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MINI-REVIEW

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Anaerobic sugar catabolism in *Lactococcus lactis*: genetic regulation and enzyme control over pathway flux

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Abstract Lactic acid bacteria and particularly *Lactococcus lactis* are widely used for the production of lactic acid in fermented foods. Control of the catabolic rate in *L. lactis*, i.e., the rate of lactic acid production, appears to be determinant for dairy product quality. While the mechanisms involved in control have not been totally elucidated, they seem to depend upon the strain and the growth conditions. Furthermore, it remains unclear whether the catabolic rate is controlled at the level of transcription, translation or enzyme activity. The recent sequencing of the *L. lactis* genome has brought novel insights to physiologic studies of the bacteria. This review discusses both genetic information and metabolic studies concerning anaerobic sugar catabolism in *L. lactis*.

Introduction

Lactococcus lactis is a homofermentative lactic acid bacterium that is widely used in the dairy industry as a starter in milk fermentation, notably in cheese manufacture. There are two subspecies of *L. lactis*, designated initially as *Streptococcus lactis* and *Steptococcus cremoris* and reclassified more recently as *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* (Schleifer et al. 1985). While *L. lactis* subsp. *lactis* is used for soft-cheese manufacture, *L. lactis* subsp. *cremoris* is preferred for hard cheeses. However it is believed that this difference in cheese type is associated with differences in the rates of catabolism rather than other aspects of each strain. Control of the catabolic rate in *L. lactis* appears to be essential for dairy product quality and therefore constitutes one of the most important challenges in cheese manufacture. The formulation of adequate starter cultures adapted to the desired properties of the final product would facilitate reliable organoleptic quality at an industrial scale. To achieve this objective, a better understanding of the central metabolic pathways and their regulations is necessary. Here, current knowledge regarding anaerobic metabolism in *L. lactis* is presented.

Lactose, which is the main milk sugar, is converted into lactic acid via the catabolism of its two hexose monomers. In light of this, the metabolic pathways involved in glucose, galactose and lactose catabolism are described in this review; the metabolism of other, marginal sugars, such as the pentoses, though interesting, will not be discussed. Likewise, emphasis has been placed upon the central catabolic pathway from the sugar to the major products derived from pyruvate (lactate and mixed acid fermentation products). The metabolism of aromatic compounds such as diacetyl, also produced from pyruvate but in low quantity, is well documented elsewhere (Platteeuw et al. 1995; Boumerdassi et al. 1997; Lopez de Felipe et al. 1997; 1998; Curic et al. 1999; Hugenholtz et al. 2000; Monnet et al. 2000; Henriksen and Nilsson 2001) and will therefore not be considered. Furthermore, exopolysaccharides production in *L. lactis* (Marshall et al. 1995; van Kramenburg et al. 1997), not exceeding a few hundred milligrams per liter even with good producing strains, is not addressed in this review since it does not significantly affect carbon flux through the central metabolism.

Control over acidification is complex, as evidenced by the wealth of genetic information, both from specific studies of individual genes and information obtained from the complete sequencing of the genome of *L. lactis* subsp. *lactis* IL1403 (Bolotin et al. 2001), as well as data concerning the biochemical mechanisms exerting control over key enzymes. Realistic targets for metabolic engineering will be correctly identified only if the biochemical and genetic information is integrated into an overall metabolic model.

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Central metabolic pathways

Most sugars are taken up by *L. lactis* either by the phosphoenolpyruvate (PEP)-dependent phosphotransferase systems (PTS) involving coupled transport and phosphorylation of the sugar, or via permease systems, in which sugar transport is followed by kinase-mediated phosphorylation of the free sugar within the cytosol (Yamada 1987). In *L. lactis*, the contribution of each transport mechanism to overall sugar uptake remains unknown. A number of genes corresponding to the various transport systems are predominantly located on the chromosome (Bolotin et al. 2001), although many of the genes coding for putative permeases have not been entirely identified in that the nature of the translocated sugar remains to be determined. Genes corresponding to specific lactose-PTS components (*lacEF*) are found on a plasmid-located operon together with the phospho-β-galactosidase (*lacG*) gene, responsible for cleavage of the phosphorylated sugar. While this operon has been extensively studied (de Vos and Simons 1988; de Vos and Gasson 1989; van Rooijen and de Vos 1990; de Vos et al. 1990), few studies have focused on genetic characterization of other transporters. Recently, genes coding for common PTS enzymes, i.e., phosphorylating enzyme (HPr) and enzyme I, were demonstrated to be organized in a chromosomal operon, *ptsHI* (Luesink et al. 1999), but the contribution of this transport system to overall sugar uptake has not been quantitatively examined.

Once phosphorylated, sugars are catabolized by relatively simple linear pathways (Fig. 1) whose composition depends upon the nature of the sugar. Many monosaccharides enter central metabolism via glucose-6-phosphate, which follows the glycolytic pathway until pyruvate. Some of the glycolytic genes have been sequenced in the last decade, including *tpi, gap* (Cancilla et al. 1995a, b) and the *las* operon consisting of the *pfk*, *pyk* and *ldh* genes (Llanos et al. 1992; 1993), while others have been identified from the complete sequencing of the *L. lactis* genome (Table 1). Glycolytic genes are generally located near the replication origin (Bolotin et al. 2001), as would be expected for highly expressed genes. Unlike in other bacteria, the *gap* and *tpi* genes are not located in an unique operon together with *pgk* but are monocistronic (Cancilla et al. 1995a, 1995b). More than one gene coding for similar glycolytic proteins has been

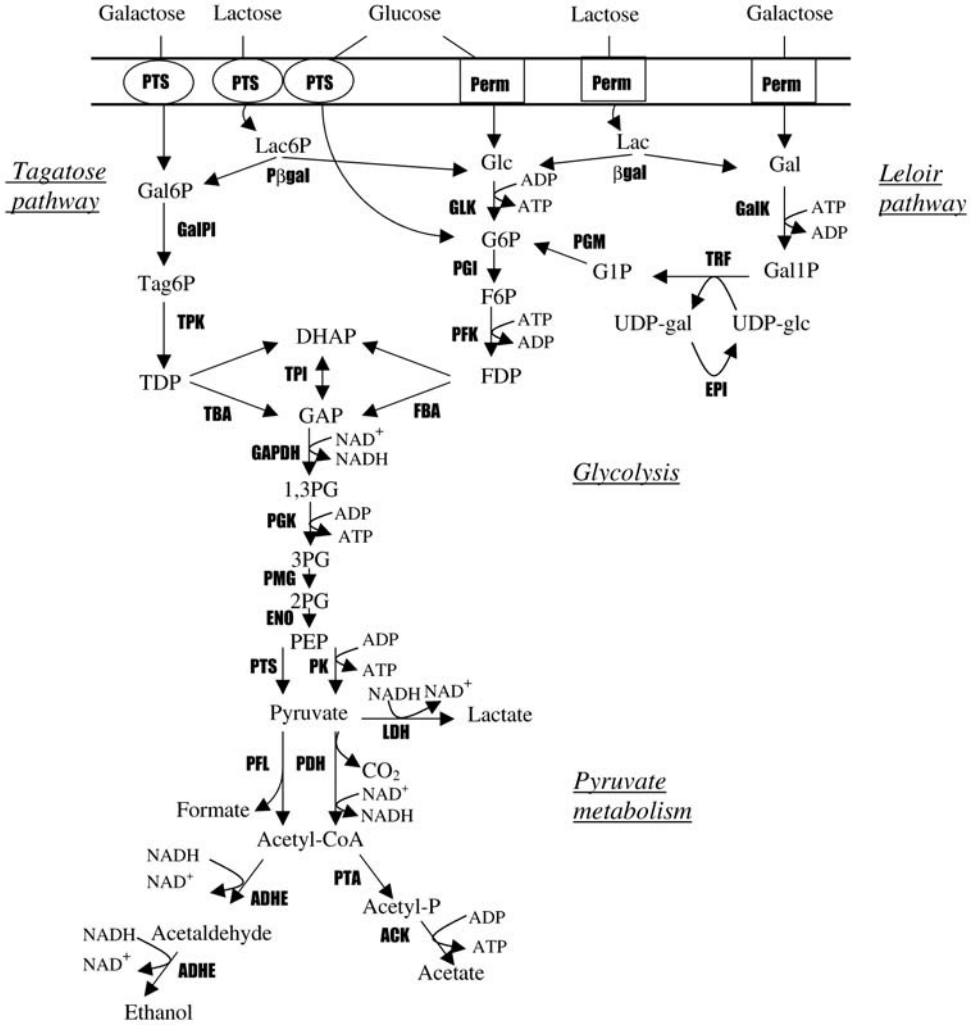

Fig. 1 Lactose, galactose and glucose metabolism in *Lactococcus lactis*: enzymes involved in central metabolic pathways. *Glc* Glucose, *G6P* glucose-6-phosphate, *G1P* glucose-1-phosphate, *F6P* fructose-6-phosphate, *FDP* fructose-1,6-diphosphate, *DHAP* dihydroxyacetone-phosphate, *GAP* glyceraldehyde-3-phosphate, *1,3PG* 1,3-diphosphoglycerate, *3PG* 3-phosphoglycerate, *2PG* 2-phosphoglycerate, *PEP* phosphoenolpyruvate, *Tag6P* tagatose-6-phosphate, *TDP* tagatose-1,6-diphosphate, *Gal* galactose, *Gal6P* galactose-6-phosphate, *Gal1P* galactose-1-phosphate, *GLK* glucokinase, *PGI* glucose-phosphate isomerase, *PFK* phosphofructokinase, *FBA* fructose-bisphosphate aldolase, *TPI* triose-phosphate isomerase, *GAPDH* glyceraldehyde-phosphate dehydrogenase, *PGK* phosphoglycerate kinase, *PMG* phosphoglycerate mutase, *ENO* enolase, *PK* pyruvate kinase. *GalPI* galactose-phosphate isomerase, *TPK* tagatose-phosphate kinase, *TBA* tagatose-bisphosphate aldolase, *GalK* galactokinase, *TRF* galactose/uridylyl transferase, *EPI* UDP-glucose epimerase, *PGM* phosphoglucomutase, *LDH* lactate dehydrogenase, *PFL* pyruvate formate lyase, PDH pyruvate dehydrogenase, *PTA* phosphotransacetylase, *ACK* acetate kinase, ADHE alcohol dehydrogenase

Table 1 Lactose, galactose and glucose metabolism in *Lactococcus lactis*: genes involved in the central metabolic pathways

Gene	Function	Reference
Glycolysis		
glk	Glucokinase	Bolotin et al. (2001)
pgiA	Phosphoglucose isomerase	Bolotin et al. (2001)
las:pfk-pyk-ldh	*Las* operon: phosphofructokinase, pyruvate kinase, lactate dehydrogenase	Llanos et al. (1992, 1993)
fbaA	Fructose-1,6-bisphosphate aldolase	Bolotin et al. (2001)
tpi	Triose phosphate isomerase	Cancilla et al. (1995a)
gap1, gap2	Glyceraldehyde-phosphate dehydrogenase	Cancilla et al. (1995b); Jamet et al. (2001a)
pgk	Phosphoglycerate kinase	Bolotin et al. (2001)
pmg	Phophoglycerate mutase	Bolotin et al. (2001)
enoA, enoB	Enolase	Bolotin et al. (2001)
Tagatose pathway		
lacABCDFEGX	Lactose operon: galactose-6-phosphate isomerase, tagatose-6-phosphate kinase, tagatose-1,6-bisphosphate aldolase, lactose PTS, P-β-galactosidase	de Vos et al. (1990); Van Rooijen et al. (1991)
Leloir pathway		
galAMKTE	Galactose operon: permease, aldose-1-epimerase, galactokinase, galactose-1-phosphate uridylyltransferase, UDP-glucose-4-epimerase	Grossiord et al. (1998)
pgmB	*β*-Phosphoglucomutase	Qian et al. (1997)
Pyruvate metabolism		
pfl	Pyruvate-formate lyase	Arnau et al. (1997)
pdhABCD	Pyruvate dehydrogenase	Bolotin et al. (2001)
pta	Phosphotransacetylase	Bolotin et al. (2001)
ackA1, ackA2	Acetate kinase	Bolotin et al. (2001)
adhE, adhA	Alcohol dehydrogenase	Arnau et al. (1998); Bolotin et al. (2001)

identified on the chromosome, as illustrated by the two *eno* genes (Bolotin et al. 2001). Both *enoA* and *enoB* are expressed simultaneously but codon-bias analysis suggested that the protein corresponding to *enoA* gene is the major glycolytic enzyme (Jamet et al. 2001). Similarly, two *gap* genes have been identified in *L. lactis* strain IL 1403 chromosome (Jamet et al. 2001), namely, *gap1* and *gap2*, the latter corresponding to the *gap* gene previously sequenced by Cancilla et al. (1995b). These duplicated genes show more than 80% homology with each other and code for two different glyceraldehyde-3-phosphate dehydrogenases offering quite similar kinetic properties (Jamet 2001). However, the interruption of *gap1,* but not of *gap2,* is lethal, suggesting that *gap1* encodes an essential glycolytic function (Jamet 2001). The role of *gap2* is not yet known.

The galactose-6-phosphate moiety of the lactose transported via the PTS-lactose enters glycolysis at the level of triose phosphate after transformation by the tagatose pathway, through galactose-6-phosphate isomerase, tagatose-6-phosphate kinase and tagatose-1,6-bisphosphate aldolase. Four genes (*lacABCD*) code for the various enzymes of the tagatose pathway; they are located on the plasmidic *lac* operon, together with genes corresponding to lactose-PTS-specific components (*lacFE*), phospho-β-galactosidase (*lacG*) and a protein with unknown function (*lacX*) (de Vos et al. 1990; van Rooijen et al. 1991). When galactose transport is mediated by galactose-PTS, the resulting galactose-6-phosphate is also metabolized by the tagatose pathway.

Galactose entering the cell via permease or liberated in the cytoplasm via hydrolysis of lactose by β-galactosidase reaches glycolysis at the level of glucose-6-phosphate following processing by the Leloir pathway, which includes galactose kinase, galactose-1-phosphate uridylytransferase, UDP-glucose 4-epimerase and phosphoglucomutase. The first three activities are encoded by *galK*, *galT* and *galE*, respectively, and constitute the *galAMKTE* operon in *L. lactis* subsp. *cremoris* MG1363, together with *galA* and *galM*, coding for galactose permease and aldose-1-epimerase (Grossiord et al. 1998). In the slow lactose-consuming *L. lactis* subsp *lactis* NCDO 2054, lactose genes coding for thiogalactosidase and β-galactosidase, *lacA* and *lacZ* respectively, are located between the *galT* and *galE* genes of the *gal-lac* operon (Vaughan et al. 1998). In *L. lactis* subsp. *lactis* IL1403, *galM, galK, galT, galE* and *LacZ* are located near the origin of replication. Regarding phosphoglucomutase activity, the *pgmB* gene was identified in *L. lactis* (Qian et al. 1997; Bolotin et al. 2001), but this gene codes for a β-enzyme that is specific for maltose metabolism during which the β-form of glucose-1-phosphate is liberated; thus, *pgmB* is probably not involved in galactose metabolism.

L. lactis is a homofermentative lactic acid bacteria in which pyruvate is converted into lactate via lactate dehy-

drogenase with subsequent regeneration of NAD^+. This enzyme is encoded by the *ldh* gene, located within the *las* operon (Llanos et al. 1992). Three other genes highly similar to *ldh* (*ldhB*, *ldhX* and *hicD*) are present in the genome of *L. lactis* but their physiological role is not yet known, although *hicD* probably corresponds to an isocaproic acid dehydrogenase (Bolotin et al. 2001). Under certain conditions, i.e., growth on galactose, *L. lactis* deviates significantly from homolactic metabolism to mixed acid production (Cocaign et al. 1996). Pyruvate is metabolized into acetyl-CoA by either pyruvate formate lyase or pyruvate dehydrogenase to yield formate or CO_2, respectively. Acetyl-CoA is then transformed either to acetate, via phosphotransacetylase and acetate kinase, or ethanol, via alcohol dehydrogenase. The various enzymes are encoded by the chromosome-located genes *pfl* and *pdhABCD* – corresponding to the subunits of pyruvate dehydrogenase- and *pta* (Table 1). Two genes putatively encoding acetate kinase *(ackA1* and *ackA2*) and alcohol dehydrogenase (*adhE* and *adhA*) have been identified but the extent to which each gene is expressed remains unclear.

Gene regulation

There is little information concerning genetic control of the central metabolic pathways. In *L. lactis,* as in *E. coli,* the *lac* operon is repressed by LacR (van Rooijen and de Vos 1990). The structure of LacR suggests a possible interaction with galactose-6-phosphate or tagatose-6-phosphate, thereby avoiding linkage with the *lac* promoter and permitting transcription of the operon. Studies on *adhE* and *pfl* promoter sequences have shown the presence of FNR boxes (Arnau et al. 1997, 1998) which are probably implicated in the regulation of gene expression under anaerobic conditions.

In gram-positive bacteria and particularly in *L. lactis,* carbon catabolite repression, which is observed when sugars such as glucose are rapidly metabolized, is mediated by a regulatory catabolite control protein, CcpA. This regulator binds to *cre* sites located upstream, inside or downstream of the promoter of genes, and transcription is consequently enhanced, diminished or aborted (Ye et al. 1994; Henkin 1996) (Fig. 2). The binding of CcpA is enhanced by interaction with the PTS subunit HPr when the latter is phosphorylated at Ser-46 in the presence of fructose-1,6-diphosphate (Saier et al. 1996). Phosphorylation of HPr at Ser-46 is mediated by a kinase/phosphatase system which has been recently demonstrated to be a single reversible enzyme in *L. lactis* (Monedero et al. 2001). The kinase reaction is mainly activated by fructose-1,6-diphosphate and inhibited by Pi, while the reverse direction is inversely controlled (Monedero et al. 2001). In *L. lactis*, the *gal* operon encoding the Leloir pathway enzymes is repressed by CcpA, while the glycolytic *las* operon and *gap1* were found to be activated by CcpA (Luesink et al. 1998; Jamet 2001). The extent to which CcpA has an effect on the global regulation of central metabolic pathways remains to be established in *L. lactis.* Simple interruption of *ccpA* cannot be used to determine whether CcpA exerts direct or indirect control over central metabolism, since indirect and pleiotropic effects have recently been observed in a *Bacillus subtilis ccpA* mutant (Tobisch et al. 1999). However, the fact that control of central metabolic pathways is co-ordinated in *L. lactis* seems to be clear. Expression of glycolytic genes and *ldh* was generally higher in response to glucose than to galactose, while genes implicated in mixed acid metabolism were expressed at a higher level in the presence of galactose (Even et al. 2001). In this recent study using novel, direct labeling of mRNA (Fontaine et al. 2001), transcript quantification was corrected by the cellular RNA con-

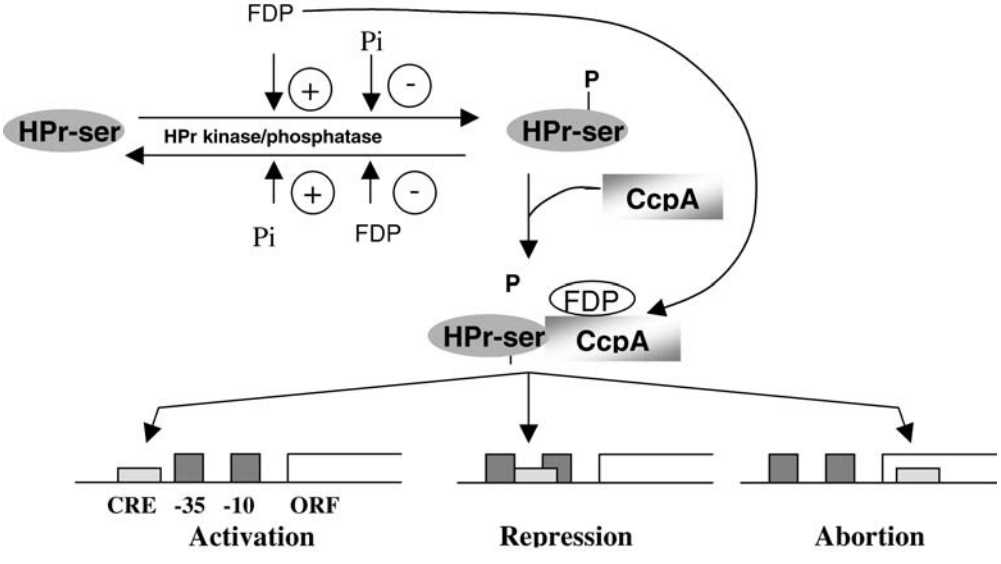

Fig. 2 Gene expression control mediated by the regulatory catabolite control protein CcpA

centration to achieve the cellular concentration rather than the simple abundance of a specific mRNA in the total RNA population. This approach was first introduced for mRNA quantification in *E. coli* by Nilsson et al. (1984); unfortunately, the method was not applied to LAB gene expression studies, in which only transcript abundance was determined since total RNA quantity was systematically standardized in protocols. Determination of the cellular concentration of the transcript rather than its abundance takes into account the fact that total RNA concentration in the cells is not constant and is dependent upon growth conditions. To date, this approach has been exploited only in expression studies on *L. lactis* for genes involved in central metabolic pathways (Even et al. 2001) or heat shock response (Hansen et al. 2001), but will no doubt be used in the near future when global transcriptome analysis is carried out.

Enzymes controlling flux through glycolysis

Various enzymes have been found to play a significant role in the control of catabolic flux in *L. lactis,* depending on the strain and nutritional conditions. Chemostat culture or carbon starvation resulted in high levels of PEP and thus allowed the identification of pyruvate kinase as a potential flux-regulating enzyme in *L. lactis* subsp. *lactis* ATCC 7962 (Thompson and Thomas 1977; Mason et al. 1981; Poolman et al. 1987b). The large pools of PEP were attributed to the inactivation of pyruvate kinase activity due to the diminished concentration of its activator (FDP) and the increased concentration of its inhibitor (Pi). Allosteric control of pyruvate kinase was extensively studied in the 1970s (Collins and Thomas 1974; Crow and Pritchard 1976), but the physiological role of the enzyme has not been further investigated using metabolic control analysis.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) exerts a major controlling influence in *L. lactis* subsp. *lactis* strain NCDO 2118 during homolactic metabolism, as evidenced in vivo by the high GAP and DHAP levels (Garrigues et al. 1997). Under such conditions, the high $NADH/NAD^+$ ratio provokes a strong inhibition of GAPDH activity, following an NADH competitive inhibition mechanism (Even et al. 1999). Modification of in vivo GAPDH activity has confirmed the major influence of this enzyme, for which an extremely high flux control coefficient has been obtained (Poolman et al. 1987a; Even et al. 1999). It should be noted that high PEP pools were also measured during homolactic metabolism (Garrigues et al. 1997), which may indicate that control of the glycolytic flux is shared between GAPDH and pyruvate kinase.

More recently, it was found that a twofold reduction of phosphofructokinase activity in *L. lactis* subsp. *cremoris* MG1363 resulted in decreased glycolytic flux during glucose metabolism, suggesting a possible role for phosphofructokinase in the control of catabolic rate (Andersen et al. 2001). However the same authors demonstrated that increasing the in vivo activity of the enzyme eight-fold did not provoke any increase in the glycolytic capacity of the strain, hence questioning the effective rate-limiting status of this enzyme. Thus, as is often found with enzymes of glycolysis, protein conservation strategies seem to have led to enzyme concentrations that are adequate but not in excess of the maximum glycolytic flux. It is interesting to note that the same strain provided with a lactose plasmid (*L. lactis* subsp. *cremoris* MG1820) showed GAPDH limitation when cultivated on lactose (Garrigues et al. 2001b). Since sugar consumption rates were similar to those observed during glucose metabolism, it is probable that the GAPDH level also exerts considerable influence on glycolytic flux in strain MG1363.

When the glycolytic flux is low, control is more probably exerted on the upper part of sugar catabolism pathways and presumably at the level of sugar transport rather than of glycolytic enzymes. In *L. lactis* subsp. *lactis* strain NCDO 2118, inefficient sugar uptake during lactose metabolism was postulated as being due to transport limitation, since the strain lacks the lactose transporter lactose-PTS (Garrigues et al. 1997). The concentrations of glycolytic intermediates in this strain are thus considerably lower than those of rapidly catabolised sugars. In the recently sequenced *L. lactis* subsp. *lactis* IL1403 strain, the same sugar transport limitation was postulated for glucose, despite high glycolytic flux, since low intracellular concentrations of glycolytic intermediates and high in vivo glycolytic enzyme capacities were found (Even et al. 2001). No distinction could be made regarding central metabolite control between *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* since two *L. lactis* subsp. *lactis* strains NCDO 2118 and IL 1403 showed different glycolytic control points, while similarities were found between *L. lactis lactis* and *L. lactis cremoris* subspecies. In *L. lactis* subsp. *lactis* strain IL1403, both permease and PTS are believed to be operative, since the genes encoding glucose-/mannose-PTS, *ptn*ABCD, have been identified on the chromosome (Bolotin et al. 2001). However, the relative in vivo activities of the two transport systems have never been determined. Furthermore, allosteric control of PTS or permease, other than the negative control exerted by HPr(Ser)P that is commonly observed in all gram-positive bacteria (Ye et al. 1994; Ye and Saier 1995, 1996), has not been examined. Thus, the rate-limiting effect of transport capacity for certain sugars can often be identified but the extent to which this is due to genetic mechanisms influencing transporter concentration, rather than high feedback control, will require additional research efforts.

Metabolic control of pyruvate metabolism

The shift from homolactic to mixed acid metabolism was first observed in carbon-limited chemostat cultures of *L. lactis* subsp. *lactis* and *cremoris*, but also under carbon-excess conditions with certain slowly metabolized sugars

such as galactose (Thomas et al. 1979, 1980). The shift under carbon-excess-conditions is actually associated with an imbalance between anabolism and catabolism, since the decreased anabolic rate due to simplification of the medium composition provokes a deviation towards homolactic metabolism during growth on galactose (Garrigues et al. 2001b). Mixed acid fermentation and the extent to which pyruvate is shifted away from lactate synthesis results from the competition that occurs between lactate dehydrogenase and pyruvate formate lyase or pyruvate dehydrogenase. Under anaerobic conditions, when pyruvate formate lyase is active, reducing equivalent equilibrium can be maintained by equimolar partitioning between acetate and ethanol with a net increase in ATP gain compared to homolactic metabolism. Under such anaerobic conditions, only ethanol would be produced (without ATP gain) if pyruvate dehydrogenase was operative, due to additional NADH formation. The stoichiometry of mixed acid metabolism in anaerobically grown *L. lactis* clearly indicates that pyruvate formate lyase is the only alternative pathway of pyruvate metabolism (Garrigues et al. 1997). Pyruvate dehydrogenase activity, which is known to be active under aerobic conditions, is generally absent during anaerobic growth (Garrigues et al. 1997). Furthermore, pyruvate dehydrogenase activity is strongly inhibited by the high NADH/NAD^+ ratio characteristic of anaerobiosis (Snoep et al. 1993). By contrast, under aerobic conditions, no formate is produced and pyruvate formate lyase is totally inactivated due to its extreme sensitivity to oxygen (Melchiorsen et al. 2000). However, no oxygenolytic cleavage of pyruvate formate lyase was detected in complex medium, since inactive enzyme without free radical is present under these conditions, ensuring total protection against oxygen (Melchiorsen et al. 2000).

For many years, the shift from homolactic metabolism towards mixed acid fermentation was believed to be controlled by a decrease in the fructose-1,6-diphosphate pool, which is an essential in vitro activator of lactate dehydrogenase (Thomas et al. 1979). More recently it was found that in vivo the fructose-1,6-diphosphate pool is always sufficient to ensure full activation of lactate dehydrogenase, even when there is no flux through lactate dehydrogenase, which is more probably controlled in vivo by the NADH/NAD^+ ratio (Garrigues et al. 1997). However, when lactate dehydrogenase activity was modeled in vivo, an apparent overcapacity was found in most cases investigated, which is consistent with the assumption that the pyruvate pool was too low to saturate the enzyme (Garrigues et al. 1997). Indeed, in genetically engineered strains in which in vivo lactate dehydrogenase activity was diminished to less than 30% of that measured in the wild-type strain, no modification of the rate of lactic acid production was observed but the intracellular pyruvate concentration was postulated to increase (Garrigues et al. 2001a). Under anaerobic conditions, homolactic metabolism occurs with fully activated lactate dehydrogenase (high NADH/NAD^+) but also requires that pyruvate formate lyase is inactive. This control is exerted in vivo by the pools of glyceraldehyde-3-phosphate and dihydroxyacetone-phosphate. These metabolites have been shown to increase in cells when the flux capacity of glyceraldehyde-3-phosphate dehydrogenase activity becomes rate-limiting, thereby provoking the subsequent inhibition of pyruvate formate lyase activity (Garrigues et al. 1997). When the control exerted by GAPDH is relaxed, the pools of these triose-phosphates diminish and control at the level of pyruvate formate lyase is released. Under such conditions pyruvate metabolism is re-directed towards mixed acid fermentation. However such a model is not suitable for *L. lactis* subsp. *lactis* strain IL1403 for which the link between GAPDH limitation and homolactic metabolism could not be established. Indeed, during the metabolism of glucose or other sugars, such as galactose, homolactic behavior was maintained despite the fact that triose-phosphates remained at low intracellular concentrations, indicating that in this strain GAPDH is not the major flux-control-

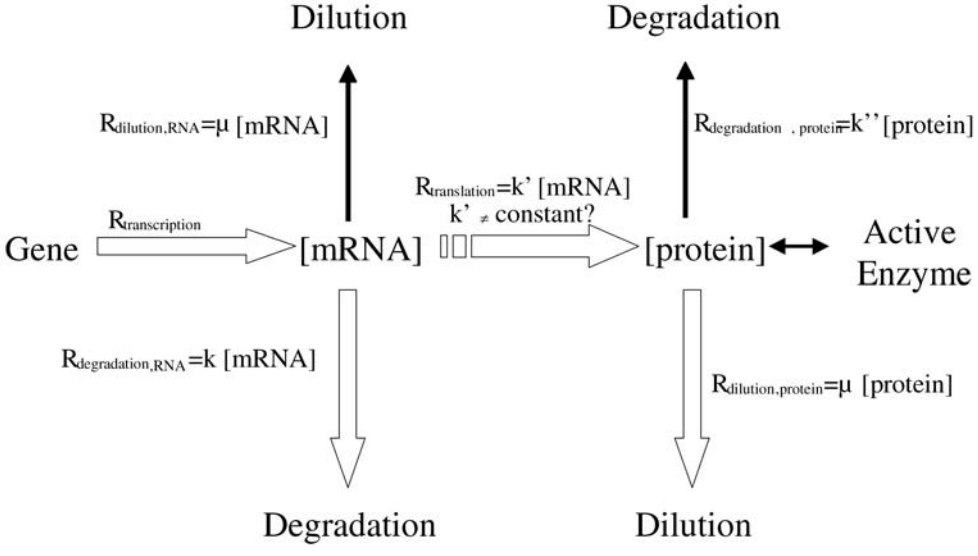

Fig. 3 From gene to enzyme. μ Growth rate, k mRNA turnover, k' translation efficiency, k'' protein turnover

ling enzyme. The absence of the shift towards mixed acid metabolism is difficult to explain, although extremely low levels of intracellular pyruvate formate lyase and alcohol dehydrogenase activities were detected, despite the presence of significant amounts of each transcript (Even et al. 2001). Clearly some other form of control is being exerted in this strain.

An integrated approach from gene to enzyme

Both the concentration of an enzyme and its allosteric regulation determine the in vivo activity promoting carbon fluxes through a metabolic pathway. Although the mechanisms of enzyme activity regulation are quite well documented, little is known about the genetic control of glycolytic enzyme concentrations: is control limited to the transcriptional level, or is there translational control as well? A good way to study the global regulation of gene expression and to identify translational regulation is to measure transcript and enzyme levels within the same study. This was recently done in *Bacillus subtilis* by a combined transcriptome and proteome analysis using DNA micro-array technology and 2D protein gel electrophoresis (Yoshida et al. 2001). While numerous data were obtained from this study, in some cases there was a lack of correlation following direct comparison of transcript and enzyme profiles. A similar conclusion could be drawn for the comparison of the transcript and enzyme profiles of the *las* operon in a wild-type and a Δ*ccpA* strain of *L. lactis* (Luesink et al. 1998). Transcripts and enzymes of the operon decreased in the *ccpA* mutant compared with the wild-type strain, but in different proportions. These data illustrate that some inconsistencies will be observed when directly comparing transcript and enzyme profiles. In fact, as proposed by Nilsson et al. (1984), cellular transcript concentrations should be compared to rates of enzyme synthesis, rather than to intracellular enzyme concentrations. Indeed, the intracellular enzyme concentration does not necessarily reflect the rate of enzyme synthesis since it results from the equilibrium between its synthesis and its disappearance (cellular dilution and protein degradation which is often negligible). Consequently, comparison of the rate of enzyme synthesis with the cellular transcript concentration should restore the predicted correlation between mRNA and protein synthesis if only transcriptional control is exerted (Fig. 3). Otherwise, regulation at the translational level must occur, as recently observed in *L. lactis* subsp. *lactis* strain IL 1403. A three-fold lower translational efficiency (k') was found with galactose than with glucose, for the various genes coding for central pathway enzymes (Even et al. 2001). Such translational regulation can be seen as an efficient manner for the cell to minimize variations in transcript levels. Furthermore, due to this variation in translation efficiency, rates of enzyme synthesis change to the same extent as the growth rate, provoking only minor changes in intracellular enzyme concentrations. However, glycolytic flux under such conditions is subject to important changes, indicating that the major form control is over enzyme activity itself, via inhibition/activation mechanisms or modification of the degree of enzyme saturation.

When taking into account the kinetic equations for the different classes of metabolites (Fig. 3), it may be ascertained that, from gene to protein, the rate of enzyme synthesis depends predominantly on the dilution rate, i.e., the growth rate (μ), during the growth period. Protein degradation rate most likely predominates during the stationary phase, particularly under stress conditions. In a similar way, the rate of gene transcription is not directly related to mRNA concentration, since both degradation and dilution rates can also modulate transcript concentration. If the dilution rate due to cell growth can generally be neglected for mRNA, then mRNA turnover becomes an important determinant of the cellular mRNA concentration. Although no results are available in lactic acid bacteria, in *E. coli* average mRNA turnover is 1 min (Neidhart et al. 1994). Meaningful analysis of gene arrays will require that this aspect is integrated into data analysis.

A comprehensive view of the global regulation of cell metabolism requires that the successive steps from the genome to the transcriptome, and then to the proteome and the metabolome are integrated into complex experimental procedures. This is the challenge at the onset of the post-genomic area. Recently, much enthusiasm has been provoked by transcriptome analysis, and certainly in the near future, by the combined analysis of transcriptome and proteome. One should bear in mind, however, that a direct comparison of raw data, and unfortunately of data often obtained under poorly defined fermentation conditions, will not result in a full description of the global regulation of gene expression. Only when these potentially powerful new metabolic analysis tools are used within carefully controlled growth environments and care is taken to avoid possible artifacts will we begin to unravel the complex physiological behavior of the deceptively simple lactic acid bacteria.

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