Development of Mutagen-Sensitive Strains of Neurospora to Detect Specific Locus Mutations

by Frederick J. de Serres*

The host-mediated assay (1) was developed originally to provide an assay for the induction of point mutations in animals treated with potentially mutagenic compounds. Since there is no simple assay that can be made on cells that are a normal component of the animal for point mutations, these investigators introduced an organism in which such an assay could be made. *Salmonella* was introduced into the peritoneum of the host, incubated for about three hours and then withdrawn for genetic analysis. By using a histidine-requiring strain (G-46) (2), chemicals capable of producing base-pair substitutions could be shown to be genetically active. Additional strains have since been developed by Ames (3) which detect a wider range of genetic alterations, and the most recent tester set (4) is an even more sensitive detector of genetic activity than the original strains.

The enhanced sensitivity of the new strains is due in part to the incorporation of a repair-deficient mutation, which results in the conversion of a higher frequency of the genetic damage produced by mutagenic treatment into observed mutation. All of this development has, in effect, made available potent tools for the detection of specific types of genetic alterations. Such strains will be extremely useful in screening programs to detect genetic activity of environmental chemicals, and the simplicity of the tests should make it possible to test large numbers of compounds quickly and efficiently. In some cases, the simple demonstration of genetic activity in such screening tests may provide adequate evidence to reject a chemical for use and to search for non-mutagenic alternatives. In other cases, we will want to know the significance of the positive test data for man. To extrapolate to man and to try to predict the relative hazard of some genetically active chemical for man, we need to have additional information of quite a different type. We need to know, for example, whether the agent will cause chromosome aberrations, somatic recombination and gene mutations as they occur in man.

With regard to gene mutations, we need to have the type of information which can only be derived from forward-mutation experiments rather than reverse-mutation experiments. We need to know the kind of increase we might expect in the frequency of mutation of unaltered genes over the entire genome. The type of data needed to make this evaluation can be obtained only from forward-mutational assays that register as mutations any type of genetic damage that

*Mutagenesis Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, N. C. 27709.

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occurs throughout the gene rather than reverse-mutational assays at specific sites within the gene. Ideally the number of such genes assayed should be large so that the data are not biased by individual differences between genes in overall base composition or base sequence.

In Neurospora, it is possible to assay for forward-mutation at specific loci as well as over the entire genome by using a two-component heterokaryon (5). By assaying for forward-mutations in the ad-3 region, we can study the induction of mutations at specific loci and relate the frequency of these events to those that occur in the remainder of the genome. In man, gene mutations result from both point mutations and chromosome deletion; and both of these types of gene mutations can be studied with a two-component heterokaryon.

In our most recent work (6; Schupbach and de Serres, unpublished) we are trying to determine whether an approach similar to that used by Ames and co-workers will make our two-component heterokaryon a more sensitive indicator of genetic activity. We have obtained six different presumptive repair-deficient strains of Neurospora to study the effect of each on the induction of point mutations at the ad-3A and ad-3B loci. The question is simply, do any of these repair-deficiencies affect the frequency of mutations at these two loci recovered after mutagenic treatment? Will we be able to identify repair-deficient strains of Neurospora in which mutation-induction is markedly enhanced as it is in Salmonella?

The strains and their sources are listed in Table 1. Each strain was back-crossed to the Oak Ridge wild-type strain to make all strains essentially isogenic. A comparison was then made of the induction of mutations in the six mutant strains and the wild type strain after ultraviolet irradiation and γ-irradiation. The results of these experiments are summarized in Table 2. As shown in Table 2, all possible results were found with the strains studied.

Two strains, upr-1 and uvs-2, are more sensitive to mutation-induction after both ultraviolet and γ-ray treatments; uvs-6 is more sensitive than wild type only after γ-irradiation. The remaining strains, uvs-3, uvs-4 and uvs-5, show either the same sensitivity as wild type or markedly reduced sensitivity. Insofar as the genetic analysis of the ad-3 mutants recovered has shown, we know that the same effect has been found on both the ad-3A and ad-3B locus in each strain (de Serres, unpublished). However, it will not be until the appropriate two-component heterokaryons have been constructed that we will know the effects of each of these three mutations on mutation-induction over the entire genome.

Since we have obtained dose-response curves for mutation induction in both the ultraviolet and γ-ray experiments, we can calculate the dose-reduction factors obtained with each strain. These values are given in

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Table 1. Origin of wild type and presumptive repair-deficient strains of Neurospora crassa.

| Mutant | Investigator |
|--------|--------------|
| uvs-2  | D. R. Stadler, University of Washington |
| uvs-3  | A. L. Schroeder, Washington State University |
| uvs-4  | A. L. Schroeder, Washington State University |
| uvs-5  | A. L. Schroeder, Washington State University |
| uvs-6  | A. L. Schroeder, Washington State University |
| upr-1  | R. W. Tuveson, University of Illinois |

Table 2. Comparison of presumptive repair-deficient strains of Neurospora crassa with wild-type after ultraviolet or γ-irradiation. *

|          | Ultraviolet | γ-Rays |
|----------|-------------|--------|
|          | Survival    | Mutation | Survival | Mutation |
| uvs-2    | s           | s       | s        | s        |
| uvs-3    | s           | r       | s        | r (?)    |
| uvs-4    | s           | r       | o        | o        |
| uvs-5    | s           | r       | o        | o        |
| uvs-6    | s           | o       | s        | s        |
| upr-1    | s           | s       | s        | s        |

* Legend: s = more sensitive; r = more resistant; o = similar to wild type.
Table 3. What these numbers mean, in effect, can be illustrated with upr–1. After mutagenic treatment, the forward-mutation frequency found in the wild-type strain was found at a 5.5-fold lower dose in the upr–1 strain after ultraviolet and a 3.3-fold lower dose after γ-irradiation.

Table 3. Dose-reduction factors for mutation-induction at the ad-3A and ad-3B loci in presumptive repair-deficient strains.

| Strain | Ultraviolet | γ-Rays |
|--------|-------------|--------|
| upr–1  | 5.5         | 3.3    |
| uvs–2  | 5.0         | 3.2    |
| uvs–6  | 0.0         | 4.0    |

These data show quite convincingly that the process of mutation-induction at specific loci is under genetic control in Neurospora and that it is possible to obtain strains that are more sensitive detectors of genetic damage than standard wild-type strains. We intend to determine whether these strains are also more sensitive to chemical mutagens, with the hope that we can develop a strain that will be more sensitive than our standard wild-type strain to chronic levels of exposure and to weak mutagens.

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