Rates of RNA and protein synthesis in the cold-sensitive mutant, crib-l

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Rates of RNA and protein synthesis in the cold-sensitive mutant, crib-l

Abstract
Rates of RNA and protein synthesis in cold-sensitive mutant

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After incubation, the samples were diluted with 3 ml of PBS buffer and the cells were pelleted and washed three times with the same solution. The liquid was finally decanted and the radioactivity in the cell pellet was measured in a Gamma counter. The results shown in Table I demonstrate that cells from both "slime" strains were able to bind radioactive Con A. The binding was competed with by an excess of unlabeled Con A or by α-methyl mannoside, but not by α-methyl glucoside. These data demonstrate that binding sites for Con A are indeed present on the cell surface of both "slime" strains. However, we cannot conclude whether the two strains differ in the number of binding sites present per unit of surface, or whether these sites have the same affinity to bind Con A. Kinetic experiments, which are beyond the scope of this note, should provide the answer.

The results reported here strongly suggest that the original "slime" obtained by UV treatment and the recombinant strain possess structural differences at the cell surface level. It is tempting to speculate on whether such differences are not related to the cr-1 mutation. Crossing tests, which are under way, should clarify this matter.

We thank Dr. L. F. Lefoil, Dr. R. Pims and other members of the Instituto de Investigaciones Bioquimicas for useful suggestions. This work was supported in part by grants from Banco de la Nacion Argentina and the Consejo Nacional de Investigaciones Cientificos y Tecnicas. The authors are Career Investigators of the latter institution. & Instituto de Investigaciones Bioquimicas "Fundacion Companoria" and Facultad de Ciencias Exactas y Naturales, Obligado 2490, 1428 Buenos Aires, Argentina.

M.J. Fraser. Enzymogenous proteases in extracts of Neurospora mycelia

activate the exonuclease associated with a putative Rec-nuclease.

Fraser 1975 In P. C. Hanawalt and R.B. Setlow (Eds.) Molecular mechanisms of radiation, New York, p. 577. It was observed that the single-strand (ss) DNase activity of wild-type (74-ORZ3-IVA) had increased 20 to 3-fold in extracts prepared in 24 hr at 37°C for more than two weeks. These activations occurred in 24 hr at room temperature (see Table I) and in 1-2 hr at 37°C. Both activities were subsequently reduced on further incubations.

Table I. Activation and inactivation of single-strand DNase (ss-DNase) and double-strand DNase (ds-DNase) activities in extracts of wild-type and uvs-3 by endogenous "serine protease"

<table>
<thead>
<tr>
<th>Extract</th>
<th>Days at Room Temp.</th>
<th>Activity (units/ml)</th>
<th>ss-DNase</th>
<th>ds-DNase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>-PMSF</td>
<td>+PMSF</td>
</tr>
<tr>
<td>Wild-type</td>
<td>0</td>
<td>87.88</td>
<td>98.83</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>336.340</td>
<td>106.101</td>
<td>66.64</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>142</td>
<td>157</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>40</td>
<td>147</td>
<td>2.1</td>
</tr>
<tr>
<td>uvs-3</td>
<td>0</td>
<td>46.46</td>
<td>40.42</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>47</td>
<td>42.44</td>
<td>1.8, 1.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>217</td>
<td>64</td>
<td>52.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>105</td>
<td>66</td>
<td>15.1</td>
</tr>
</tbody>
</table>

Aliquots of extracts of mycelia were put through 0.45 μ Millipore filters into sterile screw capped vials to avoid bacterial contamination. Crystals of solid PMSF were added to half of the vials (+PMSF). Room temperature averaged about 21°C.

37°C was inhibited by adding 2 mg/ml serum albumin to the one activity remaining was found to be a single-strand specific above.

A stable nuclease preparation has now been derived from the uvs-3 strain. When this was subjected to electrophoresis in 6 M urea-polyacrylamide gels, a very acidic protein was recovered which had both ss-DNase and ds-DNase activities. When uvs-3 nuclease preparation was treated with 3-10 μg trypsin for 30 min at 37°C, the exonuclease was activated. It thus seems likely that the activation of the ds-DNase activity observed in extracts is due to the direct action of "serine proteinase" on the enzyme rather than due to the destruction of an inhibitor. It also seems possible that the uvs-3 strain is deficient in proteinase(s) which cause these cold-sensitive mutant, cri-1.

The cold-sensitive mutant strain crib-1 has been shown to synthesize disproportionately amounts of cytoplasmic ribosomal subunits at 10°C; instead of the 2.3:1 mass ratio of 60S:37S ribosomal subunits characteristic of wild type, the mutant strain exhibits a mass ratio of approximately 7:1 (Schlitt and Russell 1974 J. Bacteriol. 120: 666-671). The aim of the experiments reported here was to compare...
the rates of RNA and protein synthesis in the wild type (74A) and the crib-l strain to determine whether or not the mutant strain is conditionally defective in the synthesis of either or both of these classes of macromolecules.

The rates of in vivo RNA and protein synthesis were determined by incubating mycelial pods in the presence of \([5^3]H\)uridine and \([4,5^{3}H]\)lysine, respectively, and measuring the amount of trichloroacetic acid (TCA)-precipitable radioactivity after selected time intervals. For both strains tested, 125-ml DeLong flasks containing 30 ml of liquid Vogel's minimal medium were inoculated to a final concentration of 10^6 conidia per ml and incubated without shaking at 25\(^\circ\) or 10\(^\circ\)C until a mycelial pad had formed on the surface of the medium. At this time the labeled precursor was added to a final concentration of 0.5 \(\mu\)Ci/ml, and the cultures were shaken for selected time intervals (5 to 60 min) at the appropriate temperature. Mycelial pods were harvested on filter paper with vacuum filtration and dried by passing large quantities of acetone through the mycelial mat. Each dried pad was weighed and then homogenized in 5 ml of 0.1 M potassium phosphate buffer, pH 7.8. The homogenate was clarified by centrifugation, the supernatant liquid was brought to 5% TCA, mixed thoroughly and allowed to stand for 20 min at 25\(^\circ\)C. The resultant precipitate was collected by centrifugation, washed, and resuspended in 5 ml of water. The particulate suspension was made homogeneous by the addition of NaOH and then the radioactivity in each sample was determined by liquid scintillation counting.

Fig. 1 compares the rates of in vivo RNA synthesis for wild type and crib-l at 25\(^\circ\)C (the permissive temperature) and at 10\(^\circ\)C (the nonpermissive temperature). The data indicate no significant difference in the rates of RNA synthesis for the two strains when incubated at 25\(^\circ\)C. However, at ICPC the rate of RNA synthesis in crib-l is substantially lower than that of wild type; indeed, in the interval between 30 and 60 min, it is approximately 4% that of wild type.

Fig. 2 compares the rates of in vivo protein synthesis for the two strains at 25\(^\circ\) and 10\(^\circ\)C. At 25\(^\circ\)C there is little difference. By contrast, at 10\(^\circ\)C a slight decrease in the rate of amino acid incorporation into protein is observed for crib-l compared with wild type. However, this difference in rates of protein synthesis is not as marked as the difference in rates of RNA synthesis of the two strains.

![Figure 1](image1.png)

**Figure 1.** The rates of \([5^3]H\)uridine incorporation into RNA by wild type (\(\bullet\)) and by crib-l (\(\bigcirc\)) at 25\(^\circ\) and 10\(^\circ\)C.

![Figure 2](image2.png)

**Figure 2.** The rates of \([4,5^{3}H]\)lysine incorporation into protein by wild type (\(\bullet\)) and by crib-l (\(\bigcirc\)) at 25\(^\circ\) and 10\(^\circ\)C.
These results suggest that the primary functional defect in the crib-l strain is in the synthesis and/or accumulation of rRNA and that the observed decrease in the rate of protein synthesis at 10°C is a secondary consequence of that defect. Since the largest fraction of the cytoplasmic RNA is rRNA, the primary lesion in crib-l may involve the production of rRNA. In eukaryotes, 3 of the 4 species of rRNA are produced from a common, high-molecular-weight precursor RNA molecule which is specifically methylated and cleaved to result in the mature species. Since the crib-l strain is also characterized by a disproportionate accumulation of ribosomal subunits, we hypothesize that crib-l has a conditional defect in the production or accumulation of 17S rRNA from the high-molecular-weight precursor molecule. - = = Biology Department, Reed College, Portland, Oregon 97202.

**LINKAGE DATA AND STOCKS**

Catcheside, D. E. A. Map location of rec-l in N. crassa. The presence in crosses of the gene rec-l+ leads to the reduction of recombination at the his-1 locus on linkage group V and at the nit-2 locus on linkage group I. Previous work has shown rec-l to be located between ad-7 and asn on linkage group V (Catcheside and Austin 1969 Am. J. Bot., 56: 685). Catcheside 1974 Aust. J. Biol. Sci., 27: 561). Subdivision of this interval by ro-4 and p.b-2 (Catcheside 1973 Neurospora News1. 20: 43; 1974 Neurospora News1. 21: 24) offered the possibility for a more precise location of rec-l with the attendant benefits for experimental manipulation of the locus.

A cross segregating for ad-7, ro-4, asn and ret-l and homozygous for his-l (K83) was made. Randomly selected spores were germinated and the genotypes of the isolates with respect to auxotrophic and morphological markers were determined. Segregants recombinant for either or both of the gene pairs ad-7, ro-4 and asn were crossed to rec-l testers of the appropriate mating type (strain f3869; cot-l (C102h); his-l (K651), int (37401), rec-l and strain f3222; cot-l (C102h); his-l (K625), int (37401), rec-l). Recombination frequencies in these crosses were determined as previously described (Catcheside 1974 Aust. J. Biol. Sci., 27: 561).

The results, summarized in Table I, indicate that rec-l is located between ro-4 and asn.

<table>
<thead>
<tr>
<th>Cross number</th>
<th>Zygote genotype and recombination</th>
<th>Parentals</th>
<th>Recombinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>7123</td>
<td>(un A + cot-l am-1 his-1) (A + a al-2 cot-l am-1 his-1)</td>
<td>ad-7 + rec-1 asn</td>
<td>124</td>
</tr>
</tbody>
</table>

Alleles: b39t, 15300, C102t, 47305, K83, K77, B38, WH137

This work was supported by The Australian Research Grants Committee. I am grateful to Mrs. J. R. Maclean for technical assistance. - = = School of Biological Sciences, Flinders University, South Australia, 5042, Australia.

Selitrennikoff, C. P. Easily-wettable, N.crassa conidio and aerial hyphae are characterized, in part, by their hydrophobic nature. For example, when a drop of water is carefully placed on the aerial hyphal mass of a wild type culture on agar slant (10 mm x 75 mm), the drop is not absorbed but remains intact suspended on top of the aerial mass. In contrast, when similar cultures of easily-wettable (eas) are soaked, the drop of water is immediately absorbed into the aerial hyphal mass. The eas strain was initially recovered from EMS-treated mycelia of strain 74-ORB-Ia and has been subsequently backcrossed to strain 74- OR23-I. A four times. eas strains are unique not only by the "water-drop test", but also by their inability to release free conidia when slant cultures are inverted and tapped (Selitrennikoff and Nelson 1973 Neurospora News1. 20: 34). However, abundant free conidia are released when cultures (or loops of aerial material) are flooded with water. Strains of eas were found not to differ from wild type with respect to general morphological appearance, vegetative growth rate, female fertility and numbers of conidio produced/mg vegetative mycelium. The eas phenotype is easily recognized in auxotroph: eas are double mutant strains (scored by "water-drop test"). Preliminary mapping data suggest linkage to fl (II).

The ease of scoring the eas phenotype in a variety of genetic backgrounds may make this marker useful in linkage studies. eas strains do not liberate free conidia into slants placed in water suspensions demonstrates their potential utility in teaching and also in laboratory work where airborne contamination is to be stringently controlled. eas strains of both mating types are available from FGSC. - = = Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706.