AN APPARENT CONNECTION BETWEEN HISTIDINE, RECOMBINATION, AND REPAIR IN NEUROSPORA

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ABSTRACT

Two mutants of Neurospora crassa, uvs-3 and mei-3, share four properties—UV sensitivity, inhibition by histidine, meiotic blockage when homozygous, and increased duplication instability (due to mitotic crossing over; to deletions or to both). The present paper shows that a third nonallelic mutant, uvs-6, exhibits the same four properties. Also, the instability of duplications in the absence of any UV-sensitive mutant is increased by the presence of histidine in the growth medium.

Several years ago, it was found that histidine strongly inhibits the growth of three ultraviolet-sensitive mutants of Neurospora crassa (uvs-3, uvs-4, uvs-5) at a concentration that only slightly inhibits wild type (SCHROEDER 1970). To the best of our knowledge, there has been no report that histidine inhibits UV-sensitive mutants in any other organism.

Among other properties of uvs-3, it was found that meiosis is blocked when uvs-3 is homozygous and that uvs-3 increases the instability of duplications (SCHROEDER 1970). The duplication-instability test that was used measures mitotic crossovers and deletions (NEWMEYER and GALEAZZI 1977, 1978). These properties of uvs-3 have been found among UV-sensitive mutants of other organisms. Increased spontaneous mitotic crossing over, sometimes accompanied by meiotic impairment in homozygous crosses, has been reported among UV-sensitive mutants of Ustilago (HOLLIDAY et al. 1976), Saccharomyces (BORAM and ROMAN 1976), and Aspergillus (JANSEN 1970; FORTUIN 1971; SHANFIELD and KÄPER 1969). In E. coli, the UV-sensitive polA mutant produces a high frequency of spontaneous deletions (COUKELL and YANOFSKY 1970).

In the accompanying paper (NEWMEYER and GALEAZZI 1978), a second Neurospora mutant, mei-3, is described, which has the same four properties.

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noted above for *uvs-3*, that is, it is UV sensitive, is inhibited by histidine, blocks meiosis when homozygous, and increases the instability of duplications.

In the present paper, we report that a third mutant, *uvs-6* (SCHROEDER, DE SERRES and SCHUPBACH 1972), which is not allelic to either *uvs-3* (SCHROEDER 1970) or *mei-3* (NEWMEYER and GALEAZZI 1978), also shares the same four properties, including the sensitivity to histidine. We also show that in the absence of any UV-sensitive mutant, and without irradiation or any other mutagenic treatment, the presence of histidine in the growth medium markedly increases the instability of duplications. Both effects of histidine were discovered in the course of using histidine-requiring mutants as markers. The mechanism is not known. However, these findings strongly suggest that histidine, or a metabolite of histidine, in some way influences repair and/or mitotic recombination in Neurospora.

**MATERIALS AND METHODS**

Routine media and genetic methods were described by PERKINS et al. (1969). Histidine was added at 0.5 mg/ml unless otherwise noted. Isolates were routinely grown at 25° or room temperature, including inocula to be used in high temperature tests.

All stocks used were as described in NEWMEYER and GALEAZZI (1978), except for *UVS-2* (STADLER and SMITH 1968).

Duplication instability was measured by two methods—escape speed and fertility. Both are summarized briefly in RESULTS. The escape-speed method for measuring instability of inhibited duplications was as described in NEWMEYER and GALEAZZI (1978), except that the criterion of escape in Figure 1 was the production of aerial hyphae, rather than the production of surface or subterranean hyphae with normal morphology. The latter criterion, used in Figure 2, gives a somewhat earlier escape time. The fertility method for measuring instability of non-inhibited duplications was as described in NEWMEYER and GALEAZZI (1977, 1978).

Significance tests were done by the Chi-square method, using a $2 \times 2$ contingency table.

Isolates were scored for sensitivity to histidine at 38.5° on slants in 12 x 75 mm tubes. Tubes were inoculated at a single point on the surface of the slant with a very small amount of dry conidia on a fine-gauge inoculating needle. Using a larger or scattered inoculum, or inoculum containing mycelia, gives less clear-cut tests.

Routine testing of isolates for UV sensitivity was done by spot tests, by the method of STADLER and SMITH (1968).

**RESULTS**

*uvs-6*: Crosses heterozygous for *uvs-6* are fertile, regardless of whether *uvs-6* is the male (conidial) or female (protoperithecial) parent, but crosses homozygous for *uvs-6* are barren, producing many perithecia but few or no ascospores. Cytological observations by RAJU and PERKINS (1978) indicate that crosses homozygous for *uvs-6* are arrested during crozier development before nuclear fusion.

The effect of histidine on growth of *uvs-6* was determined on minimal slants with and without histidine, with a tiny central inoculum of dry conidia. At 25°, histidine makes *uvs-6* grow very slowly but does not alter its nearly wild-type morphology. At 38.5°, histidine makes *uvs-6* grow as a small weak colonial; after 48 hours growth the colony diameter is 3 to 7 mm, of which only the central
1 to 3 mm is dense enough to be visible to the naked eye without holding the tube up to a light. At 38.5°, histidine also makes mei-3 and uvs-3 grow colonially; the mei-3 colonies are almost as small as the uvs-6 colonies, while the uvs-3 colonies are somewhat larger. At 38.5°, in the absence of histidine, all three mutants grow with wild-type morphology. Histidine causes a small reduction even in the growth rate of wild type, but the effect is very much less than the effect on the mutants; wild type gives vigorous noncolonial growth with abundant aerial mycelia after 48 hours on histidine at 38.5°. The difference between mutants and wild type under these conditions is so striking that it constitutes by far the easiest method of scoring isolates for the presence of uvs-6 or mei-3. (It is not a useful method for scoring uvs-3, because the high spontaneous mutation rate of this mutant makes it produce frequent slow-growing or aconidial progeny, which causes confusion.)

L-histidine HCl has routinely been used, at a concentration of 0.5 mg/ml. However, uvs-6 at 38.5°, is markedly inhibited by as little as 0.05 mg/ml L-histidine HCl, and also by D-histidine HCl or L-histidine free base, both tested at 0.5 mg/ml.

To test whether the histidine inhibition and the production of barren perithecia in crosses are due to uvs-6 rather than to some other factor in the stocks, 28 uvs-6 and 21 uvs-6+ ascospores were isolated from a cross of wild type × uvs-6. The resulting cultures were tested for histidine inhibition at 38.5°, and also for production of fertile vs. barren perithecia in crosses to uvs-6 A and uvs-6 a testers. In all cases, the histidine inhibition and the production of barren perithecia stayed with the UV sensitivity.

The effect of uvs-6 on duplication instability was measured by scoring the escape speed of inhibited duplications. Escape can occur by mitotic crossing over (homozygosis) or deletion (Newmeyer and Galeazzi 1977). The method of testing for escape is described by Newmeyer and Galeazzi (1978). Briefly, uvs-6 was crossed to the pericentric inversion In(II→IR)H4250, and duplication progeny were identified by their very abnormal morphology and inhibited growth; these abnormalities are caused by heterozygosity for the mating-type locus in the duplicated region. The duplications were examined daily to see when they escaped from the inhibition. After escape, they were scored for uvs-6. The results are shown in Figure 1. It is clear that most duplications that carried uvs-6 escaped decidedly faster than most duplications that were wild type for UV sensitivity. This indicates that uvs-6, or a neighboring gene, markedly increases duplication instability. Among many other genetic stocks that have been tested by this system, only uvs-3 and mei-3 have caused a comparable increase in duplication instability. These mutants both share other properties with uvs-6, as described above. In the case of mei-3 it has been shown that the increased instability is due to the mei-3 gene itself. Thus, in the present case we have assumed that the increased instability is due to uvs-6 itself, and not to a linked gene.

The effect of histidine on duplication instability: Use of the unlinked tol mutation made it possible to test this in two ways. The cross In(II→IR)H4250 tol × wild type was made on synthetic cross medium without histidine. (No uvs or
Figure 1.—Effect of uvs-6 on escape speed of inhibited duplications. The duplications were obtained from the cross \textit{In}(I\textit{L} \rightarrow I\textit{R})H4250 \times uvs-6 \textit{a} and grown on minimal medium. Seventy-three duplications carried \textit{uvs-6}, and 17 carried \textit{uvs-6+}. Most duplications carry \textit{uvs-6} because the \textit{uvs-6} locus is left of the center of the inversion loop. A diagram of the inversion, showing how it makes duplications, is given in Figure 2 of \textit{Newmeyer} and \textit{Galeazzi} (1978). \textit{P} for difference between \textit{uvs-6} and \textit{uvs-6+} is < 0.002. Escape speeds in this figure cannot be compared directly with those in Figure 2, because aerial rather than normal subterranean or surface growth was used as the criterion for escape. See Materials and Methods.

Meiotic mutants were present.) Ascospores from the same cross tube were isolated, some to minimal and some to minimal plus histidine. Such a cross produces two types of duplication. Those carrying \textit{tol+} are inhibited as described above. Those carrying \textit{tol} have a noninhibited phenotype, but can be identified by a minor morphological difference from wild type, and by their ability to cross with both mating types (\textit{Newmeyer} 1970). The effect of histidine on duplication instability was tested in both types of duplication.

The inhibited duplications (\textit{tol+}) were tested for duplication instability by the escape-speed method, as described above. The results are shown in Figure 2. In these tests histidine was at least as effective as \textit{mei-3} and \textit{uvs-6} in speeding the escape. The fast-escape effect seems clearly to be due to histidine or a histidine product and not to an uncontrolled variable, for the following reasons. The data are pooled results from three different batches of inhibited duplications on each medium, isolated at different times; all three batches isolated on minimal plus histidine gave primarily fast escapes, and all three batches isolated on minimal gave slower escapes. In two cases, the ascospores were isolated to the two media at the same time, heat-activated at the same time, and incubated side by side,
to minimize any environmental differences, e.g., in the CO₂ concentration or humidity in the incubator, or in exposure to light.

The effect of histidine on the instability of the noninhibited duplications (those carrying tol) was tested by the fertility method (Newmeyer and Galeazzi 1977, 1978). Noninhibited duplications are usually very barren; that is, they

**TABLE 1**

*Effect of histidine on fertility of noninhibited duplications*

<table>
<thead>
<tr>
<th>Medium</th>
<th>Fraction of crosses with &gt;1000 ascospores</th>
<th>Fraction of crosses with 20-1000 ascospores</th>
<th>Fraction of crosses with 0-20 ascospores</th>
<th>Total number of duplications tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal</td>
<td>0/48 (0%)</td>
<td>2/48 (4.2%)</td>
<td>46/48 (95.8%)</td>
<td>24</td>
</tr>
<tr>
<td>Minimal plus histidine</td>
<td>19/50 (38%)</td>
<td>19/50 (38%)</td>
<td>12/50 (24%)</td>
<td>25</td>
</tr>
</tbody>
</table>

Data are based on the number of ascospores shot in testcrosses by 21 days after fertilization. In each fraction the denominator is the total number of testcrosses; the numerator tells how many testcrosses produced the number of spores indicated in the heading. Each duplication was heterozygous for mating type and was crossed once to each of the two mating-type testers. Most of the fertile variants had the same mating-type allele as the normal sequence parent; this indicates that in most cases it was the duplicate segment in the transposed position that was lost (Newmeyer and Galeazzi 1978).
produce perithecia but few or no ascospores when testcrossed to normal sequence stocks. Fertile variants are produced when one duplicate segment is lost. These do not make visible sectors, but are detected because they produce fertile perithecia in the test crosses. The effect of histidine on the fertility of the noninhibited duplications is shown in Table 1. It is clear that many of the duplications grown on minimal plus histidine contained fertile variants, whereas, very few of the duplications grown on minimal contained detectable fertile variants.

**Histidine inhibition tests on uvs-2 and upr-1:** The UV-sensitive mutants *uvs-2* and *upr-1* were tested for inhibition by histidine at 38.5°C, using the same technique described for *uvs-6* above. Neither *uvs-2* nor *upr-1* was inhibited significantly more than wild type.

**DISCUSSION**

The results presented here, combined with those in previous papers, show that mutants at three different loci have four traits in common—UV sensitivity, inhibition by histidine, meiotic blockage when homozygous, and increased duplication instability. The results also show that histidine itself increases the instability of duplications in the absence of any UV-sensitive mutant. This has been demonstrated by two different methods, and is thus unlikely to be an artifact.

The escape-speed method of measuring duplication-instability detects mitotic crossovers and deletions. The fertility method detects deletions of a complete duplicate segment (Newmeyer and Galeazzi 1977). However, there are reasons to suspect that such deletions can result from mitotic crossovers occurring between homologous material located at chromosome tips and at the tip breakpoint of the inversion (Newmeyer and Galeazzi 1977). Thus, the effect of histidine on duplication instability may be due to an increase in either mitotic crossing over or deletion.

There are various general mechanisms that might explain the observed results. It could be that *uvs-3, uvs-6* and *mei-3*, when grown on minimal medium, contain an excess of some metabolite of histidine that causes duplication instability. If so, adding histidine to the wild type could make it behave like the mutants, thus accounting for its increased duplication instability. However, adding histidine to the mutants might produce a toxic concentration of the histidine product, thus explaining the growth inhibition. Similar reasoning would apply if exogenous histidine causes a deficit of something that is already in short supply in the mutants. Alternatively, histidine could inhibit a replication/repair pathway different from the one or ones blocked in the mutants, and the combination of two blocked pathways could cause poor replication or poor repair of spontaneous lesions, resulting in the observed growth inhibition. Or there might be a reaction that is inhibited by normal histidine levels in the mutants, but is inhibited only by high levels of histidine in the wild type.

Within this framework, some of the possible specific mechanisms are as follows. First, Penedyala and Wellman (1975) cite evidence suggesting that histidine represses adenine synthesis in Neurospora. If true, it is possible that
<table>
<thead>
<tr>
<th></th>
<th>uvs-2</th>
<th>uvs-3</th>
<th>uvs-4</th>
<th>uvs-5</th>
<th>uvs-6</th>
<th>upr-1</th>
<th>mel-3</th>
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<tr>
<td>Histidine sensitivity</td>
<td>Normal$$</td>
<td>Sensitive$</td>
<td>Sensitive$</td>
<td>Sensitive$</td>
<td>Sensitive$$</td>
<td>Normal$$</td>
<td>Sensitive†</td>
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<td>Fertility of</td>
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<td>Sterile or</td>
<td>Poor$</td>
<td>Barren$</td>
<td>Barren$</td>
<td>Normal†‡</td>
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<tr>
<td>Duplication instability</td>
<td>Normal‖</td>
<td>Increased$</td>
<td>Decreased*§</td>
<td>Decreased*§</td>
<td>Increased$$</td>
<td>—</td>
<td>Increased†‡</td>
</tr>
<tr>
<td>Dimer excision</td>
<td>None††</td>
<td>Lag followed by slow excision††</td>
<td>—</td>
<td>Normal†‡</td>
<td>Normal†‡</td>
<td>None††</td>
<td>—</td>
</tr>
</tbody>
</table>

* Possibly an artifact due to the very slow growth of these mutants.

References:
a reduced purine pool could result in less efficient replication and repair. Second, histidine could act as a chelating agent and remove a metal needed for catalyzing a reaction involved in recombination and/or repair. A third possibility derives from ideas of Parag and Parag (1975) and Holliday et al. (1976). They suggested that crossing over is repressed during vegetative growth, induced during meiosis, and also induced by radiation damage in a manner possibly analogous to the postulated induction of SOS repair in bacteria (Witkin 1976). A histidine product might stimulate this induction system.

The available information suggests that this histidine sensitivity and the other properties associated with it may occur only in mutants that are able to excise dimers. The relevant information is summarized in Table 2. (For additional properties of UV-sensitive mutants see Schroeder 1975.) Worthy and Epler (1973) showed that uvs-2 and upr-1 cannot excise dimers. We have found that uvs-2 and upr-1 are not inhibited by histidine. Furthermore, these mutants are fully fertile when homozygous (Stadler and Smith 1968; Tuveson 1972), and the one tested, uvs-2, has no effect on duplication instability (Schroeder 1974). Among the histidine-sensitive mutants, Worthy and Epler (1973) found that uvs-5 and uvs-6 excise dimers normally, and uvs-3 excises them after a lag; uvs-4 and mei-3 have not been tested.

The histidine-sensitive mutants uvs-4 and uvs-5 are perhaps anomalous. Both affect meiosis when homozygous (uvs-5 crosses are sterile, and uvs-4 crosses produce about 30% semilethal progeny, although meiotic recombination is normal (Schroeder 1970). Both mutants differ from the other histidine-sensitive uvs mutants in reducing, rather than enhancing, the escape speed of inhibited duplications, but this might be an artifact resulting from the very slow growth that is characteristic of these mutants. It is thus not clear whether or not there is a subclass of histidine-sensitive mutants that does not increase duplication instability.

We have seen no reports of histidine inhibiting UV-sensitive mutants in other organisms. Histidine-sensitive mutants have been described in Saccharomyces, but they were not tested for UV sensitivity (Meuris et al. 1967). There have also been reports (Luzzati et al. 1971; Henaut and Luzzati 1971) that histidine at 10 mg/l inhibits mitotic gene conversion in Saccharomyces. This occurs only in the presence of the ad-3 mutant, which requires both histidine and adenine because of the interconnection between adenine and histidine biosynthesis. It is not clear whether this phenomenon is independent of, or related to, our results in Neurospora.

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LITERATURE CITED


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