EFFECT OF *S*-ADENOSYLMETHIONINE AND CYCLIC ADENOSINE 3′,5′-MONOPHOSPHATE ON RNA SYNTHESIS DURING GLUCOSE-DEREPRESSION IN *SACCHAROMYCES CEREVISIAE*

Ronald E. LAW and Adolph J. FERRO

Department of Biological Sciences, University of Illinois at Chicago, Chicago, Illinois 60680, USA

Received 30 May 1977

1. Introduction

Yeast growing on a high concentration of glucose either anaerobically or aerobically are catabolite repressed. During catabolite repression, cells exhibit low rates of cyanide-sensitive respiration and are deficient in cytochromes of the mitochondrial electron transport chain [1]. Glucose-derepression can be induced under conditions of limited growth resulting from glucose or nitrogen downshift of the culture medium [1]. During glucose-derepression both the mitochondrial and nuclear genomes are engaged in transcription required for the full development of functional mitochondria and the synthesis of enzymes involved in respiration [2–6]. Evidence has accumulated which suggests that in yeast, glucose-derepression may be mediated through the action of cyclic adenosine 3′,5′-monophosphate (cAMP) [7–10].

The internal concentration of *S*-adenosylmethionine (SAM) in *Saccharomyces cerevisiae* has also been shown to be dependent on the amount of glucose supplemented to the growth medium [11]. Additionally, since the synthesis of SAM requires the transfer of the adenosyl-moiety of ATP to methionine, this compound shares a common metabolic precursor with cAMP. Since both cAMP and SAM may function as regulatory molecules during glucose-derepression, we have examined the effect of these compounds on RNA synthesis during glucose-derepression in *S. cerevisiae.*

2. Materials and methods

Saccharomyces cerevisiae 7752, a wild type diploid strain, was used in all experiments. Cells were inoculated into medium consisting of 10% glucose and 2% yeast extract (repressing medium). After 16 h, a 5% transfer was made into fresh repressing medium. The cells were then grown for 5 h, at which time they were in the mid-log phase of growth. The cells were harvested by centrifugation and resuspended at 1×10^7 cells/ml in medium containing 1% glucose, pH 4.5 (derepressing medium). Unless otherwise specified, cells were rapidly aerated at 30°C on a reciprocating shaker.

The incorporation of uridine into RNA was monitored by incubating cells (1×10^7/ml) in derepressing medium containing 5 μCi/ml of [^{3}H]uridine. After various times of incubation, duplicate aliquots (0.2 ml) were removed and added to 0.2 ml cold 10% TCA. The mixtures were precipitated at 0–4°C for 30 min and then collected on Whatman GF/C filters. The filters were washed with 3 × 10 ml cold 10% TCA and 1 × 10 ml cold 95% ethanol. The dried filters were counted in 5 ml of Triton X:toluene (1:2) in 0.4% PPO in a Beckman LS 250 scintillation counter.

The transport of [^{3}H]uridine into cells was monitored by the same procedure as above, except that whole cells were collected on millipore filters (HAWP 25 mm, 0.45 μm) and were washed with 30 ml ice-cold water.

[5,6-^{3}H]Uridine (40–60 Ci/mmol) was purchased from Schwarz-Mann. 3′,5′ Cyclic AMP was the product of Sigma Chemical Co. *S*-Adenosylmethionine was obtained from Boehringer-Mannheim Corp.

3. Results

RNA synthesis during glucose-derepression was measured by following the incorporation of [^{3}H]uri-

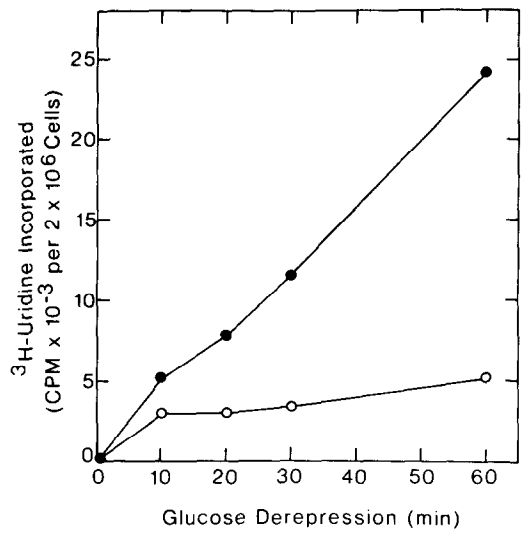

Fig.1. Effect of SAM on the incorporation of [^{3}H]uridine into RNA during glucose-derepression. The incorporation was measured in the presence (○) or absence (●) of 3 mM SAM.

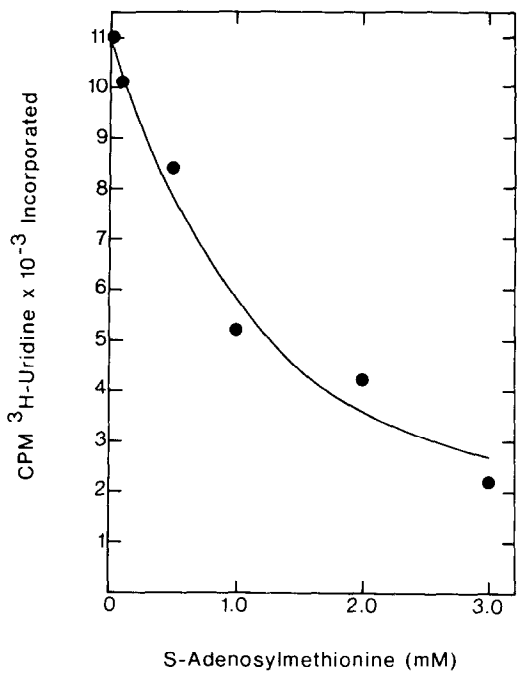

Fig.2. Effect of increasing concentrations of SAM on RNA synthesis during glucose-derepression. Cells were incubated in derepressing medium for 30 min and the incorporation of [^{3}H]uridine was measured as described in Materials and methods.

dine into acid insoluble material. For control cultures, the kinetics of incorporation of [^{3}H]uridine into RNA were linear during the 60 min incubation period (fig.1). The addition of 3 mM SAM at t_0 to the derepressing medium (1% glucose, pH 4.5), however, resulted in a greater than 75% inhibition of RNA synthesis after the 60 min incubation. The extracellular addition of SAM resulted in a leveling off of the incorporation beginning at 10 min (fig.1).

Inhibition of [^{3}H]uridine incorporation into RNA, during glucose derepression, as a function of the concentration of SAM is shown in fig.2. SAM was found to be inhibitory in the range of 0.1–3.0 mM. Fifty percent inhibition was observed at about 1.0 mM while a maximum inhibition of greater than 75% was seen in cells incubated in the presence of 3 mM SAM. This latter concentration of SAM was employed for all subsequent studies.

It was of interest to determine if the inhibitory effect on RNA synthesis by SAM was specific for cells undergoing glucose-derepression. To test this, cells were initially grown to mid-log phase in medium containing 10% glucose and 2% yeast extract. The cells were then harvested by centrifugation and resuspended at 1×10^7 cells/ml in either: (a) 1% glucose, pH 4.5 (derepressing medium) or (b) fresh medium containing 10% glucose and 2% yeast extract (repressing medium). The inhibitory effect of SAM on RNA synthesis was limited to those cells which were incubated in the 1% glucose (fig.3A). RNA synthesis was unaffected by the presence of SAM in cells which were incubated in 10% glucose and 2% yeast extract (fig.3B). It should be noted that the level of incorporation of [^{3}H]uridine into RNA was substantially higher in cells incubated in derepressing medium as compared to those incubated in repressing medium.

The effect of cAMP on RNA synthesis and its effect on the reversal of the SAM mediated inhibition of RNA synthesis during glucose derepression is shown in table 1. The addition of cAMP alone resulted in a 40% stimulation of RNA synthesis. The addition of 3 mM cAMP to cells also incubated in the presence of 3 mM SAM resulted in a restoration of RNA synthesis to approximately 50% of the control value after a two-hour incubation.

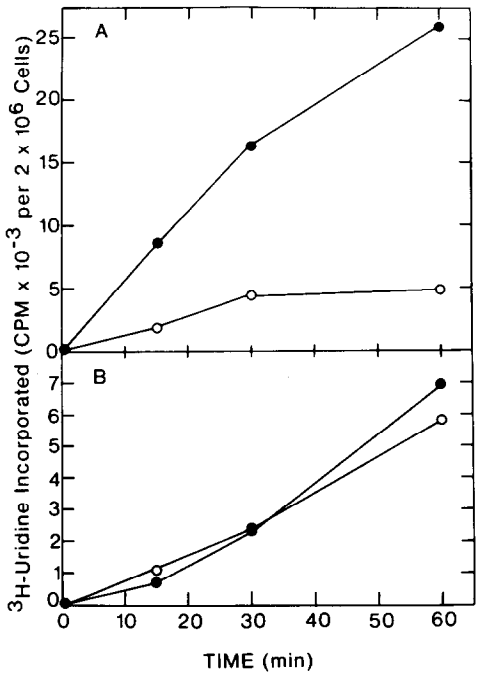

Fig.3. Effect of SAM on the incorporation of [^{3}H]uridine into RNA. Cells were grown to mid-log in repressing medium, harvested and resuspended at 1 X 10^7 cells/ml in either derepressing medium (A) or repressing medium (B) in the presence (○) or absence (●) of 3 mM SAM.

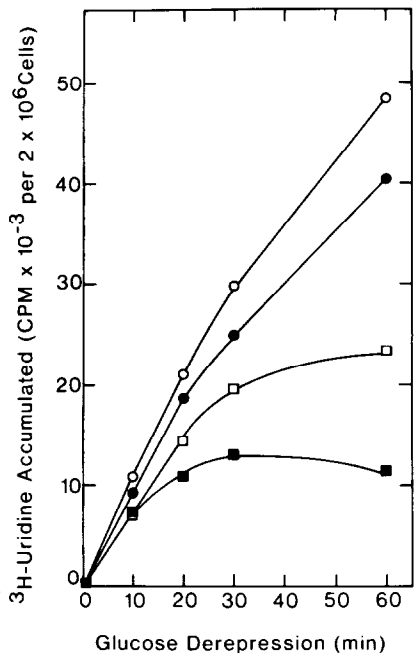

Fig.4. Effect of SAM and cAMP on the transport of [^{3}H]uridine into whole cells during glucose-derepression. Accumulation was measured with the following supplements to the derepressing medium: (●), No supplement: (○), 3 mM cAMP; (■), 3 mM SAM; (□), 3 mM cAMP and 3 mM SAM.

Table 1
Effect of *S*-adenosylmethionine and cyclic AMP on the incorporation of [^{3}H]uridine into RNA during glucose derepression

Time (min)	Radioactivity incorporated (cpm)			
	Additions to the medium			
	None	SAM	cAMP	SAM + cAMP
10	5239	3119	7707	5470
20	7836	3092	10 580	6063
30	11 666	3438	9675	11 258
60	24 306	5288	37 245	16 950
120	38 680	5803	53 095	20 586

The incubation conditions and the procedure for measuring the incorporation of [^{3}H]uridine into RNA are described under Materials and methods. *S*-Adenosylmethionine (SAM) and cyclic AMP (cAMP) were each added to the medium at zero time at 3.0 mM.

In *Schizosaccharomyces pombe*, it has been shown that cAMP modifies the transport of nucleosides and amino acids into the cell [12]. For this reason, we examined the effect of SAM and cAMP on the transport of [^{3}H]uridine into the cells during glucose derepression. The kinetics of accumulation of [^{3}H]uridine in 'derepressing' cells is shown in fig.4. Cells incubated in the presence of 3 mM SAM accumulated only 25% as much [^{3}H]uridine as controls, after incubation for 60 min. The addition of 3 mM cAMP to the incubation medium resulted in a 20% stimulation in the accumulation of [^{3}H]uridine. Incubation in the presence of both SAM and cAMP resulted in an enhancement in the accumulation of [^{3}H]uridine to a value double that of cells incubated only with SAM. A difference in the kinetics of accumulation was observed whenever SAM was present in the incubation medium.

4. Discussion

We have observed that SAM inhibits RNA synthesis during glucose-derepression in *S. cerevisiae*. The inhibitory effect of SAM was found to be specific for derepression conditions since inhibition was not observed in cells incubated in medium containing high concentrations of glucose. It should be noted, however, that the conditions for glucose-derepression employed here result in both a glucose and nitrogen downshift of the cells which severely restricts their capacity for growth and cell division. Additional experiments, therefore, will be necessary to establish whether the effect of SAM during glucose-derepression is due to derepression conditions and/or to an impairment of normal growth and cell division.

The finding that cAMP stimulates the incorporation of uridine into RNA and acts as an antagonist of the SAM-mediated inhibition of RNA synthesis is supportive of the suggestion that SAM functions as a regulatory molecule during glucose-derepression. During glucose-derepression in *S. pombe*, uridine incorporation into RNA was also markedly stimulated by exogenously supplied cAMP [9]. Subsequently, Foury and Goffeau [12] suggested that an observed stimulation of the active transport of uridine by cAMP might account for this enhanced incorporation. We have observed similar effects in derepressing cells of *S. cerevisiae*. Both the incorporation of uridine into RNA and its transport into whole cells was inhibited by SAM and stimulated by cAMP. Additionally, cAMP was found to partially reverse the inhibition of both the incorporation and accumulation of uridine in cells derepressing in the presence of SAM.

This study suggests, for the first time, that a regulatory relationship may exist between SAM and cAMP. Further investigations will be necessary to determine if this relationship is specific for glucose-derepression, or may have a more general metabolic significance.

Acknowledgment

This research was supported by Public Health Service Grant GM 23307.

References

[1] Slonimski, P. P. (1953) in: Formation des Enzymes Respiratoires chez la Levure, Masson, Paris.
[2] Polakis, E. S. and Bartley, W. (1965) Biochem. J. 97, 284–297.
[3] Polakis, E. S., Bartley, W. and Meek, G. A. (1965) Biochem. J. 97, 298–302.
[4] Polakis, E. S. and Bartley, W. (1966) Biochem. J. 99, 521–533.
[5] Witt, I., Kronav, R. and Holzer, H. (1966) Biochem. Biophys. Acta 118, 522–537.
[6] Jayaraman, J., Cotman, C., Mahler, H. R. and Sharp, C. W. (1966) Arch. Biochem. Biophys. 116, 224–251.
[7] Fang, M. and Butow, R. A. (1970) Biochem. Biophys. Res. Commun. 41, 1579–1583.
[8] Van Wijk, R. and Konijn, T. M. (1971) FEBS Lett. 13, 184–186.
[9] Foury, F. and Goffeau, A. (1973) Nature New Biol. 245, 44–47.
[10] Schlanderer, G. and Dullweg, H. (1974) Eur. J. Biochem. 49, 305–316.
[11] Schlenk, F., Zydek, C. R., Ehninger, D. J. and Dainko, J. L. (1965) Enz. Acta Biocat. 29, 283–298.
[12] Foury, F. and Goffeau, A. (1975) J. Biol. Chem. 250, 2354–2362.