Glucose-dependent, cAMP-mediated ATP efflux from *Saccharomyces cerevisiae*

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Extracellular ATP plays an important role in the physiology of multicellular organisms; however, it is unknown whether unicellular organisms such as yeast also release ATP extracellularly. Experiments are described here which show that *Saccharomyces cerevisiae* releases ATP to the extracellular fluid. This efflux required glucose and the rate was increased dramatically by the proton ionophores nigericin, monensin, carbonyl cyanide m-chlorophenylhydrazone and carbonyl cyanide p-(trifluoromethoxy)-phenylhydrazone; ATP efflux was also increased by the plasma membrane proton pump inhibitor diethylstilbestrol. The increase in the concentration of extracellular ATP was not due to cell lysis or general disruption of plasma membrane integrity as measured by colony-forming and methylene-blue-staining assays. ATP efflux was strictly correlated with a rise in intracellular cAMP; therefore, the cAMP pathway is likely to be involved in triggering ATP efflux. These results demonstrate that yeast cells release ATP in a regulated manner.

Keywords: ATP efflux, cAMP mediated, ionophores and yeast, *Saccharomyces cerevisiae*

INTRODUCTION

In most tissues and organs, extracellular adenine nucleotides have diverse physiological effects that are principally mediated by the P2 purinergic receptors (Dubyak & El-Moatassim, 1993; Chen *et al.*, 1995; Lewis *et al.*, 1995). In some cases, such as the stimulation of platelet aggregation and the mediation of pain perception, the source of extracellular nucleotide is damaged tissue. In other cases, such as with adrenal chromaffin cells, ATP is released from secretory granules during their fusion to the plasma membrane. Thirdly, ATP appears to move directly across the plasma membrane via a channel or transporter, as demonstrated by the release of ATP from cardiac myocytes and red blood cells during hypoxia (Forrester, 1990), and from smooth muscle cells upon stimulation with catecholamines (Sedaa *et al.*, 1989).

The mechanism of release of ATP from animal cells through the plasma membrane is not known. ATP efflux has been correlated with the expression in the plasma membrane of a member of the ATP-binding cassette (ABC) superfamily of proteins, the multidrug resistance efflux pump P-glycoprotein (Pgp), in cultured mammalian cells (Abraham *et al.*, 1993). The ABC protein cystic fibrosis transmembrane conductance regulator (CFTR) was shown to act as a dual ATP and Cl-channel in the plasma membrane of mammalian cells (Reisin *et al.*, 1994). Furthermore, it has been shown that one role of CFTR is to provide extracellular ATP which stimulates outwardly rectifying chloride channels in airway epithelia (Schwiebert *et al.*, 1995). However, subsequent work has not supported the idea that the CFTR is a conduit for ATP (Reddy *et al.*, 1996; Li *et al.*, 1996; Grygorczyk *et al.*, 1996). In any event, it seems that ABC proteins, which have a broad expression pattern and are present in a wide variety of organisms, including yeast, (Blight & Holland, 1990; Doige & Ames, 1993; Gotessman & Pastan, 1993; Balzi *et al.*, 1994; Bissinger & Kuchler, 1994; Dean *et al.*, 1994; Servos *et al.*, 1993) may play a role in ATP efflux (Guidotti, 1996).

It is not known whether adenine nucleotides are also released into the extracellular environment by unicellular organisms, except for cAMP which is released in large amounts from bacteria, yeasts and slime moulds.

**Abbreviations:** ABC, ATP binding cassette; CCCP, carbonyl cyanide m-chlorophenylhydrazone; CFTR, cystic fibrosis transmembrane conductance regulator; FCCP, carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone; 4-NQO, 4-nitroquinoline-N-oxide; Pgp, P-glycoprotein.
In view of the ability of all cells to release cAMP (Brunton & Heasley, 1988), we asked whether ATP release is also a feature of unicellular organisms. In this paper we describe experiments which demonstrated that ATP efflux from S. cerevisiae is likely to be mediated by the cAMP pathway because when the pH was raised to prevent the nigericin-induced cAMP response, nigericin did not stimulate ATP efflux. The basal and stimulated ATP efflux activities were not caused by cell lysis or non-specific permeability, and were observed in washed cells suspended in defined buffer and in cells growing in liquid culture. It thus appears that S. cerevisiae contains the capacity to increase ATP efflux when the intracellular cAMP concentration rises. The data suggest that yeast cells provide a good model system for studying ATP efflux; furthermore, the results suggest the possibility that extracellular ATP, ADP, or adenosine play a role in yeast physiology, possibly as intercellular signals.

**METHODS**

**Saccharomyces cerevisiae strains.** Yeast strains YMR4, YMR400 and YHH33 were obtained from Albert Hinnen (Ciba-Geigy Ltd, Basel, Switzerland). The genotypes are as follows. YMR4: MATa b1s-11,15 leu2-3,112 URA3 can Res pHO5:::ura3A1. YMR400: MATa b1s-11,15 leu2-3,112 URA3 can Res pHO5:::URA3 PHO5:::; YHH33: MATa b1s-11,15 leu2-3,112 pHO5. Yeast cells were grown and maintained on synthetic complete (2% dextrose) medium (Sherman et al., 1986). The wild-type yeast described in the text is strain CTY182 obtained from Vytais Bankaitis (University of Alabama, USA).

**Materials.** All drugs were purchased from Sigma. The HPLC columns used were Vydac nucleotide analysis column and a Hydroprop sax column. The cAMP assay system was purchased from Amersham.

**Extracellular nucleotide sample preparation.** Cells were grown to exponential phase (OD600 of 2.0) on glucose-containing medium, washed with 20 mM Tris/citrate, pH 5.5, resuspended in the appropriate cold incubation buffer at a cell density of 5.2×10^8 cells ml^-1 (OD600 of 1 corresponded to 1.6×10^7 cells ml^-1) and moved to room temperature (23–28 °C) at time zero. The samples were incubated at room temperature for the appropriate time period, after which each sample was filtered through a 0.2 μm syringe-tip filter. The filtrate was then stored at -70 °C until HPLC analysis was performed. For the experiment shown in Fig. 6, extracellular nucleotides were isolated by filtering samples of the cell culture; the filtrate was then subjected to HPLC analysis.

**Intracellular nucleotide sample preparation.** Cell suspensions were prepared and incubated as described for the extracellular ATP samples. Intracellular nucleotides were then isolated as described by Pogolotti & Santi (1982). Briefly, at the end of each incubation, the cells were pelleted, resuspended in 0.5 ml 0.6 M trichloroacetic acid and incubated at 0 °C for at least 10 min. The precipitate was removed by centrifugation, and the supernatant was extracted with freon/trietylyamine (TOA). The samples were stored at -70 °C until HPLC analysis was performed.

**HPLC analysis.** One hundred microliters of each sample was subjected to HPLC to separate the nucleotides in the sample as described by Pogolotti & Santi (1982) and the ATP and ADP were quantified. The procedure for separating nucleotides was: solvent A, 0.045 M NH4COOH, pH 4.6, in H₃PO₄; solvent B, 0.275 M NaH₂PO₄, pH 2.7, in HCOOH; gradient, 0–100% solvent B over 10 min; flow rate, 2.0 ml min^-1; detection, 254 nm. Data were quantified either by hand or by using Beckman System Gold software.

**cAMP assays.** Samples were prepared as described in ‘Intracellular nucleotide sample preparation’. cAMP assays, using the competitive binding of cAMP and [3H]cAMP to binding protein, were conducted using the ‘Cyclic AMP [3H] Assay System’ (Amersham).

**Viability assays.** Cells were grown to exponential phase, then prepared and incubated in 20 mM Tris/citrate pH 5.5, with the addition of nigericin and/or glucose as indicated in Fig. 4, at a cell density of 5.2×10^7 cells ml^-1. Following incubation at 23–28 °C for the times indicated in Fig. 4, samples of the cells were diluted in water, 200 cells were spread onto each YPD plate and the plates were incubated at 30 °C for 2 d. Colonies were then counted to determine the number of viable cells on each plate.

**Methylene blue assay.** Cells were isolated and incubated as in the previous experiments except that the incubation buffers also contained 0.01% (w/v) methylene blue. At the times indicated in Fig. 5, the cells were diluted into 0.01% methylene blue to a cell density suitable for counting in a haemocytometer; total and blue cells were counted.

**RESULTS**

**ATP efflux**

To avoid hydrolysis of extracellular ATP by periplasmic acid phosphatases (Nosaka, 1990; Vogel & Hinnen, 1990) we used the Saccharomyces cerevisiae strain YMR4, which is deficient in both the PHO5 and PHO3 genes. Preliminary experiments verified that YMR4 cells do not contain measurable extracellular ATPase activity while cells containing either acid phosphatase gene were able to hydrolyse extracellular ATP (unpublished observations).

Fig. 1 shows the time course of the efflux of ATP and ADP (a) and the intracellular amounts of ATP and ADP (b) in the presence and absence of glucose. It is clear that there is a glucose-dependent efflux of ATP from these cells. After 50 min the amount of extracellular ATP in the presence of glucose (Fig. 1a) is approximately six times the amount seen in the absence of glucose (Fig. 1a).

Having established that yeast cells release ATP, it was of interest to determine whether compounds known to be toxic to yeast would have any effect on this phenomenon. The rationale for this approach is that the Pgp, which is responsible for the extrusion of some toxins
ATP efflux from yeast

L7 1.6- 1.2- E E 5 I - 0.8 10 20 30 40 50 60

Time (min)

+ Glucose - Glucose

Fig. 1. Time course of ATP efflux. Samples were prepared as described in Methods. All incubation buffers were 20 mM Tris/citrate, pH 5.5, with or without 2% (w/v) glucose. (a) △, ▲, with glucose; ○, ●, without glucose; △, ○, extracellular ATP; ▲, ●, extracellular ADP. (b) Intracellular ATP (filled columns) and ADP (open columns) after 50 min incubation. Data represent means ± variances, n = 2.

E 5 1.6- 1.2- E E 5 I - 0.8 10 20 30 40 50 60

Time (min)

+ Glucose - Glucose

Fig. 2. Time course of nigericin-stimulated ATP efflux. Samples were prepared as described in Methods. All incubation buffers were 20 mM Tris/citrate, pH 5.5, 0.25% (v/v) ethanol, 25 μg nigericin ml⁻¹, with or without 2% glucose. (a) △, ▲, with glucose; ○, ●, without glucose; △, ○, extracellular ATP; ▲, ●, extracellular ADP. (b) Intracellular ATP (filled columns) and ADP (open columns) after 50 min incubation. Data represent means ± variances, n = 2.

from mammalian cells, seems to mediate the efflux of ATP (Abraham et al., 1993). Nigericin, a H⁺/K⁺ ionophore, was chosen because of its toxicity to yeast (Kovac et al., 1982) and because previous data indicated an interaction between nigericin and a mammalian Pgp when expressed in yeast (Boyum & Guidotti, 1997). Fig. 2(a) shows that nigericin dramatically stimulates the efflux of ATP (note the change in scale from Fig. 1). This efflux also requires the presence of glucose (Fig. 2a). These data demonstrate that the release of ATP from yeast is stimulated by nigericin, and that the stimulated efflux also requires glucose.

To further characterize ATP efflux, additional experiments were conducted with several classes of compounds (Table 1). These included the Na⁺/H⁺ exchanger monensin and the proton ionophores CCCP and FCCP, which dramatically stimulated ATP efflux; the plasma membrane proton pump inhibitor diethylstilbestrol (Serrano, 1988), which also stimulated ATP efflux; compounds which appear to interact with ABC transporters in yeast (Bissinger & Kuchler, 1994; Kuchler & Thorner, 1992; Servos et al., 1993), valinomycin, 4-nitroquinoline-N-oxide (4-NQO) and cycloheximide, which had no effect on ATP efflux; and an inhibitor of CFTR-mediated ATP efflux in mammalian cells, glybenclamide (Schwiebert et al., 1995), which had no effect on either the basal or the nigericin-stimulated efflux shown here. Fig. 3 shows that diethylstilbestrol and nigericin both affect ATP efflux in a concentration-dependent and saturable manner. At 25 μg ml⁻¹, the concentration used in these studies, the effect of nigericin was at a maximum, and the effect of diethylstilbestrol was also at a maximum at 1.9 mM. In all cases, ATP efflux required glucose.

Table 1. Effects of various compounds on ATP efflux

<table>
<thead>
<tr>
<th>Percentage of control</th>
<th>No. of assays</th>
</tr>
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<tbody>
<tr>
<td>Nigericin (0.034 mM)</td>
<td>411±1.7 3</td>
</tr>
<tr>
<td>Monensin (0.034 mM)</td>
<td>276±0.9 3</td>
</tr>
<tr>
<td>CCCP (0.034 mM)</td>
<td>337±9.1 3</td>
</tr>
<tr>
<td>FCCP (0.034 mM)</td>
<td>293±3.2 3</td>
</tr>
<tr>
<td>Diethylstilbestrol (1.9 mM)</td>
<td>134±5.2 2</td>
</tr>
<tr>
<td>Cycloheximide (0.034 mM)</td>
<td>97±1.2 2</td>
</tr>
<tr>
<td>4-NQO (0.034 mM)</td>
<td>98±5.4 2</td>
</tr>
<tr>
<td>Valinomycin (0.034 mM)</td>
<td>100±7.3 2</td>
</tr>
<tr>
<td>Glybenclamide (0.1 mM)</td>
<td>100±4.2 3</td>
</tr>
<tr>
<td>Glybenclamide (0.1 mM)</td>
<td>429±7.24 3</td>
</tr>
<tr>
<td>+ nigericin (0.034 mM)</td>
<td>429±7.24 3</td>
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Fig. 3. Dose dependence of diethylstilbestrol and nigericin- 
stimulated ATP efflux. Extracellular ATP samples were prepared 
as described in Methods. The incubation time for all samples 
was 50 min. The incubation buffer was 20 mM Tris/citrate, 
ph 5.5, 2% glucose. (a) Incubation buffers also contained 4% 
(DMSO and diethylstilbestrol at the indicated 
concentrations. Data were obtained from two to four 
dependent experiments. (b) Incubation buffers also contained 
0.25% ethanol and nigericin at the concentrations indicated. 
Data were obtained from two independent experiments. Data 
represent means ± SD.

The ATP efflux seen in acid phosphatase mutant cells is 
not specific to these cells. When wild-type yeast cells 
were used in the experiments, extracellular ATP was 
observed, but the amounts were small due to hydrolysis 
and therefore difficult to measure. For instance, when 
the experiment was conducted at pH 5.5, the amount of 
extraacellular ATP was undetectable by HPLC after a 
30 min incubation unless nigericin and glucose were 
included, in which case the extracellular ATP concen-
tration was 1.144 nmol ml⁻¹. When the same ex-
periment was conducted at pH 8.8, which partially 
inactivates the periplasmic acid phosphatases (data not 
shown), extracellular ATP was not detected in the 
absence of glucose, but its concentration was 
0.470 nmol ml⁻¹ in the presence of both nigericin and 
glucose. These results indicate that the release of ATP, 
and its stimulation by proton ionophores, is a general 
phenomenon in yeast, and not limited to cells lacking 
periplasmic acid phosphatases. Furthermore, since 
extraacellular adenosine is not metabolized or taken up 
by yeast cells (Anderson & Roth, 1976; Deeley, 1992), 
ATP release from wild-type yeast cells will lead to an 
accumulation of extracellular adenosine, which may 
then play a physiological role in addition to any role 
played by ATP.

If the effect of compounds which stimulate ATP efflux 
was to permeabilize the plasma membrane non-specifi-
cally, the relative concentrations of extracellular ATP 
and ADP would be a direct reflection of their 
intracellular concentrations. This would cause the efflux 
of ATP to appear to be glucose-dependent because the 
intracellular concentration of ATP is higher in the 
presence of glucose than in its absence (Figs 1 and 2). 
However, the extracellular concentration of ADP would 
be expected to be higher in the absence of glucose 
because its intracellular concentration was higher in the 
absence of glucose than in its presence. This is not the 

Case, however, because extracellular ADP was at very 
low levels when glucose was absent, even though the 
intracellular level of ADP increased under these con-
ditions. This point is illustrated in Figs 1 and 2: panels (a) 
show the level of extracellular ADP, and panels (b) show 
the intracellular levels of ATP and ADP at the end of the 
50 min incubation. In these experiments, the nucleotides 
quantified in panels (a) and (b) represent the amounts 
obtained from the same number of cells. In all cases, the 
efflux of ADP was higher in the presence of glucose than 
in its absence, even though the intracellular level of ADP 
was lower in the presence of glucose. Therefore, the 
observed increases in extracellular nucleotide are not a 
result of non-specific permeability.

Cell viability

The previous data concerning the glucose-dependence 
of ATP and ADP efflux suggest that nucleotide efflux is 
not due to lysis or non-specific permeability. In fact, 
incubation of cells with nigericin for up to 6 h did not
result in a decrease in the number of viable cells, as measured by a colony-forming assay, indicating that there was no cell lysis (Fig. 4). If the release of 25% of the intracellular ATP in 50 min (as seen in Fig. 2) were due to cell lysis, there should be an obvious loss of viability in the first hour in the presence of nigericin and glucose. However, there was no apparent loss of viability for more than 6 h following the addition of 25 μg nigericin ml⁻¹.

Fig. 5 shows that nigericin does not cause an increase in non-specific permeability as measured by the methylene blue assay. This assay is based on the observation that viable yeast cells exclude the dye from the cytoplasm and remain colourless while non-viable or permeable yeast cells stain blue (Hieda et al., 1984). This experiment provides a real time complement to the experiment shown in Fig. 4 because it demonstrates that nigericin does not cause a temporary and glucose-dependent increase in plasma membrane permeability. As a positive control for this assay, nystatin was shown to cause a dramatic increase in the percentage of blue cells that was independent of the presence of glucose. This is expected because nystatin is known to induce non-specific permeability of the plasma membrane (Ikehara et al., 1986).

Role of cAMP

The addition of glucose to yeast cells is known to induce an increase in intracellular cAMP (Thevelein et al., 1987b). Also, exposure of yeast to proton ionophores and diethylstilbestrol raises intracellular cAMP; this rise, unlike that caused by glucose, is due to intracellular acidification and does not occur at alkaline pH (Thevelein et al., 1987a). These data raise the possibility that ATP efflux from yeast is triggered by a rise in intracellular cAMP. The data shown in Table 2 support this hypothesis. When cells were incubated at pH 5.5, nigericin caused an initial rise in intracellular cAMP, as measured 7 min after the start of the experiment. In contrast, when cells were incubated at alkaline pH, a condition under which nigericin cannot induce intracellular acidification, the cAMP level did not rise above the level caused by glucose alone. When extracellular ATP was measured under the same conditions and at the same time point of 7 min, it was seen that nigericin induced ATP efflux to a much greater extent at pH 5.5 than at pH 8.8. The omission of glucose led to a lower level of cAMP in all cases and prevented appreciable ATP efflux in this short experiment (data not shown). The measurements of cAMP and ATP were made after a short time (7 min) because at later time points, cellular metabolism caused acidification of the medium which then allowed a nigericin-stimulated increase in intracellular cAMP and ATP efflux as was shown for the

Table 2. Role of cAMP in ATP efflux

Cells were prepared and incubated as described in Methods. All incubations included 2% glucose. pH 5.5 indicates that the incubation buffer was 20 mM Tris/citrate, pH 5.5; pH 8.8 indicates that the incubation buffer was 20 mM Tris, pH 8.8. Data were recorded after 7 min; n = 2 for cAMP, n = 1 for ATP. Data are expressed as a percentage of the amount seen in the absence of nigericin (cAMP, 27 pmol ml⁻¹ at pH 5.5 and 43 pmol ml⁻¹ at pH 8.8; ATP, 33 pmol ml⁻¹ at pH 5.5 and 27 pmol ml⁻¹ at pH 8.8). +Nigericin indicates the presence of 25 μg nigericin ml⁻¹.

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<tr>
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<th>pH 5.5</th>
<th>pH 8.8</th>
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<tbody>
<tr>
<td></td>
<td>+Nigericin</td>
<td>−Nigericin</td>
</tr>
<tr>
<td>Intracellular cAMP</td>
<td>181.2 ± 12.4</td>
<td>100.0 ± 11.3</td>
</tr>
<tr>
<td>Extracellular ATP</td>
<td>384.8</td>
<td>100.0</td>
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wild-type cells described earlier. These data show that at alkaline pH, while glucose affects both intracellular ATP and extracellular ATP, nigericin has no effect on either intracellular cAMP or extracellular ATP because it cannot acidify the cell under these conditions. This result supports the idea that cAMP is important in regulating ATP efflux.

ATP efflux during growth

The previous experiments demonstrate that yeast cells, when harvested and suspended in a defined buffer, release ATP under conditions in which the intracellular cAMP concentration rises. To address the physiological significance of this ATP efflux, it was necessary to determine whether ATP is also released from cells in actively growing cultures. Therefore, extracellular ATP was monitored during the growth of yeast cells. Fig. 6(a) shows that the growth of yeast was dramatically slowed by the addition of nigericin compared to growth in medium without nigericin. Fig. 6(a) also shows that ATP accumulated in both cultures and accumulated more rapidly in the nigericin-containing culture, even though this culture was growing much more slowly and thus contained a lower cell density at the later time points. Fig. 6(b) shows that cells eventually grew in a culture containing nigericin and that these cells released ATP to an extent similar to cells grown in medium without nigericin. The eventual growth of the cells in the presence of nigericin is not due to a simple removal of nigericin from the medium. These cells will grow without delay after dilution into fresh nigericin-containing medium (data not shown). The fact that acute exposure of the growing cells to nigericin led to increased ATP efflux (Fig. 6a) is consistent with what one would expect for a process mediated by a transient rise in intracellular cAMP. Apparently, ATP efflux and its stimulation by nigericin take place during normal growth, as well as under unusual experimental conditions. This suggests that ATP efflux is a component of yeast physiology.

DISCUSSION

It is clear from the present work that S. cerevisiae cells contain a glucose-dependent ATP release mechanism that is stimulated by proton ionophores and diethylstilbestrol, an inhibitor of the plasma membrane proton pump. The idea that ATP efflux is triggered by a rise in intracellular cAMP is supported by the following observations: glucose is absolutely required (Figs 1 and 2) and glucose is known to cause a rise in cAMP (Thevelein et al., 1987b); proton ionophores and diethylstilbestrol, which cause intracellular acidification and thereby raise the cAMP concentration (Thevelein et al., 1987a; Table 2), stimulate ATP efflux (Fig. 2 and Table 1); valinomycin, which can affect membrane potential but not intracellular pH or cAMP (Thevelein et al., 1987a), has no effect on ATP efflux (Table 1); cycloheximide, 4-NQO, and glybenclamide, which are unlikely to affect intracellular pH, have no effect on ATP efflux (Table 1); and nigericin does not stimulate ATP efflux under alkaline conditions that prevent cellular acidification and the subsequent rise in intracellular cAMP (Table 2). ATP efflux is seen in washed cells suspended in a defined buffer and in cells growing in liquid culture. The fact that cell lysis or non-specific permeability are not responsible for the increase in extracellular ATP is supported by three findings: the glucose dependence of ADP efflux (Figs 1 and 2), the maintenance of cell viability during nigericin exposure (Fig. 4) and the demonstration of plasma membrane integrity by the methylene blue assay (Fig. 5).

The glucose dependency of the protonophore effect on ATP efflux may be due to the fact that the cells are grown on glucose and are therefore glucose-repressed. It is known that glucose-repressed cells are unable to synthesize cAMP in the absence of glucose when exposed
to protonophores (Arguelles et al., 1990). The absence of a cAMP signal under these conditions would therefore result in no protonophore stimulation of ATP efflux. This is further indication of the role of cAMP in ATP efflux.

There is an apparent, but incomplete specificity of the ATP efflux mechanism. Other nucleotides, such as GTP, were sometimes detected and appeared to be released at very low levels along with ATP (data not shown). The low level of release of other nucleotide triphosphates may be a result of specificity for ATP, the very low intracellular concentration of these molecules in relation to ATP, or both. Since it is known that cAMP is released to the extracellular medium (Smith et al., 1990) and that the conditions that stimulate ATP efflux, the addition of glucose and proton ionophores, also cause a rise in intracellular cAMP, it is reasonable to speculate that ATP and cAMP may be released from the cell by the same carrier.

The increased appearance of extracellular ATP, caused by proton ionophores and diethylstilbestrol, is likely due to a stimulation of efflux rather than to interference with an ATP uptake mechanism because yeast cells are known not to have adenine nucleotide uptake activity (Anderson & Roth, 1976; Deeley, 1992). It has been shown, however, that adenine-requiring strains can satisfy the adenine requirement with adenosine if it gains access to the cytoplasm by detergent solubilization of the plasma membrane or by mutation, supporting the view that yeast cells do not normally contain an uptake mechanism for adenine nucleotides (Anderson & Roth, 1976; Deeley, 1992). One exception to this generalization is the finding that sporulating yeast cells apparently utilize extracellular nucleotides and these compounds are required for adenine auxotrophs to undergo sporulation (Jakubowski & Goldman, 1988).

The mechanism of this import into sporulating yeast cells is not known; however, both efflux and uptake may occur via non-specific permeability due to a lack of structural integrity of the plasma membrane. In contrast, the ATP efflux reported here is not a result of non-specific permeability of the plasma membrane.

Several, not necessarily exclusive, possibilities for the purpose of ATP efflux from yeast exist. One, suggested by the stimulation by various compounds, is that ATP efflux is involved in or is a consequence of a cellular detoxification mechanism, similar to one theory of Pgp activity (Abraham et al., 1993). A second possibility is that extracellular ATP is needed by an enzyme such as an ectokinase for some unknown biochemical function. A third possibility is that ATP is released as a means of regulating the intracellular ATP concentration or the local concentration of ATP at the plasma membrane. A very intriguing possibility is that extracellular ATP, or one of the products of its hydrolysis by the periplasmic acid phosphatases, ADP, AMP or adenosine, could perform an intercellular signalling function in yeast.

In summary, these studies demonstrate that yeast cells release ATP to the extracellular fluid, this release requires glucose and is stimulated by compounds that cause a rise in intracellular cAMP. This is the first step in determining whether the release of ATP from the cytoplasm and/or the presence of ATP extracellularly have a role in yeast physiology, possibly in intercellular signalling. Furthermore, the fact that yeast cells release and hydrolyse extracellular ATP opens additional questions concerning the role of the products of its hydrolysis, ADP, AMP and adenosine, as extracellular components of yeast physiology.

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REFERENCES


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