Control of Arylsulfatase in a Serine Auxotroph of *Neurospora*

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A serine auxotroph of *Neurospora* requires exogenous serine to repress the arylsulfatase, suggesting that this enzyme is repressed by cysteine and not by methionine.

The assimilation of sulfate is a process that is energetically expensive for microorganisms. No doubt for this reason, *Neurospora crassa* can vary its production of enzymes of sulfur assimilation by a factor of more than 100-fold. It was suggested some years ago by Metzenberg and Parson (7) that there might be two parallel repressor systems for enzymes of sulfur assimilation in *Neurospora*: one sensitive to cysteine or sulfide, the second sensitive to methionine. The evidence in favor of the first control system is very persuasive. Marzluf and Metzenberg (5) have concluded that the cys-3 gene produces a macromolecular inducer of several enzymes involved in the early steps of sulfur metabolism, among them the arylsulfatase. The pattern of control is roughly parallel but not coordinate. On the other hand, the existence of the hypothetical methionine-related repressor remains unproven. For a time it was thought that the genetic locus *eth-1* represented a methionine-sensitive control system (6). However, this locus is now thought to be the structural gene of the enzyme S-adenosylmethionine synthetase (E. S. Jacobson, G. Chen, and R. L. Metzenberg, manuscript in preparation; see also reference 3).

The present work represents a direct test of the hypothesis that methionine itself represses the enzymes of sulfur assimilation. Our method for limiting cysteine involved use of a "leaky" serine auxotroph. In a wide variety of species cysteine has been shown to be derived from serine (1, 2, 8, 9, 10, 11), and we have confirmed that this is the case in *Neurospora* (data not shown). When forced to grow on endogenous serine, this strain should also be starved for cysteine, even though the supply of sulfur might be adequate. Under these conditions the growth rate is 25% of that of the wild type. The data show that strain *ser-1* (G. Dubes, Ph.D. thesis, California Institute of Technology, Pasadena, 1953) is derepressed for the arylsulfatase when grown on endogenous serine with 5 mM

methionine as sole sulfur source. It can be seen (Table 1) that exogenous serine restores a 300-fold repression, whereas inorganic sulfate alone represses by 27-fold. In the wild type, it is known that the arylsulfatase is fully repressed by either exogenous methionine or inorganic sulfate (7).

We have demonstrated conditions under which the arylsulfatase of *Neurospora* is synthesized abundantly despite the presence of excess methionine. Since starvation for serine de-represses the arylsulfatase, and since cysteine is made from serine, it seems probable that the level of cysteine, rather than that of methionine, exerts a key control on sulfur assimilation. A direct test of the repression of arylsulfatase by cysteine would be very desirable. Unfortunately, exogenous cysteine strongly inhibits the growth of *Neurospora*.

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** LITERATURE CITED


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* Table 1. Repressibility of the arylsulfatase in ser-1

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<tr>
<th>Sulfur source</th>
<th>Sp act of arylsulfatase (U/mg of protein)</th>
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</thead>
<tbody>
<tr>
<td>5 mM methionine</td>
<td>24.4, 16.0</td>
</tr>
<tr>
<td>2 mM inorganic sulfate</td>
<td>0.825, 0.880</td>
</tr>
<tr>
<td>5 mM methionine + 5 mM serine</td>
<td>0.057, 0.069</td>
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* Flasks containing Fries salts without sulfate (10) were supplemented as indicated. Conidia of the *ser-1* strain were inoculated to give an absorbancy of 0.1 at 420 nm and harvested when the absorbancy had reached 1.3. The cultures were then assayed for arylsulfatase (8) and for protein (6).

† Results from duplicate cultures are shown.


