UV-induced inactivation and mutation-induction in a new two-component heterokaryon (59) homozygous for the excision-repair deficient mutant uvs-2

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Abstract

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UV-induced inactivation and mutation-induction in a new two-component heterokaryon (59) homozygous for the excision-repair deficient mutant UVS-2

To determine the effect of various mutations which confer sensitivity for UV inactivation and mutation-induction at the ad-3A and ad-3B loci, comparisons have been made between our standard auxotrophic strain 74-OR31-16A (al-2; Cot-1; pan-2) and various UV-sensitive derivatives bearing uvs-1, uvs-2, uvs-4, uvs-5, uvs-6, and uvr-1. The comparative sensitivities of these 8 strains to inactivation and the induction of ad-3 mutants are being published elsewhere (de Serres; de Serres et al.; Inoue et al. -- Mutation Res. in press). The excision-repair deficient strain containing uvs-2 was more sensitive to radiation (UV, x-rays, and \( \gamma \)-rays) and various chemical mutagens (MMM, ICR-170 and 4-NQO) than the auxotrophic strain both with regard to inactivation and the induction of ad-3A and ad-3B mutations.

In heterokaryon 12 which consists of the following two strains: 74-OR60-29A (his-2 ad-3A ad-36 nit-2; ad-2; inl) and 74-OR31-16A (al-2; cot-1; pan-2), both point mutations (ad-3A\(^{R}\) and ad-3B\(^{R}\)) and multilocus deletions (ad-3A\(^{R}\), ad-3B\(^{R}\)) and (ad-3A, ad-3B\(^{R}\)) in the ad-3 region can be recovered (de Serres and Millmy 1971, Chemical Mutagens 2:311). To determine the effect of uvs-2 on the recovery of both of these classes of mutations, a new heterokaryon (59) was constructed that has the same genotype as heterokaryon 12 except that it is homozygous for uvs-2.

The strain numbers and genotypes of this new heterokaryon are as follows: 74-OR276-40A (his-2 ad-3A ad-36 nit-2; ad-2; uvs-2; inl) and 74-OR244-3A (al-2; cot-1; uvs-2; pan-2). The sensitivity of this new heterokaryon to UV was compared with that of heterokaryon 12 for inactivation of the heterokaryotic fraction as well as for the induction of mutations in the ad-3 region. This experiment showed that, whereas heterokaryon 12 has a multihit survival curve with a broad shoulder (de Serres and Kilbey 1971, Mutation Res. 12:221), heterokaryon 59 has greater sensitivity to UV-induced inactivation with a simple exponential survival curve. A comparison of the UV exposures required to give 50% survival, for example, gives a relative biological effect (RBE) for inactivation of 46.7. A comparison of the dose-response curves for the overall induction of ad-3 mutants shows no difference between the slopes of the curves; both curves increase as the square of UV exposure. In a comparison of the UV exposures required to give comparable forward-mutation frequencies (in the range of 10^-100 x 10^-5 survivors), the higher yield of ad-3 mutations in heterokaryon 59 results in an RBE of 4.0. Genetic analysis of ad-3 mutants induced in heterokaryon 59 will reveal whether the spectrum of ad-3 mutants is qualitatively different from that found in heterokaryon 12.

These experiments report the development of a new two-component heterokaryon that is excision-repair deficient and demonstrates its expected sensitivity to both inactivation and the induction of specific locus mutations in the ad-3 region. -- Office of the Associate Director for Genetics, National Institute of Environmental Health Sciences, P. O. Box 12233, Research Triangle Park, North Carolina 27709


Comparison of nuclear DNA with whole cell DNA isolated from Neurospora crassa.

Huang 1972 Biochem Genetics 6:41). Studies on mycelial whole cell DNA (Outta 1976 Mycolagia 68:388) of numerous Neurospora strains and species exhibited two fractions: a major high G:C (52-56 G:C mol%) DNA fraction which comprised 75-80% percent of the total genome, and a minor low G:C (32-33 mol%) fraction comprising 20-25% percent of the genome. It was inferred that most of these low G:C DNA sequences were reiterated and could be partly mitochondrial and/or other non-nuclear origin. The small percentage of repeated sequences in nuclear DNA of Aspergillus could be due to lack of mitochondrial and/or other non-nuclear DNAs. We have thus compared nuclear and whole cell DNA isolated from N. crassa. Purified nuclei from conidial and mycelial cells were isolated by the procedure of Hautala et al. (1977, J. Bacteriol 130:704). DNA was isolated by a hydroxyapatite chromatography procedure described previously (1976 Mycolagia 68:388).

Unlabeled nuclear DNA and whole cell DNA were first characterized by analysis of hyperchromic shifts using a Gilford spectrophotometer (see Table 1). Whole cell DNA of both N. crassa conidial and the mycelial cells showed typically the two fractions mentioned before. Nuclear DNA from these cells contained very little, if any, of the low G:C minor fraction but was comprised almost entirely of the high G:C fraction. This observation suggested that almost all of the low G:C fraction (about 10-25% of total DNA) of N. crassa whole cell DNA was indeed non-nuclear.

Nuclear DNA from conidia was \(^{3}H\)-labeled by nick translation (Krumlauf and Marzluf 1978 Neurospora Newsletter 25:15) and sheared to 400 nucleotide piece size at 50,000 p.s.i. release pressure. These \(^{3}H\)-DNAs were denatured and allowed to reassociate to a Cot of 2.0 followed by SI nuclease treatment and fractionation with