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A restriction and modification model for the initiation and control of recombination in *Neurospora*

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Summary

It is hypothesized that the products of Neurospora *rec*$^+$ genes mask recombinators such as *cog* by modifying DNA and that unmodified recombinators act as recognition sites for an endonuclease with scission properties like those of the type I restriction enzymes found in *E. coli.* These cut the DNA in both strands at some variable distance from a recognition site. Repair of a two strand gap initiated in this way would require DNA synthesis using the information contained in the homologous DNA duplex, leading to gene conversion. Crossing over could follow from resolution of two Holliday structures formed during gap repair. The hypothesis explains the polarity in the frequency of conversion events across genetic loci, the observation that chromosomes carrying recombinators are more often converted than is the homologue, and how recombinators can initiate conversion at a distance, as suggested by the pattern of conversion events in the *his-3* locus in crosses heterozygous for the translocation TM429.

1. Introduction

Meiotic recombination appears to be initiated from specific sites that have been termed recombinators (Holliday, 1968). There is good evidence for such sites in *Neurospora crassa*, where recombination in several genes has been shown to be non-random, with conversion frequencies being higher at one end of the gene than at the other (Murray, 1963; D. G. Catcheside, 1977). In addition, variant genes are known which influence recombination in their immediate vicinity and others are known that determine recombination frequencies in each of several specific chromosomal regions.

A model for the initiation and control of recombination in *Neurospora* was deduced from the properties of the naturally occurring variant alleles of genes determining local recombination frequencies: see D. G. Catcheside (1977) for a review. In this 'repressor' model, it is envisaged that there are some 500 *recog*nition sites scattered throughout the genome at which recombination is initiated by a single strand nick. It was proposed that initiation is subject to control by repressors coded by about ten different *rec*ombination genes that each block the initiation of recombination from a subset of the *cog* sites by binding to a *con*trol site of the appropriate specificity situated adjacent to each affected *cog* site. Possible mechanisms for processing a nicked DNA duplex and its interaction with a homologous duplex to yield genetic recombinants have been described by D. G. Catcheside & Angel (1974) and by Meselson & Radding (1975).

A difficulty with single strand nick models in their simplest form is that asymmetric heteroduplex is formed only on the homologous duplex, not on the duplex first nicked. This predicts that the nicked duplex will more often be the donor of genetic information. This is not in fact the case: the duplex that carries an active recombinator is more often the recipient of information in conversion events. This difficulty can be overcome in various ways. D. G. Catcheside (1977) proposed that following the establishment of a single strand bridge, strand transfer to the duplex first nicked is driven by DNA replication from the new 5′ end on the homologous duplex, the reverse of the usual direction, resulting in the majority of asymmetric duplex being upon the initiating chromosome. Markham & Whitehouse (1982) have proposed that following recognition of the recombinator, an endonuclease cuts in *trans*, opening a single strand nick in the homologous duplex. Radding (1982) has proposed that nicking of the recombinator is followed by degradation to open a single strand gap. Strand invasion would then result in the formation of asymmetric heteroduplex DNA on the initiating duplex.

The model proposed in this paper offers a different explanation. The principal features of the model are that: (i) *cog* sites are subject to epigenetic modification by *rec* gene products and that only unmodified

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sites are recombinators, (ii) the endonuclease initiating recombination binds to the recombinator and translocates the DNA past the enzyme for a variable distance prior to making a two strand cut in the same duplex, (iii) the ends are eroded to form a gap and the gap filled by repair synthesis using information contained in the homologous duplex.

Enzyme systems with properties appropriate for the initiation of recombination in this manner are known in *Escherichia coli* type I restriction and modification systems (Yuan, Hamilton & Burckhardt, 1980), where they provide defence for the cell from invading phage or plasmid DNA. Repair of double strand gaps has recently been implicated in recombination events in Yeast (Orr-Weaver, Szostak & Rothstein, 1981; Orr-Weaver & Szostak, 1983) and a model for recombination involving gap repair that leads to the formation and resolution of two Holliday junctions has been proposed (Szostak *et al.* 1983).

2. Genes that control recombination in *Neurospora*

Four types of gene influencing the frequency of meiotic recombination have been described in *Neurospora crassa*: *rec*, *con*, *cog* and *ss* (Table-I). For reviews, see D. G. Catcheside (1977) and D. E. A. Catcheside (1981). In each case, the effect is limited to specific regions of the genome and for each of *rec*, *cog* and *ss*, two or more alleles have been found as a polymorphism in the wild and laboratory populations.

Three *rec* gene loci are known (Figure 1). *rec-1* affects allelic recombination at the *his-1* locus on linkage group V (Jessop & D. G. Catcheside, 1965) and at the *nit-2* locus on linkage group I (D. E. A. Catcheside, 1970; 1974). *rec-2* affects recombination between *his-5* and *pyr-3* on linkage group IV (Smith, 1966; 1968), between *his-3* and *ad-3* on linkage group I (D. G. Catcheside & Corcoran, 1973) and also between *his-3* alleles (D. G. Catcheside & Angel, 1974). *rec-3* affects recombination between *his-2* and *sn* on linkage group I (D. G. Catcheside & Corcoran, 1973) and also allelic recombination at the *his-2* locus (D. G. Catcheside & Austin, 1971) and at the *am* locus on linkage group V (D. G. Catcheside, 1966; Smyth, 1971; 1973). In each case, presence of the dominant *rec*$^+$ allele reduces recombination frequency about one order of magnitude within the affected regions.

con, *cog* and *ss* each influence recombination only within short segments of a linkage group adjacent to their location. Recombination between *nit-2* alleles in crosses heteroallelic for *ss* is reduced, suggesting that *ss* is involved in the pairing of DNA duplexes in this region of linkage group I (D. E. A. Catcheside, 1981). *ss* heterozygosity acts multiplicatively with *rec-1*$^+$ to reduce recombination at the *nit-2* locus about 100 fold. However, the effects of both *con* and *cog*, are dependent on *rec*.

There are two known alleles at the *cog* locus which lies between *his-3* and *ad-3* on linkage group I. In crosses homozygous *rec-2*, the dominant allele *cog*$^+$ permits higher levels of recombination between *his-3* alleles and between *his-3* and *ad-3* than does the reces-

Table 1. *Region-specific genes that influence meiotic recombination frequency in* Neurospora

Locus	Linkage group	Alleles: dominant	other	Target loci or region	Linkage group	Effect on Recombination in target region
rec-1	V	*rec-1*$^+$	*rec-1*	*his-1*	V	Presence of
				nit-2	I	dominant allele
rec-2	V	*rec-2*$^+$	*rec-2*	*his-5-pyr-3*	IV	in a cross
				his-3, *his-3-ad-3*	I	reduces
rec-3	I	*rec-3*$^+$	*rec-3*, *rec-3*^{L}	*his-2*, *his-2-sn*	I	recombination
				am	V	up to 30-fold
cog	I	*cog*$^+$	*cog*	*his-3*, *his-3-ad-3*	I	Dominant allele yields high frequency recombination in crosses homozygous *rec-2*
con[a]	V	—	—	*am*	V	Reduced by either of *rec-3*$^+$ and *rec-3*^{L}.
	I	—	—	*his-2*	I	Reduced by *rec-3*$^+$ only.
ss	I	—	*ss*^{E}, *ss*^{S}, *ss*^{C}	*nit-2*	I	Reduced by any heterozygous combination.

[a] The existence of this class of gene was postulated to account for the differential effect of *rec-3*^{L} on allelic recombination at *am* and *his-2*. There are no known allelic variants of *con*. However, the *con* sites on linkage groups I and V are non-identical since *rec-3*^{L} differentiates between them.

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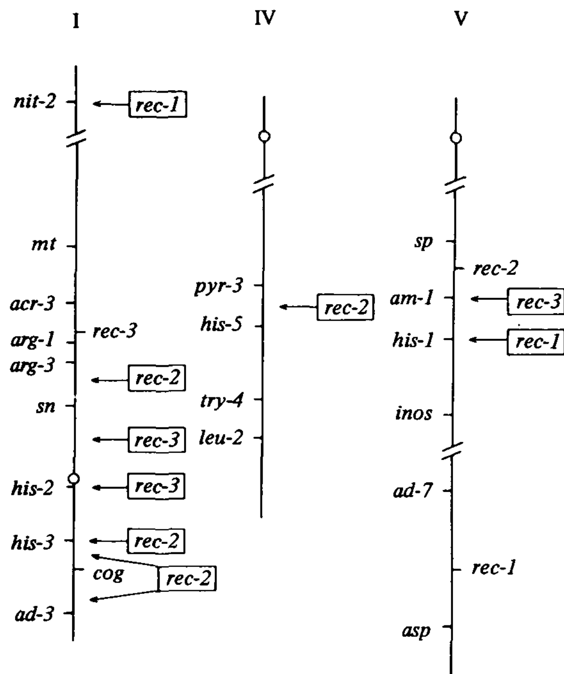

Fig. 1. A partial linkage map of *N. crassa* showing the three known *rec* loci and the loci or regions where they are known to influence recombination. From D. G. Catcheside, 1975, p. 304.

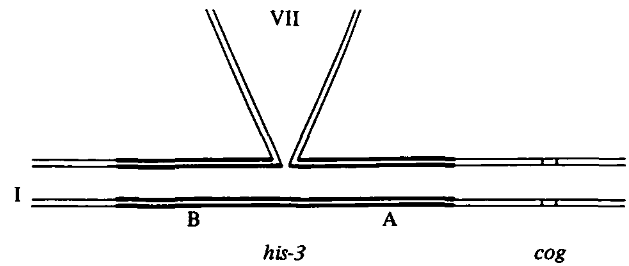

Fig. 2. Pairing relationship between a normal sequence linkage group I and the translocation chromosomes of TM429 in which a reciprocal exchange between linkage groups I and VII has occurred interrupting the *his-3* locus. In crosses to TM429, when *his-3* mutants are located in region B of the normal sequence chromosome, the yield of prototrophic recombinants is low and insensitive to reduction by *rec-2*$^+$ unless *cog*$^+$ is present in the normal sequence chromosome. For *his-3* mutants in region A, the recombination frequency is high and sensitive to reduction by *rec-2*$^+$ irrespective of which duplex contains *cog*$^+$.

sive allele *cog* (Angel, Austin & Catcheside, 1970; D. G. Catcheside & Angel, 1974). However, *rec-2*$^+$ is epistatic to *cog*$^+$. In the presence of *rec-2*$^+$, recombination frequencies in this region of linkage group I are reduced to the same low level irrespective of the presence of *cog*$^+$ (Angel *et al.* 1970).

Recognition of the existence of *con* followed from the properties of alleles at the *rec-3* locus. Three alleles are known: *rec-3*$^+$, *rec-3*^{L} and *rec-3*. Allelic recombination at the *am* locus is reduced by *rec-3*$^+$ and to a lesser extent by *rec-3*^{L}. However, unlike *rec-3*$^+$, *rec-3*^{L} does not reduce recombination between *his-2* alleles, yielding a recombination frequency similar to that of crosses homozygous *rec-3*. This indicates that there are sites adjacent to each of *am* and *his-2* that determine their sensitivity to control by *rec-3* alleles and that, although similar, the sites cannot be identical. These sites have been called *con*, and are presumed to bind the *rec-3*$^+$ gene product (D. G. Catcheside, 1975; 1977).

3. The repressor model for recombination control in *Neurospora*

In each of the three known cases, the dominant allele *rec*$^+$ reduces recombination in the two or more known target regions. This is most simply explained by proposing each *rec* gene locus has a diffusible product and that the product of the *rec*$^+$ allele actively interferes in some way with recombination which otherwise would have occurred within the target regions. The alternative, that the product of each *rec* allele stimulates recombination within its specific target region, is difficult to sustain since in that case the *rec* alleles would be expected to be dominant and give high frequency recombination. Although dominance of low frequency could be explained as negative complementation by a defective *rec*$^+$ gene product, that this should occur in the case of each of four *rec* variants: *rec-1*$^+$, *rec-2*$^+$, *rec-3*$^+$ and *rec-3*^{L} seems improbable.

Since the same low frequency of recombination is obtained at the *his-3* locus in crosses containing *rec-2*$^+$, irrespective of which of *cog* or *cog*$^+$ is present, the *cog* locus has been assumed not to be the site through which the *rec-2*$^+$ gene product exerts its regulating influence at *his-3*. Thus in addition to the *cog* locus adjacent to *his-3*, it has been presumed that there is also a *con* locus through which *rec-2*$^+$ exerts control over recombination events in this region of linkage group I (Angel *et al.* 1970). The dominance of *cog*$^+$ in stimulating recombination has been interpreted as indicating that recombination events are initiated from *cog*$^+$ and that initiation is blocked by the *rec*$^+$ product interacting with the adjacent *con* locus (Angel *et al.* 1970). This view was greatly strengthened by data obtained when the interchange strain TM429, in which the *his-3* locus is interrupted by a translocation, was crossed to *his-3* mutants with structurally normal chromosomes (Fig. 2) (D. G. Catcheside & Angel, 1974). When the mutant in the normal sequence chromosome was located on the opposite side of the translocation breakpoint from the *cog* locus, the frequency of formation of *his*$^+$ recombinants was low unless *cog*$^+$ was in the normal sequence chromosome and moreover, was only subject to control by *rec-2*$^+$ when the normal sequence chromosome carried *cog*$^+$ (D. G. Catcheside & Angel, 1974). This result prescribes that recombination events are initiated at *cog*$^+$ and specifically upon the DNA duplex that carries it. The alternative possibility, that *rec-2*$^+$ controls a function for terminating recombination events, is not tenable as cross

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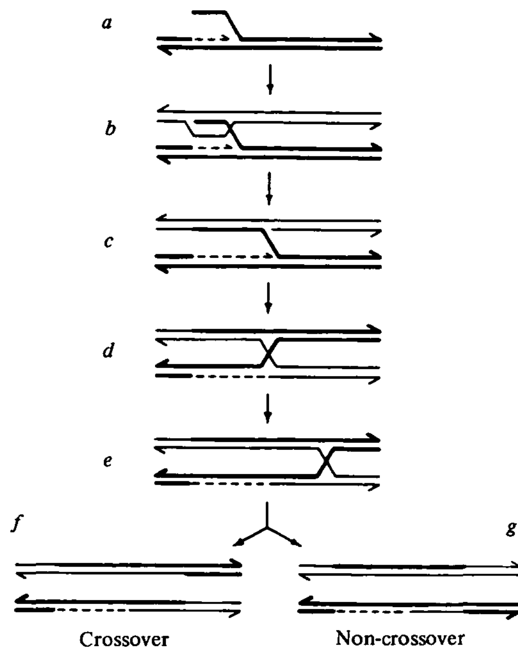

Fig. 3. Model for recombination initiated from a single strand nick. From Szostak *et al.* 1983, p. 27. (*a*) DNA synthesis displaces a single strand which invades the homologous duplex (*b*) forming asymmetric heteroduplex (*c*). Isomerization or branch migration can lead to the formation of a two strand bridge (*d*) and rotary diffusion of this Holliday junction can generate symmetric heteroduplex (*e*). Correction of mispaired bases leads to conversion. Resolution of the junction can lead to crossover (f) or non-crossover chromosomes (*g*). This model can account for the genetics of TM429 if the nick in (*a*) can be driven by DNA synthesis along the normal sequence chromosome beyond the interchange point prior to invasion of the homologous duplex.

strand bridges would be blocked from access to the cog^+ site by the interchange.

Since recombination events initiated at cog^+ must be able to pass the breakpoint, the TM429 data are also prescriptive of the events that initiate recombination. There must be a phase in which an incipient recombination event initiated at the cog^+ site can migrate along the DNA duplex prior to forming a cross strand exchange with the homologous chromosome. One possibility is illustrated in Fig. 3, proposed originally by D. G. Catcheside & Angel (1974). This is similar to the Aviemore model proposed by Meselson & Radding (1975), suggesting that recombination is initiated by scission of only one strand of the duplex. The cut strand is proposed to be displaced by DNA polymerase extending the 3′ end generated by the single strand nick, with a cross strand bridge being established by D loop formation when the displaced single strand pairs with the homologous DNA duplex. A two strand bridge necessary for symmetric recombination events can be established by isomerization (Sobell, 1974), and recombination completed by correction of mispaired bases, breakage of cross strand bridges and filling and ligation of gaps. Recombination events initiated at cog^+ could pass the interchange in a cross heterozygous for the TM429 translocation if D loop formation is delayed until the DNA polymerase driven nick has progressed along the normal sequence chromosome beyond the interchange (D. G. Catcheside & Angel, 1974).

Based on the known extent of their effect, the three known *rec* loci appear to control recombination in about 30% of the *Neurospora* genome. If all of the genome is subject to such control, then a total of about ten *rec* genes might be involved (D. G. Catcheside, 1975). The size of individually controlled segments suggests that up to 500 *cog* loci might exist each with an attendant *con* locus of the appropriate specificity to confer control by one of the *rec* loci (D. G. Catcheside, 1975).

4. The role of two strand gaps in yeast recombination

Transformation of Yeast with plasmid DNA has been shown to be stimulated up to 3000-fold by the presence in the plasmid of double strand gaps in regions of homology with the Yeast chromosome (Orr-Weaver *et al.* 1981). More recently, Orr-Weaver & Szostak (1983) have shown, using plasmids capable of autonomous replication, that repair of the double strand gap using chromosomal information yields approximately equal numbers of integrated and non-integrated plasmids, indicating that gene conversion by double strand gap repair can occur with or without crossing-over, and in this respect is similar to meiotic recombination. The integration of gapped or linear plasmids, but not of circular molecules, is blocked by the *rad52-1* mutation (Orr-Weaver *et al.* 1981). The *RAD52* gene product is also required for meiotic recombination, for UV- and gamma-ray-induced mitotic recombination, and for normal levels of spontaneous mitotic recombination (Prakash *et al.* 1980; Game *et al.* 1980).

These and other considerations have led Szostak *et al.* (1983) to propose a two strand gap model for meiotic recombination in which the principal features are: the repair of a double strand gap by two rounds of single strand repair synthesis, the formation of heteroduplex DNA in the regions flanking the zone of gap repair, and the resolution of two Holliday junctions to complete the repair event (Fig. 4). The model provides an explanation for the observation that DNA duplexes carrying recombinators such as cog^+ in *Neurospora* (D. G. Catcheside & Angel, 1974), the M26 mutant in *Schizosaccharomyces pombe* (Gutz, 1971) and YS17 in *Sordaria brevicollis* (MacDonald & Whitehouse, 1979, 1983), are usually the recipients of genetic information in conversion events, since gaps generated on the chromosome carrying the recombinator must be repaired by information from the homologous duplex. Orr-Weaver & Szostak (1985) have shown that, with the appropriate assumptions, the processing of a single strand nick or of a two strand gap in DNA can account for the genetic outcomes of meiotic recombination.

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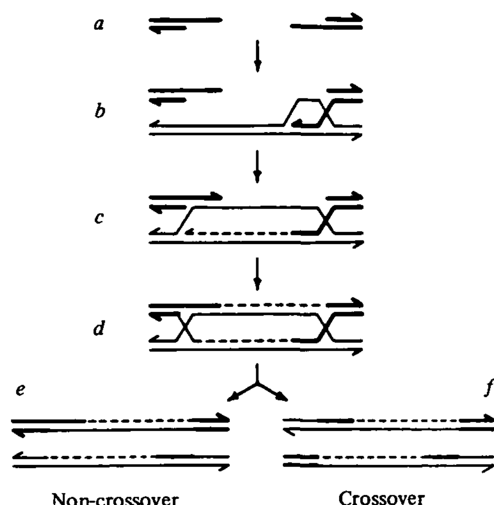

Fig. 4. Model for recombination initiated by two strand gaps. From Szostak *et al.* 1983, p. 30. The 3′ ends of the gapped molecule (*a*) act as primers for repair of the gap using information from the homologous duplex (*b*), (*c*). This leads to the formation of two Holliday junctions (*d*). Resolution can lead to non-crossover (*e*) or crossover chromosomes (*f*). A means whereby this model can account for the genetics of TM429 is outlined in Fig. 5.

5. A restriction and modification model for the control of recombination in *Neurospora*

An examination of the way in which the properties of the TM429 translocation could accommodate the initiation of recombination by two strand gaps was prompted by the indication that two strand gaps are involved in recombination in Yeast and by the fact that the simplest form of the Meselson and Radding model for recombination does not fit with the observation that DNA molecules carrying recombinators are more often recipients rather than donors of genetic information in conversion events.

TM429 shows that recombination events begun at the recombinator cog^+ regularly migrate some distance prior to the establishment of single or double strand bridges between homologous duplexes. Moreover, since recombinants can be recovered readily from crosses heterozygous for TM429 and mutations located in the normal sequence chromosome on the far side of the interchange junction from cog^+, any two strand gap cannot always be begun at the cog^+ site itself since a two strand gap extending from cog^+ to cover the mutation would destroy continuity in the only intact *his-3* allele involved in the exchange and his^+ recombinants could not occur, unless the sister chromatid also becomes involved.

Enzymes which make two strand breaks in DNA at variable distances from a host specificity sequence are well known in type I restriction and modification systems in *Escherichia coli* (Yuan & Hamilton, 1982). These restriction enzymes only cleave the DNA duplex if the host specificity sequence is not methylated. In the K restriction system, the endonuclease and methylase activities are associated. The functional enzyme is composed of two subunits specified by the gene *hsdR* that carry the endonuclease site, two subunits specified by *hsdM* that carrry the methylase site and a subunit specified by *hsdS* carrying the DNA binding site. The hetero-pentamer binds to the DNA and becomes specifically associated with the host specificity sequence. If only one strand of the DNA is methylated within the host specificity sequence, methylation of the second strand ensues. If neither strand is methylated, allosteric changes unmask a second DNA binding site that makes a second and non-specific contact with the DNA, generating a loop. Contact with the host specificity sequence is maintained but in an ATP dependent reaction, the point of contact with the second site is moved along the DNA for an indeterminate distance prior to cleavage of first one and then the second strand of the duplex. Scission thus occurs at variable distances up to several thousand bases away from the host specificity sequence. Type I restriction enzymes have hyphenated host specificity sequences comprising constant domains of three and four bases separated by a non-specified spacer. In the case of *EcoK*, the spacer is six bases. In other restriction and modification systems, notably those of type II which generally cleave the DNA at the host specificity sequence, the restriction and modification enzymes are separate entities and the host specificity sequence is uninterrupted.

The possibility that there is an enzyme which binds at cog^+ that has the DNA cleavage characteristics of type I restriction enzymes, offers an alternative to single strand scission for the initiation of recombination in Neurospora. Extension of the analogy leads also to an attractive alternative to the repressor model for the action of *rec* genes in controlling recombination. It can be supposed that *rec* gene products are modification enzymes that modify *cog* sites. Only unmodified *cog* sites would be recombinators.

The model has the following essential features: (i) each *cog* locus in the genome can exist in one of two states, modified or unmodified. In the unmodified state *cog* loci act as recombinators, when modified they do not. (ii) rec^+ genes specify modification enzymes that inactivate recombinators. (iii) An endonuclease recognises unmodified *cog* loci, binds and initiates scission of both strands of the DNA duplex at an unspecified site at an indeterminate distance up to several kilobases away from *cog* on the same duplex (Fig. 5). Modified *cog* loci do not permit endonuclease action. (iv) The initial two strand break is extended to form a gap, perhaps by a second scission by the endonuclease or by exonuclease activity. (v) The two strand gap is repaired using information from an intact homologous duplex by a mechanism such as that described by Szostak *et al.* (1983), leading to recombination.

A possible variation is single strand scission by the endonuclease. However, like all single strand scission models this would beg further explanation as to why

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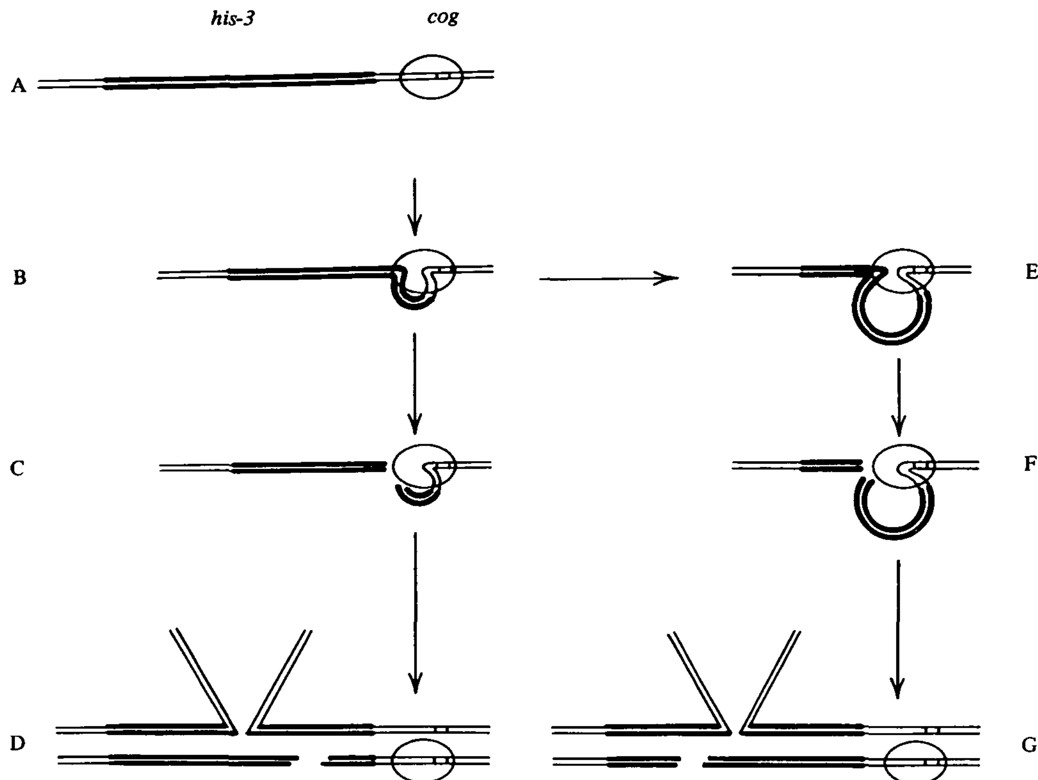

Fig. 5. A restriction and modification model for the initiation of recombination. An endonuclease binds to *cog* (A) and translocates the DNA past a second site for an indeterminate distance (B) prior to making a two strand scission (C) which is widened to form a gap (D). The two strand gap is refilled by repair synthesis using information from the homologous duplex in the manner shown in Fig. 4. The cut duplex is here shown paired with TM429. If the translocation proceeds far enough (E) the two strand gap (F) will initiate recombination beyond the interchange in a translocation heterozygote (G). It is hypothesized that the endonuclease is unable to act if *cog* is modified by an enzyme specified by *rec-2*$^+$. If each phosphodiester bond translocated past the endonuclease has a finite chance of cleavage, gap formation should decrease in frequency with distance from *cog*, explaining the polarity of conversion across *his-3*.

the duplex carrying a recombinator is more often the recipient rather than the donor of information.

A restriction and modification like system for the initiation and control of recombination might be organized in a number of ways. Since at least three and perhaps as many as ten *rec* loci exist that control recombination in different chromosomal regions, there must be several different sorts of recombinator distinguished either by differential recognition by a variety of recombinogenic endonucleases or by differential recognition by a variety of modification enzymes.

If a single species of endonuclease initiates recombination at a variety of different recombinators, the specificity site for the endonuclease would be associated with a variable region recognised differentially by the products of the various *rec* gene loci, leading to differential modification of recombinator sites even though each when unmodified is a potential binding site for the same species of recombinogenic endonuclease. In this type of model, the genes *con* and *cog* are different parts of a recombinator sequence. *con* might be buried within *cog* as the variable spacer region of a hyphenated binding site for the endonuclease.

If each type of recombinator is acted upon by a different endonuclease, the endonuclease and modification enzymes may recognize precisely the same sequence, in which case, the distinction between *con* and *cog* would disappear. In such models, modification of each type of recombinator might be effected by an enzyme coded by *rec*$^+$ which is either separate from or, like the type I restriction enzymes, associated with the endonuclease. If modification is maintained continuously, the modification enzyme would be required following each DNA replication whilst the endonuclease would function only at meiosis. If modification is imposed only prior to meiosis, again a temporal separation of modification and endonuclease action is expected. However, these considerations do not preclude the endonuclease being an additional subunit attached to the various modification proteins during the prophase of meiosis.

The differential effect of *rec-3*$^+$ and *rec-3*^{L} on recombination at *am* and *his-2* indicates that the recombinators adjacent to these two loci differ. This can be accommodated by the restriction and modification model if there are at least two permissible variants of the recombinator recognised by the *rec-3*$^+$ gene product and if the version coded by the *rec-3*^{L} allele does not recognize the variant adjacent to *his-2*.

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6. Conclusions

In its essential features, the restriction and modification model for the initiation and control of recombination in Neurospora fits with the genetics of both *rec* and *cog* loci.

The dominance of each of *rec-1*$^+$, *rec-2*$^+$ and *rec-3*$^+$ in determining low frequency of recombination in multiple specific chromosomal regions is accounted for by their products being enzymes that modify and thereby inactivate recombinators. The recessive nature of the alleles *rec-1*, *rec-2* and *rec-3* is accounted for by their products lacking the modification activity.

The dominance of *cog*$^+$ over *cog* in stimulating recombination is accounted for by its being a *cis* acting recombinator permitting endonucleolytic activity on the same DNA duplex. The recessive nature of the allele *cog* is accounted for by its being a defective recombinator which by virtue of its location *trans* to *cog*$^+$ in heterozygotes does not interfere with the more efficient initiation of recombination from *cog*$^+$.

That *rec-2*$^+$ is epistatic to *cog* is accounted for if the *rec-2*$^+$ gene product is able to modify both *cog* and *cog*$^+$, thereby inactivating both *cog* as a low efficiency recombinator and *cog*$^+$, a recombinator of higher efficiency.

The polarity of conversion which decreases with distance from recombinators in *Neurospora* is accounted for by the decreasing probability of the endonuclease cutting the DNA as distance from the *cog* binding site increases.

A restriction and modification system for the initiation and control of recombination might be organized in one of several ways. Amongst the most economical would be a single endonuclease, that recognises all classes of recombinator by recognizing a constant deoxynucleotide sequence common to all, and a number of *rec* gene specified modification enzymes each targeted at a specific class of recombinator by recognizing the version of a variable sequence characteristic of that class of recombinator. The genes *cog* and *con* of the repressor model would respectively become the constant and variable regions of a recombinator. Alternatively, the several modification enzymes might each be complexed with a common subunit, present only during the meiotic prophase, carrying an endonuclease site or unmasking an endonuclease site on the modification protein. In this case, the same protein site recognises a recombinator for both modification and endonuclease binding and the distinction between *cog* and *con* vanishes.

The restriction and modification model for the control of recombination in *Neurospora*: (i) accounts for the initiation of coversion events on *cog*$^+$ chromosomes beyond the breakpoint of the translocation TM429, (ii) provides a known mechanism for generating two stand breaks at unspecified positions close to a recombinator, (iii) explains why, in heterozygotes, the chromosome carrying the recombinator, in this case *cog*$^+$, is more often the recipient of information than the donor in conversion events, and (iii) provides an alternative to the repressor model for the initiation and control of recombination in Neurospora that is consistent with the genetics of each of *rec*, *cog* and *con* and that may be generally applicable to meiotic recombination in eukaryotes. The interpretation of *ss*, that it is concerned with the establishment of synapsis prior to the initiation of recombination, remains unchanged.

The nature of any modification imposed by *rec*$^+$ genes on *cog* sites is a matter for speculation. However, since methylation is known to occur in Neurospora rDNA (Rodland & Russell, 1984) and in amplified DNA present in transformants (Bull & Wooton, 1984), methylation, particularly at the 5 position of cytosine, is an obvious possibility for any DNA modification involved in the control of recombination.

In recent years, evidence has accumulated that gene activity is influenced by DNA methylation (see Doerfler, 1983 and Razin & Cedar, 1984 for reviews). Thuriaux (1977) has pointed out that recombination in eukaryotes appears to be confined to genes and their immediate vicinity. This follows from the observation that although the DNA content varies over a thousand fold range in organisms as diverse as fungi, Drosophila and maize, the map length of genes and the total map length of the genome is relatively constant. Holliday (1984) has proposed a unified theory relating DNA methylation, the regulation of gene activity and the purpose of meiosis. It is speculated that recombinators are either one and the same as non-methylated promoters, or have base sequences in common and that apart from the role in generating genetic diversity, meiotic recombination serves to restore defective epigenetic methylation controls of gene activity by creating hemi-methylated DNA in the hybrid regions formed by the recombination event. The hemi-methylated hybrid DNA would be generated specifically wherever a recombinator/non-methylated promoter occurred and would serve as substrate for a maintenance methylase to restore full methylation. Holliday (1984) also noted that the resemblance of nucleases initiating recombination to bacterial restriction enzymes may not be fortuitous. The proposed relationship between promoters and recombinators may not occur in Neurospora, since the *rec-3* locus which controls the recombinator effecting allelic recombination at the *am* locus, does not influence expression of the *am* gene (D. E. A. Catcheside, 1967). It may also be relevant that the hypermethylation of *am* in transformants has no apparent influence on gene expression (Bull & Wootton, 1984). This hypermethylation does not have the preference for CG sequences typical of methylation of DNA in higher prokaryotes and Bull and Wootton have speculated that it might be involved in a recombination or repair process for which amplified DNA is a target. If so, it may have no relevance to control of genes or recombination in

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single copy sequences which in Neurospora have a low 5-methyl cytosine content.

If methylation inactivates recombinators, substances that cause hypomethylation would be expected to stimulate recombination. Hypomethylation of the appropriate sequences might be achieved with 5-aza cytidine or with ethionine, dimethylsulphoxide or 3-deazaadenosine which should be less specific. Alternatively, since differential methylation is expected in *rec* and *rec*$^{+}$ strains, digestion of the DNA with appropriate pairs of isoschizomers having differential sensitivity to methylation would be expected to yield DNA fragments of different sizes. Pairs of isoschizomers that might be appropriate include *MspI-HpaII* and *MboI-Sau3A*. A modification other than by methylation also cannot be discounted.

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References

Angel, T., Austin, B. & Catcheside, D. G. (1970). Regulation of recombination at the *his-3* locus in Neurospora crassa. *Australian Journal of Biological Science* **23**, 1229–1240.

Bull, J. H. & Wootton, J. C. (1984). Heavily methylated amplified DNA in transformants of *Neurospora crassa*. *Nature* **310**, 701–704.

Catcheside, D. E. A. (1967). Regulation of the *am-1* locus in Neurospora: evidence of independent control of allelic recombination and gene expression. *Genetics* **59**, 443–452.

Catcheside, D. E. A. (1970). Control of recombination within the nitrate-2 locus of *Neurospora crassa*: an unlinked dominant gene which reduces prototroph yields. *Australian Journal of Biological Science* **23**, 855–865.

Catcheside, D. E. A. (1974). A second locus subject to recombination control by the *rec-1* gene of *Neurospora crassa*. *Australian Journal of Biological Science* **27**, 561–573.

Catcheside, D. E. A. (1981). Genes in Neurospora that suppress recombination when they are heterozygous. *Genetics* **98**, 55–76.

Catcheside, D. G. (1966). A second gene controlling allelic recombination in *Neurospora crassa*. *Australian Journal of Biological Science* **19**, 1039–1046.

Catcheside, D. G. (1975). Regulation of genetic recombination in *Neurospora crassa*. In *The eukaryote chromosome* (ed W. J. Peacock and R. D. Brock), pp. 301–312. Canberra: Australian National University Press.

Catcheside, D. G. (1977). The genetics of recombination. London: Edward Arnold.

Catcheside, D. G. & Angel, T. (1974). A histidine-3 mutant in *Neurospora crassa* due to an interchange. *Australian Journal of Biological Science* **27**, 219–229.

Catcheside, D. G. & Austin, B. (1971). Common regulation of recombination at the amination-1 and histidine-2 loci in *Neurospora crassa*. *Australian Journal of Biological Science* **24**, 107–115.

Catcheside, D. G. & Corcoran, D. (1973). Control of non-allelic recombination in *Neurospora crassa*. *Australian Journal of Biological Science* **26**, 1337–1353.

Doerfler, W. (1983). DNA methylation and gene activity. *Annual Review of Biochemistry* **52**, 93–124.

Gutz, H. (1971). Site specific induction of gene conversion in *Schizosaccharomyces pombe*. *Genetics* **69**, 317–337.

Game, J. C., Zamb, T. J., Braun, R. J., Resnick, M. A. & Roth, R. M. (1980). The role of radiation (*rad*) genes in yeast. *Genetics* **94**, 51–68.

Holliday, R. (1968). Genetic control of recombination in fungi. In *Replication and Recombination of Genetic Material* (ed. W. J. Peacock and R. D. Brock), pp. 157–174. Canberra: Australian Academy of Science.

Holliday, R. (1984). The Biological significance of meiosis. In *Controlling Events in Meiosis* (ed. C. Evans), pp. 381–394. 38th Symposium of the Society for Experimental Biology.

Jessop, A. P. & Catcheside, D. G. (1965). Interallelic recombination at the *his-1* locus in *Neurospora crassa* and its genetic control. *Heredity* **20**, 237–256.

MacDonald, M. V. & Whitehouse, H. L. K. (1979). A *buff* spore colour mutant in *Sordaria brevicollis* showing high-frequency gene conversion. 1. Characteristics of the mutant. *Genetical Research* **29**, 65–81.

MacDonald, M. V. & Whitehouse, H. L. K. (1983). A *buff* spore colour mutant in *Sordaria brevicollis* showing high-frequency gene conversion. 2. Loss of the high-frequency conversion. *Genetical Research* **41**, 155–163.

Markham, P. & Whitehouse, H. L. K. (1982). A hypothesis for the initiation of genetic recombination in eukaryotes. *Nature* **295**, 421–423.

Meselson, M. S. & Radding, C. M. (1975). A general model for genetic recombination. *Proceedings of the National Academy of Science (U.S.A.)* **72**, 358–361.

Murray, N. E. (1963). Polarised recombination and fine structure within the *me-2* gene of *Neurospora crassa*. *Genetics* **48**, 1163–1183.

Orr-Weaver, T. L., Szostak, J. W. & Rothstein, R. J. (1981). Yeast transformation: A model system for the study of recombination. *Proceedings of the National Academy of Science (U.S.A.)* **78**, 6354–6358.

Orr-Weaver, T. L. & Szostak, J. W. (1983). Yeast recombination: The association between double-strand gap repair and crossing-over. *Proceedings of the National Academy of Science (U.S.A.)* **80**, 4417–4421.

Orr-Weaver, T. L. & Szostak, J. W. (1985). Fungal recombination. *Microbiological Reviews* **49**, 33–58.

Prakash, S., Prakash, L., Burke, W., & Montelone, B. A. (1980). Effects of the *rad52* gene on recombination in *Saccharomyces cerevisiae*. *Genetics* **91**, 31–50.

Radding, C. M. (1982). Homologous pairing and strand exchange in genetic recombination. *Annual Review of Genetics* **16**, 405–437.

Razin, A. & Cedar, H. (1984). DNA methylation in Eukaryote cells. *International Review of Cytology* **92**, 159–185.

Rodland, K. D. & Russell, P. J. (1984). Methylation of Neurospora rDNA. *Neurospora Newsletter* **31**, 15.

Smith, B. R. (1966). Genetic control of recombination. I. The recombination-2 gene of *Neurospora crassa*. *Heredity* **21**, 481–498.

Smith, B. R. (1968). A genetic control of recombination in *Neurospora crassa*. *Heredity* **23**, 162–163.

Smyth, D. R. (1971). Effect of *rec-3* on polarity of recombination in the amination-1 locus of *Neurospora crassa*. *Australian Journal of Biological Science* **24**, 97–106.

Smyth, D. R. (1973). Action of *rec-3* on recombination near the amination-1 locus of *Neurospora crassa*. *Australian Journal of Biological Science* **26**, 439–444.

Sobell, H. M. (1974). Concerning the steriochemistry of strand equivalence in genetic recombination. In *Molecular Mechanisms in Genetic Recombination* (ed. R. F. Grell). New York: Plenum.

Szostak, J. W., Orr-Weaver, T. L., Rothstein, R. J. & Stahl, F. W. (1983). The double strand break model for recombination. *Cell* **33**, 25–35.

https://doi.org/10.1017/S0016672300023077 Published online by Cambridge University Press

Thuriaux, P. (1977). Is recombination confined to the structural genes of the eukaryotic chromosome? *Nature* **268**, 460–462.
Whitehouse, H. L. K. (1983). Duplex breaks in DNA as recombination initiators. *Nature* **306**, 645–646.
Yuan, R., Hamilton, D. L. & Burckhardt, J. (1980). DNA translocation by the restriction enzyme from *E. coliK*. *Cell* **20**, 237–244.
Yuan, R. & Hamilton, D. L. (1982). Restriction and modification of DNA by a complex protein. *American Scientist* **70**, 61–69.

https://doi.org/10.1017/S0016672300023077 Published online by Cambridge University Press