Glucose-induced activation of the plasma membrane H⁺-ATPase in *Fusarium oxysporum*

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Addition of glucose and other sugars to derepressed cells of the fungus *Fusarium oxysporum* var. *lini* triggered activation of the plasma membrane H⁺-ATPase within 5 min. Glucose was the best activator while galactose and lactose had a lesser effect. The activation was not prevented by previous addition of cycloheximide and it was fully reversible when the glucose was removed. The activation process *in vivo* also caused changes in the kinetic properties of the enzyme. The non-activated enzyme had an apparent K_m of about 3.2 mM for ATP whereas the activated enzyme showed an apparent K_m of 0.26 mM. In addition, the pH optimum of the H⁺-ATPase changed from 6.0 to 7.5 upon activation. The activated enzyme was more sensitive to inhibition by vanadate. When *F. oxysporum* was cultivated in media containing glucose as the major carbon source, enhanced H⁺-ATPase activity was largely confined to the period corresponding to the lag phase, i.e. just before the start of acidification of the medium. This suggests that the activation process might play a role in the onset of extracellular acidification. Addition of glucose to *F. oxysporum* var. *lini* cells also caused an increase in the cAMP level. No reliable increase could be demonstrated for the other sugars. Addition of proton ionophores such as DNP and CCCP at pH 5.0 caused both a large increase in the intracellular level of cAMP and in the activity of the plasma membrane H⁺-ATPase. Inhibition of the DNP-induced increase in the cAMP level by acridine orange also resulted in inhibition of the activation of plasma membrane H⁺-ATPase. These results suggest a possible causal relationship between the activity of *F. oxysporum* var. *lini* plasma membrane H⁺-ATPase and the intracellular level of cAMP.

Introduction

Plasma membrane H⁺-ATPases of fungi and plants participate in the creation and maintenance of electrochemical proton gradients necessary for the transport of nutrients. Internal alkalization and external acidification apparently resulting from H⁺-ATPase activity are associated with growth induction of yeast cells in glucose-containing media. Acidification of the external medium is due to metabolism of the sugar. When the extracellular pH is lower than the cytoplasmic pH, passive proton movement tends to acidify the cytoplasm. However, due to plasma membrane H⁺-ATPase activity the internal pH of yeast cells is maintained between 6.0

and 7.5 even when great variations of extracellular pH occur (Serrano, 1985, 1988). Genetic evidence has confirmed the importance of the H⁺-ATPase in both proton pumping and active nutrient transport (Serrano *et al.*, 1986a, b).

Recently, much interest has been paid to the post-translational mechanisms which regulate H⁺-ATPase activity. In *Saccharomyces cerevisiae*, it has been demonstrated that addition of glucose or related sugars to derepressed cells causes rapid activation of plasma membrane H⁺-ATPase (Serrano, 1983; Sychrová & Kotyk, 1985). Non-metabolizable sugar analogues had no effect (Serrano, 1983). Since the activation persists upon plasma membrane isolation, it seems to be the result of covalent modification. Phosphorylation of yeast H⁺-ATPase has been demonstrated by several groups

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(McDonough & Mahler, 1982; Yanagita *et al.*, 1987) and data have been presented which suggest that dephosphorylation leads to deactivation of the enzyme (Kolarov *et al.*, 1988). Recently, a correlation between activation and phosphorylation of specific sites on the protein has been demonstrated (Chang & Slayman, 1991). Addition of glucose to derepressed *S. cerevisiae* cells also induces a transient increase in the cAMP level which triggers a protein phosphorylation cascade (Thevelein, 1988). This suggests that H^+ -ATPase activation after glucose addition might be the result of a phosphorylation process, possibly catalysed by cAMP-dependent protein kinase. Conflicting evidence, however, has been presented with respect to cAMP involvement in the regulation of plasma membrane H^+ -ATPase (Mazon *et al.*, 1989; Ulaszewski *et al.*, 1989). In this paper, we report that glucose-induced activation of H^+ -ATPase is also observed in derepressed cells of the fungus *Fusarium oxysporum* var. *lini*.

It has been suggested previously that the difference in variation of the extracellular pH between *F. oxysporum* var. *lini* cultures grown in media containing either glucose or lactose as carbon source could be explained by a different degree of activation of the plasma membrane H^+ -ATPase by the two sugars (Brandão & Nicoli, 1988). In this paper, we demonstrate that the activation of plasma membrane H^+ -ATPase of *F. oxysporum* var. *lini* depends on the substrate used. In addition, evidence is presented suggesting a role for the transient sugar-induced increase in the cAMP level as the triggering event for activation.

Methods

Micro-organism and its maintenance. *Fusarium oxysporum* var. *lini* ATCC 10960 was maintained on potato dextrose agar (PDA). It was incubated at 30 °C for 7 d and then kept in a refrigerator at 4 °C for a maximum 15 d.

Culture conditions. Cells were grown in a gyratory incubator (200 r.p.m.) at 30 °C for 20 h. The culture medium contained 2% (w/v) peptone, 0.025% $MgSO_4 \cdot 7H_2O$, 0.16% NH_4Cl dissolved in a saline mixture (Fermor & Wood, 1981). The mycelium was harvested by filtration and washed with distilled water. Subsequently, the cells were transferred to a flask containing Vogel's saline medium (Vogel, 1956), in which KH_2PO_4 (37 mM) was replaced by KCl (37 mM). The cells were incubated at 30 °C for 5 h under shaking to allow derepression to occur. After being filtered and washed the cells were weighed and divided into individual samples. For the experiment on the growth kinetics of *F. oxysporum* in medium containing glucose as carbon source (Fig. 2), the cells were incubated in a New Brunswick fermenter as described before (Brandão *et al.*, 1988). The culture medium contained 2% (w/v) glucose, 0.2% peptone, 0.1% yeast extract, 0.15% KH_2PO_4 and 0.075% $MgCl_2$.

Activation conditions. In experiments on the activation of H^+ -ATPase, samples of 0.5–1.0 g (wet wt) were added to flasks containing 25 ml of 25 mM-MES buffer, pH 6.0, or 25 mM-glycine buffer, pH 5.0, for about 10 min at 30 °C before addition of organic substrates (2% g/vol. or 4% g/vol. in the case of lactose) or protonophores

Fig. 1. Effect of sugar addition on plasma membrane H^+ -ATPase activity in *F. oxysporum* cells. The sugars were added at time zero and samples were taken at the times indicated. □, ○, 2% (w/v) glucose with (□) or without (○) pre-incubation with 70 μ M-cycloheximide for 10 min. ●, Glucose removed after 5 min incubation; △, 2% (w/v) galactose; ■, 4% (w/v) lactose; ▲, control (buffer without sugar).

[2,4-dinitrophenol (DNP) 0.25–1 mM; carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) 0.1–0.5 mM]. DNP and CCCP were added in ethanol to give final ethanol concentrations of, respectively, 0.96% and 6% in the cell suspension. In the control experiments 6% ethanol was added: it had no effect on H^+ -ATPase activity or cAMP levels. To measure cAMP levels, samples of 1.65 g (wet wt) were incubated for 10 min at 30 °C in flasks containing 50 ml of the buffers described above before addition of sugars or protonophores. Samples of 2.5 ml were taken from the suspension and the cells collected as quickly as possible by vacuum filtration. The filters with the cells were immediately frozen in liquid nitrogen and subsequently used to determine cAMP levels.

Determination of H^+ -ATPase activity. The procedures used to obtain *F. oxysporum* plasma membranes and to determine H^+ -ATPase activity for the time curves of H^+ -ATPase activity *in vivo* and for determinations *in vitro* of vanadate sensitivity, K_m values and pH optimum were as described previously (Brandão & Nicoli, 1988). The buffer used for determination of the optimum pH was 50 mM-MES adjusted with Tris (Serrano, 1983).

cAMP determination. This was done as described previously (Thevelein *et al.*, 1987a).

Other determinations. Glucose consumption and dry weight were determined as described previously (Brandão & Nicoli, 1988). Protein was determined by the Lowry method with bovine serum albumin as standard.

Reproducibility of results. All experimental points are means of the values obtained in two independent experiments. The two independent experiments gave similar results.

Fig. 2. Properties of *F. oxysporum* plasma membrane H^+ -ATPase. (a) pH optimum, (b) inhibition by vanadate and (c) K_m of plasma membrane H^+ -ATPase activity. ●, Activated enzyme; ○, non-activated enzyme.

Results

Addition of different sugars to cells of *F. oxysporum* var. *lini* caused a varying degree of activation of the plasma membrane H^+ -ATPase (Fig. 1). As observed before with *S. cerevisiae* (Serrano, 1983; Sychrová & Kotyk, 1985), glucose showed the greatest activating effect. Pre-incubation of cells with $70 \mu\text{M}$ -cycloheximide before addition of glucose did not prevent the activation. If the cells were removed 5 min after glucose addition, washed and incubated again without glucose, the H^+ -ATPase activity decreased to the initial level in about 20 min.

Activation by glucose changed several properties of the H^+ -ATPase. H^+ -ATPase activity in plasma membranes of *F. oxysporum* var. *lini* cells obtained immediately before and 5 min after glucose addition was tested for pH optimum, sensitivity to vanadate and K_m value for ATP. Activation by glucose changed the pH optimum of the enzyme from 6.0 to about 7.5 (Fig. 2a), made the enzyme more sensitive to inhibition (non-competitive in both cases) by vanadate (K_i $145 \mu\text{M}$ for the activated enzyme and $226 \mu\text{M}$ for the non-activated enzyme in exp. 1; in exp. 2 $110 \mu\text{M}$ and $170 \mu\text{M}$, respectively) (Fig. 2b), and lowered its K_m value for ATP

Fig. 3. Cell growth (●), pH of the extracellular medium (○), glucose consumption (▲) and plasma membrane H^+ -ATPase activity (□) in a batch culture of *F. oxysporum* with glucose as the main carbon source.

(from 3.2 mM to 0.26 mM in exp. 1 and from 2.0 mM to 0.27 mM in exp. 2) (Fig. 2c). The V_{max} did not change appreciably [$19.8 \text{ nmol P}_i \text{ min}^{-1} (\text{mg protein})^{-1}$ in exp. 1 and $22.4 \text{ nmol P}_i \text{ min}^{-1} (\text{mg protein})^{-1}$ in exp. 2].

The growth kinetics of *F. oxysporum* var. *lini* cells in a medium containing glucose as the main carbon source are shown in Fig. 3. An increase in H^+ -ATPase activity occurred during the first 3 h with a peak corresponding to the end of the lag phase. Up to 12 h of culture, rapid growth and intense acidification of the extracellular medium occurred simultaneously. Afterwards, growth slowed and the pH of the medium stabilized at about 2.8 for up to 50 h of culture.

Addition of glucose also caused an increase in the cAMP level (Fig. 4). A cAMP increase caused by lactose and galactose could not be demonstrated reliably. Only the difference between glucose and the other sugars clearly fits with the effect on H^+ -ATPase activation (see Figs. 1 and 4). As opposed to previous results with *S. cerevisiae* (Mazon *et al.*, 1982; Purwin *et al.*, 1986; Thevelein *et al.*, 1987b; Van der Plaats, 1974) the cAMP increase observed in *F. oxysporum* is clearly lower. Therefore, we also studied the effect of the protonophores DNP and CCCP on the intracellular level of cAMP in *F. oxysporum*. Both DNP and CCCP caused pronounced cAMP increases with optimal concentrations of 0.75 mM and 0.25 mM, respectively (Fig. 5a). The effect of DNP and CCCP was strongly dependent on pH. Even with optimal concentrations, an effect was observed only at pH 5.0 (25 mM-glycine buffer) and not at all at pH 4.0 (25 mM-glycine buffer) or pH 6.0 (25 mM-MES buffer) (results not shown). Both DNP and CCCP also caused a strong and rapid activation of H^+ -ATPase (Fig. 5b). The optimum concentrations (0.75 mM for DNP and 0.25 mM for CCCP) were similar to those for the effect of DNP and CCCP on the cAMP level (results not shown).

Fig. 4. Sugar-induced cAMP increase in *F. oxysporum* cells. (a) 2% (w/v) Glucose; (b) 2% (w/v) galactose; (c) control (buffer without sugar). Sugars were added at time zero.

Fig. 5. Effect of protonophores on cAMP level (a) and plasma membrane H^+ -ATPase activity (b) in *F. oxysporum* cells. $\circ$, 0.75 mM-DNP; $\bullet$, 0.25 mM-CCCP.

Fig. 6. Inhibition by acridine orange of the stimulatory effect of 0.75 mM-DNP on the cAMP level (a) and plasma membrane H^+ -ATPase activity (b) in *F. oxysporum* cells. $\circ$, Control (no acridine orange); $\bullet$, 10 μ M-acridine orange; $\triangle$, 40 μ M-acridine orange.

Although these results show that both glucose and protonophores cause increases in the intracellular level of cAMP as well as activation of the plasma membrane H^+ -ATPase in *F. oxysporum* var. *lini* cells, the data did not allow conclusions as to whether there is a relationship between enzyme activation and cAMP increase. In an attempt to gain more evidence for such a relationship, we studied the effect of acridine orange on the increase in the cAMP level and the activation of H^+ -ATPase induced by addition of DNP. In *S. cerevisiae*, acridine orange is a potent inhibitor of *in vivo* cAMP synthesis

(Thevelein & Beullens, 1985). As shown in Fig. 6(a), previous addition of 10 μ M-acridine orange for 10 min partially inhibited the DNP-induced cAMP increase, whereas pre-incubation with 40 μ M-acridine orange completely eliminated it. DNP-induced H^+ -ATPase activation was affected by acridine orange in a very similar way (Fig. 6b). An increase in enzyme activity could still be observed when 10 μ M-acridine orange was used but the activation was completely suppressed at 40 μ M. Control experiments showed that acridine orange, in the concentrations used in the *in vivo* experiments, did

not have an inhibiting effect *in vitro* on the H⁺-ATPase activity present in *F. oxysporum* var. *lini* plasma membranes (data not shown).

Discussion

Many sugar-induced regulatory phenomena have been described in fungi, and have been studied in great detail in *S. cerevisiae* in particular. In general, with respect to the effectiveness of inducing these regulatory phenomena, one can draw a rough line between rapidly fermentable sugars such as glucose, fructose, mannose and sucrose, and other sugars which are more slowly metabolized such as galactose, maltose, lactose and trehalose. This difference is also observed for plasma membrane H⁺-ATPase activation both in *S. cerevisiae* (Serrano, 1983; Sychrová & Kotyk, 1985) and *F. oxysporum* (Fig. 1). Sugar-induced plasma membrane H⁺-ATPase activation in *Fusarium* also resembles activation of the enzyme in *Saccharomyces* in other respects. The sugar-induced shifts in the pH optimum, K_m value and vanadate sensitivity (Fig. 2) are very similar to those reported for *S. cerevisiae* (Serrano, 1983; Sychrová & Kotyk, 1985). Since these changes remain present after plasma membrane isolation, they most probably reflect covalent modification of the enzyme. The same conclusion was reached by Serrano (1983). In general, the glucose-induced response might be quite similar between *Fusarium* and *Saccharomyces*, since glucose-induced inactivation of the high-affinity glucose uptake system observed in derepressed *S. cerevisiae* cells (Bisson & Fraenkel, 1984), was also described for derepressed cells of *F. oxysporum* var. *lini* (Brandão & Loureiro-Dias, 1990).

Contradictory results have been published concerning the possible involvement of cAMP-dependent protein phosphorylation in the regulation of the plasma membrane H⁺-ATPase in *S. cerevisiae*. According to Ulaszewski *et al.* (1989), incubation of temperature-sensitive mutants defective in cAMP synthesis leads to a decrease in plasma membrane H⁺-ATPase activity. The inhibition is prevented by addition of exogenous cAMP in strains containing an additional mutation which allows suppression of the temperature-sensitive phenotype by external cAMP. In addition, it was shown by Eraso & Gancedo (1987) that incubation of yeast cells at low pH causes activation of the plasma membrane H⁺-ATPase. It is well known that intracellular acidification causes large increases in the cAMP level in yeast (Mazon *et al.*, 1982; Purwin *et al.*, 1986; Thevelein *et al.*, 1987a; Trevillyan & Pall, 1979). In *Fusarium*, stimulation of cAMP synthesis by protonophores was observed only in a very narrow pH range (pH $\pm$ 0.5) (Fig. 5a). This is

probably due to the sensitivity of the system to intracellular acidification, the efficiency of the protonophores in lowering the pH in *Fusarium* cells and also the fact that when the pH drops too low a general toxic effect may be expected. Recent experiments in our laboratory have shown that the protonophores CCCP and DNP also cause large increases in plasma membrane H⁺-ATPase activity in *S. cerevisiae* (J. Becher dos Passos, unpublished results). On the other hand, using different yeast mutants affected in the RAS/cAMP pathway M. J. Mazon's group (Portillo & Mazon, 1986; Mazon *et al.*, 1989) came to the conclusion that cAMP was not involved in the regulation of H⁺-ATPase activity. Mutants in the RAS-activating protein CDC25, however, were deficient in activation of the ATPase, which these authors concluded identified a new function for CDC25.

The glucose-induced transient increase in cAMP level reported for *Fusarium* cells in this paper (Fig. 4) extends the range of fungi in which such rapid cAMP spikes have been observed. Similar sugar-induced cAMP responses have been observed for vegetative cells of *Neurospora crassa* (Pall, 1977) and *S. cerevisiae* (Van der Plaats, 1974), and spores of *Mucor rouxii* (Dewerchin & Van Laere, 1984), *Phycomyces blakesleeana* (Van Mulders & Van Laere, 1984), *Pilobolus longipes* (Bourret, 1986) and *S. cerevisiae* (Thevelein, 1984). At the time of the initial discovery of protonophore-induced cAMP increases it was thought that plasma membrane depolarization was responsible for the observed effect (Pall, 1977; Trevillyan & Pall, 1979). Subsequent work, however, has clearly shown that intracellular acidification is the mediator of the protonophore effect on the cAMP level (Caspani *et al.*, 1985; Purwin *et al.*, 1986; Thevelein *et al.*, 1987a). Intracellular acidification might also be the mediator of the stress-induced effects on the cAMP level reported by Pall (1977) for *Neurospora* [see Thevelein (1991) for a recent extensive discussion].

Our results on sugar- and protonophore-induced activation of plasma membrane H⁺-ATPase in *Fusarium* also show a correlation with increased intracellular cAMP levels (Figs. 4 and 5). The parallel inhibition of H⁺-ATPase activation and increased cAMP levels appears to confirm the existence of a causal relationship between the two phenomena (Fig. 6). The specificity of acridine orange as an inhibitor of cAMP synthesis is not well established, however. In addition, because of the relatively small sugar-induced cAMP increases observed in *Fusarium*, it was not very meaningful to carry out the acridine orange inhibition experiment for sugar-induced activation of plasma membrane H⁺-ATPase.

Experiments with a wide range of yeast mutants affected in cAMP metabolism and/or protein phosphorylation could probably give a decisive answer to the

question of whether sugar-induced activation of plasma membrane H⁺-ATPase is mediated by cAMP as a second messenger. It cannot be excluded that the H⁺-ATPase is regulated by more than one mechanism. The effect observed by Ulaszewski *et al.* (1989) on H⁺-ATPase activity was much weaker than the effect observed on the cAMP level in the same mutants. In addition, Mazon *et al.* (1989) also observed reduced H⁺-ATPase activities at the restrictive temperature in temperature-sensitive mutants defective in cAMP synthesis. They had also shown that phorbol esters are able to stimulate plasma membrane H⁺-ATPase activity (Portillo & Mazon, 1985). Our results with *F. oxysporum*, although consistent with cAMP involvement, do not exclude the possibility of additional activation of the H⁺-ATPase by another pathway.

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References

- BISSON, L. F. & FRAENKEL, D. G. (1984). Expression of kinase-dependent glucose uptake in *Saccharomyces cerevisiae*. *Journal of Bacteriology* **159**, 1013–1017.
- BOURRET, J. A. (1986). Evidence that a glucose-mediated rise in cyclic AMP triggers germination of *Pilobolus longipes* spores. *Experimental Mycology* **10**, 60–66.
- BRANDÃO, R. L. & LOUREIRO-DIAS, M. C. (1990). Regulation of sugar transport systems in *Fusarium oxysporum* var. *lini*. *Applied and Environmental Microbiology* **56**, 2417–2420.
- BRANDÃO, R. L. & NICOLI, J. R. (1988). Participation des systèmes de captation des carbohydrates et de l'H⁺-ATPase de la membrane cytoplasmique lors de l'utilisation du glucose et du lactose par *Fusarium oxysporum* var. *lini*. *Canadian Journal of Microbiology* **34**, 979–986.
- BRANDÃO, R. L., NICOLI, J. R. & FIGUEIREDO, A. F. S. (1988). Purification and characterization of a β -galactosidase from *Fusarium oxysporum* var. *lini*. *Journal of Dairy Science* **70**, 1331–1337.
- CASPANI, G., TORTORA, P., HANOZET, G. M. & GUERRITORE, A. (1985). Glucose-stimulated cAMP increase may be mediated by intracellular acidification in *Saccharomyces cerevisiae*. *FEBS Letters* **186**, 75–79.
- CHANG, A. & SLAYMAN, C. W. (1991). Maturation of the yeast plasma membrane H⁺-ATPase involves phosphorylation during intracellular transport. *Journal of Cell Biology* **115**, 289–295.
- DEWERCHIN, M. A. & VAN LAERE, A. J. (1984). Trehalase activity and cyclic AMP content during early development of *Mucor rouxii* spores. *Journal of Bacteriology* **158**, 575–579.
- ERASO, P. & GANCEDO, C. (1987). Activation of yeast plasma membrane ATPase by acid pH during growth. *FEBS Letters* **224**, 187–192.
- FERMOR, T. R. & WOOD, D. A. (1981). Degradation of bacteria by *Agaricus bisporus* and other fungi. *Journal of General Microbiology* **126**, 377–387.
- KOLAROV, J., KULPA, J., BAIJOT, M. & GOFFEAU, A. (1988). Characterization of a protein serine kinase from yeast plasma membrane. *Journal of Biological Chemistry* **263**, 10613–10619.
- MCDONOUGH, J. P. & MAHLER, H. P. (1982). Covalent phosphorylation of the Mg²⁺-dependent ATPase of yeast plasma membranes. *Journal of Biological Chemistry* **257**, 14579–14581.
- MAZON, M. J., GANCEDO, J. M. & GANCEDO, C. (1982). Phosphorylation and inactivation of yeast fructose-bisphosphatase *in vivo* by glucose and by proton ionophores. A possible role for cAMP. *European Journal of Biochemistry* **127**, 605–608.
- MAZON, M. J., BEHRENS, M. M., PORTILLO, F. & PINON, R. (1989). cAMP- and RAS-independent nutritional regulation of plasma-membrane H⁺-ATPase activity in *Saccharomyces cerevisiae*. *Journal of General Microbiology* **135**, 1453–1460.
- PALL, M. L. (1977). Cyclic AMP and the plasma membrane potential in *Neurospora crassa*. *Journal of Biological Chemistry* **252**, 7146–7150.
- PORTILLO, F. & MAZON, M. J. (1985). Activation of yeast plasma membrane ATPase by phorbol ester. *FEBS Letters* **192**, 95–98.
- PORTILLO, F. & MAZON, M. J. (1986). The *Saccharomyces cerevisiae* start mutant carrying the *cdc25* mutation is defective in activation of plasma membrane ATPase by glucose. *Journal of Bacteriology* **168**, 1254–1257.
- PURWIN, C., NICOLAY, K., SCHEFFERS, W. A. & HOLZER, H. (1986). Mechanism of control of adenylate cyclase activity in yeast by fermentable sugars and carbonyl cyanide *m*-chlorophenylhydrazone. *Journal of Biological Chemistry* **261**, 8744–8749.
- SERRANO, R. (1983). *In vivo* glucose activation of the yeast plasma membrane ATPase. *FEBS Letters* **156**, 11–14.
- SERRANO, R. (1985). *Plasma Membrane ATPase of Plants and Fungi*. Boca Raton, Fla.: CRC Press.
- SERRANO, R. (1988). Structure and function of proton translocating ATPase in plasma membranes of plants and fungi. *Biochimica et Biophysica Acta* **947**, 1–28.
- SERRANO, R., KIELLAND-BRANDT, M. C. & FINK, G. R. (1986a). Yeast plasma membrane ATPase is essential for growth and has homology with (Na⁺ + K⁺), K⁺ and Ca⁺⁺-ATPases. *Nature, London* **319**, 689–693.
- SERRANO, R., MONTESINOS, C. & CID, A. (1986b). A temperature-sensitive mutant of the yeast plasma membrane ATPase obtained by *in vitro* mutagenesis. *FEBS Letters* **208**, 143–146.
- SYCHROVÁ, H. & KOTYK, A. (1985). Conditions of activation of yeast plasma membrane ATPase. *FEBS Letters* **183**, 21–24.
- THEVELEIN, J. M. (1984). Cyclic-AMP content and trehalase activation in vegetative cells and ascospores of yeast. *Archives of Microbiology* **138**, 64–67.
- THEVELEIN, J. M. (1988). Regulation of trehalase activity by phosphorylation-dephosphorylation during developmental transitions in fungi. *Experimental Mycology* **12**, 1–12.
- THEVELEIN, J. M. (1991). Fermentable sugars and intracellular acidification as specific activators of the RAS-adenylate cyclase signalling pathway in yeast: the relationship to nutrient-induced cell cycle control. *Molecular Microbiology* **5**, 1301–1307.
- THEVELEIN, J. M. & BEULLENS, M. (1985). Cyclic AMP and the stimulation of trehalase activity in the yeast *Saccharomyces cerevisiae* by carbon sources, nitrogen sources and inhibitors of protein synthesis. *Journal of General Microbiology* **131**, 3199–3209.
- THEVELEIN, J. M., BEULLENS, M., HONSHOVEN, F., HOEBECK, G., DETREMERIE, K., DEN HOLLANDER, J. A. & JANS, A. W. H. (1987a). Regulation of the cAMP level in the yeast *Saccharomyces cerevisiae*: intracellular pH and the effect of membrane depolarizing compounds. *Journal of General Microbiology* **133**, 2191–2196.
- THEVELEIN, J. M., BEULLENS, M., HONSHOVEN, F., HOEBECK, G., DETREMERIE, K., GRIEWEL, B., DEN HOLLANDER, J. A. & JANS, A. W. H. (1987b). Regulation of the cAMP level in the yeast *Saccharomyces cerevisiae*: the glucose-induced cAMP signal is not mediated by a transient drop in the intracellular pH. *Journal of General Microbiology* **133**, 2197–2205.
- TREVILLYAN, J. M. & PALL, M. L. (1979). Control of cyclic adenosine 3',5'-monophosphate levels by depolarizing agents in fungi. *Journal of Bacteriology* **138**, 397–403.
- ULASZEWSKI, S., HILGER, F. & GOFFEAU, A. (1989). Cyclic AMP controls the plasma membrane H⁺-ATPase activity from *Saccharomyces cerevisiae*. *FEBS Letters* **245**, 131–136.

- VAN DER PLAAT, J. B. (1974). Cyclic 3'-5'-adenosine monophosphate stimulates trehalose degradation in baker's yeast. *Biochemical and Biophysical Research Communications* **56**, 580-587.
- VAN MULDER, R. M. & VAN LAERE, A. J. (1984). Cyclic AMP, trehalase and germination of *Phycomyces blakesleeana* spores. *Journal of General Microbiology* **130**, 541-547.
- VOGEL, H. J. (1956). A convenient growth medium for *Neurospora* (Medium N). *Microbiology General Bulletin* **13**, 42-45.
- YANAGITA, Y., ABDEL-GHANY, M., RADEN, D., NELSON, N. & RACKER, E. (1987). Polypeptide-dependent protein kinase from baker's yeast. *Proceedings of the National Academy of Sciences of the United States of America* **84**, 925-929.