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Glucose triggers different global responses in yeast, depending on the strength of the signal, and transiently stabilizes ribosomal protein mRNAs

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Summary

Glucose exerts profound effects upon yeast physiology. In general, the effects of high glucose concentrations (>1%) upon *Saccharomyces cerevisiae* have been studied. In this paper, we have characterized the global responses of yeast cells to very low (0.01%), low (0.1%) and high glucose signals (1.0%) by transcript profiling. We show that yeast is more sensitive to very low glucose signals than was previously thought, and that yeast displays different responses to these different glucose signals. Genes involved in central metabolic pathways respond rapidly to very low glucose signals, whereas genes involved in the biogenesis of cytoplasmic ribosomes generally respond only to glucose concentrations of >0.1%. We also show that cytoplasmic ribosomal protein mRNAs are transiently stabilized by glucose, indicating that both transcriptional and post-transcriptional mechanisms combine to accelerate the accumulation of ribosomal protein mRNAs. Presumably, this facilitates rapid ribosome biogenesis after exposure to glucose. However, our data indicate that yeast activates ribosome biogenesis only when sufficient glucose is available to make this metabolic investment worthwhile. In contrast, the regulation of metabolic functions in response to very low glucose signals presumably ensures that yeast can exploit even minute amounts of this preferred nutrient.

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Introduction

In the wild, *Saccharomyces cerevisiae* experiences sudden transitions from famine to feast. This yeast can exploit a wide range of fermentable and non-fermentable carbon sources, but pathways for the assimilation of alternative carbon sources are repressed in the presence of glucose, the favoured growth substrate. Glucose also activates glycolysis, decreases respiratory activity, increases ribosome biogenesis and regulates growth and development. Hence, glucose exerts profound effects on the yeast cell. Not surprisingly, therefore, a complex array of signal transduction pathways co-ordinates the various metabolic and cellular responses to glucose (for reviews, see Gancedo, 1998; Carlson, 1999; Johnston, 1999; Thevelein and de Winde, 1999; Rolland *et al*., 2001). Components of these glucose signalling pathways are conserved in plant and mammalian cells (reviewed by Johnston, 1999; Rolland *et al*., 2001). Hence, budding yeast provides an excellent model for the dissection of nutrient signalling and gene regulation in eukaryotic cells.

A large number of yeast genes respond to glucose (DeRisi *et al*., 1997; Brown *et al*., 2001). For example, galactose utilization, TCA cycle and gluconeogenic genes are transcriptionally repressed by glucose, whereas glycolytic, hexose transporter and ribosomal protein genes are transcriptionally activated (Warner, 1989; Boles and Zimmermann, 1993; Gancedo, 1998; Johnston, 1999; Özcan and Johnston, 1999). In contrast, a relatively small number of yeast mRNAs are known to be regulated post-transcriptionally by glucose. These include the *SDH2*, *SUC2*, *SPO13*, *PCK1* and *FBP1* mRNAs (Lombardo *et al*., 1992; Mercado *et al*., 1994; Surosky *et al*., 1994; Cereghino and Scheffler, 1996).

We reported that yeast is exquisitely sensitive to glucose, modulating gene expression in response to very low concentrations of glucose (<0.01%; Mercado *et al*., 1994; Yin *et al*., 2000). However, several observations suggest that yeast responds differentially to different ambient glucose concentrations. In response to 0.1% glucose, transcription of the hexose transporter genes *HXT2–4* is activated, but *HXT1* transcription remains low. In contrast, *HXT1* is activated by 4% glucose, but *HXT2* and *HXT4* are repressed at this high glucose concentration (Özcan and Johnston, 1995). Also, the gluconeogenic mRNAs are

repressed by 0.01% glucose, whereas the *SDH2* mRNA only reacts to high glucose signals (Mercado *et al*., 1994; Cereghino and Scheffler, 1996; Yin *et al*., 1996; 2000). In these cases, distinct signalling pathways appear to transduce the low and high glucose signals (Özcan and Johnston, 1995; 1998; Cereghino and Scheffler, 1996; Yin *et al*., 2000).

Previously, we suggested that gluconeogenic mRNAs are exceptionally sensitive to very low glucose signals that do not affect other yeast mRNAs (Mercado *et al*., 1994; Yin *et al*., 1996; 2000). In this study, we tested this hypothesis by studying global responses to different glucose concentrations using transcript profiling. We show that, in contrast to our previous suggestions, many yeast genes are sensitive to a very low glucose signal (0.01%). Ribosome biogenesis is not as sensitive to glucose as metabolic functions, but ribosomal protein mRNA levels do respond rapidly to a low glucose signal (0.1%). We also show that transient stabilization of ribosomal protein mRNAs contributes to this response. Therefore, yeast displays differential global responses to weak and strong glucose signals.

Results and discussion

Global responses to different glucose signals

Previous global studies of glucose regulation have focused mainly on the long-term effects of high glucose signals (>1.0% glucose; Lashkari *et al*., 1997; Lopez and Baker, 2000; Brown *et al*., 2001). Other studies have analysed time courses, during which changes in glucose concentration are accompanied by changes in other parameters (DeRisi *et al*., 1997). Hence, in this study, our aim was specifically to characterize short-term changes in gene expression that occur in response to different glucose signals, because short-term changes are more likely to reflect primary responses to these signals.

We compared the transcript profiles of yeast cells harvested 30 min after exposure to very low (0.01%), low (0.1%) or high glucose signals (1.0%) with control cells (no glucose). We reasoned that, in this short period, primary glucose responses would be activated, but activation of secondary and tertiary responses to glucose addition was less likely. However, most glucose-responsive mRNAs had not reached their new steady-state levels within 30 min and, as a result, most fold changes in expression were relatively low. Therefore, we used robust statistical analyses (Beissbarth *et al*., 2000; see *Experimental procedures*) to assess the significance of each fold change for every gene. The M-CHIPS software package (Fellenberg *et al*., 2001; 2002; http://www.dkfz.de/tbi/services/mchips) provided two measures of statistical significance. Changes that were highly significant (where all replicate data points for one condition were above or below all replicates for the control condition) were coloured red (activated) or blue (repressed). Changes that were significant (where not all replicate points for one condition were above or below all replicates for the control condition, but where the means were separated by at least one standard deviation) were coloured yellow (activated) or cyan (repressed). Critically, this treatment allows rapid visual assessment of the statistical significance of individual transcript profiling data points. The complete transcript profiling data set – colour-coded tables of normalized data as well as the comprehensive list of raw data – is freely available at http://mips.gsf.de/proj/eurofan/eurofan_2/b2/. Image files (tiff format), a list of array elements (including gene/clone IDs, array location, descriptions and functional catalogue entries for each spot) and more details on the normalization strategies and algorithms are available at http://www.dkfz-heidelberg.de/funct_genome/yeast-data. html or directly from the authors. The data analysis and also the compilation of experimental as well as organism-specific annotations meet the criteria for MIAME2 compliance (Brazma *et al*., 2001).

As expected, the levels of a large number of yeast transcripts were significantly affected by glucose. A total of 352 (5.8%) genes displayed significant increases in expression of ≥twofold after exposure to high glucose signal, and 368 (6.0%) genes displayed decreases in expression of similar strength. This is consistent with previous studies (DeRisi *et al*., 1997; Brown *et al*., 2001). Significantly, many more yeast genes were repressed than were induced by the very low glucose signal (0.01%; Fig. 1). In contrast, more genes were induced than were repressed by the low (0.1%) or high (1.0%) glucose signals. Therefore, in general, yeast does not activate many functions in response to glucose unless at least modest concentrations are available. The observation that many

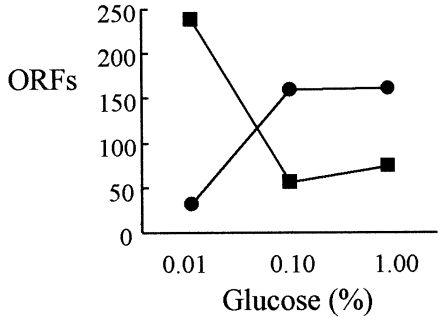

Fig. 1. Numbers of yeast genes that respond to different glucose signals. ORFs that showed ≥twofold regulation 30 min after exposure to very low (0.01%), low (0.1%) or high glucose signals (1.0%) are shown. Numbers are not cumulative, i.e. 161 genes were induced by 1.0% glucose, but not by 0.01% or 0.1% glucose: induced, circles; repressed, squares.

Table 1. Proportion of ORFs in each functional category that showed ≥twofold regulation in response to different glucose signals.[ab]

		0.01% Glucose		0.1% Glucose		1.0% Glucose	
Functional categories	All ORFs	Up	Down	Up	Down	Up	Down
1. Metabolism	17%	44%	18%	30%	14%	27%	14%
1.1 Amino acids	3%	3%	2%	7%	2%	9%	2%
1.2 Nitrogen and sulphur	1%	0%	1%	1%	0%	1%	1%
1.3 Nucleotides	2%	3%	2%	4%	2%	4%	1%
1.4 Phosphate	1%	0%	0%	0%	0%	1%	0%
1.5 Carbon	6%	38%	7%	16%	8%	9%	9%
1.6 Lipids and fatty-acids	3%	0%	4%	3%	2%	4%	2%
1.7 Vitamins and cofactors	1%	0%	1%	1%	0%	1%	0%
1.8 Secondary metabolism	0%	0%	0%	0%	0%	0%	0%
2. Energy	4%	16%	8%	11%	7%	5%	10%
3. Cell cycle and DNA processing	10%	0%	8%	5%	5%	5%	4%
4. Transcription	12%	6%	11%	6%	9%	9%	7%
5. Protein synthesis	6%	38%	5%	35%	2%	28%	2%
6. Protein fate	9%	6%	10%	4%	5%	4%	7%
7. Cellular transport	8%	19%	7%	3%	5%	5%	5%
8. Cellular communication/signal transduction	1%	0%	0%	1%	1%	1%	1%
9. Cell rescue	4%	3%	5%	4%	3%	4%	2%
10. Interaction with cellular environment	3%	3%	4%	2%	2%	2%	2%
11. Cell fate	7%	0%	8%	2%	5%	3%	4%
12. Transposable elements	2%	0%	0%	1%	0%	1%	0%
13. Cellular organization	3%	0%	4%	2%	2%	3%	2%
14. Subcellular localization	35%	78%	39%	65%	29%	57%	29%
15. Protein activity regulation	0%	0%	0%	0%	0%	0%	0%
16. Proteins with binding functions	0%	0%	0%	0%	0%	0%	0%
17. Transport facilitation	5%	19%	5%	3%	4%	5%	4%
18. Classification not yet clear cut	2%	0%	1%	2%	5%	1%	1%
19. Unclassified proteins	37%	6%	36%	15%	22%	14%	18%
Number of ORFs[c]	6450	32	238	191	294	352	368

a. ORFs can belong to more than one functional category in the MIPS database (http://mips.gsf.de/proj/yeast/CYGD/db/index.html; January 2003).
b. Expressed as percentage of number of ORFs belonging to each functional category.
c. Total number of ORFs regulated by each glucose signal are given.

glucose-sensitive functions are repressed even when yeast is exposed to very low glucose concentrations might reflect a need to repress non-fermentative functions until all remaining glucose is exhausted as yeast enters diauxie.

Using the MIPS catalogue of functional categories in yeast (http://mips.gsf.de/proj/yeast/CYGD/db/index.html), we examined the global effects of the different glucose signals upon yeast biology (Table 1). Several functional categories were enriched in the subsets of glucose-sensitive genes, compared with the genome as a whole. Carbon metabolism, transport and protein synthesis were induced by glucose. Some energy-related functions were enriched in glucose-induced subsets, whereas other energy-related functions were repressed. The increase in subcellular localization functions reflects the fact that the location of many glucose-regulated proteins has been established. (For example, glycolytic enzymes have been assigned to the functional category subcellular localization as well as to carbon metabolism.)

Significantly, differential responses to very low, low and high glucose signals were observed (Table 1). Carbon metabolism and transport functions were significantly enriched among gene sets that were sensitive to very low glucose signals, but less so for the high glucose signal. The reverse was true for amino acid metabolic functions. Energy functions were induced at very low and low glucose signals, but were repressed at high glucose signals. In contrast, protein synthetic genes were over-represented in all three glucose-induced gene subsets. This clearly indicates that yeast triggers different global cellular responses, depending upon the strength of the glucose signal. Genes involved in central carbon metabolism, hexose transport and ribosome biogenesis were subjected to more detailed analysis.

Carbon metabolism

The effects of high glucose signals upon central carbon metabolism are well characterized. Glycolytic genes are induced, whereas gluconeogenic, glyoxylate cycle and TCA cycle genes are repressed (Boles and Zimmerman, 1993; Gancedo, 1998). Hence, these genes provide a good test of the quality of our transcript profiling data set. Most glycolytic mRNAs displayed significant increases within 30 min of exposure to a high glucose signal (Fig. 2).

A

Protein	Gene	[Glucose] 0.01%	0.10%	1.00%
Gluconeogenesis				
Fbp1	YLR377c	-3.19	-3.81	-3.28
Pck1	YKR097w	-7.98	-4.80	-5.98
Pyc1	YGL062w	-1.67	-1.73	-2.42
Pyc2	YBR218c	-2.35	-2.39	-2.11
Glyoxylate Cycle				
Icl1	YER065c	-4.38	-5.31	-4.82
Mls1	YNL117w	-3.68	-3.79	-3.29
TCA Cycle				
Aco1	YLR304c	-2.82	-3.76	-8.85
Cit1	YNR001c	-1.99	-2.43	-5.77
Cit2	YCR005C	-1.43	-1.02	1.59
Cit3	YPR001w	-1.08	-1.42	-1.49
Fum1	YPL262w	-1.54	-1.64	-2.25
Idh1	YNL037c	-1.27	-1.73	-2.90
Idh2	YOR136w	-1.63	-1.58	-2.31
Idp1	YDL066w	-1.96	-1.11	1.32
Idp2	YLR174w	-4.51	-7.46	-7.84
Kgd1	YIL125w	-2.46	-2.06	-3.55
Kgd2	YDR148c	-1.89	-1.63	-2.69
Lpd1	YFL018c	-1.85	-2.10	-2.13
Lsc1	YOR142w	-1.38	-1.40	-1.63
Lsc2	YGR244c	1.06	-1.28	-2.66
Mdh1	YKL085w	-1.34	-1.49	-3.05
Osm1	YJR051w	-3.40	-1.65	-1.40
Sdh1	YKL148c	-1.32	-1.52	-2.15
Sdh2	YLL041c	-1.10	-1.31	-2.71
Sdh3	YKL141w	-1.51	-1.59	-2.47
Sdh4	YDR178w	-1.54	-1.24	-2.21

B

Protein	Gene	[Glucose] 0.01%	0.10%	1.00%
Glycolysis				
Glk1	YCL040w	1.63	1.04	-1.81
Hxk1	YFR053c	3.11	5.00	1.21
Hxk2	YGL253w	-1.11	1.42	1.39
Pgi1	YBR196c	-1.45	1.12	1.68
Pfk1	YGR240c	-1.94	-1.05	1.53
Pfk2	YMR205c	1.21	2.10	2.65
Fba1	YKL060c	2.04	3.72	4.61
Tpi1	YDR050c	1.79	2.27	3.69
Tdh1	YJL052w	1.62	2.20	2.22
Tdh2	YJR009c	1.24	1.61	1.76
Tdh3	YGR192c	-1.15	1.40	1.90
Pgk1	YCR012w	1.98	2.87	3.46
Gpm1	YKL152c	1.50	1.80	2.05
Gpm2	YDL021w	1.08	1.22	-1.18
Gpm3	YOL056w	-1.15	-1.32	-1.17
Eno1	YGR254w	1.80	2.73	4.15
Eno2	YHR174w	2.43	3.88	6.29
Pyk1	YAL038w	2.28	3.64	5.36
Pyk2	YOR347c	1.07	1.28	-1.30
Pda1	YER178w	-1.05	1.12	-1.21
Pdb1	YBR221c	-1.96	-2.63	-2.03

Fig. 2. Genes involved in carbon anabolism and catabolism display differential responses to glucose. Fold induction for each gene is shown, with statistically significant changes highlighted in colour: highly significant, red (induced) or blue (repressed); significant, yellow (induced) or cyan (repressed).
A. Gluconeogenic, glyoxylate cycle and TCA cycle genes are listed in the upper, middle and lower parts respectively.
B. Glycolytic genes.

The only exceptions were the mRNAs for Glk1, Gpm2, Gpm3 and Pyk2, all of which play minor roles in fermentation compared with their functionally redundant homologues. As expected, all gluconeogenic and glyoxylate cycle mRNAs and most TCA cycle mRNAs were repressed by the high glucose signal.

Having established that our data are consistent with previous observations, we examined the effects of weaker glucose signals upon these gene sets. As expected (Yin *et al.*, 1996; 2000), gluconeogenic mRNAs were repressed by a very low glucose signal (Fig. 2). Interestingly, glyoxylate and TCA cycle mRNAs were also very sensitive to a very low glucose signal. Furthermore, mRNAs central to the glycolytic pathway were induced by a very low glucose signal. This unexpected finding indicates that central carbon metabolism is very sensitive to glucose. Even when exposed to very low ambient glucose concentrations, yeast activates carbon catabolism and represses carbon anabolism.

Hexose transporters

The rate of glucose uptake exerts a high degree of control over growth rate in yeast (Ye *et al.*, 1999). Also, some

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Protein	Gene	[Glucose] 0.01%	0.10%	1.00%
Hxt1	YHR094c	3.52	5.18	8.97
Hxt2	YMR011w	3.03	14.99	5.47
Hxt3	YDR345c	8.70	11.38	9.05
Hxt4	YHR092c	-1.37	1.41	1.66
Hxt5	YHR096c	1.47	1.46	-1.24
Hxt6	YDR343c	7.07	10.25	3.28
Hxt7	YDR342c	9.98	12.06	4.06
Hxt8	YJL214w	-1.32	-1.37	-1.65
Hxt9	YJL219w	-1.04	1.05	-1.01
Hxt10	YFL011w	1.31	1.17	1.12
Hxt11	YOL156w	1.08	1.17	-1.00
Hxt12	YIL170w	-1.04	-1.10	-1.26
Hxt12	YIL171w	-1.28	-1.24	-1.08
Hxt13	YEL069c	1.09	1.01	-1.10
Hxt14	YNL318c	-1.29	-1.34	-1.45
Hxt15	YDL245c	-1.34	-1.00	-1.06
Hxt16	YJR158w	-1.87	-2.08	-2.12
Hxt17	YNR072w	-1.09	-1.16	-1.17
Gal2	YLR081w	1.06	1.13	1.06
Rgt2	YDL138w	-1.43	-1.54	-1.25
Snf3	YDL194w	-1.16	-1.08	-1.18

B

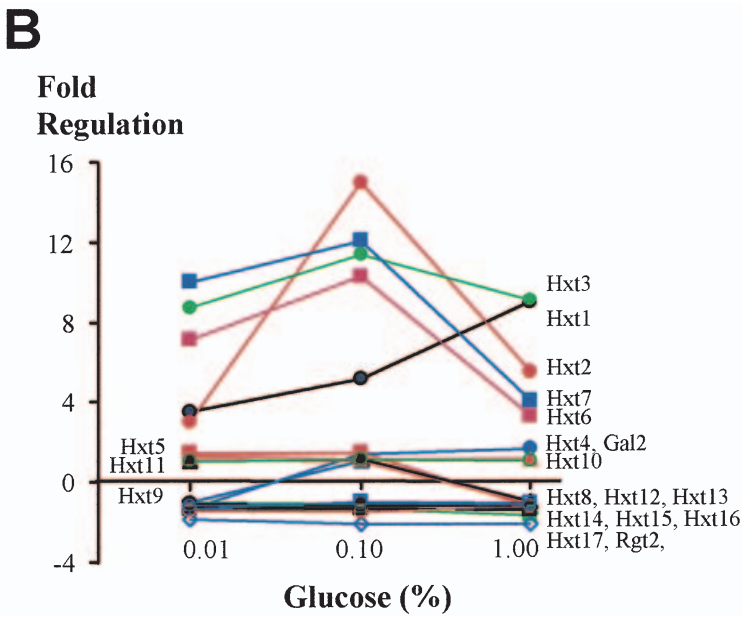

Fig. 3. Responses of hexose transporter family members to different glucose signals.
A. Fold induction for each gene is shown, with statistically significant changes highlighted in colour (*Experimental procedures*).
B. Graphical representation of fold changes.

hexose transporter genes are known to be differentially regulated by high and low glucose signals (Özcan and Johnston, 1995). Therefore, we examined the expression of the hexose transporter gene family.

The 20 members of this family displayed significantly different responses to glucose (Fig. 3). *HXT1–7* increased in response to at least one glucose concentration, whereas *HXT8–17*, *GAL2*, *RGT2* and *SNF3* did not. This is consistent with genetic and biochemical data that indicate that *HXT1–7* are the major players in glucose uptake (Kruckeberg, 1996; Özcan and Johnston, 1999). *HXT2*, *HXT6* and *HXT7* are important for growth on 0.1% glucose, whereas *HXT1*, *HXT3* or *HXT4* contribute to growth on high glucose concentrations (Reifenberger *et al.*, 1997). In contrast, *HXT8–17* are not thought to contribute significantly to glucose uptake, *GAL2* encodes a galactose transporter, and *RGT2* and *SNF3* encode glucose sensors (Kruckeberg, 1996; Özcan and Johnston, 1998; 1999). Interestingly, *HXT1–3* and *HXT6–7* responded dramatically to a very low glucose signal. Five of only seven yeast genes that increase ≥threefold after exposure to 0.01% glucose encode hexose transporters. This is consistent with the strong influence of glucose uptake upon growth rate (Ye *et al.*, 1999).

Our transcript profiling data are consistent with those of Özcan and Johnston (1995; 1999). For example, like us, these authors observed that *HXT1–5*, but neither HXT8 nor *HXT10–17*, are glucose induced. Also, like Özcan and Johnston (1995), we observed that *HXT2* responds more strongly to a low glucose signal than to a high glucose signal (Fig. 3). However, subtle differences are apparent between these data sets. For example, we observed a dramatic increase in *HXT1*, but not in *HXT4*, after exposure to 0.1% glucose: Özcan and Johnston (1995) observed the opposite. There are several possible reasons for this. Özcan and Johnston (1995) assayed β-galactosidase levels expressed from *HXT–lacZ* fusions, whereas levels of wild type transcripts were measured in this study. They analysed cells grown to mid-exponential phase, whereas we analysed cells 30 min after exposure to glucose. Our high glucose signal corresponded to 1.0%, whereas theirs was 4% glucose. Our non-fermentable carbon source was lactate, whereas theirs was galactose or raffinose. Given these significant differences in experimental design, the two data sets show a remarkably high degree of concordance. Therefore, although pregrowth on different non-fermentable carbon sources might be expected to exert minor effects upon the transcript profiles, the changes observed here with lactate-grown cells appear to reflect true physiological responses to glucose.

Ribosome biogenesis

Ribosome biogenesis involves a major investment of cellular resources and hence is tightly regulated in response to growth conditions (reviewed by Warner, 1989; 1999; Mager and Planta, 1991; Planta *et al.*, 1995). Ribosomal protein gene transcription is induced in response to high glucose signals (>1.0% glucose; Donovan and Pearson,

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Protein	Gene	[Glucose] 0.01%	0.10%	1.00%	Protein	Gene	[Glucose] 0.01%	0.10%	1.00%
Rpl1A	YPL220w	2.81	4.17	4.29	Rpl38	YLR325c	1.12	1.3	1.63
Rpl1B	YGL135w	1.89	2.39	3.72	Rpl39	YJL189w	1.54	2.03	1.31
Rpl2B	YIL018w	-1.61	-1.47	-1.36	Rpl40A	YIL148w	2.19	2.62	1.99
Rpl3	YOR063w	1.82	2.94	3.01	Rpl40B	YKR094c	2.03	2.59	1.96
Rpl4A	YBR031w	-1.02	1.05	1.52	Rpl41A	YDL184c	1.12	1.09	1.13
Rpl4B	YDR012w	1.78	2.7	3.01	Rpl42A	YNL162w	2.45	2.57	3.23
Rpl5	YPL131w	1.5	3.22	4.23	Rpl42B	YHR141c	2.01	2.12	2.27
Rpl6B	YLR448w	1.61	2.42	2.29	Rpl43A	YPR043w	2.15	2.39	1.83
Rpl7A	YGL076c	1.3	1.71	1.55	Rps0A	YGR214w	1.48	1.62	2.46
Rpl7B	YPL198w	1.39	2.47	2.89	Rps0B	YLR048w	1.24	1.55	2.35
Rpl8A	YHL033c	1.58	2.36	4.14	Rps1A	YLR441c	1.84	2.46	2.96
Rpl8B	YLL045c	1.74	2.35	3.08	Rps1B	YML063w	1.65	1.8	3
Rpl9A	YGL147c	1.7	2.33	2.9	Rps2	YGL123w	1.2	1.42	2.2
Rpl9B	YNL067w	1.54	2.37	2.4	Rps3	YNL178w	1.83	2.66	3.05
Rpl10	YLR075w	1.97	2.55	3.2	Rps4A	YJR145c	1.32	1.25	2.16
Rpl10E	YLR340w	1.66	2.61	3.24	Rps4B	YHR203c	1.72	2.25	3.58
Rpl11A	YPR102c	1.44	1.71	1.79	Rps5	YJR123w	-1.55	-1.39	-1.13
Rpl11B	YGR085c	1.59	2.08	1.92	Rps6A	YPL090c	1.86	2.32	3.35
Rpl12A	YEL054c	-2.26	1.77	2.6	Rps6B	YBR181c	1.07	1.02	1.91
Rpl12B	YDR418w	2.62	2.7	4.04	Rps7A	YOR096w	1.75	2.48	3.22
Rpl13A	YDL082w	1.39	1.62	1.81	Rps7B	YNL096c	1.43	1.91	1.85
Rpl14A	YKL006w	1.81	1.72	2.45	Rps8A	YBL072c	1.15	1.35	2.15
Rpl14B	YHL001w	-1.08	1.72	2.59	Rps8B	YER102w	2.35	3.37	4.97
Rpl15A	YLR029c	1.12	1.23	1.92	Rps9A	YPL081w	1.51	1.85	2.07
Rpl15B	YMR121c	2.01	2.48	3.21	Rps9B	YBR189w	1.17	1.58	2.93
Rpl16A	YIL133c	2.55	3.23	3.13	Rps10A	YOR293w	1.6	2.18	2.6
Rpl16B	YNL069c	1.63	2.9	3.86	Rps11A	YDR025w	1.46	2.03	3.04
Rpl17A	YKL180w	2.11	2.67	3.72	Rps11B	YBR048w	1.09	1.19	1.62
Rpl17B	YJL177w	-1.04	1.46	1.63	Rps12	YOR369c	2.5	3.59	3.36
Rpl18A	YOL120c	-1.53	-1.49	-1.16	Rps13	YDR064w	1.28	1.07	1.89
Rpl18B	YNL301c	1.78	2.41	2.84	Rps14A	YCR031c	1.9	2.7	4.35
Rpl19A	YBR084c	1.3	2	2.52	Rps14B	YJL191w	1.24	1.44	1.65
Rpl19B	YBL027w	-1.1	1.41	2.07	Rps15	YOL040c	1.92	2.13	2.82
Rpl20A	YMR242c	2.03	2.75	3.2	Rps16B	YDL083c	1.92	2.57	3.53
Rpl20B	YOR312c	1.76	2.09	2.24	Rps17B	YDR447c	-1.31	3.07	3.29
Rpl21A	YBR191w	1.13	1.15	2.25	Rps18A	YDR450w	2.09	2.36	3.16
Rpl21B	YPL079w	2.21	2.55	3.77	Rps19A	YOL121c	1.85	1.93	2.66
Rpl22A	YLR061w	1.7	1.7	1.88	Rps19B	YNL302c	1.17	1.1	1.59
Rpl23A	YBL087c	1.08	-1.12	1.34	Rps20	YHL015w	1.14	2.1	1.61
Rpl23B	YER117w	1.85	1.97	2.51	Rps21A	YKR057w	1.61	1.64	1.8
Rpl24A	YGL031c	1.37	1.56	1.66	Rps21B	YJL136c	2.44	2.39	2.51
Rpl24B	YGR148c	2.14	2.78	3.73	Rps22A	YJL190c	1.05	1.19	1.44
Rpl25	YOL127w	1.4	1.48	1.86	Rps22B	YLR367w	1.64	2.4	2.69
Rpl26A	YLR344w	1.79	2.13	2.25	Rps23A	YGR118w	1.79	1.99	2.11
Rpl26B	YGR034w	1.82	2.18	2.78	Rps23B	YPR132w	2.1	2.84	3.52
Rpl27A	YHR010w	1.6	1.93	2.1	Rps24A	YER074w	2.21	2.21	2.54

B

Protein	Gene	[Glucose] 0.01%	0.10%	1.00%
Img1	YCR046c	-1.49	-1.51	-1.34
Mrp1	YDR347w	-1.52	-1.54	-1.17
Mrp13	YGR084c	-1.01	-1.32	-1.16
Mrp17	YKL003c	-1.25	-1.41	-1.53
Mrp2	YPR166c	-1.05	-1.15	-1.17
Mrp20	YDR405w	-1.13	-1.04	1.11
Mrp21	YBL090w	1.03	-1.11	-1.45
Mrp4	YHL004w	-1.01	-1.16	-1.16
Mrp49	YKL167c	-1.10	-1.09	1.17
Mrp7	YNL005c	1.11	1.04	-1.11
Mrp8	YKL142w	-2.38	-1.82	-2.89
Mrpl10	YNL284c	-1.01	-1.31	-1.51
Mrpl11	YDL202w	1.23	1.18	-1.01
Mrpl13	YKR006c	-1.48	-1.84	-1.80
Mrpl16	YBL038w	-1.57	-1.69	1.05
Mrpl17	YNL252c	-1.02	1.09	-1.11
Mrpl19	YNL185c	-1.20	1.01	-1.06
Mrpl20	YKR085c	-2.00	-2.04	-2.03
Mrpl23	YOR150w	-1.35	-1.40	-1.60
Mrpl24	YMR193w	1.05	-1.02	-1.28
Mrpl25	YGR076c	-2.97	-5.75	-4.03
Mrpl27	YBR282w	-1.08	-1.21	-1.24
Mrpl28	YDR462w	-1.35	-1.30	-1.56
Mrpl3	YMR024w	1.09	1.06	1.03
Mrpl31	YKL138c	1.02	-1.10	-1.41
Mrpl32	YCR003w	-1.14	-1.11	-1.18
Mrpl35	YDR322w	-1.09	-1.09	1.06
Mrpl36	YBR122c	1.12	-1.06	-1.08
Mrpl37	YBR268w	-1.04	-1.03	-1.27
Mrpl38	YKL170w	-1.05	-1.13	-1.36
Mrpl4	YLR439w	-1.74	-2.07	-2.10
Mrpl40	YPL173w	1.00	-1.20	-1.27
Mrpl49	YJL096w	-1.32	-1.40	-1.53
Mrpl6	YHR147c	-1.22	-1.55	-1.71
Mrpl8	YJL063c	1.00	1.33	1.22
Mrpl9	YGR220c	-1.09	-1.29	-1.24
Mrps28	YDR337w	-1.01	-1.15	1.05
MrpS5	YBR251w	1.14	1.12	-1.03
MrpS9	YBR146w	1.20	1.39	1.21
Nam9	YNL137c	-1.27	-1.24	-1.45
Pet123	YOR158w	-1.31	-1.19	-1.29
Ppe1	YHR075c	-1.27	-1.16	-1.17
Rmi2	YEL050c	-1.00	-1.31	-1.31
Ymr31	YFR049w	-1.06	-1.17	-1.99

Fig. 4. Effects of different glucose signals upon ribosomal protein mRNAs. Fold induction for each gene is shown, with statistically significant changes highlighted in colour (*Experimental procedures*).
A. Cytoplasmic ribosomal protein mRNAs.
B. Mitochondrial ribosomal protein mRNAs.

1986; Klein and Struhl, 1994; Griffioen *et al*., 1996; Lopez and Baker, 2000). In addition, the abundance of ribosomal protein mRNAs decreases as glucose becomes exhausted during diauxic growth (DeRisi *et al*., 1997). However, the influences of low glucose signals upon these genes are largely unexplored.

Almost without exception, the levels of cytoplasmic ribosomal protein mRNAs increased significantly in response to 0.1% or 1.0% glucose, but they were not induced by 0.01% glucose (Fig. 4). In contrast, mitochondrial ribosomal protein mRNAs remained unaffected by any of these glucose signals. Clearly, these effects of glucose upon ribosome protein mRNAs are specific for cytoplasmic ribosomes. Our data confirm that ribosomal protein gene transcription responds to high glucose signals (Donovan and Pearson, 1986; Klein and Struhl, 1994; Griffioen *et al*., 1996; Lopez and Baker, 2000). Significantly, our data extend this by showing that the expression of cytoplasmic ribosomal protein genes is sensitive to a low glucose signal, but not to a very low glucose signal.

Northern blotting was performed on a subset of gluconeogenic (*PCK1* and *FBP1*), glycolytic (*PGK1*) and ribosomal protein mRNAs (*RPL3* and *RPL24*) to test further the validity of the transcript profiling data (Fig. 5). Similar responses were observed in the transcript profiles and Northern blots for all mRNAs examined. The gluconeogenic mRNAs were repressed, and the glycolytic and ribosomal protein mRNAs were induced even when exposed to 0.01% glucose. Note that *RPL3* and *RPL24* were among the few ribosomal protein mRNAs that were sensitive to a very low glucose signal (Fig. 4).

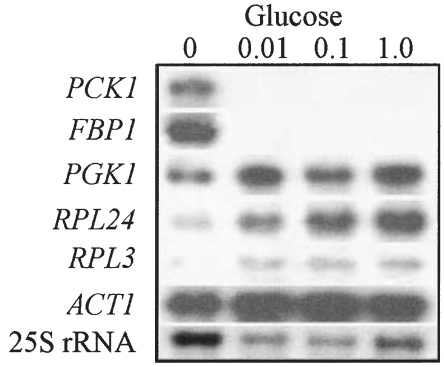

Fig. 5. Northern analysis confirms the validity of transcript profiling data for control mRNAs. Northern analysis was performed on RNA prepared from W303-1B, 30 min after glucose addition: no glucose control, 0; very low glucose signal, 0.01; low glucose signal, 0.1; high glucose signal, 1.0. Similar data were obtained for independent RNA samples.

Ribosomal protein mRNAs are transiently stabilized by glucose

Yeast ribosomal protein mRNAs are relatively abundant, and yet they have short half-lives. Consequently, about 50% of RNA polymerase II transcription is devoted to ribosomal protein genes in rapidly growing yeast cells (Warner, 1999). This author pointed out that regulation of ribosomal protein expression is important for the economics of yeast growth. Hence, in view of the rapid response of ribosomal protein mRNAs to glucose (Fig. 4), we revisited the issue of ribosomal protein mRNA stability.

As reported previously (Kim and Warner, 1983; Herrick *et al.*, 1990), we observed that the *RPL3* and *RPL24* mRNAs had short half-lives (Fig. 6A). [*RPL24* was previously called *RPL29* (Mager *et al.*, 1997).] Their half-lives of about 8 and 16 min, respectively, did not differ significantly during prolonged growth on glucose or lactate. However, when lactate-growing cells were exposed to glucose, *RPL3* and *RPL24* mRNA half-lives increased signif-

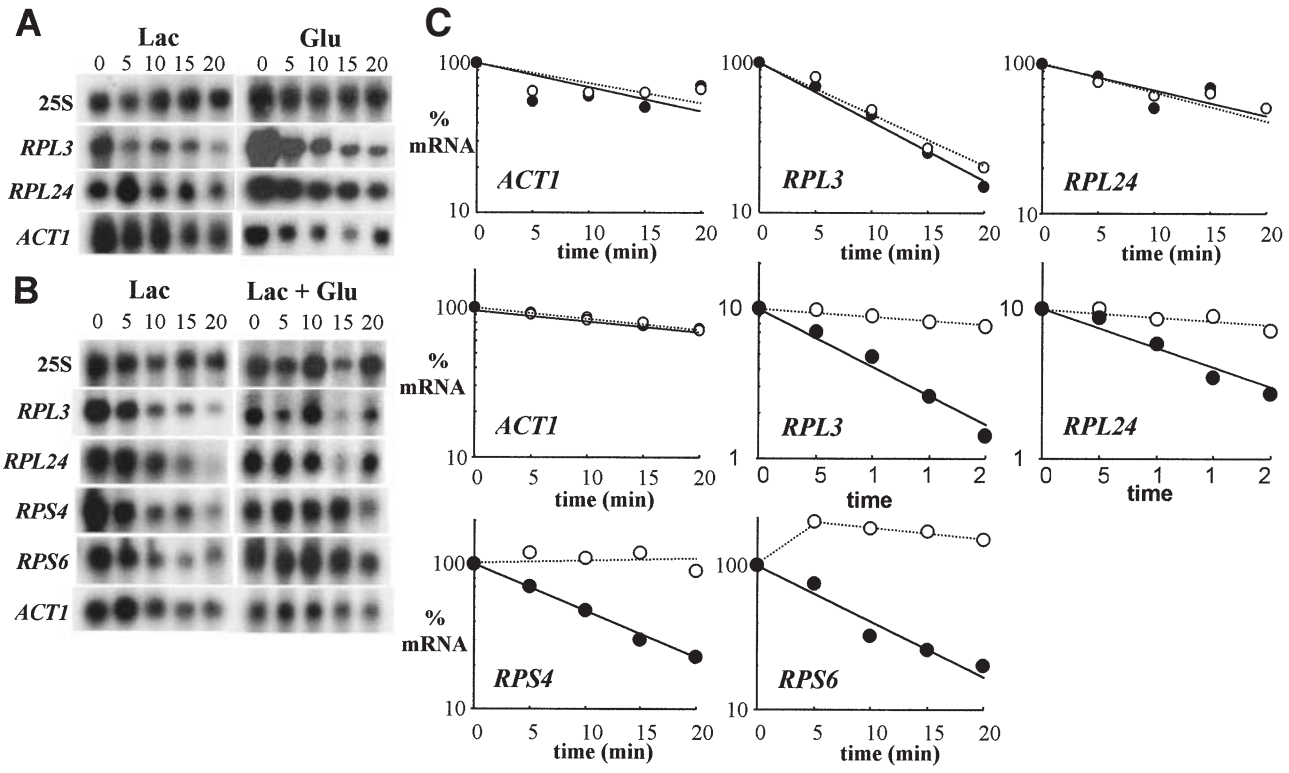

Fig. 6. Effect of glucose upon ribosomal protein mRNA stability. Northern blots are shown for ribosomal protein mRNAs, the control *ACT1* mRNA and the loading control 25S rRNA. Signals on these Northern blots were quantified by phosphorimaging, corrected for loading differences and plotted relative to the zero time point: no glucose, filled circles; glucose, open circles. mRNA levels were measured in YRP582 cells 0, 5, 10, 15 and 20 min after transcriptional shut-off.
A. Decay rates during exponential growth in YPD (Glu) or YPL (Lac).
B. Decay rates after exposure to 0.01% glucose. Cells were grown to mid-exponential phase in YPL. At $t = 0$, glucose was added to one culture (Lac + Glu) and H_2O to the control culture (Lac), and mRNA decay rates were analysed immediately thereafter.
C. Decay rates after exposure to 1.0% glucose. Cells were treated in the same way as in (B), except that glucose was added to 1.0% (Lac + Glu).

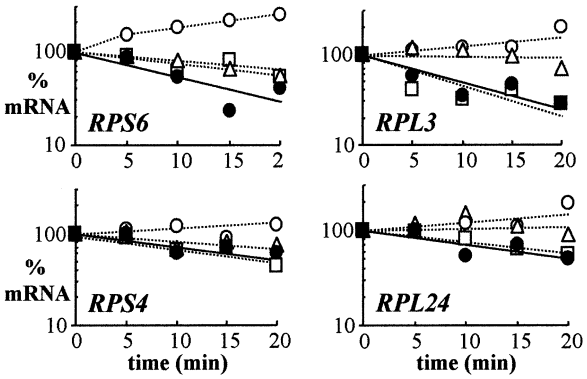

Fig. 7. Ribosomal protein mRNAs are transiently stabilized by glucose. YRP582 cells were grown to mid-exponential phase in YPL. Glucose (2%) was added, and mRNA decay rates were analysed by shutting off transcription immediately (open circles), after 1 h (open triangles) and 3 h later (open squares). H_2O was added to the control culture (filled circles). Signals were quantified as described in the legend to Fig. 6.

icantly, to about 60 and 45 min respectively (Fig. 6B). We then examined the *RPS4* and *RPS6* mRNAs to test whether mRNAs encoding small ribosomal subunit proteins behaved in a similar fashion. The *RPS4* and *RPS6* mRNAs were also stabilized after glucose addition (Fig. 6B).

The effect of glucose upon ribosomal protein mRNA stability appeared to be transient, because stabilization was observed immediately after glucose addition, but not after prolonged growth on glucose (Fig. 6). To test this further, the *RPL3*, *RPL24*, *RPS4* and *RPS6* mRNA stability was examined 0, 1 and 3 h after glucose addition (Fig. 7). Glucose addition stabilized all four mRNAs, but the half-lives of the *RPS4* and *RPS6* mRNAs returned to normal within 1 h. *RPL3* and *RPL24* mRNA half-lives returned to normal within 3 h. Hence, these ribosomal protein mRNAs are transiently stabilized after exposure to glucose.

Influence of glucose signalling upon transient stabilization of ribosomal protein mRNAs

Ribosomal protein gene transcription displays a biphasic response to a high glucose signal. The initial response, within 20 min, is dependent upon sugar phosphorylation and protein kinase A. The sustained response after 60 min depends upon metabolism of the glucose, but not protein kinase A (Klein and Struhl, 1994; Griffioen *et al*., 1996). Therefore, we tested the effects of glucose sensing and signalling mutants upon the transient stabilization of ribosomal protein mRNAs.

Transient stabilization of the *RPS6* mRNA was observed in congenic *rgt2* and *snf3* mutants, indicating that the high (Rgt2) and low glucose sensors (Snf3) are not required for this effect (Fig. 8). Glucose did not affect *RPS6* mRNA stability in a yeast strain lacking all three hexose kinases, encoded by *HXK1*, *HXK2* and *GLK1*. Transient *RPS6* mRNA stabilization was retained in a strain lacking Ras protein (Ras1/2), the regulatory subunit of protein kinase A (Bcy1), and containing a weak protein kinase A (*tpk1*, *tpk2*^{w1}, *tpk3*). In general, similar effects were observed for the *RPL3*, *RPL24* and *RPS4* mRNAs. However, there were two exceptions (not shown). First, *RPL3* and *RPL24* mRNA stabilization was blocked in the *ras1*, *ras2 bcy1*, *tpk1*, *tpk2*^{w1}, *tpk3* strain. Secondly, *RPL3* mRNA stabilization was retained in *hxk1*, *hxk2*, *glk1* cells (Wilson, 2001). These effects were reproducible. Therefore, ribosomal protein mRNAs for both small and large subunits are transiently stabilized by glucose, but different signalling pathways may mediate this stabilization. Components of the protein kinase A pathway are required for glucose stabilization of large ribosomal subunit mRNAs, as well as for the short-term activation of ribosomal protein gene transcription (Griffioen *et al*., 1996). In contrast, glucose phosphorylation, but not protein kinase A pathway components, appear to be required for glucose stabilization of small ribosomal subunit mRNAs (Fig. 8).

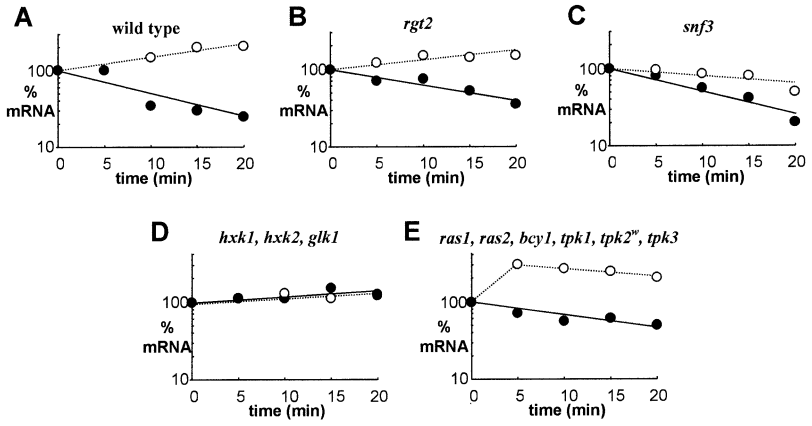

Fig. 8. Effect of glucose signalling mutations upon ribosomal protein mRNA stabilization. Strains were grown to mid-exponential phase in YPL, whereupon glucose (2%) was added, and mRNA decay rates were analysed immediately thereafter. Signals for the *RPS6* mRNA were quantified as described in the legend to Fig. 6: no glucose, filled circles; glucose, open circles.
A. DC124.
B. YM4817.
C. YM4714.
D. D308-3.
E. PD6595.
Note that *RPS6* mRNA decay rates are slower in D308-3 in the absence of glucose, presumably because this strain is relatively slow growing. Relevant genotypes are shown, and full genotypes are in Table 2.

Yeast ribosomal protein gene regulation is mediated primarily at the transcriptional level (Planta *et al*., 1995; Warner, 1999). Nevertheless, several other examples of post-transcriptional regulation have been described for yeast ribosomal protein genes. Autoregulation of *RPL32* and *RPS14* mRNA levels appears to be achieved partly by feedback inhibition of premRNA splicing (Vilardell and Warner, 1997; Fewell and Woolford, 1999). Translation of excess *RPL3* mRNA is downregulated (Pearson *et al*., 1982). Furthermore, excess Rps7, Rps10A, Rps10B or Rpl24 protein is degraded rapidly (Warner *et al*., 1985). These phenomena help to ensure the production of equimolar amounts of each ribosomal protein.

Conclusions

In conclusion, our global analyses of short-term responses to different glucose signals has shown that yeast is exquisitely sensitive to glucose. A large number of yeast genes respond to very low glucose signal (0.01%; Figs 1–4). Significantly, central metabolic functions are more sensitive to glucose than most other cellular functions. For example, glycolytic, gluconeogenic, key hexose transporter and glyoxylate and TCA cycle genes respond to very low glucose signal (0.01%; Figs 2 and 3), whereas genes involved in the biogenesis of cytoplasmic ribosomes only respond to a low glucose signal (0.1%; Fig. 4).

These observations indicate that yeast modulates its response to glucose depending upon the ambient concentration of this key nutrient. Central metabolic pathways are regulated in such a way as to take advantage of even very low glucose concentrations. In contrast, ribosome biogenesis, which requires a major investment of the cell's resources (Warner, 1999), is only stimulated if sufficient amounts of glucose are available to support growth.

We have also demonstrated that glucose regulates the expression of cytoplasmic ribosomal protein genes at a post-transcriptional level. Glucose transiently stabilizes mRNAs involved in the biogenesis of both large and small ribosomal subunits: *RPL3*, *RPL24*, *RPS4* and *RPS6* (Figs 6 and 7). Subtle differences might exist with respect to the signalling pathways that trigger the stabilization of different ribosomal protein mRNAs (Figs 7 and 8). Nevertheless, this transient stabilization of ribosomal protein mRNAs by glucose presumably promotes a rapid and cost-effective acceleration of ribosome biogenesis after a nutritional upshift (Fig. 9).

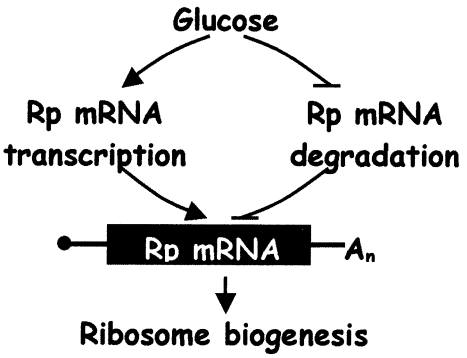

Fig. 9. Model illustrating the effects of glucose upon ribosome biogenesis. Glucose activates ribosomal protein gene transcription and transiently inhibits ribosomal protein mRNA degradation (see text). These two effects combine to increase ribosomal protein mRNA abundance, thereby accelerating ribosome biogenesis.

Experimental procedures

Strains and growth conditions

Yeast strains (Table 2) were grown at 30°C in YPD or YPL (1% yeast extract, 2% peptone containing 2% glucose or 3% lactate respectively).

MRNA analysis

Yeast mRNA decay was analysed in mid-exponential cells using published procedures (Yin *et al*., 1996; 2000). Transcriptional shut-off in YRP582 cells was achieved using the conditional *rpb1-1* mutation (Parker *et al*., 1991) or using 1,10-phenanthroline in other strains (Santiago *et al*., 1986). The *FBP1* probe was a 1.9 kb *Hin*dIII–*Xba*I fragment from pJJ5 (de la Guerra *et al*., 1988; Mercado *et al*., 1994); the *PCK1* probe was a 1.3 kb *Hin*dIII–*Sal*I fragment from pJJ1

Table 2. Strains used in this study.

Strain	Genotype	Source/reference
W303-1B	*MATalpha*, *ade2*, *his3*, *leu2*, *trp1*, *ura3*	Thomas and Rothstein (1989)
DC124	*MATalpha*, *ade8*, *can1*, *trp1*, *his3*, *leu2*, *ura3*	Toda *et al*. (1985)
D308-3	*MATalpha*, *ade1*, *trp1*, *his2*, *met14*, *hxk1*, *hxk2*, *glk1*	Lobo AND Maitra (1977)
PD6595	*MAT***a**, *ade8*, *his3*, *leu2*, *trp1*, *ura3*, *tpk1::URA3*, *tpk2^{w1}*, *tpk3::TRP1*, *bcy1::LEU2*, *ras1::HIS3*, *ras2::ADE8*	Durnez *et al*. (1994)
YM4127	*MAT***a**, *ade2*, *his3*, *leu2*, *lys2*, *trp1*, *tyr1*, *ura3*	Özcan and Johnston (1995)
YM4714	*MAT***a**, *ade2*, *his3*, *leu2*, *lys2*, *trp1*, *tyr1*, *ura3*, *snf3::hisG*	Özcan *et al*. (1996)
YM4817	*MAT***a**, *ade2*, *his3*, *leu2*, *lys2*, *trp1*, *tyr1*, *ura3*, *rgt2::HIS3*	Özcan *et al*. (1996)
YRP582	*MATalpha*, *leu2*, *ura3*, *rpb1*	Nonet *et al*. (1987)

(Stucka *et al*., 1988; Mercado *et al*., 1994); the *ACT1* probe was a 1.5 kb *Bam*HI–*Hin*dIII fragment from pSPACT9 (Bettany *et al*., 1989; Moore *et al*., 1991); the *PGK1* probe was a 1.0 kb *Xba*I–*Bgl*II fragment from pBS-PGK1 (constructed by L. Hatton in this laboratory); the *RPL3* probe was a 3.2 kb *Ava*I–*Bam*HI fragment from pTCM(3.2) (Fried and Warner, 1981); the *RPL29* probe was a 1.5 kb *Eco*RI–*Bam*HI fragment from pA13(1.5) (Mitra and Warner, 1984); the *RPS4* probe was a 0.74 kb polymerase chain reaction (PCR) product generated using the primers 5′-GCTAGAGGACCGTAT GTT and 5′-GTCGATCTTAACAGTGTC; and the *RPS6* probe was a 0.64 kb PCR product generated using the primers 5′-GCAGGAAGACTACCATAT and 5′-CAGTAGTGTCAGTTA GAC. As an internal loading control, membranes were reprobed for 25S rRNA using the end-labelled oligonucleotide 5′-CCTCCGCTTATTGATATGCTTAAG. Northern blots were autoradiographed, and then signals were quantified directly by phosphorimaging using a Fuji FLA-3000 phosphorimager (Raytek). mRNA levels were measured relative to the 25S rRNA internal control. There were variations in basal mRNA degradation rates in different strain backgrounds (Yin *et al*., 2000). However, all experiments were performed at least twice, and only reproducible observations are reported.

Transcript profiling

W303-1B cells were grown at 30°C in YPL to mid-exponential phase ($OD_{600} = 0.5$) and then subcultured into fresh YPL at 30°C and regrown to an OD_{600} of 0.5. Glucose was then added to the specified concentration, and the cells were harvested 30 min later. Water alone was added to control cells. Cells were frozen rapidly in liquid N_2, sheared mechanically using a microdismembrator (Braun Melsungen) and RNA prepared by extraction with Trizol Reagent™ (Gibco BRL), as described previously (Hauser *et al*., 1998). Hybridization probes were made by preparing [^{33}P]-first-strand cDNA using a $(dT)_{15}$ primer (Hauser *et al*., 1998). cDNA probes were denatured by incubation in 0.3 M NaOH at 65°C and hybridized to the *S. cerevisiae* arrays for 24 h at 65°C in 0.5 M $NaPO_4$, 7% SDS, 0.01 M ETDA, pH 7.2 (Church and Gilbert, 1984). Hybridization signals were detected using a Fuji FLA-3000 phosphorimager. Probes were stripped from membranes using six washes with 0.1% SDS, 5 mM $NaPO_4$, pH 7.2, at 100°C and reprobed. Phosphorimages were analysed using ARRAY VISION software (Amersham).

Data analysis

For all analyses performed, the overall signal intensity on all spots was used to normalize individual experiments. Four data points from two independent experiments were taken into account. The significance of the observed variations in transcript levels was analysed by statistical procedures (Beissbarth *et al*., 2000). The median of all signal intensities obtained on each fragment was calculated, and the significance of variations was assessed by two stringency criteria. The highly stringent 'min–max separation' is calculated by taking the minimum distance between all data points of the two conditions. The less stringent criteria, called 'standard deviation separation', is defined as the difference of the means of either data set diminished by one standard deviation. Data quality assessment, normalization and statistical analysis were performed with the M-CHIPS software package (Fellenberg *et al*., 2001; 2002) (http://www.dkfz-heidelberg.de/tbi/services/mchips) written in MATLAB (Mathworks). The complete transcript profiling data set is available in the form of both lists of the normalized results and raw data files at http://mips.gsf.de/proj/eurofan/eurofan_2/b2/. Functional categories were assigned using the MIPS catalogue (http://mips.gsf.de/proj/yeast/CYGD/db/catalog_index.html: see 'Functional Classification Catalogues'). In this catalogue, some genes are assigned to more than one functional category.

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