COINCIDENCE RELATIONS BETWEEN GENE CONVERSION AND MITOTIC RECOMBINATION IN SACCHAROMYCES[1]

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Received October 3, 1962

THE major attributes of mitotic gene conversion in Saccharomyces have been detailed by ROMAN (1956a) and by KAKAR (1961) for heteroallelic diploids, while LINDEGREN (1953), WINGE (1955), and PAPAZIAN and LINDEGREN (1960) have investigated instances of aberrant segregation ratios in meiosis. Heteroallelic repair, or gene conversion, is essentially non-reciprocal in character. Three lines of evidence presented by ROMAN (1956a) indicate that crossing-over in the conventional sense is not implicated in the conversional process. These are: (1) double mutants are not recovered from heteroallelic revertants; (2) either input allele may be recovered from a given convertant, although not necessarily with equal frequencies; (3) heteroallelic repair is not correlated with the occurrence of mitotic recombination for linked outside marker genes. While conventional crossing-over *per se* may not be associated with the mechanism responsible for conversional revertants in heteroallelic diploids, it is significant that conversion rates for specific allelic combinations are enhanced following meiosis (ROMAN, 1956a). MAGNI and VON BORSTEL (1962) have also reported that mutation rates are significantly higher among meiotic products compared to mitotically dividing cells.

JAMES (1955), and JAMES and LEE-WHITING (1955), reported that ultraviolet irradiation significantly increased the frequency of mitotic recombination, as evidenced by the occurrence of visibly sectored clones. ROMAN (1956a) confirmed this finding and also demonstrated ultraviolet stimulation of gene conversion in heteroallelic diploids. Moreover, ROMAN and JACOB (1958) found that increasing ultraviolet dosage yielded a persistent increase in conversion rates, with no concomitant increase in homozygosis rates for outside linked markers. This finding, like MITCHELL's (1957), suggests that crossing-over and conversion are separable events.

However, the present study reports evidence for the coincident association of heteroallelic repair with mitotic recombination for both linked and unlinked markers within a given diploid nucleus during the course of a single DNA replication cycle.

[1] This study was aided by Grant RG-6979 from the Public Health Service, National Institutes of Health.

MATERIALS AND METHODS

The parent clone for all experiments described here was isolated as a single diploid clone on synthetic complete media deficient for both uracil and tryptophan. For details of all media see ROMAN (1956b). The cross was made by overlaying aqueous suspensions of the haploid parents on the media specified. The parental haploids, obtained from the stock collection at the University of Washington, were as follows:

A798C *a ad-2 ad-6-15 SU-1 ma-1 ur-1 TR-2 me-1 IS-1 ga-2*

A423C α *ad-2 ad-6-21 su-1 MA-1 UR-1 tr-2 ME-1 is-1 GA-2*

The adenine-2 (*ad*), uracil-1 (*ur*), galactose-2 (*ga*), and methionine-1 (*me*) loci segregate independently of each other and exhibit no linkage to any centromere. The adenine-6 and sucrose-1 and maltose-1 loci were reported by ROMAN (1956a) to exhibit mitotic linkage. HAWTHORNE and MORTIMER (1960) placed *ad-6* at 31 centimorgans from the centromere in linkage group VII. The latter report isoleucine-1 (*is*) and tryptophan-2 (*tr*) to be located on linkage group V, nine centimorgans from each other; *is-1* is approximately 50 centimorgans from the centromere. The Sucrose-1 and Maltose-1 loci are the M_1-R_1 of WINGE and ROBERTS (1952) now designated *SU-1* and *MA-1* by the Carbondale Yeast Genetics Conference (1963).

The resultant diploid was white and adenine requiring. Any alteration at the adenine-6 locus yielding reversion to an effective wild type or intermediate allele, will yield red or pink clones, or sectored white and red or pink clones. The revertant red cells are still adenine requiring. Non-specific dominant suppressor mutations affecting both *ad-2* and *ad-6* are known (FOGEL and HURST, unpublished), but these did not arise in this study. Such suppressors, unless closely linked to *ad-2* or *ad-6*, are easily detected following tetrad analysis.

The single white diploid was further purified by reisolation as a single clone from a streak plate on synthetic complete media. The cells of the single clone were washed and appropriately diluted in sterile distilled water so that platings of 0.1 ml would yield approximately 250 clones/plate. Untreated control samples were plated immediately. In Trials I and II the remainder was treated in water (approximately 10–15 ml) on a shaker device in a Petri dish (15 × 150 mm) at 50 cm from the ultraviolet source, a General Electric 15-watt germicidal lamp. The third trial, also in water, was conducted for a dose-response curve, utilizing a Hanovia Letheray (No. 24500) over a shaker device. The incident energy measured by a Latarjet ultraviolet dosimeter was 13 ergs/mm^2/sec. In this latter experiment, dilutions were adjusted so that 0.1 ml aliquots, consecutively removed at 30, 60, 120, and 240 seconds, could be plated appropriately. Routinely, treated cells were plated immediately on synthetic complete medium (SC) in dimmed daylight to avoid photoreactivation. After incubation in the dark at 30°C for four to five days, the plates were counted and scored, usually under a dissecting microscope. Convertant clones, identified as red clones, or red-white sectored clones among a background of whites, were transferred into 5 ml sterile water and streaked on SC. Typically, seven red and seven white clones from each streaked

revertant clone were placed upon a yeast extract-peptone-dextrose (YEPD) plate (1.5 percent agar). This was incubated 24 hours and replicated, using the velvet transfer technique, to an appropriate omission series of SC deficient for single nutrilites. Unselected samples from both untreated controls and nonconvertants of the irradiated population were replica-tested to the same media. These assay mitotic recombinational events in both the untreated and irradiated control populations.

Randomly selected red and white derivative clones from the two sides of the original sector colonies were sporulated and dissected. Initially, sporulation was induced by exposing cells to liquid acetate-raffinose (POMPER, DANIELS and MCKEE 1954). A simpler, more reliable and rapid method for sporulating cultures was devised subsequently, according to the following protocol: 0.1 ml of a stationary phase culture grown in liquid YEPD is suspended in 5 ml of sterile water. A few drops of the suspension are transferred to 1.5 percent agar plates containing 0.5 percent glycerol, 0.5 percent yeast extract, and one percent peptone. Alternatively, an entire clone may be transferred directly to this solid medium by means of sterile toothpicks. Maximum sporulation is attained within two to four days at 28°C.

Verification of genotypes was achieved by ascus dissection through micromanipulation using the snail enzyme technique of JOHNSTON and MORTIMER (1958), and subsequent replica plating tests of the ascosporal clones. Only four-spored asci were dissected. During this study a convenient source of enzyme was found. Commercial importers of edible snails can supply both *Helix aperta* and *Otalla lactea*. The crop is removed and the red-brown contents dissolved in water, approximately 1 ml per crop. After storing in the refrigerator overnight, the crude enzyme solution is decanted, subjected to high speed centrifugation, 15,000 rpm for 20 minutes at 10°C, and the supernatant sterilized by Millipore filtration. The freshly prepared enzyme mixture from *H. aperta* dissolved the ascus wall within five to 10 minutes, while the preparation from *O. lactea* required one hour. These preparations deteriorate slowly under ordinary refrigeration: after a six-month storage period, the *H. aperta* enzyme dissolved ascus walls in four to six hours.

EXPERIMENTAL RESULTS

Effect of ultraviolet irradiation on heteroallelic repair (Table 1): The relationship between ultraviolet dose and conversion frequency may be seen most clearly from the results of the third experiment. The convertant frequency rises with radiation dose from 0.08 percent in controls to 1.25 percent at 240 seconds; this is consistent with data reported by ROMAN and JACOB (1958). Clones which were sectored (red and white) or whole red were scored as convertants. Two small 1/16 red sectors were observed in the control population in the first experiment, none in the second, and four in the third. In the second experiment convertants were not scored under the microscope. This contributes to the difference in conversion frequency between the first two 60-second runs since very small sectors (less than 1/16 red) would be undetected except on microscopic examination.

TABLE 1

The effect of ultraviolet irradiation on survival and heteroallelic repair

Ultraviolet treatment in seconds	Clones examined	Percent survival	Number of convertants	Percent convertants in survivors
Experiment 1:				
None	541	100	2	0.37
45	2314	82	3	0.13
60	46080	47	293	0.64
Experiment 2:				
None	7741	100	0	0.00
60	12060	45	44	0.37
Experiment 3:				
None	5270	100	4	0.08
30	5730	91	17	0.30
60	17500	83	55	0.32
120	3210	25	21	0.66
240	80	0.5	1	1.25

The frequency of convertants in the untreated population is thus taken to be about 0.08 percent. The plateau between 30 and 60 seconds in the UV-dosage versus convertant-frequency curve is tentatively regarded as genuine, since similar effects have been observed repeatedly. A precise evaluation of this finding awaits further study.

Coincidence relations between heteroallelic repair and mitotic recombination (Table 2): The observed mitotic recombination frequencies in the three different cell populations, controls, treated-nonconvertant-whites, and convertants (red or red-white sectors) are compared in Table 2. The isoleucine, methionine and galactose markers were not scored in Experiments 1 and 2. The control, unirradiated populations showed a low mitotic homozygosis frequency for the reces-

TABLE 2

Coincidence relations between heteroallelic repair and mitotic recombination

Population	Clones tested	Percent homozygosity for						
		tr	*is*	*ga*	*me*	*ur*	*SU*	*MA*
Experiments 1 & 2:								
Controls	2088	0.05	...	...	...	0.05	0.05	0.25
Treated whites	2156	0.1	...	...	...	0.74	0.65	1.3
Convertants	135	0.0	...	...	...	8.9	3.7	12.7
Experiment 3:								
Controls	2000	0.05	0.05	0.0	0.0	0.0	0.2	0.15
Treated whites:								
30 secs.	2000	0.05	0.05	0.0	0.05	0.35	0.1	0.6
60 secs.	2000	0.2	0.1	0.0	0.4	1.1	1.1	1.75
120 secs.	2000	0.8	0.3	0.15	1.8	3.55	2.45	2.15
240 secs.	77	0.0	2.6	0.0	1.3	2.6	2.6	0.0
Convertants	80	0.0	0.0	3.8	8.8	16.3	17.5	23.8

sive alleles. The treated white populations showed a two- to 16-fold increase in homozygosis frequencies over the controls, and in Experiment 3 homozygosis frequency was demonstrably dose-dependent. The *ur-1* marker exhibited maximal homozygosis, 3.6 percent following 120 seconds of irradiation. However, since approximately half the homozygotes produced by mitotic recombination are expected to be homozygous for the dominant alleles and therefore not detectable by replica plating techniques, the observed frequencies represent only 50 percent of the total homozygosis due to mitotic recombination. The apparent effect of ultraviolet irradiation is to increase the frequency of mitotic recombination. This is consistent with results reported by JAMES (1955) and by ROMAN (1956a).

The rate of increase in recessive homozygosis with irradiation dose is roughly proportional to the map distances of these markers to their respective centromeres. The order of the markers on linkage group V is centromere-isoleucine-1-tryptophan-2. The centromere-*is* distance is 49 units; the *is-tr* distance is nine units (HAWTHORNE and MORTIMER 1960). The sucrose and maltose loci (the M_1-R_1 of WINGE and ROBERTS 1952), the methionine-1 locus, and the uracil-1 locus are not linked to any centromere, although the *SU-1 MA-1* loci have been found to be mitotically linked to *ad-6* (ROMAN 1956a). The galactose-2 marker is possibly 36 units from its centromere (linkage group XII). Thus, in terms of distance from their respective centromeres, these markers are seriable in the order *ga-2*, *is-1*, *tr-2*, *me-1*, *SU-1*, *MA-1*, and *ur-1*. This corresponds to the percent of mitotic recombination in the 120-second population; on this basis methionine-1 would be predicted to be closer to a centromere (1.8 percent recombination) than *SU-1 MA-1* (2.2-2.5 percent). These observations on mitotic recombination are in agreement with those reported by PONTECORVO and KÄFER (1958) in Aspergillus.

In the convertant population, where heteroallelic repair had already occurred at the *ad-6* locus, homozygosis frequencies for the unselected markers increased five- to ten-fold over the comparable values for similarly treated non-convertant whites for most of the markers (from 2.0 percent to 17.5 percent for *SU-1*; from 3.6 percent to 16.3 percent for *ur-1*). No coincident mitotic recombinations were observed for *is* or *tr*. However, for these latter markers the sample sizes may well be limiting since only three to six recombinants for *tr-2* would have been expected among convertants. It is nonetheless clear that a selected convertant population, detected by a visible color change on nonselective media, represents a cellular population in which simultaneous or coincident mitotic recombination occurs with high frequency.

Tetrad analysis: Intragenic mitotic recombination has yielded mostly reciprocal products in Aspergillus (PRITCHARD 1960), and nonreciprocal products in yeast (ROMAN 1956a, 1956b; ROMAN and JACOB 1958). MORTIMER (unpublished) and ROMAN (1958) have found both reciprocal and nonreciprocal products among meiotic products from heteroallelic diploids. Intergenic mitotic recombination has classically yielded reciprocal products in Drosophila (STERN 1936), in Aspergillus (PONTECORVO and KÄFER 1958) and in yeast. Verifica-

tion of reciprocality for marker homozygosis in red-white sectored convertant clones was achieved in this study by ascus dissections and tetrad analysis. Of the sectored convertant clones which were uracil requiring, eight were dissected and were found reciprocal, *ur/ur* on one side of the sector, and *UR/UR* on the other. Of these, two were also reciprocally homozygous for the distally linked fermentation markers, *e.g.*, *SU-1/SU-1 ma-1/ma-1*, and *su-1/su-1 MA-1/MA-1*. Since the *SU-1 MA-1* loci are mitotically linked to *ad-6*, while the *ur-1* locus segregates independently, we observed reciprocality for both linked and unlinked markers in the same convertant clone. In a similar manner, four of the *ma/ma* cases, and one of the *su/su* cases were dissected and found to be reciprocal. Thus the reciprocal mitotic event is found associated with the nonreciprocal heteroallelic repair. Detailed evidence for the nonreciprocal character of the conversion event will be presented in a subsequent publication.

DISCUSSION

The frequencies of mitotic recombination and of gene conversion are both enhanced by ultraviolet irradiation. A given cell in which a conversion has occurred possesses a higher probability for coincident mitotic recombination than a similarly irradiated cell in which gene conversion has not occurred (Table 2). The coincidence probability is greater than can be expected on the plausible assumption that conversion and mitotic recombination are simultaneous though independent events in the same single cell. The coincidence expectations for conversion and mitotic recombination may be calculated as follows: The conversion rate in the 120-second population was 0.0066. The frequency of mitotic recombination for *ur* in this population was 3.6 percent. The probability of simultaneity on the assumption of independence is 0.00024, but the observed coincidence was 0.162, or 700 times the expectation. Similar calculations may be performed for the other loci. Clearly, the observed coincidence rates for simultaneity of mitotic recombination and gene conversion do not reflect complete independence of these two recombinational events. Therefore, gene conversion and mitotic recombination are interdependent, either upon each other or upon a common precondition, process, or event.

The observed coincidence, however, is not a mere reflection of linkage, since *ur-1* and *me-1* are not linked to *ad-6*. The mitotically linked loci *SU-1* and *MA-1* do show higher coincidence (1091 and 1690 times expectation) than the unlinked loci in the 120-second irradiated population, but the significance of this observation is obscured by the magnitude of the discrepancies for the unlinked loci.

It is not unreasonable to suppose that both the reciprocal and non-reciprocal events depend upon some common generalized cell property. In mitotically active cells a likely precursor event might be effective synapsis or point pairing during interphasic DNA replication, since recombined strands could result from exchange or copy choice between synapsed homologous chromosome segments during a four-strand stage. The effect of ultraviolet, then, could be to increase the frequency of synaptic contacts, or to extend the time of contact. If these are

interphase events, local point synapsis ("local effective pairings," PRITCHARD 1960) is much more likely than synapsis along the total length of the chromosome. A point synaptic contact could have a reciprocal or nonreciprocal result, or both, in the same cell, depending upon the number of nucleotide pair differences between regions in effective contact, or the length of the synapsed region and perhaps the timing of synapsis with relation to chromosome replication.

Under the hypothesis that the observed coincidence of these recombinational events reflects effective synapsis as a common precondition, it is not obligatory that conversion and mitotic recombination occur at the same time during the DNA replication cycle. Observations on closely linked markers in Neurospora (MURRAY 1960) and in yeast (FOGEL and HURST, unpublished) indicate that high recombination frequencies for linked outside markers may be found associated with conversion, but every conversion does not necessarily result in recombination for an outside marker. Thus, these observations may be similar to those reported here.

Lastly, it is conceivable that point pairing for short segments of homologous chromosomes is a generalized condition in some proportion (perhaps a constant proportion) of the cell population, and the effect of ultraviolet could be to enhance interactions between the segments, leading to recombinations, either conversional or reciprocal. This possibility would affect the comparisons between recombinations in unconverted cells (Table 2) but leaves unchanged the major observation, namely high coincidence of reciprocal and nonreciprocal events, which leads to the conclusion that both share a common requirement or precondition.

SUMMARY AND CONCLUSIONS

A diploid clone of Saccharomyces, in which both heteroallelic repair and mitotic recombination could be observed, was treated with ultraviolet light, plated on a complete nonselective medium, and scored for both classes of recombinational events during mitosis. An increase in both types of events with increased radiation dose was observed. A coincidence of mitotic recombinants or recessive marker homozygosis in excess of expectations due to independence was found in the convertant population. It is suggested that reciprocal and nonreciprocal mitotic recombination have a common precursor condition for their expression.

ACKNOWLEDGMENTS

The authors wish to thank Miss ELIZABETH JONES for her invaluable technical assistance, and DR. HERSCHEL ROMAN for advice, helpful criticism and for making available the stock collection of the University of Washington.

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