Influence of some environmental factors on recombination

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Influence of some environmental factors on recombination

Abstract
Influence of environmental factors on recombination

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communication, however, mhl low containing Ageing may of 0.44 M (strain E. coli). The use of heat-shock mixture fied mitochondria were suspended in AMT-sucrose, which is used to heat-shock purified by the procedure described elsewhere (Küntzel and Schäfer 1971 Nature New Biol. 231: 265). The purified mitochondria were suspended in AMT-sucrose, which is composed of 0.44 M sucrose containing 100 mM NH₄Cl, 10 mM MgCl₂ and 10 mM Tris-HCl (pH 7.5). The assay of OAT was formed according to Jenkins and Tsai (1970 Methods in Enzymology 17A: 281), except that 10 μg of Lubrol WX were included in the assay mixture (final vol-

As shown in Table 1, the OAT activity co-sedimented with mitochondria when it was centrifuged in AMT-sucrose at 12,000 x g for 10 min. In these sites, we have also observed that OAT in the Neurospora mitochondria is very unstable, in the sense that it loses more than 50% of the initial activity during overnight storage at 5°C. Whereas the present results are consistent with those obtained from mammalian systems, the question whether the OAT activity observed in the cytoplasmic fraction of Neurospora is authentic or is on artifact due to the leakage from mitochondria has not been examined.

We are grateful to Dr. Hans Küntzel for a generous supply of purified Neurospora mitochondria.


The effects on recombination of temperature of incubation of the conidial age, age of the crossing medium and heat-shock of conidia and protoperithecia were studied, using the Δ900 mutant of N. crassa. Media and general methods were those of Lomb (1966 Genet. Res. 7: 325).

Incubation of conidiating cultures at 8, 15, 17.5 or 37°C for six days prior to conidiation produced second division segregation frequency (SDSF) values not significantly different from control values (incubation at 25°C) even at the 20% level of probability. Since little cytoplasm is contributed by the conidia, any temperature-sensitive "effector compounds" (Lomb 1969 Genetics 63: 807) present in conidial cytoplasm would need to be effective in extremely low concentrations at meiosis, to be detected.

An investigation of the effect of ageing of conidial cultures on recombination indicated paternal age effects. Storage of conidial cultures at room temperature (about 20°C) for two months resulted in significantly lower SDSF values which were statistically homogeneous with each other but significantly different from the control value. Ageing may cause the following effects, amongst others: (1) Mutation of regulator genes present in conidial nuclei; this is possible as Auerbach (1959 Heredity 13: 414) estimated that the number of conidia carrying one class of mutation (recessive lethals) increased by 0.3% a week during storage. (2) Accumulation of recombination inhibitors with age; the passage of such inhibitors from conidia to protoperithecia could cause lowered SDSF's, but, like temperature-sensitive effectors, these inhibitors would need to be effective in extremely low concentrations at meiosis. A reduction in SDSF with increasing age of protoperithecia culture at conidiation was found by Lomb (1971 Genet. Res. 18: 255) but the protoperithecia do contribute nearly all the cytoplasm in the cross. The result of ageing conidia for two months at 25°C was unexpected in that it produced SDSF values homogeneous with the controls. This may be because the process of ageing differs with temperature.

The age of medium (old medium was used for both protoperithecial and conidial cultures) also seemed to affect recombination. The use of stale or freshly made but repeatedly autoclaved medium for crossing produced SDSF values significantly different from controls made using fresh medium. Ageing or repeated autoclaving probably caused alteration of the chemical composition of the medium by hydrolysis and decomposition. However, these results differ from those of Toth and Stadler (1964 Genetics 49: 577); using the Δ900 mutant of N. crassa, they observed that chemical alteration of the crossing medium resulted in significant increases in SDSF but caused no significant decrease.

Heat-shocking either protoperithecial or conidial cultures at 60°C for 60 seconds produced no significant difference in SDSF, as compared to control values. Heated sterile paraffin oil was used to heat-shock protoperithecial cultures. Conidia were heat-shocked in sterile distilled water at 60°C. Results from heat-shock experiments are in agreement with those of Mitchell (1957 Symp. on Chemical Basis of Heredity, p. 94, McElroy and Glass (edr.), Johns Hopkins Press, Baltimore).

Table 1. Localization of ornithine aminotransferase in mitochondria.

<table>
<thead>
<tr>
<th>Ept. No.</th>
<th>Preparation</th>
<th>OAT activity (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Purified mitochondria suspension (32 mg/ml AMT-sucrose)</td>
<td>232</td>
</tr>
<tr>
<td>II</td>
<td>Post-mitochondrial supernatant</td>
<td>69</td>
</tr>
<tr>
<td>III</td>
<td>Mitochondrial pellet, resuspended</td>
<td>212</td>
</tr>
</tbody>
</table>

* Same as Exp. 1, but mitochondria removed by centrifugation at 12,000 x g for 10 min. ** Mitochondrial pellet from Exp. II was resuspended in the same volume of AMT-sucrose.

The wild-type N. crassa (strain EN 5256) was used in there experiments. The mitochondria were isolated and purified by the procedure described elsewhere (Küntzel and Schäfer 1971 Nature New Biol. 231: 265). The purified mitochondria were suspended in AMT-sucrose, which is composed of 0.44 M sucrose containing 100 mM NH₄Cl, 10 mM MgCl₂ and 10 mM Tris-HCl (pH 7.5). The assay of OAT was formed according to Jenkins and Tsai (1970 Methods in Enzymology 17A: 281), except that 10 μg of Lubrol WX were included in the assay mixture (final vol-

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