Intragenic Mapping of Chemically Induced ad-7 Mutants of Schizosaccharomyces pombe

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Thirty adenine-requiring ad-7 mutants of Schizosaccharomyces pombe, induced by ethylmethanesulfonate (EMS), methylmethanesulfonate, and hydroxylamine and exhibiting low spontaneous reversion frequencies, were located by intragenic recombination analysis. Their identification as ad-7 mutants was assessed in relation to two previously mapped ad-7 mutants. Each mutant was found to occupy a distinct mutational site; the smallest recombination fraction observed between the two closest mutational sites was of the order of $0.5 \times 10^{-4}$.

The extensive studies of Leupold and Gutz (2–7) on the genetic fine structure of the ad-7 locus of Schizosaccharomyces pombe Lindner have shown that many mutational sites within the locus may be distinguished by intragenic recombination analysis. The ad-7 locus may be considered as a cistron on the basis of the lack of interallelic complementation; presumably, the enzyme involved has, therefore, a monomeric structure. It is probable that this enzyme controls one of the steps in the conversion of 5-aminimidazole ribotide to 5-amino-4-imidazole-N-succynylcarboxamide ribotide in the pathway of purine biosynthesis.

Previous intragenic analyses within the ad-7 locus have been performed with mutants induced by ultraviolet (UV) light (4–6), X rays (2, 3), and nitrous acid (3).

In the present study, a group of 30 adenine-requiring mutants, induced by ethylmethanesulfonate (EMS), methylmethanesulfonate (MMS), and hydroxylamine (HA), are analyzed.

MATERIALS AND METHODS

All mutants used were obtained by forward mutation from the wild-type 972 $h^+$ strain of S. pombe; conditions for mutagenic treatment were those reported by Loprieno (8) and Guglielminetti et al. (Mutation Res., in press).

Among 66 HA-induced, 59 EMS-induced, and 81 MMS-induced ad-7 mutants, 30 (with an absolute requirement for adenine) were chosen (11 HA, 7 EMS, 13 MMS) for recombination analysis, on the basis of their low rate of spontaneous reversion to adenine independence.

Each mutant was crossed with the wild-type strain 975 $h^+$. From these crosses, the mutants were isolated as $h^+$; the $h^-$ strains were reisolated.

Spontaneous reversion to adenine independence.
the mutants analyzed and (lower line) three of the mutants previously analyzed by Leupold (3, 6), namely, UV-273, UV-633, and UV-50. In the present analysis, five mutants (EMS-198, MMS-33, EMS-424, HA-347, and HA-192) were located in the segment UV 273 — UV 633, in which no mutants had been found previously (3, 6).

**DISCUSSION**

Several ad-7 mutants of S. pombe, induced by EMS, MMS, and HA, were scored on the basis of the accumulation of a red pigment (7); they were distinguished from the ad-6 mutants, which also form the red pigment, by genetic analysis (8). As the lowest recombination frequency so far observed for ad-7 mutants of S. pombe is of the order of $10^{-4}$ (6), some 30 ad-7 mutants induced by EMS, MMS, and HA were chosen because the spontaneous rate of their reversion to adenine independence was of the order of $10^{-6}$ or lower for both mating types, $h^+$ and $h^-$. They were employed in the present study for studies on intragenic recombination within the ad-7 locus.

The 30 mutants analyzed represent 30 different mutational sites along the locus. Previously studied mutants, induced by UV light, X rays, and nitrous acid, were found to be distributed over the whole length of the locus (47 mutational sites) with the exception of the segment of the left arm between the mutants UV-273 and UV-633. In the present study, 5 of 30 mutants were located in this segment (Fig. 2).

In general, additivity of the recombination frequencies along the map was observed; the smallest unit of recombination found for the pairs MMS-36/EMS-632 and HA-322/MMS-669 was $0.5 \times 10^{-4}$ (average of all crosses done). This indicates a total number of about 3,000 mutational sites for the ad-7 locus of S. pombe; this value is higher than that reported previously which was based on 940 mutational sites (1).

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FIG. 1. Linkage relationships of 30 ad-7 mutants of Schizosaccharomyces pombe induced by ethylmethanesulfonate (EMS), hydroxylamine (HA), and methylmethanesulfonate (MMS). Map distances are presented in terms of prototrophs per 10^6 viable ascospores plated.

\[ p = 0.1\% \]

FIG. 2. Genetic map of the ad-7 locus of Schizosaccharomyces pombe. The upper line shows the location of the mutants analyzed according to recombination frequency; three mutants previously reported (3,6) are shown on the lower line. Previously, no mutants were found between UV-273 and UV-633, whereas in the present analysis five mutants were located in this region.

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LITERATURE CITED
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