

Ritari, S. J., W. Sakami and C. W. Black. Identification

of two genes specifying foylpolylglutamate synthases.

diglutamate synthase and the other on enzyme forming higher polyglutamates. It appeared probable that one locus was me-6, since the folates of the me-6 (35809) strain had been analyzed in this laboratory and found to consist of mono and diglutamates rather than the higher polyglutamates characteristic of wild type N. crassa (Selhub 1970 Ph.D. Thesis, Case Western Reserve Univ.). The identity of the gene coding diglutamate synthase was less obvious. One possibility was that it was mac, even though mac had been considered to be on allele of me-6. Genetic studies supporting this identification had not excluded the possibility that mac and me-6 are closely linked, but non-allelic, loci (Murray 1969 Genetics 61:67).

Table 1. Polyglutamate synthase activities of extracts of strains of N. crassa with different folate substrates.

| source of extract | Glutamate- ¹⁴ C incorporated in folate* when incubated with | | | |
|-------------------|--|------------------------------------|------------------------------------|------------------------------------|
| | H ₄ PteGlu ₁ | H ₄ PteGlu ₂ | H ₄ PteGlu ₃ | H ₄ PteGlu ₄ |
| me-6(35809)A | 2.99 | -0.05 | 0.13 | 0.10 |
| mac(65108)A | 0.46 | 2.59 | - | - |
| 74-OR8-1a wt. | 5.78 | - | 2.86 | - |

*μMoles/hr/mg protein

forms of tetrahydrofolate, glutamate-¹⁴C, ATP, Mg⁺⁺, K⁺ and CoA for 1 hour under anaerobic conditions, the results shown in Table 1 were obtained. The extract of me-6(35809) was able to incorporate glutamate-¹⁴C into foylpolylglutamate with H₄PteGlu as substrate but was essential-inactive with H₄PteGlu₂, H₄PteGlu₃, and H₄PteGlu₄. The extract of mac(65108) possessed different activity: while it was able to utilize H₄PteGlu₂, it was inactive, or weakly active, with H₄PteGlu. These results indicate that the formation of the diglutamate is catalyzed by a protein coded by mac and that the ability of the extract of N. crassa to use the di-, tri- and tetraglutamates involves a single enzyme specified by the me-6 locus.

The finding that the biosynthesis of foylpolylglutamates by N. crassa involves at least two enzymes (Ritari et al. 1973 Neurospora Newsl.20, preceding note) indicated that two (or more) loci are concerned with this process, one specifying a

In the present study, the foylpolylglutamate synthase activities of N. crassa strains me-6(35809) and 65108(formerly called mac) have been assayed by the procedure of Ritari et al. (ibid.), which involves determination of the ability of the extracts of these strains to convert glutamate-¹⁴C into foylpolylglutamate-¹⁴C on incubation with various forms of THF. Extracts of the organisms were prepared with a Hughes press. Particulate matter was removed from the suspension of broken mycelia by centrifugation at 105,000 x g for 2 hours in a Spinco ultracentrifuge and the supernatant solutions were dialyzed against Tris chloride (0.1M) buffer, pH 8.1. Folates and nucleic acids were removed by passing the extracts through columns of Dowex IX4 (Cl-, pH 8.1). When the crude enzyme preparations were incubated with various

This research has been supported by USPHS, NIH Grant AM 08098 and the Cleveland Foundation.

- - - Department of Biochemistry, Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106.