The Mechanism of Untargeted Mutagenesis in UV-Irradiated Yeast

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Summary. The SOS error-prone repair hypothesis proposes that untargeted and targeted mutations in *E. coli* both result from the inhibition of polymerase functions that normally maintain fidelity, and that this is a necessary precondition for translesion synthesis. Using mating experiments with excision deficient strains of Bakers' yeast, *Saccharomyces cerevisiae*, we find that up to 40% of *cyc1-91* revertants induced by UV are untargeted, showing that a reduction in fidelity is also found in irradiated cells of this organism. We are, however, unable to detect the induction or activation of any diffusible factor capable of inhibiting fidelity, and therefore suggest that untargeted and targeted mutations are the consequence of largely different processes.

We propose that these observations are best explained in terms of a *limited fidelity* model. Untargeted mutations are thought to result from the limited capacity of processes which normally maintain fidelity, which are active during replication on both irradiated and unirradiated templates. Even moderate UV fluences saturate this capacity, leading to competition for the limited resource. Targeted mutations are believed to result from the limited, though far from negligible, capacity of lesions like pyrimidine dimers to form Watson-Crick base pairs.

Introduction

The observation that untargeted mutations (Witkin and Werumdsen 1978) can be induced in the progeny of untreated lambda phage grown in UV-irradiated *E. coli* (Jacob 1954; Devoret 1965; Ichikawa-Ryo and Kondo 1975) has been an important element in the construction of the SOS error-prone repair hypothesis (Witkin 1976; Boiteux et al. 1978), and lack of comparable information has prevented a critical examination of this model in Bakers' yeast, *Saccharomyces cerevisiae*. According to this hypothesis, untargeted and targeted mutagenesis are different consequences of the same condition induced in UV-irradiated cells; both result from the suppression of a DNA polymerase function that normally enhances fidelity. Low fidelity is required for translesion synthesis because pyrimidine dimers and other similar lesions are believed to be non-instructive (Witkin 1976), that is incapable of directing base selection during DNA chain elongation. Resumption of elongation therefore requires an undirected, more or less random, insertion of nucleotides at these sites, leading to a high probability of targeted mutation. Failure to replicate accurately at other sites gives a low probability of untargeted mutation. This method of translesion synthesis is an essential tolerance mechanism because lesions in excision repair templates or at sites of “overlapping gaps” in daughter chromosomes cannot be repaired or tolerated in any other way. Suppressing fidelity in these circumstances is therefore an adaptive process, in the evolutionary sense, and the resulting mutations the unavoidable though presumably small price paid for survival. This price is minimized by the inducible and transient nature of SOS repair.

Direct biochemical support for this model has not yet been obtained from any organism, and the indirect evidence from yeast is inconclusive or conflicting. Genetic analysis indicates some kind of relationship between UV mutagenesis and repair or recovery (Lemontt 1971; Lawrence et al. 1974; Lawrence and Christensen 1976), but also shows that induced base-pair substitution is far from random (Lawrence and Christensen 1979; Lawrence 1981). These data, together with what appears to be a low yield of mutations per dimer in excision deficient strains (Lawrence 1981), question whether these lesions are truly non-instructive. If dimerized pyrimidines do in fact retain a limited capacity to direct the insertion of the correct purines, a reduction in polymerase accuracy may not be required for translesion synthesis, though it could occur for other reasons. It is therefore of interest to determine whether untargeted mutagenesis occurs in yeast, and if so, by what mechanism.

We have examined these questions in mating experiments that are conceptually similar to the recombination experiments of Fabre and Roman (1977). Preliminary results from this work have been reported briefly elsewhere (Lawrence and Christensen 1980a, b; Lawrence et al. 1982). We find that although a high proportion, up to 40% or more, of the mutations studied were untargeted, there was nevertheless no evidence for the induced synthesis of any factor capable of reducing the fidelity of replication. We suggest that these and other data are best explained in terms of a *limited fidelity* model for UV mutagenesis, in which untargeted and targeted mutations are thought to occur in largely independent ways. It is proposed that untargeted mutations result from the limited capacity of the processes in yeast cells that are responsible for maintaining fidelity, and that these mechanisms are used during replication on both irradiated and unirradiated templates. Even moderate UV fluences are believed to saturate the capacity of these processes, leading to competition for the limited resource. Targeted mutations, on the other hand, are believed to result from the limited, though far from negligible, capacity of lesions to form Watson-Crick base-pairs, a process more similar to misreplication than misrepair. If this is correct, reduced fidelity in UV-irradiated cells is not adaptive, and mutations occur because it is physiologically infeasible or impossible to improve fidelity.

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Strain Construction. Meiotic segregants containing rad mutations were identified by spot tests for UV or gamma ray sensitivity (Lawrence and Christensen 1976) and sometimes by failure to complement tester strains. The ren3-1 allele (Lemoni 1971) was detected by the reduction it causes in arg4-17 reversion induced by UV (Lawrence and Christensen 1979b), and karl-1 mutants (Conde and Fink 1976) by low yield of prototrophic diploids in spot tests for mating type. Segregants carrying cycl alleles were identified by spectroscopic examination of whole cells at 190°C (Sherman and Slonimski 1964). Cycl-91 is an identical repeat of cycl-9 and contains an ochre triplet at the second codon of the structural gene (Sherman et al. 1975). The proline missense allele cycl-152 is an identical repeat of cycl-115 (F. Sherman, personal communication) in which the leucine 14 codon is replaced by CCT (Sherman and Stewart 1978). The multistate, non-reverting cycl-363 allele (Sherman et al. 1975) has end points at least 130 base-pairs beyond the 3' end of the structural gene (K.S. Zaret and F. Sherman, personal communication) and at least 21 base-pairs before the 5' end (Sherman and Stewart 1978). It is similar to the cycl-1 mutation, which is a physical deletion (Shalit et al. 1981), but unlike this allele does not extend into the RAD7 locus (Sherman et al. 1975). Cycl-363 has a distal end point between amino acid codon 16 and 18, and a proximal one before the initiation codon (Sherman et al. 1975). Spontaneous mutations to canavanine resistance were isolated in the two cycl-91 mutants used for the karl crosses, and shown to be can1 alleles by complementation. These strains were made p0 by overnight growth in medium containing ethidium bromide. In addition to mutations of the kinds described above, all pairs of strains used in mating experiments contained several complementing auxotrophic mutations to facilitate prototrophic selection of diploids.

Media. Composition of the various media and their uses were as described in previous papers (Lawrence et al. 1974; Lawrence and Christensen 1976; McKee and Lawrence 1979), which also give details of general methods, radiation sources and dosimetry. A major exception to these methods was the use of SG60 medium to estimate the viable titer of diploids in place of YPG, a change demanded by the need for selective growth of prototrophic diploids. SG60 contains 0.17% Difco yeast base w/o ammonium sulfate or amino acids, 0.5% ammonium sulfate, 3% glycerol, 1.5% agar plus 0.01% dextrose to stimulate growth, and any necessary nutrilites. When either exponential or stationary phase cells are irradiated in suspension, survival is identical on SG60, YPG or SD medium (unpublished data). Canavanine sulfate was used at a concentration of 60 mg/l in some media. In addition to the two reciprocally irradiated matings, control matings were also made. Mating cultures were shaken at 30°C in the dark for 4.5 to 5 h, washed and resuspended in 5.3 ml water, at a nominal concentration of about 2.3 x 10^6 cells (of all kinds)/ml. The actual cell concentration was greater by about 2 fold because haploids grow during the mating period. 0.2 ml of this suspension was spread on each of 20 SLY plates for CYC1 reversion and suitable dilutions on SG60 for diploid titer. Unirradiated samples of the unmated point mutant were also plated on these media. Although the mating suspensions were somewhat flocculant, even very brief sonication at this stage drastically reduced the yield of zygotes, and therefore they were mixed vigorously with a vortex mixer, rather than given this treatment. Cells were handled and plated under "gold" light and incubated in the dark to avoid photoreactivation. Reversion plates were scored after 10–14 days incubation at 30°C, and again a week later, at which time all putative cycl revertants were picked and grown for spectroscopic examination at 190°C (Sherman and Slonimski 1964).

Procedures for the karl experiments were similar except that cells were plated on media containing canavanine and the nutrilites required by the cycl-91 mutant. In some cases SLY without canavanine was used. Unmated cells of this type cannot grow on these non-fermentable media because they are p0 (lack mitochondrial DNA), while haploid progeny of the zygotes containing the deletion nucleus cannot grow because of auxotrophic requirements and canavanine sensitivity. Similarly, prolonged growth of the heterokaryons themselves is inhibited because the can1 mutations are recessive, and such cells are therefore sensitive to the drug. All putative cycl revertants were crossed with cycl-1 deletion strains for spectroscopic examination, and their requirements and mating type verified.

A number of preliminary experiments were carried out with exponential phase cells, harvested from 100 ml cultures that were inoculated from precultures. Apart from its general inconvenience, such a procedure was abandoned because the need for precultures and large volumes tended to give high spontaneous reversion frequencies, the induced reversion frequencies were low, and mating efficiency was no better than with early stationary phase cells. A few experiments were carried out using 40 Kards of 60Co gamma rays rather than UV, with aerated suspensions being exposed at a dose rate of 6–7 Krad min^-1.

The amount of residual growth on SLY medium was measured by scraping and washing cells from the plates either immediately after plating or after 1 or 2 days incubation at 30°C, followed by spreading 0.1 ml of suitable dilutions onto SD plates capable of supporting only the growth of diploids.

Results

Rationale

Untargeted mutations were detected, and their proportion in the total of induced events estimated, in mating experiments conceptually similar to the recombination experiments of Fabre and Roman (1977). The experimental plan entailed the irradiation of one or the other (or neither) of two haploid strains of opposite mating type, followed by mating and the detection of mutations among the diploid progeny. Haploid strains carrying the non-revertible deletion cycl-363 were crossed with haploids containing the highly revertible ochre allele, cycl-91, the mutant site of which is located within the deleted region (Sherman et al. 1975). Radiation-induced lesions can therefore be introduced either into the genome containing the revertible site