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Pentose phosphates in nucleoside interconversion and catabolism

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Ribose phosphates are either synthesized through the oxidative branch of the pentose phosphate pathway, or are supplied by nucleoside phosphorylases. The two main pentose phosphates, ribose-5-phosphate and ribose-1-phosphate, are readily interconverted by the action of phosphopentomutase. Ribose-5-phosphate is the direct precursor of 5-phosphoribosyl-1-pyrophosphate, for both *de novo* and 'salvage' synthesis of nucleotides. Phosphorolysis of deoxyribonucleosides is the main source of deoxyribose phosphates, which are interconvertible, through the action of phosphopentomutase. The pentose moiety of all nucleosides can serve as a carbon and energy source. During the past decade, extensive advances have been made in elucidating the pathways by which the pentose phosphates, arising from nucleoside phosphorolysis, are either recycled, without opening of their furanosidic ring, or catabolized as a carbon and energy source. We review herein the experimental knowledge on the molecular mechanisms by which (a) ribose-1-phosphate, produced by purine nucleoside phosphorylase acting catabolically, is either anabolized for pyrimidine salvage and 5-fluorouracil activation, with uridine phosphorylase acting anabolically, or recycled for nucleoside and base interconversion; (b) the nucleosides can be regarded, both in bacteria and in eukaryotic cells, as carriers of sugars, that are made available though the action of nucleoside phosphorylases. In bacteria, catabolism of nucleosides, when suitable carbon and energy sources are not available, is accomplished by a battery of nucleoside transporters and of inducible catabolic enzymes for purine and pyrimidine nucleosides and for pentose phosphates. In eukaryotic cells, the modulation of pentose phosphate production by nucleoside catabolism seems to be affected by developmental and physiological factors on enzyme levels.

Pentose phosphates are heterocyclic, five-membered, oxygen-containing phosphorylated ring structures, with ribose-5-phosphate (Rib-5-P) and 2-deoxyribose-5-phosphate (deoxyRib-5-P) being basal structures of ribonucleotides and deoxyribonucleotides, respectively, and 5-phosphoribosyl-1-pyrophosphate (PRPP) the common precursor of both *de novo* and 'salvage' synthesis of nucleotides. Two main pathways are involved in pentose phosphate biosynthesis (Fig. 1). In the oxidative branch of the pentose phosphate pathway, Rib-5-P is generated from glucose-6-phosphate. In the phosphorylase-mediated pathway, deoxyribose-1-

Abbreviations
CNT, concentrative nucleoside transporter; deoxyRib-1-P, deoxyribose-1-phosphate; deoxyRib-5-P, deoxyribose-5-phosphate; ENT, equilibrative nucleoside transporter; 5-FU, 5-fluouracil; PNP, purine nucleoside phosphorylase; PRPP, 5-phosphoribosyl-1-pyrophosphate; Rib-1-P, ribose-1-phosphate; Rib-5-P, ribose-5-phosphate; UPase, uridine phosphorylase.

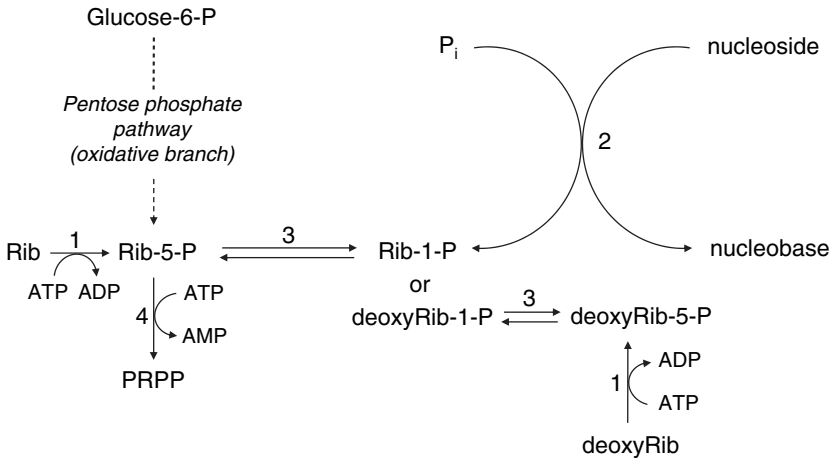

Fig. 1. Pentose phosphate synthesis. In most cells, ribose-5-phosphate (Rib-5-P) is synthesized through the oxidative branch of the pentose phosphate cycle. Alternatively, pentose phosphates are synthesized by phosphorolysis of nucleosides, either supplied by nucleic acid breakdown or transported from the external milieu. 1, ribokinase; 2, nucleoside phosphorylases; 3, phosphopentomutase; 4, 5-phosphoribosyl-1-pyrophosphate (PRPP) synthetase.

phosphate (deoxyRib-1-P) and ribose-1-phosphate (Rib-1-P) are supplied by various nucleoside phosphorylases, such as thymidine phosphorylase, uridine phosphorylase (UPase) and purine nucleoside phosphorylase (PNP) [1]. PNP deficiency causes a clinical syndrome of severe combined immunodeficiency, indistinguishable from that of adenosine deaminase deficiency [2,3]. Rib-5-P may also be formed from free ribose by the action of ribokinase. The enzyme from *Escherichia coli* has been crystallized and its genetic regulation extensively studied in bacteria [4–8]. However, the phosphorylation of free ribose by ribokinase is a less investigated pathway in mammals, even though its involvement in the elevation of PRPP, following ribose administration as a metabolic supplement for the heart and central nervous system, has been demonstrated [9,10].

The reader is referred to the numerous excellent reviews covering the different aspects of nucleoside and nucleobase metabolism [11–13]. This article focuses on the direct link between the ribose moiety of nucleosides and central carbon metabolism.

Pentose phosphates in nucleoside interconversion

PNP and UPase-mediated ribose transfer

The equilibrium of PNP-catalysed reactions is thermodynamically in favour of nucleoside synthesis [1,14]. Nevertheless, it is generally accepted that *in vivo* inosine and guanosine phosphorolysis is favoured (a) because the intracellular concentration of P_i is higher than that of nucleosides [11] and (b) as a result of the coupling of liberated hypoxanthine and guanine with hypoxanthine-guanine phosphoribosyl transferase (HPRT) and, in certain tissues, xanthine oxidase or guanase, respectively, the equilibrium of the PNP reaction is shifted towards Rib-1-P accumulation (Fig. 2). Another important factor is the absence in mammals of any kinase acting on inosine and guanosine [15–17], which further favours the channelling of purine nucleosides towards phosphorolysis. Interestingly, purine ribonucleoside kinases are also absent in *Lactococcus lactis*, hence the only pathway for purine nucleoside salvage in this bacterium is through phosphorolytic cleavage by PNP to the free nucleobase and Rib-1-P [13]. We can reasonably assume that *in vivo* PNP acts catabolically, leading to pentose phosphate formation for its further utilization in cell metabolism.

A different metabolic situation may be envisaged for UPase. The homeostasis of uridine, which regulates several physiological and pathological processes [18], is maintained by the relative activities of two enzymes: the UTP-CTP inhibited uridine kinase [19,20] and UPase. It has long been assumed that UPase, in analogy to PNP, acts catabolically, even though in 1985 Schwartz *et al.* [21] gave convincing evidence for its anabolic role in 5-fluouracil (5-FU) activation to cytotoxic compounds. More recent *in vitro* experiments have established that indeed UPase may catalyse the Rib-1-P-mediated ribosylation of 5-FU and uracil, even in the presence of excess P_i [20,22]. In normal rat tissues and in PC12 cells, this process, called the 'Rib-1-P pathway' predominates over the one-step 'PRPP pathway', as catalysed by orotate phosphoribosyltransferase, and represents the only known way for salvaging uracil [20,23]. Cao *et al.* [24] have developed a UPase gene knockout embryonic stem cell model and have shown that the disruption of UPase activity leads to a 10-fold increase in the 5-FU 50% inhibitory concentration (IC_{50}), and to a two to threefold reduction in its incorporation into nucleic acids. At least in rat brain this 'UPase-mediated anabolism' (Fig. 2) is

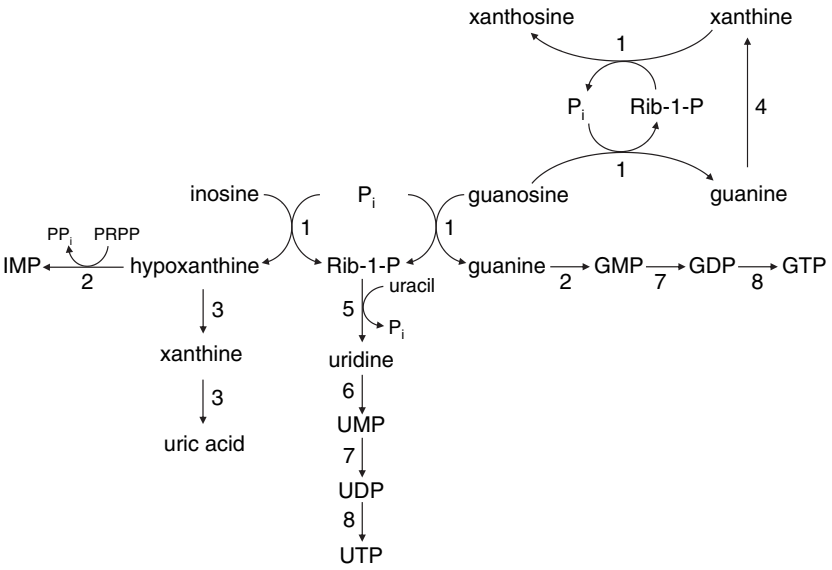

Fig. 2. Purine nucleoside phosphorylase (PNP) as a source of ribose-1-phosphate (Rib-1-P). Even though the thermodynamic equilibrium of the PNP-catalysed reaction (enzyme 1) favours nucleoside synthesis, nucleoside phosphorolysis is favoured over base ribosylation because the products hypoxanthine and guanine become substrates of virtually irreversible reactions [hypoxanthine-guanine phosphoribosyl transferase (HPRT), enzyme 2; xanthine oxidase, enzyme 3; guanase, enzyme 4], and because the intracellular concentration of P_i is higher than that of nucleosides. In the uridine phosphorylase (UPase)-mediated uracil anabolism, UPase (enzyme 5) is a linkage between purine salvage (PNP, enzyme 1; HPRT, enzyme 2) and pyrimidine salvage (uridine kinase, enzyme 6; nucleoside mono-and diphosphokinases, enzymes 7 and 8, respectively). The combined action of PNP and UPase results in the net transfer of ribose from a purine nucleoside to a pyrimidine base. The upper right part of the figure shows the process of Rib-1-P recycling for nucleoside interconversion, in which the combined action of PNP and guanase results in guanosine deamination, in the absence of a specific guanosine deaminase. Note that in this process, the ribose moiety of guanosine is transferred to xanthine, which possesses the same purine ring of guanosine.

favoured because (a) degradation of uracil to β-alanine, which would drive uridine phosphorolysis, is absent in the central nervous system (CNS) [14,25], (b) multiple consecutive phosphorylations of uridine by the ubiquitous uridine kinase and nucleoside mono- and diphosphokinases drive the Rib-1-P-mediated uracil and 5-FU ribosylation catalysed by UPase, and (c) the absence of uracil phosphoribosyltransferase in mammals [26] further channels Rib-1-P towards 5-FU and uracil ribosylation.

We can therefore assume that the Rib-1-P produced by inosine phosphorolysis may, in part, become a substrate for 5-FU activation and for uracil salvage, thus establishing a metabolic link between purine and pyrimidine salvage synthesis (Fig. 2). In bacterial systems, whether UPase can be used anabolically for uptake of uracil without any ribose donors added may be determined in mutants lacking uracil phosphoribosyltransferase (*upp pyr* mutants) [13]. In *L. lactis*, the low concentration of Rib-1-P makes the ribonucleoside synthesis unfavourable. Thus, in an *upp pyr* mutant, the irreversibility of UPase was shown by the inability of uracil to satisfy the pyrimidine requirement [27]. However, when supplied with a purine nucleoside as a source of Rib-1-P, the uracil analogue, 5-FU, is converted to 5-fluorouridine [28]. The inability to utilize uracil through UPase is also found in enteric bacteria [29]. Usually wild-type bacteria, including Gram-positive bacteria, are unable to anabolize thymine. However, thymine-requiring mutants of *E. coli* and *Salmonella typhimurium* can deoxyribosylate thymine to thymidine by thymidine phosphorylase, because their deoxyRib-1-P pools are high [30]. In these mutants, deoxyUTP accumulates and is broken down to deoxyuridine, which again is cleaved by thymidine phosphorylase to uracil and deoxyRib-1-P. The PNP-mediated ribose transfer from a nucleoside to a base analogue, with potential antiviral or antineoplastic activity, has been widely used for the *in vitro* synthesis of novel nucleoside analogues. Alternatively, a nucleoside modified in its ribose moiety may be used to obtain a new nucleoside analogue, modified in its pentose ring. The utility of this procedure was documented by Krenitski *et al.* in 1981 [31]. Since then, a large variety of new nucleoside analogues have been enzymatically synthesized. We refer to the excellent review of Bzowska *et al.* [1] for furthering the principles and techniques related to this important field of applied

enzymology. The recent introduction of thermostable phosphorylases isolated from *Sulfolobus solfataricus* and *Pyrococcus furiosus* [32,33] might offer a promising improvement.

Rib-1-P recycling

During the course of experiments designed to isolate deoxyRib-1-P formed by the reversible enzymatic phosphorolysis of deoxyguanosine catalysed by PNP, in 1952 Friedkin tried to increase the yield of deoxyRib-1-P by coupling deoxyguanosine phosphorolysis with the irreversible guanine deamination, catalysed by guanase [34]. In theory, for each mole of deoxyguanosine undergoing phosphorolysis, one mole of xanthine and one mole of deoxyRib-1-P should also be formed. However, both xanthine and deoxyRib-1-P unexpectedly disappeared. This observation led to the isolation of deoxyxanthosine, a hitherto-undescribed deoxynucleoside, which was formed by deoxyribosylation of xanthine, catalysed by PNP. The sum of the three above-reported reactions is the hydrolytical deamination of deoxyguanosine, in the absence of a specific deoxyguanosine deaminase. Years later, an enzyme system, catalysing the apparent deamination of guanosine to xanthosine, was reconstituted *in vitro*, using commercial PNP and guanase [14]. In this system, xanthine, after reaching a maximal value, decreased consistently in parallel with the increase of xanthosine. Moreover, replacement of P_i with arsenate, hindering the formation of Rib-1-P, prevented the formation of xanthosine, but not that of guanine and xanthine. The Rib-1-P recycling for guanosine deamination is operative in rat liver [14,34] and brain [35], and might be responsible for the presence of xanthosine in human serum and tissues [36].

In both the 'UPase-mediated Rib-1-P anabolism' and the 'Rib-1-P recycling for nucleoside and base interconversion', the ribose moiety of Rib-1-P, produced by the action of PNP, is transferred to a nucleobase. Nevertheless, the two processes are metabolically different. In the first, the net reaction is the transfer of ribose from a nucleoside to a base, with Rib-1-P acting as a form of activated ribose. In the second, the net reaction is the hydrolytic deamination of guanosine, with Rib-1-P acting catalytically [14] (Fig. 2). A similar Rib-1-P recycling system is operative in *Bacillus cereus* [37]. This organism does not possess any adenine deaminase, yet it can quantitatively mobilize the amino group of adenine for biosynthetic reactions by catalysing the ribosylation of adenine by adenosine phosphorylase, an enzyme distinct from PNP [38], followed by adenosine deamination and inosine phosphorolysis. Alternatively, adenosine can be phosphorylated to AMP by adenosine kinase [39]. Rib-1-P recycling also occurs in *E. coli* and *L. lactis*. In these organisms, free adenine can serve as the sole purine source. Adenine is converted into adenosine, and then into inosine and hypoxanthine using the Rib-1-P recycling process, and after conversion of hypoxanthine to inosine-5′-monophosphate (IMP), these reactions in summary result in the conversion of adenine into IMP, which serves as a precursor for guanosine-5′-monophosphate (GMP) synthesis [13]. Mammals do not possess any adenosine phosphorylase activity, therefore they cannot carry out these kinds of Rib-1-P recycling.

N-deoxyribosyltransferases

Contrary to the ribose moiety of inosine, which must be transformed by PNP into free Rib-1-P in order to be transferred to a nucleobase, the deoxyribose moiety of deoxyinosine can be transferred to a nucleobase acceptor by a single enzyme protein, the *N*-deoxyribosyltransferase, without the intermediate formation of free deoxyRib-1-P. The glycosyl transfer is stereospecific, in that only the β-anomer of the deoxynucleoside is formed. The enzyme, first discovered by McNutt in 1952 [40], is present in *Lactobacillus* species, which are devoid of nucleoside phosphorylases and hence cannot degrade or synthesize deoxyribonucleosides phosphorolytically. As they also often have a growth requirement for deoxynucleosides, it is important that these compounds are not degraded when present in the medium. The presence of the *N*-deoxyribosyltransferase and all four nucleobases found in DNA and just one deoxynucleoside ensures a supply of all four deoxynucleotides, because these bacteria possess deoxynucleoside kinase activities. The genes encoding two distinct *N*-deoxyribosyltransferases have been isolated by Kaminski [41]. The wide specificity of the two transferases for deoxynucleoside donors and base acceptors made it possible to synthesize a large number of deoxynucleoside analogues with potential antiviral and antineoplastic activity [42].

Pentose phosphates as a carbon and an energy source

As this section is devoted to the catabolism of the ribose moiety of both intracellular and extracellular nucleotides, an introduction on the reactions involved in this pathway and on the enzymes catalysing these reactions appears to be necessary (Fig. 3). Nucleoside phosphorylases play a key role in the utilization of nucleosides [1]. Based on their structural properties, nucleoside phosphorylases have been classified into

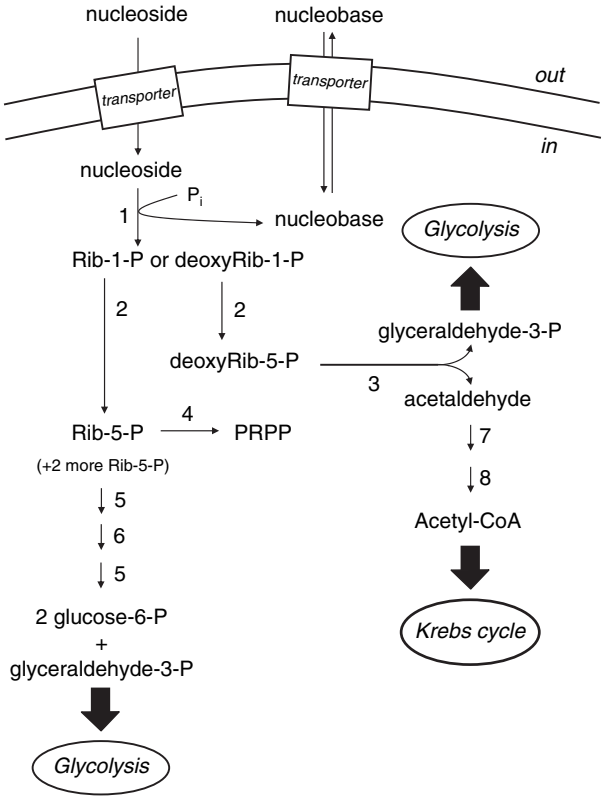

Fig. 3. The phosphorylated pentose moiety of nucleosides may be used as an energy source. Nucleosides enter the cell through specific transporters and are ultimately subjected to a phosphorolytic cleavage, catalysed by nucleoside phosphorylases (enzyme 1). After isomerization, catalysed by phosphopentomutase (enzyme 2), the destiny of the phosphorylated sugar diverges: deoxyribose-5-phosphate (deoxyRib-5-P), through deoxyriboaldolase (enzyme 3), is converted to glyceraldehyde-3-P and acetaldehyde, while ribose-5-phosphate (Rib-5-P) can be either utilized for 5-phosphoribosyl-1-pyrophosphate (PRPP) synthesis or, through the pentose phosphate pathway, can be converted into glycolytic intermediates. 4, PRPP synthetase; 5, transketolase; 6, transaldolase; 7, aldehyde oxidase; 8, acetyl-CoA synthetase.

two families: NP-I and NP-II. The NP-I family includes homotrimeric and homohexameric enzymes from both prokaryotes and eukaryotes acting on inosine, guanosine, adenosine and uridine. The NP-II family includes homodimeric proteins structurally unrelated to the NP-I family, such as bacterial pyrimidine phosphorylases and eukaryotic thymidine phosphorylase [43]. This enzyme was shown to be identical to the platelet-derived endothelial cell growth factor, a protein known to possess chemotactic activity *in vitro* and angiogenic activity *in vivo* [44]. However, stimulation of endothelial cell proliferation was soon after ascribed to the deoxyribose arising from the intracellular breakdown of thymidine, rather than to an intrinsic property of thymidine phosphorylase [45]. Phosphopentomutase catalyses the reversible reaction between Rib-1-P and Rib-5-P and between deoxyRib-1-P and deoxyRib-5-P. The enzyme has been extensively studied in bacteria [46–48]. Among eukaryotes, phosphopentomutase activity has been detected in rabbit tissues [49], human leukemic cells [50], human erythrocytes [51] and in a cell line derived from the human amnion epithelium (WISH) [52], and has been purified from rat liver [53].

The key enzyme for the catabolism of the pentose moiety of deoxyribonucleosides is deoxyriboaldolase, which cleaves deoxyRib-5-P into acetaldehyde and glyceraldehyde 3-P. Bacterial deoxyriboaldolases have been extensively studied [54–56], and many studies on the organization and regulation of the aldolase-encoding gene have been performed. The eukaryotic enzyme is known in much less detail. It has been purified from rat liver [57] and human erythrocytes [58]. More recently, the presence of deoxyriboaldolase has been reported in the liver of a number of vertebrates, as well as in human lymphocytes and some cultured cell lines [59]. The widespread distribution of deoxyriboaldolase among higher organisms points to an important role in the catabolism of deoxynucleosides.

Utilization of the pentose moiety of nucleosides in eukaryotes

In the course of pioneering experiments on nucleoside metabolism, it was demonstrated that human red cells readily catabolize inosine to hypoxanthine, while the pentose moiety is ultimately converted via the pentose phosphate pathway and glycolysis to lactate [60], thus leading to the net synthesis of ATP. Deoxyinosine is cleaved to hypoxanthine, but in this case the deoxyribose moiety is converted into acetaldehyde and glyceraldehyde 3-P by deoxyriboaldolase (Fig. 3). Glyceraldehyde-3-P is further catabolized to lactate through glycolysis, while acetaldehyde may be converted into acetyl-CoA by the action of two enzymes (aldehyde oxidase and acetyl-CoA synthetase), which are widely distributed among eukaryotes [61,62]. In WISH cells, the utilization of exogenous deoxyinosine results mainly in the catabolism of the pentose moiety, the purine ring being not appreciably salvaged [52]. Plasma inosine is the main energy source for swine and chicken erythrocytes, which lack glucose transporters [63,64].

Still a matter of debate is whether nucleosides exert their protective action by interacting with specific receptors, or after their entry into the cell and metabolic conversion to energetic intermediates. While, in some cases, the action of adenosine is receptor-mediated

[65,66], to explain the effect of its deamination product, inosine, the contribution of hitherto-unknown specific receptors has been invoked [67]. On the other hand, a number of studies report a receptor-independent mechanism of nucleoside action, which ultimately involves phosphorolytic cleavage with generation of phosphorylated sugar that is used as energy source [68–71] (Fig. 3).

Studies on the distribution of purine catabolic enzymes in the mouse alimentary tract have shown that PNP, guanase and xanthine oxidase are present at their highest levels in the proximal small intestine, and may account for the conversion of dietary purines into uric acid [72]. Metabolic studies on isolated rat intestine perfused through the lumen with uridine [73] or purine nucleosides [74] demonstrated that following absorption, nucleosides are converted into uracil or uric acid and ribose phosphate, respectively, which are released in the serosal secretion. Further studies have been performed *in vitro* on intestinal epithelial cells to examine the transcellular transport of nucleosides [75]. Purine and pyrimidine nucleosides were taken up by differentiated Caco-2 cells grown on filters and catabolized to free nucleobases, which appeared in the external medium on the opposite side of the cell monolayer. However, the destiny of the pentose moiety was not investigated.

In conclusion, nucleosides deriving from digestion of dietary nucleic acids or endogenous turnover appear as a source of phosphorylated sugar, which can sustain cellular metabolic requirements either by substituting or supplementing glucose in both aerobic and anaerobic conditions.

Regulation of nucleoside transport and catabolism in eukaryotes

Two types of nucleoside transport processes have been described in eukaryotic cells: the concentrative Na^+/nucleoside cotransport and the equilibrative nucleoside transport. These activities are mediated by transmembrane proteins belonging to two transporter families, designated concentrative nucleoside transporter (CNT) and equilibrative nucleoside transporter (ENT), respectively. For a better insight into the structural and functional properties of these transporters, the reader is referred to a number of excellent articles [76,77].

A marked variability in the expression of both CNTs and ENTs has been observed in human tissues, as well as a decreased expression in several human tumors compared with normal tissues [78]. Nutritional factors may influence the regulation of nucleoside transport [79]. A reduction in human ENT1 and mRNA levels has been observed in human umbilical vein endothelial cells exposed to high concentrations of glucose. This effect is induced via stimulation of P2Y2 purinoceptors by ATP released from cells in response to glucose [80]. An increase in CNT expression has been observed during cell proliferation induced by partial hepatectomy or in proliferating hepatoma cells [81], as well as during rat liver embryonal development [82]. Upregulation of nucleoside transporters has been associated with the action of hormones known to induce differentiation of fetal hepatocytes, such as dexamethasone and T_3 [82]. Steroid and thyroid hormones also modulate the expression of nucleoside transport in cultured chromaffin cells [83,84]. Recently, it has been demonstrated that in conditions of energy depletion induced by mitochondrial inhibition, human colon carcinoma cells increase the uptake of nucleosides, consistent with the idea that nucleosides can be used as an energy source [71].

Other signal molecules, such as cytokines and pancreatic hormones, modulate nucleoside transport by activating protein kinases. Activation of protein kinase C affects nucleoside transport in chromaffin cells [85] and neuroblastoma cells [86], while protein kinase A inhibits the equilibrative uptake of adenosine in cultured kidney cells [87] and neuroblastoma cells [86]. In human B lymphocytes, tumor necrosis factor-α activates concentrative transport and decreases equilibrative transport of uridine by activating protein kinase C [88]. Glucagon produces a rapid, transient stimulation of Na^+-dependent uridine uptake, and insulin exerts a stable, long-term induction of concentrative uridine transport, consistent with a mechanism involving the insertion of more carrier proteins into the plasma membrane [89]. An insulin-induced increase of ENT1 through activation of the nitric oxide/cGMP cascade has been demonstrated in human umbilical artery smooth muscle cells [90], thus confirming previous observations on nitric oxide modulation of nucleoside transport [91]. Conversely, insulin downregulates diabetes-elevated transport via the cAMP pathway [90].

The rapid increase in the knowledge of the diverse and complex mechanisms modulating the expression and activity of nucleoside transporters points to the importance of nucleosides to cell physiology. Available data on the modulation of nucleoside catabolism indicate the influence of developmental and physiological factors on enzyme levels. Thus, expression of deoxyriboaldolase was shown to depend on the cell cycle in rat hepatoma cells, peaking in the G2 phase [92]. The expression of purine-degrading enzymes, including 5′-nucleotidase, adenosine deaminase, PNP and

xanthine oxidase, is co-ordinately induced at the mouse maternal–fetal interface during embryonic development, as well as during postnatal maturation of the mouse gastrointestinal tract [93].

Nucleoside catabolism in bacteria

Enterobacteria

The expression of all nucleoside transport systems and nucleoside-catabolizing enzymes is inducible in enteric bacteria [94]. *E. coli* possesses both cytidine and adenosine deaminase [95,96]. Four different nucleoside phosphorylases have been found in *E. coli*: thymidine phosphorylase [97,98], and UPase [99], specific for pyrimidine nucleosides, and PNP [100] and xanthosine phosphorylase [101] specific for purine nucleosides. *S. typhimurium* expresses the same enzymes, except for xanthosine phosphorylase [102]. Enteric bacteria possess phosphopentomutase acting on both ribose- and deoxyribose-phosphates [46], and deoxyriboaldolase [55].

In *E. coli*, the enzymes and transport proteins required for nucleoside catabolism and recycling are encoded by genes belonging to the CYTR regulon. This family consists of six genes encoding nucleoside-catabolizing enzymes (thymidine phosphorylase, deoxyriboaldolase, phosphopentomutase, PNP, UPase and cytidine deaminase), and three genes encoding nucleoside transport systems (*nupG*, *nupC* and *tsx*). The expression of these transcriptional units is regulated by the CytR repressor. Deoxyriboaldolase, thymidine phosphorylase, PNP and phosphopentomutase, along with the NupG and Tsx transport systems, are separately regulated by a second DeoR repressor via an independent mechanism [103,104]. In *E. coli*, adenosine deaminase expression is induced only by adenine or hypoxanthine, while in *Salmonella* the enzyme is not inducible [102]. Finally, genes encoding xanthosine phosphorylase and xanthosine transporter are induced by xanthosine [105]. Therefore, the expression of enzymes involved in the phosphorolysis of nucleosides and in the utilization of their pentose moiety as an energy source is under the same regulation of the nucleoside transport proteins. The expression of the proteins included in the CYTR regulon is induced several-fold by nucleosides added to the growth medium. Cytidine, by interacting with the CytR repressor regulates the synthesis of all the enzymes encoded by the regulon, which are far more than those required to catabolize cytidine. It has been speculated that cytidine might serve as a signal for the presence of both ribo- and deoxyribo-nucleosides, indicating that carbon sources are available for the cell [102]. In *E. coli*, adenosine can also function as an inducer of CYTR but, being rapidly catabolized, this nucleoside is unable to be effective in wild-type cells. In *S. typhimurium*, uridine also functions as a CYTR inducer [102]. This regulation ensures the efficient transport and catabolism of any available nucleoside. As a consequence, *E. coli* can grow on nucleosides as a sole carbon and energy source [102,106]. Nucleoside catabolism and pentose-phosphate utilization is not only regulated through specific repressors, but is also dependent on the presence of glucose as a carbon source. In fact, the CytR repressor-regulated operons and genes of xanthosine catabolism are under control of catabolite repression [107]. On the other hand, the induction of DEOR regulon is not subject to catabolite repression, being independent of the cAMP level in the cell [102]. As a consequence, deoxynucleosides are catabolized also in the presence of glucose in the medium, while ribonucleosides are readly catabolized only when the source of primary sugar is exhausted. In this regard, it is interesting to note that the true inducing compound for the DeoR repressor is deoxyRib-5-P. In enteric bacteria, the inhibition exerted by glucose on the uptake of a different carbon source (inducer exclusion) and, in the absence of glucose, the positive regulation of catabolic gene expression by a complex of cAMP and the CAP protein, are the two main mechanisms of catabolite repression. Both these mechanisms are mediated by $EIIA^{glc}$ protein, a component of the glucose phosphotransferase transport system [107].

Bacilli

B. cereus, similarly to enteric bacteria, is able to grow on nucleosides as the sole carbon and energy source. Also in this micro-organism the expression of enzymes of purine catabolism is regulated by a mechanism triggered by metabolites present in the growth medium. *B. cereus* expresses 5′-nucleotidase and adenosine deaminase, as well as phosphopentomutase and deoxyriboaldolase. Furthermore, *B. cereus* and *B. subtilis* express two phosphorylases, one specific for inosine and guanosine (PNP) and the other specific for adenosine (adenosine phosphorylase) [38,108]. In *B. cereus*, 5′-nucleotidase and adenosine phosphorylase are constitutive enzymes, while adenosine deaminase is induced by adenine [109]. PNP and phosphopentomutase are induced by pentose- and deoxypentose- phosphates [47,110]. Finally, aldolase is induced by deoxynucleosides [111]. As a consequence of these regulatory events, nucleosides are readily catabolized inside the cell, yielding free bases and glycolytic

intermediates. When *B. cereus* is grown in the presence of 10 mM purine nucleoside as the sole carbon and energy source, the ribose moiety is fully utilized, yielding bacterial growth comparable to that obtained in the presence of 20 mM glucose, while the free base can be almost quantitatively recovered in the external medium. Despite the presence in *B. cereus* of the specific adenosine phosphorylase, the major catabolic fate of adenosine is its deamination into inosine. Adenosine taken up from the external medium is cleaved by the phosphorylase, a constitutive enzyme, yielding adenine, which in turn causes a 20-fold increase in the expression of adenosine deaminase. This enzyme can therefore be considered as the true catabolic enzyme [111]. In *E. coli*, adenosine is deaminated, rather than phosphorolytically cleaved. This is probably a result of the toxic effects exerted by high concentrations of both adenine and adenosine on growing cells [112]. The expression of transport systems has not been studied in *B. cereus*, but measurements have been performed of the rate of nucleoside disappearance and base accumulation in the external medium, in suspensions of bacteria grown beforehand in the presence or absence of inducers of the catabolic pathway. It has been observed that the rates of nucleoside disappearance and of intermediate and base accumulation were entirely in agreement with the pattern and extent of enzyme expression, implying that the transport systems were not limiting [109]. This strongly suggests that, as mentioned for enteric bacteria, in *B. cereus* the expression of proteins involved in the transport of nucleosides is induced with the same mechanisms described for the enzymes of nucleoside catabolism. In *B. cereus*, the expression of all proteins involved in nucleoside catabolism is under the control of catabolite repression [109,110], demonstrating that also in this micro-organism exogenous nucleosides are perceived as energy and carbon sources alternative to glucose, rather than as nucleic acid precursors. In Gram-positive bacteria, catabolite repression is exerted through a mechanism distinct from that described for enteric bacteria. Thus, in *B. subtilis*, negative control of expression of catabolic genes and operons in the presence of glucose and other well-metabolisable carbon sources is the major mechanism of catabolite repression [113].

While ribose phosphate may be recycled for base salvaging or nucleotide *de novo* synthesis, deoxyribose phosphate can undergo only a catabolic fate. Deoxyriboaldolase is the key enzyme allowing deoxyribose phosphate to enter the carbohydrate metabolism. Deoxyriboaldolase purified from bacterial sources exhibits homogeneous molecular and functional features, is apparently characterized by the lack of physiological effectors and appears to be regulated exclusively at transcriptional level [56]. The transcription rate of deoxyriboaldolase is increased not only when deoxynucleosides or even DNA are present in the growth medium, but also as a function of oxygen supply [59]. In fact, a decrease in oxygen supply determines an increase in the expression of deoxyriboaldolase and in the rate of deoxyribose utilization through anaerobic glycolysis as a consequence of the low energy yield of sugar fermentation.

The catabolism of purine and pyrimidine nucleosides in *B. subtilis* shows several differences with respect to both *B. cereus* and *E. coli*. *B. subtilis* possesses cytidine deaminase and three distinct nucleoside phosphorylases: a PNP active on inosine and guanosine, a phosphorylase specific for adenosine similar to that described in *B. cereus* and a phosphorylase specific for pyrimidine nucleoside [102]. Finally, *B. subtilis* expresses phosphopentomutase and deoxyriboaldolase [102]. The genes for enzymes of purine and pyrimidine catabolism are located in two operons: the first encoding phosphopentomutase and PNP, and the second encoding deoxyriboaldolase, pyrimidine phosphorylase and a protein involved in the transport of pyrimidine nucleosides. In addition there are two single genes for cytidine deaminase and adenosine phosphorylase whose expression is unresponsive to the presence of nucleosides in the growth medium [114]. On the contrary, transcription of the operon containing the genes of PNP and phosphopentomutase is increased by the presence of both ribo- and deoxyribo-nucleosides in the growth medium. The operon is negatively regulated by a protein which recognizes both Rib-5-P and deoxy-Rib-5-P as signals for the operon derepression. The operon is also subjected to catabolite repression [115]. The operon which encodes deoxyriboaldolase, pyrimidine phosphorylase and a pyrimidine nucleoside transporter is negatively regulated by a *deoR* gene product. The regulatory protein binds deoxyRib-5-P as a signal for the operon derepression [116]. Moreover, the expression of this DEOR operon is subjected to catabolite repression by glucose [117]. Therefore, with the exclusion of cytidine deaminase and adenosine phosphorylase, all the enzymes involved in nucleoside catabolism and pentose utilization in *B. subtilis* are inducible and their expression depends on the availability of a primary carbon and energy source. When glucose is lacking and deoxyRib-5-P accumulates in the cell as a signal for nucleoside availability, the pyrimidine transporter, pyrimidine phosphorylase, PNP, phosphopentomutase and deoxyriboaldolase are readily transcribed, leading to complete utilization of the deoxyribose moiety of nucleosides as a carbon and

energy source. On the contrary, when Rib-5-P accumulates in the cell, only the transcription of PNP and phosphopentomutase is increased, and the nucleoside transport system seems to be unaffected. This observation explains why *B. subtilis* can grow in the presence of thymidine as a carbon and energy source, but cannot grow on inosine as the sole carbon source. It has been suggested that the limiting factor in the catabolism of nucleosides in this organism is the purine nucleoside transport system [115].

In conclusion, bacteria possess a battery of transport systems and catabolic enzymes for purine and pyrimidine nucleosides, which are regulated at the transcriptional level by mechanisms similar to those devoted to the transport and the utilization of sugars alternative to glucose. When a suitable carbon and energy source is available, the relatively low rate of expression of nucleoside transport systems and catabolic enzymes ensures enough material for nucleoside, base and phosphorylated pentose salvaging and recycling. In this case, exogenous nucleic acid and endogenous RNA turnover may be considered as a reserve of building blocks for anabolic purposes. When the primary carbon source is exhausted and an internal increase of phosphorylated pentose signals exogenous nucleic acid availability, the whole pathway assumes a catabolic role. As a consequence, the pentose moiety is utilized to sustain the cell energy requirement, while the base is either expelled from the cell or partially utilized as a nitrogen source or as a precursor for nucleic acid synthesis. In this case, exogenous nucleic acids are perceived as a carbohydrate polymer analogous to glycogen. Therefore, in bacteria, nucleosides may well be considered as carriers of sugar, and nucleoside phosphorylases as sugar-activating enzymes, because they yield phosphorylated pentoses at no expense of ATP. These mechanisms allow bacteria to grow utilizing nucleic acids arising from decaying tissues or organisms, or excreted by living cells.

It is interesting to underline that, while in bacteria the induction of catabolic enzymes and transporters exerted by deoxynucleosides is a widespread phenomenon, in some cases also independent of catabolite repression, the regulation of ribonucleoside catabolism differs among different species and is always dependent on catabolite repression, thus confirming that, as stated above, ribonucleosides are regarded as carriers of sugar. On the other hand, it might be speculated that catabolism of deoxynucleosides play not only a role in energy supply but also in defending the cell from foreign DNA. In fact, in *B. cereus*, deoxyriboaldolase is induced 24-fold by 0.5 $mg \cdot mL^{-1}$ of whole eukaryotic DNA [59].

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