REVIEW ARTICLE

Glucose control in *Saccharomyces cerevisiae*: the role of *MIG1* in metabolic functions

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Overview

Industrial cultivation media, such as molasses, wort, agricultural waste and lignocellulose hydrolysates, contain a mixture of metabolizable carbohydrates. These carbohydrates are taken up by cells in a certain order with intermittent lag phases due to a set of mechanisms controlled by glucose, referred to hereafter as glucose control. Thus, the presence or uptake of glucose has a negative impact upon the metabolism of other sugars. Glucose repression reduces the transcription rate of repressible genes, and is the most thoroughly investigated mechanism of glucose control (Fig. 1).

In this review the different mechanisms of glucose control in *Saccharomyces cerevisiae* are first discussed, focusing on the mechanism of glucose repression. Next, the function of the regulatory protein Mig1 in the signalling cascade of glucose repression is explained. The impact of Mig1-mediated glucose repression on metabolic functions is then discussed. It will be made clear that glucose repression is not confined to the above-mentioned example of mixed sugar metabolism. The last part of the review summarizes the effects glucose repression has on single metabolic functions. These components are then linked to a holistic view with the aim of understanding the effects of Mig1 on overall metabolism. Against this background possible physiological impacts of deletion/disruption of the *MIG1* gene are discussed.

Glucose control in *S. cerevisiae*

The transcription of a number of genes is repressed when *S. cerevisiae* is cultivated on rapidly fermentable sugars, i.e. glucose, fructose and mannose; the transcription of genes essential for the catabolism of slowly fermentable sugars is repressed, as well as gluconeogenic and mitochondrial functions (Gancedo, 1992; Ronne 1995). The Crabtree effect, i.e. ethanol formation in the presence of oxygen in strains of *Saccharomyces* and

related genera, can partially be explained by the lack of sufficient capacity for respiration resulting in overflow of pyruvate into ethanol fermentation reactions and partially by repression of mitochondrial function (de Deken, 1966; Fiechter *et al.*, 1981; Käppli, 1986). Since glucose is the most repressing sugar, the term 'glucose repression' is often employed. In some publications, glucose repression is used in terms of control of gene expression. In this review glucose repression is defined as transcriptional glucose repression, which together with mRNA degradation rate and translational efficiency controls gene expression. Besides the regulation of gene expression, glucose control includes two further regulatory mechanisms, namely accelerated protein degradation (carbon catabolite inactivation) and inhibition of the target enzyme (Fig. 1).

The concentration of a specific mRNA species in a cell is positively correlated with transcription efficiency and negatively with mRNA degradation. An increase in mRNA degradation in the presence of glucose, i.e. shorter half-lives of distinct mRNA populations, as a post-transcriptional mechanism has been reported (Federoff *et al.*, 1983; Lombardo *et al.*, 1992; Cereghino & Scheffler, 1996). The mechanism and control of

Fig. 1. Negative glucose control mechanisms.

mRNA turnover, however, has not been given as much attention as glucose repression, but has been reviewed recently (Caponigro & Parker, 1996).

Analogously to mRNA concentration, the concentration of a specific protein depends on translation efficiency and protein degradation. Knowledge about the impact of glucose on the translation rate in *S. cerevisiae* strains is limited (Parets Soler *et al.*, 1987; Gallie, 1996), but translational efficiency and mRNA turnover seem to be intimately linked (Caponigro & Parker, 1996). More attention has been paid to the post-translational modification of proteins triggered by glucose, generally referred to as carbon catabolite inactivation (Holzer, 1976). Carbon catabolite inactivation affects enzymes of peripheral functions, for example maltose permease and galactose permease (Görts, 1969; DeJuan & Lagunas, 1986), as well as enzymes of central functions such as the gluconeogenic enzymes fructose-1,6-bisphosphatase and phosphoenolpyruvate carboxykinase (Holzer, 1976). Vacuolar and proteasomal proteolysis have recently been identified as possible mechanisms of degradation, the former particularly for carrier proteins (Schork *et al.*, 1994; Riballo *et al.*, 1995; Horak & Wolf, 1997). Finally, glucose can inhibit the activities of various enzymes that are specifically related to sugars, as shown for enzymes that hydrolyse maltose and melibiose (Lazo *et al.*, 1978; Siro & Lövgren, 1978). Maltase has been reported to have a K_m value of 25 mM for maltose, but it is competitively inhibited by glucose, for which it exhibits a low K_i value of about 1 mM (Siro & Lövgren, 1978). The existence of various glucose control mechanisms makes it clear that glucose repression is only one of the mechanisms within the framework of glucose control. This is important to keep in mind when results from genetically engineered strains are to be interpreted.

The cascade of glucose repression and the role of *MIG1*

The two best investigated glucose signalling pathways are the Ras-adenylate cyclase pathway and the main glucose repression pathway. The former has been reviewed by Thevelein (1994), the latter additionally by Entian & Barnett (1992), Gancedo (1992), Trumbly (1992), Ronne (1995), and Entian & Schüller (1997). The Ras-adenylate cyclase pathway is operative in glucose-derepressed cells and acts transiently after glucose has been added. Sugar phosphorylation by any hexokinase (Hxk1, Hxk2, Glk1) leads to an accumulation of cAMP via the Ras-cAMP pathway, inducing a massive increase of cAMP-dependent protein kinase, which modifies a number of proteins post-translationally.

The main glucose repression pathway has the long-term function of keeping the cells repressed. It also includes the sensing of glucose, followed by a signal transfer and finally the physical binding of a repressor (downstream effector) to the promoter of the repressed gene. The key elements are the sensing hexokinase PII (Hxk2), the serine/threonine protein kinase Snf1 and the DNA-binding repressor Mig1. Knowledge about this repres-

Fig. 2. Key elements involved in the main glucose repression pathway.

sion cascade stems from genetic research, where derepression and repression mutants helped to identify the genes involved. Kinases seem to play a crucial role in this cascade, such as hexokinase PII (encoded by *HXX2*, which is identical to *HEX1*), which phosphorylates rapidly fermentable sugars and exhibits protein kinase activity, and a protein kinase that acts further downstream (encoded by *SNF1*, which is identical to *CAT1*), as illustrated in Fig. 2. Snf1 together with Snf4 (Cat3), is part of a protein kinase complex. It is activated by phosphorylation under derepressing conditions, and inactivated by dephosphorylation under repressing conditions (Jiang & Carlson, 1996; Wilson *et al.*, 1996). The phosphorylation correlates with a high AMP:ATP ratio, indicating the role of adenine nucleotides in glucose signalling (Wilson *et al.*, 1996; Hardie & Carling, 1997). The gene function of *GLC7*, as well as those of *REG1* (*HEX2*) and *REG2* are known for their antagonism to Snf1; they code for the protein phosphatase 1 complex and are therefore assumed to dephosphorylate Snf1 (Neigeborn, 1987; Tu & Carlson, 1995; Frederick & Tatchell, 1996; Jiang & Carlson, 1996). Moreover, Reg1 has been reported to be involved in the signal transfer for stimulated mRNA degradation (Cereghino & Scheffler, 1996). The precise interrelation of elements in the upper part of the signalling cascade is less well understood and will not be discussed further.

In 1990 Nehlin & Ronne cloned the *MIG1* gene (multicopy inhibitor of *GAL* gene expression). It is identical to *SSN1* (Vallier & Carlson, 1994) and to *CAT4* (Schüller & Entian, 1991). Its gene product is a C_2H_2 zinc finger protein, which resembles the mammalian Egr and Wilms' tumour proteins, the CreA repressor in *Aspergillus nidulans* and the Mig1 repressor in *Kluyveromyces lactis* (Nehlin & Ronne, 1990; Dowzer & Kelly, 1991; Cassart *et al.*, 1995). The *MIG1* promoter has been reported to be auto-regulated (Lundin *et al.*, 1994). Transcriptional repression is effected by a protein complex consisting of Ssn6, Tup1 and Mig1. Whilst Ssn6 and Tup1 confer the actual repression, the DNA-binding Mig1 directs them to the respective promoters (Keleher *et al.*, 1992; Treitel & Carlson, 1995) (Fig. 2). Mig1 is increasingly phosphory-

Table 1. Impact of the *MIG1* gene product on the expression of other genes

Part of metabolism affected	Gene name(s)	Function of gene product	EC no.	(Putative) Mig1-binding site	Physiological effect after <i>MIG1</i> :		Reference(s)
					Disruption	Overexpression	
Peripheral	<i>FPS1</i>	Osmosensitive glycerol uptake/efflux facilitator		Yes	Yes	NT	Lundin <i>et al.</i> (1994); Luyten <i>et al.</i> (1995); Klein <i>et al.</i> (1996)
	<i>GAL1</i>	Galactokinase	2.7.1.6	Yes	Yes	Yes	Nehlin & Ronne (1990); Nehlin <i>et al.</i> (1991); Lundin <i>et al.</i> (1994)
	<i>GAL3</i>	Inducer function protein		Yes	NT	NT	Lundin <i>et al.</i> (1994)
	<i>GAL4</i>	Transcriptional activator		Yes	Yes	Yes	Nehlin & Ronne (1990); Nehlin <i>et al.</i> (1991); Lundin <i>et al.</i> (1994)
	<i>GAL5</i> (PGM2)	Phosphoglucumutase 2	5.4.2.2	Yes	NT	NT	Fu <i>et al.</i> (1995)
	<i>GLK1</i>	Glucokinase 1	2.7.1.2	Yes	NT	NT	Sierkstra <i>et al.</i> (1992); de Winde <i>et al.</i> (1996)
	<i>HXK1</i>	Hexokinase 1	2.7.1.1	Yes	NT	NT	Sierkstra <i>et al.</i> (1992)
	<i>HXT2,3</i>	Hexose transporter	}	Yes	Yes	NT	Özcan & Johnston (1995); Reifemberger <i>et al.</i> (1995); Özcan & Johnston (1996); Walsh <i>et al.</i> (1996)
	<i>HXT4</i> (LGT1)	glucose sensor					
	<i>SNF3</i>	Glucose sensor, hexose transporter					
					Yes	NT	Wendell & Bisson (1994); Özcan & Johnston (1995); Reifeberger <i>et al.</i> (1995); Walsh <i>et al.</i> (1996)
	<i>MALR</i>	Regulatory protein		Yes	Yes	Yes	Lundin <i>et al.</i> (1994); Hu <i>et al.</i> (1995)
	<i>MALS</i>	Maltase	3.2.1.20	Yes	Yes	Yes	Lundin <i>et al.</i> (1994); Hu <i>et al.</i> (1995)
	<i>MALT</i>	Maltose transporter		Yes	Yes	Yes	Lundin <i>et al.</i> (1994); Hu <i>et al.</i> (1995)
	<i>MEL1</i>	Melibiose	3.2.1.22	Yes	NT	NT	Lundin <i>et al.</i> (1994)
	<i>SUC2</i>	Invertase	3.2.1.26	Yes	Yes	Yes	Nehlin & Ronne (1990); Lundin <i>et al.</i> (1994)
Central	<i>CAT8</i>	Regulatory protein Cat8p, derepressor of <i>FBP1</i> , <i>PCK1</i> , <i>ICL1</i> and <i>ACSI</i>		Yes	Yes	NT	Hedges <i>et al.</i> (1995); Kratzer & Schüller (1997)
	<i>COX5B</i>	Cytochrome- <i>c</i> oxidase subunit Vb	1.9.3.1	Yes	NT	NT	Hodge <i>et al.</i> (1990); de Winde & Grivell (1993)
	<i>CYT1</i>	Cytochrome <i>c</i> ₁		Yes	NT	NT	Oechsner <i>et al.</i> (1992); de Winde & Grivell (1993)
	<i>FBP1</i>	Fructose-1,6-bisphosphatase	3.1.3.11	Yes	No	No	Mercado <i>et al.</i> (1991); Mercado & Gancedo (1992); Lundin <i>et al.</i> (1994)
	<i>GGI1</i> (BYPI-3, CIFI, FDP1, TPS1, TSS1)	Subunit of trehalose-6-phosphate synthase, regulator of carbon catabolite inactivation, controller of glucose flux into glycolysis	2.4.1.15	Yes	NT	NT	Bell <i>et al.</i> (1992); González <i>et al.</i> (1992); Hohmann <i>et al.</i> (1992); Lundin <i>et al.</i> (1994)
	<i>HAP4</i>	Activation factor for transcription of genes involved in the oxidative phosphorylation, tricarboxylic acid cycle and gluconeogenesis		Yes	No	NT	Forsburg & Guarente (1989); Gancedo (1992); de Winde & Grivell (1993); Lundin <i>et al.</i> (1994); Rosenkrantz <i>et al.</i> (1994); Schlaepfer <i>et al.</i> (1994); Crawford <i>et al.</i> (1995)
	<i>ICL1</i>	Isocitrate lyase	4.1.3.1	Yes	No	NT	Schöler & Schüller (1993)
	<i>MIG1</i>	Repressor protein		Yes	NT	NT	Lundin <i>et al.</i> (1994)
	<i>MLS1</i>	Malate synthase	4.1.3.2	Yes	NT	NT	Schöler & Schüller (1993)
	<i>NTH1</i>	Neutral trehalase	3.2.1.28	Yes	NT	NT	Kopp <i>et al.</i> (1993)
	<i>PCK1</i>	Phosphoenolpyruvate carboxykinase	4.1.1.49	Yes	No	No	Mercado & Gancedo (1992)
	<i>PDC1</i>	Pyruvate decarboxylase (isoenzyme 1)	4.1.1.1	Yes	NT	NT	Lundin <i>et al.</i> (1994)
	<i>QCR8,9</i>	Ubiquinol-cytochrome- <i>c</i> oxidoreductase subunits VIII and IX	1.10.2.2	Yes	NT	NT	Maarse <i>et al.</i> (1988); de Winde & Grivell (1993)

NT, Not tested.

lated with decreasing glucose availability, possibly by Snf1 protein kinase, thus standing under negative control of Snf1 (Treitel & Carlson, 1995; DeVit & Johnston, 1996). The putative phosphorylation site as well as the effector domain of Mig1 were identified with the help of deletion mapping (Östling *et al.*, 1996). Apart from Mig1-redundant repressors, other probable DNA-binding proteins that guide Ssn6-Tup1 to different promoters are Rox1, α 2-Mcm1, α 1- α 2 and Rgt1 for hypoxic, MATa-specific, haploid-specific and glucose-induced genes, respectively (Keleher *et al.*, 1992; Deckert *et al.*, 1995; Özcan *et al.*, 1996).

There are generally three strategies that help to reveal Mig1-mediated repression: the first is a search for

putative Mig1-binding sites on the promoters of glucose-repressible genes, and the consensus motif has been suggested to be (G/C)(C/T)GG(G/A)G (Nehlin & Ronne, 1990). The existence of such a consensus sequence on a gene promoter is, however, no more than an indication that Mig1 might actually bind.

A second and qualitatively better strategy is provided by assays that prove Mig1 binding to the alleged promoter sequence *in vitro* (Nehlin & Ronne, 1990). In a footprint assay, the endonuclease DNase I hydrolyses DNA that is not covered by bound Mig1, the undigested sequence representing the putative binding sequence. In an oligonucleotide gel shift experiment, Mig1 is incubated with a DNA fragment that contains a putative Mig1-

binding site and that can be generated by saturation mutagenesis. If the mobility of the DNA fragment in an electrophoresis gel is decreased, Mig1 must have bound to it. The agarose gel shift assay is based on the observation that protein (Mig1)-binding to DNA causes a bend in the DNA and that the relative electrophoretic mobility of the protein-DNA complex is dependent on the protein-binding site on the respective DNA strand. By examining the mobility of various protein-DNA complexes obtained by cleaving DNA with different restriction enzymes the localization of the specific binding site can therefore be identified. These assays are performed *in vitro*, and therefore do not necessarily reflect *in vivo* affinities. In the context of Mig1 binding to, and affinity for, a particular DNA sequence, it must be stressed that the degree of repression is dependent on this DNA sequence, implying that glucose repression is not a pure on/off mechanism (Lundin *et al.*, 1994).

The third strategy is to investigate the physiological consequences of *MIG1* deletion/disruption and/or *MIG1* overexpression. Again, no absolute evidence can be provided; an example is the unchanged physiology of a $\Delta mig1$ mutant, which could well be explained by a Mig1-redundant protein, i.e. a protein that is similar to Mig1 and that can partly substitute for it. Recently, a second repressor, Mig2, 71% identical to Mig1, as well as a related protein with unknown function, Yer028, have been identified (Lutfiyya & Johnston, 1996). Mig2 has been shown to bind to some of the Mig1-binding sites. A double deletion mutant $\Delta mig1 \Delta mig2$ yielded a considerably higher derepression than the single $\Delta mig1$ mutant for *SUC2* expression, but not for *PCK1*, *FBP1*, *HXT2* and *GAL1* (Lutfiyya & Johnston, 1996). In view of the lack of knowledge about these redundant functions, the focus of this review will be kept on the function of *MIG1*. The eventuality of Mig1-related activation of transcription as well as a binding of activators on to the Mig1 consensus sequence will be discussed below.

Mig1-controlled metabolic functions

Since *MIG1* plays a pivotal role in glucose repression for many metabolic functions, they will be discussed one at a time. Metabolic functions can be grouped into central metabolism, which comprises glycolysis, the tricarboxylic acid cycle, fermentative and respirative functions, the glyoxylate shunt and gluconeogenesis, and the peripheral functions, such as the catabolism of sugars to the level of glucose-6-phosphate. Table 1 gives a survey of genes that are proposed to be under Mig1-mediated glucose repression.

Metabolism of disaccharides and trisaccharides

The disaccharides sucrose and melibiose and the trisaccharide raffinose are hydrolysed outside the cell membrane into monosaccharides, which are then taken up by the cell. Secretion of hydrolytic enzymes is essential because there are no major transport systems for

melibiose and raffinose. Since beet molasses contains 40–50% sucrose and up to 2% raffinose, extracellular sugar hydrolysis is important in industrial baker's yeast production, where beet molasses often is utilized as the main carbon source.

Invertase (EC 3.2.1.26) hydrolyses sucrose into glucose and fructose (Fig. 3). *SUC2* is the most commonly found structural gene in laboratory strains. *SUC2* transcription is repressed both by the complete absence of glucose and by high glucose concentrations, but requires low levels of glucose for maximal transcription (Özcan *et al.*, 1997). A *MIG1* deletion in a haploid strain resulted in a ninefold increase of *SUC2* mRNA levels when glucose was used as carbon source (Nehlin & Ronne, 1990). A *MIG1* disruption in an industrial baker's yeast strain as well as in another laboratory strain also led to a significant glucose derepression (Klein *et al.*, 1996; Olsson *et al.*, 1997). Nehlin & Ronne (1990) reported that *MIG1* overexpression results in an aggravated repression of *SUC2* expression and proved by DNase I footprinting that Mig1 binds to the *SUC2* upstream region, identifying possible recognition sequences. A second significant contribution to repression mediation is given by Mig2, as shown in single and double deletion mutants (Lutfiyya & Johnston, 1996). Thirdly, *SUC2* mRNA stability is decreased in the presence of glucose (Cereghino & Scheffler, 1995). Besides extracellular sucrose hydrolysis, direct sucrose uptake has been reported, playing an important role under repressing conditions (Santos *et al.*, 1982; Mwesigye & Barford, 1996).

Melibiose (EC 3.2.1.22) hydrolyses melibiose into glucose and galactose (Fig. 3). It is encoded by the *MEL* genes, of which *MEL1* is the most thoroughly investigated (Sumner-Smith *et al.*, 1985; Naumov *et al.*, 1996). *MEL1* is normally present in *Saccharomyces carlsbergensis*, but not in *S. cerevisiae* and belongs to the coordinately galactose-inducible and glucose-repressible *GAL/MEL* gene family (see also following section). There is evidence that *MEL1* expression is under Gal4 control (Post-Beittenmiller *et al.*, 1984; Johnston, 1987), whose expression is under Mig1 control (Nehlin *et al.*, 1991). Moreover, there are good indications that Mig1 directly binds to the *MEL1* upstream sequence, as verified for example by DNase I footprinting (Lundin *et al.*, 1994).

Maltose metabolism is important for brewing and bread-making. Malt brewer's wort contains 50–60% maltose, while plain dough mainly is composed of starch, which is made available to the yeast in the form of maltose, after hydrolysis by amylases. In contrast to the above-mentioned extracellular sugar hydrolysis, maltose is first taken up via maltose permease and then hydrolysed intracellularly by maltase (EC 3.2.1.20) into two units of glucose (Fig. 3). Maltose permease is encoded by *MALT*. It plays a crucial role for maltose metabolism, being essential not only for maltose uptake, but also indirectly for maltose induction of the *MAL* genes: *MAL* genes cannot be induced if maltose cannot

Fig. 3. Synopsis of (putative) Mig1-repressed genes and the metabolic role of their expression products; genes are given in brackets when they are under control of a Mig1-repressed regulator such as MalR, Gal4, Cat8 or Hap4.

be transported into the cell. Maltase is encoded by *MALS*. The third gene involved is *MALR*, which encodes a regulatory zinc finger protein essential for maltose induction and glucose repression of *MALT* and *MALS* expression.

Hu *et al.* (1995) identified Mig1-binding sites on promoters of *MAL* genes and quantified the glucose derepression caused by *MIG1* deletion as well as the increase in repression that is caused by *MIG1* overexpression. Glucose control was shown to be partly mediated by, and partly independent of, Mig1. The latter contribution could be due to other repressors (glucose repression), but is certainly due to other glucose control mechanisms, for example catabolite inactivation of maltose permease and the stimulated degradation of *MALS* transcripts (Görts, 1969; Federoff *et al.*, 1983). The Ras-cAMP pathway has been shown to be involved in the stimulated degradation of *MALT* mRNA and maltose permease inactivation (Wanke *et al.*, 1997). Since Mig1 regulates the transcription of the structural genes both directly, and indirectly via MalR, *MAL* gene control can be referred to as dual-level control.

Galactose metabolism

Galactose utilization and *GAL* gene regulation have been excellently reviewed by Johnston & Carlson (1992) and also by Lohr *et al.* (1995). Galactose is taken up by galactose permease (encoded by *GAL2*), phosphorylated by galactokinase (EC 2.7.1.6, encoded by *GAL1*), and further converted to glucose-6-phosphate by enzymes of the Leloir pathway, which are encoded by *GAL7*, *GAL10* (Fig. 3) and *GAL5* (*PGM2*) (encoding the major isozyme of phosphoglucomutase, EC 5.4.2.2, which, in the reverse direction, is pivotal for glycogen and trehalose anabolism). The transcription of *GAL2*, *GAL1*, *GAL7*, *GAL10* and *GAL5* is activated by the binding of the zinc finger transcription activator Gal4 on to their upstream sequences. Concomitant binding of Gal80 to the activation region prevents the transcriptional activation and is neutralized by the galactose-dependent binding of the regulatory protein Gal3, or, less efficiently, by that of the related Gal1 protein, to Gal80 (Johnston, 1987; Oh & Hopper, 1990; Zenke *et al.*, 1996; Yano & Fukasawa, 1997). Phosphorylation of Gal4 seems to be necessary for its competency to activate *GAL* transcription (Mylin *et al.*, 1990; Sadowski *et al.*, 1996).

Both *GAL4* and *GAL1* are repressed by Mig1, as proven biochemically by DNase I footprinting, and physiologically by *MIG1* deletion and *MIG1* overexpression (Nehlin & Ronne, 1990; Nehlin *et al.*, 1991). Thus, *GAL1* is under dual-level control (Mig1 and Gal4), whereas *GAL2*, *GAL7* and *GAL10* are at least under indirect *MIG1* control. Moreover, Mig1 has been shown to bind to the *GAL3* promoter and to have a consensus sequence on the *GAL5* promoter (Lundin *et al.*, 1994; Fu *et al.*, 1995). Interestingly, Gal5 has been reported to be post-translationally modified in response to glucose and galactose, however without apparent changes in enzymic activity (Fu *et al.*, 1995). Thus, the physiological

significance of this observation remains unclear. As concerns the overall glucose control, analogously to maltose permease, galactose permease is subject to catabolite inactivation (DeJuan & Lagunas, 1986; Horak & Wolf, 1997).

Metabolism of glucose, fructose and mannose

The sugars glucose, fructose and mannose are often referred to as rapidly fermentable sugars, with fructose and mannose being able to exert 'glucose repression' in many cases, but to a lesser extent than glucose (Gancedo, 1992; Thevelein, 1994). As to the uptake system for these sugars, the gene products of *HXT1-17* and *SNF3* are possible putative transporters for their facilitated diffusion (Celenza *et al.*, 1988; Reifemberger *et al.*, 1995; Kruckeberg, 1996; Fig. 3). Hxt1 through Hxt4, Hxt6 and Hxt7 are strongly suggested to mediate hexose uptake (Reifemberger *et al.*, 1995). Amongst the above-mentioned putative transporters, Snf3 and Hxt2 have so far been shown to be involved in regulation of glucose transport and in glucose sensing (Ko *et al.*, 1993; Wendell & Bisson, 1994; Liang & Gaber, 1996; Walsh *et al.*, 1996). *MIG1* deletion mutants have shown that *HXT2*, *HXT4* (= *LGT1*) and *SNF3* are subject to strong Mig1-mediated glucose repression, whereas Mig1 has a lower repressive impact on *HXT3* expression (Özcan & Johnston, 1995). The weak glucose repression of *HXT3* stands in contrast to a general induction of *HXT3* by glucose. It could be explained by a fine-tuned Mig1-mediated repression, i.e. a balance between glucose induction and repression gives the appropriate gene expression. As concerns *HXT2* and *HXT4* transcription, maximum transcription occurs at low glucose concentrations, since Rgt1-mediated repression is operative under complete absence of glucose and Mig1 mediates repression at high glucose concentrations (Özcan & Johnston, 1996; Özcan *et al.*, 1996). Full derepression of *HXT2* in the presence of glucose has been reported for a $\Delta mig1 \Delta mig2 \Delta yer028$ triple mutant (Lutfiyya & Johnston, 1996). In conclusion, glucose repression is mediated by Mig1 (*Mig2*, *Yer028*) to *SNF3*, *HXT2* and *HXT4*, whose gene products in turn may act as glucose sensors and be involved in the regulation of glucose transport. The glucose transport system has been shown to be subject to catabolite inactivation (Busturia & Lagunas, 1986).

Uptake of rapidly fermentable sugars is followed by phosphorylation by hexokinase PI or hexokinase PII (EC 2.7.1.1) or glucokinase (specific for glucose and mannose, EC 2.7.1.2), encoded by *HXK1*, *HXK2*, and *GLK1*, respectively. The *HXK2* gene plays a major role in the signalling cascade of the main glucose repression pathway (Fig. 2); the steady state level of *HXK2* mRNA is high in the presence of glucose and drastically reduced in the presence of ethanol, glycerol or galactose (Herrero *et al.*, 1995). The opposite is the case for *HXK1* and *GLK1* mRNAs, raising the question as to the physiological task of Hxk1 and Glk1, especially under repressing conditions (Herrero *et al.*, 1995). The implication is likely to be a rapid initial response of glucose

control for example via the Ras-adenylate cyclase pathway (transcriptional repression, mRNA degradation, catabolite inactivation), preceding a sustained long-term response in the form of glucose repression, where Hxk2 is involved (de Winde *et al.*, 1996; Sanz *et al.*, 1996). There is evidence that Mig1 is involved in glucose repression of *HXK1* and *GLK1* (Sierkstra *et al.*, 1992; de Winde *et al.*, 1996).

The function of the *GGI1/TPS1* gene is essential for growth on glucose since it directly or indirectly affects the influx of glucose into the glycolytic pathway, for which Thevelein & Hohmann (1995) have proposed three models. *Ggi1/Tps1* has been shown to be a positive regulator of fructose-1,6-bisphosphatase and maltose permease inactivation and of the stimulated mRNA degradation of *FBP1*, *PCK1* and *MALT* transcripts (Bell *et al.*, 1992; Wanke *et al.*, 1997; Dr Zhikang Yin, personal communication). There is a putative Mig1-binding sequence on the *GGI1/TPS1* promoter (González *et al.*, 1992), but *in vitro* binding with Mig1 could not be confirmed in oligonucleotide gel shift experiments (Lundin *et al.*, 1994).

Trehalose metabolism and glycerol influx/efflux

Glycogen and trehalose are the main storage carbohydrates in yeast. While glycogen is suggested to function as a main energy source, trehalose is recognized as a stress protectant that is essential for preservation and survival (Panek, 1991). The two key reactions in trehalose anabolism are catalysed by the trehalose-6-phosphate synthase/phosphatase complex, whose components have been reported to be subject to glucose repression and catabolite inactivation (François *et al.*, 1991; Thevelein & Hohmann, 1995). Mig1 is hypothesized to bind to the promoter of one encoding gene, *GGI1/TPS1* (González *et al.*, 1992) (Fig. 3). Moreover, there is evidence that the constitutive expression of *MAL* genes accelerates trehalose synthesis during growth on glucose (Petit & François, 1994). Therefore, a dual-level control of *GGI1/TPS1*, both directly by Mig1 and indirectly by gene products of *MAL* genes, can be assumed.

Trehalose is hydrolysed to glucose by neutral trehalase (EC 3.2.1.28), encoded by *NTH1* (Nwaka *et al.*, 1995), on whose promoter a possible Mig1-binding site has been found (Kopp *et al.*, 1993). A similar putative function of Mig1 on genes for two antagonizing reaction steps (*GGI1/TPS1* and *NTH1*) might implicate futile cycling in the absence of glucose and/or in $\Delta mig1$ mutants if there were not any further glucose control mechanism. Trehalose-6-phosphate synthase and neutral trehalase, however, have been shown to be, respectively, down- and up-regulated by post-translational modification in response to glucose (François *et al.*, 1991; Panek, 1991).

FPS1 encodes a channel protein that facilitates glycerol uptake and efflux (Luyten *et al.*, 1995) (Fig. 3). An oligonucleotide gel shift experiment proved Mig1 binding to a consensus sequence on the *FPS1* promoter

(Lundin *et al.*, 1994). This finding bears relevance for the anaerobic physiology of *S. cerevisiae* where glycerol secretion is necessary for the oxidation of excessive NADH. A deletion of *MIG1* would allow more glycerol to be secreted if secretion via *Fps1* were the rate-controlling step. Two $\Delta mig1$ strains were shown to secrete significantly higher amounts of glycerol compared to their parental strains (Klein *et al.*, 1996). This finding, however, does not exclude the possible influence of Mig1 on the other genes in the glycerol production pathway, such as *GPD1*.

Gluconeogenesis and glyoxylate shunt

Due to the unidirectional glycolytic reactions of phosphofructokinase and pyruvate kinase, the functions of *FBP1*, encoding fructose-1,6-bisphosphatase (EC 3.1.3.11), and *PCK1*, encoding phosphoenolpyruvate carboxykinase (EC 4.1.1.49), are essential for gluconeogenesis (Fig. 3). Both enzymes are subject to catabolite inactivation (Holzer, 1976). Both *FBP1* and *PCK1* have three consensus sequences on their promoters: for Mig1, for the activating Hap2/Hap3/Hap4/Hap5 complex (see below), and for the derepressing zinc finger Cat8 protein. Cat8 is related to Gal4, requires phosphorylation for activation (hypothetically by Snf1) and its expression is under the control of Mig1 (itself controlled via phosphorylation by Snf1) (Mercado *et al.*, 1991; Mercado & Gancedo, 1992; Hedges *et al.*, 1995; Randez-Gil *et al.*, 1997). Neither *MIG1* overexpression nor *MIG1* deletion had a significant effect on *FBP1* and *PCK1* expression (Mercado *et al.*, 1991; Mercado & Gancedo, 1992). The exceptionally high sensitivity of the mRNAs of these genes towards glucose can at least be partly explained by stimulated mRNA degradation, and has been shown to be independent of Mig1 (Mercado *et al.*, 1994; Yin *et al.*, 1996).

In the glyoxylate shunt, isocitrate and acetyl-CoA are converted to succinate and malate, without losing CO₂, in contrast to the tricarboxylic acid cycle. Preceding gluconeogenesis, this reaction sequence is the essential link between acetyl-CoA and oxaloacetate when yeast is growing on non-fermentable substrates, such as ethanol or acetate, and drastically repressed in the presence of glucose (Gancedo & Serrano, 1989). The glyoxylate shunt is catalysed by two enzymes: isocitrate lyase (EC 4.1.3.1, encoded by *ICL1*) and malate synthase (EC 4.1.3.2, encoded by *MLS1*) (Fig. 3). Both genes contain putative Mig1-binding sites in their promoter regions (Schöler & Schüller, 1993). Moreover, the expression of *ICL1* and of *ACS1* depends on the derepressing CAT8 gene function (Hedges *et al.*, 1995; Kratzer & Schüller, 1997). *ACS1* encodes the glucose-repressible acetyl-CoA synthetase (EC 6.2.1.1), which is essential for the entry of ethanol/acetate into the tricarboxylic acid cycle.

Fermentative functions, tricarboxylic acid cycle and respiratory functions

The main carbon flux splits at the level of pyruvate into the tricarboxylic acid cycle and alcoholic fermentation

(Fig. 3). Activities of the enzymes in each of the two branches can be assumed metabolically to control the ratio of the bifurcating fluxes. Pyruvate decarboxylase (EC 4.1.1.1) converts pyruvate to acetaldehyde, the first step in ethanol formation. The most important gene relative to growth on glucose is *PDC1* (Hohmann, 1991, 1993). DNase I footprinting revealed that Mig1 binds on to the promoter of *PDC1* (Lundin *et al.*, 1994), which seemingly stands in contradiction to the finding that *PDC1* expression is increased in the presence of glucose (Schmitt *et al.*, 1983). This issue will be further discussed below.

The first step of the tricarboxylic acid cycle is catalysed by mitochondrial citrate synthase (EC 4.1.3.7), encoded by *CIT1* and *CIT3* (Fig. 3). The expression of the *CIT1* gene requires an activation complex that contains Hap4, which is under *MIG1* control (Rosenkrantz *et al.*, 1994). The conversion from α -ketoglutarate to succinate is catalysed by the α -ketoglutarate dehydrogenase complex, whose components are encoded by *KGD1*, *KGD2* and *LPD1*. All three genes have been shown to be activated by Hap4 (de Winde & Grivell, 1993). The dihydrolipoyl dehydrogenase (encoded by *LPD1*) is also a component of the pyruvate dehydrogenase complex; thus the repression of one gene affects two enzyme complexes (Fig. 3).

Oxidative phosphorylation takes place in the mitochondria. The transcription of a number of genes that encode components of the respiratory chain and of the mitochondrial gene expression machinery is induced by oxygen and repressed by fermentable carbon sources (de Winde & Grivell, 1993). The genes *HAP2*, *HAP3*, *HAP4* and *HAP5* code for proteins that form a promoter-binding, activating complex and that regulate different respiratory functions, Hap4 appearing to be limiting in this complex (Johnston & Carlson, 1992). This complex activates transcription of a number of respiratory genes, such as *CYC1*, *CYB2*, *CYT1*, *QCR2,7,8*, *COX4,5A*, *5B*, *6*, *HEM1* and *YHG*. Furthermore, Hap4-binding sequences have been identified on the promoters of *QCR1*, *RIP1*, *QCR6,9*, *COX6B* and *HEM2* (de Winde & Grivell, 1993; Schlaepfer *et al.*, 1994; Crawford *et al.*, 1995). This enumeration of (putatively) controlled mitochondrial genes underlines the pivotal role the Hap2/Hap3/Hap4/Hap5 complex plays in the expression of these genes. *HAP4* overexpression has been reported to result in a more oxidative metabolism with a higher specific growth rate and an increase in cell mass yield by 45% (Blom *et al.*, 1997).

HAP4 has a Mig1-binding site on its promoter as verified by oligonucleotide gel shift experiments (Lundin *et al.*, 1994); moreover *HAP2* and *HAP4* have been shown to be glucose-repressible (Pinkham & Guarente, 1985; Forsburg & Guarente, 1989). It has, however, been reported that a *MIG1* deletion did not result in a derepression of *HAP4*, suggesting a redundant repression mechanism (Lundin *et al.*, 1994). Finally, Mig1-binding sites (direct control) have been identified on the promoters of the genes *CYT1*, *COX5B* and *QCR8,9*

(Maarse *et al.*, 1988; Hodge *et al.*, 1990; Oechsner *et al.*, 1992; de Winde & Grivell, 1993).

Possible putative impacts of *MIG1* deletion/disruption: a holistic view

Potential candidates for Mig1-mediated glucose repression are those genes that bear the Mig1 consensus sequence on their promoters, that have promoters on to which Mig1 has been shown to bind *in vitro*, and/or that show a physiological effect in $\Delta mig1$ or *MIG1^c* mutants. These genes have been discussed previously and are summarized in Table 1.

A synopsis is given in Fig. 3 for the main carbon pathways in the cell and for genes that encode key enzymes or effectors and that stand under direct or indirect Mig1-mediated repression. An interesting feature of Mig1-mediated repression is dual-level control. Mig1 mediates repression by binding on to the promoter of both the functional gene and of the relative regulator gene, such as *MALR*, *GAL4*, *CAT8* and *HAP4*, which respectively encode the regulatory protein for *MAL* genes, the *GAL*-gene activator, a derepressor for gluconeogenic genes and a subunit of the transcriptional activator for mitochondrial genes (Nehlin *et al.*, 1991; de Winde & Grivell, 1993; Hedges *et al.*, 1995; Hu *et al.*, 1995). Such a dual-level control is a potential amplifier of glucose repression.

The controlled genes can be divided into peripheral functions, i.e. metabolism of saccharides to glucose-6-phosphate as well as further influx/efflux functions, and central functions, such as gluconeogenesis, the glyoxylate shunt, fermentative functions, the tricarboxylic acid cycle and oxidative phosphorylation. As can be concluded from Table 1, *MIG1* deletion has a greater impact on peripheral functions than on central metabolism. However, a deletion/disruption of *MIG1* is not able to eliminate glucose repression entirely of galactose, maltose and sucrose metabolism, as substantial Mig1-independent glucose control mechanisms do exist for the *GAL*, *MAL* and *SUC* systems (Nehlin & Ronne, 1990; Nehlin *et al.*, 1991; Hu *et al.*, 1995). These results are relevant for industrial yeast processes because glucose, which is most commonly present in industrial carbon sources, delays the uptake of the sugars and unnecessarily prolongs the production process. An effective alleviation of glucose control would help to achieve a better process economy for the cultivation of baker's yeast, alcohol fermentation and bread-making.

The central functions putatively affected by Mig1 have not been investigated as thoroughly as the peripheral ones, but the data presented give a sound indication that glucose repression, which is well-known for gluconeogenesis, the glyoxylate shunt and respiration (Fiechter *et al.*, 1981), is partly mediated by Mig1. The finding that *CAT8* is derepressed in a $\Delta mig1$ strain, whereas Cat8-controlled *FBP1*, *PCK1* and *ICL1* are not, gives a clear hint of the existence of other repressors such as Mig2 or

a post-translational modification of an effector such as the phosphorylation of the derepressor Cat8 (Hedges *et al.*, 1995; Rande-Gil *et al.*, 1997). Moreover, control mechanisms other than glucose repression, for example higher mRNA degradation, a decreased translational efficiency or carbon catabolite inactivation, are operative, as discussed above (Fig. 1).

The presence of a Mig1-binding site on the promoter of the *PDC1* gene, which is known to be induced by glucose, seems contradictory and could point to an activating function of Mig1 or to activating zinc finger proteins that bear resemblance to Mig1. One assumption of Mig1 mediating glucose induction could be consistent with the finding that the fusion protein LexA-Mig1 (and also LexA-Mig2) activated transcription of a *lexA* operator-controlled reporter gene when the *SSN6* gene was absent (Treitel & Carlson, 1995; Lutfiyya & Johnston, 1996). A second assumption is that of effectors that outcrowd Mig1 from its binding site, as supported by the existence of *Msn2* and *Msn4* (Lundin *et al.*, 1994; Martínez-Pastor *et al.*, 1996); *MSN2* overexpression resulted in an occupation of Mig1-binding sites leading to an increased invertase expression (Schmitt & McEntee, 1996). A third reconciling hypothesis, as in the case of *HXT3*, could be that a weak Mig1-mediated repression counteracts general glucose induction, thus functioning as a fine-tuning controller of gene expression. This hypothesis fits in well with the finding of *HXT2* and *HXT4* transcription repression, which is mediated by Rgt1 when no glucose is present and by Mig1 at high glucose concentrations (Özcan & Johnston, 1996; Özcan *et al.*, 1996). Concerning the phosphorylation step that follows sugar uptake, one may also speculate about the indirect effect Mig1 has via the control of *HXK1* and *GLK1* expression on cell physiology.

From a holistic point of view it makes sense that the essential central metabolism of an organism stands under a more rigid control and exhibits a more complex control network than is the case for non-essential peripheral functions. Consequently, metabolic engineering of central functions is likely to be a greater challenge than that of peripheral ones. As to peripheral functions, success has already been achieved in partially derepressing galactose, maltose and sucrose metabolisms (Nehlin *et al.*, 1991; Hu *et al.*, 1995; Klein *et al.*, 1996; Olsson *et al.*, 1997). For central functions, industrial interest could, for example, lie in the creation of strains that exhibit higher specific growth rates for baker's yeast or recombinant protein production, or of distiller's yeast strains that give higher ethanol yields.

REFERENCES

- Bell, W., Klaassen, P., Ohnacker, M., Boller, T., Herweijer, M., Schoppink, P., van der Zee, P. & Wiemken, A. (1992). Characterization of the 56-kDa subunit of yeast trehalose-6-phosphate synthase and cloning of its gene reveal its identity with the product of *CIF1*, a regulator of carbon catabolite inactivation. *Eur J Biochem* **209**, 951–959.
- Blom, J., Teixeira de Mattos, J. M. & Grivell, L. A. (1997). Carbon-source control of respiratory function in *Saccharomyces cerevisiae*. In *European Research Conferences: Control of Metabolic Flux, Approaches for Understanding the Control of Flux in Yeasts and Fungi*, Giens, France, 14–18 June 1997 (programme and abstracts), p. 27.
- Busturia, A. & Lagunas, R. (1986). Catabolite inactivation of the glucose transport system in *Saccharomyces cerevisiae*. *J Gen Microbiol* **132**, 379–385.
- Caponigro, G. & Parker, R. (1996). Mechanisms and control of mRNA turnover in *Saccharomyces cerevisiae*. *Microbiol Rev* **60**, 233–249.
- Cassart, J.-P., Georis, I., Östling, J., Ronne, H. & Vandenhaute, J. (1995). The MIG1 repressor from *Kluyveromyces lactis*: cloning, sequencing and functional analysis in *Saccharomyces cerevisiae*. *FEBS Lett* **371**, 191–194.
- Celenza, J. L., Marshall-Carlson, L. & Carlson, M. (1988). The yeast *SNF3* gene encodes a glucose transporter homologous to the mammalian protein. *Proc Natl Acad Sci USA* **85**, 2130–2134.
- Cereghino, G. P. & Scheffler, I. E. (1996). Genetic analysis of glucose regulation in *Saccharomyces cerevisiae*: control of transcription versus mRNA turnover. *EMBO J* **15**, 363–374.
- Crawford, M. J., Sherman, D. R. & Goldberg, D. E. (1995). Regulation of *Saccharomyces cerevisiae* flavohemoglobin gene expression. *J Biol Chem* **270**, 6991–6996.
- Deckert, J., Perini, R., Balasubramanian, B. & Zitomer, R. S. (1995). Multiple elements and auto-repression regulate Rox1, a repressor of hypoxic genes in *Saccharomyces cerevisiae*. *Genetics* **139**, 1149–1158.
- DeJuan, C. & Lagunas, R. (1986). Inactivation of the galactose transport system in *Saccharomyces cerevisiae*. *FEBS Lett* **207**, 258–261.
- de Deken, R. H. (1966). The Crabtree effect: a regulatory system in yeast. *J Gen Microbiol* **44**, 149–156.
- DeVit, M. & Johnston, M. (1996). Analysis of the regulation of the Mig1 glucose repressor. In *Yeast Genetics and Molecular Biology*, Madison, WI, USA, 6–11 August 1996 (programme and abstracts), p. 282.
- Dowzer, C. E. A. & Kelly, J. M. (1991). Analysis of the *creA* gene, a regulator of carbon catabolite repression in *Aspergillus nidulans*. *Mol Cell Biol* **11**, 5701–5709.
- Entian, K.-D. & Barnett, J. A. (1992). Regulation of sugar utilization by *Saccharomyces cerevisiae*. *Trends Biochem Sci* **17**, 506–510.
- Entian, K.-D. & Schüller, H.-J. (1997). Glucose repression (carbon catabolite repression) in yeast. In *Yeast Sugar Metabolism*, pp. 409–434. Edited by F. K. Zimmermann & K.-D. Entian. Lancaster, Basel: Technomic.
- Federoff, H. J., Ecclesall, T. R. & Marmur, J. (1983). Carbon catabolite repression of maltase synthesis in *Saccharomyces cerevisiae*. *J Bacteriol* **156**, 301–307.
- Fiechter, A., Fuhrmann, G. F. & Käppli, O. (1981). Regulation of glucose metabolism in growing yeast cells. *Adv Microb Physiol* **22**, 123–183.
- Forsburg, S. L. & Guarente, L. (1989). Identification and characterization of HAP4: a third component of the CCAAT-bound HAP2/HAP3 heteromer. *Genes Dev* **3**, 1166–1178.
- François, J., Neves, M.-J. & Hers, H.-G. (1991). The control of trehalose biosynthesis in *Saccharomyces cerevisiae*: evidence for a catabolite inactivation and repression of trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase. *Yeast* **7**, 575–587.

- Frederick, D. L. & Tatchell, K. (1996).** The REG2 gene of *Saccharomyces cerevisiae* encodes a type 1 protein phosphatase-binding protein that functions with Reg1p and the Snf1 protein kinase to regulate growth. *Mol Cell Biol* **16**, 2922–2931.
- Fu, L., Bounelis, P., Dey, N., Browne, B. L., Marchase, R. B. & Bedwell, D. M. (1995).** The posttranslational modification of phosphoglucosyltransferase is regulated by galactose induction and glucose repression in *Saccharomyces cerevisiae*. *J Bacteriol* **177**, 3087–3094.
- Gallie, D. R. (1996).** The cap and poly(A) tail function synergistically to regulate mRNA translational efficiency. *Genes Dev* **5**, 2108–2116.
- Gancedo, J. M. (1992).** Carbon catabolite repression in yeast. *Eur J Biochem* **206**, 297–313.
- Gancedo, C. & Serrano, R. (1989).** Energy-yielding metabolism. In *The Yeasts*, vol. 3, pp. 205–259. Edited by A. H. Rose & J. S. Harrison. New York: Academic Press.
- González, M. I., Stucka, R., Blázquez, M. A., Feldmann, H. & Gancedo, C. (1992).** Molecular cloning of C1F1, a yeast gene necessary for growth on glucose. *Yeast* **8**, 183–192.
- Görts, C. P. M. (1969).** Effect of glucose on the activity and the kinetics of the maltose-uptake system and of α -glucosidase in *Saccharomyces cerevisiae*. *Biochim Biophys Acta* **184**, 299–305.
- Hardie, D. G. & Carling, D. (1997).** The AMP-activated protein kinase. Fuel gauge of the mammalian cell? *Eur J Biochem* **246**, 259–273.
- Hedges, D., Proft, M. & Entian, K.-D. (1995).** CAT8, a new zinc cluster-encoding gene necessary for derepression of gluconeogenic enzymes in the yeast *Saccharomyces cerevisiae*. *Mol Cell Biol* **15**, 1915–1922.
- Herrero, P., Galíndez, J., Ruiz, N., Martínez-Campa, C. & Moreno, F. (1995).** Transcriptional regulation of the *Saccharomyces cerevisiae* HXK1, HXK2 and GLK1 genes. *Yeast* **11**, 137–144.
- Hodge, M. R., Singh, K. & Cumsy, M. G. (1990).** Upstream activation and repression elements control transcription of the yeast COX5b gene. *Mol Cell Biol* **10**, 5510–5520.
- Hohmann, S. (1991).** Characterization of PDC6, a third structural gene for pyruvate decarboxylase in *Saccharomyces cerevisiae*. *J Bacteriol* **173**, 7963–7969.
- Hohmann, S. (1993).** Characterisation of PDC2, a gene necessary for high level expression of pyruvate decarboxylase structural genes in *Saccharomyces cerevisiae*. *Mol Gen Genet* **241**, 657–666.
- Hohmann, S., Huse, K., Valentin, E., Mbonyi, K., Thevelein, J. M. & Zimmermann, F. K. (1992).** Glucose-induced regulatory defects in the *Saccharomyces cerevisiae* *byp1* growth initiation mutant and identification of MIG1 as a partial suppressor. *J Bacteriol* **174**, 4183–4188.
- Holzer, H. (1976).** Catabolite inactivation in yeast. *Trends Biochem Sci* **1**, 178–181.
- Horak, J. & Wolf, D. H. (1997).** Catabolite inactivation of the galactose transporter in the yeast *Saccharomyces cerevisiae*: ubiquitination, endocytosis, and degradation in the vacuole. *J Bacteriol* **179**, 1541–1549.
- Hu, Z., Nehlin, J. O., Ronne, H. & Michels, C. A. (1995).** MIG1-dependent and MIG1-independent glucose regulation of MAL gene expression in *Saccharomyces cerevisiae*. *Curr Genet* **28**, 258–266.
- Jiang, R. & Carlson, M. (1996).** Glucose regulates protein interactions within the yeast SNF1 protein kinase complex. *Genes Dev* **10**, 3105–3115.
- Johnston, M. (1987).** A model fungal gene regulatory mechanism: the GAL genes of *Saccharomyces cerevisiae*. *Microbiol Rev* **51**, 458–476.
- Johnston, M. & Carlson, M. (1992).** Regulation of carbon and phosphate utilization. In *The Molecular and Cellular Biology of the Yeast Saccharomyces*, pp. 193–281. Edited by E. W. Jones, J. R. Pringle & J. R. Broach. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Käppli, O. (1986).** Regulation of carbon metabolism in *Saccharomyces cerevisiae* and related yeasts. *Adv Microbiol Physiol* **28**, 181–209.
- Keleher, C. A., Redd, M. J., Schultz, J., Carlson, M. & Johnson, A. D. (1992).** Ssn6-Tup1 is a general repressor of transcription in yeast. *Cell* **68**, 709–719.
- Klein, C. J. L., Olsson, L., Rønnow, B., Mikkelsen, J. D. & Nielsen, J. (1996).** Alleviation of glucose repression of maltose metabolism by MIG1 disruption in *Saccharomyces cerevisiae*. *Appl Environ Microbiol* **62**, 4441–4449.
- Ko, C. H., Liang, H. & Gaber, R. F. (1993).** Roles of multiple glucose transporters in *Saccharomyces cerevisiae*. *Mol Cell Biol* **13**, 638–648.
- Kopp, M., Müller, H. & Holzer, H. (1993).** Molecular analysis of the neutral trehalase gene from *Saccharomyces cerevisiae*. *J Biol Chem* **268**, 4766–4774.
- Kratzer, S. & Schüller, H.-J. (1997).** Transcriptional control of the yeast acetyl-CoA synthetase gene ACS1 by the activators CAT8 and ADR1 and the repressor UME6. *Yeast* **13** (Special issue), S98.
- Kruckeberg, A. L. (1996).** The hexose transporter family of *Saccharomyces cerevisiae*. *Arch Microbiol* **166**, 283–292.
- Lazo, P. S., Ochoa, A. G. & Gascón, S. (1978).** α -Galactosidase (melibiase) from *Saccharomyces carlsbergensis*: structural and kinetic properties. *Arch Biochem Biophys* **191**, 316–324.
- Liang, H. & Gaber, R. F. (1996).** A novel signal transduction pathway in *Saccharomyces cerevisiae* defined by Snf3-regulated expression of HXT6. *Mol Biol Cell* **7**, 1953–1966.
- Lohr, D., Venkov, P. & Zlatanova, J. (1995).** Transcriptional regulation in the yeast GAL gene family: a complex genetic network. *FASEB J* **9**, 777–787.
- Lombardo, A., Cereghino, G. P. & Scheffler, I. E. (1992).** Control of mRNA turnover as a mechanism of glucose repression in *Saccharomyces cerevisiae*. *Mol Cell Biol* **12**, 2941–2948.
- Lundin, M., Nehlin, J. O. & Ronne, H. (1994).** Importance of a flanking AT-rich region in target site recognition by the GC box-binding zinc finger protein MIG1. *Mol Cell Biol* **14**, 1979–1985.
- Lutfiyya, L. L. & Johnston, M. (1996).** Two zinc-finger-containing repressors are responsible for glucose repression of SUC2 expression. *Mol Cell Biol* **16**, 4790–4797.
- Luyten, K., Albertyn, J., Skibbe, W. F., Prior, B. A., Ramos, J., Thevelein, J. M. & Hohmann, S. (1995).** Fps1, a yeast member of the MIP family of channel proteins, is a facilitator for glycerol uptake and efflux and is inactive under osmotic stress. *EMBO J* **14**, 1360–1371.
- Maarse, A. C., de Haan, M., Bout, A. & Grivell, L. A. (1988).** Demarcation of a sequence involved in mediating catabolite repression of a gene for the 11 kDa subunit VIII of ubiquinol-cytochrome c oxidoreductase in *Saccharomyces cerevisiae*. *Nucleic Acids Res* **16**, 5797–5811.
- Martínez-Pastor, M. T., Marchler, G., Schuller, C., Marchler-Bauer, A., Ruis, H. & Estruch, F. (1996).** The *Saccharomyces cerevisiae* zinc finger proteins Msn2p and Msn4p are required for transcriptional induction through the stress response element (STRE). *EMBO J* **15**, 2227–2235.

- Mercado, J. J. & Gancedo, J. M. (1992). Regulatory regions in the yeast *FBP1* and *PCK1* genes. *FEBS Lett* 311, 110–114.
- Mercado, J. J., Vincent, O. & Gancedo, J. M. (1991). Regions in the promoter of the yeast *FBP1* gene implicated in catabolite repression may bind the product of the regulatory gene *MIG1*. *FEBS Lett* 291, 97–100.
- Mercado, J. J., Smith, R., Sagliocco, F. A., Brown, A. J. P. & Gancedo, J. M. (1994). The levels of yeast gluconeogenic mRNAs respond to environmental factors. *Eur J Biochem* 224, 473–481.
- Mwesigye, P. K. & Barford, J. P. (1996). Mechanism of sucrose utilization by *Saccharomyces cerevisiae*. *J Gen Appl Microbiol* 42, 297–306.
- Mylin, L. M., Johnston, M. & Hoper, J. E. (1990). Phosphorylated forms of *GAL4* are correlated with ability to activate transcription. *Mol Cell Biol* 10, 4623–4629.
- Naumov, G. I., Naumova, E. S., Turakainen, H. & Korhola, M. (1996). Identification of the α -galactosidase *MEL* genes in some populations of *Saccharomyces cerevisiae*: a new gene *MEL11*. *Genet Res* 67, 101–108.
- Nehlin, J. O. & Ronne, H. (1990). Yeast *MIG1* repressor is related to the mammalian early growth response and Wilms' tumour finger proteins. *EMBO J* 9, 2891–2898.
- Nehlin, J. O., Carlberg, M. & Ronne, H. (1991). Control of yeast *GAL* genes by *MIG1* repressor: a transcriptional cascade in the glucose response. *EMBO J* 10, 3373–3377.
- Neugeborn, L. & Carlson, M. (1987). Mutations causing constitutive invertase synthesis in yeast: genetic interactions with *snf* mutations. *Genetics* 115, 247–253.
- Nwaka, S., Kopp, M. & Holzer, H. (1995). Expression and function of the trehalase genes *NTH1* and *YBR0106* in *Saccharomyces cerevisiae*. *J Biol Chem* 270, 10193–10198.
- Oechsner, U., Hermann, H., Zollner, A., Haid, A. & Bandlow, W. (1992). Expression of yeast cytochrome *c1* is controlled at the transcriptional level by glucose, oxygen and haem. *Mol Gen Genet* 232, 447–459.
- Oh, D. & Hopper, J. E. (1990). Transcription of a yeast phosphoglucomutase isozyme gene is galactose inducible and glucose repressible. *Mol Cell Biol* 10, 1415–1422.
- Olsson, L., Larsen, M. E., Rønnow, B., Mikkelsen, J. D. & Nielsen, J. (1997). Silencing *MIG1* in *Saccharomyces cerevisiae*: effects of antisense *MIG1* expression and *MIG1* gene disruption. *Appl Environ Microbiol* 63, 2366–2371.
- Östling, J., Carlberg, M. & Ronne, H. (1996). Functional domains in the *Mig1* repressor. *Mol Cell Biol* 16, 753–761.
- Özcan, S. & Johnston, M. (1995). Three different regulatory mechanisms enable yeast hexose transporter (*HXT*) genes to be induced by different levels of glucose. *Mol Cell Biol* 15, 1564–1572.
- Özcan, S. & Johnston, M. (1996). Two different repressors collaborate to restrict expression of the yeast glucose transporter genes *HXT2* and *HXT4* to low levels of glucose. *Mol Cell Biol* 16, 5536–5545.
- Özcan, S., Leong, T. & Johnston, M. (1996). Rgt1p of *Saccharomyces cerevisiae*, a key regulator of glucose-induced genes, is both an activator and a repressor of transcription. *Mol Cell Biol* 16, 6419–6426.
- Özcan, S., Vallier, L. G., Flick, J. S., Carlson, M. & Johnston, M. (1997). Expression of the *SUC2* gene of *Saccharomyces cerevisiae* is induced by low levels of glucose. *Yeast* 13, 127–137.
- Panek, A. D. (1991). Storage carbohydrates. In *The Yeasts*, vol. 4, pp. 655–677. Edited by A. H. Rose & J. S. Harrison. New York: Academic Press.
- Parets Soler, A., Casanova, M., Gozalbo, D. & Sentandreu, R. (1987). Differential translational efficiency of the mRNAs isolated from derepressed and glucose repressed *Saccharomyces cerevisiae*. *J Gen Microbiol* 133, 1471–1480.
- Petit, T. & François, J. (1994). Accumulation of trehalose in *Saccharomyces cerevisiae* growing on maltose is dependent on the *TPS1* gene encoding the UDPglucose-linked trehalose synthase. *FEBS Lett* 355, 309–313.
- Pinkham, J. L. & Guarente, L. (1985). Cloning and molecular analysis of the *HAP2* locus: a global regulator of respiratory genes in *Saccharomyces cerevisiae*. *Mol Cell Biol* 5, 3410–3416.
- Post-Beittenmiller, M. A., Hamilton, R. W. & Hopper, J. E. (1984). Regulation of basal and induced levels of the *MEL1* transcript in *Saccharomyces cerevisiae*. *Mol Cell Biol* 4, 1238–1245.
- Randez-Gil, F., Bojunga, N., Proft, M. & Entian, K. D. (1997). Glucose derepression of gluconeogenic enzymes in *Saccharomyces cerevisiae* correlates with phosphorylation of the gene activator Cat8p. *Mol Cell Biol* 17, 2502–2510.
- Reifenberger, E., Freidel, K. & Ciriacy, M. (1995). Identification of novel *HXT* genes in *Saccharomyces cerevisiae* reveals the impact of individual hexose transporters on glycolytic flux. *Mol Microbiol* 16, 157–167.
- Riballo, E., Herweijer, M., Wolf, D. H. & Lagunas, R. (1995). Catabolite inactivation of the yeast maltose transporter occurs in the vacuole after internalization by endocytosis. *J Bacteriol* 177, 5622–5627.
- Ronne, H. (1995). Glucose repression in fungi. *Trends Genet* 11, 12–17.
- Rosenkrantz, M., Kell, C. S., Pennell, E. A. & Devenish, L. J. (1994). The *HAP2,3,4* transcriptional activator is required for derepression of the yeast citrate synthase gene, *CIT1*. *Mol Microbiol* 13, 119–131.
- Sadowski, I., Costa, C. & Dhanawansa, R. (1996). Phosphorylation of Gal4p at a single C-terminal residue is necessary for galactose-inducible transcription. *Mol Cell Biol* 16, 4879–4887.
- Santos, E., Rodríguez, L., Elorza, M. V. & Sentandreu, R. (1982). Uptake of sucrose by *Saccharomyces cerevisiae*. *Arch Biochem Biophys* 216, 652–660.
- Sanz, P., Nieto, A. & Prieto, J. A. (1996). Glucose repression may involve processes with different sugar kinase requirements. *J Bacteriol* 178, 4721–4723.
- Schlaepfer, I. R., Mattoon, J. R. & Bajsász, G. (1994). The sequence and potential regulatory elements of the *HEM2* promoter of *Saccharomyces cerevisiae*. *Yeast* 10, 227–229.
- Schmitt, A. P. & McEntee, K. (1996). Msn2p, a zinc finger DNA-binding protein, is the transcriptional activator of the multistress response in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* 93, 5777–5782.
- Schmitt, H. D., Ciriacy, M. & Zimmermann, F. K. (1983). The synthesis of yeast pyruvate decarboxylase is regulated by large variations in the messenger RNA level. *Mol Gen Genet* 192, 247–252.
- Schork, S. M., Bee, G., Thumm, M. & Wolf, D. H. (1994). Catabolite inactivation of fructose-1,6-bisphosphatase in yeast is mediated by the proteasome. *FEBS Lett* 349, 270–274.
- Schöler, A. & Schüller, H.-J. (1993). Structure and regulation of the isocitrate lyase gene *ICL1* from the yeast *Saccharomyces cerevisiae*. *Curr Genet* 23, 375–381.
- Schüller, H.-J. & Entian, K.-D. (1991). Extragenic suppressors of yeast glucose derepression mutants leading to constitutive synthesis of several glucose-repressible enzymes. *J Bacteriol* 173, 2045–2052.

- Sierkstra, L. N., Nouwen, N. P., Verbakel, J. M. A. & Verrips, C. T. (1992). Analysis of glucose repression in *Saccharomyces cerevisiae* by pulsing glucose to a galactose-limited continuous culture. *Yeast* **8**, 1077–1087.
- Siro, M.-R. & Lövgren, T. (1978). On the properties of α -glucosidase and the binding of glucose to the enzyme. *Acta Chem Scand Ser B Org Chem Biochem* **32**, 447–451.
- Sumner-Smith, M., Bozzato, R. P., Skipper, N., Davies, R. W. & Hopper, J. E. (1985). Analysis of the inducible *MEL1* gene of *Saccharomyces carlsbergensis* and its secreted product, α -galactosidase (melibiase). *Gene* **36**, 333–340.
- Thevelein, J. M. (1994). Signal transduction in yeast. *Yeast* **10**, 1753–1790.
- Thevelein, J. M. & Hohmann, S. (1995). Trehalose synthase: guard to the gate of glycolysis in yeast? *Trends Biochem Sci* **20**, 3–10.
- Treitel, M. A. & Carlson, M. (1995). Repression by *SSN6-TUP1* is directed by *MIG1*, a repressor/activator protein. *Proc Natl Acad Sci USA* **92**, 3132–3136.
- Trumbly, R. J. (1992). Glucose repression in the yeast *Saccharomyces cerevisiae*. *Mol Microbiol* **6**, 15–21.
- Tu, J. & Carlson, M. (1995). *REG1* binds to protein phosphatase type 1 and regulates glucose repression in *Saccharomyces cerevisiae*. *EMBO J* **14**, 5939–5946.
- Vallier, G. V. & Carlson, M. (1994). Synergistic release from glucose repression by *mig1* and *ssn* mutations in *Saccharomyces cerevisiae*. *Genetics* **137**, 49–54.
- Walsh, M. C., Scholte, M., Valkier, J., Smits, H. P. & van Dam, K. (1996). Glucose sensing and signalling properties in *Saccharomyces cerevisiae* require the presence of at least two members of the glucose transporter family. *J Bacteriol* **178**, 2593–2597.
- Wanke, V., Vavassori, M., Thevelein, J. M., Tortora, P. & Vanoni, M. (1997). Regulation of maltose utilization in *Saccharomyces cerevisiae* by genes of the RAS/protein kinase A pathway. *FEBS Lett* **402**, 251–255.
- Wendell, D. L. & Bisson, L. F. (1994). Expression of high-affinity glucose transport protein Hxt2p of *Saccharomyces cerevisiae* is both repressed and induced by glucose and appears to be regulated posttranslationally. *J Bacteriol* **176**, 3730–3737.
- Wilson, W. A., Hawley, S. A. & Hardie, D. G. (1996). Glucose repression/derepression in budding yeast: SNF1 protein kinase is activated by phosphorylation under derepressing conditions, and this correlates with a high AMP:ATP ratio. *Curr Biol* **6**, 1426–1434.
- de Winde, J. H. & Grivell, L. A. (1993). Global regulation of mitochondrial biogenesis in *Saccharomyces cerevisiae*. *Progr Nucleic Acid Res Mol Biol* **46**, 51–91.
- de Winde, J. H., Crauwels, M., Hohmann, S., Thevelein, J. M. & Winderickx, J. (1996). Differential requirement of the yeast sugar kinases for sugar sensing in establishing the catabolite-repressed state. *Eur J Biochem* **241**, 633–643.
- Yano, K.-i. & Fukasawa, T. (1997). Galactose-dependent reversible interaction of Gal3p with Gal80p in the induction pathway of Gal4p-activated genes of *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* **94**, 1721–1726.
- Yin, Z., Smith, R. J. & Brown, A. J. P. (1996). Multiple signalling pathways trigger the exquisite sensitivity of yeast gluconeogenic mRNAs to glucose. *Mol Microbiol* **20**, 751–764.
- Zenke, F. T., Engels, R., Vollenbroich, V., Meyer, J., Hollenberg, C. P. & Breunig, K. D. (1996). Activation of Gal4p by galactose-dependent interaction of galactokinase and Gal80p. *Science* **272**, 1662–1665.