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Glucose-Induced Regulatory Defects in the *Saccharomyces cerevisiae byp1* Growth Initiation Mutant and Identification of *MIG1* as a Partial Suppressor

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***Saccharomyces cerevisiae byp1-3* mutants displayed a long lag phase when shifted from a nonfermentable carbon source to a medium containing glucose. The *byp1-3* mutation also caused several defects in regulatory phenomena which occur during the transition from the derepressed state to the repressed state. As opposed to wild-type cells, the addition of glucose to cells of the *byp1-3* mutant grown on nonfermentable carbon sources did not induce a cyclic AMP signal. Fructose-2,6-bisphosphate formation and inactivation of fructose-1,6-bisphosphatase were severely delayed, but trehalase activation was not affected. In addition, the induction of pyruvate decarboxylase both at the level of activity and that of transcription was very slow compared with that in wild-type cells. These pleiotropic defects in glucose-induced regulatory phenomena might be responsible for the very long lag phase of *byp1-3* cells and the inability of ascospores to initiate growth after germination on glucose media. Screening of a yeast gene library for clones complementing the *byp1-3* phenotype resulted in the isolation of a truncated form of the previously described zinc finger transcription repressor *MIG1*. The entire *MIG1* gene and the truncated form suppressed even on a single-copy vector the growth initiation defect but not the regulatory abnormalities of the *byp1-3* mutant. *MIG1* is not allelic to *byp1-3*.**

The addition of glucose to derepressed cells of the yeast *Saccharomyces cerevisiae* causes a series of rapid changes in enzyme activity due to posttranslational modification (12, 20) followed by slower changes which are due to repression (6) or induction (e.g., reference 17) at the transcriptional level. Identification of the glucose-induced signaling pathways triggering these events has attracted a lot of attention. Evidence has been obtained that cyclic AMP (cAMP)-dependent protein phosphorylation is involved in rapid inactivation of fructose-1,6-bisphosphatase, isocitrate lyase, and the galactose and high-affinity glucose carrier and in rapid activation of trehalase and phosphofructokinase 2 (22). The addition of glucose to derepressed yeast cells causes a rapid signal-like spike in the cAMP level (21). Incubation of temperature sensitive mutants in cAMP synthesis at the restrictive temperature or cAMP-requiring mutants in the absence of cAMP abolishes the glucose-induced cAMP signal and also the inactivation of fructose-1,6-bisphosphatase (27) and the activation of trehalase and phosphofructokinase 2 (7). This has been taken as evidence that the glucose-induced cAMP signal triggers phosphorylation of the enzymes by causing activation of cAMP-dependent protein kinase. Under these conditions, however, not only is the cAMP signal abolished, but the basal cAMP level in the cells is also much lower than that in wild-type cells. It has never been investigated whether the absence of the cAMP signal or the very low basal cAMP level is responsible for the absence of the changes in enzyme activity.

The induction of a cAMP signal in derepressed yeast cells by the addition of glucose or related fermentable sugars is mediated by a specific signaling pathway (for a recent review, see reference 21). The yeast RAS proteins and the RAS-activating protein CDC25 are essential components of the signal transduction pathway leading from glucose to cAMP (13, 28). Knowledge about the upstream part of the pathway is still limited; the affinity of the glucose receptor is relatively low (apparent K_m, 15 to 20 mM) (3); as far as sugar metabolism is concerned, only sugar kinase activity and no further metabolism of glucose is required for induction of the cAMP signal; and the pathway appears to contain a glucose-repressible protein (1, 3, 14, 31). Recently, several mutants which are deficient in induction of the cAMP signal by glucose, e.g., the *fdp1* mutant (30), the *lcr1* mutant (2), and, as shown in this paper, the *byp1-3* mutant, have been identified.

The *byp1-3* mutation belonged to one of three complementation groups identified by Breitenbach-Schmitt et al. (4) in a screening for mutations which block growth of single *pfk1* or *pfk2* mutants (*PFK1* and *PFK2* are the structural genes for phosphofructokinase) on glucose. In such *byp1 pfk* double mutants, and less in the *pfk* or *byp1* single mutant, hexose-monophosphates and sedoheptulose-7-phosphate, a metabolite of the pentosephosphate pathway, accumulate at unusually high levels (4). However, *byp1* was not allelic to any of the structural genes for glycolytic enzymes, and none of the enzymes of the pentosephosphate pathway was affected in the *byp1-3* mutant (4). Interestingly, the *byp1-3* mutant also accumulated vast amounts of fructose-1,6-bisphosphate, the product of the phosphofructokinase reaction, which was virtually absent in the *pfk1 pfk2* and *pfk byp1-3* double

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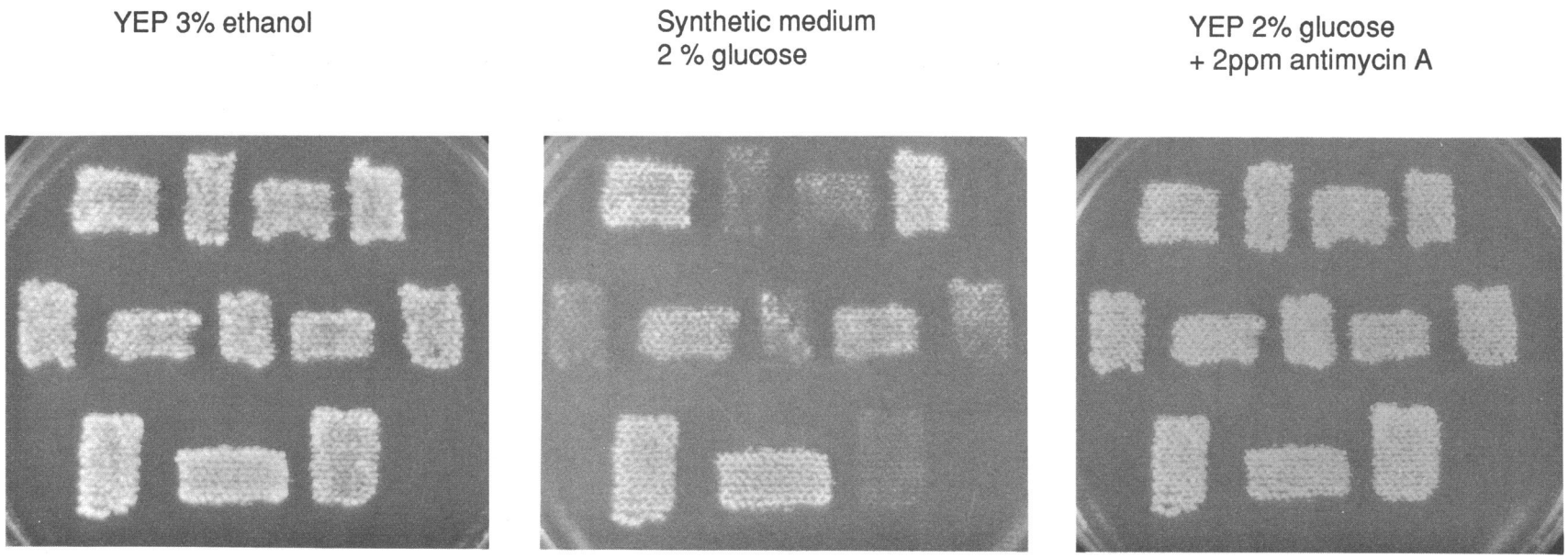

FIG. 1. Growth of *byp1-3* mutants and suppression by *MIG1* after replica plating from YEPE. Strains in each panel (from left): upper row, YSH1.1.-6B (wild type), YSH3.131.-6A (*byp1-3*), YSH3.131.-6D (*byp1-3*), and a heterozygous diploid wild type/*byp1-3*; middle row, homozygous diploid *byp1-3*/*byp1-3* (constructed from YSH3.131.-6A and YSH3.131.-6D), strain YSH3.131.-6A (*byp1-3*) transformed with *MIG1* on multicopy and single-copy plasmids, and strain YSH3.131.-6D (*byp1-3*) transformed with *MIG1* on multicopy and single-copy plasmids; bottom row, strains H174 (*mig1*Δ), the heterozygous diploid *byp1-3*/*mig1*Δ, and the haploid double mutant *byp1-3 mig1*Δ.

TABLE 1. Ethanol formation in wild-type strains and in *byp1-3* mutants[a]

Strain	Genotype	Ethanol formation (μmol/OD_{600}) after the following minutes[b]:		
		30	60	120
UTL-7A	Wild type	3.1 ± 0.3	5.7 ± 0.3	9.7 ± 0.4
ENY.WA-1A	Wild type	3.7 ± 0.2	7.2 ± 0.5	11.4 ± 0.9
YSH3.131.-6A	*byp1-3*	0.2 ± 0.2	0.4 ± 0.3	0.5 ± 0.3
YSH5.59.-4B	*byp1-3*	0.2 ± 0.2	0.4 ± 0.2	0.8 ± 0.1
YSH3.131.-6A pKHD8	*byp1-3*(ptr*MIG1*[c])	0.4 ± 0.2	0.9 ± 0.2	2.0 ± 0.6

[a] Cells were grown overnight on YEPE medium, diluted into fresh YEPE, and grown to an optical density at 600 nm (OD_{600}) of 0.5. Cells from 5-ml cultures were collected by centrifugation and resuspended in 5 ml of YEPD medium.
[b] At the times indicated, 1-ml aliquots were assayed for ethanol formation. Values are the means of five experiments.
[c] Plasmid containing the *MIG1* gene.

mutants (4). These and additional data (4) suggest that the *BYP1* gene product is needed for proper induction of the flux through the upper part of glycolysis.

The *byp1-3* mutation was crossed out of the original *pfk1* background and was backcrossed three times with strain YSH 1.1-6B (*MATα leu2-3,112 trp1-92 ura3-52*). When replica plated onto YEP (2% peptone, 1% yeast extract) or synthetic media with 2% glucose, *byp1-3* cells showed a lag phase that was prolonged compared with that of wild-type cells. On YEP 3% ethanol (YEPE) or YEP 2% glucose-plus-antimycin A plates, *byp1-3* and wild-type cells grew equally well (Fig. 1). In liquid YEPE no difference between the growth curves of wild-type and those of *byp1-3* cells was observed, but in liquid YEP-dextrose (YEPD) containing between 0.2 and 8% glucose, the *byp1-3* cells did not start growing within 28 h. In YEPD plus antimycin A, the *byp1-3* cells grew slowly and entered stationary phase at a density much lower (having divided just about four times) than that of the wild type. The growth characteristics of the heterozygous diploid (*BYP1/byp1-3*) were like those of the wild type, while the homozygous diploid (*byp1-3/byp1-3*) had the *byp1-3* phenotype. It was able to sporulate. Spore germination characteristics of *byp1-3* strains on YEPE, YEPD, and YEPD plus antimycin A were similar to the growth characteristics of vegetative *byp1-3* cells on these media. Microscopic investigation revealed that the *byp1-3* spores germinated on YEPD, giving rise to microcolonies of about 30 cells which stopped dividing further.

The growth initiation defect of the *byp1-3* mutant on glucose was clearly correlated with a defect in fermentation. After a shift from ethanol to glucose medium, the fermentation rate was very low compared with that of wild-type cells (Table 1). The addition of 100 mM glucose (Fig. 2A) or 100 mM fructose (not shown) to derepressed cells of the *byp1-3* mutant did not induce the typical cAMP signal observed for wild-type cells (24). The slow increase in the cAMP level observed in some experiments is probably an acidification artifact, as was observed previously, e.g., in cells of the *cat3* mutant (1). When glucose (Fig. 2A) or fructose (not shown) was added in Tris buffer (pH 7) or in YEP-glycerol, the slow cAMP increases nearly disappeared. Intracellular acidification as triggered by the addition of 2 mM 2,4-dinitrophenol to cells of the *byp1-3* mutant caused a huge increase in the cAMP level (results not shown). These results show that cAMP synthesis is not defective per se and that the *BYP1* gene product is required for stimulation of the RAS-adenylate cyclase pathway by fermentable carbon sources but not for stimulation of the pathway by intracellular acidification. They confirm a model that previously proposed that the upstream activation pathway of RAS-adenylate cyclase consists of an initial part which is specific for stimulation by fermentable sugar followed downstream by a second part which can also be stimulated by intracellular acidification (21). The addition of antimycin A together with glucose had no significant effect on the cAMP level in the time period in which the cAMP signal is observed (results not shown). It remains unclear how antimycin A suppresses the *byp1-3* growth initiation defect.

The addition of glucose to *byp1-3* cells grown in ethanol did not induce the typical increase in the fructose-2,6-bisphosphate level observed for wild-type cells (Fig. 2B). In addition, the basal level of fructose-2,6-bisphosphate before the addition of glucose was significantly higher than that in wild-type cells (Fig. 2B). The reason for this is not clear. The addition of glucose to derepressed yeast cells causes inactivation of fructose-1,6-bisphosphatase triggered by two distinct processes: (i) a rapid partial inactivation caused by cAMP-dependent protein phosphorylation (12) and (ii) a slower complete inactivation caused by proteolytic degradation (26). The addition of glucose to derepressed cells of the *byp1-3* mutant caused only a slight decrease in fructose-1,6-bisphosphatase activity compared with that in wild-type cells (Fig. 2C). Apparently, both the glucose-induced phosphorylation and proteolytic degradation processes are deficient in the mutant. Investigation of glucose-induced trehalase activation in derepressed cells of the *byp1-3* mutant showed that glucose triggered activation of trehalase comparable to that observed for wild-type cells (Fig. 2D). Similar results were obtained with other yeast strains which display a normal basal cAMP level but lack the glucose-induced cAMP signal (29). This appears to indicate that glucose-induced activation of trehalase does not require the glucose-induced cAMP signal but requires only a basal cAMP level. Since the cells used in our experiments are harvested in exponential phase, they should contain an adequate internal nitrogen supply, and since nitrogen-source-induced activation of trehalase is not mediated by a cAMP increase but is also dependent on glucose (10, 23), the alternative pathway activating trehalase in the *byp1-3* mutant might be the nitrogen-signaling pathway. A recent model for this pathway implies dependency on a basal level of cAMP (21).

Glucose induction of pyruvate decarboxylase synthesis (17) in cells of the *byp1-3* mutant was much slower than that in wild-type cells (Fig. 3A). This was due to a lack of induction of pyruvate decarboxylase at the mRNA level (Fig. 3B). Glucose repression (6) as measured by invertase activity or by the level of mitochondrial respiration was not affected (results not shown).

A yeast genomic library constructed in a single-copy vector (19) was screened for a plasmid which complements

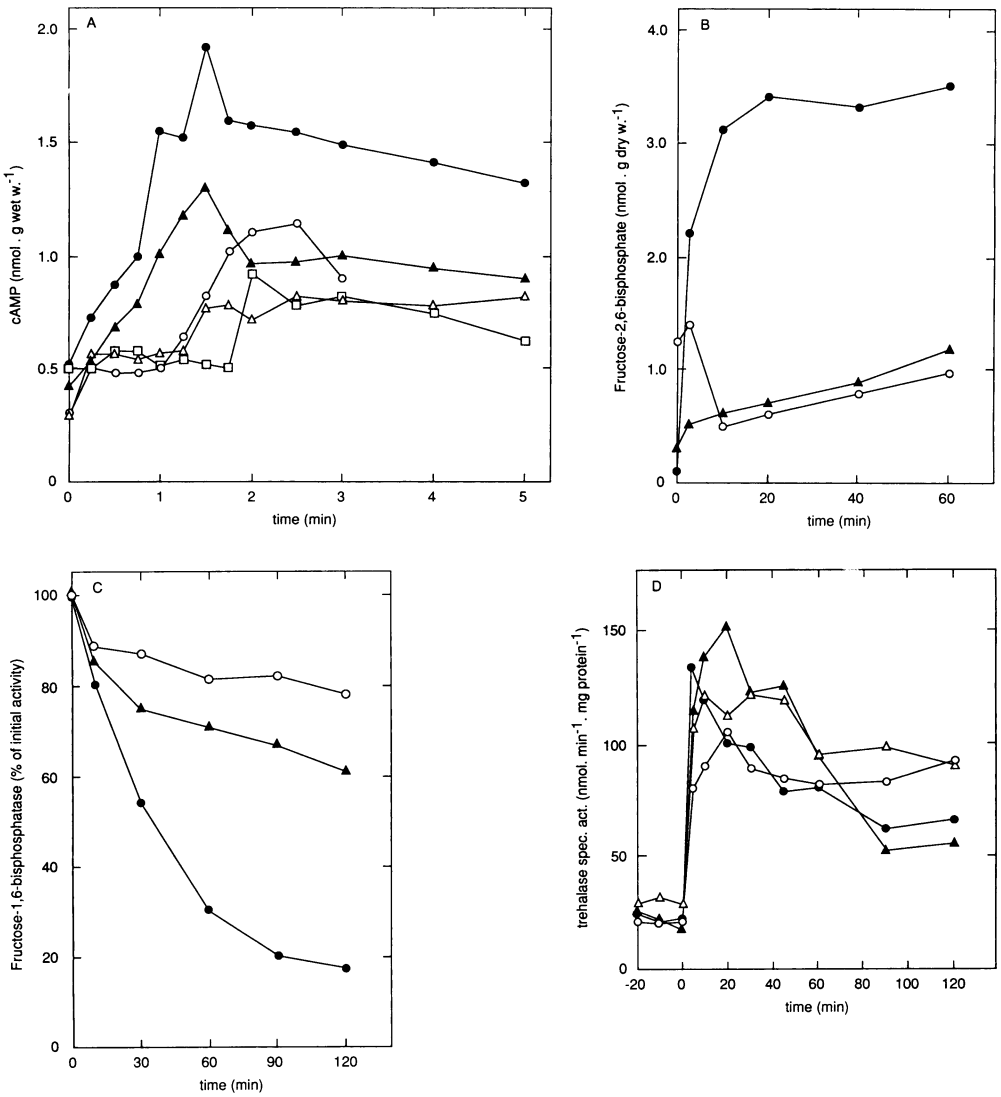

FIG. 2. Glucose-induced posttranslational regulatory effects. (A) cAMP levels after the addition of 100 mM glucose to cells of the *byp1-3* mutant (○, △, and □) and the corresponding wild type (● and ▲). The cells were suspended in 25 mM MES [2-(*N*-morpholino)ethanesulfonic acid] buffer (pH 6) (○ and ●), 25 mM Tris buffer (pH 7) (△, ▲), or YEPG (□). (B) Fructose-2,6-bisphosphate levels (32) in cells of the wild-type strain (●), the *byp1-3* mutant (○), and the *byp1-3* mutant transformed with the truncated *MIG1* gene (▲) after the addition of glucose (at 0 min) to derepressed cells. (C) Fructose-1,6-bisphosphatase activity (9) in cells of the wild-type strain (●), the *byp1-3* mutant (○), and the *byp1-3* mutant transformed with the truncated *MIG1* gene (▲) after the addition of glucose (at 0 min) to derepressed cells. (D) Glucose-induced activation of trehalase (25) in the *byp1-3* mutant (○ and △) and the corresponding wild type (● and ▲). The cells were suspended in 25 mM Tris buffer (pH 7) (○ and ●) or YEP-glycerol (△ and ▲).

the growth defect of the *pfk1Δ byp1-3* mutant. One plasmid suppressed the growth defect of the *pfk1Δ byp1-3* double mutant and that of the *byp1-3* mutant in both a single- and a multicopy vector. The suppressing subclone contained a single open reading frame coding for 386 amino acids which terminated after 6 amino acids at a fortuitous stop codon within the vector. Thus, we had isolated a truncated gene. Comparison of this sequence with the sequence of the recently identified MIG1 protein showed that we had isolated a truncated version of *MIG1* which codes for a protein of 504 amino acids. The MIG1 protein functions as a transcriptional repressor for genes regulated by glucose repression, like the *GAL* genes and the *SUC2* gene, which codes for invertase, and it binds to the DNA by virtue of two zinc fingers located close to the N terminus (16).

The complete version of *MIG1* also suppressed the *byp1-3* growth defect, and again, suppression was clearly more effective with the multicopy vector but also significant with the single-copy plasmid (Fig. 1). Moreover, the truncated version of *MIG1* could complement the glucose repression deficiency of invertase synthesis in a *mig1Δ* strain (not shown), indicating that the last 124 amino acids of MIG1 are not necessary for its function in glucose repression.

A cross of *byp1-3* with a *mig1Δ* strain (16) showed that both genes are distinct and that *MIG1* is a suppressor of the *byp1-3* mutation. Interestingly, the *mig1Δ byp1-3* double mutants grew even more slowly on glucose than the single *byp1-3* mutant (Fig. 1), while no growth impairment on glucose has been observed for the *mig1Δ* mutant (Fig. 1) (16). Inactivation of fructose-1,6-bisphosphatase, the in-

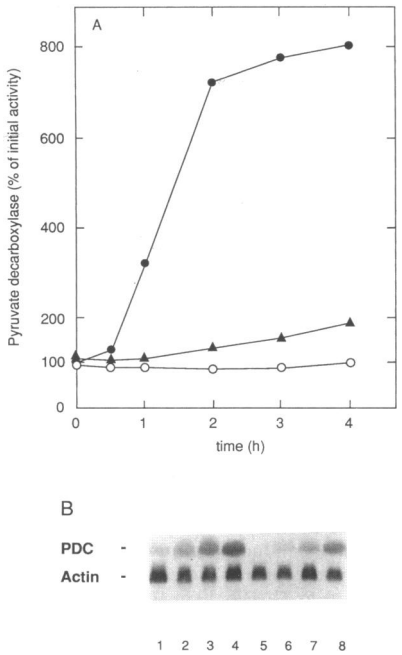

FIG. 3. Glucose-induced regulatory effect at the level of transcription. (A) Pyruvate decarboxylase activity (18) in cells of the wild-type strain (●), the *byp1-3* mutant (○), and the *byp1-3* mutant transformed with the truncated *MIG1* gene (▲) after the addition of glucose (at 0 min) to derepressed cells. (B) Northern (RNA) blot analysis of PDC induction in the *byp1-3* mutant and in the wild type. Shown is induction in the wild type (lanes 1 to 4) and in *byp1-3* (lanes 5 to 8) 0, 15, 30, and 60 min after the addition of glucose. Probes were *PDC1* (17) and actin (8), the latter as the constitutive control.

crease in the level of fructose-2,6-bisphosphate, and the induction of pyruvate decarboxylase synthesis were also not affected in the *mig1*Δ mutant.

Neither the truncated nor the complete *MIG1* gene restored any of the regulatory defects observed for the *byp1-3* mutant. Glucose-induced cAMP signaling (not shown), the glucose-induced increase in the fructose-2,6-bisphosphate level (Fig. 2B), glucose-induced inactivation of fructose-1,6-bisphosphatase (Fig. 2C), and glucose induction of pyruvate decarboxylase synthesis (Fig. 3A) were as they were in the untransformed *byp1-3* mutant. The restoration of ethanol production was partial (Table 1). Thus, growth on glucose was the only observed suppressed parameter. This situation is similar to the suppression of the growth defect of the *fdp1* mutant by *FPS1* even on a single-copy vector. *FPS1* also does not suppress any of the complex regulatory defects of the *fdp1* mutant (30). The FDP1 product is also involved in glucose-induced regulatory phenomena, and both the *fdp1* and *byp1-3* mutants are defective in growth initiation on glucose, although this effect is much more severe in the *fdp1* mutant.

We have recently also cloned the real *BYP1* gene (11). A deletion mutant constructed by using the cloned gene has growth characteristics similar to those of the *byp1-3* mutant described in this paper (11). The predicted amino acid sequence of *BYP1* has no similarities to that of *MIG1* or that of any other gene in the current data bases but is nearly identical to the sequence of *CIF1*, which has been entered into the data base recently but which has not been published. The *cif1* mutant grows on glucose but not on fructose (15), and *cif1* had been reported to be allelic to *fdp1* (5). The gene which we have cloned and identified as *BYP1* has been cloned and identified independently as complementing the growth defect of *fdp1* (11). A detailed characterization of the *BYP1* gene will be presented elsewhere.

The results show that the *BYP1* gene product is required for several regulatory effects observed after the addition of glucose to derepressed yeast cells. Either BYP1 is required for or it constitutes itself an important component located close to the origin in the signal transduction pathway(s) induced by glucose. The data obtained with the *byp1-3* mutant support the idea that glucose-induced cAMP signaling is required for fructose-1,6-bisphosphatase inactivation, for induction of pyruvate decarboxylase synthesis, and for the increase in the fructose-2,6-bisphosphate level, but they appear to contradict its requirement for the activation of trehalase. Since *MIG1* restores the growth defect of the *byp1-3* mutant, it appears that the observed glucose-induced regulatory events do not seem to be truly essential for growth initiation on glucose, a situation similar to the restoration by *FPS1* of growth on glucose in the *fdp1* mutant (30).

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REFERENCES

1. **Argüelles, J. C., K. Mbonyi, L. Van Aelst, M. Vanhalewyn, A. W. H. Jans, and J. M. Thevelein.** 1990. Absence of glucose-induced cAMP signaling in the *Saccharomyces cerevisiae* mutants *cat1* and *cat3* which are deficient in derepression of glucose-repressible proteins. Arch. Microbiol. **154:**199–205.
2. **Becher dos Passos, J., M. Vanhalewyn, R. L. Brandão, I. M. Castro, J. R. Nicoli, and J. M. Thevelein.** Glucose-induced activation of plasma membrane H^+-ATPase in mutants of the yeast *Saccharomyces cerevisiae* affected in cAMP metabolism, cAMP-dependent protein phosphorylation and the initiation of glycolysis. Biochim. Biophys. Acta, in press.
3. **Beullens, M., K. Mbonyi, L. Geerts, D. Gladines, K. Detremerie, A. W. H. Jans, and J. M. Thevelein.** 1988. Studies on the mechanism of the glucose-induced cAMP-signal in glycolysis- and glucose repression-mutants of the yeast *Saccharomyces cerevisiae*. Eur. J. Biochem. **172:**227–231.
4. **Breitenbach-Schmitt, I., H. D. Schmitt, J. Heinisch, and F. K. Zimmermann.** 1984. Genetic and physiological evidence for the existence of a second glycolytic pathway in yeast parallel to the phosphofructokinase-aldolase reaction sequence. Mol. Gen. Genet. **195:**536–540.
5. **Charlab, R., D. E. de Oliveira, and A. D. Panek.** 1985. Investigation of the relationship between *sst1* and *fdp* mutations in yeast and their effect on trehalose synthesis. Braz. J. Med. Biol. Res. **18:**447–454.
6. **Entian, K. D.** 1986. Glucose repression: a complex regulatory system in yeast. Microbiol. Sci. **3:**366–371.
7. **François, J., E. Van Schaftingen, and H. G. Hers.** 1984. The mechanism by which glucose increases fructose-2,6-bisphosphate concentration in *Saccharomyces cerevisiae*. A cyclic-AMP-dependent activation of phosphofructokinase 2. Eur. J. Biochem. **145:**187–193.
8. **Gallwitz, D., and J. Sures.** 1980. Structure of a split yeast gene: complete nucleotide sequence of the actin gene in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **77:**1546–2550.
9. **Gancedo, J. M., and C. Gancedo.** 1971. Fructose-1,6-diphosphatase, phosphofructokinase and glucose-6 phosphate dehy-

drogenase from fermenting and non-fermenting yeast. Arch. Microbiol. **76:**132–138.
10. **Hirimburegama, K., P. Durnez, J. Keleman, E. Oris, R. Vergauwen, H. Mergelsberg, and J. M. Thevelein.** Submitted for publication.
11. **Hohmann, S., L. Van Aelst, B. Bulaya, and J. M. Thevelein.** Unpublished results.
12. **Holzer, H.** 1984. Mechanism and function of reversible phosphorylation of fructose-1,6-bisphosphatase in yeast, p. 143–154. *In* P. Cohen (ed.), Molecular aspects of cellular regulation, vol. 3. Elsevier, Amsterdam.
13. **Mbonyi, K., M. Beullens, K. Detremerie, L. Geerts, and J. M. Thevelein.** 1988. Requirement of one functional *RAS* gene and inability of an oncogenic *ras* variant to mediate the glucose-induced cyclic AMP signal in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:**3051–3057.
14. **Mbonyi, K., L. Van Aelst, J. C. Argüelles, A. W. H. Jans, and J. M. Thevelein.** 1990. Glucose-induced hyperaccumulation of cyclic AMP and defective glucose repression in yeast strains with reduced activity of cyclic AMP-dependent protein kinase. Mol. Cell. Biol. **10:**4518–4523.
15. **Navon, G., R. G. Shulman, T. Yamane, T. R. Eccleshall, K.-B. Lam, J. J. Baronofsky, and J. Marmur.** 1979. Phosphorus-31 nuclear magnetic resonance studies of wild-type and glycolytic pathway mutants of *Saccharomyces cerevisiae*. Biochemistry **18:**4487–4499.
16. **Nehlin, J. O., and H. Ronne.** 1990. Yeast MIG1 repressor is related to the mammalian early growth response and Wilms' tumour finger proteins. EMBO J. **9:**2891–2898.
17. **Schmitt, H. D., M. Ciriacy, and F. K. Zimmermann.** 1983. The synthesis of yeast pyruvate decarboxylase is regulated by large variations in the messenger RNA level. Mol. Gen. Genet. **192:**247–252.
18. **Schmitt, H. D., and F. K. Zimmermann.** 1982. Genetic analysis of the pyruvate decarboxylase reaction in yeast glycolysis. J. Bacteriol. **151:**1146–1152.
19. **Sengstag, C., and A. Hinnen.** 1987. The sequence of the *Saccharomyces cerevisiae* gene PHO2 codes for a regulatory protein with unusual amino acid composition. Nucleic Acids Res. **15:**233–246.
20. **Thevelein, J. M.** 1988. Regulation of trehalase activity by phosphorylation-dephosphorylation during developmental transitions in fungi. Exp. Mycol. **12:**1–12.
21. **Thevelein, J. M.** 1991. Fermentable sugars and intracellular acidification as specific activators of the RAS-adenylate cyclase signaling pathway in yeast. Relation with nutrient-induced cell cycle control. Mol. Microbiol. **5:**1301–1307.
22. **Thevelein, J. M.** The RAS-adenylate cyclase pathway and cell cycle control in *Saccharomyces cerevisiae*. *In* L. Grivell (ed.), Antonie van Leeuwenhoek J. Microbiol., special issue. Molecular biology of yeasts, in press.
23. **Thevelein, J. M., and M. Beullens.** 1985. Cyclic AMP and the stimulation of trehalase activity in the yeast *Saccharomyces cerevisiae* by carbon sources, nitrogen sources and inhibitors of protein synthesis. J. Gen. Microbiol. **131:**3199–3209.
24. **Thevelein, J. M., M. Beullens, F. Honshoven, G. Hoebeeck, K. Detremerie, J. A. den Hollander, and A. W. H. Jans.** 1987. Regulation of the cAMP level in the yeast *Saccharomyces cerevisiae*: intracellular pH and the effect of membrane depolarizing compounds. J. Gen. Microbiol. **133:**2191–2196.
25. **Thevelein, J. M., A. J. Van Laere, M. Beullens, J. A. Van Assche, and A. R. Carlier.** 1983. Glucose-induced trehalase activation and trehalose mobilization during early germination of *Phycomyces blakesleeanus* spores. J. Gen. Microbiol. **129:** 719–726.
26. **Tortora, P., M. Birtel, A. G. Lenz, and H. Holzer.** 1981. Glucose-dependent metabolic interconversion of fructose-1,6-bisphosphatase in yeast. Biochem. Biophys. Res. Commun. **100:**688–695.
27. **Tortora, P., N. Burlini, G. Caspani, and A. Guerritore.** 1984. Studies on glucose-induced inactivation of gluconeogenetic enzymes in adenylate cyclase and cAMP-dependent protein kinase yeast mutants. Eur. J. Biochem. **145:**543–548.
28. **Van Aelst, L., E. Boy-Marcotte, J. H. Camonis, J. M. Thevelein, and M. Jacquet.** 1990. The C-terminal part of the CDC25 gene product plays a key role in signal transduction in the glucose-induced modulation of cAMP level in *Saccharomyces cerevisiae*. Eur. J. Biochem. **193:**675–680.
29. **Van Aelst, L., P. Durnez, M. Vanhalewyn, and J. M. Thevelein.** Unpublished results.
30. **Van Aelst, L., S. Hohmann, F. K. Zimmermann, A. W. H. Jans, and J. M. Thevelein.** 1991. A yeast homologue of the bovine lens fibre MIP gene family complements the growth defect of a *Saccharomyces cerevisiae* mutant on fermentable sugars but not its defect in glucose-induced RAS-mediated cAMP signaling. EMBO J. **10:**2095–2104.
31. **Van Aelst, L., A. W. H. Jans, and J. M. Thevelein.** 1991. Involvement of the *CDC25* gene product in the signal transmission pathway of the glucose-induced RAS-mediated cAMP signal in the yeast *Saccharomyces cerevisiae*. J. Gen. Microbiol. **137:**341–349.
32. **van Schaftingen, E., B. Lederer, R. Bartrons, and H. G. Hers.** 1982. A kinetic study of pyrophosphate:fructose 6-phosphate phosphotransferase from potato tubers. Eur. J. Biochem. **129:** 191–195.