UvA-DARE (Digital Academic Repository)

Carbon catabolite regulation of transcription of nuclear genes coding for mitochondrial proteins in the yeast *Kluyveromyces lactis*

Mulder, W.; Scholten, I.H.J.M.; Grivell, L.A.

DOI

[10.1007/BF00309786](https://doi.org/10.1007/BF00309786)

Publication date

1995

Published in

Current Genetics

[Link to publication](#)

Citation for published version (APA):

Mulder, W., Scholten, I. H. J. M., & Grivell, L. A. (1995). Carbon catabolite regulation of transcription of nuclear genes coding for mitochondrial proteins in the yeast *Kluyveromyces lactis*. *Current Genetics*, 28, 267-273. <https://doi.org/10.1007/BF00309786>

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

ORIGINAL PAPER

Wietse Mulder · Inge H.J.M. Scholten
Leslie A. Grivell

Carbon catabolite regulation of transcription of nuclear genes coding for mitochondrial proteins in the yeast *Kluyveromyces lactis*

Received: 7 February 1995

Abstract Promoter regions of the *KIQCR7*, *KIQCR8* and *KICYC1* genes, coding for subunits of the bc_1 -complex and cytochrome *c* respectively, in the short-term Crabtree-negative yeast *Kluyveromyces lactis* differ markedly in sequence from their *Saccharomyces cerevisiae* counterparts. They have, however, conserved very similar configurations of binding-site motifs for various transcription factors known to be involved in global and carbon-source regulation in *S. cerevisiae*. To investigate the carbon source-dependent expression of these genes in *K. lactis*, we have carried out medium-shift experiments and determined transcript levels during the shifts. In sharp contrast to the situation in *S. cerevisiae*, the level of expression in *K. lactis* is not affected when glucose is added to a non-fermentable carbon-source medium. However, the genes are not constitutively expressed, but become significantly induced when the cells are shifted from glucose to a non-fermentable carbon source. Finally, induction of transcriptional activation does not occur in media containing both glucose and non-fermentable carbon sources.

Key words *Saccharomyces cerevisiae* · *Kluyveromyces lactis* · Transcriptional regulation · Catabolite repression

Introduction

Growth of the yeast *Saccharomyces cerevisiae* on a fermentable carbon source such as glucose leads to

dramatic changes in the pattern of gene expression, including the induction of genes involved in glycolysis and the repression of genes involved in other catabolic pathways (recently reviewed by Entian and Barnett 1992; Gancedo 1992; Trumbly 1992). This response is intimately bound up with the yeast cell's ability to sense and assimilate nutrients. For a limited number of genes, both genetic and biochemical analysis have yielded information on the chain of events that is triggered following uptake of glucose by the cell, leading eventually to changes in transcription. A major effect of glucose in *S. cerevisiae* is the repression of respiratory function. Although glucose effects on mRNA stability and translation have been reported (Lombardo et al. 1992), the primary mechanism for repression is down-regulation of the transcription of nuclear genes encoding mitochondrial respiratory chain proteins (Forsburg and Guarente 1989a). One common feature of the promoters of these genes is the presence of binding sites for the transcription factors HAP2/3/4, which form a heterotrimeric complex capable of activating transcription in the absence of glucose (Forsburg and Guarente 1989b). HAP2 and 3 are homologues of the mammalian NF-Y/CCAAT-binding proteins and are synthesised constitutively (Chodosh et al. 1988; Li et al. 1992). In contrast, synthesis of HAP4, the component of the complex thought to be responsible for activation, is glucose-repressible and the low levels of this protein in glucose-repressed cells may be a major factor in restricting transcription from promoters of genes for respiratory components to basal levels (Forsburg and Guarente 1989b). The promoters of these nuclear genes, encoding mitochondrial proteins, often contain additional *cis*-acting elements, including glucose-responsive elements and binding sites for the general transcription factors ABF1, CPF1 and RAP1 (reviewed by Diffley 1992). For the *ScQCR8* gene, encoding subunit VIII of the mitochondrial ubiquinol cytochrome *c* oxidoreductase (QCR) or bc_1 -complex, ABF1 has been shown to be necessary for maintaining basal

W. Mulder¹ · I.H.J.M. Scholten · L.A. Grivell (✉)
Section for Molecular Biology, Institute for Molecular Cell Biology,
Biozentrum Amsterdam, University of Amsterdam, Kruislaan 318,
1098 SM Amsterdam, The Netherlands.

¹ Present address: Wageningen Agricultural University, Section for
Molecular Genetics of Industrial Micro-organisms, Dreijenlaan 2,
6703 HA Wageningen, The Netherlands

Communicated by L. Frontali

transcription rates under glucose repression and for a rapid and efficient upswing of transcription on escape from glucose-repression. CPF1, whose binding site overlaps with that for ABF1, appears to act as a negative regulator of transcription (De Winde and Grivell 1992). In order to extend our understanding of the individual contributions of the various factors to transcriptional activation, we have undertaken a study of the *QCR* counterpart genes, and the corresponding transcription factors, in the yeast *Kluyveromyces lactis*.

In terms of the highly conserved sequences of its proteins, *K. lactis* is closely related to *S. cerevisiae*. In terms of regulatory factors, however, work so far has uncovered an informative divergence which has shed light on transcriptional regulatory mechanisms in both of these yeasts (Mylin et al. 1991; Czyz et al. 1993; Zenke et al. 1993; Mulder et al. 1994 a, d).

We have in previous papers reported the isolation of the *KIQCR7* (Mulder et al. 1994 c) and *KIQCR8* genes (Mulder et al. 1994 b), respectively encoding subunits VII and VIII of the *bc*₁-complex of *K. lactis*, and shown that the promoters of both genes lack overall DNA sequence similarity with their *S. cerevisiae* counterparts, but share binding sites for the transcription factors HAP2/3/4, ABF1 and CPF1.

In the present paper we examine the carbon source-dependent expression of *K. lactis* genes encoding components of the respiratory chain. Despite the similarity in configuration of binding sites for the various transcription factors in the promoter regions, we find that transcription in *K. lactis* is inducible on non-fermentable carbon sources when glucose is absent. Contrasting to the situation in *S. cerevisiae*, addition of glucose to a non-fermentable carbon-source medium does not affect the level of expression.

Materials and methods

Strains and growth conditions. *K. lactis* strain CBS2359 was used in all experiments and cells of this strain were grown overnight in 10 ml of WO medium (0.67% yeast nitrogen base, 2% D-glucose) at 30 °C. These cultures were used to inoculate 40 ml or 500 ml of YP media (1% yeast extract, 2% Bacto-peptone) supplemented with either 5% D-glucose (YPD5), 2% D-glucose (YPD2), or 2% (w/v) ethanol and 2% glycerol (YPEG), depending on the kind of experiment, and were again grown overnight to a *D*₆₀₀ of 1.0–2.0 as measured on a CamSpec spectrophotometer. For medium-shift experiments, the 40-ml YPD2 culture was subsequently pelleted for 5 min at 5000 rpm (Sorvall) at 25 °C and resuspended in 1 ml of sterile water (30 °C); 250-μl aliquots were added to both 40 ml of YPD2 and 40 ml of YPD5 and the remainder was added to 40 ml of YPEG. The cultures were allowed to grow for an additional 4–5 h and thereafter harvested, transferred to a 1.5-ml reaction tube, frozen in liquid nitrogen and kept at –20 °C until RNA was isolated. For time-course medium-shift experiments, the 500-ml YP overnight culture was pelleted as described above. The pellet was resuspended in sterile water and added to 500 ml of fresh YP medium at 30 °C and containing the appropriate amount and type of carbon source; 10-ml aliquots were withdrawn at fixed time points, added to 10 ml of water (0 °C) and stored on ice to immediately stop cell metabolism.

The *D*₆₀₀ was measured and cells were harvested and treated as stated above. In some experiments, the shift was achieved without change of medium by the addition of an appropriate volume of a stock solution containing either 50% D-glucose or 20% ethanol/20% glycerol to a final concentration of respectively 5% glucose and 2% ethanol/2% glycerol.

Northern blotting. RNA isolation was performed essentially as described by De Winde and Grivell (1992) and spectrophotometrically quantified at 260 nm (CamSpec); 15 μg of RNA was electrophoresed on a 1.2% non-denaturing agarose gel in 0.25 × TBE buffer (1 × TBE: 89 mM Tris, 89 mM boric acid, 2.5 mM EDTA at pH 8.3) and subsequently blotted to a Hybond N filter (Amersham) according to procedures recommended by the manufacturer. Hybridisation and washing conditions were used as described (De Winde and Grivell 1992). Blots were exposed to either Kodak X-Omat S- or X-Ar-films for various time periods at –70 °C. As DNA probes we used: a 500-bp *EcoRI*-*HindIII* fragment of plasmid pART6, containing the *KICYC1* gene (Freire-Picos et al. 1993); a 2100-bp *KpnI*-*KpnI* fragment containing the chromosomal-linked *KLAPA2* and *KIQCR7* genes (Mulder et al. 1994 c); a 840-bp *HindIII*-*SalI* fragment derived from pJH1 possessing the *ScQCR8* gene (De Winde and Grivell 1992); a 335-bp *HindIII*-*HindIII* fragment containing the *KIQCR8* gene; a 540-bp *KpnI*-*PstI* fragment containing the *KIFPS1* gene and a 1600-bp *SalI*-*EcoRI* fragment containing both *KIFPS1* and *KIQCR8*, all three being derived from plasmid pK1#11.1 (Mulder et al. 1994 b); and a 900-bp *EcoRI*-*HindIII* fragment of plasmid pK17, containing the *K. lactis* actin gene (Deshler et al. 1989).

Results

Medium-shift experiments

In initial experiments designed to characterise carbon-source dependency of gene expression in *K. lactis*, conditions similar to those routinely used in our laboratory for the study of *S. cerevisiae* (De Winde and Grivell 1992) were found to yield irreproducible responses. Better control of responses was obtained by use of the protocol described in Materials and methods. Essential features of this protocol are that high cell densities are avoided and all cultures have the same environmental history. Figure 1 shows the results

Fig. 1 Medium-shift experiment. *K. lactis* strain CBS2359 was grown in a 40-ml YPD2 culture, which was pelleted and subdivided over 40 ml of medium containing the carbon source indicated below the panels. RNA was isolated after 5 h growth at 30 °C. For each sample 15 μg of RNA was loaded four times on a 1.2% non-denaturing agarose gel, electrophoresed and blotted. The blot was cut into four identical pieces and these were hybridised with DNA probes as indicated.

obtained when wild-type *K. lactis* cells were pre-cultured in 2% glucose and transferred to either 5% glucose, 2% glucose, or ethanol/glycerol. Similar to the corresponding *S. cerevisiae* genes, expression of the *KIQCR7*, *KIQCR8* and *KICYC1* genes is significantly induced on a non-fermentable carbon source. However, the expression under glucose growth conditions is, in particular for the *KIQCR7* and *KIQCR8* genes, relatively high compared to the situation in *S. cerevisiae*. In contrast, but like *S. cerevisiae*, constitutive expression is observed for the *K. lactis* actin gene and for the *KIAPA2* and *KIFPS1* genes, which are closely-linked to *KIQCR7* (Mulder et al. 1994c) and *KIQCR8* (Mulder et al. 1994b) respectively. The insensitivity of expression of the *KIFPS1* and *KIAPA2* genes to a change of carbon source, coupled with the fact that their sequences are present on the same DNA-probe fragments as the corresponding *QCR* genes, makes their transcripts highly suitable and convenient internal standards in Northern blot analysis. They were therefore used as such in subsequent experiments.

Glucose fails to affect the level of expression of *K. lactis* genes coding for respiratory chain components following a glucose shift

To investigate what effects the addition of glucose would have on the level of expression of the genes encoding for respiratory chain components, a glucose-shift experiment was performed. Cells were grown in YPEG and glucose was added to a final concentration of 5%. Both growth and transcript levels were determined at fixed time-points after the shift. As shown in Fig. 2A, expression of the *KIQCR7*, *KIQCR8* and *KICYC1* genes is not significantly affected by the addition of glucose, a response which is in marked contrast to the situation in *S. cerevisiae* (Zitomer et al. 1979; Maarse et al. 1988; De Winde and Grivell 1992; Gancedo 1992). In this latter organism the level of expression of the counterpart genes drops to almost undetectable levels within 30 min using similar experimental conditions. The slight differences in expression between the various time-point samples observable in

Fig. 2A, B Medium shift by addition of glucose to an ethanol/glycerol-containing medium. *K. lactis* strain CBS2359 was grown as described in the text. RNA was isolated from each time-point sample and growth was spectrophotometrically monitored at 600 nm (panel B). At time point zero the culture had a *D* of 0.56 and glucose was added to a final concentration of 5%. Approximately 15 µg of RNA was triplicate loaded on a 1.2% agarose gel and electrophoresed. The blot was cut in three identical pieces and hybridised with DNA probes as indicated (panel A).

Fig. 2 A are due to small differences in RNA loading, as already seen on the ethidium bromide-stained gel. The absence of any effect of glucose on the level of expression in *K. lactis* is consistent with the report of De Deken (1966) who observed that synthesis of enzymes of the respiratory chain of several yeast species, including *K. lactis*, is not repressed by glucose. At the level of transcription, therefore, our results, support the earlier findings of De Deken (1966).

Transcription of the *KIQC7*, *KIQC8* and *KICY1* genes is strongly induced on non-fermentable carbon sources

The absence of glucose repression in *K. lactis* does not necessarily mean that the *KIQC7*, *KIQC8* and *KICY1* genes are constitutively transcribed. As in

Fig. 3 Medium shift from fermentable to non-fermentable carbon source. *K. lactis* strain CBS2359 was grown in 500 ml of YPD2 medium and the medium was changed for YPEG as detailed in text. Approximately 15 µg RNA at each time-point sample was quadruplicate-loaded onto a 1.2% agarose gel and electrophoresed. After blotting the blot was cut into four identical pieces and hybridised with DNA-probes as indicated.

S. cerevisiae, repression and activation of transcription are likely to be distinct and opposing processes, as is well illustrated by the complex regulation of the *GAL4* gene (Griggs and Johnston 1993) and the *HEM1* gene (Keng and Guarente 1987). In these cases induction of expression is the net result of transcriptional activation superimposed on the absence of repression. As already shown in Fig. 1 the genes studied here are more highly expressed on a non-fermentable carbon source than on glucose. We decided to investigate this transcriptional activation in more detail by performing a shift to a non-fermentable carbon source in which the YPD2 medium was quantifiably removed and changed for YPEG. Figure 3 shows that the transcript levels of all three genes increase significantly in amount within 2–30 min after the shift and continue to increase steadily up to 90 min. As in *S. cerevisiae*, expression of the *KIAPA2* and *KIFPS1* genes is only slightly affected by the change of carbon source (Anderson et al. 1989; Plateau et al. 1990).

Glucose blocks the induction of transcription on non-fermentable carbon sources

(Mazzoni et al. 1992) have reported that expression of the *K. lactis ADH4* gene is specifically induced by ethanol, even in the presence of glucose. To check whether a similar mechanism underlies the apparent absence of effect of glucose on the level of expression of the genes for respiratory components, a medium shift entailing a complete substitution of the YPEG medium by YPD5 was carried out. As is clear from Fig. 4,

Fig. 4 Medium shift from non-fermentable to fermentable carbon source. *K. lactis* strain CBS2359 was grown in 500 ml of YPEG and the medium was changed for YPD5 as described in the text. After 4 h, ethanol and glycerol were added to a final concentration of 2% each. RNA was isolated and approximately 15 µg was triplicate-loaded onto a 1.2% agarose gel and electrophoresed and subsequently blotted. The blot was cut into three identical pieces and hybridised with DNA probes as indicated. Note that the time-point sample 60 min after the EG addition contains more RNA than the other samples, as was determined by the ethidium bromide-stained agarose gel

transcripts of both *KIQCR7* and *KIQCR8* are unaffected by the change of medium and this situation remains unchanged even if a mixture of ethanol and glycerol is subsequently added to the medium. Since this latter behaviour contrasts with the marked induction seen on the shift to a non-fermentable carbon source, as described above, we conclude that induction on ethanol/glycerol medium can only occur in the absence of glucose. A similar response has recently been described by Lodi et al. (1994) for the carbon source dependent expression of the *K. lactis* *DLD1* gene. We conclude that the transcriptional regulation of the *QCR8* genes is yeast-species specific. Despite the facts that the promoters share, *cis*-acting elements at similar positions, and the yeast species possess conserved corresponding *trans*-acting factors, the complex interplay between these elements and factors determines the difference in the rate of transcription. Deletion analysis had already shown that *K. lactis* contains factor(s) distinct from those binding to the defined UAS that are capable of activating the transcription of the *KIQCR8* gene (Mulder et al. 1994 b). Whether these additional factors have homologs in *S. cerevisiae*, and whether these are interchangeable, has yet to be elucidated. As will be further discussed in the following section these differences, when taken together with differences in the nutrient-signalling pathways, are likely to determine overall species-specific transcriptional behaviour.

Discussion

Results presented above clearly show that, in *K. lactis*, genes encoding three mitochondrial proteins are not glucose-repressible. They are, however, inducible by growth on non-fermentable C sources and this induction is prevented by glucose. This behaviour contrasts with that of the corresponding genes in *S. cerevisiae*, but is in itself not surprising; as early as 1966, levels of various respiratory enzymes in *K. lactis* were shown to be insensitive to glucose-repression (De Deken 1966). Our results confirm these observations at the level of transcription, but demonstrate in addition that the corresponding genes do become transcriptionally induced on non-fermentable carbon sources. Surprising, however, is the fact that for the homologous *QCR8* genes both the pattern and level of expression are strongly yeast species-specific, despite the relatively well-conserved promoter structure between the two yeasts: binding sites are present for the transcription factors HAP2/3/4, ABF1 and CPF1 and these factors, despite significant differences in sequence, are even functionally interchangeable (Gonçalves et al. 1992; Mulder et al. 1994 a, d).

Although unexpected, these findings are in line with two previous observations concerning the involvement of the HAP2/3 factors in the carbon-source response of

K. lactis. First, in contrast to *S. cerevisiae*, *K. lactis* mutants disrupted in the gene for either protein continue to transcribe genes of the respiratory complex subunits and second, such mutants retain respiratory function (Mulder et al. 1994 a; C.C. Nguyen and M. Bolotin-Fukuhara, personal communication). Taken together, these observations point to multiple differences in responses to nutrient-sensing mechanisms between the two yeast. The dependence of *K. lactis* on respiratory function, even whilst growing on glucose, accounts for the drastic decrease in growth rate displayed by cells carrying disruptions in genes for subunits of complex III of the respiratory chain (Mulder et al. 1994 b, c).

In contrast to *S. cerevisiae*, *K. lactis* has been classified as a so-called Crabtree-negative yeast, indicating that glucose is not metabolised to ethanol in the presence of oxygen, (Crabtree 1929). This classification requires qualification, since the Crabtree-effect, as originally defined, concerns only the short-term fermentative response of cells to glucose (Petrik et al. 1983; Van Urk et al. 1989), whereas the term has come to be loosely applied to long-term responses to glucose during growth under steady state conditions. These latter responses are probably better referred to as the Warburg effect (Racker and Spector 1981). *K. lactis* is a short-term Crabtree-negative yeast, but during steady state growth on glucose, the cells do produce some ethanol under aerobic conditions (Shain et al. 1992; Wésolowski-Louvel et al. 1992) and, as we also demonstrate in this report, the respiratory pathway is not repressed. Both fermentative and oxidative metabolic pathways co-exist (De Deken 1966; Mazzoni et al. 1992) and thus we propose to classify *K. lactis* as a short-term Crabtree-negative and a long-term Crabtree-, or Warburg-positive yeast. De Deken (1966) has suggested that the Crabtree effect is related to, or mediated by, glucose repression, while other authors have suggested that the effect is attributable to a limited capacity of the respiratory chain (Rieger et al. 1983; Käppeli and Sonnleitner 1986). Neither view is likely to be correct. First, Van Urk et al. (1989) have shown that the respiratory capacities of Crabtree-negative and -positive yeasts are very similar. Second, Sierkstra et al. (1993) have demonstrated that in *S. cerevisiae* a Crabtree effect is also exerted by the non-repressing sugar galactose. These authors therefore propose that the metabolic changes observed at the onset of the Crabtree effect derive from changes in flux through the glycolytic and pentose-phosphate pathways, resulting in an increased flux of NADH into NAD. The basis for the presence or absence of the (short-term) Crabtree effect therefore possibly lies in the regulation of glucose uptake, the level of glycolytic activity, the activity of the enzyme glucose-6-phosphate dehydrogenase, and in the cell's ability to balance the activity of pyruvate dehydrogenase (PDH) relative to pyruvate decarboxylase (PDC) (Wenzel 1994). The genes encoding

subunits of either of these latter enzymes may therefore turn out to be candidates for regulation at this branch point of respiratory and fermentative metabolism. In line with this expectation, the promoter of the *ScLPD1* gene, encoding one of the subunits of PDH in *S. cerevisiae*, possesses binding sites for the transcription factors ABF1, CPF1, HAP2/34, GCN4 and RAP1 (Bowman et al. 1992; Sinclair et al. 1994). Characterisation of the corresponding *K. lactis* genes, encoding subunits of the PDH and PDC complexes, and elucidation of the extent and mechanism of the carbon source-control of expression will be of great interest.

The reported species-specific response of carbon source-dependent expression is probably due to the differences outlined above, most likely acting in combination with other components of the nutrient-signalling pathway (reviewed by (Gancedo 1992) that collectively influence the activity of these transcription factors differently in the two yeasts. Here too, the isolation and characterisation of the *K. lactis* homologs of these components, such as SNF1/SNF4, SSN6/TUP1 and MIG1, will be highly informative.

Clearly, more detailed comparisons will be necessary to gain a better understanding of transcriptional responses in both yeasts and of the roles played by individual factors.

Acknowledgements The authors thank M.A. Freire-Picos for providing the plasmid containing the *KICYC1* gene. We are grateful to M. Bolotin-Fukuhara for the communication of unpublished results. This work was supported in parts by grants from the Netherlands Foundation for Chemical Research (SON) and financial aid from the Netherlands Organisation for the Advancement of Research (NWO).

References

- Anderson MS, Yarger JG, Burck CL, Poulter CD (1989) Farnesyl diphosphate synthetase. Molecular cloning, sequence, and expression of an essential gene from *Saccharomyces cerevisiae*. *J Biol Chem* 264:19176–19184
- Bowman SB, Zaman Z, Collinson LP, Brown AJP, Dawes IW (1992) Positive regulation of the *LPD1* gene of *Saccharomyces cerevisiae* by the HAP2/HAP3/HAP4 activation system. *Mol Gen Genet* 231:296–303
- Chodosh LA, Olesen J, Hahn S, Baldwin AS, Guarente L, Sharp PA (1988) A yeast and a human CCAAT-binding protein have heterologous subunits that are functionally interchangeable. *Cell* 53:25–35
- Crabtree (1929) Observations on the carbohydrate metabolism of tumors. *Biochem J* 23:536
- Czyz M, Nagiec MM, Dickson RC (1993) Autoregulation of *GAL4* transcription is essential for rapid growth of *Kluyveromyces lactis* on lactose and galactose. *Nucleic Acids Res* 21:4378–4382
- De Deken RH (1966) The Crabtree effect: a regulatory system in yeast. *J Gen Microbiol* 44:149–156
- De Winde JH, Grivell LA (1992) Global regulation of mitochondrial biogenesis in yeast: ABF1 and CPF1 play opposite roles in regulating expression of the *QCR8* gene, encoding subunit VIII of the mitochondrial ubiquinol cytochrome *c* oxidoreductase. *Mol Cell Biol* 12:2872–2883
- Deshler JO, Larson GP, Rossi JJ (1989) *Kluyveromyces lactis* maintains *Saccharomyces cerevisiae* intron-encoded splicing signals. *Mol Cell Biol* 9:2208–2213
- Diffley JFX (1992) Global regulators of chromosome function in yeast. *An van Leeuwenhoek Int J Gen Mol Microbiol* 61:25–33
- Entian KD, Barnett JA (1992) Regulation of sugar utilization by *Saccharomyces cerevisiae*. *Trends Biochem Sci* 17:506–510
- Forsburg SL, Guarente L (1989 a) Communication between mitochondria and nucleus in the regulation of cytochrome genes in the yeast *Saccharomyces cerevisiae*. *Annu Rev Cell Biol* 5:153–180
- Forsburg SL, Guarente L (1989 b) Identification and characterization of HAP4: a third component of the CCAAT-bound HAP2/HAP3 heteromer. *Genes Dev* 3:1166–1178
- Freire-Picos MA, Rodriguez-Torres AM, Ramil E, Cerdan ME, Breunig KD, Hollenberg CP, Zitomer RS (1993) Sequence of a cytochrome *c* gene from *Kluyveromyces lactis* and its upstream region. *Yeast* 9:201–204
- Gancedo JM (1992) Carbon catabolite repression in yeast. *Eur J Biochem* 206:297–313
- Goncalves PM, Maurer K, Mager WH, Planta RJ (1992) *Kluyveromyces* contains a functional ABF1-homologue. *Nucleic Acids Res* 20:2211–2215
- Griggs DW, Johnston M (1993) Promoter elements determining weak expression of the *GAL4* regulatory gene of *Saccharomyces cerevisiae*. *Mol Cell Biol* 13:4999–5009
- Käppeli O, Sonnleitner B (1986) Regulation of sugar metabolism in *Saccharomyces*-type yeast: experimental and conceptual considerations. *Crit Rev Biotechnol* 4:299–325
- Keng T, Guarente L (1987) Constitutive expression of the yeast *HEM1* gene is actually a composite of activation and repression. *Proc Natl Acad Sci USA* 84:9113–9117
- Li XY, Mantovani R, Hooft van Huijsduijnen R, Andre I, Benoist C, Mathis D (1992) Evolutionary variation of the CCAAT-binding transcription factor NF-Y. *Nucleic Acids Res* 20:1087–1091
- Lodi T, O'Connor D, Goffrini P, Ferrero I (1994) Carbon catabolite repression in *Kluyveromyces lactis*: isolation and characterization of the *KIDL1* gene encoding the mitochondrial enzyme D-lactate ferri-cytochrome *c* oxidoreductase. *Mol Gen Genet* 244:622–629
- Lombardo A, Cereghino GP, Scheffler IE (1992) Control of mRNA turnover as a mechanism of glucose repression in *Saccharomyces cerevisiae*. *Mol Cell Biol* 12:2941–2948
- Maarse AC, De Haan M, Bout A, Grivell LA (1988) Demarcation of a sequence involved in mediating catabolite repression of the gene for the 11 kDa subunit VIII of the ubiquinol cytochrome *c* oxidoreductase in *Saccharomyces cerevisiae*. *Nucleic Acids Res* 16:5797–5811
- Mazzoni C, Saliola M, Falcone C (1992) Ethanol-induced and glucose-insensitive alcohol dehydrogenase activity in the yeast *Kluyveromyces lactis*. *Mol Microbiol* 6:2279–2286
- Mulder W, Scholten IHJM, De Boer RW, Grivell LA (1994 a) Sequence of the HAP3 transcription factor of *Kluyveromyces lactis* predicts the presence of a novel 4-cysteine zinc-finger motif. *Mol Gen Genet* 245:96–106
- Mulder W, Scholten IHJM, Nagelkerken B, Grivell LA (1994 b) The isolation and characterisation of the *FPS* and *QCR8* genes coding for farnesyl phosphate synthetase and the 11-kDa subunit VIII of the *bc₁*-complex of *Kluyveromyces lactis*. *Biochem Biophys Acta* 1219:719–723
- Mulder W, Scholten IHJM, Van Roon H, Grivell LA (1994 c) The isolation and characterisation of the *APA2* and *QCR7* genes coding for Ap₄A phosphorylase II and the 14-kDa subunit VII of the *bc₁*-complex of *Kluyveromyces lactis*. *Biochem Biophys Acta* 1219:719–723
- Mulder W, Winkler AA, Scholten IHJM, Zonneveld BJM, De Winde JH, Steensma HY, Grivell LA (1994 d) Centromere promoter factors (CPF1) of the yeasts *Saccharomyces cerevisiae* and *Kluyveromyces lactis* are functionally exchangeable, despite low overall homology. *Curr Genet* 26:198–207

- Mylin LM, Gerardot CJ, Hopper JE, Dickson RC (1991) Sequence conservation in the *Saccharomyces* and *Kluyveromyces* GAL11 transcription activators suggests functional domains. *Nucleic Acids Res* 19:5345–5350
- Petrik M, Käppeli O, Fiechter A (1983) An expanded concept for the glucose effect in the yeast *Saccharomyces uvarum*: involvement of short- and long-term regulation. *J Gen Microbiol* 129:43–49
- Plateau P, Fromant M, Schmitter JM, Blanquet S (1990) Catabolism of bis(5'-nucleosidyl) tetraphosphates in *Saccharomyces cerevisiae*. *J Bacteriol* 172:6892–6899
- Racker E, Spector M (1981) Warburg effect revisited: merger of biochemistry and molecular biology. *Science* 213:303–307
- Rieger M, Käppeli O, Fiechter A (1983) The role of a limited respiration in the complete oxidation of glucose by *Saccharomyces cerevisiae*. *J Gen Microbiol* 129:653–661
- Shain DH, Salvatore C, Denis CL (1992) Evolution of the alcohol dehydrogenase (*ADH*) genes in yeast: characterization of a fourth *ADH* in *Kluyveromyces lactis*. *Mol Gen Genet* 232:479–488
- Sierkstra LN, Nouwen NP, Verbakel JMA, Verrips T (1993) Regulation of glycolytic enzymes and the Crabtree-effect in galactose-limited continuous cultures of *S. cerevisiae*. *Yeast* 9:787–795
- Sinclair DA, Kornfeld GD, Dawes IW (1994) Yeast intragenic transcriptional control: activation and repression sites within the coding region of the *Saccharomyces cerevisiae* *LPD1* gene. *Mol Cell Biol* 14:214–225
- Trumbly R (1992) Glucose repression in the yeast *Saccharomyces cerevisiae*. *Mol Microbiol* 6:15–21
- Van Urk H, Bruinenberg PM, Veenhuis M, Scheffers WA, Van Dijken JP (1989) Respiratory capacities of mitochondria of *Saccharomyces cerevisiae* CBS8066 and *Candida utilis* CBS621 grown under glucose limitation. *Ant van Leeuwenhoek Int J Gen Mol Microbiol* 56:
- Wenzel TJ (1994) Function and regulation of the pyruvate dehydrogenase complex from the yeast *Saccharomyces cerevisiae*. Ph.D thesis, University of Leiden
- Wésolowski-Louvel M, Goffrini P, Ferrero I, Fukuhara H (1992) Glucose transport in the yeast *Kluyveromyces lactis*. I. Properties of an inducible low-affinity glucose transporter gene. *Mol Gen Genet* 233:89–96
- Zenke FT, Zachariae W, Lunkes A, Breunig KD (1993) Gal80 proteins of *Kluyveromyces lactis* and *Saccharomyces cerevisiae* are highly conserved but contribute differently to glucose repression of the galactose regulon. *Mol Cell Biol* 13:7566–7576
- Zitomer RS, Montgomery DL, Nichols DL, Hall BD (1979) Transcriptional regulation of the yeast cytochrome *c* gene. *Proc Natl Acad Sci USA* 76:3627–3631