Editorial

Metabolic, Pathological, and Therapeutic Perspectives Intracellular 5'-Nucleotidases

5'-Nucleotidases have long been considered as merely catabolic enzymes, catalyzing the entry step of purine and pyrimidine nucleotide breakdown. New insight into the functional role of the 5'-nucleotidases followed the recognition that brain and blood red cells maintain their nucleotide pools by salvaging purine and pyrimidine rings mainly in the form of imported nucleosides, rather than by synthesizing nucleotides de novo from simple precursors, and that a cross talk exists between the extracellular nucleoside generation from exported nucleoside-triphosphate catabolism and intracellular imported nucleoside anabolism to nucleoside-triphosphates. During this recycling process the quantitative and qualitative intracellular balance of nucleosides is maintained by the reciprocal modulation of nucleoside kinases and of at least three 5'-nucleotidases: the ADP activated AMP preferring cN-1, the ATP-ADP activated IMP-GMP preferring cN-II, the UMP-CMP preferring cN-III, and their respective subtypes. One of the aims is to provide a common basis for the study of the relationship between Biochemistry of cytosolic 5'-nucleotidases and other related disciplines, such as Physiology, Pharmacology, and Therapy.

Accumulating evidence indicates that the phosphotransferase activities of human cN-II and cN-III play an important role in the process of nucleoside interconversion, as well as in the process of a series of approved nucleoside analogs activation to antitumoral and antiviral drugs. Another important aim is to further our knowledge on the interaction of nucleoside analogs with enzymes of nucleoside and nucleotide metabolism, including cytosolic nucleotidases, for the development of novel specific inhibitors.

The importance of cytosolic 5'-nucleotidases in humans has been shown long ago by the discovery of hereditary nonspherocytic hemolytic anemia. In this disorder, the inherited deficiency of cN-III activity leads to accumulationof pyrimidine metabolites in red cells. More recently cN-II iperactivity has been shown in erythrocytes of patients with Lesch-Nyhan syndrome, and in colon carcinoma.

In this special issue these different aspects of the involvement of intracellular 5'-nucleotidases in a series of physiopathological processes that regulate the intracellular pools of nucleosides and nucleotides, and serve as the starting points for the development of new antiviral and anticancer drugs are considered and discussed.

The first review by *Ipata and Balestri*, mainly devoted to non experts in the flied, addresses the problem of the role of intracellular 5'-nucleotidases. The presence in human genome of at least seven genes for 5'-nucleotidases suggests that these enzymes, endowed with different nucleoside-monophosphate specificity, do not perform only merely catabolic functions. The main metabolic problem with cytosolic 5'nucleotidases is the following: why are nucleosides produced by the action of 5'-nucleotidases only to be converted back to nucleoside-monophosphates, at the expense of ATP? It is now well established that the intracellular balance of nucleotides, which perform a myriad of intra- and extracellular functions, is maintained by the metabolic modulation of at least 3 metabolic cycles, in which 5'-nucleotidases play a major role: i) the adenosine kinase and AMP preferring 5'-nucleotidase dependent "adenosine cycle", ii) the the uridine kinase and UMP preferring 5'-nucleotidase dependent "uridine cycle", iii) the IMP-GMP preferring 5'-nucleotidase, purine nucleoside phosphorylase, and hypoxanthine-guanine phosphoribosyltransferase dependent "oxipurine cycle".

5'-Nucleotidases exhibit numerous functions in the CNS, including termination of nucleoside triphosphate signaling, cell adhesion, synaptogenesis, cell proliferation, and intracellular and extracellular formation of nucleosides. The uneven distribution of nucleosides in the brain strongly suggests that 5'-nucleotidases, as well as nucleoside transporters and receptors, have region-specific functions in the human brain. The interaction between regional nucleoside levels and the activities of nucleoside metabolic enzymes, with emphasis on cytosolic 5'-nucleotidases are discussed by *Kovács, Dobolyi, Kékesi and Juhász*. Indeed, alterations in the regulation of nucleoside homeostasis may induce pathological changes, resulting in central nervous system diseases. Conversely, several CNS diseases such as epilepsy may be treated by modulating the nucleoside system, which is best achieved by modulating 5'-nucleotidases.

The relative activities of anabolic nucleoside kinases and catabolic 5'-nucleotidases maintain the function of cytosolic and mitochondrial substrate cycles, that regulate the intracellular pools of active nucleotides. In his review *Eriksson* discusses the expression patterns, the levels of mRNA, protein and activities of 4 nucleoside kinases, i.e. the cytosolic deoxycytidine- and thymidine kinases, and the mitochondrial thymidine- and deoxyguanosine kinases, and of 6 intracellular 5'-nucleotidases cN-IA, cN-IB, cN-II, cN-III. cdN, and mdN. Based on the catalytic and regulatory properties of these enzymes, Eriksson evaluates their role as primary controllers of the accumulation and breakdown of anti viral and anti cancer nucleoside analogs, and the possible efficacy and side effects of new nucleotide drug candidates.

The contribution by *Skladanowski* is a survay of cytoplasmic and mitochondrial 5'-nucleotidases with respect to their ability to dephosphorylate known purine and pyrimidine nucleoside 5'- monophosphate analogs, acting as antiviral and antineoplastic agents. The ability of 5'-nucleotidases to transfer the phosphate group of a natural (deoxy)monophosphate to a (deoxy)nucleoside analog, as well as their role in determining clinical resistance to nucleoside analog-based drugs, are also highlighted. Thus, 5'-nucleotidases may have a dual role: They either dephosphorylate a nucleoside-monophosphate analog, thus

causing drug resistance, or phosphorylate a nucleoside-analog, acting *de facto* as a kinase, or as an activating agent. The antiproliferative analogs of natural nucleotides discussed by Skladanowski as potential substrates of 5'-nucleotidases include Z-nucleotides, arabinofuranoside nucleotides, 8-aza- and 7-CH purine-nucleotides, dideoxypentose and sulfur containing nucleotides, N-amidophosphates, 2' or 3'-phosphates. 5'-Nucleotidases include plasma membrane-bound eN, cytosolic cN-II, cN-III and cdN, and mitochondrial mdN.

Most studies aimed at elucidating the pathological significance of an enzyme are based on the results of studies on its main catalytic and regulatory features. The results of many biochemical studies discussed in *Itoh*'s review on cytosolic 5'-nucleotidase cN-II point for the pivotal role of this enzyme in regulating the intracellular pool of IMP. This nucleoside monophosphate is synthesized either "de novo" from simple precursors, or by salvaging preformed hypoxanthine. Degradation of adenine nucleotides may also be a source of IMP. Being the common precursor for both AMP and GMP synthesis, IMP might play a pivotal role in maintaining the qualitative and quantitative balance of purine nucleotides, for the stability of genomic information and for the interaction with purine receptors. The results of a series of comparative biochemical studies on cN-II, discussed by Itoh, support this hypothesis. Another cytosolic 5'-nucleotidase, cN-I, preferentially hydrolyzes AMP. However, in contrast to the ubiquitous expression of cN-II, the tissue distribution of cN-I is limited. Interestingly, in rat liver and rat polymorphonuclear leucocytes, in which cN-I is not expressed, AMP dephosphorylation is catalyzed by cN-II. Finally, even in tissues in which cN-I is highly expressed, a significant amount of AMP is deaminated to IMP.

Cytosolic 5'-nucleotidase (cN-II) regulates the intracellular availability of IMP, GMP and, directly or indirectly, of AMP. As a consequence this ubiquitous enzyme may be involved in a number of cellular mechanisms, such as regulation of nucleotide precursors for nuclear and/or mitochondrial DNA replication, nucleotide degradation, metabolism of antitumoral purine prodrugs, mechanisms of drug resistance. The results obtained by modifying the level of cN-II expression in different cell models, including yeast cells, on adenosine production, purine and pyrimidine drug toxicity, and nucleotide pools homeostasis are discussed by *Tozzi , Pesi and Allegrini*. Their review underlines that cN-II is not only involved in antitumoral drug resistance and in maintenance of purine and pyrimidine nucleotide pools, but also in the cell response to energy depletion through modulation of AMP kinase.

The contribution by *Jordheim and Chaloin* takes into consideration the main features of the structure and the kinetics of cN-II, with emphasis on its phosphotransferase activity and its allosteric regulation. Available data, and *in vitro* studies on cancer cell models performed by the Authors, and on samples from cancer patients, suggest a pivotal role of cN-II as a therapeutic drug target in anticancer treatment. Thus, the inhibition of its enzymatic activity induces cell death, while the exposure to increasing concentrations of inhibitors over a long time induces the development of resistant cell lines. The role of cN-II in the phosphorylation of unnatural nucleosides, i.e. in the process of pro-drug nucleoside analogs activation, remains uncertain. However, the high K_m of cN-II for IMP and GMP, the two main phosphate donors, markedly decreases in the presence of ATP and other allosteic effectors, thus favouring the phosphorylation of poorly metabolized nucleoside analogs. Based on the well known dependence of cN-II activity on the formation of a phosphoenzyme, and on the crystal structure of the enzyme protein, 5'-modified nucleosides that inhibit the release of inorganic phosphate, and ribonucleotide analogs that tightly bind to the active site, without being dephosphorylated have been developed and used as research tools. As stated by the Authors, the study of cN-II inhibition in animal models "constitutes an appealing approach to increase the activity of cancer treatment".

5'-Nucleotidase cN-III and 5'(3')-nucleotidase cdN are characterizes by ubiquitous distribution in mammalian tissues and by strict specificity towards pyrimidine nucleoside monophosphates. A nonspherocytic hemolytic anemia, characterized by abnormally high levels of pyrimidine compounds in erythrocytes, is associated to hereditary cN-III deficiency. The major findings on cN-III and cdN, with particular emphasis on the relationship between their structure and function, as well as on their roles in normal and pathological conditions, as well as the possible therapeutic approaches in detoxifying common antiviral and antineoplastic drugs are discussed by *Magni, Amici and Orsomando*. The catalytic mechanism of both hydrolytic and phosphotransferase activities, possessed by both enzymes, are discussed also in the light of recent solution of both cN-III and cdN three-dimensional structures.

Piero Luigi Ipata
Guest Editor
Professor Emeritus of Biochemistry
University of Pisa
Italy
Tel: +390502211462
Fax: +390502211460
Email: plipata@biologia.unipi.it

Maria Grazia Tozzi
Guest Editor
Associate Professor
Department of Biology
Unit of Biochemistry
University of Pisa
Italy
Tel: +039-050-2211457
Fax: +039-050-2211460
Email: mtozzi@biologia.unipi.it