A NEUROSPORA CRASSA MORPHOLOGICAL MUTANT SHOWING REDUCED ADENYLATE CYCLASE ACTIVITY

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SUMMARY: A Neurospora crassa morphological mutation at the crisp locus is related to a low specific activity of adenylate cyclase. This observation was made in two different strains: FGSC No. 329 and FGSC No. 326, both carrying the crisp mutation (allele N. B 123). Crosses of these strains to others having the wild type allele yield isolates with the crisp phenotype all of them having low adenylate cyclase specific activity in crude extracts. The other phenotypes obtained showed wild type levels of specific activity. Addition of 3',5' dibutyryl cyclic AMP to the FGSC No. 329 cultures increased four-fold mycelial growth rate and stimulated the formation of aerial hyphae. Simultaneously, uniform conidiation on the surface of the slant was inhibited by the cyclic AMP derivative.

Previous papers from this laboratory demonstrated the occurrence of a membrane-bound adenylate cyclase in a slime mutant of Neurospora crassa (1-4). The physiological role of cyclic 3',5'-AMP in fungi has not been studied in detail, but at least in Neurospora, it seems related with glycogenolysis (5, 6). However in another simple eucaryotic organism, Dictyostelium discoideum, cyclic AMP promotes cell aggregation, the first step of ameboid cell differentiation towards the organized multicellular fruiting body (7, 8). A similar effect was recently found in conidia suspensions of Aspergillus niger (9).

The control of the morphological phenotype in Neurospora is not well understood. About one hundred mutations affecting conidiation or morphology of hyphae or ascus have been isolated (10). However most of them cannot be related with a primary enzymatic defect. The few exceptions are: 1) mutations at the col-2 (colonial), ballon and frost loci, which have a deficient glucose 6-phosphate dehydrogenase activity (11, 12); and 2) the mutations at the rg-1 and rg-2 (ragged) loci, having a reduced phosphoglucomutase activity (13, 14). In the case of the col-2

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mutation the morphological phenotype has been correlated with an altered NADPH level and an over stimulation of fatty acid synthesis (15, 16).

This paper reports that a Neurospora crassa morphological mutation at the cr-1 (crisp) locus is related to a low specific activity of adenylate cyclase. The culture of a strain carrying this mutation in the presence of dibutyryl cyclic AMP restores some growth patterns and morphological characteristics of wild type strains.

METHODS

Neurospora was grown in liquid or solid Vogel's minimal medium (17) containing 2% sucrose (w:v) and 2.5 μg/ml biotin. When required the cultures were supplemented with 0.5 mg/ml l-arginine or 50 μg/ml nicotinamide. Conditions for the culture of slime strains were previously described (1).

Mycelial elongation was measured by the race tube method of Ryan et. al. (18) modified by Gillie (19).

Enzymatic extracts were prepared by homogenization of mycelial pads in 1 mM $NaHCO_3$ (2 ml per g of wet mycelium), using a glass-teflon homogenizer. The extract was centrifuged at 900 x g for 6 min and the supernatant ("crude extract") was used for enzyme assays or in turn it was centrifuged at 105,000 x g for 60 min in order to obtain the precipitate of "crude membranes". Further purification of crude membranes was performed as previously described (1). Assays of adenylate cyclase activity were performed at 0.5 mM ATP and $MnCl_2$ as previously described (2).

Some strains used in this work were a generous gift of Dr. W.N.Ogata (Fungal Genetics Stock Center, Arcata), others were from our collection or were obtained by crosses performed according to standard procedures. The slime strain 326 was obtained from the 327 heterocaryon. Strain nomenclature was that recommended by Barrat and Ogata (20). The genetic markers of the different strains used throughout this work correspond to the following alleles: fz (fuzzy): no number; sg (spontaneous germination): no number; arg-1 (arginine): B369; cr-1 (crisp): B123; aur (aurescent): 34508; os-1 (osmotic): B 135; nic-3 (nicotinic acid): Y31881; and cot (colonial temperature sensitive): C 102 (t). Allele numbers of markers in FGSC No. 327 heterocaryon are not listed in the Neurospora Stock List (20).

RESULTS AND DISCUSSION

This study began as a consequence of the observation that enzymatic extracts prepared from the FGSC No. 326 Neurospora crassa slime strain

exhibited a reduced adenylate cyclase activity. In crude mycelial extracts from this strain the specific activity of the enzyme was 2 to 3 percent of those found in wild-type strains (Table I, expt. 1).

The FGSC No. 326 slime strain is derived by mutagenesis from FGSC No. 329 strain, and it has a complex genotype: fz; sg; arg-1; cr-1; aur; os-1 (21); where fz, sg, and os-1 are the genes responsible of the slime

Table 1. Adenylate cyclase activity in crude extracts from mycelia or slime cells of different Neurospora crassa strains.

Conditions were as those described under "Methods".

Expt.	Strain	Genotype	Morphological phenotype	Adenylate cyclase activity (pmoles/min per mg protein)
1	FGSC No.326	fz;sg;os-1;arg-1; cr-1;aur	slime	1.5
	FGSC No.329	os-1;arg-1;cr-1;aur	crisp[a]	1.3
	FGSC No.1118	fz;sg;os-1	slime	27.5
	FGSC No.1119	(fz;sg;os-1) + (os-1;arg-1;cr-1;aur)	osmotic	32.0
	FGSC No.327	(fz;sg;os-1;arg-1;cr-1; aur) + (al-2;nic-1;lys-3; os-1)	osmotic	43.0
	FGSC No.951	os-1	osmotic	44.0
	FGSC No.810	os-1	osmotic	28.0
	FGSC No.424	W.T. (Em. 5256)	wild type	40.0
	FGSC No.627	W.T. (Em. 5397)	wild type	73.0
	BAT 9-5	nic-3;cot-1	wild type at 25°	33.0
2	Segregants from cross FGSC No.326 x FGSC No.810		crisp[a]	1.0-2.0[b]
			Other phenotypes (osmotic or fuzzy-osmotic)	19.0-83.0[b]
3	Segregants from cross FGSC No.329 x BAT 9-5		crisp[a]	0.25-2.5[b]
			Other phenotypes (osmotic or wild type)	26.0-59.0[b]

[a]Osmotic morphology was masked in the crisp phenotype.

[b]Given values represent the lowest and the highest specific activities observed.

phenotype and the others including os-1 correspond to the FGSC No. 329 parental strain. The latter is a mycelial strain having the "crisp" phenotype defined as an "early uniform conidiation on surface of slant" (22) which is caused by the cr-1 mutation (23). Crude mycelial extracts from this strain also showed a low specific adenylate cyclase activity.

The heterocaryon FGSC No. 327 carrying mutant and wild type cr-1 alleles exhibited wild type levels of adenylate cyclase activity and osmotic mycelial morphology. Therefore it seems that the characters of the reduced adenylate cyclase activity and crisp morphology behave as recessive.

Crosses of the strains carrying the cr-1 mutation to others having the wild type allele provided evidence that could indicate a relationship between this mutation and a reduced adenylate cyclase activity in cell-free extracts.

1) Crosses of FGSC No. 326 slime strain to the FGSC No. 810 os-1 strain yielded isolates with crisp phenotype, all of them having a reduced adenylate cyclase activity (Table I; expt. 2). All other phenotypes obtained from this cross: osmotic or fuzzy-osmotic (21) carrying or not the aur mutation did not exhibit a reduced adenylate cyclase activity.

2) Crosses of the FGSC No. 329 crisp strain to the BAT-9-5 (nic-3; cot), a strain having a wild type mycelial morphology at 25° yielded isolates of the crisp or wild type phenotypes associated or not with the aurescent and/or osmotic mutation. Enzymatic extracts prepared from all isolates showing a crisp morphology exhibited a low specific adenylate cyclase activity (Table I, expt. 3). The other phenotypes obtained had wild type adenylate cyclase specific activity in the corresponding extracts.

Some facts indicated that the detection of a reduced adenylate cyclase activity in extracts prepared from the 329 strain is not a consequence of a somehow special condition selected for the enzyme assay and/or preparation.

1) The enzyme activity was negligible over a wide range of equimolar concentrations of ATP and $MnCl_2$ (0.1 to 5 mM). 2) An increase of Mn^{++} concentration over that forming the complex with ATP did not improve the enzyme activity. 3) When Mg^{++} was used instead of Mn^{++}, no activity was detected. 4) Under the standard conditions for the adenylate cyclase assay the enzyme activity was negligible throughout all the purification steps: "crude extract", "crude membranes" or "purified membranes".

If the molecular basis of "crisp" morphology in cr-1 mutants is the

consequence of a reduced synthesis of cyclic AMP, it could be expected that addition of dibutyryl cyclic AMP to the FGSC No. 329 cultures might restore in this strain some of the growth characteristics of a wild type strain. As can be seen in Fig. 1 the rate of mycelial elongation of the

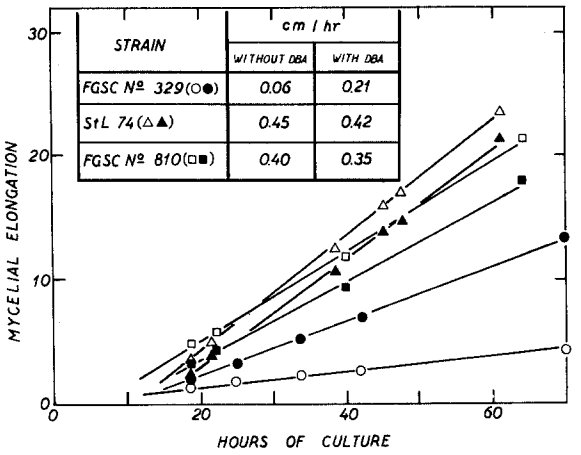

Fig. 1. Effect of dibutyryl cyclic AMP on the rate of mycelial elongation in different strains of N. crassa: FGSC No. 329 (○,●); StL 74 (△,▲), and FGSC No. 810 (□,■). A conidial suspension was inoculated on solid Vogel's minimal medium containing sucrose, biotin and l-arginine. In addition media were supplemented (closed symbols) or not (open symbols) with dibutyryl cyclic AMP (DBA) to a final concentration of 5 mM. Incubations were carried out at 30°C. Other conditions were as those indicated under Methods.

FGSC No. 329 strain is about seven times lower than those of strains having the wild type cr-1 allele. Supplementation of the cultures with dibutyryl cyclic AMP (about 5 mM) increased about 3-4 times the elongation rate of the FGSC No. 329 strain but slightly inhibited those of the latter strains.

Fig. 2 shows the modification induced by cyclic AMP and dibutyryl cyclic AMP on the morphological phenotype of cultures of the FGSC No. 329 strain carried out in solid medium. It can be observed that in a medium containing 5 mM dibutyryl cyclic AMP, conidia at the surface of the slant was no longer produced. On the contrary the formation of aerial hyphae and conidiation at the top of the slant was stimulated by the nucleotide. At the same concentration (5 mM) cyclic AMP resulted less efficient in bringing about similar effects as its dibutyryl derivative. On the other

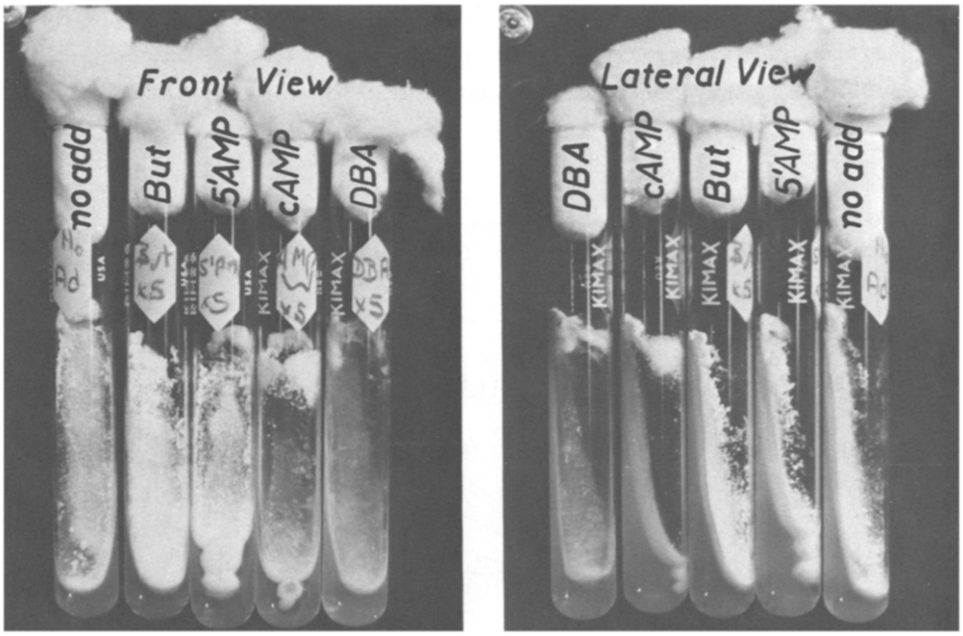

Fig. 2. Effect of cyclic AMP and dibutyryl cyclic AMP on morphology of FGSC No. 329. A conidial suspension was inoculated on slants of solid Vogel's minimal medium containing sucrose, biotin and l-arginine. Media were supplemented with the following additions: 5 mM cyclic AMP (cAMP); 5 mM dibutyryl cyclic AMP (DBA); 5 mM 5'-AMP; or 5 mM sodium butyrate (But). Incubation was carried out at 30°C for four days. A control slant was incubated under the same conditions but without additions (no add).

hand sodium butyrate or 5'-AMP did not elicit any action on the culture morphology.

The results reported in this paper indicate that cyclic AMP would play an important role on the control of Neurospora crassa morphology. High intracellular levels of cyclic AMP favour mycelial elongation and tend to depress uniform conidiation. All the evidence suggests that the mutation at the cr-1 locus in the FGSC No. 329 strain is responsible for the reduced specific activity of adenylate cyclase.

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