According to the Holliday model (Holliday, 1970) of hetero-duplex formation, the direction of a DNA strand cut determines whether it undergoes to recombination or gene conversion. *In vitro* experiments have demonstrated that when a supercoiled DNA was incubated with homologous double strand linear DNA in the presence of the enzymes, Rec BCD, they form a primary complex. If Rec A is added, they convert to an intermediate complex. Here, Rec BCD have DNA helicase activit and 3' to 5' exonuclease activity until they meet certain nucleotide sequences on a DNA molecule, like the kai sequence of λphage, E. coli, Neurospora and others. RuvA and RuvB complex also show DNA helicase activity. All of these help to move Holliday structure to loci where recombination occurs (Takagi,M. et al. 1994). RecG protein, which has RNA helicase activity, was found to function for the migration of Holliday structure. Helicase mutants are deficient in DNA repair and homologous recombination.

Until the 1980s, the involvement of RNA(s) in recombination was never mentioned, but the finding of RNA fragment(s) in the complex of DNA-protein, obtained by mild treatment of pachytene chromatin with *Micrococcal* nuclease or DNase I, suggested the presence of a functional RNA(s). Pachytene chromosomes are much more resistant to various nucleases than mitotic

chromosomes, but some of the sensitive regions contained a small RNA(s) bound with protein and/or DNA (Hotta, 1986). A double strand RNA was found in relation with homologous recombination in yeast meiosis and germ cell production (Watanabe, 1994 and Yamashita, 1998). Involvement of specific RNase was found in microsporocytes of rape and lily. RNA helicase and DEAD box protein with Asp-Glu-Ala-Asp as a consensus sequences were universally detected in the reproductive tissue. For example, vas protein from *Drosophilla* oocytes, An3 protein from Xenopus oocytes and PL10 protein from their testes are DEAD box protein. Mutants with An3 found in yeast to man and Ste13 of fission yeast (gene for 58kDa protein with 8 DEAD boxes) suppress their meiosis but not mitosis. Dhh1 of budding yeast, Me31B of Drosophila and p54/Rck of man are all homologous to Ste13 and their products bind with an RNA and show a RNA helicase activity. These are all supportive evidence to show an RNA involvement for establishing homologous recombination.

Transcription and recombination: In general, a transcribing region of DNA, if a damage occurs, it can be repaired actively by polymerase β in somatic cells. Plasmids containing the promoter of murine mammary tumor virus that becomes neomycin aminoglycoside (G418) if the recombination takes place, were introduced into ovary cells (K1c) in which transcription was induced using dexamethazone (dex). In the presence of dex. the observed recombination frequency increased six fold (Nikoloff and Reynolds, 1990). The loci expressed in germ cells and a number of repetitive DNA elements including SINEs and LINEs are metabolically active. Thus, it is possible to use such segments of DNA for gene-transfer, particularly during meiosis.

Spermatocytes exposed to poliovirus RNA take up the viral RNA followed by reverse transcription and the copied DNAs integrate into the nuclear genome. This has been confirmed by PCR after extensive RNase treatment. The presence of reverse transcriptase in the nuclear scaffold (Pittoggi, 2000) has been demonstrated by immunological studies and by sequencing of nuclease-sensitive regions of spermatogonial DNA. Almost all sequences obtained here contained retrotransporsons like ORF2 of LINE1. LINE1 has two ORFs, one codes for 40kDa RNA binding protein (ORF1) and the other (ORF2), codes for reverse transcriptase. These suggest the use of germ cells for integration of DNA into the loci that are active in transcription or hypersensitive to DNase. Although pollen-mediated gene transfer has not been successful, the presence of transporson-like sequences in microsporocytes of lily suggests the possibility of such methods of gene transfer. Introduction of DNA into pollen is achieved by DNA-coated iron particle bombardment. The pollen containing iron particles can be separated using a magnet and placed on the top of pistils for fertilization. Sperm and pollen nuclei have been considered inactive, but recent studies show that they are active in some parts, although much less than oocyte- and ova- nuclei, and that they are useful for transfection and transformation.

Meiosis and environment: Any unfavorable environment tends to induce meiosis by acting directly on the cells and/or indirectly through the body. Nutrient starvation in yeast induces meiosis and the formation of spores, as long as the genome is capable. In higher organisms, their meiosis depends on the temperature of tissue and organs in which meiosis takes place. In general, meiosis occurs at much lower temperatures than that at which somatic cells replicate at the best rate. The temperature of mammalian testicles is several degrees lower than the abdominal cavity. In females, ovary is located in the abdominal cavity and it's temperature is expected to be the

same as the body. However, the temperature of the follicle is lower than the surrounding ovary tissue and becomes even lower at ovulation, by 3℃. When this temperature difference is small or reversed, ova are sterile *in vivo* and in artificial insemination. In bull frogs, their somatic growth best at 18-30℃ occurs, but germinal breakdown suppressed. In hibernating frog, germinal breakdown takes place during winter.

At higher temperatures, the expression of P34cdc fails and HF-MPF, which replaces MPF, does not function. Then, the ova becomes sterile due to meiotic degradation (Grinsted, 1985). A meiosis associated heat shock protein, HSP70 and the gene called LIM18 were analyzed (Minami, 2000). The former is localized in cytoplasm to act as a chaparon while the latter localizes in nucleus and is sensitive to higher temperature. These proteins together with some other proteins control chromosome pairing and homologous recombination. Also, if the temperature is too low, cellular spindle fibers break down, leading to failure of chromosome segregation in meiosis I. Male meiosis of rice becomes abnormal in drought, resulting in male sterility while female organs differentiate normally and maintain their fertility. This male sterility occurs due to the inhibition of invertase in the anther. Invertase controls water through carbohydrate metabolism and protects the whole body and female parts by sacrificing male parts in drought. Most of the reports indicate that the female parts are better protected than the male parts, structurally and metabolically.

Conclusion: Recombination, whether in somatic cells or in meiotic cells, homologous or non-homologous, is a major factor of evolution. It is also a usable system for developing new genetic traits and for the treatment of diseases. It is essentially insertion or exchange of DNA segment(s) through scission, exchange and repair. The molecular recombination hot spots are; the presence of ① transient left handed helices or zDNA structure, ② binding sequences of recombination enzyme, ③ high transcription and sensitive sites to nuclease (Fan,1995), ④ certain repeated sequences, like HIS4,AGR4, DNT11, DNT42 of yeast, and ⑤ miniature inverted repeats, SINE, LINE and transposable elements (Bureau, 1996). At the chromosome level, hot spots are telomeres and subtelomeres, regions with telomere-like sequences, chromosomal fragile sites and the particulara sites of genes. The spots can be used to insert or remove certain genes for genetic manipulation. The highest recombination has been observed during meiotic prophase, when necessary recombination factors are synthesized and the expression of many somatic genes is suppressed.

References:

1. Carpenter, A.T.C. Recombination nodules and synaptonemal complex in recombination defective females of Drosophila. Chromosoma 75:259-263 (1979).
2. Fan, Q., Xu, F. and Petes, T.D. meiosis-specific double-strand DNA breaks at the HIS4 recombination hot spot in yeast Saccharomyces cerevisiae: Control in cis and trans. Mol. Cell Biol. 15:1679-1688 (1995).
3. Fernandez, J.L., Gosalvez, J. and Goyanes, V. High frequency of mutagen-induced chromatid exchanges at interstitial telomere-like DNA sequenceblocks of Chinese hamster cells. Chromosome Res. 3: 281-284 (1995).
4. Furukawa,K. and Hotta, Y cDNA cloning of a germ cell specific lamin B3 from mouse spermatocyte and its function and ectopic expression in somatic culture cells. EMBO J. 12: 97-105 (1993).
5. Furukawa,K., Inagaki,H., Naruge,T., Tabata,S., Tomita,T., Yamaguchi,M. and Nagahama,Y. cDNA cloning and functional characterization of meiosis specific protein (MNS1) with apparent nuclear association.

Chromosome Res. 2: 99-113 (1994).
6. Grinsted,J., Kjer,J.J., Blendstrup,K. and Pedersen,J.F. Is low temperature of follicular fluid prior to ovulation necessary for normal oocyte development? Fertil Steril 43: 34-39 (1985).
7. Hays,S.L., Firmenich,A.A. and Berg,P. Complex formation in yeast double-strand break repair: Participation of Rad51, Rad52, Rad55 and Rad57 proteins. Proc. Natl. Acad. Sci., U.S.A. 92: 6925-6929 (1995).
8. Hino,O., Tabata,S., and Hotta,Y. Evidence for increased in vitro recombination with insertion of human hepatitis B virus DNA. Proc. Natl. Acad. Sci., U.S.A. 88:9248-9252 (1991).
9. Hotta,Y. and Stern,H. Analysis of DNA synthesis during meiotic prophase. J. Mol. Biol. 55: 337-355 (1971)
10. Hotta,Y., Tabata,S., Bouchard,R.A., Pinon, R. and Stern,H. General recombination mechanisms in extracts of meiotic cells. Chromosoma 93: 140-151 (1985).
11. Hotta Y., Furukawa,K. and Tabata,S. Meiosis specific transcription and functional proteins. Adv. Biophys. 31: 101-115. (1995).
12. Meslsohn,M. and Stahl,F.W. The replication of DNA in E.coli. Proc. Natl. Acad. Sci., U.S.A 44: 671-682 (1958)
13. Minami,M., Hiratsuka,R., Ogata,S-i., Takase,H., Hotta,Y. and Hiratsuka,K. Characterization of a meiosis-associated heat shock protein 70. Plant Biotech. 17: 145-153 (2000).
14. Nairz,K. and Klein, mre11S-a yeast mutation that blocks double-strand-break processing and permits non-homologous synapsis in meiosis. Genes Dev. 11:2272-2290 (1997).
15. Nikoloff,J.A. and Reynolds,R.J. Transcription stimulates homologous recombination in mammalian cells. Mol. Cell. Biol. 10: 4837-4845 (1990).
16. Plug,A.W., Xu,J., Reddy,G., Golub,E.I. and Ashley,T. Presynaptic association of Rad51 protein with selected sites in meiotic chromatin. Proc. Natl. Acad., Sci. U.S.A. 93:5920-5924 (1996).
17. Stern,H. and Hotta,Y. DNA metabolism during pachytene in relation to crossing over. Genetics 78: 227-235 (1974).
18. Takagi,M., Iwasakai,H. and Shinagawa,H. Structural requirements of substrate DNA for binding to and cleavage by RuvC, a Holliday junction resolvase. J.Biol. Chem. 269: 15132-15139 (1994).
19. Taylor,H. DNA replication in mammalian cells. Ed.by Becker,Y. Nijohoff, Netherland.
20. Terasawa,M., Shinohara,A., Ogawa,H., Hotta, Y. and Ogawa,T. Localization of RecA-like recombination proteins on chromosomes of lily at various meiotic stages. Genes & Develop. 9:925-834 (1995).
21. Watanabe,Y. and Yamamoto,M. *S. pombe* mei2+ encodes and RNA binding protein essential for premeiotic DNA synthesis and meiosis I, which cooperates with a novel RNA species meiRNA. Cell 78:487-498 (1994).
22. Watson, J.D. and Crick, F.H.C. Molecular structure of nucleic acids. A structure for deoxynucleic acids. Nature 171:737-738 (1953).
23. Whitehouse, H.L.K. Genetic recombination: Unfolding, the mechanisms. Wily, New York (1982).
24. Yamashita,A., Watanabe,Y., Nukina,N. and Yamamoto,M RNA-associated nuclear transport of the meiotic regulator Mei2P in fission yeast. Cell 95: 115-123 (1998).