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Cyclic AMP deficiency, modifier-mutations, and instability of the cr-1 phenotype

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Abstract

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The morphological mutant cr-1 (crisp) of Neurospora crassa is severely deficient in adenylyl cyclase activity (Terenzi et al. 1974 Biochem. Biophys. Res. Commun. 58: 990). When cyclic AMP was added to the culture medium of the mutant, it partially restored wild-type morphology (Terenzi et al. 1976 J. Bacteriol. 126: 91); therefore, the enzymatic deficiency moy be directly involved in the developmental failure associated with cr-1. Because of these properties, cr-1 mutants represent on interesting system to study the role of cyclic AMP in eucaryotic cells. Earlier work (Garnjobst and Tatum 1970 Genetics 66: 281) demonstrated thot cr-1 cultures accumulate spontaneous mutations which modify the crisp phenotype. There mutations were found in aged cultures and also appeared during vegetative propagation of cultures recently iroloted from ascospores. Crisp-modifier mutations hod a clear-cut effect on the morphology of homocaryons, but hod no visible effect in heterocaryons, until a significant proportion of double mutant nuclei was reached. Thus, the presence of modifier mutations could interfere in onyottemptto characterize the cr-1 mutant biachemicolly.

In a recent study (Terenzi et al. 1979, in press) we demonstrated that cr-1 mutant strains are "noble to grow on several carbon sources, including glycerol, mannitol ond arabinose. This pleiotropic deficiency was overcome by the addition of cyclic AMP to the culture medium. This con be observed in Table 1, where it is shown that the growth yield of the cr-1 strain (FGSC #488) in glycerol supplemented medium was greatly enhanced by cyclic AMP. On the other hand, the nucleotide does not affect the growth of the wild type, or that of the mutant in glucose supplemented medium. Spontaneous mutations were also found to overcome the nutritional deficiencies of the cr-1 mutant (Table 1). These mutations, which occurred at a very high frequency, partially suppressed the abnormal morphology of cr-1. Taking advantage of the nutritional differences between cr-1

TABLE 1

Medium	cAMP (1 mM)	Growth of cultures (mg total protein (a)) St. L. 74A	crisp-I *	crisp-mod (b)
2% glucose	-	17.1	16.6	15.7
2% glucose		18.0	15.1	14.3
1% glycerol	-	10.8	0.4	12.1
1% glycerol		8.9	6.3	11.2

(a) - Cultures were grown on 10 ml of Vogel's liquid medium supplemented as indicated. Incubations carried out at 30° C for 48 hr. Mycelia collected ond precipitated with cold 10% TCA. After centrifugation the mycelial pellet was extracted with 1 N NaOH at 100° C, and recentrifuged. Protein was determined in the supernatant by the method of Lowry et al. (1951, J. Biol. Chem. 193: 265).

(b) - This strain was isolated from a cr-1 (8123) culture (*FGSC #488) grown in glycerol medium and reisolated several times by plating on glycerol supplemented medium.

and a-l-modified strains, we have studied the rate of incidence of the spontaneous modifier mutations. The procedure that we devised should be useful to check cr-1 stocks for the presence of modifiers.

All cultures were prepared, using standard petri dishes, in Vogel's medium supplemented with glucose (2%), or glycerol (1%). The cr-1 cultures employed were established from ascospores of a cross of cr-1 (FGSC # 488, allele 8123) X St. L. 74A wild type. The presence of the modifier mutation was tested for by plating a conveniently diluted conidiol suspension on minimal medium supplemented with glucose or with glycerol. Colonies were counted after 48 hr at 32° C. The number of colonies developing in glycerol-supplemented medium, expressed as a percent of the viable population (No. colonies in glycerol/No. colonies in glucose x 100), was regarded as the frequency of crisp-modifiers present in the culture. As Figure 1 shows, the proportion of glycerol-utilizing conidio increased dramatically in aging cultures. In thot experiment, the cultures were all inoculated simultaneously ond were from a single cr-1 isolate. In a different experiment we studied fifty-two separate cr-1 isolates obtained from the cross cr-1 x wild-type. At different times, conidiol samples from each culture were tested in glucose and in glycerol media. Seven days after isolation, growth in glycerol was negativefor all the cultures; after fifteen doys, twelve cultures (23%) gave a positive response. This number increased to twenty-one (40%) at the 22nd day, and, after a month all cultures produced conidia able to develop in glycerol medium. We conclude that old cultures of the cr-1 mutant inevitably contain modifier mutations.

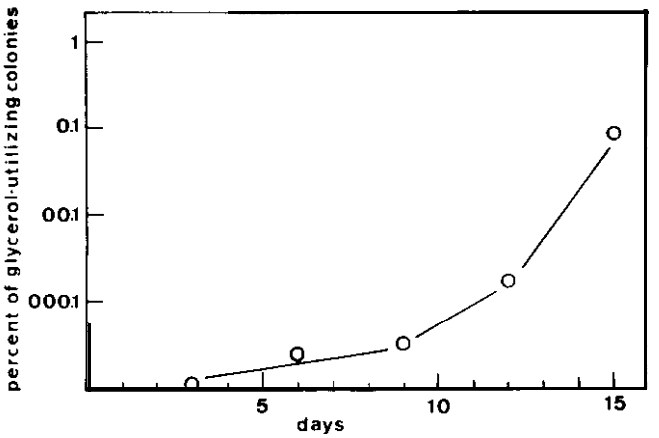

Figure I.-- Increase in the proportion of glycerol-utilizing conidio in aging cr-1 cultures. Each experimental point represents one slant, from a group of five, which hod been simultaneously inoculated with 0.05 ml of a single conidial suspension.

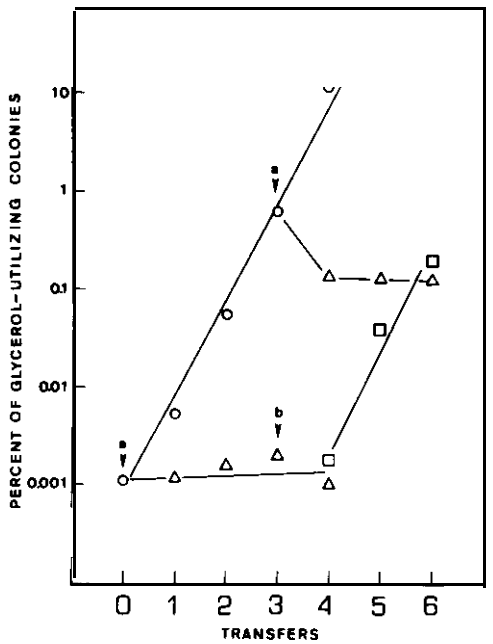

Figure 2. -- Increase in the proportion of glycerol-utilizing conidio during serial transfers of a cr-1 strain, in medium supplemented (A), or not (o, □) with 2mM dibutyryl cyclic AMP. Arrows indicate when cultivation in the presence of cAMP was initiated (a), or discontinued (b). Time interval between transfers was five days.

Figure 2 shows the exponential increase in the proportion of glycerol-utilizing conidia in a cr-1 strain which was propagated by repeated transfers. According to Garnjobst ond Tatum, spontaneous cr-I-modifiers were not observed in wild-type strains; and, although we have occassionally observed w-l-modified phenotypes among the progeny of cr-1 x wild-type crosses, they were not very common. Therefore, we suspected that the extremely fast appearance of the modifier in cr-1 cultures might be related to the mutant deficiencyof adenylyl cyclase activity. In support of this view, it was observed that when the cr-1 strain was propagated in cyclic AMP-supplemented medium, the proportion of glycerol-utilizing conidio did not increase (Figure 2). This effect of cyclic AMP was observed at both a low (0.001%) and a high (1%) proportion of modifier in the heterocaryon. When cyclic AMP was withdrawn, a rapid increase in the number of glycerol-utilizing conidio occurred.

The nutritional advantages provided to the cr-I mutant by the modifier mutation do not seem to contribute to the rapid selection of the latter in any obvious way; i.e., cr-I cultures were propagated in glucose-supplemented medium, in which wild type, cr-1, and modified-a-l growth rates ore the some (Table 1). Moreover, the effects of the modifier on cr-1 morphology only became apparent when a high proportion (over 10%) of nuclei contained the modifier. Nevertheless, the rate of increase of modifiers during the vegetative propagation of a cr-I strain (Figure 2), was linear over four orders of magnitude. Garnjobst ond Tatum found that the spontaneous crisp-modifiers represented at least five different loci. It remains to be established whether the cr-1-modifien thot con be selected on the basis of the nutritional requirements occur at a single or several genetic loci.

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