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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, far seven days. After this period, a scarcely condiating, 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-'.

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

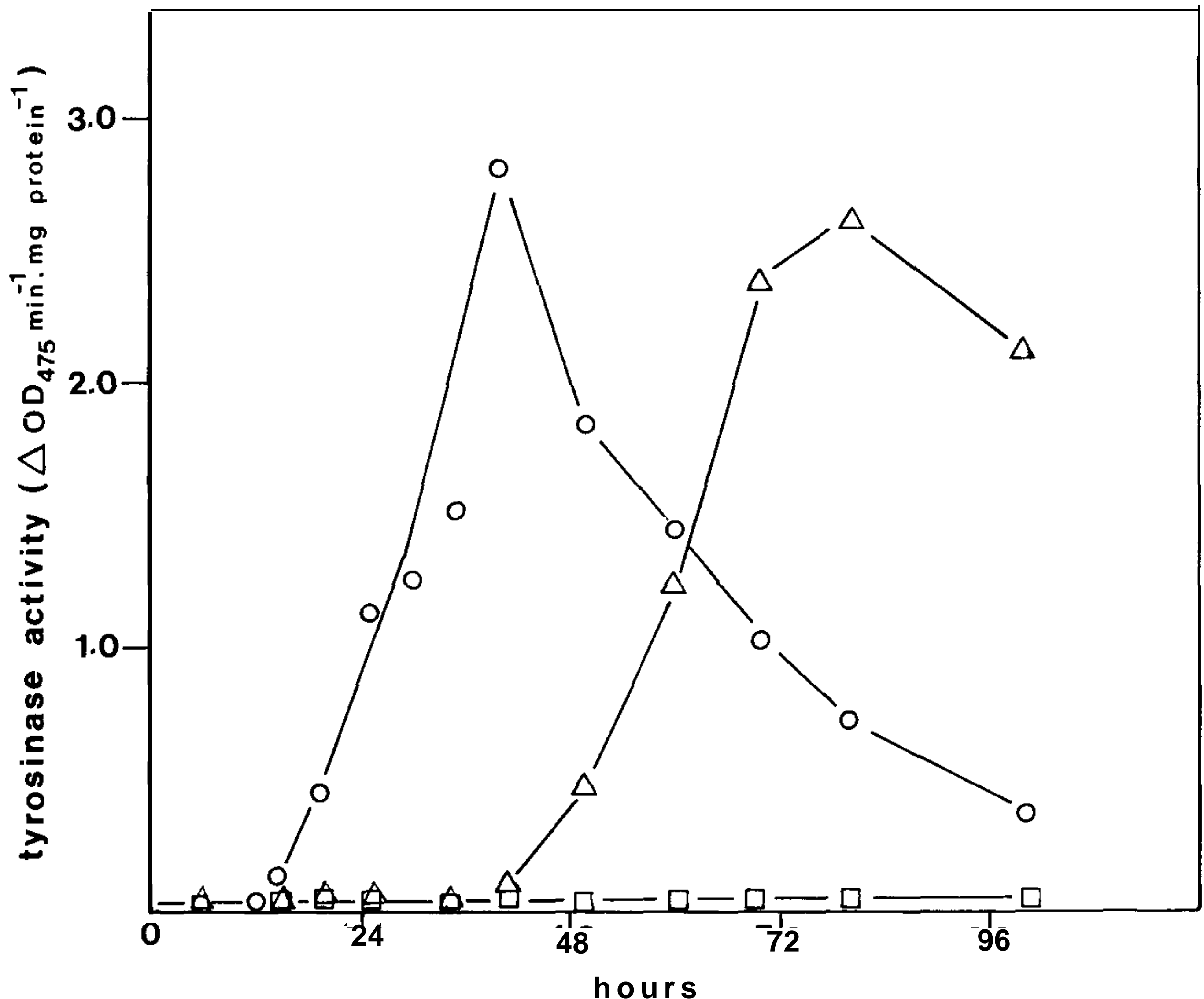

Figure 1. -- Derepression of tyrosinase in seven day-old cultures of high-fertile strains of mating type A. At zero time the cultures were treated as follows: ( ), addition of conidia of mating type a; (r), starvation in phosphate buffer; (£), addition of conidia of mating type A, or no treatment.

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 impaired tyrosinase derepressio". 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 Ribeirao Preto, Universidade de Sao Paulo, 14100 Ribeirao Preto, Estado de Sao Paula, Brasil.