tions found in the serum of some cancer patients, and possibly other clinical states such as viral hepatitis. However, it should be mentioned that in mice we regularly observe moderate plasma lactic dehydrogenase elevation in certain stages of the development of radiation-induced leukemia and chemically induced primary tumors where, in most instances, we have been unable to detect the presence of transmissible agents (*4*).

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4. We are indebted to Dr. William Money for providing the pituitary tumor employed in some of these studies, and to Editha Huerto, David Bardell, James D. Loveless, and Mary Anne Fitzmaurice for valuable assistance. Appreciation is expressed for the counsel of Dr. C. Chester Stock and Dr. Gilbert Dalldorf. This study was partially supported by a grant from the U.S. Public Health Service (National Cancer Institute).

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Transreplication and Crossing Over in Sordaria fimicola

Abstract. A study of the segregation of markers closely linked to the *gray* ascospore color locus in *Sordaria fimicola* reveals that there is a high incidence of crossing over very near the locus when it transreplicates, which is much more pronounced in 5:3 than in 6:2 asci. Also, a single 7:1 and several aberrant 4:4 asci are described. At a different spore color locus, transreplication yields only 6:2 ratios, while other spore color loci fail to transreplicate altogether.

Transreplication or "gene conversion" is easily detected in asci of *Sordaria fimicola* heterozygous for loci affecting ascopore color (*1*). Recently (*2*), a linkage group has been worked out for the chromosome bearing the *gray* (*g*) color marker, and several of the mutant loci are near the *g* locus (Fig. 1). This has made possible a study of the relationship between crossing over in the *g* region and transreplication at that locus. The present report is based on a study of 146 aberrant asci.

An abnormal ratio for spore color appears among asci heterozygous for the *g* locus with a frequency of 1 in 800 to 1000 asci, regardless of the combination of other markers involved. Most of the aberrant asci have 6:2 and 5:3 spore color ratios. These are generally believed to have resulted from DNA miscopying during pairing prior to meiotic separation of chromosomes in the ascus (Fig. 2). The frequency of the two types is very nearly the same, the 5:3 asci being only slightly more abundant. However, transreplication does not proceed with equal frequency to the mutant and wild type alleles, the $g \rightarrow g+$ event occurring about five times more frequently than the $g+ \rightarrow g$ event.

An attempt to explain the 5:3 octads requires utilization of an eight-strand model of paired homologs at meiotic prophase, such as that recently proposed by Taylor (*3*). A single half-chromatid temporarily switching from its own strand to copy for a short distance off the homologous strand can readily account for the 5:3 ratio (Fig. 2*A*) without the complication of chromosome breakage, rejoining, and obligately increased crossing over required by Taylor's explanation of the 6:2 event.

An examination of the data on crossing over in the region of transreplication shows that none of the linked loci is altered in character by the transreplication event at *g*. Furthermore, the frequency of crossing over in the *mi-cor* interval of 4.4 units, which includes the *g* locus, is much higher in the aberrant asci—especially those with a 5:3 ratio—than in normal ones, and these crossovers in most cases involve the transreplicating strand. This relationship has been previously explained on the basis of intimate chromosomal pairing within a restricted region, under which condition both crossing over and transreplication are favored. The present studies support this concept as well as the hypothesis that the two are separate events and not obligately interdependent, since a number of aberrant asci fail to show crossing over in the area of transreplication.

By variously deploying the linked markers among the strains crossed, it was found that the transreplication-related crossing over was occurring very close to the *g* locus and within the *mi-cor* interval. For example, among 61 asci with a 5:3 ratio, 41 (67 percent) showed crossing over in the *mi-cor* interval (4.4 units), 26 showed a crossover between *g* and *cor* (3.4 units), and 29 between *mi* and *g* (1 unit), with 14 (23 percent) of

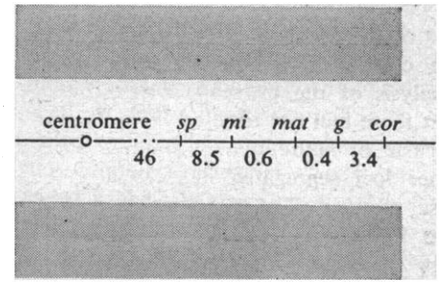

Fig. 1. Part of *g* linkage group showing morphological markers. Abbreviations: *sp*, spotty; *mi*, milky; *mat*, matted—all referring to mycelial characters; *g*, gray ascospore character; *cor*, corona, referring to the ring that appears near the center of the colony.

these having crossovers in both intervals simultaneously where only 0.14 percent of the asci would be expected to show double crossovers. When the marker *mat* just 0.4 crossover unit to the left of *g* was used, it was discovered that transreplication-related crossing over on that side was limited to the shorter interval.

In 6:2 asci the frequency of crossing over in the region of transreplication, though somewhat higher than normal, is less than half that found in 5:3 asci. This significant difference is not predicted by previous explanations of aberrant tetrads.

Six 4:4 asci, each with a pair of

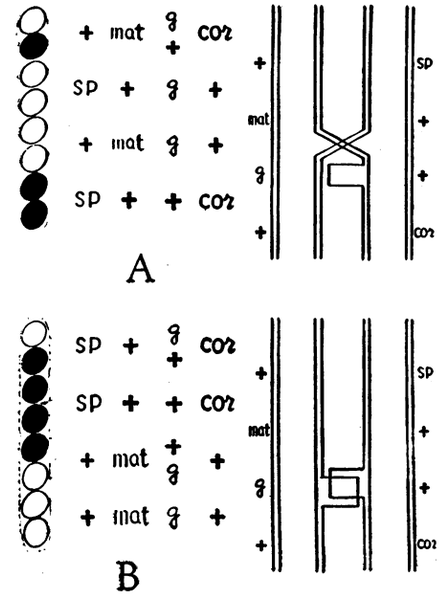

Fig. 2. Two types of aberrant asci with explanatory diagrams. (*A*), 5*g*:3*g*+ ascus, whose progeny can be explained by a half-chromatid miscopying at the *g* locus and a crossover between *mat* and *g*. (*B*), Aberrant 4:4 ascus that is most simply explained by reciprocal miscopying by two homologous half-chromatids.

spores heterogeneous for color in each end of the ascus, were obtained during the course of this study (Fig. 2*B*). Analysis of the progeny demonstrated that these had not resulted from drastic nuclear or spore rearrangement, since other loci segregated in normal pairwise manner. The data indicate that two half-chromatids of paired homologs switched over in opposite directions and miscopied alleles simultaneously. We are referring to this as "reciprocal double transreplication."

A single $7g+:1g$ ascus was found. In this ascus other loci segregated normally. The unusual ratio is believed to have resulted from the combination of a 6:2 and a 5:3 event, but the two are difficult to explain as products of the same meiotic process. In view of this and the fact that 6:2 and 5:3 asci show a marked difference with regard to related crossing over, it may be that the two events have a different explanation, at least as to time of occurrence. If a 5:3 event occurred in the crozier within a diploid nucleus or between homologs of an intimately associated dikaryon which then divided once before producing the ascus nucleus, such an event would allow for a second DNA replication in the ascus and result in a 6:2 ratio. A 5:3 event in the ascus nucleus superimposed upon the foregoing occurrence could then give a 7:1 ratio. The data presently available are insufficient to determine whether this hypothesis is correct. If it should prove valid, then it would logically follow that transreplication in general is the result of miscopying by half-chromatids.

We have obtained several spore color mutants whose loci show no evidence of transreplication, possibly because they represent deletions or other chromosomal aberrations that are not subject to miscopying. On the other hand, one of our spore color mutants (*m*), when crossed with wild type, gives rise to 6:2 asci at the rate of about 1 in 1500, but no 5:3 asci have been found.

It is hoped that future studies may help explain these differences that have been observed among loci that are able to transreplicate and that they may further elucidate the underlying mechanism of the process (*4*).

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17 May 1961

Stimulation of Uterine Contraction by Extracts of the Cockroach, Periplaneta

Abstract. Blood and extracts of gut of the American cockroach, *Periplaneta americana*, powerfully stimulate contractions of the isolated rat uterus. The contractions are characterized by long latency and a prolonged relaxation phase having superimposed rhythmic contractions. Evidence indicates that the stimulating factor is not acetylcholine, 5-hydroxytryptamine, histamine, or substance P. The active factor is heat labile and nondialyzable.

Stimulation of the rat uterus by blood of the roach, *Periplaneta americana*, was encountered by Barton Browne *et al.* (*1*). To determine the origin and characteristics of the stimulating principle, extracts were prepared from brain, nerve cord, muscle, gut, and blood of roaches, and were tested on the isolated rat uterus preparation. The roach tissues were ground in a small mortar with Tyrode's solution, then filtered. Undiluted blood was obtained by centrifugation of the roaches (*2*).

In each experiment, one uterine horn of a rat was suspended in a 7-ml bath containing low-calcium Tyrode's solution (*3*), and its isotonic contractions were recorded by a conventional lever exerting a slight load on the uterus. The muscle was stimulated at 4-min intervals by application of 0.5 to 2.0 μg of carbamylcholine chloride (carbachol). Extracts were applied 4 min after the routine stimulation and were allowed to remain in the bath for 60 to 90 sec. Thereafter, washing was repeated at 60-sec intervals until the next application of carbachol.

Only gut extracts and blood showed the characteristic stimulating activity, with gut showing greater activity per unit weight and being used in most experiments. Uteri responded to extracts from as little as one-tenth of a single washed gut. Figure 1 shows typical responses. The latent period after introduction of the extract into the bath varies from 45 to 120 sec, depending upon dosage. Maximum contraction is followed by a long period of slow relaxation, with spontaneous contractions superimposed. Higher doses, equivalent to extracts of several washed guts, cause uteri to go into short contracture, followed by relaxation phases of up to 1 hr. The degree of uterine contraction obtainable far exceeds the maximum contraction obtainable by application of carbachol.

The active principle was differentiated from acetylcholine and histamine. Both rat colon and blood pressure were insensitive to extracts of ten roach guts. Atropine, sufficient to render uteri insensitive to carbachol, did not reduce the contractions elicited by gut extracts. Uteri responding strongly to extract equivalent to one gut were insensitive to 100 μg of histamine, whereas the arterial pressure of the rat and cat showed a significant fall to only 1 to 2 μg of histamine but did not respond to large amounts of the extract.

5-Hydroxytryptamine (5-HT) and

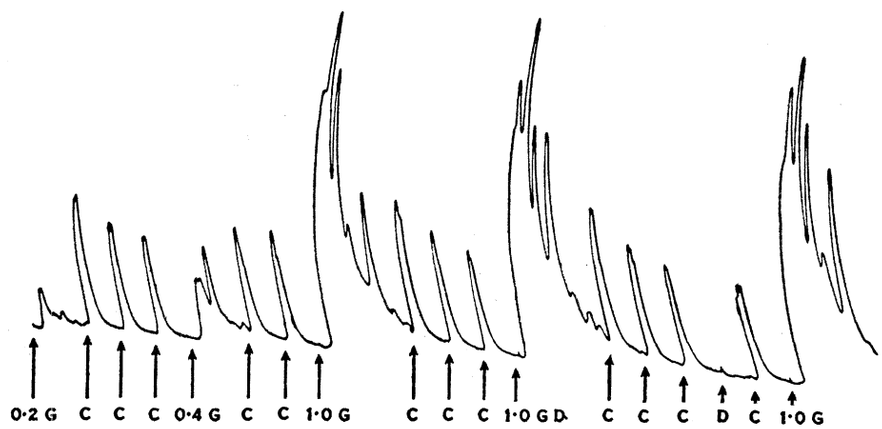

Fig. 1. The effects of dialyzed and nondialyzed extracts of roach gut upon isolated rat uterus. C, 1 μg carbachol; 0.2 G and 1.0 G, extracts equivalent to 1/5, 2/5, and 1 roach gut, respectively; 1.0 GD, dialyzed extract equivalent to 1 gut; D, dialyzate equivalent to 5 guts.