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Minireview

Regulation by degradation, a cellular defense against deoxyribonucleotide pool imbalances

Chiara Rampazzo, Cristina Miazzi, Elisa Franzolin, Giovanna Pontarin, Paola Ferraro, Miriam Frangini, Peter Reichard, Vera Bianchi*

Department of Biology, University of Padova, Via Ugo Bassi 58B, 35131 Padova, Italy

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ABSTRACT

Deoxyribonucleoside triphosphates (dNTPs) are the precursors used by DNA polymerases for replication and repair of nuclear and mitochondrial DNA in animal cells. Accurate DNA synthesis requires adequate amounts of each dNTP and appropriately balanced dNTP pools. Total cellular pool sizes are in the range of 10–100 pmoles of each dNTP/million cells during S phase, with mitochondrial pools representing at most 10% of the total. In quiescent or differentiated cells pools are about 10-fold lower both in the cytosol and mitochondria. Contrary to what may be expected on the basis of the roughly equimolar abundance of the 4 nitrogen bases in DNA, the four dNTPs are present in the pools in different ratios, with pyrimidines often exceeding purines. Individual cell lines may exhibit different pool compositions even if they are derived from the same animal species. It has been known for several decades that imbalance of dNTP pools has mutagenic and cytotoxic effects, and leads to "mutator" phenotypes characterized by increased mutation frequencies. Until 10 years ago this phenomenon was considered to affect exclusively the nuclear genome. With the discovery that thymidine phosphorylase deficiency causes destabilization of mitochondrial DNA and a severe multisystemic syndrome the importance of dNTP pool balance was extended to mitochondria. Following that first discovery, mutations in other genes coding for mitochondrial or cytosolic enzymes of dNTP metabolism have been associated with mitochondrial DNA depletion syndromes. Both excess and deficiency of one dNTP may be detrimental.

We study the mechanisms that in mammalian cells keep the dNTP pools in balance, and are particularly interested in the enzymes that, similar to thymidine phosphorylase, contribute to pool regulation by degrading dNTP precursors. The role of some relevant enzymes is illustrated with data obtained by chemical or genetic manipulation of their expression in cultured mammalian cells.

Contents

Abbreviations: ADA, adenosine deaminase; 5BU, 5-Bromo uracil; AdR, deoxyadenosine; cdN, cytosolic 5′-deoxynucleotidase; cNI, cytosolic 5′-nucleotidase I; cNII, cytosolic 5′-nucleotidase II; cpm, counts per minute; dCK, deoxycytidine kinase; dGK, deoxyguanosine kinase; dNDP, deoxynucleoside diphosphate; dNMP, deoxynucleoside monophosphate; dNTP, deoxynucleoside triphosphate; GdR, deoxyguanosine; ko, knockout; mdN, mitochondrial 5′-deoxynucleotidase; mt, mitochondrial; NDP, ribonucleoside diphosphate; NTP, ribonucleoside triphosphate; pmol, picomole; PNP, purine nucleoside phosphorylase; ssDNA, single-stranded DNA; TdR, thymidine; TK1, cytosolic thymidine kinase; TK2, mitochondrial thymidine kinase; TP, thymidine phosphorylase; UdR, deoxyuridine.

* Corresponding author. Tel.: +39 0498276282; fax: +39 0498276280.
E-mail address: vbianchi@bio.unipd.it (V. Bianchi).

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1. Introduction

The size and composition of deoxyribonucleoside triphosphate (dNTP) pools affect the genetic stability of mammalian cells because fidelity of DNA synthesis requires dNTPs in adequate and balanced amounts [1,2]. The mutagenic consequences of dNTP pool imbalance were recognized about three decades ago in cell lines where genetic defects of dNTP metabolism produced large perturbations of the pools [2–4]. Such pool imbalances increased mutation frequencies at multiple loci destabilizing the nuclear genome. Subsequent studies clearly demonstrated at the molecular level that the type of mutation reflected the dNTP present in excess in the cellular pool [5]. Although the mutagenic consequences of precursor imbalance were firmly established for nuclear DNA, only ten years ago came the first demonstration that the stability of the mitochondrial (mt) genome is similarly affected [6]. Interestingly, the first enzyme deficiency found associated with mtDNA alterations was that of a catabolic cytosolic enzyme, thymidine phosphorylase (TP), whose deficiency supposedly lead to thymidine triphosphate (dTTP) buildup inside the cells upsetting the dNTP pool balance [6]. This genetic condition causes a severe progressive multiorgan syndrome, mitochondrial neurogastrointestinal encephalomyopathy (MNGIE). Other mt diseases were later found to be due to genetic inactivation of mt and cytosolic enzymes of dNTP synthesis, suggesting that both excess and deficiency of precursors impair mtDNA (reviewed in [7]). Both human genetic diseases and the pathological phenotypes of knockout mouse models are valuable sources of information about the physiological relevance of individual enzymes participating in dNTP synthesis and regulation. Here we will use this information to complement and corroborate the conclusions drawn from experiments with cultured mammalian cells.

2. Two pathways in the enzyme network for dNTP regulation

It is now recognized that both nuclear and mitochondrial DNA synthesis need balanced supplies of dNTPs. The total amounts of precursors needed for the replication of the two genomes are however very different, as mtDNA corresponds to a small percentage of a cell's total DNA. The synthesis of dNTPs is tuned to the needs of nuclear DNA replication, that, in contrast to mtDNA replication, is restricted to the S phase of the cell cycle. At the transition between G1 and S several enzymes of dNTP synthesis are induced and the pools expand about 5- to 10-fold remaining high until DNA replication is completed [1]. Outside S phase and in cells that have stopped dividing dNTP pool sizes are strongly reduced [8,9].

Two pathways produce dNTPs in mammalian cells, i.e. cytosolic de novo synthesis and salvage of deoxynucleosides, the latter based on two parallel sets of enzymes located in the cytosol and in the mt matrix (Fig. 1).

2.1. De novo synthesis of deoxynucleotides

De novo synthesis is responsible for the bulk of dNTP synthesis during S phase and is based on the activity of ribonucleotide reductase [1,10]. Through its complex and unique allosteric regulation this enzyme catalyzes a balanced reduction of all four ribonucleoside diphosphates to the corresponding deoxynucleotides, that are then phosphorylated to the triphosphates to be used for DNA synthesis. Active ribonucleotide reductase is a heterotetramer containing two copies of a large subunit (protein R1) and two of a small subunit (R2 or p53R2). Protein R2 contains in its sequence a signal marking it for proteasome-dependent degradation in late mitosis [11]. Until the discovery of p53R2 [12,13], dNTP de novo synthesis was believed to be S-phase specific and operate exclusively in cycling cells. The amino acid sequence of p53R2 is instead devoid of the destruction signal and p53R2 is a stable protein that can be detected in all phases of the cell cycle and during quiescence [14]. Together with R1, a long-lived protein, p53R2 carries out de novo synthesis in non-dividing cells [15]. Although the absolute level of this synthesis is very low compared to that occurring in S phase,

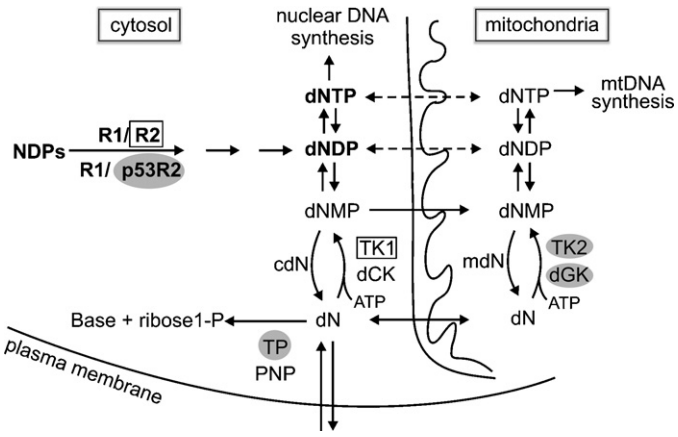

Fig. 1. The enzyme network for dNTP synthesis and regulation in mammalian cells. Deoxynucleotides are synthesized de novo in the cytosol (symbols in bold) and by two parallel salvage pathways in the cytosol and in mitochondria. The R1/R2 isoform of ribonucleotide reductase catalyzes reduction of ribonucleoside diphosphates (NDPs) during S phase and the R1/p53R2 isoform outside S phase and in non-dividing cells. Deoxynucleosides (dN) are phosphorylated in the cytosol by dCK and TK1, in mitochondria by dGK and TK2. The monophosphates (dNMP) are either converted to dNTPs by two additional phosphorylation steps or dephosphorylated by 5′-deoxynucleotidases (cdN and mdN) that participate in substrate cycles with the kinases. Deoxynucleosides are either exchanged across the plasma membrane and the inner mt membrane by equilibrative or concentrative nucleoside carriers or degraded by nucleoside phosphorylases (TP and PNP). Mitochondria import dTMP by a dedicated transporter [9]. The transporters responsible for the exchange of other deoxynucleotides between mitochondria and cytosol are still poorly defined. Boxed symbols for R2 and TK1 indicate the cell-cycle regulation of the two proteins. Shaded symbols highlight enzymes whose mutations cause mtDNA abnormalities in humans.