The effect of the recombination-3 gene on hirtidine-5.

The recombination-3 gene described by D. G. Catcheside (1966 Austral. J. Biol. Sci. 19: 1039) controls the frequency of recombination between pairs of auxotrophic amination alleles in such a way that crosses bearing the dominant rec-3+ allele in one or both parents give frequencies of protrophic recombinants that are around 15 times lower than those in crosses homozygous for the recessive rec-3 allele. The recombination-3 gene does not control recombination at the histidine-1 locus which is linked to amination on chromosome V or at the histidine-3 locus on chromosome I (Jha 1967 Genetics 57: 365), indicating that its effect is locus specific. Since rec-3 is linked to mating type in linkage group I, its effect on the histidine-5 gene in linkage group IV could be easily tested.

The tests measured recombination between the his-5 alleles K553 and K512. The K553 a rec-3 stock isolated from the wild type Em a rec-3 was crossed to each of five K512 A stocks isolated from a cross of K512 a of unknown rec-3 constitution with the wild type Em A; rec-3+ . Frequencies of prototrophic recombinants arising in the progeny of these five crosses ranged from 7.4 to 11.7 per 105 ascospores. Since rec-3 is only 12 map units from mating type, the probability that at least one of the crosses bears the dominant rec-3+ allele is 0.999 or unity if the K512 a stock is rec-3+. Five isolates of K512 of mating type A were isolated from a cross of a with a rec-3 stock cot-l (C102); am (47305), isolate no. 3675 supplied by D. G. Catcheside. Each of these five isolates were crossed to the K553 a; rec-3 stock and the frequency of histidine prototrophs in the progeny was determined. Frequencies ranged from 8.4 to 12.6 per 105 ascospores. The probability that at least one of the five crosses was homozygous for rec-3 is 0.999. It may be confidently assumed therefore that recombination-3 differences do not control recombination between K553 and K512, or if they do then the effect is only very slight.

Since ret-3 controls recombination frequency between all pairs of amination alleles tested, the absence of any detectable control of recombination between the his-5 auxotrophs K553 and K512 adds considerable weight to the supposition that control by rec-3 is locus specific. Further tests will be needed to determine whether rec-3 controls recombination at loci other than amination

Kapoor, M. and D. Bray. Catabolite effects on some enzymes of Neurospora

During an experiment designed to study the effect of growth conditions on the activity and synthesis of glutamine synthetase, several interesting observations were made. Neurospora crassa (Y 8743m) (FGSC #37) was used as a source of enzymes in this study. All cultures were prepared in Vogel's minimal medium with sucrose or glucose as a carbon source, and mycelial powders were obtained as described in the communication on phosphofructokinase in this issue of the Neurospora Newsletter. The cultures were grown for 30 hours at 28°C. There was no autolysis in cultures with low concentration of sucrose. At the end of 30 hours all of the sugar in the medium was exhausted, a very small amount remaining. Crude extracts were prepared by extracting at 3°C lyophilized mycelial powder in 0.05 M phosphate buffer (5 x 10^{-4} M in EDTA and 10^{-4} M in β-mercaptoethanol) pH 7.5, for 30 minutes, straining the mixture through four layers of cheesecloth and centrifuging the supernatant at 27,000 x g for 15 minutes. The residue was discarded and the supernatant was used without further purification.

Table 1. Effect of sucrose on some enzymes of Neurospora.

<table>
<thead>
<tr>
<th>Sucrose concentration</th>
<th>Specific activity (OD/mg protein)</th>
<th>GDH-D</th>
<th>GDH-T</th>
<th>GluN-S''ase</th>
<th>PK</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1%</td>
<td>0.75</td>
<td>0.05</td>
<td>0.005</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>0.75</td>
<td>0.05</td>
<td>0.000</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5%</td>
<td>0.56</td>
<td>0.37</td>
<td>0.18</td>
<td>1.30</td>
<td></td>
</tr>
<tr>
<td>0.53</td>
<td>0.38</td>
<td>0.17</td>
<td>1.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1%</td>
<td>0.45</td>
<td>0.70</td>
<td>0.32</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td>0.43</td>
<td>0.70</td>
<td>0.32</td>
<td>2.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1%</td>
<td>0.23</td>
<td>0.81</td>
<td>0.41</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>0.24</td>
<td>0.79</td>
<td>0.41</td>
<td>2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5%</td>
<td>0.25</td>
<td>0.86</td>
<td>0.42</td>
<td>1.75</td>
<td></td>
</tr>
<tr>
<td>0.15</td>
<td>0.86</td>
<td>0.42</td>
<td>2.00</td>
<td></td>
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</tr>
<tr>
<td>2%</td>
<td>0.12</td>
<td>0.98</td>
<td>0.45</td>
<td>2.59</td>
<td></td>
</tr>
<tr>
<td>0.19</td>
<td>0.98</td>
<td>0.44</td>
<td>2.30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Activities of glutamine synthetase (GluN-S''ase), NAD-specific glutamate dehydrogenase (GDH-D), NADP-specific glutamate dehydrogenase (GDH-T) and pyruvate kinase (PK) were determined in extracts of mycelia obtained from cultures grown in different concentrations of sucrose. Arrays of the activities of the two glutamate dehydrogenases were performed with a Gilford model 2000 recording spectrophotometer by following the initial decrease in OD at 340 μm accompanying the reductive amination of α-ketoglutarate in the presence of ammonia and reduced NAD or reduced NADP (K Kapoor and Smith 1968 Can. J. Microbiol. 14: 609). GluN-S''ase was assayed by measuring the formation of Y-glutamyl hydroxamate from L-glutamate and hydroxylyamine in the presence of ATP (K Kapoor and Bray 1968 Biochemistry 7: 3583). PK was measured by following the decrease in OD at 340 μm in the following reaction mixture at 25°C: Tris-HCl, pH 8.0, 100 μmole; MgCl2 10 μmole; ADP 1 μmole; reduced NAD 0.14 μmole; PEP 0.6 μmole; LDH (Sigma) 100 μg and enzyme preparation in a total volume of 3 ml.

Table 1 shows the specific activities of these enzymes in crude extracts of Neurospora mycelium at concentrations varying from 0.1% to 2.5%. Glutamine synthetase is not repressed by sucrose and neither is pyruvate kinase; both these enzymes show an increase in specific activity in the presence of sucrose up to 1.5% but no further increase was noted at 2% and 2.5% sucrose. A study of the response of the two GDH's towards sucrose in the growth medium revealed a dramatic feature of regulation of GDH-T and GDH-D. Whereas GDH-D is subject to catabolite repression by sucrose and glucose, GDH-T is induced under the same conditions, thus demonstrating a reciprocal relationship between these two enzymes. It is already known that in the presence of glutamate or ammonia in the medium GDH-D is induced with a simultaneous repression
of GDH-T (Sanwal and Lata 1962 Arch. Biochem. Biophys. 98: 420). It has been suggested that GDH-D is primarily a catabolic enzyme and that GDH-T serves an anabolic function in the cell. Our studies are in agreement with this suggestion in so far as it is GDH-D alone that is subject to catabolite repression and that GDH-T is induced under the same conditions. - - - Department of Biology, University of Calgary, Calgary, Alberta, Canada.

Van Winkle, W. B. Preliminary observations on the ultrastructure of the slime mutant. The growth and gross morphological features of slime have been presented by Emerson (1963 Genetics 34: 162). The heterocaryon (\(\text{rz, spore-1}, \text{cr, or, or-1} + \text{al-5, nic-1, lys-3-xd})\) (FGSC 327), as well as the methods for sustaining slime, were kindly supplied by W. V. Woodward. Growth on agar facilitated the isolation of the hyphlets. Hyphlets 24-48 hours old were fixed in 2.5% glutaraldehyde and postfixed in \(\text{OsO}_4\), both buffered with 0.1 M Sorenson's buffer. Following staining in aqueous uranyl acetate and ethanol dehydration, specimens were embedded in Araldite for electron microscopy.

Electron microscopic examination reveals that the majority of hyphlets from spheroplasts are devoid of cell walls. The absence of cell walls no doubt allows for good fixation by the glutaraldehyde. Cells are found to be multinucleate with obvious connections between the nuclear envelope and the rough endoplasmic reticulum. Prominent granular nucleoli are present, usually one per nucleus. To date, only rough endoplasmic reticulum has been observed in slime. Occasionally, the endoplasmic reticulum is found in large lamellar arrangements of 3-5 layers of membranous structures. Oblique or glancing sections of endoplasmic reticulum show numerous polyribosomes associated with the membranes.

Mitochondria of the typical elongate form are very common; however, some do exist in doughnut-like configurations. The cristae of the mitochondria terminate in square ends or in bulbous shaper. Serial sections have revealed what appear to be several mitochondria in single sections are actually different lobes of single large mitochondria. In young hyphlets myelin whorls involving both the inner and outer mitochondrial membranes have been observed. Some mitochondria are seen to have "buds" of the outer membrane extending into the surrounding cytoplasm.

An outstanding feature of slime hyphlets is the abundance of dense granular organelles, morphologically identical to microbodies (peroxisomes). These organelles range in diameter from 0.13 \(\mu\) to 0.18 \(\mu\) and, in many cases, are dumbbell-shaped and associated with rough endoplasmic reticulum sections. Ultrastructural demonstration of the presence of various enzymes in peroxisomes, mitochondria and endoplasmic reticulum is currently being attempted. (Robert Welch Foundation Grant F-060.) - - - Department of Zoology, University of Texas at Austin, Austin, Texas 78712.

Bakerspigel, A. Migrating and dividing nuclei in somatic cells of Neurospora. Recent HCl-Giemsa and Azure A-SO2 stained preparations have provided new observations on dividing nuclei in somatic cells of Neurospora crassa:

1) In contrast to the report by Wilson (1966 Neurospora News. 10: 6), division does not always occur perpendicular to the longitudinal axis of the cell. In fact, nuclei in several stages of division can be found lying parallel to the longitudinal axis of germinating conidia and hyphal cells.

2) During the final reparation of sister nuclei (telophase) two lagging chromosomes can be observed. One end of each of these chromosomes is attached to the nucleus. Their free ends are swollen or bulbous.

3) During early nuclear division the chromosomes appear to be looped, hooked or curved at one end. A ring-shaped chromosome has also been observed at this stage. The free ends of at least three chromosomes in metaphase complexes were observed to have swollen ends.

4) A migrating nucleus in a hypha can be oval in shape. A long slender intranuclear strand can be observed attached to the nuclear membrane. This strand may be a "Y"-shaped and extends away from the septal pore through which the nucleus migrated, terminating in a small, densely stained body which is assumed to be the centriole. Such a strand may be composed of two portions, one of which is twice the length of the other. The regions to which these strands are attached appear to be composed of tiny granules situated opposite each other on the nuclear membrane. In contrast to some previous reports, none of these strands were observed to extend in opposite directions.

5) Although chromosomal complexes and individual chromosomes were observed, no classical alignment of these chromosomes on a metaphase plate was noted. Wilson reported that a spindle occurs only between separating chromatids. Present observations suggest that a "spindle apparatus", similar to that described by the writer in Trichophyton mentagrophytes (Robinow and Bakerspigel 1965 p. 119-161. In Ainsworth and Sussman (eds), The Fungi, Vol. 1, Academic Press, New York), may operate in dividing, somatic nuclei of \(N. \) crassa. - - - Department of Clinical Microbiology, Victoria Hospital, London, Ontario, Canada.

Morgan, D. H. Arginaseless mutants of Neurospora. The selection procedure for arginase mutants described by R. H. Davis, (1968 Report of the Fourth Neurospora Information Conference, Neurospora News. 13: p. 13) was arrived at independently by the author while working in N. H. Horowitz's laboratory at Pasadena. Our procedures differed in only two respects: 1) I used Vogel's "N", with ammonia as nitrogen source, for the filtration and plating medium whereas Davis anticipated complications involving arginine uptake in the presence of ammonia and therefore used a nitrate minimal.