Carbohydrate and energy-yielding metabolism in non-conventional yeasts1

Carmen-Lisset Flores 2, Cristina Rodríguez, Thomas Petit 3, Carlos Gancedo *

Instituto de Investigaciones Biomédicas Alberto Sols C.S.I.C.-UAM, Unidad de Bioquímica y Genética de Levaduras, 28029 Madrid, Spain

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Abstract

Sugars are excellent carbon sources for all yeasts. Since a vast amount of information is available on the components of the pathways of sugar utilization in *Saccharomyces cerevisiae* it has been tacitly assumed that other yeasts use sugars in the same way. However, although the pathways of sugar utilization follow the same theme in all yeasts, important biochemical and genetic variations on it exist. Basically, in most non-conventional yeasts, in contrast to *S. cerevisiae*, respiration in the presence of oxygen is prominent for the use of sugars. This review provides comparative information on the different steps of the fundamental pathways of sugar utilization in non-conventional yeasts: glycolysis, fermentation, tricarboxylic acid cycle, pentose phosphate pathway and respiration. We consider also gluconeogenesis and, briefly, catabolite repression. We have centered our attention in the genera *Kluyveromyces*, *Candida*, *Pichia*, *Yarrowia* and *Schizosaccharomyces*, although occasional reference to other genera is made. The review shows that basic knowledge is missing on many components of these pathways and also that studies on regulation of critical steps are scarce. Information on these points would be important to generate genetically engineered yeast strains for certain industrial uses. ß 2000 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Non-conventional yeast; Carbohydrate metabolism; Respiration; Tricarboxylic acid cycle; Catabolite repression

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* Corresponding author. Fax: +34 (91) 585 4587; E-mail: cgancedo@iib.uam.es

1 This article is dedicated to the memory of Niko van Uden, an enthusiastic yeast researcher and a cultivated person.
2 On leave of absence from Departamento de Bioquímica, Facultad de Biología, Universidad de La Habana, Cuba.
3 Present address: DSM Bakery Ingredient Division, 26000 MA Delft, The Netherlands.

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1. Introduction

Yeast are microorganisms for which we have some of the oldest bibliographic references. Already in Exodus 12 and 13 there are mentions of leaven, likely a mixture of different yeast species and bacteria similar to that found nowadays in those rare places where bread is still baked by truly artisanal means [1,2]. For historical reasons, one yeast species, *Saccharomyces cerevisiae*, has dominated the scientific stage and has become a synonym for yeast. However, *S. cerevisiae* is but one among the almost 800 yeast species described in a recent taxonomy treatise [3]. It is logical to think that many of these different species may also be interesting for a variety of reasons ranging from their use in specific technological applications to the threat of the infections caused by some of them. It is perhaps time for zymologists to dedicate more attention to those non-conventional yeasts (NCY) or non-*Saccharomyces* yeasts. In fact, some species have already attracted researchers in the last years on different grounds: *Kluyveromyces lactis* as a possible utilizer of the residual whey in dairy industries, some methylotrophic yeasts for their efficiency as hosts for the production of heterologous proteins or for their special qualities for the study of peroxisome biogenesis, *Yarrowia lipolytica* for its ability to grow on particular substrates and its high protein excretion capacity, not to mention *Candida albicans* or *Cryptococcus neoformans* due to their relevance in some health issues.

In spite of the importance of this group of yeasts, basic knowledge about enzymes of fundamental pathways, their regulation and the genes encoding them is relatively scarce.

Sugars are utilized by all known yeasts. The common theme in their metabolism is the conversion of glucose 6-phosphate or fructose 6-phosphate to pyruvate through the common glycolytic trunk (Fig. 1). The metabolic destiny of pyruvate is then different depending on the yeast species and the cultivation conditions. In *S. cerevisiae* (the conventional yeast) glucose and related sugars cause a strong impairment in respiratory capacity (Crabtree effect) [4,5] and therefore, even in the presence of air, *S. cerevisiae* ferments sugar in batch cultures. Some other yeasts, so-called ‘Crabtree positive’, also behave in this way. However, in most cases in aerobic conditions, pyruvate is oxidized to CO$_2$ through the tricarboxylic acid (TCA) cycle. Glycolysis and the TCA cycle are therefore central pathways of metabolism. They perform a dual role in organisms: to produce energy and reducing equivalents in the form of ATP, NADH or NADPH and to provide building blocks to synthesize other biomolecules.

The purpose of this review is to survey the existing knowledge on these pathways and related ones in someNCY and contrast it with what is established in *S. cerevisiae*. It will become apparent that, in a great number of cases, the absence of information is the dominant note and we hope that this awareness will provide an invitation to fill in the corresponding gaps. Detailed reviews concerning different aspects of carbohydrate and energy metabolism in *S. cerevisiae* are available to which the reader may usefully refer [6–9].

We have restricted this review to a number of species of the genera *Kluyveromyces*, *Pichia*, *Candida*, *Yarrowia* and *Schizosaccharomyces*, although occasional references to other genera will be made. This choice was dictated by the already well-established importance of species of these genera in some area of research or technology. To place *Schizosaccharomyces* among the non-conventional yeasts may be a matter of opinion; researchers working on cell
cycle or morphogenesis may consider it as quite conventional. Some background information and a treatment of several aspects of cultivation, and molecular biology techniques pertaining to certain NCY may be found in [10].

2. Uptake of sugars

"Don't talk to me of permeability, this is the last resort of the biochemist who cannot find any better explanation" M. Stephenson (quoted in [11])

"The realization that the membranes are not sievelike bags but barriers to a large extent impermeable to glucose, makes it obvious that some sort of mechanism is likely to intervene to enable an adequate supply of glucose to reach the intracellular glycolytic machinery” A. Sols [12]

2.1. Common hexoses

Cell membranes are not freely permeable for a variety of solutes, sugars among them. Therefore, transport is the first step in carbohydrate metabolism, except in those cases in which a di- or tri-saccharide is hydrolyzed outside the cell. Transport across the membrane is carried out by specific transporters, sometimes called permeases. In S. cerevisiae, a family of glucose transporters with \( K_m \) ranging from 1 to 100 mM, has been characterized. The transcription of the genes encoding these transporters is finely regulated by the glucose concentration of the medium (for reviews see [13,14]). Transport of the common monosaccharides, glucose, fructose or mannose in S. cerevisiae is a facilitated diffusion process; however, the situation may be different in other yeasts.

In K. lactis glucose transport appears to proceed by facilitated diffusion and two genes encoding transporters with different affinities for glucose have been identified in this yeast: \( HGT1 \) that encodes a high affinity glucose transporter, \( K_m = 1 \) mM, [15] and \( RAG1 \) that encodes a transporter with low affinity for glucose, \( K_m = 20-50 \) mM [16]. Overexpression of \( HGT1 \) could not restore the wild-type phenotype to a \( rag1 \) mutant indicating that \( HGT1 \) and \( RAG1 \) have distinct functions in glucose transport [15]. Wild-type strains of \( K. \) lactis are divided into two groups with regard to their different sensitivity to the respiratory inhibitor antimycin A: those that grow in glucose in the presence of the inhibitor are called \( Rg^+ \) (resistance to antimycin on glucose) and those that do not, are termed \( Rg^- \). Wild-type \( Rg^+ \) strains possess \( RAG1 \) and \( HTG1 \), while \( Rg^- \) strains have lost \( RAG1 \) [17]. \( RAG1 \) seems to have arisen by a recombination event between two genes, \( KHT1 \) and \( KHT2 \), that encode low affinity glucose transporters and are found in tandem in some strains. The sequence of \( RAG1 \) is identical to that of \( KHT1 \) except for an arginine and a phenylalanine, the last two C-terminal amino acids, which are exchanged for the last three amino acids of Kht2 (alanine—methionine—leucine) [18].

Expression of \( HGT1 \) does not vary with culture conditions [15] while transcription of \( RAG1 \) that is undetectable during growth in glycerol increases during growth in glucose [17]. It is not known which signal(s) trigger the induction of \( RAG1 \) expression [19], but an extended glycolysis does not seem to be required since a \( rag2 \) mutant, defective in phosphoglucose isomerase did not show decreased levels of \( RAG1 \) mRNA [20,21]. Several genes affect the expression of \( RAG1 \) and \( HGT1 \). Mutations in \( RAG5 \) encoding the unique hexokinase of \( K. \) lactis [22] abolish expression of \( RAG1 \) [19] and decrease the level of the 2.0-kb mRNA species of \( HGT1 \) [15]. Mutations in \( RAG4 \), a gene of unknown function, eliminated transcription of \( RAG1 \) [19] but increased the 2.0-kb mRNA species of \( HGT1 \) [15]. A \( rag4 \) \( rag5 \) double mutant showed a level of \( HGT1 \) transcript similar to that of the wild-type, suggesting that \( RAG4 \) is epistatic over \( RAG5 \) for the regulation of the transcription of \( HGT1 \) [15]. The \( RAG8 \) gene, an apparently essential gene that shows a high degree of identity with the two casein kinases I of \( S. \) cerevisiae [23], also abolished expression of \( RAG1 \) [19], but did not affect that of \( HGT1 \) [15]. These results show that different controls distinctly affect the transcription of both genes. \( RAG1 \) expression was unaffected in a \( hgt1 \) null mutant and vice versa. A double mutant \( rag1 \) \( hgt1 \) showed a greatly reduced level of glucose uptake, but still grew in glucose, although it stopped growth earlier than the wild-type [15]. It seems, therefore, that some alternative system with low glucose affinity is able to transport this sugar in the double mutant.

A gene from C. albicans that restores growth in glucose of a \( S. \) cerevisiae strain deleted in the seven physiologically relevant glucose transporters has been isolated and named \( CaHGT1 \). The gene encodes a protein of 545 amino acids with 12 transmembrane domains and a 51% identity with the \( K. \) lactis glucose transporter, Hgt1. The transcript levels of \( CaHGT1 \) were enhanced in response to a variety of drugs suggesting an implication of the encoded protein in drug resistance [24].

In Candida utilis, the popular ‘fodder yeast’, glucose appears to be transported by a proton symport when the organism is grown at low glucose concentration [25,26], but a facilitated diffusion operates when the cells are grown at higher glucose concentrations [25].

Weierstall et al. [27] isolated three genes encoding glucose transporters from the xylose fermenting yeast \( Pichia stipitis \) with \( K_m \) values around 1 mM. Expression of one of these genes, \( SUT1 \), was induced by glucose while the expression of the others, \( SUT2 \) and \( SUT3 \), was constitutive.

A transport system for glucose with two components with \( K_m \) 3 and 10 mM respectively, was found in \( Y. \) lipolytica. These components were present with the same activity independently of the glucose concentration in the medium [28].

In Schizosaccharomyces pombe Höfer and Nassar [29] identified hexose transport as a \( H^+ \)-symport; since then
a family of hexose transporters has been identified with six components called Ght1 to Ght6 [30]. Ght5 is the most abundant of these transporters. Ght1, Ght2 and Ght5 exhibited higher activities with glucose than with fructose as substrate [30]. Mehta et al. [31] isolated two S. pombe mutants deficient in glucose transport; one of them has, apparently, an altered affinity for glucose and both present defects in the regulation of the expression of the enzymes of the pentose phosphate pathway. No information is available on the gene(s) affected in these mutants.

2.2. Other sugars

Galactose seems to be transported in K. lactis by the same protein that transports lactose, since mutations in the LAC12 gene encoding the lactose transporter abolish growth both in lactose and galactose [32].

S. pombe is able to grow on gluconate and -much better – in ð-glucoronolactone. Gluconate is taken up by a proton motive-force driven transport [33]. The transporter protein Ght3 (see above) is the one implicated in gluconate transport [30]. Transport of gluconate is repressed by glucose and its derepression seems dependent on the activity of a gene named gtl1+ [34]. Overexpression of this gene shortened the time of derepression and increased the transport activity with respect to the wild-type. Glucose inactivates rapidly and reversibly the transport in a process that implicates cAMP [35].

2.3. Disaccharides

Metabolism of disaccharides is preceded by a hydrolysis to their monomers. Depending on the yeasts species, and the nature of the sugar, this hydrolysis may occur outside the cell membrane, in the periplasmic space, or inside the cell after the transport of the disaccharide.

Sucrose is hydrolyzed in most cases by an external invertase repressed by glucose. In K. lactis, the gene KINV1 encoding invertase has been isolated. It presents high amino acid homology with the invertase from S. cerevisiae and with the inulinase – a fructofuranosidase that hydrolyzes sucrose – from Kluyveromyces marxianus [36]. Genes encoding periplasmic invertases have been isolated from C. utilis, [37] and S. pombe [38].

In C. albicans, sucrose is transported into the cell by a sucrose inducible H+ symport [39] and hydrolyzed by an intracellular ð-glucosidase, also able to hydrolyze maltose [39]. This enzyme is encoded by the CambAL2 gene [39] whose expression is induced by sucrose and maltose and repressed by glucose [40]. While no maltose transport has yet been described in C. albicans, maltose is transported in C. utilis by a proton symport as in S. cerevisiae [26]. In contrast, in S. pombe, maltose is hydrolyzed by an external maltase; details on this enzyme are not available [31].

Lactose uptake in K. lactis occurs by an inducible active transport system [41], encoded by the LAC12 gene [42] whose expression is induced by lactose or galactose [32]. Once transported, lactose is hydrolyzed by a ß-galactosidase, also induced by lactose, encoded by the LAC4 gene [43].

3. The common glycolytic trunk

3.1. From intracellular sugar to trioses phosphate

The intracellular hexoses enter the glycolytic pathway after a phosphorylation step. Glucose, fructose and man- nose are phosphorylated by hexokinases; a glucokinase can phosphorylate glucose and mannose and a galactokinase phosphorylates galactose. The enzymatic equipment for hexose phosphorylation varies among different yeasts, although no physiological explanation for the differences has been found. While S. cerevisiae possesses two hexokinases and a glucokinase [44], K. lactis has only one hexokinase encoded by the RAG5 gene. rag5 mutants are not only unable to grow in glucose or fructose, but also unexpectedly, in lactose [22]. While the transcription rates of the genes HXK1, HXK2 and GLK1 that encode the hexokinases and the glucokinase of S. cerevisiae are controlled by glucose [45], the levels of RAG5 mRNA did not vary significantly between cultures in glucose or in glycerol [22].

In Candida tropicalis, two hexokinases and a glucokinase were found in chromatographic studies. The levels of the hexokinases were higher when the yeast was cultured in glucose or fructose than when grown in alkanes [46]. In Y. lipolytica a hexokinase and a glucokinase were detected [46,47]. The gene encoding the hexokinase has an intron of 436 bp located 39 bp after the adenine of the first ATG [47]. Taking into account the kinetic characteristics of both enzymes and the fact that disruption of the YHIXK1 gene does not significantly affect growth in glucose, it has been suggested that the major glucose phosphorylating capacity in vivo is due to glucokinase [47].

Two hexokinases are found in S. pombe, a major one, hexokinase 2, with a Km for glucose around 0.1 mM, the usual range for hexokinases, and a minor one, hexokinase 1, that has a low affinity for glucose (Km 9 mM) [48]. Hexokinases and glucokinase show, in general, extensive homology in sequence [49]; however, hexokinase 1 from S. pombe is peculiar in having a serine in the glucose binding site at a position where an asparagine is usually present. The low affinity for glucose of this enzyme is due, in part, to this substitution [50]. The physiological role of this minor hexokinase is not clear.

In all cases studied, the genes encoding hexokinases from NCY were able to complement the hox2 mutation from S. cerevisiae [22,47,48].

Most hexokinases are inhibited by trehalose-6-P, a compound that plays an important role in the regulation of glycolysis in S. cerevisiae [51]. The hexokinase of Y. lipopo-
lytica shows the strongest inhibition by trehalose-6-P yet found (K_i 3.6 mM) and has been used in a method for the quantitative determination of this compound [52]. The hexokinases from S. pombe are however, peculiar with regard to the inhibition by trehalose-6-P: hexokinase 2 is not inhibited, and hexokinase 1 is only poorly inhibited [48]. Apparently the regulation of glycolysis in this yeast has different characteristics from that of S. cerevisiae.

While it is known that hexokinase 2 from S. cerevisiae plays a role in the catabolite repression of certain genes, not much information on the participation of hexokinases on catabolite repression inNCY is available (see Section 11).

Phosphoglucone isomerase converts glucose-6-P into fructose-6-P. In K. lactis the gene RAG2 encodes phosphoglucone isomerase. A K. lactis rag2 mutant is able to grow in glucose, but does not produce ethanol and this growth is dependent upon an active respiration [20]. This behavior contrasts with the lack of growth in glucose found in the equivalent pgI mutant in S. cerevisiae [53] and indicates that in K. lactis, the capacity of the pentose phosphate pathway is enough for glucose utilization. Although Goffrini et al. [20] reported that the rag2 mutant did not produce ethanol during growth in glucose, Gonzalez-Siso et al. [54] detected a significant ethanol production and postulated that the dependence on respiratory activity for growth in glucose of the rag2 mutant is related to the need of function of the external mitochondrial NAD(P)H dehydrogenase for reoxidation of the NADPH produced in the hexosephosphate pathway.

Phosphofructokinase catalyzes the phosphorylation of fructose-6-P to fructose-1,6-P2. The activity of the enzyme is affected by a large variety of effectors and has been the paradigm for ‘multimodulated enzymes’ [55]. Phosphofructokinase received much attention in S. cerevisiae because it was thought to be the ‘bottleneck’ of glycolysis. This view has been weakened by the finding that overexpression of the genes encoding phosphofructokinase did not increase fermentation in S. cerevisiae [56,57] and by the realization that the control of a pathway may be rarely ascribed to a single step [58]. In all yeast species in which the genetics of phosphofructokinase have been studied, two genes have been found. In K. lactis the enzyme is an octamer composed of two different types of subunits of molecular masses 119 and 102 kDa [59] encoded by the genes KlPFK1 and KlPFK2 [60]. Mutants lacking KlPFK1 or KlPFK2 grew in glucose, although no phosphofructokinase activity was detected in vitro, a behavior similar to that observed in S. cerevisiae [61]. However, in contrast with the behavior of pfk1 pfk2 mutants in S. cerevisiae, Klpfk1 pfk2 mutants that lack both subunits of phosphofructokinase still grow in glucose if respiration is functional, indicating again that the activity of the pentose phosphate is enough for glucose utilization (see behavior of the rag2 mutant). This view is strengthened by the fact that a disruption of the gene KITALI, that encodes a transaldo-
The corresponding gene from K. lactis has been cloned and disrupted; in contrast with the behavior of a tpi mutant of S. cerevisiae, the mutant from K. lactis lacking the gene encoding triose phosphate isomerase is able to grow in glucose [69]. The growth is not possible in the presence of the respiratory inhibitor antimycin A, a behaviour similar to that shown by the rag2 and pfk1 pfk2 mutants (see above).

In the step catalyzed by glyceraldehyde-3-phosphate dehydrogenase, 1,3-bisphosphoglycerate is formed from glyceraldehyde-3-phosphate. This is an important step in glycolysis, since the oxidation of the aldehyde generates an energy-rich acyl phosphate bond. The NADH generated in the reaction has to be reoxidized for glycolysis to proceed (see Section 6). In S. cerevisiae, three genes named TDH1, TDH2 and TDH3 encode three different isoenzymes, with TDH3 accounting for 50–60% of the enzyme found in the cell [70]. Mutants with only TDH1 are not viable and those lacking either TDH2 or TDH3 grow more slowly in glucose than the wild-type [70]. A gene, KlGAP1, encoding glyceraldehyde-3-P dehydrogenase has been cloned from K. lactis. This gene and the one encoding alcohol dehydrogenase 1 are transcribed in opposite directions and separated by only 1.2 kb, suggesting that they share common elements in the promoter [71]. In contrast to the single gene reported in K. lactis, a family of three genes encoding glyceraldehyde-3-phosphate dehydrogenases is found in K. marxianus [72]. KmGAP1 is transcribed at similar rates in different carbon sources and apparently encodes a protein that is localized in the cell wall. Transcription of GAP2 is increased in glucose, while transcription of GAP3 was not detected in the conditions used [72].

In C. albicans, a glyceraldehyde-3-phosphate dehydrogenase, encoded by the gene TDH1 [73], also appears in the cell wall and is able to bind to laminin and fibronectin [74].

In P. pastoris the GAP gene, that encodes a glyceraldehyde-3-phosphate dehydrogenase with a molecular mass of 35.4 kDa, is constitutively expressed. The promoter of this gene has been used to direct the heterologous production of proteins in this yeast as an alternative to the commonly used AOX1 promoter [75].

In the reaction catalyzed by 3-phosphoglycerate kinase the energy-rich acylphosphate from 1,3-bisphosphoglycerate is used to produce ATP. In S. cerevisiae, the PGK1 gene that encodes phosphoglycerate kinase, is one of the most highly expressed and as such it has been widely studied both to understand why this is so and to use its promoter to direct expression of foreign proteins [76]. Genes encoding 3-phosphoglycerate kinase have been cloned from several NCY. In K. lactis the corresponding gene encodes a protein of 416 aa with strong codon bias [77]. In Candida maltosa, a gene encoding a protein of similar size has been cloned. Its expression was higher in glucose than in alkalans or oleic acid and its promoter has been used to direct expression of heterologous proteins [78]. In contrast with C. maltosa, the gene encoding 3-phosphoglycerate kinase in C. albicans is similarly expressed under various growth conditions. A certain amount of the protein has been found attached to the cell wall [79].

In Y. lipolytica, expression of the PGK1 gene was higher on gluconeogenic substrates than on glycolytic ones [80]. Curiously, strains with a disrupted PGK1 gene were proline auxotrophs when grown in glucose. No immediate explanation exist for this phenotype that has not been observed, to our knowledge, in other yeasts.

Phosphoglycerate mutase catalyzes the interconversion between 3- and 2-phosphoglycerate. In contrast with the protein of S. cerevisiae which is a tetrameric enzyme, the phosphoglycerate mutase of S. pombe appears to be monomeric; it lacks an amino acid segment at the C-terminus that in S. cerevisiae has been implicated in catalytic activity [81], and also a stretch of 24 aa that in S. cerevisiae appears to be involved in interactions between sub-units. Another loop also involved in these types of interactions is absent in the S. pombe protein [82].

Enolase catalyzes the dehydration of 2-phosphoglycerate to phospho-enol-pyruvate. Two genes, ENO1 and ENO2, encode the subunits of this homodimeric enzyme in S. cerevisiae [83]. While transcription of ENO1 is constitutive, that of ENO2 is stimulated about 20-fold by glucose, suggesting a differential participation of the iso-enzymes in glycolysis or in gluconeogenesis [84]. Although it was reported that C. albicans contained only one gene (ENO1) encoding enolase, a protein of 440 amino acids [85], Postlethwait and Sundstrom [86] later found two enolase gene loci that could be distinguished by the localization of particular restriction sites in their 3’flanking regions, while the promoter and coding region were identical [86]. There have been also discrepancies regarding expression of the enolase encoding gene(s) in C. albicans. The corresponding mRNA level was reported to be 6 and 13 times higher in glucose than in ethanol [85]; however, other authors did not find differences between glucose and pyruvate grown cells [86]. This discrepancy could be due either to a lack of normalization to a reference RNA in the first case, or to specific differences between ethanol and pyruvate, although this seems unlikely.

In S. pombe a gene, enol1+, which could encode an enolase has been identified; the corresponding 1.4-kb transcript is more abundant during growth in glucose than during growth in gluconeogenic substrates [87].

Pyruvate kinase catalyzes the second ATP forming reaction in the glycolytic pathway. In S. cerevisiae, two genes exist that encode two different proteins. One of these, encoded by the PYK1 gene, is strongly activated by fructose-1,6-bisphosphate, while the other one encoded by the PYK2 (YOR347c), does not respond to this compound. This last enzyme is repressed by glucose and in certain conditions may allow function of glycolysis at a reduced rate [88]. There are no reports of the existence
of more than one pyruvate kinase encoding gene in NCY. Only the genes encoding pyruvate kinases from *S. pombe* and *Y. lipolytica* have been cloned [89,90]. Overexpression of the *pyk1* gene from *S. pombe* has allowed purification of the enzyme that behaves as a dimer [89]. The organization of the *YLPYK1* gene from *Y. lipolytica* is curious as it presents two introns separated by a short stretch of 45 bp [90,91].

In general, pyruvate kinase from diverse origins presents a sigmoidal kinetics with respect to PEP; fructose-1,6-bisphosphate activates the enzyme and converts its kinetic to an hyperbolic one. In contrast, the enzyme from *C. utilis* [92] and *Y. lipolytica* [93] are not activated by fructose-1,6-bisphosphate and in this last yeast the kinetics towards PEP is hyperbolic [93].

4. Metabolic alternatives of intracellular pyruvate

The two quantitative major fates of the pyruvate produced in glycolysis are either its oxidation to CO₂ or its transformation to ethanol. In most yeasts under aerobic conditions, oxidation will be predominant, while transformation to ethanol takes place in anaerobic conditions or at high glucose concentrations even in aerobic conditions in those yeasts that present a Crabtree effect (for a review see [94]).

4.1. Pyruvate dehydrogenase

Oxidation of pyruvate to CO₂ occurs via the TCA cycle. To enter the TCA cycle, pyruvate undergoes an oxidative decarboxylation catalyzed by pyruvate dehydrogenase, a mitochondrial multienzyme complex that is formed by three different components: pyruvate dehydrogenase (E1), dihydrolipoamide acetyl transferase (E2) and dihydrolipoamide dehydrogenase (E3). The E1 element, in turn, consists of two subunits E1α and E1β. The respective genes in *S. cerevisiae* are named *PDA1* [95] or *PDHa1* [96], *PDHβ1* [97], *LAT1* [98] and *LPD1* [99]. The localization of the pyruvate dehydrogenase complex implies that cytosolic pyruvate must be transported into the mitochondrion. A mitochondrial pyruvate carrier was purified from *S. cerevisiae* by Nalecz et al. [100], but up to now the gene encoding it has not been identified.

In *K. lactis*, disruption of the gene *KIPDA1*, that encodes the E1α subunit of the complex, led to a decrease of the specific growth rate in glucose in minimal medium, while growth in rich medium or in ethanol was not affected. Also ethanol was produced in aerobic batch cultures of the mutant [101]. Expression of *KIPDA1* was partly repressed during growth in glucose by the product of the *Rag3* protein [102] a homologue of the *PDC2* gene of *S. cerevisiae* that controls transcription of pyruvate dehydrogenase [103]. The E1β subunit of the pyruvate dehydrogenase complex has been cloned from *S. pombe*, but no studies on the effects of its disruption have been reported [104]; however, the same authors found that mutants from the fission yeast with reduced pyruvate dehydrogenase activity were auxotrophic for arginine or glutamine [105]. In *P. stipitis*, the activity of pyruvate dehydrogenase is constitutive [106].

4.2. Pyruvate decarboxylase

In *S. cerevisiae*, several genes encoding pyruvate decarboxylase have been found although *PDC1* normally accounts for about 80% of the enzymatic activity. Enzyme activity increases in glucose due to enhanced transcription of the *PDC1* and *PDC5* genes [107]. The product of the *PDC2* gene is involved in the control of expression of the structural *PDC* genes [107]. In contrast, in *K. lactis* only the gene *RAG6* appears to encode pyruvate decarboxylase [108]. Contrary to what happens in mutants from *S. cerevisiae* lacking pyruvate decarboxylase, *K. lactis* *rag6* mutants grew in glucose at the same rate as the wild-type. This fact raises the question of the origin of the acetyl CoA needed for biosynthesis in the cytoplasm in this mutant (see Section 4.3) and highlights once more the different lifestyles of these two yeasts. Transcription of *RAG6* is stimulated by glucose and autoregulated. The product of the gene *RAG3* is necessary for glucose induction [109]. Mutations in *RAG1*, *RAG5* or *RAG2* reduce the induction by glucose of *RAG6* [108].

In *C. albicans*, the gene encoding pyruvate decarboxylase has not been yet cloned, but a homologue of Sc*PDC2* has been found [110]. *CuPDC2* complemented the defect of *S. cerevisiae* *pdc2* mutants [110] in contrast with what happened with *RAG3* that was ineffective [109].

In *P. stipitis* two genes encoding pyruvate decarboxylase have been found, but detailed studies on their physiological roles are not available. The *PsPDC1* gene has an 81-bp stretch that is absent from other known *PDC* genes [111].

4.3. The pyruvate bypass

Acetyl CoA can also be formed in the cytosol through the so-called pyruvate-bypass, that involves the synthesis of acetyl CoA through the concerted action of pyruvate decarboxylase, acetaldehyde dehydrogenase and acetyl CoA synthetase. These reactions and transport of the so formed acetyl CoA to the mitochondria could in principle ‘by-pass’ the action of pyruvate dehydrogenase (94), see Fig. 1. The physiological significance of this by-pass is shown by the absence of growth of *S. cerevisiae* *pdc* mutants in mineral medium with glucose unless ethanol or acetate is added [112] and by the observation that *acs1* *acs2* mutants devoid of acetyl CoA synthetase activity do not grow in glucose [113].

The enzymes participating in the pyruvate bypass are localized in the cytosol in *S. cerevisiae* [114,115] but no
information about their localization in other yeasts is available.

The fact that in K. lactis, disruption of the gene KlPDA1, causes production of ethanol in aerobic batch cultures indicates that either the pyruvate bypass does not produce enough acetyl CoA in those conditions or that the mitochondria has a limited capacity to import acetyl CoA from the cytosol. Since addition of low concentrations of L-carnitine restored the aerobic growth in glucose the second possibility appears the most likely [101].

5. The TCA cycle

Acetyl CoA generated either by pyruvate dehydrogenase or by the pyruvate bypass, is the link between glycolysis and the TCA cycle. Acetyl CoA reacts with oxaloacetate in a reaction catalyzed by citrate synthase to produce citrate. Three genes encoding functional citrate synthases have been identified in S. cerevisiae: CIT1 and CIT3 that encode mitochondrial enzymes and CIT2 that encodes the isoenzyme involved in the glyoxylate cycle [116,117]. In C. tropicalis a gene, CtCIT, encoding a 45-kDa polypeptide has been identified. This gene has an intron of 79 bp in the region encoding the N-terminal part of the protein; this intron can be compartmentalized in S. cerevisiae [118].

The next step of the pathway is the conversion of citrate to isocitrate catalyzed by aconitase, a 4Fe-4S cluster containing protein, that has not been much studied. In S. cerevisiae, the gene ACO1 encoding it, is synergistically repressed by glucose and glutamate and its disruption led to glutamate auxotrophy [119]. Its activity is dependent on the function of the gene YFH1 that encodes frataxin, a protein that seems implicated in the regulation of mitochondrial iron transport and that is implicated in the human degenerative disease Friedreich ataxia [120].

Isocitrate dehydrogenase transforms isocitrate into 2-oxoglutarate (α-ketoglutarate). In S. cerevisiae an NAD⁺-dependent isocitrate dehydrogenase, whose two different subunits are encoded by the genes IDH1 and IDH2 [121,122], participates in the TCA cycle [123]. Three differently compartmentalized NADP⁺-dependent isocitrate dehydrogenases, encoded by the genes IPD1, IPD2 and IPD3 exist. At least some of them are important in the production of NADPH [124]. S. pombe mutants lacking an NAD⁺-dependent enzyme are glutamate auxotrophs [125].

Apparently no other studies on genes encoding enzymes implicated in the TCA cycle have been done inNCY. This lack of information contrasts with the importance of this pathway in the aerobic metabolism of these yeasts.

5.1. Anaplerotic reactions

Intermediates from the TCA cycle are removed during growth for biosynthetic purposes; therefore, reactions that replenish the cycle are needed to keep it running. In yeasts growing in a minimal medium with a sugar as carbon source and NH₄⁺ as nitrogen source, pyruvate carboxylase is the main anaplerotic reaction (see Fig. 1). Two genes PYC1 and PYC2 encode isoenzymes of pyruvate carboxylase in S. cerevisiae [126,127], these proteins are located in the cytosol as in C. utilis, in contrast with the situation in other fungi and in mammals [128]. One unique gene encoding pyruvate carboxylase has been isolated from P. pastoris. Mutants lacking this enzyme do not grow in glucose when ammonium is the nitrogen source but can grow when glutamate or aspartate is added [129]. The situation is different from that of S. cerevisiae where glutamate cannot substitute aspartate for growth of a pyruvate carboxylase mutant, likely due to the repression exerted by sugars on glutamate dehydrogenase (C. Gancedo, unpublished results). An interesting observation is that of van Dijk et al. [130] who found that pyruvate carboxylase appears implicated in the assembly of alcohol oxidase in Hansenula polymorpha. Up to now, no indications are available about the possible mechanism of participation of this protein in the process.

Another important anaplerotic device is the glyoxylate cycle required for growth in minimal medium in carbon sources of less than three carbon atoms, such as ethanol or acetate. Two characteristic enzymes of the cycle are isocitrate lyase and malate synthase. Isocitrate lyase catalyzes the cleavage of isocitrate to succinate and glyoxylate and malate synthase catalyzes the condensation of glyoxylate with a molecule of acetyl CoA.

The gene ICL1 encodes isocitrate lyase in S. cerevisiae [131], where the protein is not located in the mitochondria [132,133]. The isocitrate lyase from C. tropicalis and that of Y. lipolytica have putative peroxisomal targeting signals [134,135]. The gene ChiCL1, encoding isocitrate lyase from C. tropicalis has been expressed to relatively high levels from its own promoter in S. cerevisiae growing in acetate, glyceral, lactate, ethanol or oleate [136]. Mutants of Y. lipolytica lacking isocitrate lyase, are unable to grow on acetate, ethanol or fatty acids, but utilize succinate or glyceral [137]; curiously growth is also impaired in glucose [135].

Malate synthase was purified from C. tropicalis [138]. Two identical genomic regions – except for one amino acid replacement in the encoded protein – were found, although only one RNA species was detected [139]. A gene, MASI, encoding the enzyme from Hansenula (Pi- chia) polymorpha has been cloned [140].

The enzyme from C. tropicalis, a homotetrameric protein of molecular mass 250 kDa, was localized in the matrix of the peroxisomes [138], but the protein from H. polymorpha does not have a conventional peroxisomal targeting sequence [140]. There is no report of the existence of more than one malate synthase in NCY as it happens in S. cerevisiae, where two genes MLS1 and DAL7 encode
two different enzymes, one involved in carbon and the other in nitrogen metabolism [141].

*S. pombe* lacks the enzymes of the glyoxylate cycle and this could explain the lack of growth in ethanol of this yeast [142,143].

6. Oxidation of NADH and energy generation

Catabolic reactions generate NADH either in the cytosol or in the mitochondrion. During fermentation, NADH produced in glycolysis is reoxidized to NAD⁺ by the formation of ethanol; NADH arising in reactions different from glycolysis is regenerated mainly by the production of glycerol. During aerobic growth, ‘external’ mitochondrial NADH dehydrogenases participate in the reoxidation of cytosolic NADH. In *S. cerevisiae* they are encoded by two genes called either *NDH1* and *NDH2* [144] or *NDE1* and *NDE2* [145]. The existence of this external dehydrogenases seems consistent with the apparent absence of a malate aspartate shuttle in this yeast [145]. In *Y. lipolytica*, a gene named *YNDI2*, encodes an external NADH oxidoreductase [146]. NADH generated in reactions occurring in the mitochondrion is regenerated by a NADH dehydrogenase located in the inner mitochondrial membrane facing the matrix space. The protein is encoded by the gene *NDII* in *S. cerevisiae* [147]. During growth on reduced substrates like ethanol, the t-glycerol-3-phosphate shuttle (see Fig. 1) appears to be important to maintain an adequate redox balance in the cytosol [148].

Reoxidation of NADH by the respiratory chain is the main source of energy for cells with a respiratory metabolism. This reoxidation is coupled to the production of ATP in the process known as oxidative phosphorylation. Although we will not discuss in detail oxidative phosphorylation, it should be mentioned that while in *S. cerevisiae* phosphorylation at site I of the electron transfer chain is inadequate redox balance in the cytosol [148].

The ATP generated in the mitochondrion is delivered to the cytosol via specific ADP/ATP translocators. In *S. cerevisiae*, three genes encoding mitochondrial ADP/ATP translocators have been identified: *AAC1* [151], *AAC2* [152] and *AAC3* [153]. The major protein involved in translocation appears to be Aac2, but Aac3 is necessary for anaerobic growth [153]. The role of Aac1 is not completely clear; curiously, it has been implicated in the vacuolar accumulation of the red pigment formed by ade2 mutants [154]. Gene homologues to those encoding ATP translocators in *S. cerevisiae* have been cloned from *K. lactis* [155], *Candida parasilopsis* [156] and *S. pombe*. In this last yeast, the gene has been termed *anc1* [157]. There is only one gene encoding an ATP/ADP translocator in *K. lactis* and *S. pombe*. A null mutation of *AAC2* in *K. lactis* made the yeast unable to grow on glycerol, galactose, maltose and raffinose [158] and null *anc1* mutants of *S. pombe* were unable to grow under oxygen limitation [159]. In some conditions, e.g. during anaerobic growth, ATP should be translocated into the mitochondria. Due to the expression pattern of the ATP/ADP translocators in *S. cerevisiae* it has been hypothesized that *AAC3* is the one that carries out this function [160].

It ought to be mentioned here, that a number of yeasts possess a respiration pathway alternative to the cytochrome pathway. This pathway is specifically inhibited by hydroxamic acids, but is not inhibited by cyanide [161,162]. Cyanide insensitive respiration is mediated by an alternative oxidase that accept electrons from the ubiquinone pool and transfer them to oxygen that is reduced to water. However, this electron flow is not accompanied by synthesis of ATP [163].

The existence of the alternative respiration pathways in *K. lactis* was demonstrated by Heritage et al. [164]. In the yeast *Hansenula anomala*, the alternative oxidase is a mitochondrial protein of 36 kDa [165] whose synthesis is induced by antymycin A and cyanide or by sulfur-containing amino acids; a cDNA encoding it has been isolated [166,167]. The gene *AOX1* encodes the alternative oxidase in *C. albicans*, the putative protein has about 44 kDa [168]. It is a non-essential gene and it is functional in *S. cerevisiae*, a genus that lacks the alternative respiratory pathway. *Y. lipolytica* possesses the pathway [169] whose activity appears to increase during the transition from logarithmic to stationary growth phase in glucose cultures [170]. In *P. stipitis*, the alternative pathway seems important during oxygen-limited xylose fermentations [171].

Since the alternative pathway is absent in *S. pombe* this yeast has been used to express the alternative oxidase from the plant *Sauromatum guttatum* under the control of the thiamine-repressible *nmt1* promoter [172]. Expression of the plant oxidase decreased slightly the growth rate of the yeast in glucose, but did not affect growth yield; however, both parameters were severely affected during growth in glycerol. The effect was due to competition for ubiquinone between the plant oxidase and the normal cytochrome pathway [173].

The physiological role of the alternative respiratory pathway in yeasts is still not clearly understood; it could act as an energy overflow device or be related to particular stress situations. The alternative respiration pathway and its underlying molecular basis and regulation is a topic that deserves further consideration.

7. The pentose phosphate pathway

The pentose phosphate pathway is an important metabolic route implicated in the production of NADPH for biosynthetic reactions and of ribose 5-phosphate for synthesis of nucleic acid and nucleotide cofactors; it also provides erythrose-4-phosphate for the synthesis of aromatic amino acids. The first reactions of the pathway are two
oxidative reactions that are physiologically irreversible, while the other ones are non-oxidative and reversible. The partition of hexose metabolism between the glycolytic and the pentose phosphate pathway occurs at the level of glucose 6-phosphate (see Fig. 1). It has been observed that the nitrogen source of the medium influences the amount of sugar directed to the pentose phosphate pathway. In S. cerevisiae, growth in a medium supplemented with amino acids decreased the flux through the pentose phosphate pathway [174]. In those yeasts that use nitrate as nitrogen source, an increase of the carbon flux through the pentose phosphate pathway shall be expected due to the increased NADPH requirement caused by the operation of nitrate and nitrite reductase; indeed this has been found in C. utilis [175].

Glucose-6-phosphate dehydrogenase directs glucose into the pentose phosphate pathway by catalyzing the oxidation of glucose-6-phosphate to 6-phospho-δ-gluconolactone. Glucose-6-phosphate dehydrogenase has been purified from S. pombe [176] and has $K_m$ values of 0.66 mM for glucose-6-P and 44 μM for NADP$^+$. The enzyme from C. utilis has been purified [177], it has a molecular weight of 11 or 22 kDa depending on pH and the presence of Mg$^{2+}$. Another group purified the enzyme from a strain of Pichia jadinii (synonym of C. utilis) and found its N-terminal amino acid acetylated and a 15 amino acid stretch matured in Ser and Thr (411–425 in a protein of 495 residues) that is absent in other related glucose-6-P dehydrogenases [178]. The significance of this stretch, if any, is unknown.

Mutants in the gene ZWF1 from S. cerevisiae that lack glucose-6-phosphate dehydrogenase activity, turned out to require an organic sulfur source [179]. This requirement may be explained by the existence of two steps in methionine biosynthesis that require NADPH. It could be interesting to test the phenotype of similar mutants in NCY to ascertain the existence of alternative, effective pathways of NADPH provision.

The hydrolysis of 6-phosphate-δ-gluconolactone to 6-phospho-gluconate occurs spontaneously at neutral pH, but at a very slow rate. A lactonase that accelerated this rate was partially purified from baker’s yeast by Brodie and Lipmann [180]; however, no further studies from a strain of Pichia jadinii (synonym of C. utilis) and found its N-terminal amino acid acetylated and a 15 amino acid stretch in Ser and Thr (411–425 in a protein of 495 residues) that is absent in other related glucose-6-P dehydrogenases [178]. The significance of this stretch, if any, is unknown.

In those yeasts able to use gluconate, 6-phospho-gluconate may be also formed by the action of 6-phospho-gluconate kinase, an enzyme that has been functionally characterized in S. pombe [183].

6-Phospho-gluconate dehydrogenase catalyzes the oxidative decarboxylation of 6-phospho-gluconate to ribulose 5-phosphate. The enzyme has been purified from S. pombe as a tetramer and its kinetic characteristics studied [184]. In C. utilis the enzyme appears to be a dimer of 100 kDa with high affinity for its substrates ($K_m$ 55 μM for 6-P-gluconate and 20 μM for NADP$^+$) [185]. In S. cerevisiae, mutants lacking this activity are unable to grow in glucose, but elimination of the glucose-6-phosphate dehydrogenase activity allowed growth in this sugar [186]. The authors concluded that the accumulated 6-phospho-gluconate was toxic for the cell.

Ribulose-5-P can be either isomerized to ribose-5-P or epimerized to xylulose-5-P. The first reaction is catalyzed by ribose-5-P isomerase. This enzyme has been purified from C. utilis by Domagk and Doering [187] and by Horitsu et al. [188]. The data about molecular mass and subunit composition differ between these authors. Ribulose-5-P epimerase catalyzes the epimerization of ribulose 5-phosphate to xylulose 5-phosphate. No data for this step in NCY are available at this time.

Transketolase catalyzes two reactions in the pentose phosphate pathway in which a glycolaldehyde (two carbon) moiety is transferred from a ketose donor to an aldose acceptor. In both reactions, the donor is xylulose 5-phosphate, but the acceptor is in one case ribose 5-phosphate and in other erythrose 4-phosphate. Two genes encoding transketolases have been isolated from S. cerevisiae, TKL1 and TKL2. Transketolase has been purified from C. utilis and shown to have high affinity both for xylulose-5-P ($K_m$ 80 μM) and ribose-5-P ($K_m$ 430 μM) [189]. A gene, KITKLI, encoding transketolase has been isolated from K. lactis. It complements an S. cerevisiae tkl1 tkl2 mutant for its requirement of aromatic amino acids. The amino acid sequence of KITKLI appeared to be more related to the corresponding prokaryotic transketolases than to those of eukaryotes [190]. The corresponding gene, TKTI, from P. stipitis has also been cloned and used to enhance xylose utilization by strains of S. cerevisiae [191] (see below). It should be mentioned that in methylotrophic yeasts, a transketolase reaction catalyzed by a different enzyme (dihydroxyacetone synthase) is responsible for the formation of dihydroxyacetone and glyceraldehyde-3-P from xylulose-5-P and formaldehyde. The gene encoding this enzyme from Hansenula anomala has a high degree of homology with the transketolase of S. cerevisiae which functions in the pentose phosphate pathway [192].

Transaldolase catalyzes the reversible formation of glyceraldehyde-3-P and sedoheptulose-7-P from erythrose-4-P and fructose-6-P. Mutants from K. lactis lacking this enzyme, encoded by the gene KITALI, have been instrumental to highlight an important difference between this yeast and S. cerevisiae concerning the capacity of the pentose phosphate pathway for glucose utilization. It was shown that K. lactis double mutants lacking phosphofructokinase and transaldolase activities could not grow in glucose, while those lacking only phosphofructokinase could [62]. This result indicates that K. lactis can utilize glucose exclusively via the pentose phosphate pathway, in contrast to the situation in S. cerevisiae, and is consistent with the behavior of rag2 mutants (see Section 3).
An enzyme that could be related with the pentose phosphate pathway, but that, up to now, lacks a clear physiological function, is the glucose dehydrogenase from S. pombe. This enzyme produces gluconate in a reaction dependent of NADP+ and could, in principle, allow metabolism of glucose through the hexose monophosphate pathway [48,183]. However, the finding that mutants lacking hexokinase activity do not grow in glucose is not easily accommodated with this proposed function [48].

7.1. The genes of xylose metabolism

Xylose is a pentose that is a quantitatively important constituent of hemicellulose and as such is a sugar with high biotechnological potential. It can be used for the production of xylitol, a non-cariogenic sweetener with low caloric value or for the production of ethanol. However, the yeast with the best fermentation capacity, S. cerevisiae does not metabolize xylose, although it grows on xylulose. Since some NCY grow in xylose, attempts have been made to express in S. cerevisiae the genes required to transform xylose into xylulose. Utilization of xylose implies a reduction to xylitol by an NAD(P)H dependent xylose reductase followed by an oxidation to xylulose by an NAD+-dependent xylitol dehydrogenase. Xylulose is then phosphorylated by xylulose kinase and enters the pentose phosphate pathway (Fig. 1). It has been postulated that fermentation of xylose to ethanol requires the action of an NADH xylose reductase to avoid a serious redox imbalance in the yeast [193].

Xylose reductases utilize NADPH or NADH; it is not completely clear if some yeasts produce different proteins with distinct coenzyme specificity [194]. The gene XYL1 encoding xylose reductase has been cloned from several yeasts. A single gene exists in K. lactis that is expressed constitutively; its disruption leads to inability to grow in xylose [195]. The gene has also been cloned from P. stipitis [196]. Expression of this gene integrated at about 40 copies in the genome of S. cerevisiae, resulted in efficient production of xylitol [197]. The xylose reductase from Candida shehatae and Candida tenuis, have been purified [198,199] and the XYL1 gene from Candida guilliermondii has been cloned [200]. A genomic region with high sequence similarity with the C-terminal region of genes encoding xylose reductases has been found in C. albicans [201].

Xylitol dehydrogenase is encoded in P. stipitis by the gene XYL2 [196]. Although in S. cerevisiae, ORF YLR070c has the characteristics of an NADH-dependent xyitol dehydrogenase, this activity is only measurable when the gene is overexpressed [202]. Expression of the P. stipitis XYL1 and XYL2 genes allows S. cerevisiae to utilize xylose; however, an efficient fermentation with high production of ethanol appears to require overexpression of xylulokinase [196,203,273].

8. Ethanol metabolism

Production of ethanol during growth in sugars by NCY is not as important as it is in S. cerevisiae due to their predominantly aerobic metabolism. Nevertheless, in anaerobiosis, ethanol is produced by those NCY able to thrive in these conditions. On the other hand, ethanol may be used as a carbon and energy source by most yeasts. Central to the production or the utilization of ethanol are alcohol dehydrogenases, enzymes that catalyze the reversible reduction of acetaldehyde to ethanol. The kinetic and regulatory characteristics of these enzymes, the regulation of the transcription of the genes that encode them, or their subcellular localization determine the direction of the reaction catalyzed under physiological conditions. However, in many cases, the precise physiological role of some alcohol dehydrogenases is far from being clearly established.

In S. cerevisiae, four genes encode isoenzymes of alcohol dehydrogenases. ADH1 encodes the isoenzyme prominent during glucose fermentation and is induced by glucose [204], while ADH2 is repressed by glucose and is implicated in the utilization of ethanol. The physiological role of the other isoenzymes is not completely clear [205].

In S. pombe, mutants partially deficient in alcohol dehydrogenase were isolated by Megnet [206] by selection of strains able to grow in glucose in the presence of 1 mM allyl alcohol. This work seems to be the first one that tried to isolate mutants in a step of the glycolytic chain. A gene encoding the S. pombe alcohol dehydrogenase was isolated 16 years later [207].

Four genes encoding alcohol dehydrogenases have been characterized in K. lactis and the proteins encoded studied. KlAdhI and II are NAD+-dependent, cytosolic enzymes. KlAdhIII and IV are mitochondrial and may use NAD+ or NADP+. These enzymes also showed activity with other aliphatic alcohols [208]. The genes KLADH1 and KLADH2 are preferentially expressed in glucose grown cells [209]. On the contrary, the KLADH3 gene is expressed at low glucose concentration and strongly repressed by ethanol, while KLADH4 is induced by it [210].

In C. albicans, a gene, CaADH1, that encodes an alcohol dehydrogenase has been isolated. The corresponding mRNA was less abundant during growth in glucose or ethanol than during growth in other carbon sources [211].

In the xylose-utilizing yeast P. stipitis, two cytoplasmic alcohol dehydrogenases have been identified, encoded by genes called PsADH1 and PsADH2 [212,213]; unfortunately the numbers assigned to the genes by these two groups are not the same. PsADH1 expression (gene name from [212]) increases about 10 times during anaerobic growth and disruption of the gene brings about an increase of PsADH2 expression on non-fermentable carbon sources [212].

In Y. lipolytica an NAD+-dependent alcohol dehydrogenase which acts better on long chain alcohols has been identified. Three other NADP+-dependent dehydrogenases
were also detected [214]. These authors made the interesting observation that Y. lipolytica is extremely tolerant, up to 1 M, to allyl alcohol. This result contrasts with what has been found in other genera like S. pombe [206].

Aldehyde dehydrogenases are important enzymes not only for the metabolism of acetaldehyde produced from ethanol, but also for that of toxic aldehydes produced in some stress situations. In S. cerevisiae the information about the number of these enzymes and the corresponding genes was confused. Navarro-Aviño et al. [215] have proposed a logical nomenclature and tried to clarify the situation. According to these authors, five related ORFs exist in the sequenced S. cerevisiae genome: YMR170c, YMR169c, YOR374w, YER073w and YPL061w. They proposed to name the corresponding genes encoding them ALD2, ALD3, ALD4, ALD5 and ALD6. The proteins Ald2, Ald3 and Ald6, are cytoplasmic while Ald4 and Ald5 are mitochondrial. Ald2 and Ald3 use NAD⁺ as cofactor, are stress-induced and repressed by glucose; Ald4 uses NAD⁰ or NADP⁰ and is also repressed by glucose, while Ald5 uses NADP⁺ and is constitutive. Ald6 uses NADP⁺, is constitutive and activated by Mg²⁺. Ald4 and Ald6 seem important for growth in ethanol [216] and other data point to an important role of some of the aldehyde dehydrogenases in the synthesis of cytoplasmic acetyl CoA [215]. No information on aldehyde dehydrogenases from NCY is available.

The transformation of acetate into acetyl CoA is catalyzed by acetyl CoA synthetase (see Section 4.3). In S. cerevisiae, two genes encoding acetyl CoA synthetases have been isolated. Disruption of one of them, ACS1, caused inability to grow in acetate, but not in ethanol[217], while disruption of the other, ACS2, caused inability to grow in glucose [113,218]. The subcellular localization of the enzyme(s) is not known. In Y. lipolytica, mutants lacking acetyl CoA synthetase, encoded by the gene ICL2, did not grow in acetate. Interestingly, mutants in the gene encoding ICL1 that encodes isocitrate lyase, increased the amount of acetyl CoA synthetase [219]. Acetyl CoA synthetase has also been detected in S. pombe; although this yeast does not grow in acetate, the levels of the enzyme increased in its presence [220].

9. Glycerol metabolism

As already stated, during anaerobiosis, reoxidation of most NADH is carried out in yeasts by alcohol dehydrogenase. However, production of glycerol under anaerobic conditions is a secondary, but important, way to regenerate NAD⁺ (see Section 6). In S. cerevisiae, mutants lacking alcohol dehydrogenase, still produce ethanol, but increase their glycerol production during glucose fermentation [221]. The situation is different in K. lactis, where a mutant lacking alcohol dehydrogenase activity did not accumulate much glycerol [222]. Glycerol production also plays another important role, namely it allows adaptation of the yeast to situations of osmotic stress.

Glycerol formation requires the sequential action of a NADH glycerophosphate dehydrogenase and a glycerophosphatase. In S. pombe – as in S. cerevisiae [223] – two genes have been identified that encode two NADH glycerophosphate dehydrogenases. One of them, gpd1⁺, is transcribed in response to osmotic upshift, while the other one, gpd2⁺, is constitutively transcribed although to a low level [224]. Curiously, deletion of gpd2⁺ caused a requirement for histidine and lysine [225]. In S. cerevisiae, two genes, GPP1 and GPP2, that encode α,β-glycerol-3-P phosphatases with relatively low affinities, Kₘ about 3–4 mM, for their substrates, have been cloned. Transcription of GPP2 is under the control of the HOG pathway and is induced by high osmolarity [226]. No information on these enzymes is available from NCY.

Glycerol may be also utilized as a carbon source under aerobic conditions by many yeasts. Two different pathways of glycerol catabolism have been identified in yeasts. In S. cerevisiae or C. utilis glycerol is phosphorylated by a glycerol kinase and the L-glycerol-3-phosphate formed is oxidized by a mitochondrial glycerol phosphate ubiquinone oxidoreductase [227,228]. In S. pombe dehydrogenation of glycerol by an NAD⁺-dependent glycerol dehydrogenase forms dihydroxyacetone which is then phosphorylated by a dihydroxyacetone kinase [229,230]. Genes encoding these enzymes have also been found in S. cerevisiae; they are expressed in response to high salt concentration in the medium [231].

One interesting point related to glycerol metabolism is that of its transport. In S. cerevisiae, glycerol produced intracellularly leaves the cell through a channel protein encoded by the gene FPS1 [232]. If the medium is hyperosmotic, the activity of the channel is reduced and glycerol remains predominantly in the cell [233]. Uptake of glycerol may be by passive diffusion, although active transport systems have been described in some yeasts acting, mainly, in conditions of osmotic stress [234,235].

10. Gluconeogenesis

NCY are able to grow in different non-carbohydrate carbon sources. In order to synthesize the sugar phosphates necessary for the synthesis of several cell components, many glycolytic steps, or all of them, need to be reversed depending on the non-sugar carbon source used. Most of the glycolytic reactions are reversible under physiological conditions; however, two reactions cannot be reversed due to the unfavorable concentrations of substrates found in the cell. These are the reactions catalyzed by phosphofructokinase and pyruvate kinase. Therefore, to synthesize fructose 6-phosphate and phospho-enol-pyruvate during gluconeogenesis, specific ‘gluconeogenic’ enzymes catalyze reactions that are different from a simple
reversal of those catalyzed by phosphofructokinase or pyruvate kinase.

Fructose-1,6-bisphosphatase catalyzes the hydrolysis of fructose-1,6-bisphosphate to fructose-6-P. This reaction is the terminal step of gluconeogenesis and therefore the corresponding enzyme is required for metabolism of every non-sugar carbon source. The gene encoding this enzyme has been isolated from *S. pombe* [236] and from *K. lactis* [237] and the enzyme has also been purified from *C. utilis* [238]. The protein from *K. lactis* contains a short amino acid insert sequence near the binding site of the allosteric inhibitor AMP absent in the *S. cerevisiae* protein. This amino acid stretch seems to be responsible for the lower sensitivity to AMP inhibition of this enzyme with respect to that of *S. cerevisiae* [237]. The expression of the gene is usually repressed in the presence of glucose, although differences in the degree of repression are observed among species. In the case of *S. pombe*, the pathway of repression implicates a cAMP signalling pathway [239] (see Section 11).

The enzyme responsible for the synthesis of phosphoenol-pyruvate is phosphoenolpyruvate carboxykinase that synthesizes it from oxaloacetate in an ATP-dependent reaction. In this respect, the yeast enzymes identified so far, differ from those of other organisms that require GTP. In addition to the gene *PCK1* isolated from *S. cerevisiae* [240,241] genes encoding this enzyme have been isolated from *K. lactis* [242] and *C. albicans* [243] and present a high sequence homology with the gene from *S. cerevisiae*. In this yeast, the enzyme is localized in the cytosol [244] a difference with the localization in some mammalian species. No data exist about its localization in NCY. Transcription of the *C. albicans* gene is repressed by glucose [243], a situation similar to that found in *S. cerevisiae* [241,245].

Isocitrate lyase and malate synthase that may be also necessary during gluconeogenesis when the organisms grow on 2C or 1C carbon sources are considered in Section 5.1.

An enzyme that may be also considered as gluconeogenic, is the malic enzyme that catalyzes the oxidative decarboxylation of malate to pyruvate using NAD(P)⁺. This enzyme is important during growth in malate and has been identified in *S. pombe* [246] and *Zygosaccharomyces bailii* [247,248]. In *Z. bailii* the enzyme seems constitutive [248], while in *S. pombe*, transcription of the gene *mae2*⁺, encoding malic enzyme is induced when cells are grown in high concentrations of glucose or under anaerobic conditions [249]. It has been suggested that this enzyme may play a role in the provision of pyruvate or in maintaining the redox potential under certain conditions [249,250].

It should be remembered that growth on certain gluconeogenic substrates, for example organic acids, needs the operation of specific transporters to bring these compounds into the cell. Also, a series of transporters that interconnect metabolically the mitochondria and the cytosol need to be operative during gluconeogenesis. We have not dealt with those proteins in this review, but they are important components for the operation of the respective pathways.

11. Catabolite repression

In *S. cerevisiae* metabolism of easily used sugars causes repression of the transcription of genes that encode enzymes necessary for the use of alternative carbon sources; this process is known as catabolite repression. Although we will not go into a deep treatment of catabolite repression, some important findings will be considered. A great amount of work on the mechanism of catabolite repression has been done in *S. cerevisiae* and a plethora of genes implicated in it has been identified (for a review see [251]). Much less work exists on catabolite repression in NCY.

In different yeasts, glucose produce an increase in the intracellular concentration of cAMP [143], but in *S. cerevisiae* cAMP only affects the repression of some genes and even for those, redundant regulatory mechanisms exist [252]. In contrast, in *S. pombe*, catabolite repression of the *fbp1*⁺ gene, encoding fructose-1,6-bisphosphatase, is dependent on a cAMP signalling pathway. Several genes named *git* (glucose insensitive transcription) participate in the repression process. One of them, *git2*⁺/*cyr1*⁺, encodes an adenylate cyclase. Loss of function of *git2*⁺, causes constitutive *fbp1*⁺ transcription, and addition of cAMP to the growth medium restores the repression [239]. Interestingly, the cAMP signalling pathway does not seem important for catabolite repression of the *invl*⁺ gene encoding invertase [38].

Hexokinase II is implicated in *S. cerevisiae* in catabolite repression of several genes, among then *SUC2* that encodes invertase [253]. Mutations in *RAG5*, the *K. lactis* gene encoding hexokinase, did not affect repression of the gene encoding invertase [22], but decreased transcription of *RAG1* which encodes the low affinity glucose transporter [19]. In *C. utilis*, high hexokinase activity is not related to glucose repression; in fact, hexokinase activity seemed inversely proportional to glucose repression, since cells growing in a chemostat at low concentration of glucose had a higher level of hexokinase than in repressed cells [254].

A gene that plays a central role in catabolite repression in *S. cerevisiae* is *SNF1* that encodes a protein kinase that participates in a complex necessary for growth in non-fermentable carbon sources [251]. Mutants unable to grow in galactose, melibiose, maltose, raffinose, glycerol, ethanol or lactate have been isolated in *K. lactis* and named *fog* (fermentative and oxidative growth negative) [255]. These mutants were unable to derepress several glucose repressible enzymes. Identification of the *FOG1* and *FOG2* genes revealed that they were related by their mechanism of action. *FOG2* is homologous to *S. cerevisiae*
SNF1 and can complement the corresponding snf1 mutant. FOG1 encodes a protein with sequence similarity to Gal83, a protein implicated in S. cerevisiae, in the interaction of Snf1 and the regulatory protein Snf4 [251]. However, no evidence for the existence of an equivalent of Snf4 in K. lactis is available. While S. cerevisiae gal83 mutants did not show a noticeable phenotype, K. lactis foga1 mutants are unable to grow in a series of substrates different from glucose [255]. This could be explained by the fact that in S. cerevisiae the Gal83 functions can also be performed by the proteins Sip1 or Sip2 that are absent in K. lactis [251].

In contrast with the situation in other yeasts, SNF1 is an essential gene in C. albicans or C. tropicalis, but not in Candida glabrata [256–258].

Another central gene in catabolite repression in S. cerevisiae is MIG1 which encodes a C2H2 zinc finger repressor [259]. Mig1 exerts its repressive effect by recruiting to the transcription start in the DNA a complex in which the protein Tup1 is the actual repressor [260]. The Mig1 homolog from K. lactis plays a role in the repression of lactose metabolism [261], but does not participate in the repression of invertase [36]. In C. albicans, no effects of CaMIG1 on repression of CaMAL2 that encodes an α-glucosidase or CaGAL1 that encodes galactokinase, have been found [201]. On the contrary, in S. pombe, an intact src1+ gene, a homolog of MIG1, is required for repression of inv1+ [38]. Genes encoding proteins similar to Tup1 have been cloned in K. lactis and in S. pombe [262]. The gene found in K. lactis complemented the tup1 phenotype in S. cerevisiae [262]. Two genes were found in S. pombe, tup1I+ and tup12+; disruption of both caused a defect in glucose repression of fbp1+ [262]. In C. albicans, TUP1 has not been related with catabolite repression, but its disruption gives rise to a filamentous morphology in all growth conditions tested [263].

12. Plasma membrane H⁺-ATPase

Although not directly related to the energetic metabolism, we think it is necessary to mention plasma membrane H⁺-ATPase in this review. The activity of this proton pump is critical for different processes, among them transport of several nutrients and maintenance of intracellular pH. The enzyme from S. cerevisiae, encoded by the PMA1 gene, is an essential protein and has been studied in great detail as it is subject to several regulatory mechanisms at different levels [264].

In S. pombe two genes, pma1+ and pma2+, that encode plasma membrane H⁺-ATPases have been isolated. The most abundant of these proteins with a molecular mass of 105 kDa is that encoded by pma1+ [265,266]. The pma2+ gene is weakly expressed and is not essential for growth; it encodes a protein about the same size as Pma1 (molecular mass 119,216 Da). When the coding region of pma2+ is expressed from a strong promoter, it is able to complement the lethal phenotype of an S. pombe pma1+ mutant [266]. In S. pombe, as it happens in S. cerevisiae, the activity of the plasma membrane H⁺-ATPase is increased by glucose [267]. This activation is likely due to a phosphorylation and could implicate the MAP kinase encoded by the spn1+/pmk1+ and the protein phosphatase encoded by the pzh1+ gene. This is suggested by the fact that an spn1−/pzh1− mutant exhibits a lower level of H⁺-ATPase activity that cannot be activated by glucose [268].

In K. lactis a gene (KIPMA1) encoding a plasma membrane H⁺-ATPase has been isolated [269]. The protein is 899 amino acids long and is structurally related to other H⁺-ATPases except for the presence of a non-homolog N-terminal domain. A change from G to A at position 699 resulted in the substitution of a highly conserved methionine by isoleucine and yielded a protein with a low capacity to pump protons [269].

The plasma membrane H⁺-ATPase has been also purified from C. albicans [270]. In contrast with what happens in S. cerevisiae or in S. pombe, the enzyme from C. albicans responds weakly to glucose and its activity appears to change mainly during morphogenetic changes [271].

13. Concluding remarks

This review was written as an attempt to systematize the existing knowledge on some central metabolic pathways in NCY. Although a certain amount of information is available on carbon and energy-yielding metabolism in NCY, there are abundant gaps of knowledge in this area. It is clear that different yeasts species have been selected through evolution to occupy different ecological niches. Therefore, it is not surprising to find among NCY a diversity in the nature of the components or the regulation of the pathways examined. The pioneering ideas of Albert Kluvyer about unity and diversity in the metabolism of microorganisms presented in his classical lecture ‘Eenheid en verscheidenheid in de stofwisseling der microben’ [272] are nicely illustrated by these organisms: a similar basic design for the metabolic pathways with differences in sugar transport systems, equipment of sugar phosphorylating enzymes, intracellular localization of certain proteins, or in the regulation of the pathways.

The differences between NCY and the classical ‘yeast’ S. cerevisiae do not allow an immediate translation of the available knowledge from this organism to NCY. Certainly, the wealth of information available from S. cerevisiae will be a good starting point when considering NCY; however, it shall be kept in mind that these organisms may present surprising and interesting variations.

The completion of the sequence of the S. cerevisiae genome and the availability of disruptions, or easy methods to perform them, in practically every gene has opened enormous possibilities to identify and isolate genes of in-
terest from NCY, thus opening the doors to the performance of reverse genetics in these organisms. Such a study of the components of central pathways in NCY will be fruitful and rewarding both from a pure and an applied point of view. Regulatory problems in carbon and energy metabolism have not been much studied in NCY, although data in the literature indicate important differences in many cases, both among them and with respect to *S. cerevisiae*. Precise knowledge of regulatory mechanisms is of extreme interest when trying to modify organisms for specific uses, or when using some genes encoding regulated proteins to be expressed in other yeasts. This knowledge is also important when trying to model the behavior of NCY systems for industrial settings.

A topic that needs deeper study is that of the relations between the different organelles – mitochondria, peroxisomes, nucleus – and the cytosol. Characterization of transport systems implicated in the traffic of a series of molecules is of crucial importance to understand the metabolic implications of this traffic. Some NCY in which particular organelles are more prominent than in *S. cerevisiae* will be the objects of choice for such studies.

Another area of interest in some NCY yeasts is the relationship between energy metabolism and differentiation processes. In fact this topic is being pursued vigorously at this moment, particularly in those cases where the differentiation process is related with pathogenicity.

Readers, particularly non-specialists, will benefit from a uniform nomenclature of genes among different NCY. Genes encoding proteins with the same function are often called differently by scientists working with different yeast species; hexose transporters are HXT in *S. cerevisiae*, but ght* or std* in *S. pombe*, hexokinases are IIXK in *S. cerevisiae*, but RAG in *K. lactis*; ACS is acetyl CoA synthetase in *S. cerevisiae*, but ICL2 in *Y. lipolytica*; trehalose 6-P synthase is TPS1 in most yeasts, but GGS1 in *K. lactis*; FOG1 and FOG2 in *K. lactis* are GAL83 and SNFI, respectively, in *S. cerevisiae*, to name but a few examples. Perhaps it is time to reach a consensus on how to avoid this chaos.

It should be kept in mind, that some problems presented by NCY will not be solved without a detailed, general appreciation of the organism. This will require the combined knowledge of modern molecular biology tools and that of the more classical physiology.

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