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# ROLE OF CYCLIC AMP IN MITOCHONDRIOGENESIS IN YEAST

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## Summary

Cyclic AMP prevents the release of proteins from mitochondria (prelabelled with radioactive aminoacids) during glucose repression in yeast cells and chloramphenicol has no effect on this process. Using specific antisera, the release of the mitochondrially synthesised membrane factor of ATPase during repression and the blocking of this by cAMP has been demonstrated.

## Introduction

In yeast cells subject to glucose repression, there occurs not only an inhibition of mitochondriogenesis, but also breakdown of pre-existing mitochondria (1). On exhaustion of glucose, there occurs a derepression and this derepression phase has been used as a system for the study of mitochondriogenesis. A series of observations suggest that cAMP may have a specific role in the derepression process. For example, it is known (2) that cAMP levels of repressed yeast are much lower than that of derepressed cells. Fang and Butow (3) have shown that addition of cAMP to repressed cells results in the derepression of oxygen uptake. Chandrasekaran and Jayaraman (4) demonstrated that cAMP stimulated mitochondrial protein synthesis, with reference to cytochrome oxidase subunits and Mahler and Lin (5) have demons-

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Abbreviations:

cAMP - 3'5' cyclic adenosine monophosphate; PMS - post mitochondrial supernatant; CHI - cycloheximide; CAP - chloramphenicol

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trated the derepressing effect of cAMP on aminolevulinic acid dehydratase enzyme.

Comparatively little information is available on the repression process itself. Dharmalingam and Jayaraman (6) have raised the possibility that induction of vacuolar lytic enzymes might be responsible for the breakdown of mitochondrial membranes. We have recently studied the release of mitochondrial proteins during repression. In this communication we present evidence to show that cAMP prevents this release and this action does not involve mitochondrial protein synthesis.

## Materials and Methods

### Chemicals

cAMP was purchased from Boehringer. Chloramphenicol was from Parke Davis, India. All other chemicals were of analytical grade. $C^{14}$-Chlorella hydrolysate (42 mCi/mgC) was from Bhabha Atomic Research Centre, India.

### Maintenance and growth of the organism

The diploid strain of Saccharomyces cerevisiae (NCIM 3095) used is from National Collection of Industrial Microorganism, Pune, India. The maintenance of the organism and growth conditions have been described previously (6). Under the conditions of growth in the repression medium, the cells remain repressed (as evidenced by the basal level activities of the oxygen uptake and electron transport enzymes) for the first 2.5 hours. Thereafter, there is an increase in the respiratory capacity. This phase is referred to as derepression phase.

Isolation of Mitochondria Mitochondria were isolated following the method of Jayaraman et al., (1).

ATPase was assayed following the method of Tzagoloff et al.,(7).

Protein was estimated by the method of Lowry et al., (8) using bovine serum albumin as standard.

### Immunoprecipitation of ATPase

The method of Tzagoloff and Meagher (9) was followed to immunoprecipitate ATPase from mitochondrial and post mitochondrial supernatant. Mitochondria were treated with Triton X100 at a final concentration of 0.1% for 5 mins. After spinning at 20,000 x g for 10 mins, the supernatant was treated with ATPase antiserum at 30°C for 1 hour and then centrifuged at 7000 g for 15 mins. The precipitate was washed with Tris-HCl (10 mM, pH 8.3). A titration of Triton X100 extract with antiserum to ATPase complex was carried out. From this the optimal ratio of the antiserum to mitochondrial protein was calculated.

Labelling of cells

Yeast cells were exposed to $C^{14}$ chlorella hydrolysate (0.5 uCi/ml) in presence of cycloheximide (50 ug/ml) for 30 mins under derepressed conditions to label selectively the mitochondrially synthesized proteins. The cells were harvested and after suitable washing with saline, were transferred to several 250 ml flasks (containing 50 ml each of repression medium + cycloheximide) at a concentration of 4 mg wet weight/ml medium. Additions were made to this medium as indicated just before the transfer of cells. At appropriate intervals, cells from individual flasks were harvested and fractionated. The TCA-precipitable radioactivity in the mitochondrial and PMS fractions were determined.

Results and Discussion

The distribution of acid-precipitable radioactivity between the mitochondrial and PMS fractions, at various time intervals after transfer of prelabelled cells to the repression medium is shown in Fig.1. Within 30 min of transfer, radioactivity in the mitochondrial fraction is decreased by about two fold (curve 1) and thereafter shows a slight increase. In the PMS fraction, on the other hand, there is an immediate increase followed by a decrease (curve 2). The patterns are inverse although not correlatable quantitatively.

Since CHI was present in the medium throughout, both during prelabelling and subsequent exposure to the repression medium, the above results could tentatively be interpreted to mean a leakage of mitochondrially synthesized proteins from the cytosol and their subsequent reintegration. The release of mitochondrial proteins into the cytosol on exposure to the repression medium, was considerably reduced when cAMP (5 mM) was included in the medium (curve 3, Fig.1). The initial increase in radioactivity in the PMS fraction is somewhat reduced (curve 4, Fig.1) but the 'reintegration' takes place faster.

Since cAMP has been shown to enhance mitochondrial protein synthesis (4), the effect of chloramphenicol on the observed effect of cAMP was studied. It can be seen that CAP has not abolished

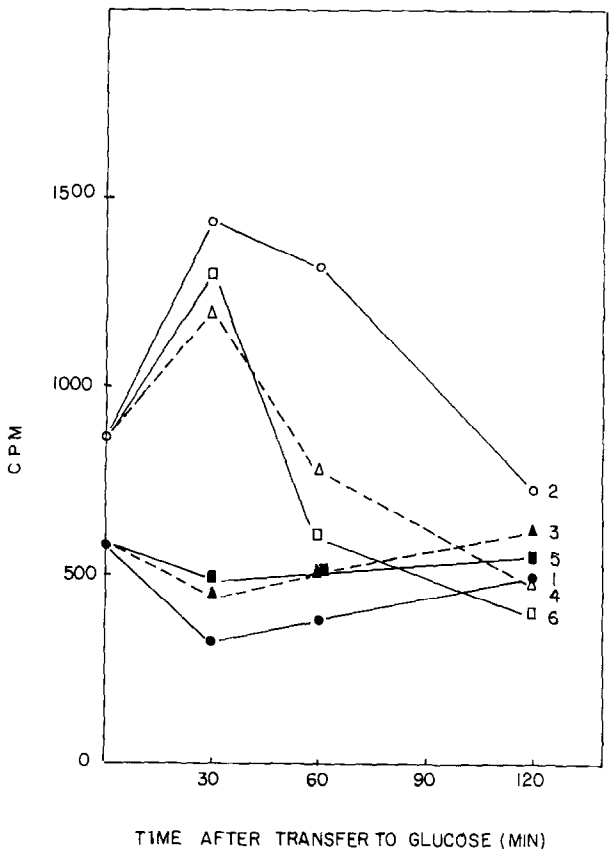


Figure 1 Release of mitochondrial proteins:
Cells pre-labelled with $C^{14}$ chlorella hydrolysate were transferred to repression medium at 0 time. At time points indicated, acid precipitable radioactivities in mitochondrial and PMS fractions were measured (Refer text for identification of lines and symbols).

the cAMP effect (curves 5 and 6, Fig.1). Addition of CAP at 15, 30, 45 ..... min after transfer to repression medium also showed no effects (data not given).

The distribution of a specific mitochondrial marker enzyme, ATPase, between the mitochondrial (curve 1) and PMS fractions (curve 2) was next studied (Fig.2A). An inverse relationship similar to that given in Fig.1 was obtained. The high 'zero time' value of PMS is due to the fact that the enzyme in its soluble form shows more activity than when membrane bound, so that quantitation is difficult (unpublished results). As can

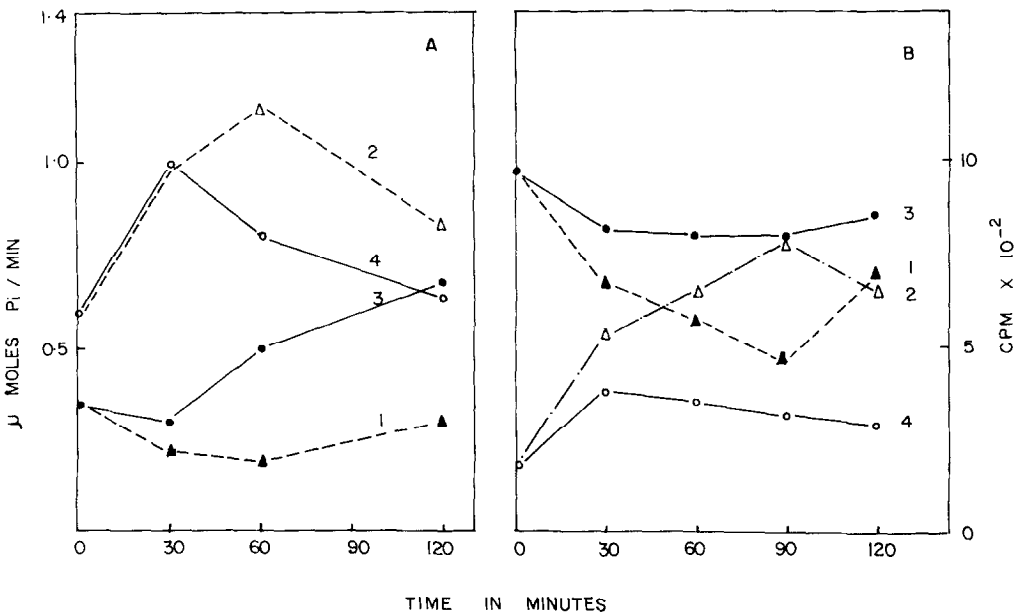


Fig.2A
Distribution of ATPase activity in mitochondrial and PMS fractions

Fig.2B
Distribution of radioactivity in immunoprecipitates obtained after treatment with ATPase antisera in mitochondrial and PMS fractions
(see text for identification of lines and symbols)

be seen from the curves 3 and 4 in Fig.2A, presence of cAMP overcomes the effect of repression medium.

ATPase enzyme consists of the F1-ATPase synthesised in the cytosol and the membrane factor synthesized in the mitochondria (10, 11). The results of the above experiments could be due to the release of the F1-ATPase only. To check this, specific antisera against ATPase complex was used. Prelabelled cells were transferred to repressed media and at different time points, the mitochondrial and PMS fractions were immunoprecipitated using the antisera. The radioactivity in the immunoprecipitate obtained from the mitochondrial fractions shows a decline upto 90 min and thereafter a recovery (curve 1, Fig.2B). The drop

is steeper than observed in any other parameters measured earlier. cAMP abolishes this effect (curve 3; Fig.2B). The inverse pattern of the PMS fraction is also clear (curves 2 and 4; Fig.2B).

Since CHI was present throughout, the radioactivity in the immunoprecipitate obtained against the antisera used, should be contributed only by the mitochondrially synthesized membrane-factor. Thus this experiment conclusively demonstrates the release of mitochondrially synthesized protein into the PMS during the early stages of repression. Cyclic AMP prevents this release.

As the effect of cAMP seems not to be due to its influence on mitochondrial protein synthesis (Fig.1) alternative explanations must be sought. Recent experiments in this laboratory indicate that cAMP reduces the glucose uptake of yeast cells by 50% and it also prevents the induction of phospholipase by glucose. It is thus possible that cAMP acts by reducing the internal glucose concentration or the degree of repression. Subsequently reintegration of the released proteins into the mitochondria is clearly shown by the results in Fig.2B. However a quantitative evaluation of this must await details about the half life of the released proteins in the cytosol, factors that influence the reintegration etc.

Acknowledgement

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References

1 Jayaraman,J., Cotman,C., Mahler,H.R. and Sharp,C.W. (1966) Arch. Biochem. Biophys. 116, 224

2 Van wijk,R. and Konifin,J.M. (1971) FEBS Lett. 13, 124

3 Fang,M. and Butow,R.A. (1970) Biochem. Biophys. Res. Commun. 41, 1579

4 Chandrasekaran,K. and Jayaraman,J. (1978) FEBS Lett. 87, 52

5 Mahler,H.R. and Lin,C.C. (1978) Biochem. Biophys. Res. Commun. 83, 1039

6 Dharmalingam,K. and Jayaraman,J. (1971) Biochem. Biophys. Res. Commun. 45, 1115

7 Tzagoloff,A. (1970) J. Biol. Chem. 245, 1545

8 Lowry,O.H., Rosebrough,N.J., Farr,N.L. and Randall,R.J. J. Biol. Chem. 193, 265

9 Tzagoloff,A. and Meagher,P. (1972) J. Biol. Chem. 247, 594

10 Senior,A.E. (1973) Biochim. Biophys. Acta 301, 249

11 Tzagoloff,A., Rubin,M.S. and Sierra,M.F. (1973) Biochim. Biophys. Acta 301, 71

12 Chandrasekaran,K. and Jayaraman,J. (1980) Eur. J. Biochem. (in press)