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Deficient Cyclic Adenosine 3',5'-Monophosphate Control in Mutants of Two Genes of *Neurospora crassa*

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Strains of *Neurospora crassa* mutant in either of two genes, Crisp-1 (*cr1*) and Frost (*fr*), showed no increase of cyclic adenosine 3',5'-monophosphate (cyclic AMP) levels when subjected to several treatments which produce large increases of cyclic AMP in wild-type *Neurospora*. Evidently, the previously reported deficiencies of adenylate cyclase in these mutants were sufficient to block the normal increases. This fact suggests that both mutants could be used to help determine which control phenomena involve cyclic AMP and to interrupt the control of established cyclic AMP-regulated functions. Earlier studies had suggested an interdependence of the cyclic AMP level and the electric potential difference across the plasma membrane of *Neurospora*. Present experiments, therefore, employed several strains with the *cr1* mutation to test for possible roles of cyclic AMP in recovery and oscillatory behavior of the *Neurospora* membrane potential. The results showed all such phenomena to be normal in the adenylate cyclase-defective strains, which demonstrates that variations of cyclic AMP are not obligatorily involved in the apparent control processes. Evidence is also presented that the induction of both glucose transport system II and the alternative oxidase do not require elevated cyclic AMP levels.

Biological properties controlled by specific regulatory systems have been studied by using several approaches. The most powerful single approach is to fix a regulatory parameter at a specific level and determine how potentially regulated functions are influenced by such fixing. For example, voltage clamp experiments have been performed to help determine the regulatory role of membrane potential on neuron properties (8). In regulatory systems using cyclic adenosine 3',5'-monophosphate (cyclic AMP) or guanine nucleotides, mutants with constant low levels of such nucleotides have been used to help determine the nucleotides' roles in cellular regulation (1, 2, 11). An interruption of the control sequence with such mutants is particularly valuable in studies of negative feedback loops, which, because of their circular nature, are especially difficult to study.

The cyclic AMP control system in *Neurospora crassa*, considered here, has been proposed but not proven to be involved in regulating such diverse processes as morphology and conidiation (15, 22, 25), glycogen metabolism (21), and cell surface properties (10, 24). The properties of adenylate cyclase (5), cyclic AMP phosphodiesterase (14), and cyclic AMP-dependent protein kinase (11a) of the organism have been characterized. In wild-type *Neurospora*, cyclic AMP levels are elevated in response to a number of treatments which have been shown to depolarize the plasma membrane of *Neurospora* (10, 24). Mutants of two different genes of *Neurospora*, Crisp-1 (*cr1*) and Frost (*fr*), have been shown to have low or aberrant adenylate cyclase activities and low basal cyclic AMP levels (12, 13, 22, 23). No studies have yet been reported on whether these mutants can elevate their endogenous cyclic AMP levels in response to treatments which increase cyclic AMP in wild-type *Neurospora*.

The results reported here show that both Crisp-1 and Frost mutants are blocked in their cyclic AMP responses, being largely defective in their ability to increase cyclic AMP levels. The mutants were used to determine if any of a number of regulated responses required cyclic AMP changes to occur normally. Specifically, we examined the hypothesis that cyclic AMP may be involved in the homeostatic control of the plasma membrane potential, with cyclic AMP levels being elevated by plasma membrane depolarization and acting, in turn, to help restore the normal electrical potential. The results indicate that it is not.

MATERIALS AND METHODS

All strains of *N. crassa* used in these experiments are listed in Table 1. Those strains obtained from the

Fungal Genetics Stock Center (Arcata, Calif.) are listed with their FGSC numbers. The *cr1*(B123) strain used was obtained from Alice Schroeder of Washington State University, Pullman. A Frost strain, *fr*(B110), was isolated carrying a spontaneous modifier mutation which improves its linear growth on agar media and its ability to form conidia. This modified Frost strain was picked up during vegetative transfer of strain FGSC 103.

The *cr1*(B74) *poky* strain was obtained from a cross of a *poky f a* strain, previously designated NSF *f*, used as a perithecial parent, with strain FGSC 826. It was not determined whether the *cr1 poky* strain obtained from the cross and used in subsequent experiments carried the *f* marker of its perithecial parent, but its relatively good growth both on slants and in liquid media suggests that it does. The perithecial parent NSF *f* has been used earlier in extensive studies of its growth (9), mitochondrial function (9), and electrical properties (6).

Mycelia for cyclic AMP determinations were grown in liquid shaking cultures (10). Mycelia were grown and treated in Vogel medium containing 2% sucrose as described earlier (10) except that L-lysine transport effect studies were performed with shaking cultures grown and still suspended in Vogel medium N plus 2% sucrose, devoid of NH_4NO_3 but containing 0.2% L-proline as the sole nitrogen source. Collection of mycelia, extraction of cyclic AMP, purification of the extracts, and assay for cyclic AMP were also as described earlier (10) except for modification due to the following problem. It was found that substantial activity in the cyclic AMP radioimmunoassay came from the trichloroacetic acid used in the extraction of the cyclic AMP from the *Neurospora* mycelia. This was a particularly serious problem in studies of the cyclic AMP-deficient mutants *cr1* and *fr*. Consequently, two alternative modifications of the extraction-purification-assay procedures were used. In the first, column blanks were run in parallel to the mycelial extracts; 2-ml amounts of 5% trichloroacetic acid were placed on alumina columns (see reference 10), the columns being washed, eluted, processed, and assayed for cyclic AMP activity by the same procedures used for normal mycelial extracts (10). The apparent cyclic AMP activity from these column blanks (approximately 60 fmol/100 μl) was subtracted from the apparent cyclic AMP activity of the *Neurospora* extracts before calculating the amount of cyclic AMP activity derived from the *Neurospora* mycelia. In the second modification, 1 N perchloric acid was used in place of 5% trichloroacetic acid used earlier (10) to extract the cyclic AMP. Purification on alumina columns then followed the procedure described earlier (10) except that 0.3 ml of 0.1 M ammonium acetate was placed onto each column before it was eluted with 2 ml of 0.1 M ammonium acetate. Subsequent processing and assay procedures were unchanged. The second alternative was used for the data in Fig. 2a and 3 and for cyclic AMP measurement on *cr1*(B74) *poky*. The first alternative was used for all other cyclic AMP data.

TABLE 1. *Neurospora* strains

Strain	Allele, isolate no.	FGSC no.
Wild type 74A		
Wild type RL21a		
cr1	B74	826
cr1	B122	804
cr1 al2	B123, 15300	
cr1	C-Ex-11-67	814
cr1 poky	B74, no number	
fr	B110	103
fr mod	B110, no number	

Electrical potential studies with microelectrodes were performed on mycelia grown on scratched cellophane, using methods described earlier (19). To obtain large enough hyphae for such studies, Crisp-1 mutant strains were grown on medium supplemented with 30 mM cyclic AMP. Cellophane strips on which such Crisp-1 mycelia were growing were washed and placed into cyclic AMP-free media for at least 1 h before electrial measurements were made. Because the half-life of cyclic AMP in *Neurospora* appears to be less than 1 min (10), it seems likely that essentially all exogenously supplied cyclic AMP was removed before any electrical experiments were performed. The Frost strain (FGSC 103) mycelia for electrical studies were grown on agar medium supplemented with 0.1 mM linolenic acid.

Media used during electrical studies included the following (19): (i) 0.3× minimal medium supplemented with 2.3 mM $CaCl_2$ (DMM + Ca) with or without 1% glucose and (ii) dimethyl glutaric buffer medium adjusted to pH 5.8 with KOH containing 1 mM $CaCl_2$ and 1% glucose (DMG) (21). Biochemical reagents were obtained from Sigma Chemical Co., St. Louis, Mo.

RESULTS

The Frost (*fr*) mutant has been reported to have a partially defective adenylate cyclase (13). In agreement with earlier reports (12, 13), there were substantial basal cyclic AMP levels in Frost mycelia (Fig. 1). However, cyclic AMP levels showed little or no increase in response to either 2,4-dinitrophenol or nystatin treatment, both of which give large cyclic AMP increases in the wild type (Fig. 1) (10, 24). It appears, consequently, that the *fr* mutant shows a much more striking deficiency in the control of cyclic AMP levels than in the maintenance of basal levels.

Crisp-1 (*cr1*) mutants showed little or no cyclic AMP under normal conditions and showed no measurable increase in cyclic AMP levels when treated with 2,4-dinitrophenol (Fig. 2). In these experiments, three different allelic *cr1* mutants were tested, B74, B122, and B123. The wild type, in contrast, showed large increases in cyclic AMP levels with dinitrophenol treatment. The *cr1* mutants showed a similar lack of cyclic AMP increase when treated with nystatin, in contrast to the wild type, which showed large cyclic AMP increases (Fig. 3 and 4). Little or no cyclic AMP was found after

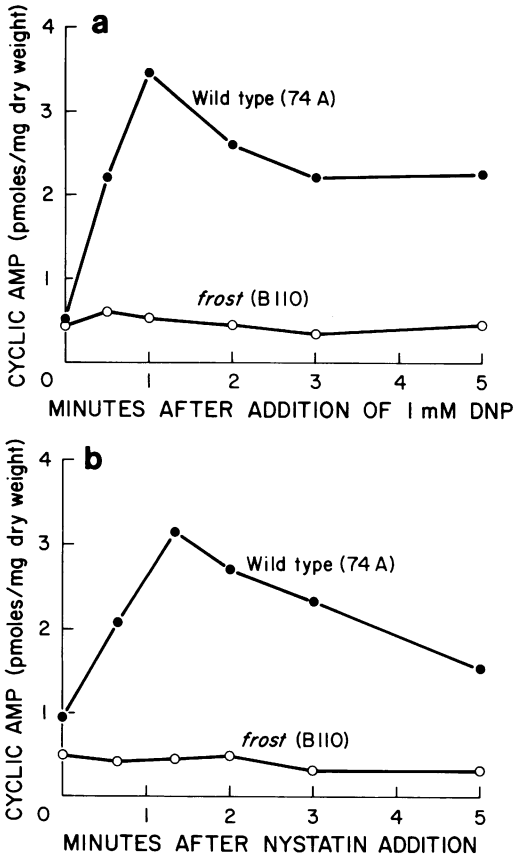

FIG. 1. *Cyclic AMP levels on treatment with 1 mM 2,4-dinitrophenol (DNP) (a) or 3 μg of nystatin per ml (b). In (b), the morphologically modified Frost strain was used.*

nystatin treatment with three *cr1* strains, B74, B123 (Fig. 3 and 4), and B122 (data not shown). A wide variety of nystatin concentrations producing progressively larger and more rapid increases in the wild type produced no apparent increase in *cr1* (Fig. 4).

A third type of depolarizing treatment previously shown to produce cyclic AMP increases in the wild type (10) is the addition of a transport substrate under conditions of high transport activity. L-Lysine addition to ammonia-deprived wild type produced a large cyclic AMP increase, but no cyclic AMP was measurable in two *cr1* strains (Fig. 5). A similar lack of response was found with a third strain, *cr1*(B123) (data not shown).

Consequently, little or no cyclic AMP was measured in *cr1* stains under three conditions which are thought to depolarize in different ways (17) and which produce the largest reported cyclic AMP increases in the wild type. Although each of these conditions depolarizes the plasma membrane, it is not clear that depolarization per se is responsible for the subsequent cyclic AMP changes.

One possible explanation for the lack of response to dinitrophenol or nystatin treatment in Frost or Crisp-1 strains might be that these mutants are abnormally resistant to the two agents used. However, the mutants showed similar plasma membrane depolarizations with each treatment to those shown in the wild type (Fig. 6 and 7). In the dinitrophenol studies (Fig. 6), the two wild-type strains, the *fr* mutant, and two *cr1* mutants were studied. All showed similar

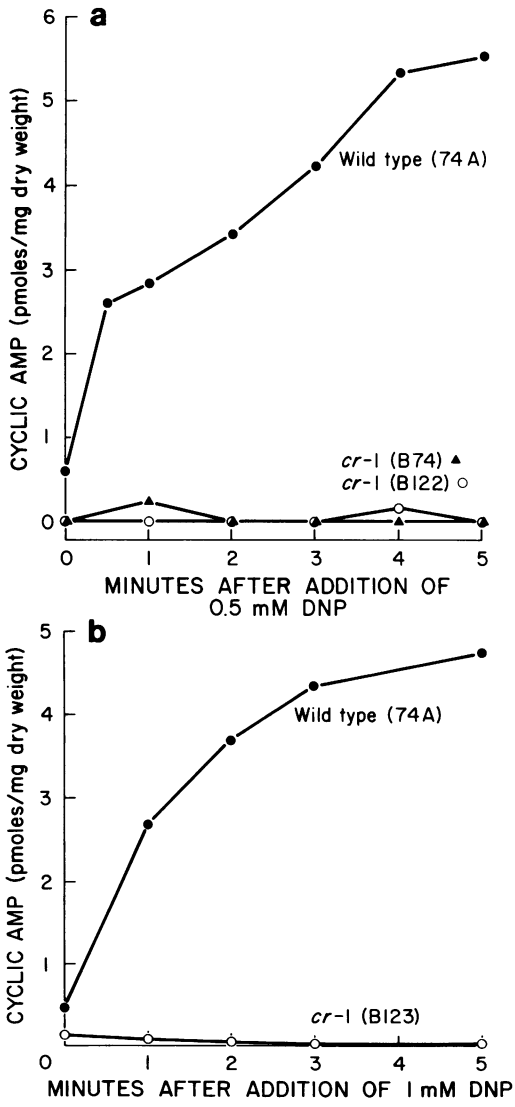

FIG. 2. *Cyclic AMP levels on treatment with 2,4-dinitrophenol (DNP).*

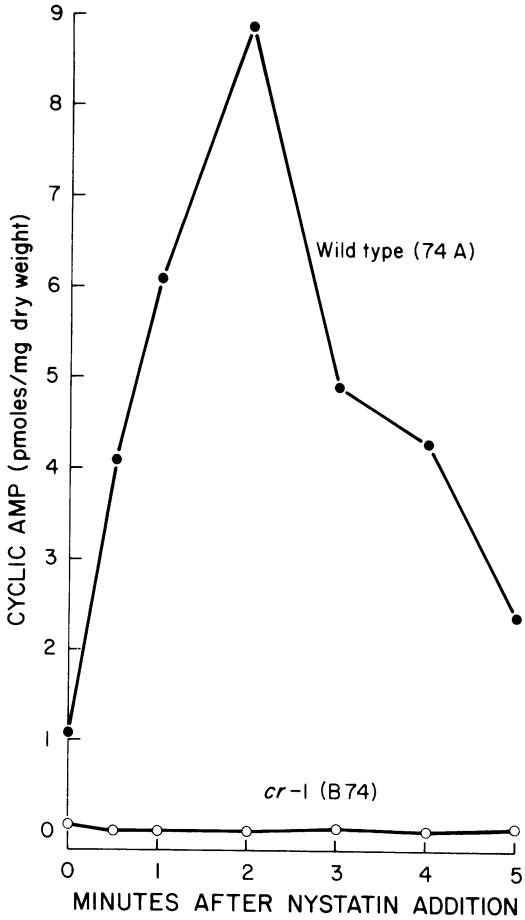

FIG. 3. *Cyclic AMP levels on treatment with 3 μg of nystatin per ml.*

rapid depolarizations on dinitrophenol addition. Washout of the dinitrophenol produced a two-phase recovery of the potential (Fig. 6). The timing of the membrane potential recovery was somewhat variable from one experiment to another, and no apparent differences were seen between mutants and wild types in either rate of depolarization or recovery.

Wild type, *cr1*, and *fr* showed similar depolarizations on nystatin treatment (Fig. 7). Nystatin depolarization is irreversible, so no washout experiment could be performed.

It may be inferred from the dinitrophenol and nystatin experiments that *cr1* and *fr* are not abnormally resistant to these agents and consequently that the lack of subsequent cyclic AMP increase is due to a blockage of some subsequent step which normally leads to the increased cyclic AMP levels. Consequently, both mutants can be used to interrupt the cyclic AMP control sequence in *Neurospora*. Because the treatments studied above produced the largest known cyclic AMP increases in wild-type *Neurospora* but no detectable increase in the mutants studied, it seems likely that mutant responses to other cyclic AMP-elevating treatments will also be missing. It should be noted that the experiments described above were performed with liquid-grown cultures. *cr1* cultures grown on solid me-

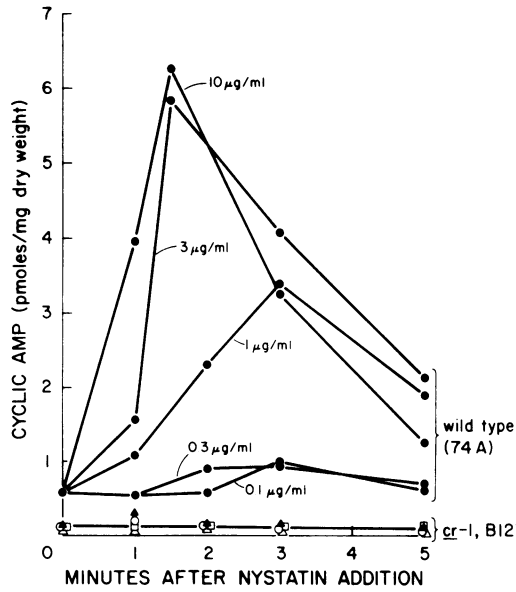

FIG. 4. *Cyclic AMP levels on treatment with various concentrations of nystatin. The cr1(B123) strain was treated with 0.1 (△), 0.3 (△), 1 (○), and 10 (□) g of nystatin per ml.*

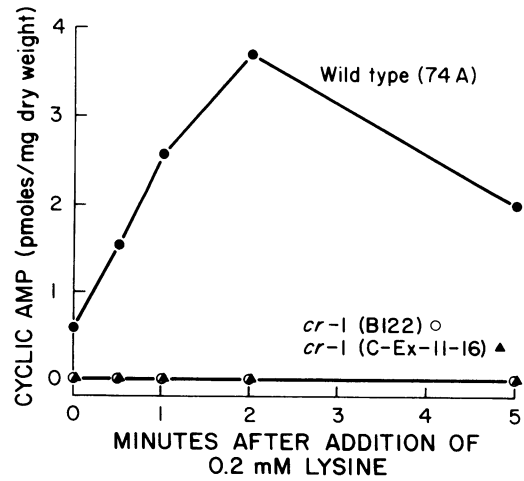

FIG. 5. *Cyclic AMP levels during transport of L-lysine. The lysine was added to mycelia grown and present in nitrogen-free medium N supplemented with 0.2% (wt/vol) proline.*

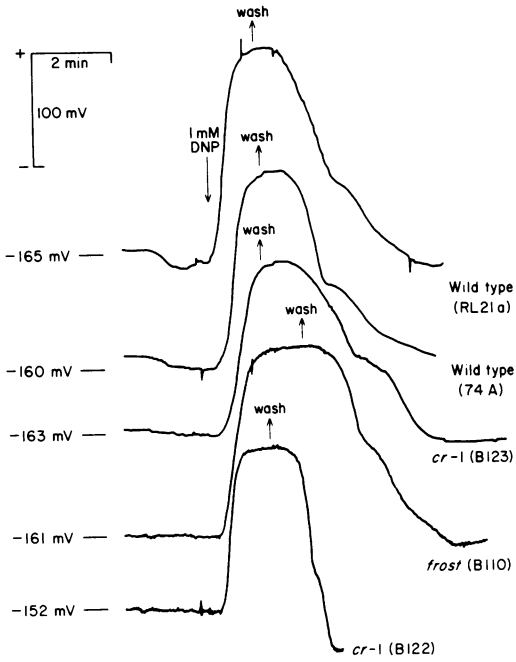

FIG. 6. *Depolarization on addition of 1 mM 2,4-dinitrophenol (DNP). DMM + Ca plus 1% glucose was the medium used.*

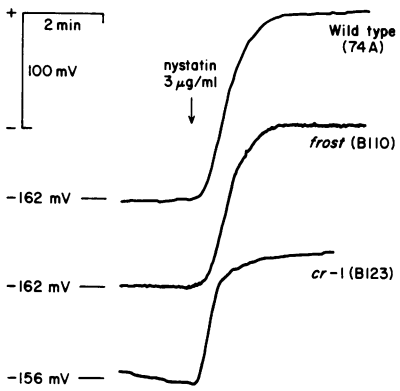

FIG. 7. *Depolarization on addition of 3 μg of nystatin per ml. DMM + Ca plus 1% glucose was the medium used.*

dium show morphological aberrations which appear to be caused by depressed cyclic AMP levels and which are largely corrected by exogenous cyclic AMP and cyclic AMP analogs (12, 22, 23), suggesting that such solid-grown cultures must also show a similar cyclic AMP deficiency to that found in liquid-grown cultures.

The experiments which follow provide examples of specific responses in *Neurospora* mycelia which were tested by using the *cr1* mutants to determine if those responses may be cyclic AMP mediated. The specific experiments performed were chosen, in part, because of earlier observations that membrane depolarizing treatments produce increases in cyclic AMP levels (10, 24). It seems possible, consequently, that cyclic AMP might control the membrane potential in homeostatic fashion, depolarization producing cyclic AMP increases which in turn might produce an increase in the membrane potential. As reviewed earlier (16), cyclic AMP has been reported to increase the plasma membrane potential of many types of animal cells. In the case of *Neurospora*, there are several types of treatment in which the potential deviates from the resting potential due to some treatment and then spontaneously changes back towards the initial resting potential. For example, when carbon-starved mycelia, which have an active glucose transport system II, are given glucose or a transported glucose analog, they depolarize, but subsequently the potential partially recovers even though the transport flux continues unabated (18). The timing of the recovery corresponds with the timing of the elevated cyclic AMP levels produced by the same treatment (10).

To test if the potential recovery under these conditions is cyclic AMP mediated, similar experiments on 3-*O*-methyl-D-glucose transport depolarization were performed with two *cr1* mutant strains and wild-type 74A (Fig. 8). The results showed a sequence of depolarization followed by partial recovery similar to that previously found in wild-type RL21a (17, 18). It may be inferred that cyclic AMP has no required role in producing a recovery of the potential under these conditions. The responses of wild-type 74A did appear to be somewhat more rapid than the responses seen in the two *cr1* strains (Fig. 8). This may have been due to the fact that *cr1* hyphal growth was considerably more dense than that of the wild-type strain, allowing much more rapid exchanges of media in the wild type.

A second response which may be cyclic AMP mediated is the oscillation of the membrane potential in a *poky*, mitochondrion-deficient strain of *Neurospora* on treatment with cyanide. The oscillations provide clear evidence for the involvement of a feedback control system (7). Certain drugs reported to influence cyclic AMP levels in *Neurospora* influence the oscillations, leading to the suggestion that cyclic AMP may be involved in the control system producing the oscillation (6, 17). To test that possibility, *cr1*(B74) was crossed into a *poky* genetic background and a double mutant was obtained. Mycelia of the double mutant had no measurable cyclic AMP in the radioimmune assay. When the double mutant was studied electrically, it

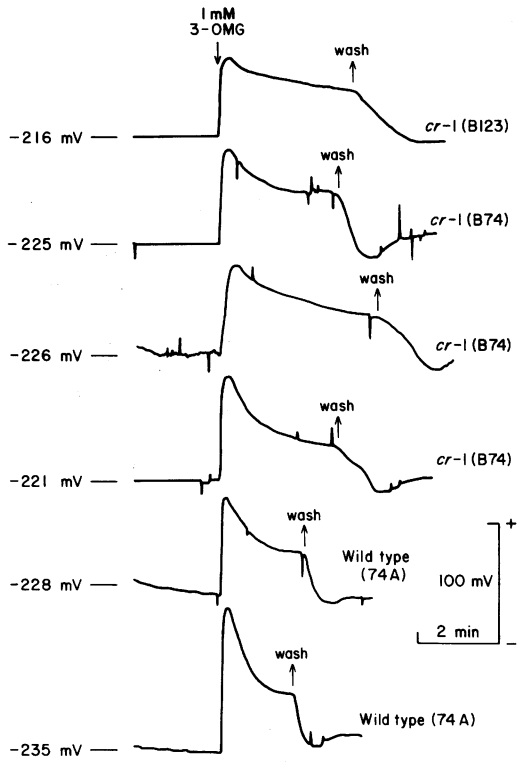

FIG. 8. *Depolarization and partial recovery on transport of 1 mM 3-O-methyl-D-glucose (3-OMG). The mycelia had been deprived of glucose or other carbon sources for over 1 h before addition of 3-OMG. The medium was DMM + Ca devoid of glucose.*

was found that cyanide produced a similar range of oscillations to that reported earlier in *poky* (6). In Fig. 9, the top four oscillations were obtained in the *poky* strain carrying the *cr1*(B74) mutation and the lower two oscillations were obtained with *poky f*, wild type at the *cr1* locus. No apparent differences were seen in the oscillations produced by the two strains. It may be inferred that cyclic AMP has no ongoing, essential role in producing the oscillations and that, specifically, cyclic AMP oscillations are not required to produce the potential oscillations.

Finally, D. Sanders and C. L. Slayman (unpublished data) have found that acetate and certain other carboxylic acids hyperpolarize the plasma membrane of *Neurospora* but that the potential rapidly drops to or below the resting potential. These changes occurred in similar fashion in both Crisp-1 and Frost mutants (Fig. 10), so cyclic AMP seems to have no required role in the process.

DISCUSSION

Both the *cr1* and the *fr* mutants of *N. crassa* are deficient in control of their cyclic AMP levels. This deficiency is particularly severe in the *cr1* mutants. Although the *cr1* mutants were shown earlier to be deficient in adenylate cyclase activity (22, 23), it is not known if they are mutant in a structural gene for adenylate cyclase. The above results show that the *cr1* adenylate cyclase is so deficient that they have little or no cyclic AMP even under the three treat-

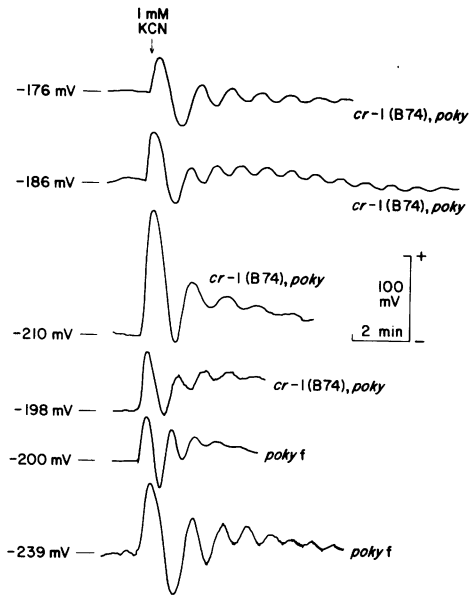

FIG. 9. *Membrane potential oscillations on addition of 1 mM KCN. All strains carried the poky mitochondrial mutation and were mutant (top four tracings) or wild type (bottom two tracings) at the cr1 locus as indicated. DMG was the medium used.*

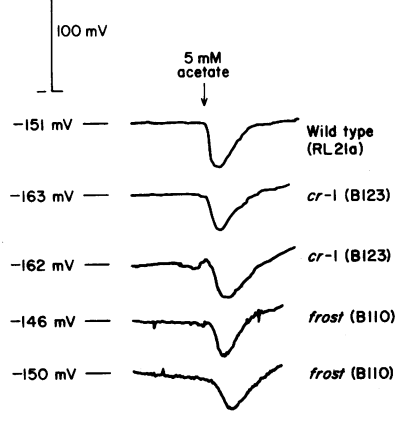

FIG. 10. *Hyperpolarization and spontaneous recovery on addition of 5 mM sodium acetate. The medium used was DMM + Ca plus 1% glucose.*

ments which produce the largest known increases in cyclic AMP in wild-type *Neurospora*.

The *fr* mutant has substantial, almost normal basal cyclic AMP levels but appears to be mainly deficient in the ability to elevate those levels. In *fr*, the adenylate cyclase deficiency is thought to be an indirect effect of the mutation, possibly because of changes in the plasma membrane (13). It seems likely that the treatments which elevate cyclic AMP levels in the wild type fail to do so in *fr* because adenylate cyclase control in this mutant is aberrant. Consequently, the major control in the wild type under the conditions studied is probably at the level of the synthesis of cyclic AMP by adenylate cyclase rather than at the level of degradation or excretion of cyclic AMP. The *fr* mutation may be particularly useful in studying adenylate cyclase control in *Neurospora*.

Of the two types of mutants studied herein, probably the *cr1* mutants will be more useful for in vivo studies of cyclic AMP action in *Neurospora*, because their cyclic AMP deficiency is more profound and because several mutant alleles of the *cr1* gene are available but only one *fr* mutant allele is known.

The data presented here provide confirming evidence that increases in cyclic AMP activity measured in the wild type are due to real increases in cyclic AMP levels and are not due to an increase of some other compound which has activity in the radioimmune assay for cyclic AMP. The adenylate cyclase-deficient mutants would not, in general, be expected to be deficient in responses not involving cyclic AMP, but clearly are deficient in the cyclic AMP activity increases found in the wild type.

A comparison of the properties of the wild type and *cr1* and, in some cases, *fr* mutants reveal that elevated cyclic AMP levels have no essential role in the control or maintenance of several different properties, including the following.

(i) The resting potential of the plasma membrane, as shown by the normal resting potential of *cr1* and *fr* mutants (generally about −150 to −180 mV in the medium, DMM + Ca plus glucose, used in many of these experiments).

(ii) Changes in the membrane potential under several different conditions (Figs. 8 through 10). Specifically no evidence was found that cyclic AMP may increase the potential in *Neurospora* as it has been reported to do in various animal cells (16).

(iii) The induction of the cyanide-insensitive alternative oxidase in *Neurospora*, as shown by the growth of the *poky* strain carrying the *cr1* mutation; growth of *poky* is thought to depend on the alternative oxidase (4, 9, 20). It is also demonstrated by the relatively mild depolarization of the *cr1 poky* mutant produced by cyanide treatment. In the wild type, where there is little cyanide-insensitive respiration, a much more complete depolarization is produced by cyanide than is produced in either *poky* (6) or *cr1 poky* (Fig. 9). It is also confirmed by respiration studies of *cr1* strains with an oxygen electrode (M. L. Pall, C. L. Slayman, and J. Zimmerman, unpublished data).

(iv) Derepression of the glucose transport system II of *Neurospora*, as shown by the substantial depolarization in glucose-deprived *cr1* mutants on addition of 3-*O*-methyl-D-glucose (Fig. 8). Similar depolarization was also obtained with glucose addition (data not shown). This depolarization has been shown to be produced by the glucose or analog transport flux mediated by glucose transport system II (18). C. W. Slayman and T. S. Kobilka (personal communication) have shown that suspension cultures of a *cr1*(B122) mutant grown in the absence of exogenous cyclic AMP can be normally induced for glucose transport system II by depriving them of glucose. The behavior of the glucose transport system II of *Neurospora* differs from those of several sugar transport systems of *Escherichia coli* (3) where derepression on glucose removal requires elevated cyclic AMP levels.

One of the striking features of the cyclic AMP literature is the large number of phenomena which have been proposed to be controlled by cyclic AMP. The mutants studied here in *Neurospora* should be of great value in distinguishing those properties which may be greatly affected by changes in cyclic AMP from those which show normal responses in the absence of such changes. Concentrating on the former group will greatly aid the focus of future in vivo and in vitro studies of cyclic AMP control on *Neurospora*.

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LITERATURE CITED

1. **Cashel, M., and J. Gallant.** 1974. Cellular regulation of guanosine tetraphosphate and guanosine pentaphosphate, p. 733–745. *In* M. Nomura, A. Tissiers, and P. Lengyel (ed.), Ribosomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
2. **Coffino, P., H. R. Bouren, P. A. Insel, K. L. Melmon, G. Johnson, and J. Vigne.** 1978. Studies of cyclic AMP action using mutant tissue culture cells. In Vitro **14**:140–145.
3. **De Crombrugghe, B., R. L. Perlman, H. E. Varmus, and I. Pastan.** 1969. Regulation of inducible enzyme synthesis in *Escherichia coli* by cyclic adenosine 3',5'-monophosphate. J. Biol. Chem. **244**:5828–5835.

4. **Edwards, D. L., and E. Rosenberg.** 1976. Regulation of cyanide-insensitive respiration in *Neurospora*. Eur J. Biochem. **62**:217-221.
5. **Flawia, M. M., and H. N. Torres.** 1972. Adenylate cyclase activity in *Neurospora crassa*. I. General properties. J. Biol. Chem. **247**:6873-6879.
6. **Gradmann, D., and C. L. Slayman.** 1975. Oscillations of an electrogenic pump of the plasma membrane of *Neurospora*. J. Membr. Biol. **23**:181-212.
7. **Higgins, J.** 1967. Oscillating reaction. Ind. Eng. Chem. **59**:19-62.
8. **Hodgkin, A. L.** 1964. The conduction of the nerve impulse, p. 56-61. Charles C Thomas, Publisher, Springfield, Ill.
9. **Lambowitz, A. M., and C. W. Slayman.** 1971. Cyanide-resistant respiration in *Neurospora crassa*. J. Bacteriol. **108**:1087-1096.
10. **Pall, M. L.** 1977. Cyclic AMP and the plasma membrane potential in *Neurospora crassa*. J. Biol. Chem. **252**: 7146-7150.
11. **Perlman, R., and I. Pastan.** 1969. Pleiotropic deficiency of carbohydrate utilization in an adenyl cyclase deficient mutant of *E. coli*. Biochem. Biophys. Res. Commun. **37**:151-157.

11a. **Powers, P. A., and M. L. Pall.** 1980. Cyclic AMP-dependent protein kinase of *Neurospora crassa*. Biochem. Biophys. Res. Commun. **95**: 701-706.

12. **Rosenberg, G., and M. L. Pall.** 1979. Properties of two cyclic nucleotide-deficient mutants of *Neurospora crassa*. J. Bacteriol. **137**:1140-1144.
13. **Scott, W. A.** 1976. Adenosine 3',5'-cyclic monophosphate deficiency in *Neurospora crassa*. Proc. Natl. Acad. Sci. U.S.A. **73**:2995-2999.
14. **Scott, W. A., and B. Solomon.** 1973. Cyclic 3',5'-AMP phosphodiesterase of *Neurospora crassa*. Biochem. Biophys. Res. Commun. **53**:1024-1030.
15. **Scott, W. A., and B. Solomon.** 1975. Adenosine 3',5'-cyclic monophosphate and morphology in *Neurospora crassa*: drug-induced alterations. J. Bacteriol. **122**:454-463.
16. **Siggins, G. R.** 1977. Electrophysiological effects of cyclic nucleotides on excitable tissues, p. 317-336. *In* H. Cramer and J. Schultz (ed.), Cyclic 3',5'-nucleotides: mechanisms of action. John Wiley and Sons, London.
17. **Slayman, C. L.** 1977. Energetics and control of transport in *Neurospora crassa*, p. 69-86. *In* A. Jungreis, T. K. Hodges, A. Kleinzeller, and S. G. Schultz (ed.), Water relations in membrane transport in plants and animals. Academic Press, Inc., New York.
18. **Slayman, C. L., and C. W. Slayman.** 1974. Depolarization of the plasma membrane of *Neurospora* during active transport of glucose: evidence for a proton-dependent cotransport system. Proc. Natl. Acad. Sci. U.S.A. **71**:1935-1939.
19. **Slayman, C. L., and C. W. Slayman.** 1979. Whole cells for the study of transport linked to membrane potential: *Neurospora crassa*. Methods Enzymol. **55**:656-666.
20. **Slayman, C. W., D. C. Rees, P. P. Orchard, and C. L. Slayman.** 1975. Generation of adenosine triphosphate in cytochrome deficient mutants of *Neurospora*. J. Biol. Chem. **250**: 396-408.
21. **Tellez-Iñon, M. T., and H. N. Torres.** 1970. Interconvertible forms of glycogen phosphorylase in *Neurospora crassa*. Proc. Natl. Acad. Sci. U.S.A. **66**:459-463.
22. **Terenzi, H. F., M. M. Flawiá, M. T. Tellez-Iñón, and H. N. Torres.** 1976. Control of *Neurospora crassa* morphology by cyclic adenosine 3',5'-monophosphate and dibutyryl cyclic adenosine 3',5'-monophosphate. J. Bacteriol. **126**:91-99.
23. **Terenzi, H. F., M. M. Flawiá, and H. N. Torres.** 1974. A *Neurospora crassa* morphological mutant showing reduced adenylate cyclase activity. Biochem. Biophys. Res. Commun. **58**:990-996.
24. **Trevillyan, J. M., and M. L. Pall.** 1979. Control of cyclic adenosine 3',5'-monophosphate levels by depolarizing agents in fungi. J. Bacteriol. **138**:397-403.
25. **Turian, G., and E. Khandjian.** 1973. Action biphasique de l'AMP-cyclique sur la conidiogenèse de *Neurospora* et la carotenogenèse en général. Ber. Schweiz. Bot. Ges. **83**:345-350.