Derepression of tyrosinase by sexual stimulation

A. K. Cruz

H. F. Terenzi

Follow this and additional works at: https://newprairiepress.org/fgr

This work is licensed under a Creative Commons Attribution-Share Alike 4.0 License.

Recommended Citation

Derepression of tyrosinase by sexual stimulation

Abstract
Derepression of tyrosinase by sexual stimulation

This research note is available in Fungal Genetics Reports: https://newprairiepress.org/fgr/vol28/iss1/4
The mutant may synthesize limited amounts of methionine after prolonged growth by virtue of the folate pool. Homocysteine methylation in *N. crassa* is about 70 times more rapid with 5-CH₃H₄PteGlu₂ than the corresponding monoglutamate (Burton et al. 1969 Biochem J. 111: 793). The met-6 mutation could alter the properties of a single folylpolyglutamate synthetase as noted for some multiple auxotrophs of animal cell lines (Taylor and Hanna 1979 Arch. Biochem. Biophys. 197: 36) or possibly prevent the formation of an enzyme catalyzing H₄PteGlu₂ → H₄PteGlu₆ (Ritari et al. 1973). An adequate assessment of these possibilities requires enzyme purification combined with verification of the polyglutamate products.

---

**TABLE 2**

The nature of conjugated folate produced by wild type and mutant extracts

<table>
<thead>
<tr>
<th>Strain</th>
<th>Elution volume from DEAE cellulose (ml)</th>
<th>Glutamate-[³H] incorporation (nmole)</th>
<th>H₄PteGlu[2-¹⁴C] incorporation (nmole)</th>
<th>Ratio³H:¹⁴C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>43 - 47</td>
<td>0.40</td>
<td>0.38</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>53 - 58</td>
<td>1.70</td>
<td>0.78</td>
<td>2.18</td>
</tr>
<tr>
<td></td>
<td>65 - 74</td>
<td>5.40</td>
<td>1.06</td>
<td>5.10</td>
</tr>
<tr>
<td>met-6 mutant</td>
<td>43 - 48</td>
<td>2.10</td>
<td>2.00</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>53 - 58</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>65 - 74</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
</tr>
</tbody>
</table>

Assays were performed as in Table 1 but included ¹⁴C-labelled H₄PteGlu (3400 dpm/nmole). Polyglutamates were eluted from columns of Bio-Rad Cellex D using the gradient elution method of Taylor and Hanna 1977 Arch. Biochem. Biophys. 181: 331.

n.d. = not detected.

---

Cruz, A. K. and H. F. Terenzi

**Derepression of tyrosinase by sexual stimulation.**

Neurospora cultures undergoing sexual differentiation synthesize tyrosinase and accumulate melanin in the fruiting bodies (Hirsch 1954 Physiol. Plantarum 7: 72). In contrast, vegetative cultures do not form significant amounts of tyrosinase, except when starved or treated with protein synthesis inhibitors (Horowitz et al. 1970 J. Biol. Chem. 245: 2784). Horowitz and coworkers suggested that tyrosinase expression is under negative control by a metabolically unstable protein repressor. However, it must be kept in mind that most studies dealing with the regulation of Neurospora tyrosinase have been carried out in vegetative cultures submitted to nonphysiological treatments to promote derepression of the enzyme. To our knowledge, no attempt has been made to study physiological conditions of tyrosinase derepression when it occurs in response to mating. In this note an experimental approach is described that permits observation of the biochemical responses of a "female receptor" mycelium after being stimulated by cells of the opposite mating type. In addition preliminary evidence is presented suggesting that tyrosinase derepression following sexual stimulus represents a phenomenon different from that which occurs under conditions of starvation.
Protoperithecial parents strains of elevated fertility were selected from crosses on the basis of high production and rapid maturation of perithecia. These strains differed phenotypically from wild type in that tyrosinase synthesis was constitutive in late stationary cultures made in liquid Vogel medium (after 100 h of incubation). On the other hand constitutive tyrosinase synthesis was not observed in Westergaard liquid medium. Cultures were prepared in standard petri dishes containing 10 ml of Westergaard liquid medium. After inoculating with conidia of either mating type (105 conidia/ml of medium) all cultures were incubated at 25°C in the dark, without shaking, for seven days. After this period, a scarcely conidiating, colorless mycelial mat formed on the surface of the liquid medium. This "female receptor" mycelium was then evenly covered with 2 ml of a suspension of conidia from the opposite mating type (107 conidia/ml). Alternatively, the mycelium was submitted to starvation: the culture medium was removed by aspiration, the mycelium was gently rinsed with sterile distilled water, and finally resuspended in 10 ml of 0.1 M sodium phosphate buffer, pH 6.0. Undisturbed cultures, and cultures which received conidia from the same mating type, served as controls. All cultures were reincubated as before, and at predetermined time intervals duplicate samples were collected and processed for tyrosinase determination. Tyrosinase activity was measured in the 20,000 x g supernatants of crude mycelial extracts, according to the spectrophotometric method described by Horowitz et al. 1960 J. Mol. Biol. 2: 96. Activity is expressed as increase in absorbancy at 475 nm min⁻¹ mg protein⁻¹.

The time course of development of tyrosinase activity of mated cultures, and of cultures submitted to starvation, is shown in Fig. 1. After a lag of approximately 15 h, tyrosinase activity increased sharply in mated cultures, reached a peak around the 40th hour and then gradually decreased. This response was

![Figure 1](image-url)

Figure 1 -- Derepression of tyrosinase in seven day-old cultures of high-fertile strains of mating type A. At zero time the cultures were treated as follows: (○), addition of conidia of mating type Ω; (□), starvation in phosphate buffer; (□), addition of conidia of mating type A or no treatment.
sex-specific, because it was not observed in cultures which received conidia from the same mating type. Addition of cycloheximide (50 µg/ml) at the time of mating abolished tyrosinase derepression (data not shown).

In the absence of mating, the composition of the external medium impairs tyrosinase derepression. This was apparent because cultures submitted to starvation in phosphate buffer showed effective tyrosinase derepression (Fig 1). Thus, it might be concluded that tyrosinase synthesis was released from the repression effect of some environmental factor(s) (nutrients or microelements?) by the sexual stimulus. Reasoning in terms of Horowitz's repressor theory, one interpretation of the data is that starvation derepressed tyrosinase by reducing the rate of synthesis, and consequently, the concentration of the repressor protein. On the other hand, mating would promote the inactivation or destruction of the repressor. It is interesting to observe that the mating-dependent tyrosinase derepression occurred much earlier than that induced by starvation.

Significant derepression of tyrosinase was not observed when conidia of the opposite mating type were added to cultures of wild type strains (for instance St. Lawrence 74A). Nevertheless, these cultures were efficiently derepressed by starvation. Thus, it seems that high fertility of the "female receptor" strain was required to obtain a marked mating response.

Mated mycelium of liquid cultures formed only a few perithecia, but darkened rapidly and excreted a brownish pigment into the culture medium, presumably phenolic compounds. Starved cultures which exhibited an elevated tyrosinase activity did not excrete a brownish pigment.

This experimental approach may be of potential significance for the study of the biochemical phenomena accompanying sexual differentiation in Neurospora. (Supported by Grants from FAPESP (75/779) and FINEP (B 39/79/245/00/00)). - - - - Departamento de Fisiologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, 14100 Ribeirão Preto, Estado de São Paulo, Brasil.

In vivo DNA-specific labelling is not possible in Neurospora because it lacks thymidine kinase (necessary for the conversion of thymidine to dTMP) and is unable to take up exogenous dTMP (Fink, R. M and Fink, R. 1961 BBRC 6: 7). This problem has been overcome in Saccharomyces cerevisiae by the isolation of (tup) mutants capable of dTMP uptake (Brendel, M W et al. 1975 Methods in Cell Biology 11: 287; Wickner, R. B. 1975 Methods in Cell Biology 11: 295). These mutants were selected by their ability to grow on medium containing dTMP when aminopterin (APT) and sulfanilamide (SAA) are added to block endogenous synthesis of dTMP from dUMP. It is also necessary to supplement the above selective growth medium with adenine, glutamic acid, glycine and methionine since APT + SAA also block purine and amino acid synthesis. Two kinds of mutants can grow on such a selective medium one of which is essentially a class of drug-resistant mutants (called asr for resistance to aminopterin and sulfanilamide) and the other which is capable of dTMP uptake (called tup mutants) and behaves as dTMP auxotrophs in the selective medium.

Here we report the isolation of tup mutants of Neurospora. They were obtained on a selective medium which consisted of minimal plating medium (Davis, R. W., deSerres, F. 1970 Methods in Enzymology 17A: 79) supplemented with casaminoacids (1 g/l), yeast extract (2.5 g/l), adenine (20 mg/l), methionine (80 mg/l), glutamic acid (80 mg/l), glycine (80 mg/l), APT (20 mg/l), SAA (5 mg/l) and dTMP (10 mg/l). Plating of mutagenized (25 mM nitrosoguanidine for 30 min.) conidia of strain uvs-3 (FGSC #1627) produced 19 asr colonies; only three of these were dTMP auxotrophs since these did not grow when dTMP was omitted from the selective medium. In contrast, none of the 177 asr mutants obtained from a similar treatment and plating of microconidia strain pefl; cot-1 a were dTMP auxotrophs. The three mutants behaved in an identical manner and exhibited the following characteristics:

1. They had a characteristic colonial morphology similar to that of the frost mutant (Garnjobst and Tatum 1967, Genetics 57: 579) on the selective medium.

2. At 37°C, the mutant had an absolute requirement for dTMP; however, at 25°C, the growth in the absence of dTMP was only about 5-10% of that in the presence of dTMP.

3. On sorbose-free medium dTMP did not stimulate growth at either 25°C or 37°C (i.e., slow growth at 25°C, no growth at 37°C).

4. Initial analysis of the crosses ORA (wild type) x asr revealed 5 ascospore isolates (out of 23 tested) with the tup phenotype. In addition, three ascospore isolates showed only asr phenotype. Since the asr mutants were used as the male parent, these observations establish the nuclear nature of these mutants.