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THE CONTROL OF KARYOGAMY IN SOMATIC CELLS OF *USTILAGO VIOLACEA*

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SUMMARY

The rate of nuclear fusion (karyogamy) in the haploid smut fungus, *Ustilago violacea*, designated D, can be determined accurately from the frequency of diploid formation. The spontaneous value of D depends mainly on the age of the cells and the temperature, varying from 10^{-5} to 10^{-2}. Ultraviolet light (u.v., 254 nm) induces high rates of fusion, up to 100 % of the survivors being diploid after very high doses. That this increase is caused by *induction* and not by *selection* is shown by the absolute increase in the number of diploids obtained under some conditions. The high value of D after a u.v. treatment is reduced by conditions which favour photoreactivation or dark repair. Acriflavine, an inhibitor of dark repair, maintains high values of D, while caffeine which also inhibits dark repair abolishes the u.v. induction entirely.

Ultraviolet-sensitive mutants located at 4 loci were tested for their effect on spontaneous and u.v.-induced karyogamy. One mutant (uvs_4) showed complete absence of somatic karyogamy even after high doses of ultraviolet. Mutant uvs_2 had no effect on the value of D, while mutants uvs_1 and uvs_3 showed low spontaneous karyogamy, but could be induced by low doses of u.v. to yield large numbers of diploids.

It is suggested that an inhibitor of karyogamy is formed in somatic cells and destroyed prior to premeiotic karyogamy. The accuracy of the transcription of this inhibitor seems to be impaired by ultraviolet, but can be restored following DNA repair. The absence of somatic karyogamy in uvs_4 mutants may indicate that these strains contain an altered organelle involved in the control of karyogamy, e.g. the spindle pole body situated on the nuclear membrane, and known to contain DNA. Alteration of such an organelle could produce pleiotropic effects including u.v. sensitivity and inhibitor production or action.

INTRODUCTION

Many fungi spend most of their life-cycles as dikaryons or multikaryons in which the nuclei co-exist and rarely fuse. In these same species, however, at a particular stage of sexual development the nuclei come together in pairs and fuse forming a diploid nucleus which promptly undergoes a meiotic division. There is clearly a control mechanism operating to prevent karyogamy in somatic cells and to induce it to occur regularly in sexual cells. The nature of this control mechanism is completely unknown. We report here experiments which indicate that most probably an inhibitor is produced by somatic cells which prevents karyogamy. We also report preliminary indications that karyogamy may depend on the stage in the nuclear cycle of the 2 nuclei prior to fusion.

The yeast-like, uninucleate cells of *Ustilago violacea* are very suitable for investigations of this sort, as the frequency of somatic karyogamy can be determined directly and accurately by a simple selection procedure. The method avoids the problems that

occur in filamentous fungi, where clonal distribution of nuclei and the indirect measurement of the frequency of nuclear fusion through conidial frequencies may lead to large inaccuracies (Roper, 1966). This is particularly the case when treatments which may affect the viability as well as the frequency of karyogamy are being tested.

The method used in *U. violacea* involves the selection on minimal medium of the rare diploid products formed by nuclear fusion between 2 complementary auxotrophic strains (Day & Jones, 1968). In this species cells of opposite mating-type conjugate by forming a tube between them, and establish a binucleate conjugated pair of cells. Such cells cannot form dikaryotic hyphae on minimal medium as this stage is obligately parasitic (Fischer & Holton, 1957; Day & Jones, 1968). Thus only diploid colonies formed after nuclear fusion grow on this medium. On complete medium, however, each conjugant buds separately and restores the haploid phase. A mixed colony (mosaic) grows from each conjugated pair of cells on complete medium. The frequency of nuclear fusion is calculated from the fraction of conjugated cells that give rise to diploid colonies. This system is illustrated in Fig. 1 (p. 621).

Most of the experiments reported here concern the effect of ultraviolet light on the frequency of somatic karyogamy, an observation first made by Ishitani, Ikeda & Sakaguchi (1956) with the filamentous fungi, *Aspergillus sojae* and *A. oryzae* and confirmed by ourselves for *Ustilago violacea* in a brief report (Clements, Day & Jones, 1969). This paper describes more detailed investigations of this effect of ultraviolet including the use of u.v.-sensitive mutants (Day & Day, 1970) and conditions which favour repair of u.v.-damaged DNA (photoreactivation and dark repair). In addition we report the effects of the inhibitors of dark repair (excision repair) caffeine and acriflavine which seem to bind to the repair polymerase enzyme and the damaged DNA respectively (Horowitz & Metzenberg, 1965; Grigg, 1968). These studies implicate a somatic repressor of karyogamy and thus provide a working hypothesis for future investigations.

MATERIALS AND METHODS

Stocks

The stocks used in these experiments were the strains 1.C2 (yellow colonies, *y*; histidine requirement, his_1; a_1 mating type) and 2.716 (wild type pink colour; lysine requirement, lys_1; and a_2 mating type) and the double auxotroph 2.D3729S (orange colonies, *or*; lysine requirement, lys_3; inositol requirement, $inos_1$; mating type a_3). The origin of the first two of these stocks is described in Day & Jones (1968, 1969). The double auxotroph 2.D3729S was derived by recombination between 1.C3 and 2.729 (Day & Jones, 1969) followed by the u.v.-induction of the orange colour colony marker. In addition we used stocks carrying the above markers and one of several mutants sensitive to ultraviolet light (*uvs* mutants). The origin and properties of these *uvs* mutants are described in Day & Day (1970).

Culture of stocks

The composition of complete and minimal media is given in Day & Jones, 1968. The cells of each strain were incubated on solid complete medium at 22 °C for 3–4 days in most experiments.

Cell fusion

Cells of 1.C2 (yellow) and 2.716 (pink) or 1.C2 and 2.D3729S (orange) were mixed together with a sterile wire on the surface of a plate of 2 % water agar. The cells were incubated at 20–22 °C for 24 h after which there would usually be about 50–60 % conjugation. The cells were resuspended in sterile water and the concentrations of conjugated cells and single cells were estimated with a haemocytometer counting chamber. The concentration of the suspension was adjusted to about $1–5 \times 10^6$ conjugated cells/ml. This mixed suspension of single and conjugated cells was used in the experiments to determine the frequency of nuclear fusion.

Nuclear fusion

In order to estimate the frequency of nuclear fusion (D), 0·1 ml of the suspension was plated on minimal medium to determine the number of diploids that were formed (d). Only diploid and haploid revertants, which are easily distinguished by cell and colony characteristics (Day & Jones, 1968), can grow on this medium. D is derived from the equation $D = \frac{d}{n_0}$, where n_0 is the number of conjugated cells plated on minimal medium. The value of n_0 can be taken directly from the haemocytometer count if the viability of the cells is not impaired by a treatment, as

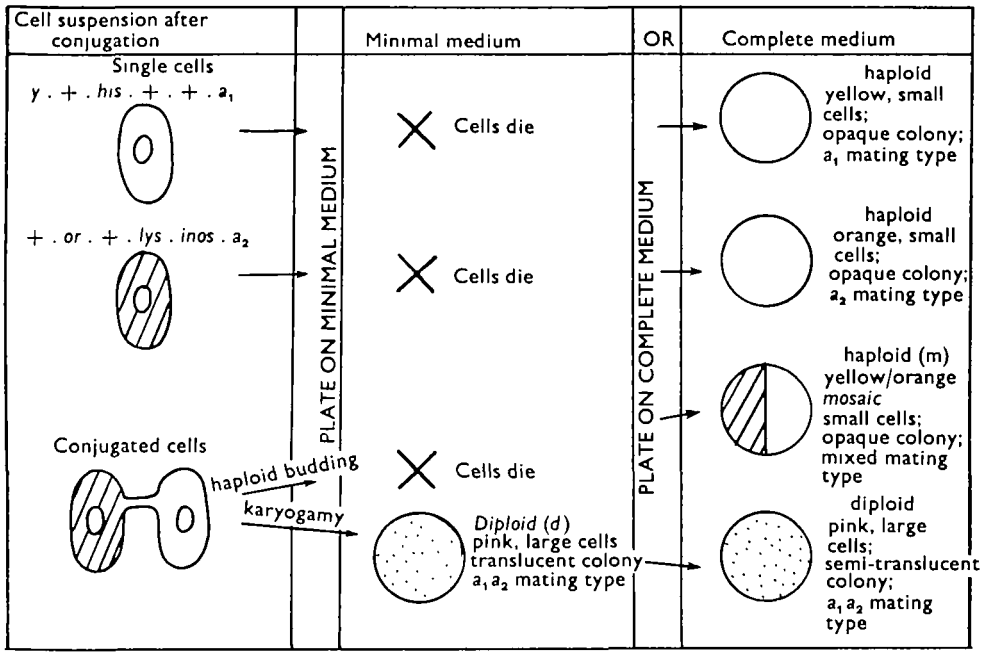

Fig. 1. Method for selecting diploid colonies on either complete medium or minimal medium.

we have determined that close to 100 % of conjugated cells are viable. However, when a treatment which affects viability is used, n_0 must be determined by a plate count. In such cases, a sample from the suspension of conjugated cells was plated on *complete* medium after appropriate dilution. All cells can grow on this medium, but colonies derived from haploid budding of conjugated cells can be distinguished by their two-tone (yellow/pink or yellow/orange) colour. Such colonies were described as *mosaics* (Clements *et al.* 1969). Rare diploid colonies on

complete medium can also be distinguished as they have the wild-type pink phenotype. The frequency of nuclear fusion on minimal medium (D_{min}) is then derived from the equation $D = \frac{d}{m \times c}$, where d = the number of diploids on minimal medium, c = the dilution factor on complete medium, and m = the number of mosaic colonies on complete medium (Clements *et al.* 1969). This system is illustrated in Fig. 1, and obviously depends on the assumption that the survival of conjugated cells is not affected by the medium used. This has been demonstrated to be true.

After inoculation of the plate with the conjugated cells, they were incubated at 20 °C for either 10 (complete medium) or 20 days (minimal medium) before the numbers of mosaic and diploid colonies were counted. The colonies were scored with the aid of a New Brunswick colony counter. Generally 5 replicates of each treatment were made and the counts averaged. The identification of diploid cells and colonies is described in Day & Jones (1968).

Chemicals and nuclear fusion

The effect of chemicals on nuclear fusion was investigated by exposing the conjugated cells to the chemical for the duration of their growth on complete and minimal medium. In most experiments the chemical was incorporated directly in the agar, e.g. caffeine at 0·05 % and acriflavine at 0·01 %. Camphor treatment was given by exposure to the vapour from a small crystal taped to the lid of the Petri dish.

A range of some 40 antimicrobial agents was screened by placing sterile disks containing known amounts of antibiotic (Fisher Scientific Co.) in the centre of the plate. The plates were scored for zones of increased or decreased diploid formation round the disk.

Irradiation and nuclear fusion

The mixed suspension of conjugated and single cells was irradiated with ultraviolet (254 nm) from a Camag TL900 UV lamp with 8-W G8T5 fluorescent tube giving a dose of about 2·5 J m^{-2} s^{-1} at 12 cm distance. Precautions were taken to avoid uncontrolled photoreactivation. The effect of irradiation and photoreactivation on survival in these stocks has been described elsewhere (Day & Day, 1970).

In the photoreactivation (PR) treatments, samples of the irradiated cells were further exposed to light from four 40-W natural 39P 'Quick-Start' fluorescent tubes at a distance of 10 cm for 0, 15 or 30 min, before plating out (Clements *et al.* 1969).

A phenomenon similar to 'liquid holding recovery' (Jagger, 1964) and termed starvation recovery (SR) has been reported for *U. violacea* (Day & Day, 1970). The effect of SR on nuclear fusion was examined by plating the irradiated cells on water agar for 24 h before transferring the water agar layer to plates of complete or minimal media. The effects of caffeine and acriflavine on SR were investigated by incorporating these chemicals into the water agar and into the subsequent nutritive medium. Appropriate controls were analysed for each of these treatments, and again the results of each experiment are derived from the averages of 5 replicates. Each experiment was repeated at least once, generally twice.

RESULTS

The spontaneous rate of nuclear fusion

The spontaneous rate of nuclear fusion is remarkably constant in different combinations of stocks. Thus in an earlier report we found that the value of D_{min} at 22 °C for 14 different combinations of auxotrophs varied from 1·6 to $5{\cdot}2 \times 10^{-4}$ with a mean of $3{\cdot}36 \times 10^{-4}$ (Day & Jones, 1968). These values were for cells incubated for 3 days on complete medium before conjugation. However, we have found that the culture 2.D3729S tends to have a reduced level of spontaneous karyogamy so that values of

around $8–10 \times 10^{-5}$ are more common for combinations involving this stock and 1.C2, whereas the higher value is obtained for the 1.C2 and 2.716 combination. Very low values of D were obtained from some u.v.-sensitive mutants (see below and Fig. 6).

The effect of environmental factors on spontaneous karyogamy

Medium. As diploids form spontaneously at a low frequency, it is easier to detect them with a selective method, such as that employing auxotrophic strains and minimal medium, than with a total isolation method on complete medium. However, we have

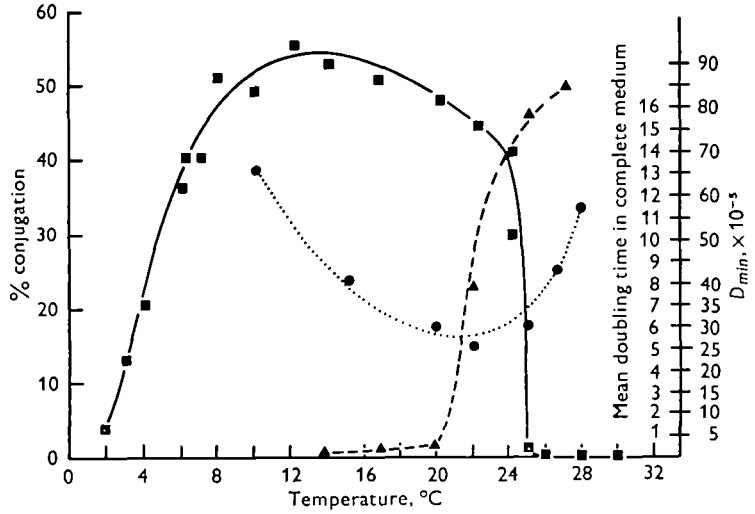

Fig. 2. The effect of temperature on conjugation (plasmogamy) (■), karyogamy (▲) and duration of the cell cycle (mean doubling time in complete medium) (●).

made some observations of the frequency of nuclear fusion on complete medium (D_{com}) by screening large numbers of plates. Diploids on this medium are a clear pink, quite distinct from the orange and yellow colonies of the parental haploids. It is therefore necessary only to check cell size in order to confirm that a pink colony is diploid and not a haploid revertant. We have found that D_{com} is generally higher by a factor of about 10-fold than the frequency on minimal medium (D_{min}).

Temperature. Fig. 2 shows a comparison of the effect of different temperatures on cell fusion, nuclear fusion (D_{min}) and growth rates. The rate of nuclear fusion increased markedly with increasing temperature from about 5×10^{-5} at 20 °C to 9×10^{-4} at 27 °C. This is quite different from the curves for cell fusion (conjugation) and cell cycle duration which show peaks at 12 and 22 °C respectively. The cells are very sensitive to high temperatures and die at about 29 °C. Most of the experiments reported below were carried out at 22 °C.

Age. The age of the haploid stocks used for cell fusion affects D_{min} significantly. Fig. 3 summarizes the values of D_{min} obtained in many experiments in which stocks of different ages have been used. A clear increase in D_{min} from about 10^{-4} to about

10^{-2} is evident as the cells pass from log phase to late stationary phase. Experiments are being carried out to determine whether or not this age effect is a cell cycle effect in which karyogamy occurs more readily in G_1 cells than in other cell cycle stages, as we have shown that stationary phase cells are arrested in G_1 (Day & Cummins, 1973).

Light. Treatments in which nuclear fusion has been allowed to occur in the dark, or in fluorescent room lighting have had no effect on D_{min}.

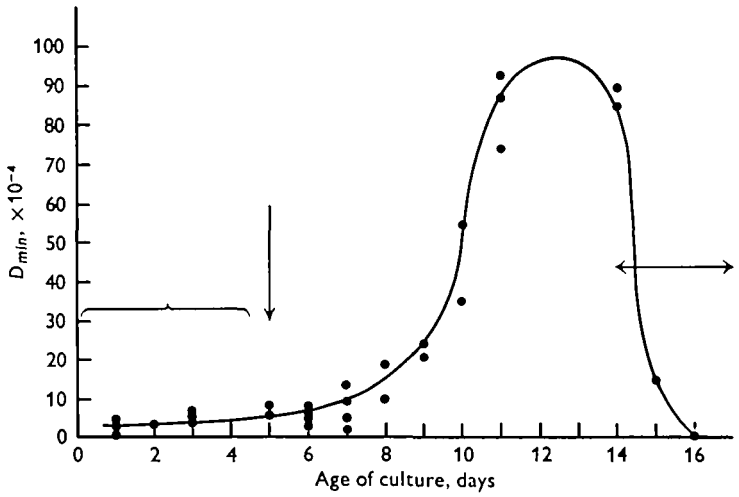

Fig. 3. The effect of age of the haploid partners on karyogamy in the conjugated cells. The cells were sampled at different times from a culture in liquid complete medium, allowed to conjugate and the frequency of karyogamy on minimal medium was determined (D_{min}). Curled brace indicates period of log phase; the vertical arrow, stationary phase; and the double-ended arrow, death of culture.

The effect of chemicals on karyogamy

Camphor has been reported to increase the frequency of karyogamy in *Aspergillus sojae* (Ishitani *et al.* 1956; Roper, 1966). We have allowed cells to undergo nuclear fusion in an atmosphere of camphor vapour for 4, 6 or 23 h. A small increase in D_{min} from 7×10^{-5} in the untreated population to $3{\cdot}3 \times 10^{-4}$ after 23-h treatment was observed.

A range of some 40 antibiotics have been screened for an effect on karyogamy. None showed any significant effects on D_{min}. The effect of chemicals such as acriflavine and caffeine are discussed below in relation to the effect of ultraviolet light.

The effect of ultraviolet light on karyogamy

Ultraviolet, photoreactivation and karyogamy. Fig. 4A shows the effect of ultraviolet and visible light on D_{min}. Binucleate sporidial pairs were irradiated with different doses of ultraviolet, and 2 aliquots from each u.v. treatment were immediately irradiated for either 15 or 30 min with photoreactivating light. Survival curves (Fig. 4B) and the value of D_{min} (Fig. 4A) were determined for each treatment at 22 °C.

The figure shows that increasing the u.v. dose increases D_{min} in a linear manner from about 8×10^{-4} in the unirradiated control cells to almost 1×10^{0} (i.e. 100%) at

the highest dose of u.v. used. At this dose of ultraviolet (450 J m^{-2}) the survival was less than 0·01 %. A 15-min dose of photoreactivating light (PR) significantly reduced the value of D_{min} for any u.v. dose, but had little effect on control cells not irradiated with ultraviolet. Increasing the PR dose to 30 min reduced still further the values of D_{min} obtained. These PR treatments also significantly increased the percentage survival of sporidia, as has been demonstrated earlier (Day & Day, 1970).

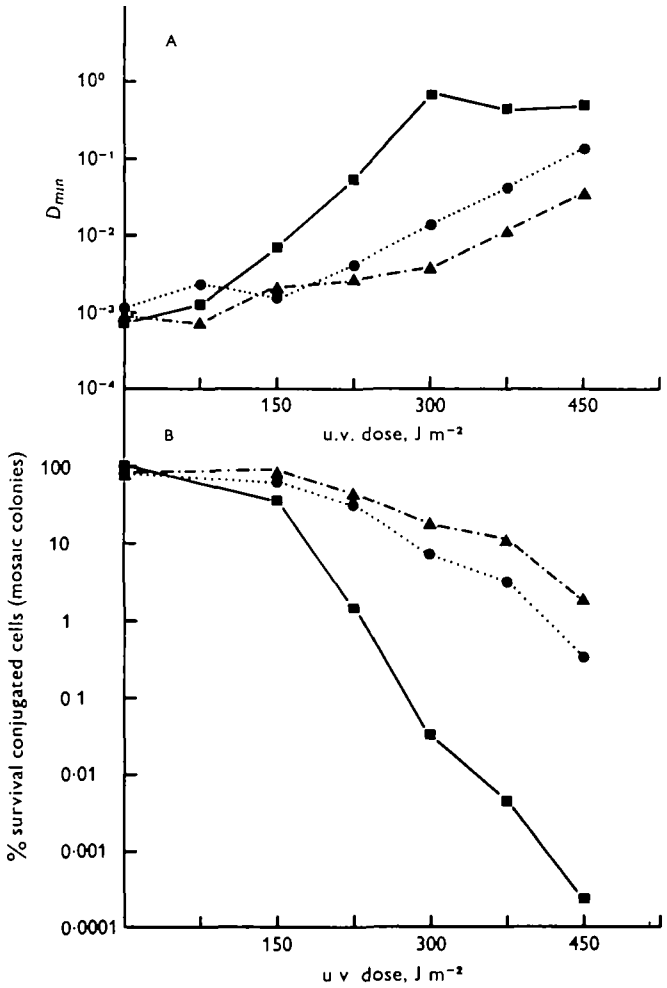

Fig. 4. The effect of u.v. and photoreactivation on survival and karyogamy in conjugated cells. A, effect on karyogamy; B, effect on survival. The u.v. doses were followed by either no photoreactivation (■), 15 min of visible light (●), or 30 min of visible light (▲).

The value of D_{min} after irradiation depends on 2 factors: the actual number of diploids obtained and the survival of the binucleate cells. Values of D_{min} increased with increasing irradiation largely because the percentage survival decreased sharply, while the actual number of diploids stayed constant or decreased slowly (Fig. 5: 22 °C). Thus it is not easy to distinguish between two mechanisms by which ultraviolet could

affect D_{min}. (1) The ultraviolet *induction* of nuclear fusion in which the increased rate of karyogamy at high doses is more or less balanced by the decrease in survival of the cells. And (2) the *selection* by ultraviolet of a highly u.v.-resistant fraction of the binucleate population which is the only fraction capable of karyogamy. The best distinction between these fundamentally different hypotheses is to demonstrate that the actual number of diploids obtained after an irradiation treatment is higher than that obtained from an unirradiated control. This would only be possible if ultraviolet

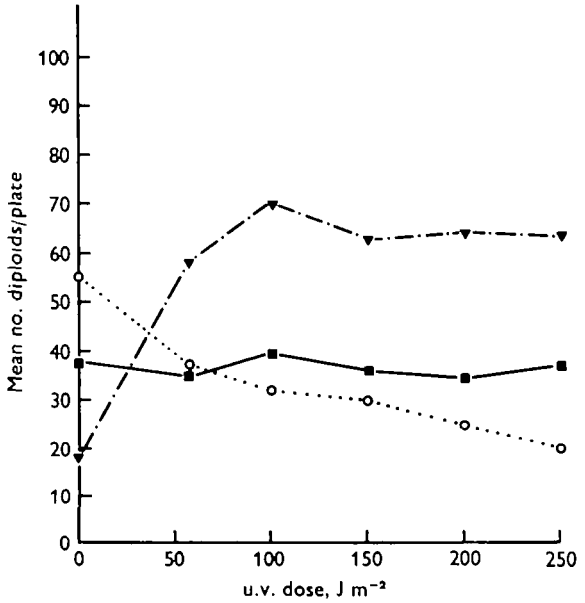

Fig. 5. The effect of u.v. on the absolute number of diploids formed at various temperatures: 20 °C, ▼; 22 °C, ■; and 25 °C, ○. A standard inoculum of conjugated cells was placed on each of a series of plates of minimal medium. The plates were then given various doses of u.v. and incubated at different temperatures. The number of diploids on each plate is the mean of 5 replicates.

induces karyogamy. In the experiment shown in Fig. 4 which was conducted at 22 °C the number of diploids observed remained more or less constant as the u.v. dose was increased. However, we have shown that at 20 °C a 3- to 4-fold increase in the number of diploids recovered is obtained when the u.v. dose is increased from 0 to 100 J m^{-2} (Fig. 5). At lower temperatures (17° C) karyogamy is rare, and the actual number of diploids obtained remains constant as the ultraviolet dose is increased, while at higher temperatures (25 °C) the actual number of diploids tends to decrease slowly as the dose is increased. The result at 20 °C (which we have observed in 3 different experiments) can only be explained by the induction hypothesis, i.e. that ultraviolet induces karyogamy. Even the results at other temperatures support this hypothesis, as the alternative selection hypothesis would require that the 'diploid-forming' fraction be almost completely resistant to u.v. doses that normally kill 99·999 % of cells. Survival curves of the binucleate conjugated cells do not show such a highly resistant fraction (e.g. Fig. 4B).

We are confident therefore that ultraviolet induces karyogamy, although of course it may *also* select fractions predisposed to nuclear fusion.

Since percentage survival is not significantly affected at any of these temperatures, we deduce from the numbers of diploids recovered that the optimum effect of ultraviolet on D_{min} is at 20 °C and that increasing the temperature to 22 and 25 °C slightly decreases the value of D_{min} for any particular u.v. dose. This is in contrast to the effect of temperature on D_{min} in unirradiated cells where the optimum is at least 27 °C (cf. Figs. 2 and 5).

Table 1. *The effect of post-irradiation treatment on D_{min} and survival in U. violacea*

	Treatment				
	Control	30 min PR	SR	SR + caffeine	SR + acriflavine
(*a*) D_{min} no u.v.	6×10^{-5}	8×10^{-5}	5×10^{-5}	9×10^{-5}	7×10^{-5}
(*b*) D_{min} 225 J m^{-2} of u.v.	2×10^{-1}	8×10^{-2}	5×10^{-2}	5×10^{-4}	$1{\cdot}5 \times 10^{-1}$
Increase in D_{min} (*b*/*a*)	$3{\cdot}3 \times 10^{3}$	10^{3}	10^{3}	5·5	$2{\cdot}15 \times 10^{3}$
% Survival, no u.v.	100	85	100	100	90
% Survival, 225 J m^{-2} of u.v.	2	35	22	6	5

As the effect of ultraviolet on karyogamy is photoreactivable we conclude that the primary u.v.-lesions responsible must be pyrimidine dimers in DNA (Smith & Hanawalt, 1969). If so, mechanisms which repair dimers in DNA may affect D_{min}. We report below the effect of starvation recovery treatments in the presence or absence of dark-repair inhibitors such as caffeine and acriflavine.

Ultraviolet, dark repair and karyogamy. Many irradiated cells recover viability when they are inoculated to water agar for 24 h before plating on a nutrient medium (starvation recovery, SR). We have applied this method to the analysis of the effect of u.v. on karyogamy. Aliquots of control and irradiated binucleate conjugated cells were incubated on water agar for 24 h before being plated out on nutrient media. Other cells were incubated directly on nutrient medium immediately after irradiation. The values of D_{min} obtained in control and SR-treated cells at different ultraviolet doses are shown in Table 1. The table also shows the effect on D_{min} of the inhibitors of dark repair, caffeine, and acriflavine. The chemicals were included in the water agar during the SR treatment and also in the nutrient medium during the formation and growth of diploid colonies.

The value of D_{min} for any u.v. dose is significantly reduced during SR, while the value in unirradiated cells is hardly affected. Acriflavine treatment inhibits dark repair and does not allow SR to occur. Under these conditions high D_{min} values are

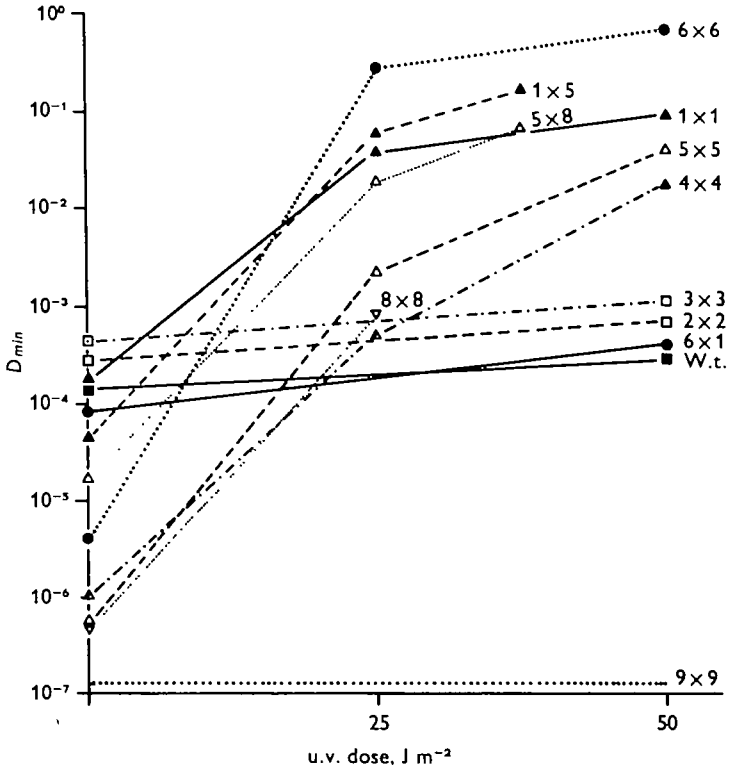

Fig. 6. The effect of u.v.-sensitive mutants on spontaneous and u.v.-induced karyogamy. The frequency of karyogamy in various combinations of u.v.-sensitive mutants was determined at 2 different u.v. doses as well as in unirradiated controls. W.t. = wild type combination (1.C2 × 2.716), 6 × 1, etc., refer to the u.v.-sensitive mutants used; e.g. this refers to the combination, allele u6 (in cell of mating type a_1) × allele u1 (in cell of mating type a_2).

obtained. Thus karyogamy in cells treated with u.v. + SR + acriflavine is almost as high as in cells treated with ultraviolet alone.

However, caffeine had the interesting effect of abolishing the u.v. induction of karyogamy, even though it has been shown to effectively inhibit dark repair in *U. violacea* (Table 1 and Day & Day, 1970). This effect has been observed in 3 different experiments.

As both photoreactivation and dark repair affect karyogamy, an obvious next step was to isolate u.v.-sensitive mutants (*uvs*) defective in one or both of these processes.

Ultraviolet-induced somatic karyogamy in u.v.-sensitive mutants. Nine u.v.-sensitive mutants (numbered u1–u9) have been isolated, classified into complementation groups and arranged in 4–5 loci by meiotic recombination tests (Day & Day, 1970). The effects of these mutants on PR, SR and caffeine-inhibited SR have been reported (Day & Day, 1970). Mutants uvs_1 (alleles u1, u4, u5 and 8) and uvs_3 (allele u6) showed no SR and were therefore described as deficient in dark repair. Mutants uvs_2 (alleles u2 and u3) and uvs_4 (allele u9) had normal SR but may be slightly deficient in PR.

Stocks of 1.C2 and 2.D3729S carrying 1 of these 9 u.v.-sensitive alleles were

synthesized. The values of D_{min} for 8 homoallelic combinations (omitting allele u7) were determined in unirradiated conditions and also after a standard u.v. dose. The value was also determined for some heteroallelic and intergenic combinations (Fig. 6). The experiment has been repeated with essentially the same results. It is clear from Fig. 6 that both the normal and u.v.-induced values of D_{min} vary in these u.v.-sensitive mutants. Thus mutants carrying uvs_4 (allele u9) appear to be incapable of somatic karyogamy, while those carrying uvs_1 and uvs_3 (u1, u4, u5, u8 and u6) differ greatly from the wild type, in that they have a lower than normal spontaneous D_{min}, but are induced to form diploids at high frequency after doses of ultraviolet that hardly affect the wild type. On the other hand mutant uvs_2 did not differ from wild type in either spontaneous or u.v.-induced karyogamy. Heteroallelic combinations of alleles in uvs_1 tended to show values for D_{min} that were similar to or slightly greater than the values for homoallelic combinations. Intergenic combinations of these recessive alleles behaved as expected in that they were indistinguishable from the wild type.

Sexual karyogamy in wild type and u.v.-sensitive strains. The strains mentioned in this paper have been mixed in many different combinations, inoculated into the host plant, and the sexually produced diploid brandspores examined (Day & Jones, 1968, 1969). The combinations used included all homoallelic combinations of *uvs* alleles, most heteroallelic combinations as well as many wild type × *uvs*, and wild type × wild type combinations. In all cases including $uvs_4 \times uvs_4$, which shows complete absence of any somatic karyogamy, the resulting brandspores were normal in appearance and development as judged by segregation of the markers (see Day & Day, 1970). Thus allele uvs_4 does not prevent sexual karyogamy.

DISCUSSION

The results presented here confirm previous reports that ultraviolet can induce karyogamy in somatic cells of fungi (Ishitani *et al.* 1956; Clements *et al.* 1969). The alternative hypothesis that u.v. merely selects a resistant fraction predisposed to karyogamy is ruled out by the absolute increases in the numbers of diploids obtained following u.v. doses in *Aspergillus sojae* (Ishitani *et al.* 1956) and in *U. violacea* at temperatures 20–22 °C.

There are several ways in which u.v. could influence karyogamy. We can tentatively exclude one obvious method, the induction of mutants at 'controller' genes by analysing u.v.-induced diploids. Our preliminary investigations of haploids derived from such diploids show a variety of mutations as expected. There was no tendency for these haploids to show a higher rate of karyogamy than the original haploids. The reversal of the u.v. effect on karyogamy by visible light implicates DNA as the primary site through which the effect is mediated. Thus hypotheses involving direct effects of u.v. on nuclear membrane structure may be eliminated.

In mammalian somatic cell studies karyogamy seems to occur only when the cell cycles of 2 nuclei are synchronous and in the mitotic phase (Macklin, 1916; Harris, 1970). Such a control system clearly does not operate in this fungus, which like many other basidiomycetes can grow as a stable dikaryon with synchronous nuclear divisions

(Fischer & Holton, 1957). However, it is certainly possible that there may be other phases in the cell cycle during which nuclear fusion is possible. The optimum phases could be different in the 2 genetically different nuclei which fuse, just as they are different for the 2 mating-types during plasmogamy (Cummins & Day, 1973). Our results indicating that karyogamy increases with age of the cells suggest that the G_1 cells of both mating types are the most capable of karyogamy as stationary phase cells are arrested in G_1 (Day & Cummins, 1973). It is an interesting correlation that the cell cycle control of plasmogamy in *Ustilago* is such that the nuclei are not automatically synchronized in the dikaryon (Cummins & Day, 1973) and karyogamy is rare, while in the yeast *Saccharomyces cerevisiae* the control of plasmogamy is such that the nuclei are actively synchronized in the G_1 phase (Hartwell, 1973) and karyogamy occurs immediately.

It is possible that these differences between mammalian cells, *Ustilago* and *Saccharomyces* may also be related to the type of nuclear division that occurs. Somatic nuclear division in fungi is difficult to interpret (Day, 1972) but in most species it is clearly intranuclear (i.e. occurring inside an intact nuclear membrane). However, *Ustilago* and the 'false yeasts' are an exception to this as the nuclear membrane does breakdown in localized regions for a short time during division in budding cells (McCully & Robinow, 1972; N. H. Poon & A. W. Day, in preparation). As it is not clear what kind of division occurs in the *hyphal* dikaryotic stage, we cannot yet conclude that *Ustilago* has the ability to keep separate 2 nuclei with partially disrupted membranes during their synchronous division (see also Discussion in Johnson & Rao, 1971).

The most likely manner in which ultraviolet affects karyogamy seems to be through the transcription of regular genes. As u.v. is clearly not the normal inducer of karyogamy under natural conditions, we conclude that it acts by interfering with normal regulation of this function. Control of karyogamy could be exerted through either positive or negative control systems. If under positive control we may postulate that a specific inducer of karyogamy is formed in the binucleate cells predestined to form the diploid sexual spore (brandspore), and that this inducer is normally absent from somatic cells.

Alternatively, a negative control could involve a specific repressor of karyogamy in somatic cells and its absence from the young prezygotic stages. Both these possibilities are likely to be over-simplifications as for instance both positive and negative control may be involved, and the level of action of the control systems might be on other developmental stages, leading to obligatory karyogamy. The results presented here suggest, however, that the primary control mechanism is likely to be a negative one, that is involving a repressor or inhibitor of somatic karyogamy. Thus it seems unlikely that u.v. doses of this magnitude would derepress an inducer without interfering with transcription of this gene.

If an inhibitor of somatic karyogamy is normally transcribed in somatic cells we can explain our results in the following way. High doses of u.v. induce large numbers of thymine dimers in DNA (Setlow, 1966). These dimers affect the accuracy and amount of transcription (Day & Cummins, 1974) as well as nucleolar-mediated transport (Harris, 1970). Thus insufficient repressor may be produced to prevent karyogamy.

Conditions which favour repair (photoreactivation or 'dark' repair mechanisms) would increase transcription and transport of the inhibitor mRNA through repair of dimers and would therefore lower the rate of karyogamy. Similarly agents which inhibit repair, for example acriflavine and caffeine, would be expected to produce D_{min} values similar to or greater than those for u.v. alone. Acriflavine does have this effect, but caffeine unexpectedly abolished the u.v. effect on D, so that the values for D_{min} remained about constant for ultraviolet doses from zero to the maximum. As caffeine is a rather non-specific inhibitor this observation may be related to its inhibition of enzymes other than dark-repair ones; for example, it is known to inhibit phosphodiesterases and thus maintain high levels of cyclic AMP in the cell.

Genes uvs_1 and uvs_3 which have been shown to be lacking in dark repair ability are predicted to fail to repair damage in the inhibitor gene which would explain their high frequency of karyogamy following even small doses of ultraviolet. Why these strains should also have very low spontaneous values of D_{min}, indicating that they are normally more efficiently inhibited than the wild type strains, is not clear. Gene uvs_2 is not affected in dark-repair ability, nor in karyogamy and its function is unknown. Mutants carrying gene uvs_4 were the most interesting strains in that they cannot form somatic diploids with or without ultraviolet treatment, even though karyogamy in sexual cells still occurs normally. This gene does not affect dark repair ability and its function is unknown (Day & Day, 1970). The gene uvs_1 in *Aspergillus nidulans* has also been reported to suppress somatic karyogamy but in addition it causes abortion of meiosis (Lanier *et al.* 1968).

The function of these genes which affect karyogamy and ultraviolet sensitivity simultaneously remains unknown. Perhaps the best guess is that the effect is a direct one on the structure of a nuclear or cytoplasmic organelle involved in karyogamy, for example the nuclear membrane itself or the spindle pole body. Any change in the structure and functioning of such organelles would be expected to have pleiotropic effects. Thus the spindle pole body is located on the nuclear membrane, contains DNA (Zickler, 1973; N. H. Poon & A. W. Day, in preparation), replicates during *S*-phase, appears to be attached to the chromosomes, and acts as a microtubule-organizing centre and possibly a nuclear-membrane organizing centre (Day, 1972; N. H. Poon & A. W. Day, in preparation). We consider it a likely location for at least some of the genes controlling behaviour of the whole nucleus including the localized breakdown of the membrane which occurs at the time of mitosis, and the fusion of nuclei.

In summary, the control of karyogamy is a necessity for many organisms, but the mechanisms by which it is achieved are unknown. Our results support a model in which somatic karyogamy is inhibited by a specific gene product. The location of the appropriate genes, the nature of the inhibitor, its site of action, its production during the cell cycle, and its effect at different temperatures remain to be discovered. The conjugated binucleate cells of *U. violacea* allow quantitative investigations of this process, and permit tests of the negative control model suggested here, through the use of synchronous cultures, conditional mutants, inhibitors of transcription, translation, and replication, and radioactive isotopes.

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REFERENCES

CLEMENTS, L. L., DAY, A. W. & JONES, J. K. (1969). The effect of ultraviolet light on nuclear fusion in *Ustilago violacea*. *Nature, Lond.* **223**, 961–963.

CUMMINS, J. E. & DAY, A. W. (1973). The cell cycle regulation of mating-type alleles in a smut, *Ustilago violacea*. *Nature, Lond.* **245**, 259–260.

DAY, A. W. (1972). Dominance at a fungus mating type locus. *Nature, New Biol.* **237**, 282–283.

DAY, A. W. & CUMMINS, J. E. (1973). Temporal allelic interaction, a new kind of dominance. *Nature, Lond.* **245**, 260–261.

DAY, A. W. & CUMMINS, J. E. (1974). Transcription and translation of the sex message in *Ustilago violacea*. I. The effect of ultraviolet light. *J. Cell Sci.* **14**, 451–460.

DAY, A. W. & DAY, L. L. (1970). Ultraviolet light sensitive mutants of *Ustilago violacea*. *Can. J. Genet. Cytol.* **12**, 891–904.

DAY, A. W. & JONES, J. K. (1968). The production and characteristics of diploids of *Ustilago violacea*. *Genet. Res.* **11**, 63–81.

DAY, A. W. & JONES, J. K. (1969). Sexual and parasexual analysis of *Ustilago violacea*. *Genet. Res.* **14**, 195–221.

FISCHER, G. W. & HOLTON, C. S. (1957). *Biology and Control of the Smut Fungi*. New York: Ronald Press.

GRIGG, G. W. (1968). Caffeine-death and a theory of chromosome breakage: an excision repair suicide model. In *Replication and Recombination of Genetic Material* (ed. W. J. Peacock & R. D. Brock), pp. 120–133. Canberra: Australian Academy of Sciences.

HARRIS, H. (1970). *Cell Fusion*. Oxford: Clarendon Press.

HARTWELL, L. (1973). Synchronization of haploid yeast cell cycles, a prelude to conjugation. *Expl Cell Res.* **76**, 111–117.

HOROWITZ, N. H. & METZENBERG, R. L. (1965). Biochemical aspects of genetics. *A. Rev. Biochem.* **34**, 527–564.

ISHITANI, C., IKEDA, Y. & SAKAGUCHI, K. (1956). Hereditary variation and recombination in Koji-moulds (*Aspergillus oryzae* and *A. sojae*) VI. Genetic recombination in heterozygous diploids. *J. gen. appl. Microbiol.* **2**, 401–430.

JAGGER, J. (1964). Photoreactivation and photoprotection. *Photochem. Photobiol.* **3**, 451–465.

JOHNSON, R. T. & RAO, P. N. (1971). Nucleo-cytoplasmic interaction in the achievement of nuclear synchrony in DNA synthesis and mitosis in multinucleate cells. *Biol. Rev.* **46**, 97–155.

LANIER, W. B., TUVESON, R. W. & LENNOX, J. E. (1968). A radiation sensitive mutant of *Aspergillus nidulans*. *Mutation Res.* **5**, 23–31.

MACKLIN, C. C. (1916). Binucleate cells in tissue culture. *Contributions to Embryology*, vol. **13**, p. 69. (Carnegie Instn Wash. Publ. No. 224).

MCCULLY, E. K. & ROBINOW, C. F. (1972). Mitosis in heterobasidiomycetous yeasts. II. *Rhodosporidium* sp. (*Rhodotorula glutinis*) and *Aessosporon salmonicolor* (*Sporobolomyces salmonicolor*). *J. Cell Sci.* **11**, 1–31.

ROPER, J. A. (1966). The parasexual cycle. In *The Fungi*, vol. 2 (ed. G. C. Ainsworth & A. S. Sussman) chapter 18. New York: Academic Press.

SETLOW, J. K. (1966). The molecular basis of biological effects of ultraviolet radiation and photoreactivation. *Curr. Topics Radiat. Res.* **2**, 195–248.

SMITH, K. C. & HANAWALT, P. C. (1969). *Molecular Photobiology, Inactivation and Recovery*. New York and London: Academic Press.

ZICKLER, D. (1973). Evidence for the presence of DNA in the centrosomal plaques of *Ascobolus*. *Histochemie* **34**, 227–238.

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