Deoxynucleoside triphosphate (dNTP) synthesis and destruction regulate the replication of both cell and virus genomes

Bruce Stillman[1]

*Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724*

Biochemical reactions, even those as complex as replicating the DNA genome of cells, follow the principle that the process is regulated by both the substrate concentration and by the enzymes that mediate the process. Deoxynucleoside triphosphates (dNTPs), the substrates for DNA polymerizing enzymes, have long been known to be limited in their concentration in cells because the enzyme that synthesizes deoxynucleotides from ribonucleotides, ribonucleotide reductase (RNR), is synthesized and enzymatically activated as cells enter the S phase (1, 2). RNR, discovered by Peter Reichard 52 y ago (3), converts all four ribonucleotide diphosphates (rNDPs) to the respective deoxynucleoside disposphates (dNDPs), which are then rapidly converted to dNTP. Low levels and activity of RNR provide sufficient dNTPs for mitochondrial DNA synthesis and for DNA repair in noncycling cells and during the G1 phase of the cell-division cycle in proliferating cells, but RNR levels and activity are hugely increased as cells commit to replicate DNA during the S phase of the cell-division cycle or following extensive DNA repair (4). Indeed, RNR is one of the most highly regulated enzymes known. The mammalian enzyme synthesizes all four dNDPs in a cycle, is allosterically activated by dATP, dTTP, and dGTP to balance the relative levels of the four dNTPs (dCTP, dTTP, dGTP and dATP), and is feed-back–inhibited by dATP, because dATP is the last dNTP to be made in the cycle of synthesizing all four dNTPs by a single RNR enzyme (1). Specific inhibitory proteins (in yeasts) also control RNR activity and RNR subunit levels are regulated by cell cycle-dependent transcription of the genes encoding the subunits and by subunit protein stability (4, 5). On the basis of these observations, one might expect that dNTP synthesis by RNR should be sufficient to control how and when genome DNA replication occurs because RNR is only maximally active during the S phase. However, recent studies, including those emerging from far-afield studies of how HIV replication is restricted to certain cell types (6, 7), have uncovered a new control of dNTP levels, dNTP destruction. The sterile alpha motif and HD-domain containing protein 1 (SAMHD1) protein is a deoxynucleoside triphosphohydrolase that cleaves dNTPs to the respective deoxynucleoside and a triphosphate (8). In PNAS, Franzolin et al. (9) show that dNTP destruction by SAMHD1 also contributes to dNTP concentration control during the cell-division cycle of proliferating cells, thereby affecting both DNA replication and cell-cycle progression.

SAMHD1 contains two recognized domains, a SAM (sterile alpha motif) domain of unknown function, and a HD domain that contains catalytic aspartic acid and histidine residues that form the catalytic core of the enzyme (8). SAMHD can only hydrolyze dGTP when each dNTP is provided individually, but it can hydrolyze dTTP, dCTP, and dATP when dGTP is present as a cofactor. dGTP most likely acts as an allosteric activator of the dimeric enzyme (8), although a recent report suggests that the enzyme may function as a tetramer (10). The observation that SAMHD1 can degrade dGTP alone and that the same dNTP can allosterically activate the triphosphohydrolase may be one mechanism to balance the concentrations of all four dNTPs in the cell. It is possible that the levels of dNTP are determined by the affinity of dGTP to the allosteric site of SAMHD1.

Franzolin et al. (9) demonstrate that SAMHD1 is intimately involved in the control of dNTP levels, not only in noncycling cells where the enzyme is abundantly expressed, but also in cycling cells. Their observation
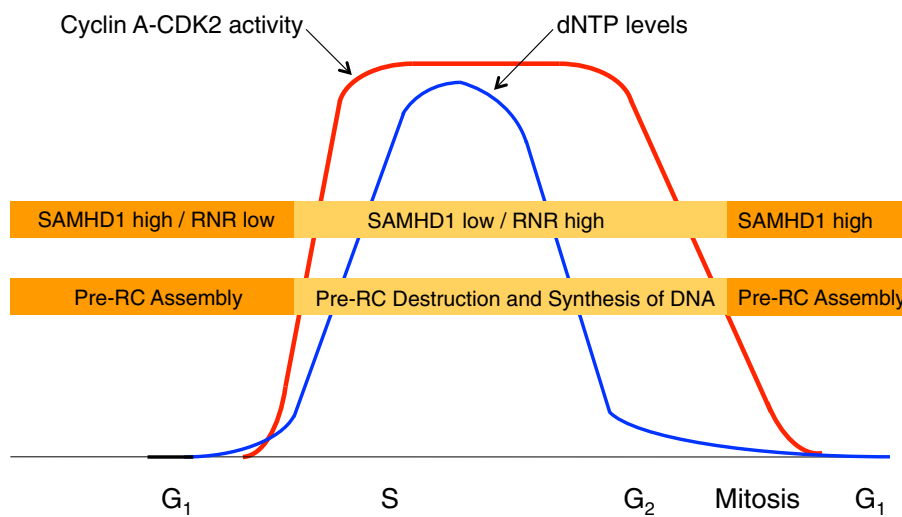
Fig. 1. A generic eukaryotic cell division cycle showing cyclin A-CDK2 activity and the relative levels of dNTPs. The dNTP-synthesizing enzyme activity of RNR and the relative activity of the dNTP triphosphohydrolase activity of SAMHD1 alternate out of phase with each other. Possibly, cyclin A-CDK2 phosphorylates SAMHD1 and promotes its destruction via ubiquitin-mediated proteolysis, allowing dNTP synthesis by RNR to be coupled to DNA replication during the S phase. This cycle parallels the cycle of assembly of prereplicative complexes (pre-RC) in the G1 phase and its destruction as cells enter into the S phase, a process driven by cyclin A-CDK2.

up-ends the previous notion that the synthesis of dNTPs by RNR was the main mechanism that regulated the intracellular concentration of dNTPs during the cell cycle. SAMHD1 is present in the nucleus of G1-phase cells, whereas RNR subunits are prominent in the cytoplasm, increasing their levels in S-phase cells (9). Depletion of SAMHD1 levels in cycling cells increased the dNTP concentration in non–S-phase cells and caused an arrest in the G1 phase. Interestingly, deregulation of the feedback inhibition of RNR in yeast cells caused elevated dNTP levels and an arrest in the G1 phase, so dNTPs levels have a direct effect on control of cell-cycle progression (11). Uncontrolled and high dNTP concentrations are known to be mutagenic for genome replication (12), which is most likely why cells go to great lengths to couple intimately the concentration of all four dNTPs to DNA synthesis during the S phase.

The gene encoding SAMHD1 was discovered as an IFN-γ–induced gene in mouse peritoneal macrophages (13). Induction of SAMHD1 in differentiated cells now makes sense because only low levels of dNTP would be required in nonproliferating cells to maintain mitochondria and for DNA repair. It is probable that high dNTP levels may cause problems with maintenance of mitochondrial function, which might occur in patients with Aicardi–Goutières Syndrome (AGS), a genetically inherited inflammatory encephalopathy that clinically resembles congenital virus infections and certain types of autoimmunity (14). AGS mutations in the SAMHD1 gene reduce either catalytic activity or the allosteric activation by dGTP, both causing an increase in intracellular dNTP levels, which may contribute to defective differentiation of innate immune cells.

Of interest is the observation that SAMHD1 restricts certain lentiviruses, including HIV1, from replicating in noncycling cells because the levels of dNTP are not sufficient for the reverse transcriptase to copy the incoming RNA template. Some lentiviruses, such as HIV2 and the Simian Immunodeficiency Virus, carry in a protein called Vpx that causes the degradation of SAMHD1, thereby allowing an increase in dNTPs and copying of the RNA genome into DNA (6, 8, 15). The K_m for different reverse transcriptases vary and contribute to the host cell-specificity for virus replication, a process influenced by the presence or absence of SAMHD1 (16). The phenotype of AGS is consistent with SAMHD1

mutations causing higher dNTP levels that, in turn, could lead to more robust virus infection for viruses that have a DNA polymerase with a K_m that requires increased dNTP levels. However, only viruses that encode their own DNA polymerizing enzymes will replicate in noncycling cells, because the cellular machinery that replicates host DNA is not active. Once the potent dNTP triphophohydrolase activity of SAMHD1 is removed, the virus polymerase can replicate the virus genome. Thus, DNA viruses, such as herpes simplex virus type 1 and vaccinia virus, which encode their own DNA polymerases, can replicate in noncycling cells if SAMHD1 is removed (17).

The striking observation by Franzolin et al. (9), that in cycling cells SAMHD1 is not present in the S phase, suggests that it is degraded by ubiquitin-dependent proteolysis as cells transit from the G1 phase into the S phase. The SAMHD1 protein can be phosphorylated by cyclin A-CDK2 (18). This kinase is activated at the G1-to-S phase transition in human cells and is responsible for initiation of actual DNA synthesis from prereplicative complexes that have been assembled during the G1 phase at all origins of DNA replication (19). One possibility is that phosphorylation of SAMHD1 primes Ub-mediated proteolysis of the enzyme at the G1-to-S phase transition (Fig. 1).

Recent studies have shown that SAMHD1 is phosphorylated at a CDK site, T592 (18, 20). Mutations that alter or mimic phosphorylation at this residue lost their ability to restrict HIV replication. These mutants

retained their ability to hydrolyze TTP in the presence of dGTP and did not alter dNTP levels in cells (20). Based on these observations, the possibility was raised that the ability of SAMHD1 to restrict retrovirus replication was not because of its ability to degrade cellular dNTPs. However, this conclusion must now be tempered in light of the recent results from Franzolin et al. (9), because the dNTP levels in cells expressing wild-type versus mutant SAMHD1 were measured in noncycling cells (PMA-stimulated U937 myeloid cells). In contrast, the ability of the wild-type and mutant proteins to restrict retroviral replication was measured in cycling cells. Perhaps in the noncycling cells the dNTP phosphohydrolase activity is not affected by phosphorylation because the kinase is absent, or the relevant E3-ligase that mediated Ub-dependent degradation of SAMHD1 is not expressed. In cycling cells, however, both cyclin A-CDK2 and the E3-ligase could induce destruction of SAMHD1, increasing dNTP levels and allowing virus replication. Clearly, future studies are needed to explore how SAMHD1 levels are controlled in both cycling and noncycling cells. Importantly, the observation that only G1-phase cycling cells express SAMHD1 will have to be taken into consideration in interpreting results on how SAMHD1 activity affects both genome and virus DNA replication and how dNTPs may affect cellular function in innate immunity. Despite 52 y of investigating dNTP metabolism, there appears to be much more to do!

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