Influence of UV-Light on Gene-Conversion in Neurospora

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1. Crosses between wild type (dark ascospores) and the asco-mutant (light ascospores) were set up and the spores collected as unordered octads. The analysis of 1317 such octads revealed 69 with abnormal segregation of the asco-marker (5.2%). If crosses were irradiated with UV at a dose equivalent to 66% conidial killing, an increase of the number of aberrant octads was found. This increase was most pronounced if the crosses were exposed to the irradiation from one day before to 2 days after plasmogamy, when up to 8.7% aberrant octads were obtained. No effect was observed from 4 days after plasmogamy until the end of the sexual cycle.

2. The relative frequency of the 8 possible types of aberrant octads was analyzed separately for experiments without and for experiments with UV-irradiation. The distribution of these frequencies was the same for unirradiated and irradiated crosses, a finding which suggests that the conversion mechanism acting spontaneously and that acting upon UV-irradiation may be identical. In both cases, there was a strong bias in favour of octads of the 2 : 6 type (giving wild spores first) as compared to such of the 6 : 2 type, also the frequency of the 0 : 8 type was higher than that of the 8 : 0 type. Hence, the conversion mechanism in this cross "prefers" to act from the wildtype to the mutant allele.

The phenomenon of gene-conversion or non-reciprocal recombination is mainly observed in higher organisms amenable to tetrad-analysis. There have been several efforts to explain its causes in molecular terms (see e.g.1, 2) but experimental proof for the hypotheses put forward is scanty.

Recent work in phages seems to indicate that those hypotheses invoking heteroduplex formation and correction of mismatched bases are in essence valid3, 4. The question remains however, whether such work is relevant to the phenomenon in higher organisms. Hence, data obtained with the mould Neurospora crassa and bearing on the incidence of several types of non-reciprocal tetrads in unirradiated as well as UV-irradiated crosses seem of interest in this context.

Materials and Methods

1. Neurospora crassa strains: Wildtype 74-OR 23-1 A and mutant asco 37402 a (same locus as lys-5). The mutant was obtained from the Fungal Genetics Stock Center, Dartmouth College, Hanover, New Hampshire, U.S.A. It is characterized by colourless (light) ascospores, in contrast to the dark coloured spores of the wildtype.

2. Growth media: Glycerol complete agar5, buth with Vogels minimal6 instead of Fries'. Liquid glucose complete, composed as glycerol complete, but without agar and with 1% glucose instead of glycerol.

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WESTERGAARDS grossing medium7. Lysine was supplemented at 100 γ/ml where required. Conidial suspensions were obtained as described earlier8.

3. Crossing procedure: All crosses were set up on plates, each containing 30 ml of crossing medium. The plates were first inoculated by spreading 0.5 ml of a mycelial suspension of the asco strain on the agar surface. 3 days later, when a mycelial lawn had formed, conidia of the wildtype were dusted onto it. Incubation of the plates was then continued, usually for 11 days, after which time perithecia had formed and ejection of spores begun. Incubation was at 25 °C, and at darkness to avoid photoreactivation8. During inoculation and for handling before and after UV-irradiation yellow light was used.

4. Collecting and classifying unordered tetrads: Our general procedure followed STRICKLAND9, DEAN10 and PERKINS11, i.e. groups of eight spores are collected on 4% agar water slabs placed into each lid of a series of Petri dishes within a distance of 1 mm of the ostioles of the ripe perithecia. Exposition times ranged from 10 sec to several minutes. Criteria for scoring the ascospore octads thus obtained were: 8 spores in a safe distance to all neighbouring spores and unambiguous dark or light coloration of the spores. Groups of eight which contained any grayish spores were omitted. To avoid variations due to aging of crosses octads were only collected during the 1st and 2nd day after the beginning of spore discharge.

5. UV-irradiation: The UV source was a sterisol F 1140 EO, original Hanau, NN 15/44 VK, with a maximal emission at 254 nm. Plates were exposed at a distance of 40 cm for 3 minutes. This dose, if administered to conidial suspensions of the wildtype gave ca. 66% killing, as checked by colony counts. On transfer of irradiated conidia into liquid glucose complete and 24 hours incubation, the mycelial dry weight obtained was reduced by 58 ± 3.8% as compared to unirradiated controls.
Table 1. Octads obtained from UV-irradiated crosses, and from unirradiated controls. Data from 5 independent experiments each.

### Results

1. **Spontaneous rate of gene conversion**

To determine the spontaneous rate of conversion from *asco* to wildtype and vice versa under the here chosen experimental conditions, 1317 octads obtained from unirradiated crosses were analyzed. Of them, 1248 or 94.8% showed the normal segregation of 4 dark and 4 light spores. 69 or 5.2% however, were of several aberrant types, among which that with 2 black and 6 white spores predominated. Similar results had been obtained before during a laboratory class on fungal genetics in this department, where 1086 octads were scored and 5.7% aberrants found.

2. **Rate of gene-conversion after UV-irradiation**

Conversion rates for crosses irradiated at different days after inoculation of the *asco* partner are given in Table 1. A total of 12,593 complete octads were classified in 5 independent experiments, carried through under identical conditions. Since in experiments 1 through 3 irradiation after day 7 did not influence conversion rate, in experiments 4 and 5 scoring efforts were concentrated on plates irradiated 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 days after inoculation of the *asco* partner.
ated at the earlier days. The data obtained reveal a definite increase of the rate of aberrant octads as compared to the spontaneous rate, if UV-irradiation was applied at any day from one day before to 2 days after the transfer of wildtype conidia to the asco plates. This effect of UV is statistically highly significant, since \( p = 0.022 \) for irradiation on the 2nd day, referring to the unirradiated controls, resp. 0.0007 (3rd day), 0.0002 (4th day) and 0.001 (5th day).

In Fig. 1 the average number of aberrant octads obtained is plotted against the day at which UV was administered, counting from the inoculation of the asco strain.

![Fig. 1. Frequency of aberrant octads (in % of octads scored) as correlated to the day of UV-irradiation of the crosses. The asco strain was inoculated first, the wildtype 3 days later. Average from 5 independent experiments. Standard errors are indicated.](image)

3. Relative frequency of individual types of aberrant octads

Formally, there are eight classes of aberrant octads, i.e. giving the number of dark spores first and that of the light spores second: 8:0, 7:1, 6:2, 5:3, 3:5, 2:6, 1:7, 0:8. The relative frequency of these eight types offers a criterium to judge the following question: Are spontaneous gene conversions and those found after UV-irradiation caused by the same or by different molecular mechanisms? If the relative frequency of the eight types for UV-irradiated crosses was distinctly different from that for unirradiated crosses, different molecular mechanisms would have to be invoked. Fig. 2 gives the distribution for both sets of experimental conditions. No significant difference of the suggested kind was found. Hence one and the same molecular mechanism seems to be involved in both cases.

The similarity between the relative frequencies of aberrant types found in both sets of experimental conditions held even then, when among the total aberrant octads obtained after UV-irradiation, those from plates irradiated after day 7 were disregarded. Since after that day, UV does not increase the rate of aberrant octads, these octads were surmised as being of spontaneous origin.

As additional fact, considering reciprocal pairs of aberrant types, it can be seen that the rate of conversion from wildtype to asco is mostly in excess of that in the opposite direction. I.e. only 5:3 and 3:5 octads occurred with roughly equal frequency. Type 2:6 octads however were far in excess of type 6:2 octads, and type 0:8 octads far more frequent than type 8:0.

4. Timing of gene-conversion during the sexual cycle

To check for a differential timing of the induction of individual types of aberrant octads during the sexual cycle, Table 2 was prepared. Formally, 2:6 and 6:2 types might be thought as originating at an earlier stage (4 strands) than 3:5 and 5:3 types (eight strands). If so, the relative number of such types should vary with the day of irradiation, and 2:6 and 6:2 types should occur at maximum rate for earlier days of irradiation than 3:5 and 5:3 types. As can be seen from Table 2, no difference of the suggested kind was found.
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Table 2. Relative frequency of the different types of aberrant octads obtained from crosses that had been UV-irradiated at separate days. a) Day after inoculation of ascospore strain, at which irradiation was administered. b) Individual types of aberrant octads, giving wildtype spores first. Figs. in Table 2 are in % of total aberrants obtained for the respective days.

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Discussion

The data reported bear on several aspects of the phenomenon of gene-conversion.

1. The marked increase of the overall rate of gene-conversion by UV-irradiation of the crosses is in line with the findings of others, who have reported an increase of recombination and gene-conversion rates in several micro-organisms upon UV-irradiation (see I.c.12, 13). The effect of UV on both recombination and gene-conversion points to a common step, at which UV acts. It has been suggested that single strand breaks, produced by endonucleases, recognizing UV-damaged regions in DNA, may be promoting recombination. According to the WHITEHOUSE model1, 2 they would also favour gene-conversion.

2. The analysis of the relative frequencies of the 8 possible types of aberrant octads, obtained either spontaneously or after UV-irradiation, represents a new approach to discriminate between several types of molecular mechanisms that could lead to gene-conversion. The distribution of these frequencies may be considered as a fingerprint, in which different molecular mechanisms should differ. Based on this criterion the events leading spontaneously to gene-conversion are closely related to those set in action on UV-irradiation.

Our method should be a valuable tool for judging the molecular effects of other agents, e.g. chemicals, that increase conversion rates.

3. The asymmetry observed in the frequencies of reciprocal types of aberrant octads points to a bias of the presumed repair enzyme or endonuclease, in correcting a mismatched base pair. Such asymmetries in the predominant direction of conversion have been observed in other fungi (see I.c.14, 15). They suggest that it is the mismatched basepair itself which sets in train the excision repair process16, and that a distinct one (resp. 2) of the 4 possible bases is excised preferentially, its mate being conserved.

4. The amount of the UV effect was shown to be markedly dependent on the age of the crosses when they were exposed to the irradiation. This finding may be considered a potential means to identify those cellular stages, at which gene-conversion actually occurs, be it that only one such stage is existing, or that 6:2 and “post-meiotic segregations” or 5:3 are induced at two separate stages.Singleton17, in crosses set up under comparable conditions, did not observe pachytene stages of prophase I before 3 days after fertilization. Since in our study, the UV effect was most pronounced one day after fertilization, it seems that gene-conversion is initiated shortly after plasmogamy and certainly before pachytene.

Further experiments will be needed to clarify the problems pointed out here.

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