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Cyclic Guanosine 3',5'-Monophosphate in the Dimorphic Fungus *Mucor racemosus*

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The dimorphic fungus *Mucor racemosus* was found to contain the cyclic nucleotide guanosine 3',5'-monophosphate (cGMP). Approximately equivalent amounts of the compound were found in ungerminated spores, yeastlike cells, and mycelia. Germinating spores contained severalfold higher amounts of cGMP than the other cell forms. cGMP levels did not change significantly during the morphogenetic conversion of yeast to mycelia. Added exogenous cGMP or the dibutyryl derivative did not influence cell morphology in any way and did not alter the effect that cyclic adenosine 3',5'-monophosphate has upon cell morphology.

The cyclic nucleotide adenosine 3',5'-monophosphate (cAMP) has been reported to effect the morphogenesis of many microorganisms, including fungi (9, 10, 14, 15, 21, 22), protozoans (4), and bacteria (3, 17). High levels of cAMP were found in yeastlike cells of the dimorphic fungus *Mucor racemosus*, and low levels were found in the mycelial form (10). Furthermore, the addition of exogenous dibutyryl cAMP influenced the cells to grow in a yeastlike form under conditions otherwise conducive to mycelial growth (10).

Cyclic guanosine 3',5'-monophosphate (cGMP) is the only other cyclic nucleotide thus far found in living cells (8). It has been proposed that cGMP acts in an opposing direction to the regulatory effects of cAMP (5, 6). Recent evidence has been accumulated to support this notion (1, 2, 12, 13, 16, 23). The present work establishes the presence of cGMP in cells of *M. racemosus* and tests the hypothesis that cGMP acts in opposition to the effects of cAMP on the morphogenesis of the organism.

M. racemosus 1216B was used throughout these experiments. The organism was grown in a complex medium (YPG) described previously (10) or in a defined minimal medium (DM; pH 4.5) consisting of glucose (20 g/liter), alanine (1.5 g/liter), sodium glutamate (1.5 g/liter), $(NH_4)_2SO_4$ (1.0 g/liter), and yeast nitrogen base (Difco) (0.5 g/liter). Inocula and growth conditions were described previously (10). The morphogenetic change from yeast to mycelia was effected by shifting the atmosphere from CO_2 to air (11). Cells were rapidly collected from cultures on membrane filters (Millipore Corp.; pore size, 0.45 μm), immediately placed into 0.1 N HCl, and quickly frozen in an ethanol-dry ice slurry. After the cells were frozen and thawed three times, they were suspended with a vortex mixer. The membrane filters were removed, and the cells were sedimented by centrifugation. The supernatant fluid was assayed for cAMP and cGMP using radioimmunoassay kits supplied by Schwarz/Mann (Orangeburg, N.Y.). The procedure was essentially that of Steiner et al. (18-20), with the modification of Weinryb et al. (24) used to collect the nucleotide antibody complex. The cGMP antibody showed no cross-reactivity with guanosine 5'-triphosphate or cAMP at levels obtained from the extracted cells. Complete destruction of all material reacting with the cGMP antibody occurred during incubation of extracts with cyclic nucleotide phosphodiesterase under the conditions described by Larsen and Sypherd (10). The assay gave a linear response to increasing amounts of purified cGMP and material from extracted cells. The cAMP assay was validated earlier (11). Cellular protein was measured by a procedure described previously (11).

Essentially two kinds of experiments were performed: (i) determination of the effects of added exogenous cGMP upon cellular morphology and (ii) measurement of intracellular cyclic nucleotide concentrations. Added exogenous cGMP had no apparent effect on *Mucor* dimorphism. The substance neither antagonized or mimicked the effects of cAMP on cellular morphology. The compound could not induce CO_2-grown cells to form mycelia or cells placed in air to grow as yeast. Cells responded to added cAMP in the presence of cGMP in a normal fashion, i.e., by growing as yeasts (Ta-

ble 1). Both the dibutyrated and free-acid forms of cGMP were tested over a 0.3 to 3 mM concentration range in YPG and DM media with identical results. At present we have no evidence that either form of the nucleotide penetrates the cells.

cGMP has been detected in all mammalian tissues examined and in many other phyla (7), but has not been studied previously in any of the phycomyces, including *Mucor*. Assay for intracellular cGMP showed that the nucleotide was present in all morphological forms of *Mucor* examined. No correlation could be made between cGMP levels and yeast-mycelial dimorphism. The intracellular levels of cGMP changed very little after a CO_2-to-air shift (Fig. 1). A transient increase in the amount of intracellular cGMP was observed during the germination of sporangiospores in both YPG and DM media (Fig. 2). The pattern mimics that reported for intracellular cAMP during germination of *Mucor* sporangiospores (11) and may possibly reflect some regulatory phenomenon involved in morphogenetic processes occurring during spore germination. The levels to which cGMP concentrations returned after germination were roughly similar for all growing forms examined (Fig. 2). cAMP:cGMP ratios were calculated in all of the above experiments but indicated no meaningful pattern and are not shown. On the basis of the data gathered in this study, the hypothesis that cGMP acts in an

TABLE 1. *Effect of exogenous cGMP on the cellular morphology of Mucor*[a]

Culture conditions	Cell morphology
CO_2	Yeast
CO_2 + cGMP	Yeast
Air	Mycelia
Air + cAMP	Yeast
Air + cGMP	Mycelia
Air + cAMP + cGMP	Yeast

[a] Cultures were grown under a CO_2 atmosphere (yeastlike morphology) on YPG or DM until the early exponential phase of growth. The cultures were then divided into several parts, which were varied in terms of atmospheric composition and cyclic nucleotide additions. The general culture conditions are summarized in the table. cAMP was always in the dibutyrated form and used at a 3 mM concentration. cGMP was tested in the concentration range of 0.3 to 3 mM in both the dibutyrated and free-acid forms. The cultures were incubated for an additional 24-h period, after which cell morphology was examined with a phase-contrast microscope. The results listed were obtained in both growth media, with both the dibutyrated and free-acid forms of cGMP, and at all concentrations of cGMP used.

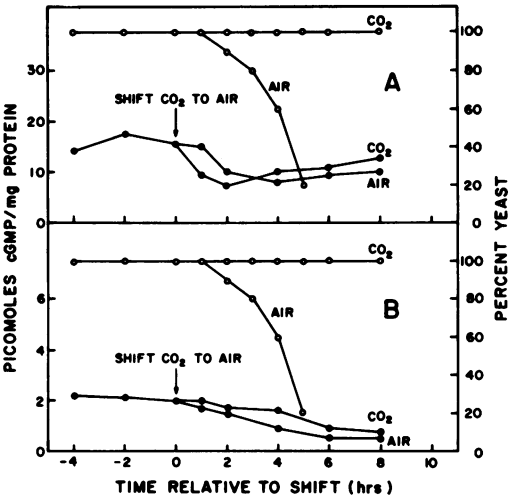

FIG. 1. *Intracellular concentration of cGMP during the morphogenetic conversion of yeastlike cells to mycelia in M. racemosus. The cells were grown under a CO_2 atmosphere until the early exponential phase of growth. The culture was then divided in half, and one part was shifted to an air atmosphere. (A) YPG medium; (B) DM medium. Symbols: ○, percentage of yeastlike cells; ●, intracellular cGMP concentration.*

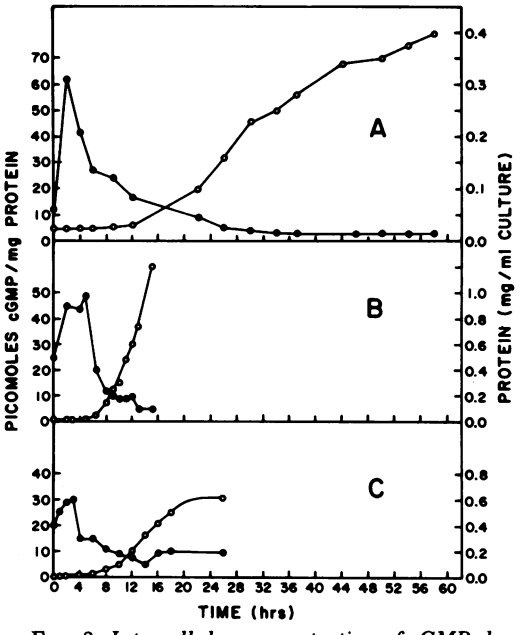

FIG. 2. *Intracellular concentration of cGMP during germination of sporangiospores and vegetative growth of yeastlike and mycelial forms of M. racemosus. (A) Germination and (yeastlike) growth under CO_2 in YPG medium; (B) germination and (mycelial) growth under air in YPG medium; (C) germination and (mycelial) growth under air in DM medium. Symbols: ○, growth as cellular protein per culture volume; ●, intracellular cGMP concentration.*

antithetical fashion with cAMP to control the cellular morphology of the dimorphic fungus *M. racemosus* cannot be supported.

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