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Review

Visualizing nucleic acid metabolism using non-natural nucleosides and nucleotide analogs☆

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ABSTRACT

Nucleosides and their corresponding mono-, di-, and triphosphates play important roles in maintaining cellular homeostasis. In addition, perturbations in this homeostasis can result in dysfunctional cellular processes that cause pathological conditions such as cancer and autoimmune diseases. This review article discusses contemporary research areas applying nucleoside analogs to probe the mechanistic details underlying the complexities of nucleoside metabolism at the molecular and cellular levels. The first area describes classic and contemporary approaches used to quantify the activity of nucleoside transporters, an important class of membrane proteins that mediate the influx and efflux of nucleosides and nucleobases. A focal point of this section is describing how biophotonic nucleosides are replacing conventional assays employing radiolabeled substrates to study the mechanism of these proteins. The second section describes approaches to understand the utilization of nucleoside triphosphates by cellular DNA polymerases during DNA synthesis. Emphasis here is placed on describing how novel nucleoside analogs such as 5-ethynyl-2′-deoxyuridine are being used to quantify DNA synthesis during normal replication as well as during the replication of damaged DNA. In both sections, seminal research articles relevant to these areas are described to highlight how these novel probes are improving our understanding of these biological processes. This article is part of a Special Issue entitled: Physiological Enzymology and Protein Functions.

1. The importance of nucleoside metabolism

Nucleosides and their corresponding nucleotides play important roles in cell physiology by functioning as both nutrients and modulators of cellular homeostasis [1]. For example, nucleoside triphosphates such as ATP and GTP are used as energy sources in various biological reactions ranging from muscle contraction to processes involving cell signaling [2]. In addition, nucleosides such as adenosine and guanosine as well as their corresponding cyclic nucleoside monophosphates (cAMP and cGMP) modulate a wide variety of cellular events by functioning as ligands for purinergic receptors and transducers of endocrine signals [3]. For example, cAMP is a second messenger that mediates a wide range of intercellular effects including glucose, glycogen, and lipid metabolism [4]. Likewise, adenosine participates in numerous processes ranging from the maintenance of cardiovascular tone to modulating immune responses [5]. For instance, adenosine binding to the A_1 receptor leads to a reduction in cAMP levels [6] which increases K^+ efflux in cardiac tissue to cause cell hyperpolarization. Another important role for adenosine is as an inflammatory modulator [7] in which increases in the extracellular levels of adenosine can activate the A_{2a} receptor on the surface of immune cells [8]. Finally, pyrimidine and purine nucleosides comprise the monomeric building blocks of DNA and RNA and thus play central roles in the storage and expression of genetic information [9]. Collectively, these examples highlight the importance of pyrimidine and purine nucleosides in many physiological processes ranging from cardiovascular activity to neurotransmission to cellular proliferation [10–12].

Nucleoside metabolism also plays important roles in the initiation and progression of several pathological conditions. Diseases such as cancer, autoimmune disorders, and viral infections, for example, require significantly high levels of DNA synthesis to sustain their hyperproliferative state. As such, these conditions require large amounts of nucleoside uptake for cellular survival and propagation. The supply for this high demand is typically obtained though salvage pathways that rely on the activity of various nucleoside transporters. Equally important, the increased reliance on nucleoside uptake and metabolism provides an important focal point for therapeutic intervention against these conditions [13]. For example, azidothymidine (AZT) is a pyrimidine analog in

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which the 3′-hydroxyl (–OH) moiety required for elongating DNA is replaced with an azide (N_3) group. This simple substitution makes AZT refractory to elongation by viral polymerases [14–16] and makes it an effective nucleoside reverse transcriptase inhibitor (NRTI) that targets viral replication in individuals infected with the human immunodeficiency virus (HIV) [17]. In addition to AZT, other NRTIs such as didanosine, stavudine, and tenofovir are used to combat HIV replication. The basis for their pharmacological effects also relies heavily on modifications to the (deoxy)ribose moiety (Fig. 1) [3]. Modifications to the sugar moiety allow the corresponding deoxynucleoside triphosphates to function as non-obligate chain terminators to inhibit DNA synthesis [18–21]. Nucleoside analogs are also important therapeutic agents against numerous types of cancers including leukemia, lymphoma, and certain solid tumors such as pancreatic and lung cancers. Indeed, approximately 20% of all anti-cancer drugs are classified as anti-metabolites which inhibit DNA synthesis [22]. Nucleoside analogs including gemcitabine and fludarabine (Fig. 1) target enzymes such as ribonucleotide reductase and DNA polymerases that play essential roles in DNA replication [23]. As described above, the pharmacological activity of these anti-cancer agents is also predicated on modifications to the (deoxy)ribose moiety.

This review article describes current research efforts applying small molecules as mechanistic probes to understand the complexities of nucleoside metabolism at the cellular level. A key focal point of this article is describing how biophotonic nucleosides are replacing conventional assays using radiolabeled substrates to study the mechanisms by which intracellular concentrations of various nucleosides and deoxynucleosides are regulated and how defects in this regulation can perturb cellular homeostasis. Emphasis is placed in two important areas. The first section describes classic and contemporary approaches to monitor nucleoside transporter activity while the second section describes approaches to understand nucleotide utilization during the replication of normal and damaged DNA.

2. Nucleoside transport

In general, the de novo biosynthesis of nucleosides and nucleotides is an energetically costly process that is restricted to selected cell types [24]. In terms of cellular energy requirements, it is more efficient for cells to obtain nucleosides by salvage pathways which take advantage of nucleoside recycling [25,26]. The first step in this process is the transport of a nucleoside past the cell membrane (Fig. 2). This seemingly simple task is in fact remarkably challenging as nucleosides and nucleotides are rather hydrophilic and thus show negligible permeability across hydrophobic cell membranes [27]. To facilitate uptake, cells use specific proteins to catalyze movement of nucleosides from the extracellular milieu into their cytosol [28]. There are two types of cellular process used in nucleoside transport. The first is an equilibrative transport system which allows for bi-directional movement of nucleosides, following a concentration gradient to achieve nucleoside influx

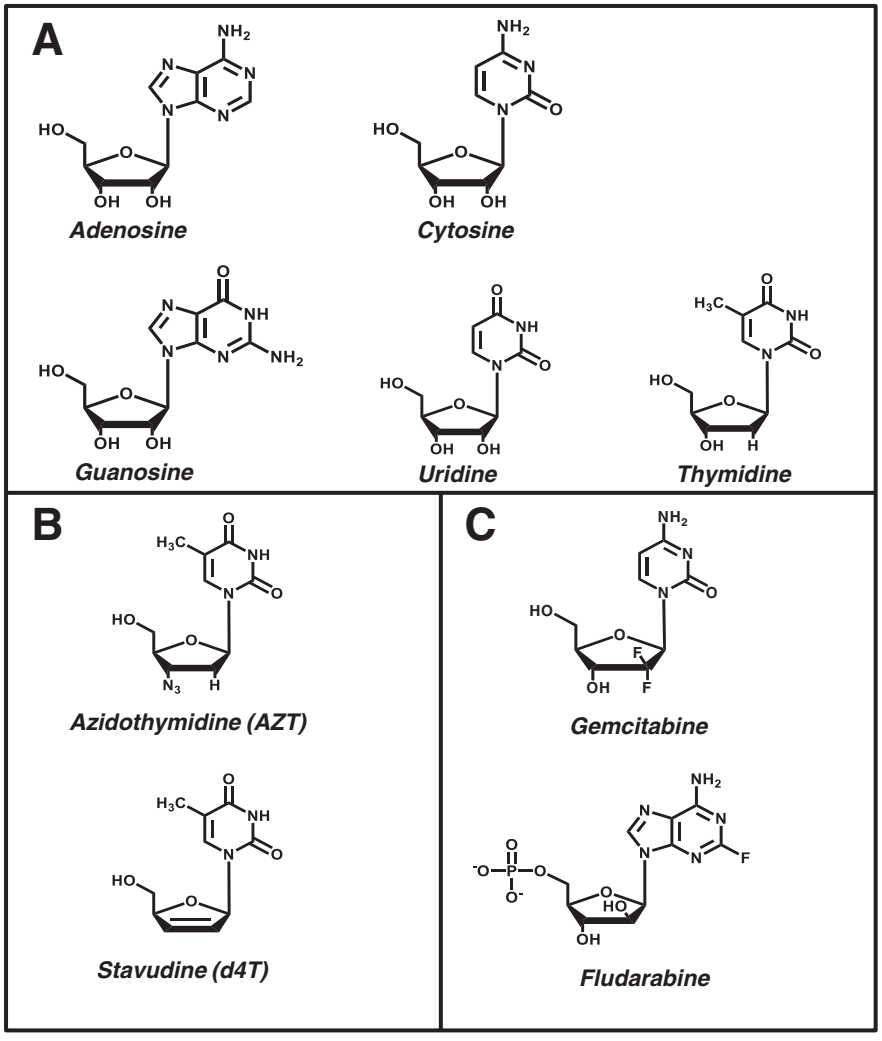

Fig. 1. (A) Structures of natural nucleosides (adenosine, cytosine, guanosine, and uridine/thymidine). (B) Structures of representative nucleoside analogs that function as anti-viral agents. (C) Structures of representative nucleoside analogs that function as anti-cancer agents.