CONTROLS OF GENETIC RECOMBINATION IN HIGHER FUNGI

Por *Celia Dubovoy**

Recombination is one of the least understood genetic phenomena. From the classical works of Morgan and Sturtevant (1911, 1913) we know that linkage between genes is konwn to reflect their presence on the same choromosome and the frequency of recombination depends exclusively on the physical distance between them. Morgan postulated that the closer two genes were to each other the less likely they were to recombine.

Several posterior studies showed that recombination was not so easy to explain and was highly affected by environmental and mainly genetical factors.

Studies in yeasts, *Neurospora* and *Schizophyllum* proved the existance of genes controlling recombination. Genetic control of recombination of higher fungi has been studied following mainly two parameters, 1) Magnitude of effect and 2) Extent of genome affected.

According to this, Simchen and Stamberg (1969) have given two sorts of genetic regulation of recombination in higher fungi. The "coarse control" shared also with prokaryotes in which the controlling genes affect the whole genome giving an all or none effect (allelic differences are seen as complete lack of occurrence of recombination). Like the rec-gene of *Escherichia coli* which brings a complete breakdown in recombination, or the asynaptinemal mutants in fungi and higher plants.

The other sort of control called "fine control" is exclusive of eukaryotes and has been found in yeasts, *Schizophyllum* and *Neurospora*. In this sort of control there are frequent genetic differences which affect recombination by changing its frequency between small regions or often between alleles of the same cistron, but do not prevent recombination of occurring.

A study of "fine control" plus two other sorts of recombination control which the author considers highly probable according to the parameters given by Simchen and Stamberg (1969) but hard to prove, will be discussed in the present work.

In fungi, several studies have been done in the genetical aspects of intracistronic and intercistronic recombination.

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Studies on recombination were based on the frequency of prototrophs formed amongst the progeny of crosses between pairs of auxotrophic alleles. I think unfortunately this is not a very accurate criterion because it does not take in account back mutations or presence of supressors of the original auxotrophic mutations, and gives a certain amount of error in this study.

Abbreviations used in the present work

rec 1, 2 etc...-Genes controlling recombination
his-1, 2 etc...-Different histidine requiring mutants
am-1, 2 etc...-Amination mutants requiring glutamic-deshydrogenase
inos-Inositol requiring mutants
nit-2.-Mutant not utilizing nitrate due to the lack of nitrato-reductase.
arg-3.-Mutant requiring arginine
pyr-3.-Mutant requiring pyrimidine
ad-3.-Mutant requiring adenine
sn.-Morphological mutant of *Neurospora crassa* called "snowflake"
NADPGH.-Glutamate deshydrogenase linked to niacin adenine diphosphate.

Jessop and Catcheside (1965) studied the *his-1* locus of *Neurospora crassa* and found a very big difference in recombination of alleles between different stocks tested. Crosses could be divided in those with a high and those with a low frequency of prototrophs, the factor difference was about ten. Genotipically observations were explained by assuming two allelic genes rec and rec$^+$; rec being recessive to rec$^+$ so that only crosses in which both parents were rec gave high frequencies of recombination and crosses with rec$^+$ always gave low frequencies. The dominant allele rec$^+$ may be regarded as the wild type.

Total reliance can not be given in the actual frequency of prototrophs even if the cross is rec x rec for the reasons outlined above, and asides because the sites of difference between to rec parents may be so close together that recombination is rare. In general, they found that between the high and low groups respectively, the prototroph frequencies shown between different stocks of the same *his-1* alleles are similar, but seldom they are significantly different, this may show the possible influence of other genetic factors.

The genetic factor had no discernible effect in the flanking markers *am* and *inos*. Recently it has been proven that the action of rec-1 is not exclusively in his-1 but it controls as well the gene controlling nitratoreductase located in another linkage group; gene assigned as *nit-2*. Perhaps the segments that are jointly controlled have some common role in a particular fitness character.

Catcheside (1968), also found a second gene in *Neurospora crassa* rec-3 controlling recombination in the *am-1* locus in the same way in which rec-1 controls recombination in *his-1*. The rec-3 affects all of the nine combinations of *am*. The degree of effect of rec-3 differs between different pairs of *am* alleles, having a much smaller depressing effect of recombination on *am-6* and

am-7. This differences probably are not only due to actual allele differences themselves but to other possible differences between genes due to use of different original stocks. Rec-3$^+$ does not affect flanking markers. As with rec-1 initially it was only known that rec-3 controls *am-1,* recently it has been proven that it controls *his-2* as well as nonallelic recombination between *sn his-2* and nonallelic recombination in the *arg-3 his-2* region (Catcheside 1973).

Jha (1967), studied the *his-3* locus founding a gene rec-4$^+$ resembling rec-1$^+$ and rec-3$^+$ in reducing allelic recombination between all combinations of *his-3* alleles, it is dominant and does not affect recombination between flanking markers. The prototroph frequency in presence of rec-4$^+$ is reduced to one half or on third.

Smith (1966), found a regulator which affects nonallelic recombination rec-2$^+$; it affects the region *pyr-3 his-5* reducing recombination from 14% to 5%. Subsequently Catcheside found that the same gene reduces recombination in *his-3* as well as other nonallelic recombinations *arg-3 sn* and *his-3 ad-3*.

Simchen (1967) studied the recombination in the *A* incompatibility factor of *Schizophyllum commune* concluding that there is also a similar control as in *Neurospora* with a rec$^+$ gene dominant. Rec$^+$ proved to be linked to the *A* factor and accounts for the major differences in recombination, but not all his data can be explained by a rec-gene, it looks that they are minor effects from other genes, recombination frequencies do not fall only in high and low but show a certain range.

Koltin and Stamberg (1973) found a gene controlling *B* factor recombination in *Schizophyllum commune*. It controls recombination between α and β subunits of the *B factor*. This gene is linked to the *B* factor itself approximately at nine map units from *B*. It was called *B* β rec-1 and does not affect recombination in an unlinked region (between subunits of the *A* factor) or in a region contiguous to the *B* factor (between *B* α and the morphological marker *dome-2)*.

Friis and Herschel (1968) showed that the mating type alleles in yests affect recombination in other loci like *ad*. All the preceding studies raise the following questions:

1) Are factors at a single locus responsible for the effect on recombination or the control might be polygenic?

2) Is the effect of the rec genes specific for small regions of the genome or could it control even the whole genome?

3) If it is specific for some regions of the genome though they might be in different linkage groups, are those regions necessarily controlled in the same way by the same controlling gene?

4) What is the effect of rec in different environments or physico chemical conditions like a whole range of temperatures etc. This, will bring some answers to the role that rec genes play in ecology particularly in a rapidly changing environment?

5) Could natural selection directly operate in this genes?

6) What is the relationship of the control of recombination and the breed-

ing system of fungi? Is the presence of dominant genes controlling low recombination favorable?

7) How do the rec genes operate at the molecular level, analysis of this, may offer an excellent way of clarifying the complexity of crossing over by dissecting it into its many components?

The first question can be answered in part by studies done by Stamberg (1968) and Simchen (1968) in *Schizophyllum*. They found that sometimes instead of recombination being high and low, it has a whole range and this could certainly speak in favor of the possibility of a polygenic control.

In answer to the second question I think that centainly in the so called "fine control" the controlling genes are confined to small regions of the genome but this, may be erroneous just because the geneticist for convenience concentrates upon small regions of the genome. As it has been outlined above the more the amount of regions analysed, the findings of more regions controlled by the same controlling element has been put in evidence. Though for instance it has been proven in *Neurospora* that rec-1 doesn't affect *am* which rec-3 affects and viceversa, asides not affecting flanking markers; nevertheless considering the parameters given by Simchen and Stamberg (1969) to divide recombination in two sorts, I deffinitely think that a priori with those parameters, there might be four possible types of recombination.

Analysing this idea in a small diagram we have the following:

Magnitude of effect	*Extent of genome affected*	*Type of control*
1) all or none	complete	Coarse
2) quantitative	partial	Fine
3) all or none	partial	Unknown
4) quantitative	complete	Unknown

The first two types of control are the ones already mentionad, the third and fourth type of control may well exist but they are difficult to prove particularly the last one since it will imply studying all the genes to see if there is a change in recombination in all of them, task which no geneticist can undertake, no matter how diligent he works. Nevertheless it is deffinitely not ruled out the possibility of those two unknown sorts of control.

Considering again the "fine control" it is hard to ascertain if genes that are controlled by the same controlling element have some common role with regard to a particular character.

As it has been verified recently that the same controlling gene controls different regions of the genome, it is possible that the number of controlling genes is not very big.

The third question can be answered in part by studies done by Simchen (1967). He found that in *Schizophyllum commune* recombination in the incompatibility *A* factor is variable and some inbred lines showed significant

increase in recombination while others did not. The *B* factor behaved in a totally different way, all the inbred lines showed a slight decrease in recombination. Though in this case the controlling region shows different effects, it is not always necesarily that the same controlling element will affect different regions in a different way, in many cases it may affect them similarly. Actually there may be a variety of responses in a complex "fine control" composed of interrelated elements, which will occur for environmental adjustements.

In answer to the fourth question, the only environmental factor that has been studied is temperature and it is known from Stamberg (1968) that it does have a major control in the recombination process of *Schizophyllum*. That may affect different components of the "fine control" which in itself may be a mechanisms by which the organism will respond to rapid release of variability in a changing environment. The effects of temperature on recombination in *Schizophyllum* are highly region specific and genome specific, e. g. recombination at 30°C for $A\,\alpha - A\,\beta$ in different crosses may be higher, lower or the same as in 22°C (Stamberg, 1968; Stamberg and Simchen 1970).

Many other factors should be studied like presence of certain anions or cations, water balance, recombination over a whole range of pH, etc. This will reflect how the organism can respond to stress and rapid environmental change by his "fine control" of recombination. All this considerations may answer the fifth question, I think it is very probable that natural selection operates directly in the rec genes, by modifying the linkage relationships when it needs adjustement, at times following changes in ecology or in the structure of a population.

In answer to the sixth question, I think actually the relationship between the control of recombination and breeding is very important. In *Schizophyllum commune* probably since the genes that increase recombination are recessive they are selected against in nature. With this, the outbreeding potential of the population increases, against inbreeding which is disadvantageous. Changes in the breeding system could bring changes in recombination.

In *Schizophyllum* and other fungi outbreeding may keep together particularly beneficial gene complexes.

In answer of the last question we can say that probably the rec genes determine the structure of an enzyme which participates on one of the events of recombination or it determines the structure of a regulatory substance which activates or represses the enzyme.

The localization of the effects of the "fine control" in short chromosomal regions implies that some sort of specific recognition will exist between the controlling and controlled elements.

Whitehouse (1966) has suggested that the controlling elements of recombination and transcription are the same. Catcheside (1968) gives evidence to the existence of different regulators for recombination and transcription. On studying the am^+ gene which specifies glutamate deshydrogenase linked to NADP he found that the enzyme is repressed by glutamate plus ammonium

or urea and that repression of these compound occurs in equal extent in strains that are rec-3$^+$ and rec-3. So, it appears that the regulator controlling the transcription of *am* gene is not the substance specified by rec-3$^+$. If the rec-3$^+$ would have been a repressor of transcription of *am-1* NADPGDH in rec-3 strains this shouldn't be repressed by conditions which repress rec-3$^+$. Even if the regulators are different it is interesing to know if the two regulators share the same operator, or if there are two operators (one of regulation of recombination and the other of transcription), it will be interesting to know how these are arranged with respect to one another and to the genes controlled. The analysis of regulator as well as operator mutants if they can be found offers an extreme interesting study giving a way of dissecting the process of crossing over. Certainly, in this case, concentration should be on a very small region of the genome.

Detailed study of genetic an other secondary variations which affect recombination is essential to the understanding of the complex molecular process involved in recombination.

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SUMMARY

A review of the literature of genetic control or recombination is made, particularly of the "fine control" of genetic recombination in higher fungi. The regulating genes called rec$^+$ are dominant in all cases, in reducing recombination in small chromosome regions sometimes distant from their own locations. Some genes control recombination in two or more chromosomal segments, the advantage of such a system of recombination for adjustement during ecological changes is discussed as well as the advantage of the system for the breeding process. A possible model of action for the rec genes at the molecular level is given and possibilities for future studies in recombination are outlined. Two other ways of recombination that are possible but hardly to prove experimentally are presented.

RESUMEN

Se presenta una revisión de parte de la literatura sobre el control de recombinación, particularmente el "control fino" de recombinación en hongos superiores. Los genes reguladores denominados rec$^+$ son siempre dominantes en la reducción de la recombinación en pequeñas regiones del cromosoma localizadas, en ocasiones, distantes del gene al que regulan. Algunos genes, controlan recombinación en dos o más segmentos cromosómicos. La ventaja de dicho sistema de control de recombinación para el ajustamiento durante cambios ecológicos es discutida, así como la ventaja del sistema en el proceso de apareamiento. Se presenta un posible modelo de acción de los genes rec a nivel molecular y se indican posibilidades para estudios futuros de recombinación, así como otras dos formas posibles de control de recombinación, pero difíciles de ser probadas experimentalmente.