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Indications for an inducible component of error-prone DNA repair in yeast

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Summary In a thermoconditional mutant of mutagenic DNA repair (*rev2*^{ts} = *rad5–8*) of *Saccharomyces cerevisiae* recovery of survival and mutation frequencies can be monitored by incubating UV-irradiated cells in growth medium at a permissive temperature (23°C) before plating and a shift to restrictive temperature (36°C). Inhibition of protein synthesis with cycloheximide during incubation at permissive conditions blocks this *REV2* dependent recovery process in stationary phase *rev2*^{ts} cells, whereas it can be reduced but not totally abolished in exponentially growing cells. These results indicate a strict dependence on post-irradiation protein synthesis in stationary phase cells and argue for a considerable constitutive level and only limited inducibility in logarithmic phase cells. The UV inducibility of the *REV2* coded function in stationary phase cells could be confirmed by analysis of the dose-response pattern of the *his 5–2* reversion: in stationary phase *rev 2*^{ts} cells, the quadratic component of the biphasic linear-quadratic induction kinetics found at 23°C, which is interpreted as the consequence of induction of mutagenic repair, is eliminated at 36°C.

Considerable evidence in favour of the SOS hypothesis exists now in *Escherichia coli*. According to this concept, "induced" (in the sense of enhanced) mutagenesis is mostly due to misrepair where several cell functions are coordinatively "inducible" (in the sense: dependent on de-novo synthesis), and their action results in an increased cell survival at the price of higher mutability (for a recent review on DNA repair in *E. coli* c.f. Schendel, 1981).

The validity of such a concept for eukaryotic cells is still subject to discussion. Even regarding UV-induced mutagenesis in the yeast *Saccharomyces cerevisiae*, a genetically well characterized, simple eukaryote, the situation is far from clear. In mating experiments carried out in excision-deficient strains, Lawrence & Christensen (1982) proved the induction of mutations in an unirradiated genome after mating with an irradiated cell (untargeted mutagenesis). Since this process is dependent on nuclear fusion, they argued against an inducible "mutagenic factor" which is cytoplasmatically transferable to an unirradiated nucleus. Other data, however, have been accumulated suggesting indirectly an involvement of inducible repair functions in excision-proficient yeast. Haynes & Eckardt (1979*b*) correlated a mechanistic interpretation with a stochastic description of survival and mutation data of yeast (Haynes & Eckardt, 1979*a*): the assumption of both a constitutive and an inducible component of error-prone DNA repair would explain the biphasic linear-quadratic dose-response pattern of UV-induced reversion frequencies normally found in excision-proficient stationary phase yeast. Liquid holding experiments provided further evidence (Eckardt *et al.*, 1978). If UV-irradiated yeast cells were held under non-growth conditions for 3 days in the presence of cycloheximide inhibiting cytoplasmic protein synthesis before plating, the quadratic component of the mutation frequency curve was largely abolished and a nearly linear dose dependence was found.

Here we report experiments where the temperature-dependent response of a repair mutant was used for further characterization of mutagenic DNA repair in yeast. A thermoconditional allele of the repair mutant *rev2* alias *rad5* (*rad5–8* = *rev2*^{ts}) has been used which shows enhanced mutagen sensitivity as well as reduced mutagenicity at 36°C (the restrictive temperature) as compared to 23°C (the permissive temperature) (Siede & Brendel, 1982). Due to the locus-specific activity of the *REV2* gene product (for review on mutagenesis in yeast c.f. Lemontt, 1980) the reduction of induced mutation frequencies at 36°C is restricted to certain ochre alleles.

The time dependent activity of the *rev2*^{ts} coded function can be detected by incubating UV-irradiated cells in growth medium at 23°C before plating and temperature shift to 36°C: survival and mutation frequencies increase during incubation at permissive conditions until the enhanced temperature causes cessation of the function of the thermosensitive protein.

Experiments are reported where this *rev2*^{ts} mediated repair process was modified by inhibiting protein synthesis with cycloheximide. Furthermore, the dose-dependent patterns of reversion frequencies in this mutant at 23°C and 36°C were

investigated. These results will be discussed regarding the possible UV inducibility of the *REV2* coded component of error-prone repair.

Materials and methods

The haploid strain of *Saccharomyces cerevisiae* used in these studies has the following genotype:

WS8004/17α *rad5–8* (=*rev2*^{ts}) *ade2-1 his5-2*

arg4-17 lys

True locus-specific reversion of the ochre allele *his5-2* or *arg4-17* is indicated by unsuppressed expression of the third ochre allele *ade2-1* causing red pigmentation of the colonies.

Details of the experimental procedures have been described elsewhere (Siede *et al.*, 1981).

Results

The influence of cycloheximide on the *REV2* dependent recovery of survival during the incubation at permissive conditions was investigated in stationary and logarithmic phase cells (Figure 1). In case of stationary phase cells, the presence of 5 $\mu g\,ml^{-1}$ cycloheximide (also present for a 1 h preincubation period before UV irradiation) in the growth medium was sufficient to block post-irradiation protein synthesis (Siede *et al.*, 1983) and also to prevent any significant *REV2* dependent increase in survival (Figure 1a). At the same UV dose, the response of logarithmic phase cells was different (Figure 1b). 100 $\mu g\,ml^{-1}$ cycloheximide was required to inhibit protein synthesis in such cells (Siede *et al.*, 1983) but even under these conditions a residual *REV2* dependent repair activity remained clearly detectable. The preincubation period in buffer containing cycloheximide had no significant effect on the *REV2* dependent recovery with (or without) cycloheximide present after irradiation. It is also notable that at restrictive temperature logarithmic phase *rev2*^{ts} cells were more UV-sensitive than stationary phase cells while the reverse response is typical for the repair-competent wild-type.

Furthermore, we investigated whether the dependence on post-irradiation protein synthesis of the *rev2*^{ts} coded function also holds true with respect to UV-induced mutagenesis in stationary phase cells (Figure 2). Without cycloheximide, recovery of survival seemed to be only roughly correlated with the increase in mutation frequencies. Pre-mutational lesions at the *his5-2* or *arg4-17* locus were mostly fixed within about 6 h at 23°C, whereas the *REV2* dependent repair of pre-lethal UV damage proceeded for more than 9 h at 36°C. Furthermore, at 36°C, no significant increase

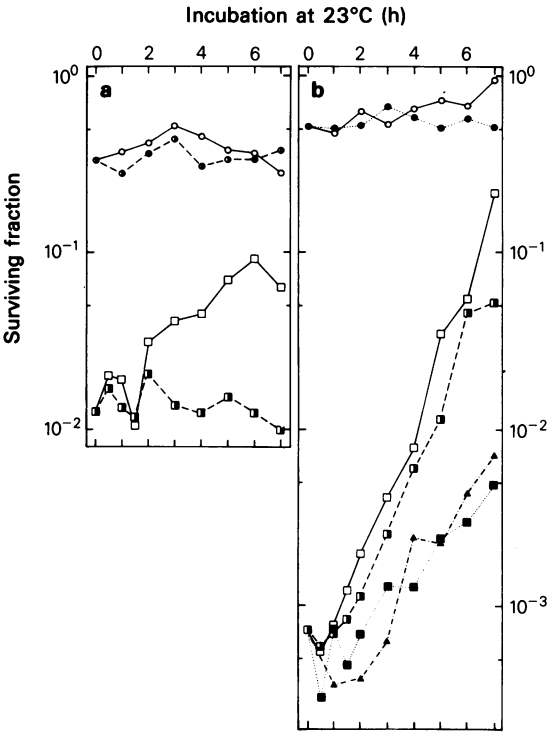

Figure 1 (a, b) Thermoconditional survival after treatment with 60 $J\,m^{-2}$ of *rev2*^{ts} cells in stationary (a) and logarithmic phase (b) as a function of incubation time at 23°C before plating. Different symbols stand for different temperatures after plating and different cycloheximide concentrations during incubation: plating temperatures were 23°C as control at full-time permissive conditions (○ ◑ ●) and 36°C (other symbols); no (○ □), 5 $\mu g\,ml^{-1}$ (◑ ◨) or 100 $\mu g\,ml^{-1}$ (● ■) cycloheximide were present during the incubation periods in liquid growth medium. Except for one case (▲) cells were incubated in phosphate buffer containing the cycloheximide 1 h before irradiation, also in case of the later-on cycloheximide-free control (no influence of different cycloheximide concentrations during preincubation).

in survival was found within the first 2 h of incubation whereas the enhancement of *arg4-17* reversion frequencies was evident.

At 40 $J\,m^{-2}$, in the presence of cycloheximide no significant *REV2* dependent increase in the reversion frequencies of the ochre alleles *his5-2* and *arg4-17* was detectable.

These results can be taken as indications for the UV inducibility of the *REV2* coded function in repair and mutagenesis. Therefore, we investigated whether the inactivation at 36°C of the *rev2*^{ts} coded function reduced the quadratic component of the dose-response pattern of reversion frequencies as

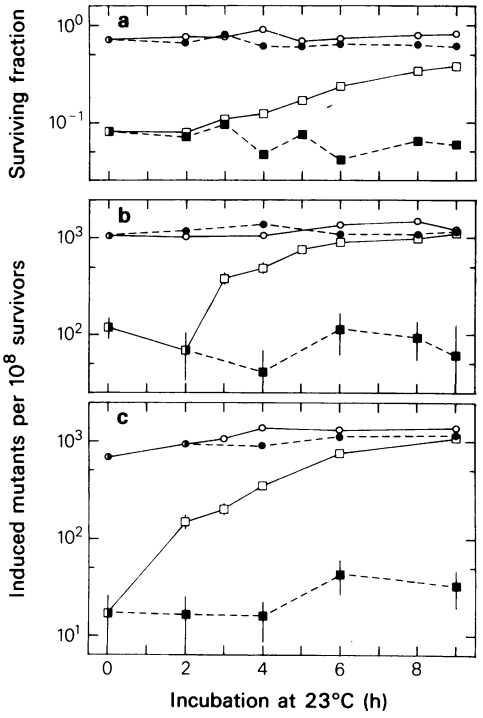

Figure 2 (a–c) Thermoconditional survival (a), locus-specific *his5-2* (b) and *arg4-17* (c) reversion after treatment of stationary phase *rev2*^{ts} cells with 40 J m^{-2} UV as a function of incubation time at 23°C before plating. Plating temperatures were 23°C (○ ●) and 36°C (□ ■), respectively; during incubation no (○ □) or 5 μg ml^{-1} (● ■) cycloheximide were present. The data shown are the mean of 3 experiments. Vertical lines represent standard deviations of the single experimental points.

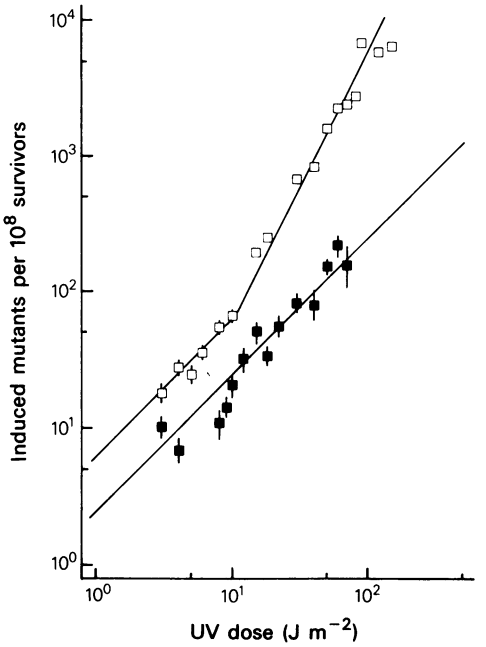

Figure 3 Dose dependence of thermoconditional UV-induced *his5-2* reversion frequencies in stationary-phase *rev2*^{ts} cells. Plating temperatures were 23°C (□) and 36°C (■). Data of several experiments were pooled. Lines were fitted by eye assuming a slope of 1 for the closed symbols and for the open symbols up to 10 J m^{-2}, and a slope of 2 for the open symbols at higher doses.

would be predicted by the model of Haynes & Eckardt (1979*b*) for the elimination of an UV-inducible mutational process. At permissive temperature, in the *rev2*^{ts} strain *his5-2* reversion frequencies followed the typical linear-quadratic induction kinetics found in repair-competent wild-type yeast whereas at 36°C no indications for a quadratic component could be shown (Figure 3). This temperature difference had no influence on the induction kinetics in a wild-type strain (Siede, unpublished data).

Discussion

The use of a thermoconditional mutant of mutagenic DNA repair in yeast allows the analysis of the process of UV-induced locus-specific reverse mutation by measuring relevant biological endpoints, although this process is almost inaccessible biochemically.

In stationary phase cells of a *rev2*^{ts} strain the *REV2* dependent recovery of survival and mutation frequencies can be abolished by inhibiting cytoplasmic protein synthesis with cycloheximide. This indicates that the *REV2* dependent process is dependent on post-irradiation protein synthesis which might imply that one or more factors newly synthesized after irradiation are required for the action of the *REV2* coded function. Hence an important condition for a model of mutagenesis including inducible components is fulfilled in the case of stationary phase cells.

It is a particular advantage of the mutant used that the dose-response pattern can be studied with reasonable accuracy since induced mutation is not completely absent at restrictive conditions. Whereas at 23°C linear-quadratic kinetics for reversion of ochre alleles is found in stationary phase *rev2*^{ts} cells, at 36°C the dose response curve is linear. This is taken as a further indication for an UV-inducible mutational process being inactive at 36°C. The temperature shift in this mutant has virtually the

same effect as liquid-holding with cycloheximide in the wild type (Eckardt *et al.*, 1978). The fact that, within the dose range of linear mutation induction, the reversion frequencies at 36°C are somewhat lower as compared to 23°C might be an indication for a partial involvement of the *REV2* dependent step in the constitutive (or dose-independent) component of mutagenesis. It should be noted that in the incubation experiment shown in Figure 2 an UV dose has been used which is probably too high to detect such a constitutive activity as expected from the mutation kinetics.

The strict dependence of the *REV2* coded function on post-irradiation protein synthesis in stationary phase cells does not hold true for logarithmic phase cells where a considerable, though not full activity of this process was found even in the presence of high concentrations of cycloheximide ($100\,\mu g\,ml^{-1}$ as compared to $5\,\mu g\,ml^{-1}$ for stationary phase cells). It seems unlikely that this effect is due to replication going on without protein synthesis in the already initiated cells. First, there is now good evidence (from pedigree studies and sector analysis: James & Kilbey, 1978; Eckardt *et al.*, 1980) that the mutational process in yeast acts mostly prereplicatively in excision-proficient cells. Second the *REV2* dependent recovery under inhibition of protein synthesis is not accelerated if the preincubation period of 1 h in buffer containing cycloheximide prior to irradiation is omitted. This preincubation should lead to termination of replication at least in a part of the initiated cells. However, preincubation has no influence which also implies a low turnover rate of the level of $rev2^{ts}$ coded protein in logarithmic phase cells.

In summary, the data shown can be best explained by assuming the UV inducibility of the *REV2* dependent process in stationary phase cells. At least for reversion of the *his5-2* allele, this pathway represents the main inducible mutational function. In logarithmic phase cells this process is partly turned on even without previous UV irradiation possibly because the *REV2* dependent process also plays a role in replication.

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References

ECKARDT, F., MOUSTACCHI, E. & HAYNES, R.H. (1978). On the inducibility of error-prone repair in yeast. In *ICN-UCLA Symposia on Molecular and Cellular Biology*, (Eds. Hanawalt *et al.*) New York: Academic Press.

ECKARDT, F., TEH, S.J. & HAYNES, R.H. (1980). Heteroduplex repair as an intermediate step of UV mutagenesis in yeast. *Genetics*, **95**, 63.

HAYNES, R.H. & ECKARDT, F. (1979*a*). Analysis of dose-response patterns in mutation research. *Can. J. Genet. Cytol.*, **21**, 277.

HAYNES, R.H., ECKARDT, F. (1979*b*). Complexity of DNA repair in a simple eucaryote. In *Proc. 6th Int. Congress of Radiation Research*, (Eds. Okada *et al.*) Tokyo: Toppan Printing Co., p. 454.

JAMES, A.P. & KILBEY, B.J. (1978). The timing of UV mutagenesis in yeast: a pedigree analysis of induced recessive mutation. *Genetics*, **87**, 237.

LAWRENCE, C.W. & CHRISTENSEN, R.B. (1982). The mechanism of untargeted mutagenesis in UV-irradiated yeast. *Mol. Gen. Genet.*, **186**, 1.

LEMONTT, J.F. (1980). Genetic and physiological factors affecting repair and mutagenesis in yeast. In *DNA Repair and Mutagenesis in Eucaryotes*, (Eds. Generoso *et al.*) New York: Plenum Press.

SCHENDEL, P.F. (1981). Inducible repair systems and their implications for toxicology. *CRC Crit. Rev. Toxicol.* **8**, 311.

SIEDE, W. & BRENDEL, M. (1982). Mutant gene $snm2\text{-}1^{ts}$, conferring thermoconditional mutagen sensitivity in *Saccharomyces cerevisiae*, is allelic with *RAD5*. *Curr. Genet.*, **5**, 93.

SIEDE, W., ECKARDT, F. & BRENDEL, M. (1983). Analysis of mutagenic DNA repair in a thermoconditional repair mutant of *Saccharomyces cerevisiae*. *Mol. Gen. Genet.*, **190**, 406.