KlADH3, a gene encoding a mitochondrial alcohol dehydrogenase, affects respiratory metabolism and cytochrome content in Kluyveromyces lactis

Michele Saliola1, Ilaria De Maria1, Tiziana Lodi2, Alessandro Fiori1 & Claudio Falcone1
1Dipartimento di Biologia Cellulare e dello Sviluppo, Università di Roma ‘La Sapienza’, Rome, Italy; and 2Dipartimento di Genetica, Antropologia e Evoluzione, Università degli Studi di Parma, Parma, Italy

Correspondence: Michele Saliola, Department of Developmental and Cell Biology, University of Rome ‘La Sapienza’, Piazzale Aldo Moro 5, 00185 Rome, Italy. Tel.: +39 06 4991254; fax: +39 06 49912351; e-mail: Michele.saliola@uniroma1.it

Abstract

A Kluyveromyces lactis strain, harbouring KlADH3 as the unique alcohol dehydrogenase (ADH) gene, was used in a genetic screen on allyl alcohol to isolate mutants deregulated in the expression of this gene. Here we report the characterization of some mutants that lacked or had highly reduced amounts of KlAdh3p activity; in addition, these mutants showed alterations in glucose metabolism, reduced respiration and reduced cytochrome content. Our results confirm that the KlAdh3p activity contributes to the reoxidation of cytosolic NAD(P)H feeding the respiratory chain through Klnid1p, the mitochondrial internal transdehydrogenase. The low levels of KlAdh3p in two of the mutants were associated with mutations in KlSDH1, one of the genes of complex II, suggesting signalling between the respiratory chain and expression of the KlADH3 gene.

Keywords
Kluyveromyces lactis; ADH; allyl alcohol; NADPH.

Introduction

In the yeast Kluyveromyces lactis, four genes coding for alcohol dehydrogenase (ADH) isoymes are present (Saliola et al., 1990, 1991; Shain et al., 1992). KlADH1 and KlADH2 encode two cytosolic activities, which are more expressed in glucose-utilizing cells, responsible for the production of ethanol during fermentation (Saliola et al., 1995). By contrast, KlADH3 and KlADH4, encoding two mitochondrial activities, are not involved in ethanol production and, at least in batch cultures, show mutually exclusive regulation. In fact, KlADH3 is expressed in the presence of respiratory carbon sources, with the exception of ethanol, which seems to repress this gene at the transcriptional level. In contrast, KlADH4 is specifically induced by ethanol and not by other respiratory carbon sources (Saliola et al., 1995; Mazzoni et al., 1992). Unlike other known ADHs, both mitochondrial isoforms of K. lactis can use NADH and NADPH, suggesting involvement of these activities in the redox balance of both cofactors (Bozzi et al., 1997). It has recently been suggested that, in both Saccharomyces cerevisiae and K. lactis, mitochondrial ADH activities could be part of an ethanol – acetaldehyde shuttle responsible for mitochondrial – cytosolic NAD(P)H redox balance (for a review see Bakker et al., 2001) that feeds the external (Nde1p, Nde2p) or the internal (Ndi1p) mitochondrial transdehydrogenases (Bakker et al., 2000; Overkamp et al., 2002; Tarrio et al., 2005). In the case of K. lactis, the presence of two mitochondrial ADH activities with opposite regulation suggests different metabolic functions that need to be clarified. Although the presence of a cis-acting element responsible for ethanol induction in the KlADH4 promoter (Saliola et al., 1999; Mazzoni et al., 2000) and for ethanol repression in the KlADH3 promoter (unpublished data) confirmed the opposite regulation of the two genes, no trans-acting factors have been identified so far.

In S. cerevisiae, the isolation of mutants resistant to the toxic allyl alcohol led to the identification of both structural and regulatory ADH genes (Lutsdorf & Megnet, 1968; Ciriacy, 1975, 1979; Denis, 1984), contributing to the genetic dissection of the ADH system in this yeast. Following a similar approach, we have already obtained one mutant not expressing KlADH4 (Mazzoni et al., 2005) and, in this paper, we report the isolation of allyl alcohol-resistant mutants from a K. lactis strain that harboured KlADH3 as the unique ADH gene. Among these mutants, we focused our attention on those with or without highly reduced...
KLAdh3p activity. Interestingly, study of the metabolism in these mutants showed a correlation between the expression of KLADH3, cellular respiration and cytochrome content.

Materials and methods

Strains, media and culture conditions

The following K. lactis strains were used in this work. MS12-8: MATa, metA1, ade2, kladh1 :: URA3, kladh2 :: URA3, kladh4 :: KIURA3, KLADH3 (this work). PM4-4B: MATa, ade1, ade2, uraA1 (Alberti et al., 2000). MS7-62: MATa, lysA1, argA1, uraA1, kladh1 :: URA3, kladh2 :: URA3, kladh3 :: URA3, kladh4 :: KIURA3 (Saliola et al., 1994). MW278-20C: MATa, uraA1, ade2, leu2, lac4–8 (Fiori et al. 2000). MW278-20C/Klsdh1Δ: Klsdh1 :: URA3KanMx4 (Saliola et al., 2004). MS12-8, the strain used for the selection of allyl alcohol-resistant (Aar+) mutants, was obtained by crossing MS6-117, a mutant expressing KLADH3 as a single ADH gene (Saliola et al., 1994), with PM4-4B (Alberti et al., 2000) and selecting as previously described (Saliola et al., 1994). MS12-8 was preferred to MS6-117 in the allyl alcohol screening because the latter mutant showed very low transformation efficiency.

Cultures were grown under shaking at 28 °C in YP (1% Difco yeast extract, 2% Difco Bacto-peptone) or in minimal medium (6.7 g L−1 Difco yeast extract, 2% Difco Bacto-peptone) supplemented with different carbon sources at the concentration specified in the text. Geneticin (G418) was used on YPD (YP+C1 ampicillin). Minimal medium was supplemented with auxotrophic requirements as needed at a final concentration of 10 μg mL−1. Mating and sporulation were performed in 5% malt extract (Difco) plates, while tetrads were separated with a Singer micromanipulator.

Escherichia coli strain DH5α was used for the propagation of plasmid DNA. Cultures were grown at 37 °C on LB medium (0.5% yeast extract, 1% Difco tryptone, 0.5% NaCl, supplemented with 100 μg mL−1 ampicillin).

Selection of allyl alcohol-resistant (Aar+) mutants

MS12-8 cultures were grown in rich medium (YPD) to the late exponential phase and plated on Petri dishes containing YPD and 1 mM allyl alcohol at a concentration of 10^7 cells per plate. Resistant clones, grown after 5–7 days, were replicated on YPD plates containing 2 mM allyl alcohol to eliminate cell adaptation to this compound. Resistant clones were then grown under nonselective conditions for 20 generations and then replicated again on YPD plates containing 2 mM allyl alcohol to confirm the Aar+ phenotype.

Assay methods

The electrophoretic conditions and the ADH staining assay have been described previously (Lutsdorf & Megnet, 1968; Mazzoni et al., 1992; Saliola & Falcone, 1995).

The succinate dehydrogenase complex (SDH) staining assay was performed as previously described (Saliola et al., 2004).

Glucose, ethanol, acetate, acetaldehyde and succinate concentrations in culture supernatants were measured using commercial kits from R-Biopharma (Darmstadt, Germany) according to the manufacturer’s instructions.

Cell respiration

Respiration rate was measured at 30 °C using a Clark-type electrode according to the method of Ferrero et al. (1981), following the conditions previously described (Saliola et al., 2004).

Quantitative determination of cytochromes was performed according to the method of Lodi & Ferrero (1993).

General methods

DNA manipulation, plasmid engineering and other techniques were performed using standard procedures. Yeast transformation was performed by electroporation with a Biorad Gene-Pulser apparatus following the method described by Becker & Guarente (1991). The pKD1-derived plasmid pKL (Saliola & Falcone, 1995), conferring resistance to geneticin, and the centromeric plasmid Kcplac13 (kindly provided by Dr Micheline Wesolowski-Louvel) were used to introduce the KLSDH1 genes into the MS12-8/1, MS12-8/2, MS12-8/3 and MW278-20C/Klsdh1Δ strains, and the KLADH3 gene (Saliola & Falcone, 1995) into the MS12-8/4 strain. KLSDH1 was amplified from the p3AS plasmid of K. lactis containing the entire gene (Saliola et al., 2004). The preparation of total RNA has been previously described (Saliola & Falcone, 1995).

Results

Selection of KLADH3 expression mutants

Allyl alcohol can be used as a substrate by ADH and converted into acrolein, a reactive aldehyde that is very toxic to cells (Kehrer & Biswal, 2000). It is known that allyl alcohol allows the selection of yeast mutants with reduced ADH activity (Ciriaacy 1975; 1979; Denis, 1984) and, using this compound, we performed a screening with the aim of isolating mutants deregulated in the expression of KLADH3.

To avoid interference by the other ADH genes, we constructed the MS12-8 strain carrying deletions in KIADH1, KIADH2 and KIADH4 and harbouring KLADH3 as the unique ADH gene. MS12-8 samples of 10^7 cells were plated...
on YPD medium containing 1 mM allyl alcohol and grown until resistant clones appeared. These clones were transferred to plates containing an increased concentration of allyl alcohol to avoid the selection of false resistant clones, as described in Materials and methods. Afterwards, we analysed the cellular extracts of the resistant clones for the presence of KlAdh3p by native acrylamide gel electrophoresis and staining for ADH activity, as previously described (Lutsdorf & Megnet, 1968; Mazzoni et al., 1992; Saliola & Falcone, 1995). Of 98 clones showing the allyl alcohol-resistant (Aar\(^{+}\)) phenotype, about 80\% were discarded because they had normal levels of KlAdh3p and might represent other classes of mutants, including those altered in their permeability to allyl alcohol. The remaining clones showed complete absence or highly reduced amounts of KlAdh3p activity and, in consequence, they were unable to grow in minimal medium containing ethanol. To distinguish mutations in the structural KlADH3 gene from those in regulatory genes, the selected clones were crossed with MS7-62, a strain lacking all four ADH genes (Saliola et al., 1994), and the resulting diploids were tested for their ability to grow in minimal medium containing ethanol. As shown in Fig. 1a, only three diploids were able to grow on ethanol, indicating that the mutations harboured by the haploid Aar\(^{+}\) strains, namely MS12-8/1, MS12-8/2 and MS12-8/3, occurred in genes other than KlADH3. The other mutants, which were unable to grow on ethanol in both the haploid and diploid backgrounds, might represent mutants in structural and/or regulatory regions of KlADH3. MS12-8/4 (Fig. 1a) is one representative of this class of mutants lacking KlAdh3p (Fig. 1b, lanes 8 and 9), in that it regained a normal level of activity and growth in ethanol when transformed with a plasmid harbouring the KlADH3 gene (not shown). Figure 1b shows a comparison of the ADH isozyme profile of the parental strain with those of the three mutant clones that expressed reduced levels of activity in the haploid background (lanes 1, 3 and 5) and regained normal levels in the diploid state (lanes 2, 4 and 6). As can be seen, the level of KlAdh3p in the three diploids was similar to that in the parental strain (lane 7), indicating that the KlADH3 gene harboured by these mutants was functional and could be activated by complementing gene(s) present in the adh null strain. We then analysed the transcription of the KlADH3 gene in the four mutants. Total RNAs prepared from cultures grown until the late exponential phase in YPD medium were blotted and hybridized to a KlADH3-specific probe (Saliola et al., 1995). As shown in Fig. 1c, all mutants showed highly reduced amounts of the KlADH3 transcript (lanes 2–5) compared to the parental strain (lane 1), indicating that the observed reduction of the KlAdh3p activity was the consequence of the lowered transcription of the gene. In the case of MS12-8/4, which was devoid of KlAdh3p activity, we can hypothesize that the mRNA present in this mutant harboured a mutation that abolished the activity of the protein. All four mutants, when grown on glucose plates containing allyl alcohol, showed increased resistance to this compound, in the order MS12-8/4, MS12-8/1, MS12-8/2 and MS12-8/3 (Fig. 2), according to the amount of KlAdh3p present in each mutant.

**Physiological analysis of the mutants**

The four mutants were further analysed for their ability to grow in synthetic media containing different carbon sources. This analysis showed that the MS12-8/1, MS12-8/2 and MS12-8/3 mutants were not able to grow in the presence of respiratory carbon sources (Fig. 3), although MS12-8/1 showed residual growth on lactate, while MS12-8/4 showed reduced growth with all respiratory substrates.
(Fig. 3) and, due to the absence of KlAdh3p, could not utilize ethanol (Fig. 1a). We also followed the growth of the mutants in glucose medium. As can be seen in Fig. 4a, with the exception of MS12-8/4, all mutants showed lower growth rates; this was most evident in the case of MS12-8/1, followed by MS12-8/2 and MS12-8/3, with reductions in the biomass yield to about one-fifth and one-third of that of the parental strain, respectively. Since all mutants were impaired in fermentation, with an absence of cytosolic ADH activities, we analyzed their respiratory capabilities by measuring oxygen consumption. Cultures were grown in YPD until the late exponential phase and starved for 24 h before determination of respiration rate; oxygen consumption was measured after feeding cells with glucose, ethanol or lactate, as previously described (Saliola et al., 2004). As shown in Fig. 4b, we found that the respiration rates in the four mutants were significantly reduced with all tested carbon sources, although to different extents. The highest reduction was observed with lactate, while with glucose and ethanol the respiration rate in the mutants was 70% that of the parental strain on average. As expected, in the case of the MS12-8/4 mutant, which lacked all four ADH activities, no respiration was observed with ethanol. To investigate further the nature of the reduced respiration rate observed in the mutants, we measured the cytochrome content within the cells. As can be seen in Fig. 4c, the cytochrome content in MS12-8/1, MS12-8/2 and MS12-8/3 was one-third that of the parental strain and about two-thirds that in MS12-8/4. These results showed that, in each mutant, the reduction in cytochrome content strictly correlated with the reduction in respiration rate.

**Metabolite determination**

We also analyzed metabolism in these mutants by measuring the consumption of glucose and the amounts of acetaldehyde, ethanol, acetate and succinate that accumulated in culture supernatants after 35 h of growth in YPD. As shown in Table 1, all mutants accumulated high amounts of glucose intermediates compared to the parental strain. In more detail, MS12-8/4, like the parental strain, did not consume all the glucose present in the medium and produced the same amount of ethanol (0.32 g L\(^{-1}\)). The absence of KlAdh3p in this mutant indicated that ethanol should be produced through an ADH-independent pathway, and we have already reported that K. lactis adh null strains, devoid of ADH activity, accumulated similar amounts of ethanol (Saliola et al., 1994). In addition, MS12-8/4, as well as the other three mutants, accumulated acetaldehyde, but this never reached the toxic concentration of 0.3 g L\(^{-1}\) (Remize et al., 1999). Even more altered metabolism was observed in MS12-8/1, MS12-8/2 and MS12-8/3, which, although producing lower biomass yields, exhausted glucose completely after 35 h of growth and accumulated high amounts of intermediates. These metabolites were more abundant in the two latter strains, where ethanol and acetate reached approximately 1 g L\(^{-1}\). Moreover, MS12-8/1 and MS12-8/2

**Fig. 2.** Allyl alcohol resistance. Cells of the parental (pt) and mutant strains (m1 to m4) were grown in YPD to about 10^8 mL\(^{-1}\); 5-μL aliquots of 10-fold culture dilutions were spotted onto agar plates containing YPD 1% and YPD 1% plus 2 mM allyl alcohol and grown at 28 °C for 2 days and 4 days, respectively.

**Fig. 3.** Utilization of carbon sources. Cells of the parental (pt) and mutant strains (m1 to m4) were grown in YPD to about 10^8 mL\(^{-1}\); 5-μL aliquots of 10-fold culture dilutions were spotted onto agar plates containing synthetic medium and glucose (SD), glycerol (SG) or lactate (SL) as carbon sources and grown at 28 °C for 4 days.
accumulated appreciable amounts of succinate, which was essentially undetectable in the parental strain.

**The mutations carried by MS12-8/1 and MS12-8/2 are allelic to KlSDH1**

To characterize the mutations present in the Aar<sup>+</sup> mutants, a genetic analysis was performed by mating MS12-8, MS12-8/1 (m1), MS12-8/2 (m2) and MS12-8/3 (m3) with MW278-20C, a K. lactis reference strain harbouring all four ADH genes. The diploids obtained were then sporulated and the asci subjected to tetrad analysis. We observed a 2<sup>1</sup>:2<sup>1</sup> meiotic segregation of the growth phenotype on minimal media containing glycerol or ethanol, indicating the presence of a single mutation in all three mutants (not shown).

The glycerol/ethanol minus phenotype was in all cases independent of the number of ADH genes present in each spore. Moreover, tetrad analysis of the diploids obtained by mating the m1 and m2 strains with MW278-20C revealed a linkage between the mutated locus and the KlLEU2 gene. Among 43 asci analysed from the former diploid, we scored 38 parental ditype (PD) and five tetratype (T). The distance between the two loci, calculated according to Perkins (1949), was estimated to be 5.8 cM. A comparison of the K. lactis genetic map (Wesolowski-Louvel & Fukuhara, 1995) with the genomic DNA sequence for the Klura3–rag2 loci indicated that 1 cM corresponded to about 7.2 kbp, and therefore we located the mutation present in MS12-8/1 roughly 42 kbp away from the KlLEU2 gene. Almost identical results were obtained from 22 asci (20 PD and 2 T) derived from the m2/MW278-20C diploid. The KlSDH1 gene is located at a distance of about 42 kbp from the KlLEU2 gene, and we have already reported that a Klsdh1<sup>D</sup> mutant of K. lactis, lacking the flavoprotein subunit of the SDH, accumulated succinate during growth on glucose (Saliola et al., 2004). On this basis, we verified the presence of SDH in all mutants by activity staining on native gels (Saliola et al., 2004). As shown in Fig. 5a, MS12-8/1 (m1) and MS12-8/2 (m2) completely lacked activity, while MS12-8/3 (m3) and MS12-8/4 (m4) showed activity levels similar to that of the parental strain (pt). When we introduced the KlSDH1 gene into MS12-8/1 and MS12-8/2, we observed the recovery of SDH activity as well as the ability to grow in the presence of respiratory carbon sources (not shown).
3 and the parental strain MS12-8 with MW278-20C/Klsdh1Δ. Despite many efforts, diploids with the Klsdh1Δ strain were obtained only with the parental and the MS12-8/3 strain. Finally, to overcome the problem, we were able to obtain diploids for the other two mutants only by mating MW278-20C/Klsdh1Δ with the m1 and m2 strains that harboured the KISDH1 gene. Growth of these two diploids for over 20 generations in nonselective conditions (YPD) allowed the isolation of single colonies that had lost the plasmid, SDH activity and the ability to grow on respiratory carbon sources. The results showed that the diploids m1 and m2 × Klsdh1Δ were able to grow on respiratory carbon sources only if they harboured the KISDH1 gene (Fig. 6a), indicating that both strains harboured a mutation in the KlSDH1 locus. Southern analysis revealed the presence of a deletion of about 0.6 kbp in the KlSDH1 locus of the m1 strain (Fig. 6b), while no apparent defects were observed in m2 (not shown). Nevertheless, the KISDH1 gene amplified from MS12-8/2 could not restore the growth on respiratory carbon sources when introduced into either the MS12-8/1 or the MW278-20C/Klsdh1Δ strain. Sequence analysis of the amplified fragment is in progress to identify the mutation present in this mutant.

Discussion

A K. lactis strain expressing KIADH3 as the unique ADH gene was used in a genetic screening on allyl alcohol to isolate mutants with altered expression of this gene. Following this approach, we isolated mutants with reduced (MS12-8/1 to MS12-8/3) or absent (MS12-8/4) KlAdh3p activity. This activity, which can use both NAD(H) and NAD(P)H as cofactor, shows the highest affinity among K. lactis ADHs toward ethanol (Bozzi et al., 1997; Brisdelli et al., 2004), while it would not appear to play a significant role in the production of this metabolite. In fact, MS12-8/4, the mutant lacking KlAdh3p, was unable to grow on ethanol as the sole carbon source but still accumulated ethanol in amounts similar to those produced in MS12-8, the isogenic parental strain that expressed KlADH3. We have already reported that adh null strains of K. lactis accumulated ethanol (Salio et al., 1994), which, therefore, should be produced by noncanonical ADH activities. Recently, the presence of a cytosolic NADPH-dependent ‘acetaldehyde reductase’ has been reported in K. lactis, and this could be responsible for the observed production of ethanol (Overkamp et al., 2002). It has also been suggested that this activity and the canonical cytosolic ADHs, together with the mitochondrial NAD(P)-dependent ADHs, constitute an ethanol–acetaldehyde shuttle (Bakker et al., 2000) responsible for the mitochondrial reoxidation of cytosolic NAD(P)H (Overkamp et al., 2002). Moreover, it has been shown that KIADH3 is one of the components of this shuttle and that this gene is strongly induced in glycolytic mutants (Overkamp et al., 2002). It follows, therefore, that the ethanol accumulated in the MS12-8 strain can flow into mitochondria, where it is converted to acetaldehyde by KlAdh3p and shuttled again to the cytoplasm, while the redox NAD(P)H potential produced feeds the respiratory chain mainly through KINdi1p, the mitochondrial internal transdehydrogenase.
(Overkamp et al., 2002; Tarrio et al., 2005). The scheme in Fig. 7a, modified from Overkamp et al. (2002), shows the role played by KlAdh3p in the parental strain. The absence of KlAdh3p in MS12-8/4 could lead to inactivation of the acetaldehyde–ethanol shuttle, followed by the accumulation of acetaldehyde and acetate (Fig. 7b). The reduction in the amount of NAD(P)H produced within mitochondria and available for KlNdi1p, which follows the absence of KlAdh3p, could account for the 30% reduction in oxygen consumption and cytochrome content observed in this mutant. Since the growth of MS12-8/4 in glucose was not significantly affected, compared to its isogenic parental strain, we could argue that the respiration rates measured in this mutant were sufficient to allow almost normal growth in the presence of this carbon source. It has been demonstrated that, unlike the case with S. cerevisiae, glucose can be efficiently dissimilated in glycolytic mutants of K. lactis through the pentose phosphate (PP) pathway, in that mitochondria can directly oxidize the NADPH produced in this bypass (Goffrini et al., 1989; Jacoby et al., 1993; González Siso et al., 1996, 2000; Overkamp et al., 2002). Furthermore, it has been recently reported that KlNde1p, the external mitochondrial transdehydrogenase of K. lactis, can accept NADPH as cofactor, pointing to this protein as the major candidate for the reoxidation of this molecule (Tarrio et al., 2005). In MS12-8, which can definitely be considered a glycolytic mutant for the absence of the fermentative ADHs, both the PP and ethanol–acetaldehyde shuttles could be activated, feeding reducing equivalents to KlNde1p and KlNdi1p, respectively (Fig. 7a) (Overkamp et al., 2002; Tarrio et al., 2005). The reduced growth of the MS12-8/4 mutant in the presence of glycerol and other respiratory carbon sources (Fig. 3), a condition in which the oxidative part of the PP shunt is not operative, suggests that KlAdh3p significantly contributes to the reoxidation of cytosolic NAD(P)H also during respiratory metabolism. MS12-8/1, MS12-8/2 and MS12-8/3, the other Aar+ mutants that we isolated, showed a substantial reduction in the KlAdh3p activity and metabolic defects even more significant than those of MS12-8/4. In particular, these mutants were unable to utilize respiratory carbon sources and showed reduced growth also on glucose. The rapid consumption of glucose in these strains indicated the presence of mutations that lead to inefficient utilization of this carbon source, with consequent reductions in energy and biomass yields. In fact, these mutants, even more than MS12-8/4, had highly reduced respiratory activity and cytochrome content, and increased accumulation of ethanol, acetate and acetaldehyde (Fig. 7b). Moreover, MS12-8/1 and MS12-8/2 produced succinate, and we have already reported that a klsdh1Δ mutant of K. lactis, lacking SDH activity, accumulated this compound during growth on glucose. On the basis of this observation and from genetic analysis, we found that the MS12-8/1 and MS12-8/2 mutants harboured mutations in the KISDH1 locus leading to the absence of SDH activity. The introduction of the KISDH1 gene in both mutants led to full recovery of growth on all carbon sources and normal levels of respiration, as well as of KlAdh3p. All of these data suggest that the reduced respiration level observed in these mutants could be the consequence of reduced reoxidation of reduced equivalents, leading to respirofermentative metabolism (Tarrio et al., 2005) with the accumulation of intermediates such as ethanol and acetaldehyde (Fig. 7b). The isolation of allyl alcohol-resistant mutants with an alteration of the redox ratio has already been described in yeast (Wills & Phelps, 1978; Wills & Martin, 1980). We would underline that the absence of activity of SDH, one of the respiratory chain.

Fig. 7. Redox balance in the MS12-8 parental and mutant strains. Glucose flux in MS12-8 is distributed between glycolysis’ pyruvate dehydrogenase (PDH) and PP pathways (a). The inactivation of the ethanol–acetaldehyde shuttle (b) in the MS12-8/4 mutant may be compensated by the PDH/PP pathways. The absence of SDH in MS12-8/1 and MS12-8/2, as well as the unknown mutation of MS12-8/3, leads to increased flux through the PP pathway, while the accumulation of high amounts of intermediates (see Table 1) may explain how the reoxidation of NAD(P)H occurred (b). Cytoplasmic NAD(P)H, according to Overkamp et al. (2002), can be directly oxidized by Kluyveromyces lactis mitochondria. G6P, glucose 6-phosphate; PYR, pyruvate; PP, pentose phosphate; NDE and NDI, external and internal transdehydrogenases; PDH, pyruvate dehydrogenase.

© 2006 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved
complexes, resulted in reduced transcription of KlADH3, suggesting signalling between the respiratory chain and the expression of this gene. It is known that, in yeast cells, changes in the functional state of mitochondria can influence the expression of nuclear genes, a phenomenon known as mitochondrial retrograde signalling (Butow & Avadhani, 2004). The fact that the MS12-8/3 mutant, which showed the same respiratory deficiency as MS12-8/1 and MS12-8/2, also had reduced amounts of the KlADH3 transcript, seems to confirm this relationship, indicating that the reduced efficiency of the respiratory chain, more than the absence of SDH activity per se, influences KlADH3 regulation. We suggest that alterations in the NAD(P)H/NAD(P) ratio could be the signal for the regulation of KlADH3. Transcriptional analysis in our mutants of KinDE1, KindI1 and KlZWF1 (the gene coding for the glucose-6-phosphate dehydrogenase, which contributes to maintaining the redox balance between cytoplasm and mitochondria) will clarify if these genes are also under the control of this signalling pathway. On the other hand, the identification of the mutation carried by MS12-8/3 and analysis of KlAdh3p in mutants of the transdehydrogenase genes, complexes III and IV of the respiratory chain, could contribute further to our understanding of the existence of the signalling but also the physiology of this yeast with respect to respiratory metabolism.

Acknowledgements

We would like to thank Dr Claudia Getuli for her help with the genetic analysis. This work was funded by the grant Ateneo 2004 from the University of Rome, La Sapienza. We would like to thank the Istituto Pasteur, Fondazione Cenci Bolognetti for providing Ilaria De Maria with a short fellowship.

Author Contributions

Michele Saliola and Ilaria De Maria contributed equally to this work.

References


