IMP2, a gene involved in the expression of glucose-repressible genes in *Saccharomyces cerevisiae*

Tiziana Lodi, Paola Goffrini, Iliana Ferrero and Claudia Donnini

Author for correspondence: Claudia Donnini. Tel: +39 521 905602. Fax: +39 521 905604.
e-mail: molgen@IPRUNIV.CCE.UNIPR.IT

Istituto di Genetica,
Università di Parma, Viale
delle Scienze, 43100 Parma,
Italy

Two mutants carrying different deletions of the *IMP2* coding sequence of *Saccharomyces cerevisiae*, $\Delta T1$, which encodes a protein lacking the last 26 C-terminal amino acids, and $\Delta T2$, which completely lacks the coding region, were analysed for derepression of glucose-repressible maltose, galactose, raffinose and ethanol utilization pathways in response to glucose limitation. The role of the *IMP2* gene product in the regulation of carbon catabolite repressible enzymes maltase, invertase, alcohol dehydrogenase, NAD-dependent glutamate dehydrogenase (NAD-GDH) and L-lactate:ferricytochrome-c oxidoreductase (L-LCR) was also analysed. The *IMP2* gene product is required for the rapid glucose derepression of all above-mentioned carbon source utilization pathways and of all the enzymes except for L-LCR. NAD-GDH is regulated by *IMP2* in the opposite way and, in fact, this enzyme was released at higher levels in both *imp2* mutants than in the wild-type strain. Therefore, the product of *IMP2* appears to be involved in positive and negative regulation. Both deletions result in growth and catalytic defects; in some cases partial modification of the gene product yielded more dramatic effects than its complete absence. Moreover, evidence is provided that the *IMP2* gene product regulates galactose- and maltose-inducible genes at the transcriptional level and is a positive regulator of maltase, maltose permease and galactose permease gene expression.

Keywords: *Saccharomyces cerevisiae*, glucose derepression, transcriptional regulation

INTRODUCTION

The compartmentalization of biosynthetic and degradative pathways into distinct subcellular organelles is characteristic of eukaryotic cells. Mitochondria contribute to the biosynthesis of several metabolites and control various cellular processes besides those involved in energy production (for reviews, see Wilkie *et al.*, 1983; de Vries & Marres, 1987; Attardi & Schatz, 1988). In the yeast *Saccharomyces cerevisiae* the interactions between mitochondria and the nucleus are mainly considered in terms of genetic contributions to mitochondrial biogenesis (for reviews, see Attardi & Schatz, 1988; Grivell, 1989; Tzagoloff & Dieckmann, 1990). There are also indications

that the expression of a few nuclear genes strictly depends on either the state or the quality of mitochondria (Parikh *et al.*, 1987; Wang & Brandriss, 1987; Partaledis & Mason, 1988). Moreover, two nuclear genes that control interorganelle communication between mitochondria, peroxisomes and the nucleus have been identified (Liao & Butow, 1993).

We have approached the study of the involvement of mitochondria in various aspects of cell metabolism through the identification of nuclear genes that are required for growth on various carbon sources in the absence of functioning mitochondria (*IMP* genes) (Algeri *et al.*, 1981; Donnini *et al.*, 1992a, b). *IMP1*, the first gene identified in this way, was shown to be allelic to *GAL2*. The ability of *gal2* null mutants to grow on galactose under respiratory-sufficient, but not under respiratory-deficient conditions, argued that galactose metabolism does not obligatorily involve the induction of the sugar-specific transport system coded by *GAL2* and that

Abbreviations: ADH, alcohol dehydrogenase; EB, ethidium bromide; L-LCR, L-lactate:ferricytochrome-c oxidoreductase; MIC, minimum inhibitory concentration; NAD-GDH, NAD-dependent glutamate dehydrogenase.

mitochondria-dependent function(s) can substitute for the activity of this system. In particular, galactose uptake depends on mitochondrial supply of ATP (Donnini *et al.*, 1992a).

The *IMP2* gene was originally identified as required for maltose, galactose and raffinose utilization in the presence of mitochondrial inhibitors such as ethidium bromide (EB) or erythromycin. Under other conditions, the *imp2* mutation can cause pleiotropic defects in cell growth and development, such as poor growth on nonfermentable carbon sources, increased sensitivity to heat shock, and failure of homozygous diploid mutants to sporulate (Donnini *et al.*, 1992b). These findings indicate that *IMP2* contributes to a number of yeast cellular processes. The *IMP2* gene was cloned by complementation and sequenced, and the deduced amino acid sequence provided a few clues as to its possible cellular function; the predicted *IMP2* protein contains a long stretch of acidic amino acids and two putative nuclear localization signals (Donnini *et al.*, 1992b). Furthermore, the low codon bias index of the *IMP2* gene (CBI = 0.08) (T. Lodi, unpublished results) is indicative of a low level of expression and is consistent with the idea that *IMP2* may correspond to a regulatory gene.

All of the functions affected by the *imp2* mutation are controlled by carbon catabolism (Donnini *et al.*, 1992b). This observation together with the similarities of the pleiotropic defects exhibited by *imp2* mutants and mutants affected in intermediary elements necessary for derepression (Gancedo, 1992; Johnston & Carlson, 1992) imply that the *IMP2* gene product may be involved in the derepression of glucose repressible genes in response to a low glucose concentration. Through the molecular and physiological characterization of various deletion mutants here, we demonstrate that the *IMP2* gene does indeed play such a regulatory role.

METHODS

Strains, media and growth conditions. *Saccharomyces cerevisiae* strains used in this work include: KD-45B (α *leu2 his1 IMP2*; our collection); its *rho*⁻ derivative, obtained by three successive treatments on 2% (w/v) glucose in the presence of EB (20 µg ml⁻¹); the Δ T1 (*imp2-ΔT1::LEU2*) derivative, obtained as described by Donnini *et al.* (1992b); 45-4A (a *trp1 his1 ura3 imp2-1*), which carries a previously identified spontaneous mutation (Donnini *et al.*, 1992b). Yeast-rich medium (YP) contained Bactopeptone (2%, w/v), yeast extract (1%, w/v) and various carbon sources as specified in the text (2%, w/v, each); glucose was used at 5% (w/v) or 0.1% to produce either repressing or derepressing conditions. Exponential-phase cells grown on a reciprocating shaker (110 r.p.m.) at 28 °C in the presence of the indicated carbon sources were collected at 4 °C, washed twice with cold distilled water and shifted to YP media supplemented with the carbon sources indicated in the text. At fixed times (as specified in the text) samples of cells were withdrawn and the OD₆₀₀, enzyme activities or mRNA content determined. Heat-shock sensitivity was determined as described by Donnini *et al.* (1992b). *Escherichia coli* JM83 [*ara Δ(lac-proAB) rpsL (= strA) φ80 lacZ ΔM15*] and MC1066 strains (Casadaban *et al.*, 1983) grown in LB or M9 broth (Maniatis *et al.*, 1982) plus ampicillin (150 µg ml⁻¹) were used for plasmid amplification.

Transformation. Yeast transformation was performed as described by Ito *et al.* (1983). Transformants were selected on mineral minimal medium containing 2% (w/v) glucose plus the required amino acids at a final concentration of 40 µg ml⁻¹ each. Transformation of *E. coli* was performed as described by Mandel & Higa (1970).

Plasmids. Plasmids pFL44 and Yep13 were kindly provided by F. Lacroute and B. Guiard (Centre de Genetique Moleculaire du CNRS, 91198 Gif-sur-Yvette Cedex, France); plasmid pUC19 was purchased from Pharmacia. The plasmids pI-7 and pFL44/P were derived from the vectors pUC19 and pFL44, respectively, by filling in the *Pst*I site. The plasmid pFM1 was constructed by cloning the 2.1 kb *Sal*I-*Xba*I *IMP2* fragment (Donnini *et al.*, 1992b) into the vector pFL44/P. pFM1, linearized by *Pst*I digestion and deleted of the 1.6 kb *Pst*I-*Pst*I fragment containing the *IMP2* ORF, was utilized for transformation of the spontaneous mutant 45-4A in the gap repair experiment (see Results).

Gene disruption. The 2.1 kb *Sal*I-*Xba*I fragment containing the *IMP2* gene (Donnini *et al.*, 1992b) was cloned into the vector pUC19 (pI-7). The 1.6 kb region between *Pst*I sites, containing the complete ORF, was then deleted and replaced by the 4.1 kb *Pst*I-*Pst*I fragment of Yep13 containing the *LEU2* gene (pII-DL). pII-DL was cleaved at *Hind*III and *Xba*I sites and used to transform the haploid strain KD-45B (*leu2 IMP2*) to leucine prototrophy. Stable Leu⁺ Imp⁻ cells were selected on synthetic medium lacking leucine and gene disruption was confirmed by Southern analysis (data not shown).

Preparation of cell-free extracts. Cells were harvested by centrifugation, washed twice with distilled water, resuspended in 1.25 vols extraction buffer (prepared differently for each enzymic determination) and disrupted by vortexing at 4 °C in the presence of an equal volume of 0.5 mm diameter glass beads. The resulting suspension was then centrifuged at 5000 g for 5 min at 4 °C, the pellet was discarded, and the supernatant was used for enzyme activity measurements.

Enzyme assays. L-Lactate:ferricytochrome-*c* oxidoreductase (L-LCR) (EC 1.1.2.3) was assayed as described by Ferrero *et al.* (1984) in the presence of 50 mM L-lactate (lithium salt). NAD-dependent glutamate dehydrogenase (NAD-GDH) (EC 1.4.1.3) and total α -glucosidase (maltase, EC 3.2.1.20; isomaltase, EC 3.2.1.10) activities were assayed as described by Grisolia *et al.* (1964) and Zimmermann *et al.* (1977). Alcohol dehydrogenase (ADH) (EC 1.1.1.1) activity was measured by following the reduction of NAD at 340 nm (Hanson & Jacobsen, 1984). Extracellular invertase (EC 3.2.1.26) was assayed in whole cells (Celenza & Carlson, 1984) as described by Goldstein & Lampen (1975).

Isolation and analysis of DNA. Plasmids were purified by standard methods (Maniatis *et al.*, 1982). A previously reported method was used for the preparation of yeast DNA (Nasmyth & Reed, 1980). Agarose gel electrophoresis and Southern analysis were carried out as described by Maniatis *et al.* (1982).

Nucleotide sequence analysis. The sequence of the *imp2-1* allele was determined with the dideoxy chain-termination method (Sanger *et al.*, 1977) using Sequenase Version 2 (USB). Overlapping deletion subclones were produced by digestion with exonuclease III and S1 nuclease starting from opposite ends of the DNA insert. The sequence of both strands was determined by double-stranded plasmid DNA sequencing using the 17-nucleotide universal and reverse primers.

RNA analysis. Total RNA isolation and formamide agarose gel electrophoresis were carried by standard methods (Sherman *et al.*, 1982). Blotting to Hybond-N filter and hybridization to the appropriate 32 P-labelled DNA probes were performed by standard techniques (Maniatis *et al.*, 1982).

RESULTS

Effect of carbon sources and mitochondrial function on *IMP2* gene transcription

Variations of the steady state levels of the *IMP2* transcript in response to either changes of carbon sources or the functional state of the mitochondria were analysed by DNA:RNA hybridization using a 1 kb *HindIII*–*PstI* *imp2* fragment (Donnini *et al.*, 1992b) as a probe (Fig. 1). The cellular level of a 900 nt RNA strand, corresponding in size to the ORF of *IMP2*, was found to be essentially the same in all the carbon sources we have tested (Fig. 1a) and was independent of the functional state of the mitochondria (Fig. 1b).

Complete deletion of the *IMP2* coding sequence

An *IMP2* gene disruption which generated a haploid strain, $\Delta T1$ (*imp2*– $\Delta T1::LEU2$), carrying an *imp2* allele that encodes a protein lacking the last 26 C-terminal amino acids (Fig. 2), was previously isolated by Donnini *et al.* (1992b). This mutation repressed growth on galactose, maltose and raffinose for up to 3 d in the presence of EB. However, in contrast to a spontaneous *imp2*–1 mutant, $\Delta T1$ grew under all conditions after 5 d incubation (data not shown). Therefore, to obtain a

Fig. 2. Schematic representation of *IMP2* and mutant alleles. The *IMP2* ORF is shown as a black box. The inserted *LEU2* gene is shown as an open box. Positions of restriction sites are indicated: H, *HindIII*; P, *PstI*; S, *SalI*; X, *XbaI*.

mutant completely lacking the *IMP2* coding region a second gene disruption was constructed (see Methods). The haploid strain with the *IMP2* gene completely deleted was named $\Delta T2$ (*imp2*– $\Delta T2::LEU2$) (Fig. 2). Similarly to the growth behaviour of the spontaneous mutants carrying the *imp2*–1 allele, this mutant was unable to grow on maltose, galactose and raffinose in the presence of 20 μ g EB ml $^{-1}$. The minimum inhibitory concentration (MIC) of EB was found to depend on the type of the *imp2* allele, both in the presence of the above mentioned carbon sources as well as in media supplemented with 2% glucose (Table 1). Similar results were obtained with erythromycin, an inhibitor of mitochondrial protein synthesis (data not shown). No differences between mutants and wild-type were observed in the presence of the respiration inhibitor antimycin A or with the mitochondrial ATPase inhibitor oligomycin (data not shown). The strain carrying a partial deletion of the *IMP2* gene ($\Delta T1$) exhibited a MIC value of EB intermediate between that of the wild-type and that of the entirely deleted mutant ($\Delta T2$); the heat-shock sensitivity of the three strains was graded in a similar way (data not shown).

Molecular characterization of the spontaneous *imp2*–1 mutation

The different phenotypic behaviour for growth on carbon sources between $\Delta T1$ and $\Delta T2$, the last being similar to the spontaneous *imp2*–1 mutant, prompted us to establish the nature of the spontaneous mutation. The chromosomal allele *imp2*–1 was isolated on a plasmid by transforming the spontaneous mutant 45-4A (*imp2*–1 *ura3*) with a replicating vector containing a double-strand gap corresponding to the ORF of the gene (pFM1) (see Methods). To isolate 'gap-repaired' plasmids that had retrieved the appropriate chromosomal information, *Ura* $^{+}$ unstable *Imp* $^{-}$ strains were selected and the insert from the plasmid carrying the chromosomal allele *imp2*–1 was sequenced. One modification was detected in the ORF; at position +523, a glutamine codon (CAA) was changed to a nonsense codon (TAA) leading to the production of the truncated protein of 174 amino acids (Fig. 2).

Fig. 1. DNA:RNA blot analysis of *IMP2* transcripts. Total cellular mRNA was prepared from strain KD-45B. Cells were collected in the mid-exponential phase of growth in the different conditions described below. (a) Glucose, 2% (w/v) (lane 1), galactose (lane 2), maltose (lane 3), raffinose (lane 4). Each lane contained 20 μ g total mRNA. (b) Glucose, 2%, in respiratory-sufficient (RS) and respiratory-deficient (RD) conditions. The respiratory-deficient conditions were obtained as described in Methods. Each lane contained 40 μ g total mRNA. Hybridization was carried out with the nick translated plasmid containing *IMP2* (see the text) as a probe. Subsequent hybridization with a probe specific for yeast actin RNA confirmed that equal amounts of RNA were loaded in all lanes (data not shown).

Table 1. Dependence of EB MIC on allelic status of the *IMP2* gene

MIC was determined on plates of YP medium, containing 2% (w/v) glucose (G), maltose (M), galactose (Ga) or raffinose (R), spread with 1×10^5 cells, after 5 d incubation at 30 °C.

Relevant genotype	MIC ($\mu\text{g ml}^{-1}$)			
	G	M	Ga	R
<i>IMP2</i>	120	40	60	50
<i>imp2-ΔT1::LEU2</i>	70	30	30	30
<i>imp2-ΔT2::LEU2</i>	50	20	20	20

Derepression and induction of various carbon catabolite pathways in *IMP2* deletion mutants

The subsequent physiological analyses were carried out on the two differently deleted mutants, $\Delta T1$ and $\Delta T2$, and their isogenic wild-type strain. To verify whether the *IMP2* gene is required for derepression of carbon utilization pathways, we measured the growth rates of wild-type and both *imp2* deletion strains first cultured at high glucose concentration (5%) and then switched to YP media supplemented with raffinose, galactose, maltose or ethanol (2% each) (Fig. 3). The wild-type strain exhibited

lag times of 4 h, 2 h and 3 h on raffinose, galactose, and maltose or ethanol, respectively (Fig. 3a, b, c, d). Under all conditions, much longer growth delays were measured for both *imp2* mutants. In addition, both mutants usually showed lower cell yields at stationary phase than those of the wild-type. In all cases, the adaptive lag of $\Delta T1$ was shorter than that of $\Delta T2$ except when grown with ethanol (32 h and 24 h, respectively) (Fig. 3d). Therefore, the *IMP2* gene appears to influence derepression under all tested conditions.

Both mutants and the wild-type displayed very similar growth curves in all conditions when cells were pregrown either under derepressing conditions (ethanol) or under inducing conditions (galactose, maltose or ethanol) (data not shown). Therefore, *IMP2* does not appear to influence induction or growth efficiency on various carbon sources.

It is known that alteration of mitochondrial function results in a delay in adaptation to growth on galactose, maltose and raffinose by affecting cell permeability (Wilkie *et al.*, 1983). To determine if the negative effect on sugar utilization was due to an inhibitory effect of the *imp2* mutation on mitochondria, we analysed the growth behaviour of the *rho⁻* *IMP2* isogenic strain KD-45B. Under growth conditions identical to those previously applied to the *imp2* mutants, this strain exhibited a lag of 38 h on raffinose, 20 h on galactose and 6 h on maltose

Fig. 3. Kinetics of derepression/induction. Strains KD-45B *rho⁺* ($\diamond$), KD-45B *rho⁻* ($\blacklozenge$), $\Delta T1$ (∇) and $\Delta T2$ ($\circ$) were grown to mid-exponential phase on YP at high glucose (5%, w/v) and then shifted to 2% (w/v) raffinose (a), galactose (b), maltose (c) and ethanol (d). Samples were taken at the indicated time points for OD_{600} measurements.

Table 2. Enzyme activities of glucose-repressible enzymes during derepression

Enzyme units are expressed in nmol substrate min⁻¹ (mg protein)⁻¹. All values are means of three independent experiments. In no case was the variation higher than 15%.

Relevant genotype	Maltase			L-LCR			NAD-GDH			ADH		
	HG*	M†	M‡	HG*	LG†	LG‡	HG*	LG†	LG‡	HG*	E†	E‡
<i>IMP2</i>	0	993	3644	3	92	128	25	114	166	390	1638	28058
<i>imp2-ΔT1::LEU2</i>	0	0	3846	2	95	103	25	229	343	413	231	27802
<i>imp2-ΔT2::LEU2</i>	0	0	2612	6	100	117	13	213	369	370	155	21998

* Cells grown with 5% (w/v) glucose (HG) were harvested during exponential phase and enzyme activities determined.

† Cells grown with 5% glucose were transferred on 2% maltose (M), 2% ethanol (E) or 0.1% glucose (LG); 4 h after the transfer cells were collected, crude extracts prepared and enzymic activities determined.

‡ Cells were harvested during exponential growth on 2% maltose (M), 2% ethanol (E) or 0.1% glucose (LG).

Table 3. Secreted invertase activity during glucose derepression

All values are means of three independent experiments. In no case was the variation higher than 15%.

Relevant genotype	Time after transfer (h)*	Secreted invertase activity [nmol min ⁻¹ (mg dry weight) ⁻¹]				
		HG†	LG‡	R‡	Ga‡	E‡
<i>IMP2</i>	2	0	66	59	56	43
	4		110	102	99	72
	6		108	113	98	112
<i>imp2-ΔT1::LEU2</i>	2	0	31	0	0	0
	4		33	0	0	0
	6		26	0	0	0
<i>imp2-ΔT2::LEU2</i>	2	0	21	0	0	7
	4		29	0	0	14
	6		25	0	0	19

* At these times the cells were collected and extracellular invertase was assayed.

† Cells grown with 5% (w/v) glucose (HG) were harvested during exponential phase and enzymic activities determined.

‡ Cells grown with 5% glucose were transferred to a medium containing 0.1% glucose (LG), raffinose (R), galactose (Ga) or ethanol (E).

(Fig. 3a, b, c, respectively). Noticeably, the latter two delay times of the *rho*⁻ mutant are considerably shorter than the corresponding values for both *imp2* mutants.

Effect of different *imp2* alleles on the expression of glucose-repressible enzymes

The effect of glucose deprivation on the levels of maltase, L-LCR, NAD-GDH, ADH and secreted invertase was examined.

Induction of maltase requires a shift from high glucose to maltose (Vanoni *et al.*, 1989). In contrast to wild-type

cells, no maltase activity was detectable in either mutant 4 h after the transfer (Table 2). Maltase was eventually expressed, albeit with different delays, and it could be detected in both mutant strains during exponential growth on 2% maltose. Its activity in strain *ΔT2* was reduced by about 30% compared to strain *ΔT1* and the parental strain (Table 2).

L-LCR activity is detectable under nonrepressing conditions (low glucose, ethanol), induced by lactate and repressed by glucose (Somlo, 1965; Lodi & Guiard, 1991). As shown in Table 2, no marked differences in L-LCR activity were observed between mutants and wild-type cells first grown at high glucose (5%) and then shifted to a low glucose medium (0.1%, a nonrepressing/noninducing condition).

We determined cytoplasmic ADH activity in cell-free extracts from mutants and wild-type strains along the transition from a high glucose medium to an ethanol-supplemented medium. Under these two distinct experimental conditions, the activity measured at high glucose can be ascribed to fermentative ADH1, the cytoplasmic isoenzyme preferentially expressed in the presence of glucose (Denis *et al.*, 1983), whereas any increase of activity observed upon shifting to an ethanol medium would result from derepression of oxidative ADH2 (Denis *et al.*, 1983), the cytoplasmic isoenzyme repressed by glucose and induced by ethanol and glycerol (Lutstorff & Megnet, 1968). Comparable levels of ADH activity were present in all strains at a high glucose concentration (Table 2). In contrast, a differential response was observed 4 h after shifting to an ethanol-supplemented medium. Under these conditions, ADH activity increased in the wild-type KD-45B, whereas it was appreciably lower in cell extracts from either mutant. During exponential growth on 2% ethanol, the levels of ADH activity were high and very similar in *ΔT1* and wild-type strains, although the enzyme activity of strain *ΔT2* was 20% down.

NAD-GDH is a key enzyme that catalyses the oxidative deamination of glutamate to ammonia thus providing a link between nitrogen and carbon metabolism. This

Fig. 4. DNA:RNA blot analysis. Strains KD-45B (lane 1), $\Delta T1$ (lane 2) and $\Delta T2$ (lane 3) were grown in YP at high glucose (5%, w/v) to mid-exponential phase and then shifted to maltose (a, b) and galactose (c) for 4 h. Total RNA was then extracted and 20 μ g was loaded in each lane. Hybridizations were carried out with *MALS* (encoding maltase) in (a), *MALT* (encoding maltose permease) in (b) and *GAL2* (encoding galactose permease) in (c). Hybridizations were also performed to detect transcripts from the actin gene (*ACT1*) as a control.

enzyme activity is controlled via a nitrogen circuit as well as through carbon catabolite repression (Holzer & Schneider, 1957; Eraso & Gancedo, 1984; Miller & Magasanik, 1990). We tested the influence of *IMP2* on the glucose regulatory circuit of NAD-GDH by transferring both wild-type and mutants cells pregrown on high glucose to a low glucose (0.1%) medium. As shown in Table 2, the enzyme was similarly repressed by high glucose in all strains. Twice as much NAD-GDH activity was found in the mutants as compared to the wild-type strain upon derepression and this ratio was maintained during exponential growth on low glucose.

The activity of the secreted enzyme invertase, whose synthesis is only regulated by glucose repression (Carlson & Botstein, 1982), was analysed in whole cells (either wild-type and mutants) transferred from high glucose medium (5%) to low glucose (0.1%). As shown in Table 3, invertase activity was detectable 2 h after switching in both mutants though at lower activity values than in the parental strain. We then examined the possibility that a long adaptive lag, with respect to invertase derepression, occurred when either mutant strain was switched from a high glucose to a raffinose-supplemented medium. Both mutants failed to express invertase activity during the first 6 h after transfer to the raffinose medium and a similar defect was also evident in the case of a shift to galactose (Table 3). Surprisingly, upon transfer to ethanol-supplemented medium strain $\Delta T2$, but not strain $\Delta T1$, derepressed invertase activity to a final level corresponding to about one-sixth of the activity of the wild-type KD-45B strain (Table 3). Derepression of secreted invertase

thus appears to depend on the carbon source and on the allelic status of the *IMP2* gene. In contrast, roughly similar levels of invertase activity were measured in both mutant and wild-type strains during exponential growth on all of the carbon sources we tested (data not shown).

***IMP2* is required for derepression of *GAL2*, *MALS* and *MALT* genes**

To investigate a possible regulatory role of *IMP2* at the transcriptional level, we examined the expression of three genes involved in sugar utilization by DNA:RNA hybridization.

The utilization of the disaccharide maltose depends on any one of five unlinked loci (*MAL1*–*MAL4*, *MAL6*) (Vanoni *et al.*, 1989) and in *Mal*⁺ strains, maltase (*MALS*) and maltose transport activities (*MALT*) are coordinately induced by maltose and catabolite repressed by glucose (Vanoni *et al.*, 1989). *imp2* mutants were found to be defective in derepressing the 1.9 kb mRNA encoding maltase (Fig. 4a) and the 1.9 kb mRNA encoding maltose permease (Fig. 4b) within 4 h after transfer to maltose. It is also worth noting that trace amounts of maltase mRNA could be detected in strain $\Delta T2$, but not in the $\Delta T1$ mutant. Nevertheless, maltase activity was not detectable at this time (Table 2) nor 6 h after the transfer (data not shown) in cell extracts from either mutant.

Genes encoding enzymes involved in galactose catabolism (*GAL1*, *GAL2*, *GAL3*, *GAL7* and *GAL10*) are transcriptionally repressed by glucose and induced by galactose

(Johnston, 1987). Both mutants were unable to derepress the 1.7 kb mRNA corresponding to galactose permease (*GAL2*) 4 h after transfer to galactose (Fig. 4c). As in the case of maltase mRNA, traces of galactose permease mRNA could be detected in strain $\Delta T2$, but not in the strain $\Delta T1$ RNA preparation.

DISCUSSION

Glucose repression is a global regulatory mechanism that controls a large number of genes. Several elements involved in either glucose repression or derepression have been identified (for recent reviews see Gancedo, 1992; Johnston & Carlson, 1992; Trumbly, 1992). *IMP2* belongs to the latter group: it is required for the rapid glucose derepression of maltose, galactose, raffinose and ethanol utilization pathways. The finding that *IMP2* contributes to, but is not absolutely required for, derepression of these pathways could indicate that a functionally redundant protein may partially compensate for the loss of *IMP2*. In particular, the inability to derepress these pathways in the presence of the anti-mitochondrial drugs EB and erythromycin when the *IMP2* product is lacking implies that the level or functionality of this alternative activity may be limited somehow by the biosynthetic alteration of mitochondrial competence.

The steady state levels of the *IMP2* transcript are not influenced either by the presence or absence of glucose in the culture medium in which the cells are grown or by the particular respiratory conditions. This indicates that *IMP2* expression is regulated neither by catabolite repression nor by mitochondrial functioning.

To analyse the regulatory role of the *IMP2* gene we constructed a deletant mutant ($\Delta T1$) lacking the C-terminal region coding for the last 26 amino acids. Since this mutant displayed a growth phenotype less dramatic than that of the previously isolated *imp2-1* mutant a second completely deleted strain ($\Delta T2$) was constructed. Its growth behaviour on different carbon sources was similar to the *imp2-1* mutant. Sequence analysis of the *imp2-1* mutant allele demonstrated that it carries a nonsense codon that reduces the protein to about a half. These results can explain why the phenotype of the *imp2-1* mutant is more severe than that caused by the 26 amino acid C-terminal deletion in mutant $\Delta T1$.

mRNA analysis data indicated that the *IMP2* gene product appears to act positively to derepress transcription of maltase (*MALS*), maltose permease (*MALT*) and galactose permease (*GAL2*) genes. The precise mechanism of action remains to be established; however, delayed transcription of the *GAL2*, *MALS* and *MALT* genes upon derepression accounts for the absence of detectable levels of maltase, as well as for various defects observed with mutant cells during growth resumption in the presence of either galactose or maltose.

An intriguing result concerns the different behaviour of the two mutants with respect to: (i) invertase derepression in ethanol-supplemented medium (Table 3); (ii) adaptive lags after transfer to ethanol (Fig. 3d); and (iii) amounts of

maltase and galactose permease mRNAs measured 4 h after shifting from high glucose to maltose and galactose, respectively (Fig. 4a, c). In all the above cases the partial modification of the gene product causes more dramatic effects than its complete absence. A possible working hypothesis is that the *IMP2* gene product may function as a part of a larger protein complex. According to this model, the absence of this component could alter the complex in a way that allows the expression of some genes, perhaps as a consequence of changes in the binding affinity to DNA.

It is worth noting that both mutants were unable to quickly derepress invertase on either raffinose and galactose, but were able to derepress the enzyme when transferred to a low-glucose medium, albeit at levels lower than the wild-type. The levels of repressible enzymic activities, even those that reach a peak in the presence of an inducer such as L-LCR, are usually higher after a shift on low glucose than on other carbon sources (data not shown), probably due to the better energetic contribution. The increase in invertase activity to maximal levels within the first 6 h after transfer to a low glucose medium in the *IMP2* strain, coupled with low levels of invertase in the two mutants under the same conditions, is consistent with the possibility that alterations of the hypothesized complex may allow gene expression. The inability of *imp2* mutants to rapidly derepress both invertase on raffinose and oxidative ADH on ethanol can account for the long lags observed after the shift to these carbon sources.

NAD-GDH is regulated by *IMP2* in an opposite way to other enzymes; in fact, its activity is present at levels higher than the wild-type in *imp2* mutants. We thus conclude that the *IMP2* gene product may be involved not only in positive, but also in negative regulation. Regulatory proteins exerting both positive and negative effects on gene expression are well documented (for reviews see Herskowitz, 1989; Verdier, 1990). Moreover, carbon catabolite repression mutants of *S. cerevisiae* have been described which at high glucose are concomitantly hyper-repressed for mitochondrial functions such as L-LCR, D-lactate:ferricytochrome-*c* oxidoreductase and cytochrome *aa₃*, and derepressed for NAD-GDH (Donnini *et al.*, 1990).

IMP2 does not control derepression of the nuclear gene encoding the glucose-repressible mitochondrial enzyme L-LCR, indicating that the former gene does not play a general role in catabolite repression.

The effect of *imp2* mutations on sugar utilization could result from its influence on mitochondrial function. Indeed, mitochondrial mutants have an impaired capacity for sugar utilization (Mahler & Wilkie, 1978; Wilkie *et al.*, 1983) and we cannot exclude that *IMP2* may also regulate mitochondrial functions. Nevertheless, the stronger impact of *imp2* mutations with respect to *rho*⁻ mutation during adaptation to galactose and maltose growth argues that the action of *IMP2* can not be simply ascribed to an effect on mitochondrial competence, if any, but rather involves a direct effect on the expression of specific genes.

The finding that *imp2* partially impairs growth on lactate and glycerol (Donnini *et al.*, 1992b) but not on ethanol (this study) could be due to a different contribution of the *IMP2* gene product to the transcriptional control of glucose-repressible genes involved in the utilization of these carbon sources. However, the wild-type parental strains utilize ethanol more efficiently than glycerol and lactate. The better energetic contribution of ethanol could counterbalance an eventual effect of *imp2* mutation.

The results of the present work demonstrate that *IMP2* has a role in the derepression of a number of genes. However, the fact that *imp2* mutants (i) are defective in both sporulation and heat-shock sensitivity (Donnini *et al.*, 1992b), (ii) are more sensitive to biosynthetic mitochondrial inhibitors also on glucose and (iii) have levels of NAD-GDH activity higher than those of the parental strain strongly argue that the *IMP2* gene product is involved in other regulatory functions besides derepression.

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