Recombination of Nonchromosomal Mutations: A Three-Point Cross in the Green Alga Chlamydomonas reinhardi

Abstract. *The heterothallic, unicellular green alga* Chlamydomonas reinhardi *possesses both chromosomal and nonchromosomal systems of inheritance. Mutations belonging to the latter system are capable of recombination. The experiments reported show that double and triple recombinants can be obtained from crosses employing three phenotypically distinct mutations and that certain pairs of mutants recombine more freely than others.*

The unicellular, heterothallic, green alga *Chlamydomonas reinhardi* possesses a nonchromosomal system of inheritance as well as a conventional chromosomal system (*1*). Mutations belonging to the former system are stable and several distinct phenotypes have been described. In crosses these mutations are transmitted uniparentally via the mating type plus parent (mt^+) in over 90 percent of all zygotes, but the remaining zygotes are exceptional in that they exhibit biparental transmission of nonchromosomal mutations derived from both parents. Since segregation of nonchromosomal mutations in the progeny of exceptional zygotes occurs during both meiotic and postmeiotic mitotic divisions, it does not appear that meiosis plays any special role in the segregation of these mutants.

Experiments have been published showing that nonchromosomal mutations can assort among the progeny of exceptional zygotes to form stable recombinants. Sager and Ramanis (*2*) found that nonchromosomal mutations to streptomycin resistance (*sr-2*) and dependence (*sd*) behave as if they are tightly linked, as do a pair of nonchromosomal mutations to acetate dependence (ac_1 and ac_2), whereas the acetate-requiring mutants appear to assort independently of the mutations to streptomycin resistance and dependence. Gillham (*3*) presented evidence for formal linkage between an *sr-2* mutation and a nonchromosomal mutation to neamine resistance (*nr-2*) in both coupling and repulsion crosses, and also demonstrated the occurrence of reciprocal recombinants in both kinds of crosses. Recently, nonchromosomal mutations resistant to 100 μg of spectinomycin per milliliter (*spr-1*) have been isolated following mutagenesis of wild-type cells with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (*4*). The *spr-1* mutants have been used together with the *nr-2* and *sr-2* mutants to perform, for the first time, crosses involving three phenotypically distinct nonchromosomal mutations. The results of the crosses suggest that *spr-1* is much more tightly linked to *nr-2* than either mutation is to *sr-2*.

Crosses were made in reciprocal directions between neamine-resistant, spectinomycin-sensitive, streptomycin-resistant stocks (*nr-2 sps sr-2*) and neamine-sensitive, spectinomycin-resistant, streptomycin-sensitive stocks (*ns spr-1 ss*) by standard procedures (*5*), and analyzed by one of two methods. In the first case the progeny of 16 exceptional zygotes from two crosses were analyzed by a nonselective method described previously (*3*). Zygotes from each cross were germinated on nonselective minimal-acetate medium (*6*) and allowed to form colonies which were then replica-plated first to minimal-acetate medium and second to a medium selective for the nonchromosomal mutations carried by the mt^- parent (*6*). Exceptional zygote-colonies were located by cell growth on the plate of selective medium, and isolated from the nonselective minimal-acetate replica, and the component cells were replated on minimal-acetate medium. After these cells had formed colonies they were replica-plated to a series of selective media (*6*) which permitted classification of the six possible recombinant categories as well as the parental genotypes. Among the progeny of the 16 exceptional zygotes analyzed in this way, recombination between *sr-2* and the other two markers was detected in ten instances, but no recombination was observed between *spr-1* and *nr-2* (Table 1). Of the exceptional zygotes producing recombinants, two produced reciprocal recombinants (that is, *nr-2 sps ss* and *ns spr-1 sr-2* cells, six produced only the *ns spr-1 sr-2* recombinant, and two gave rise to the *nr-2 sps ss* recombinant.

These results made it quite clear that the nonselective method would prove too laborious for the identification of recombination between *spr-1* and *nr-2*, and so a selective method was devised which made the analysis of a large number of exceptional zygotes possible. Zygotes were germinated as before on minimal-acetate medium, but in this instance the resulting colonies were replica-plated directly to a series of selective media (*6*). The media chosen

Table 1. Recombination of nonchromosomal mutations among the progeny of exceptional zygotes in two crosses of the type *nr-2 sps sr-2* mt^- × *ns spr-1 ss* mt^+. Independently isolated *spr-1* mutations were employed in the crosses and zygote germination was over 90 percent in both crosses. In cross 1 the percentage of exceptional zygotes was 1.05 and in cross 2 this percentage was 1.98. Of the six possible recombinant genotypes, only the reciprocal recombinants *nr-2 sps ss* and *ns spr-1 sr-2* were recovered, and only these recombinants are listed in the table.

Zygote number	Progeny cells examined	Percent progeny cells in each category			
		Parental genotypes		Recombinant genotypes	
		ns spr-1 ss	*nr-2 sps sr-2*	*nr-2 sps ss*	*ns spr-1 sr-2*
		Cross 1			
1	25	84.0	16.0	0.0	0.0
2	33	85.0	15.0	0.0	0.0
3	40	82.5	17.5	0.0	0.0
4	48	70.8	25.0	0.0	4.2
5	27	89.0	11.0	0.0	0.0
6	19	79.0	15.8	5.2	0.0
7	32	81.3	0.0	3.1	15.6
8	70	72.8	24.3	0.0	2.9
9	19	79.0	15.7	0.0	5.3
10	29	96.7	0.0	0.0	3.3
11	53	98.2	1.8	0.0	0.0
12	30	76.7	10.0	3.3	10.0
13	39	97.5	2.5	0.0	0.0
		Cross 2			
1	77	79.3	3.9	0.0	16.9
2	126	95.4	2.4	0.0	2.4
3	215	1.5	93.2	5.6	0.0

Table 2. Detection of recombination in three-point crosses by the selective method. Zygote germination in these crosses was 88 percent or better. Crosses 3 and 4 employed an *spr-1* mutation distinct from the one used in the other crosses.

Cross	Cross number	Exceptional zygotes		Number of exceptional zygotes producing recombinants		
		Percent	Total	*nr-2 spr-1 ss*	*ns spr-1 sr-2*	*nr-2 spr-1 sr-2*
nr-2 sps sr-2 mt⁻ × *ns spr-1 ss mt⁺*	1	2.55	172	0	112	0
	2	6.00	482	4	333	6
ns spr-1 ss mt⁻ × *nr-2 sps sr-2 mt⁺*	3	3.42	31	0	17	2
	4		57	0	21	0
	5	2.60	39	1?	24	1
Totals			781	4–5	507	9

permitted detection of the nonrecombinant *mt⁻* genotypes (*nr-2 sps sr-2* or *ns spr-1 ss*) as well as one member of each of the three possible pairs of recombinants (*nr-2 spr-1 ss*, *ns spr-1 sr-2*, and *nr-2 spr-1 sr-2*), but it was impossible to arrange the media in such a way as to allow selection of the other three recombinants as well (*ns sps sr-2*, *nr-2 sps ss*, and *ns sps ss*).

Recombinants belonging to each of the possible classes were found when the selective method was employed (Table 2) and, in most cases, the uniparental inheritance of each of the markers was checked in further crosses. The results substantiated those obtained by the first method, since recombination between *sr-2* and the other two markers occurred among 65 percent (507/781) of the zygotes analyzed, whereas only 1.77 percent (14/781) of the exceptional zygotes were recombinant for *spr-1* and *nr-2*. The recovery of representatives of all three possible classes of recombinants suggests that one of these may arise by a double event and opens the possibility that mapping of nonchromosomal markers may soon be feasible.

Although mutation cannot be ruled out absolutely as an explanation for the rare *nr-2 spr-1* recombinants, it should be noted that a spontaneous *nr-2* mutation has never been isolated and that the spontaneous frequency of spectinomycin-resistant mutants has been estimated on two separate occasions in wild-type stocks and found to vary between 0.76 and 4.5×10^{-6} mutations per wild-type cell (*7*). Furthermore, even if rare mutations could explain the existence of the *nr-2 spr-1* recombinants, that would in no way alter the conclusion that these mutations are very tightly linked vis-à-vis *sr-2*. On the other hand, it could be argued that the strength of linkage is a reflection of selection, either inter- or intracellular, for or against certain combinations of nonchromosomal mutations. Thus, there would be strong selection against any genotype containing *nr-2* and *spr-1* together, but much weaker selection against genotypes in which the *spr-1* is combined with *sr-2*. The only way in which this argument can be countered is by pointing out that the selective method should detect rare genotypes with a rather high efficiency. Thus, Gillham (see *1*) showed that colonies of *ss* cells containing as little as 0.1 to 1.0 percent *sr-2* cells could be detected by replica-plating. Since the selective method measures only the presence or absence of a particular recombinant in a colony, and there are about 10^4 cells per colony, the rarity of exceptional zygote-colonies containing *nr-2 spr-1* recombinants can only mean that these mutations recombine very rarely or that selection against this recombinant type is extreme. A final source of error could arise from the clear asymmetry existing in the frequency of reciprocal recombinants as well as parental genotypes [Table 1 and (*3*)]. It is conceivable that if all possible recombinants could be detected by the selective method, the linkage between *spr-1* and *nr-2* would prove to be looser than it appears. However, it is doubtful that this is an important source of error since no recombination was observed between *spr-1* and *nr-2* among the 16 exceptional zygotes sampled by the nonselective method, even though all four classes of progeny recombinant for these markers could have been scored.

All of the above considerations point up the flaws inherent in the analysis of recombination of nonchromosomal mutations which arise as a result of the unfortunate, but necessary, indirectness of the methods employed. Nevertheless, the qualitative conclusions that can be drawn from the experiments are significant. It is apparent that recombinant genotypes belonging to each of the three possible recombinant classes can arise among the progeny of exceptional zygotes in a three-point cross involving nonchromosomal mutations, and there is no reason to suspect that mutation is implicated in the process. It is also evident that *sr-2* recombines more freely with the other two mutations than they do with each other unless some extreme form of selection is operative against *spr-1 nr-2* recombinants. Since it has been argued previously (*3*) that *sr-2* and *nr-2* are linked in a formal sense, it follows that all three mutations are linked. The recent demonstration by Sager and Ramanis (*8*) that ultraviolet light increases the frequency of exceptional zygotes to over 50 percent and the discovery by Gillham (*3*, *7*) that a high proportion of diploids transmits nonchromosomal mutations biparentally and that these mutations segregate and recombine during vegetative division should now make the quantitative analysis of recombination between nonchromosomal mutations possible, for it is now feasible to study the process in pedigrees.

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References and Notes

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2. ——— and Z. Ramanis, *ibid.* **50**, 260 (1963); *ibid.* **53**, 1053 (1965).
3. N. W. Gillham, *ibid.* **54**, 1560 (1965).
4. ———, *Genetics* **52**, 290 (1965).
5. W. T. Ebersold and R. P. Levine, *Z. Vererbungslehre* **90**, 74 (1959).
6. The plating media used in these experiments were derived from the basal minimal medium of R. P. Levine and W. T. Ebersold [*Z. Vererbungslehre* **89**, 631 (1958)] and supplemented with 2 g of sodium acetate per liter [MA* (minimal acetate) medium]. The selective media employed in these experiments were made from MA supplemented with streptomycin (MS), spectinomycin (MSP*), or neamine (MN). Antibiotics were filter-sterilized and added to a final concentration of 100 mg/liter after the media had been autoclaved and allowed to cool. The three possible pairs of doubly supplemented media (that is, MSSP*, MSPN*, and MSN*) and the one triply supplemented medium (MSSPN*) were also employed in the experiments. The entire series of media was used in the nonselective method, but only those media marked with an asterisk were used in the selective method.
7. N. W. Gillham, in preparation.
8. R. Sager and Z. Ramanis, *Proc. Nat. Acad. Sci. U.S.* **58**, 931 (1967).
9. Supported by NIH grant GM 13199.

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