DNA Precursor Pools and Ribonucleotide Reductase Activity: Distribution between the Nucleus and Cytoplasm of Mammalian Cells

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Nuclear and whole-cell deoxynucleoside triphosphate (dNTP) pools were measured in HeLa cells at different densities and throughout the cell cycle of synchronized CHO cells. Nuclei were prepared by brief detergent (Nonidet P-40) treatment of subconfluent monolayers, a procedure that solubilizes plasma membranes but leaves nuclei intact and attached to the plastic substratum. Electron microscopic examination of monolayers treated with Nonidet P-40 revealed protruding nuclei surrounded by cytoskeletal remnants. Control experiments showed that nuclear dNTP pool sizes were stable during the time required for isolation, suggesting that redistribution of nucleotides during the isolation procedure was minimal. Examination of HeLa whole-cell and nuclear dNTP levels revealed that the nuclear proportion of each dNTP was distinct and remained constant as cell density increased. In synchronized CHO cells, all four dNTP whole-cell pools increased during S phase, with the dCTP pool size increasing most dramatically. The nuclear dCTP pool did not increase as much as the whole-cell dCTP pool during S phase, lowering the relative nuclear dCTP pool. Although the whole-cell dNTP pools decreased after 30 h of isoleucine deprivation, nuclear pools did not decrease proportionately. In summary, nuclear dNTP pools in synchronized CHO cells maintained a relatively constant concentration throughout the cell cycle in the face of larger fluctuations in whole-cell dNTP pools. Ribonucleotide reductase activity was measured in CHO cells throughout the cell cycle, and although there was a 10-fold increase in whole-cell activity during S phase, we detected no reductase in nuclear preparations at any point in the cell cycle.

How are DNA precursors distributed within the compartments of eucaryotic cells? This question bears directly upon the extent to which DNA replication in these cells is coordinated with the synthesis of its precursors, the deoxyribonucleoside 5'-triphosphates (dNTPs). In procaryotic systems, notably T-even phage-infected bacteria, there is evidence that dNTPs generated at replication sites are preferentially incorporated into replicating DNA (reviewed in reference 13). This apparently involves the action of complexes of enzymes of dNTP synthesis juxtaposed to the replication apparatus, a process that channels precursors to their sites of utilization.

If eucaryotic cells channel dNTPs to DNA in a similar fashion, then dNTP biosynthesis should occur primarily in the nucleus, and its attendant enzymes should be located there as well. Indeed, others (22) have reported a nuclear location of several enzymes of dNTP biosynthesis, although contrary observations have also been reported (7).

We have approached the question by trying to measure pool sizes of DNA precursors in the major compartments of cultured mammalian cells, realizing, of course, that steady-state pool data cannot definitively answer questions of this type but can only point to answers. In previous studies (2, 3), Bestwick and co-workers showed that mitochondria contain physically and kinetically distinct dNTP pools. Because of the porous nature of the nuclear membrane, conventional subcellular fractionation procedures almost certainly redistribute small molecules between nuclear and cytoplasmic compartments. This problem was partly circumvented by Skoog and Bjursell (25), who isolated Chinese hamster ovary

(CHO) cells by fractionation in nonaqueous solvents. They found an increase in the relative nuclear dNTP pools in cells in S phase as compared with those in cells in G1 phase and concluded that the cell must have a mechanism to concentrate dNTPs against a gradient. Subsequently, Bestwick et al. (3) also reported a nonrandom pattern of dNTP distribution between nuclear and cytoplasmic compartments in HeLa cells. More recently, Nicander and Reichard (16) used labeling kinetics to study dNTP compartmentation in 3T6 mouse fibroblasts. They concluded that the dCTP pool is compartmentalized. The dTTP pool, by contrast, appears to be a single equilibrated pool.

Although this evidence circumstantially indicates that dNTPs are compartmentalized, we do not know whether distinct dNTP pools actually exist or under what conditions they might equilibrate. In this study, we addressed these questions by use of a technique that allows rapid isolation of nuclei from cultured cells, under conditions where redistribution of nucleotides during isolation is minimized. This technique also allowed determination of the intracellular distribution of the enzyme ribonucleotide reductase.

MATERIALS AND METHODS

Reagents. Radioactively labeled deoxyribonucleoside triphosphates obtained from ICN Pharmaceuticals, Inc., Irvine, Calif., were: [8-³H]dATP (27 Ci/mmol), [methyl-³H]dTTP (50 Ci/mmol), [5-³H]dCTP (30 Ci/mmol), and [8-³H]dGTP (5 Ci/mmol). Tri-*N*-octylamine was also obtained from ICN Pharmaceuticals. Freon-113(1,1,2-trichlorotrifluoroethane) was obtained from Matheson Scientific, Inc., Elk Grove Village, Ill. The alternating copolymers poly(dA-dT) and

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FIG. 1. CHO cell nuclear dNTP pools versus time of treatment with NP-40-containing nuclear isolation buffer. Cells were treated with the NP-40-containing buffer for 10, 20, or 30 s, and the nuclear dNTPs were then extracted and quantitated.

poly(dI-dC) were obtained from P-L Biochemicals, Inc., Milwaukee, Wis. Unlabeled nucleotides were obtained from Sigma Chemical Co., St. Louis, Mo. Highly purified *E. coli* DNA polymerase I (3,700 U/mg of protein) was purchased from Worthington Diagnostics, Freehold, N.J. Propidium iodide and Hoechst H33258 were obtained from Calbiochem Behring, La Jolla, Calif. Nonidet P-40 (NP-40) was obtained from Particle Data Laboratories Ltd., Elmhurst, Ill. All other chemicals were reagent grade.

Cell source and growth conditions. The CHO-K1 cells used in these experiments were obtained from Mark Meuth, Imperial Cancer Research Fund, Mill Hill Laboratories, England. HeLa cells were obtained from the American Type Culture Collection, Rockville, Md. Both cell lines were maintained in Eagle minimal essential medium with nonessential amino acids (Irvine Scientific), supplemented with 5% fetal bovine serum (GIBCO Laboratories, Grand Island, N.Y.) and penicillin-streptomycin (penicillin base, 10,000 U/ml; streptomycin, 10,000 µg/ml, used at 5 ml/liter; GIBCO). Cells were routinely checked for mycoplasma contamination (5).

Isolation of nuclei. Nuclei were isolated essentially as described by Rapaport et al. (21). The medium in a 100-mm culture dish was removed by aspiration, followed by addition of 2.5 ml of NP-40 buffer (1% NP-40, 30 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid], 200 mM sucrose, 40 mM NaCl, 5 mM MgCl₂, 5 mM EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid]; pH 8.0). The buffer was removed by aspiration after 10 s, and the remaining cell contents were washed in 5.0 ml of ice-cold phosphate-buffered saline (PBS; 0.11 M NaCl, 2.0 mM KCl, 2.5 mM Na₂HPO₄, 1.2 mM KH₂PO₄; pH 7.2). The PBS was aspirated after about 5 s of washing, and the nuclei remaining on the plate were extracted for analyses of dNTP content.

dNTP analysis. Nucleotide extraction and analysis were done by methods developed in this laboratory (19), which in turn represent modifications of procedures in widespread use. Both whole cells and nuclei were extracted first with 60% aqueous methanol. The contents of three 100-mm culture dishes were pooled for whole-cell assays, and the

contents of four dishes were pooled for nuclear assays. Each methanol extract was taken to dryness, and the contents were reextracted with 0.5 N trichloroacetic acid, followed by neutralization with tri-*N*-octylamine, as described by Garret and Santi (10). We found this second extraction with trichloroacetic acid to give less interference with the enzymatic dNTP assay than did the perchloric acid extraction described in our previous study (19).

Each extract was assayed for content of each of the four dNTPs by the DNA polymerase-based enzymatic assay (19), which measures incorporation of a limiting dNTP into an alternating copolymer template [poly(dA-dT) or poly(dI-dC)] by *Escherichia coli* DNA polymerase I in the presence of an excess of the labeled complementary dNTP. Each reaction mixture contained, in a total volume of 100 µl, 45 mM Tris hydrochloride (pH 8.3), 4.5 mM MgCl₂, 0.9 mM 2-mercaptoethanol, 10 µM ³H-labeled complementary dNTP (10 µCi/ml), 0.1 U of *E. coli* DNA polymerase I, 20 µg of bovine serum albumin, and an aliquot of extract containing between 1 and 40 pmol of the unknown dNTP. After 60, 75, and 90 min of incubation (37°C), 25-µl aliquots were spotted onto Whatman no. 3 filter paper disks. The disks were dried, washed four times with 5% trichloroacetic acid–1% sodium pyrophosphate and twice with 95% ethanol, and then dried and counted.

Cell cycle analysis. Nuclei were prepared and stained with propidium iodide, and the green fluorescence distribution was examined in an Epics V cell sorter (Coulter Electronics, Inc., Hialeah, Fla.) (29).

Cell synchronization. CHO-K1 cells were synchronized by isoleucine deprivation (30). The medium was aspirated from exponentially growing cells, and each 100-mm plate was washed twice with 10 ml of PBS. Minimal essential medium lacking isoleucine and supplemented with 5% dialyzed fetal bovine serum was added to each plate. Cells were kept in this medium for 30 h, at which time isoleucine-deficient

FIG. 2. HeLa cell nuclear dNTP pools versus length of PBS wash. After 10 s of NP-40 treatment, isolated HeLa nuclei were washed with ice-cold PBS for 15 or 30 s. The dNTP pools subsequently extracted and quantitated were compared with those from the nuclei washed for 5 s (the usual length of the PBS wash) and whole-cell dNTP pools.

FIG. 3. Scanning electron micrograph of CHO whole cells and rapidly isolated nuclei (prepared as described in Materials and Methods). (A) Whole cell at $\times 6,000$ magnification. (B) Nuclei at $\times 6,000$ magnification. Bar length is 1 μm .

medium was removed, and fresh complete medium with 5% fetal bovine serum was added.

DNA measurements. DNA was measured by the method of Erwin et al. (8).

Enzyme assays. Ribonucleotide reductase was assayed as described by Slabaugh et al. (27). Lactate dehydrogenase was assayed as described by Schwert and Winer (24). The assay of citrate synthase was performed by the method of Srere (28).

Electron microscopy. For examination of whole cells and NP-40-prepared nuclei, CHO-K1 cells were grown on plastic disks on tissue culture plates. Nuclei were isolated as routinely done for nucleotide extraction. Samples were washed with PBS and fixed in 2.5% glutaraldehyde in 0.1 M NaH_2PO_4 (pH 7.4) for approximately 15 min at room temperature. The fixative was aspirated, and the samples were dehydrated with graded ethanol. Samples were dried to the critical point and shadowed in platinum-palladium (60:40).

RESULTS

Evaluation of the nuclear isolation procedure. The nuclear isolation procedure used in these experiments was both rapid and quantitative. Briefly, the procedure involved aspiration of medium, 10 s of swirling the NP-40-containing buffer on the cells, aspiration of the NP-40-containing buffer, a 5-s PBS wash of the resulting nuclei on the plate, aspiration of the PBS, and addition of 60% methanol for extraction of nucleotides. The entire procedure, which required less than 30 s to complete, took place with the tissue culture plate on ice and all solutions at 4°C . To determine nuclear recovery, we measured DNA remaining on plates after treatment with NP-40-containing buffer for times varying from 10 to 60 s. Even after 60 s of exposure to the nuclear isolation buffer, all of the DNA remained on the plate, indicating quantitative nuclear recovery. We examined the stability of dNTP pools within CHO nuclei isolated by this method by varying the

FIG. 4. HeLa whole-cell (●) and nuclear (○) dNTP pools versus cell density. Pools were measured from 100-mm plates of densities between 1.25×10^6 and 5.5×10^6 cells per plate. Results are composites from three separate experiments.

length of treatment with the NP-40-containing nuclear isolation buffer. Pools extracted after 10, 20, or 30 s of treatment with the nuclear isolation buffer were similar to each other, but these nuclear pools differed substantially from whole-cell pools (Fig. 1). In a similar experiment with HeLa cells, we found that lengthening the PBS wash after nuclear isolation to 15 and 30 s did not reduce the size of the dNTP pools isolated (Fig. 2). While we cannot rule out the possibility of some dNTP leakage from nuclei within the first 10 s of NP-40 treatment, we consider this unlikely for reasons presented in the Discussion section. Therefore, our experiments suggest that the rapidly isolated nuclei have dNTP pools that are stable during the time needed for their isolation and extraction.

Examination of isolated CHO cell nuclei under the scanning electron microscope (Fig. 3) shows protruding nuclei surrounded and covered by cytoskeletal remnants that reveal the outline of the original cell. The extent of cytoplasmic contamination of NP-40-prepared nuclei was assessed by assay of lactate dehydrogenase, a soluble cytoplasmic enzyme. Less than 5% of the whole-cell activity was found associated with the nuclei in either HeLa cells or CHO-K1 cells. Twenty-five to 30% of the whole-cell level of citrate synthase, a mitochondrial matrix enzyme, remained attached to the plates after NP-40 treatment, indicating that nuclei were contaminated with mitochondria. However,

mitochondrial dNTP pools are small enough (3) that this should not significantly affect measured nuclear pools.

HeLa whole-cell and nuclear dNTP pool size versus cell density. HeLa whole-cell and nuclear dNTP concentrations decreased as density increased (Fig. 4). Cell cycle analysis (data not shown) revealed a similar decrease in the percentage of cells in S phase. Interestingly, changes in nuclear dNTP pool levels were similar to changes in the corresponding whole-cell dNTP pool levels; the percentage of each dNTP within the nucleus remained nearly constant between densities of 1.25×10^6 and 5.5×10^6 cells per plate. The fraction of each dNTP found in the nucleus, however, differed among the four dNTPs. For example, at all densities examined, about 20% of the dTTP and 40% of the dATP were found in the nucleus. The nuclear concentrations of dTTP and dATP were equal, however, even though the whole-cell dTTP content was 50% greater than that of dATP.

CHO cell synchronization. A representative cell cycle analysis of synchronized CHO cells is shown in Fig. 5. After 30 h of isoleucine starvation, approximately 90% of the cells were blocked in the G1/G0 phase of the cell cycle. Six hours after release of this block, the cells were still in G1 phase. At 10 to 12 h postrelease, the cells were distributed as a single broad peak in S phase. Between 15 and 18 h after the addition of complete medium, the cells were in the G2/M phase of the cell cycle. Nuclear dNTP values measured at this point may be underestimates of true values, as cells undergoing mitosis would have no nuclear membrane. Eighteen to 21 h after release of the isoleucine block, the cells had divided and were back in G1 phase.

dNTP pools from synchronized CHO whole cells and nuclei. We prepared nuclear and whole-cell extracts for analysis of the dNTP pools from times throughout the synchronization procedure. The results shown in Fig. 6 represent the average dNTP values determined in three synchronization experiments. The most striking change is the ninefold increase in the dCTP whole-cell pool between the G1- and S-phase times. This large increase is probably due to the absence of dCMP deaminase in these CHO cells and has been observed before (11, 26).

The dCTP and dGTP whole-cell pools did not change for the first 6 h after the addition of complete medium to the isoleucine-starved cells. This indicates that the dCTP and dGTP whole-cell pool levels of isoleucine-starved and G1-phase cells are similar. The dATP and dTTP whole-cell pool levels, however, increased slightly during the first 6 h after release of the isoleucine block, even though the cells remained in G1 phase. The dATP and dTTP whole-cell pools increased only slightly from 6 to 10 h postrelease and decreased slightly at 12 h. At 15 h postrelease, the whole-cell dATP and dTTP pools rose slightly, but they decreased by 18 h, at the conclusion of S phase.

The nuclear dNTP pool levels fluctuated less than the whole-cell dNTP pools throughout the cell cycle. After 30 h of isoleucine starvation, the nuclear dNTP pools remained essentially equivalent to those measured before the isoleucine block, whereas the whole-cell pools decreased, thereby increasing the sizes of the relative nuclear pools. (Note the difference in scales for nuclear and whole-cell dNTP pool levels.) At 6 h postrelease, the relative nuclear pools were close to the values one would expect if the dNTPs were distributed proportionally to the volumes of the compartments. The relative nuclear dNTP pools in G1 phase were as follows: dATP, 30%; dTTP, 30%; dCTP, 45%; and dGTP, 40%. Skoog and Bjursell (25) estimated that the nucleus represents 30% of the total cellular volume in G1

FIG. 5. Cell cycle analysis of synchronized CHO cells. Nuclei from synchronized CHO-K1 cells were stained with propidium iodide and examined for DNA content. (A) Cell cycle distribution after 30 h of isoleucine deprivation. (B) At 12 h after release of isoleucine block, cells are in S phase. (C) At 15 h postrelease, cells are dividing. (D) At 21 h after release, cells are back in G1 phase. In each case, 15,000 cells were counted.

phase and 25% in S phase. During S phase, the nuclear dGTP, dATP, and dTTP pool sizes remained at nearly the same relative distributions between nucleus and cytoplasm, while the dCTP relative nuclear pool size decreased from 45% in G1 phase to 9% in S phase. Possible reasons for this unequal distribution will be discussed.

Ribonucleotide reductase. To investigate the subcellular location of this enzyme, we harvested whole cells and nuclei from synchronized CHO cells. After being treated with NP-40 and washed with PBS, nuclei were scraped into additional PBS and collected by low-speed centrifugation. Nuclei and whole cells were suspended in buffer and sonicated. Our measurements (Fig. 7) were similar to results reported by others (6, 18) in that we observed a 10-fold increase in ribonucleotide reductase activity in the extracts of S-phase cells when compared with the whole-cell extracts of G1-phase cells. At no point in the cell cycle did we find ribonucleotide reductase activity associated with rapidly isolated nuclei. This absence of reductase activity is not due to the presence of an inhibitor in nuclear extracts, because addition of nuclear extracts to the whole-cell extracts showed no inhibition of whole-cell activity. Another possibility, that the NP-40 in the nuclear isolation buffer irreversibly inactivated the enzyme, is extremely unlikely. Whole-cell extracts assayed in the presence of 1% NP-40 (the extraction concentration) gave 40% of the control activity. At a level representing that probably present after the PBS wash, namely, 0.01% NP-40, the activity was 75% of the control level. These analyses of enzyme activity comple-

ment and extend the observations of Engström et al. (7), who localized the M1 subunit to the cytoplasm by an immunocytochemical method.

DISCUSSION

In this study, our aim was to examine dNTP compartmentation in mammalian cells. In particular, we wanted to determine the degree to which dNTP pools in the cytoplasm and nucleus exist as distinct entities. The nuclear isolation method used in these studies was both rapid and reliable, in that the dNTP pools thus isolated were relatively stable during the isolation procedure. While we cannot eliminate the possibility that extensive redistribution of dNTP pools occurs during the 10-s NP-40 treatment (i.e., leakage from the nuclei), we consider this unlikely for several reasons. (i) Random leakage of nucleoside triphosphates should yield symmetry in nuclear-to-whole-cell pool ratios, since all triphosphates should leak at comparable rates. However, as noted earlier, large asymmetries were observed; in HeLa cells, the relative nuclear dATP content was twice that of dTTP, while in CHO cells, the ratio of nuclear to whole-cell dCTP varied by fivefold during the cell cycle. (ii) The nuclear-to-whole-cell pool ratios that we observed for dNTPs were comparable to those we observed for ribonucleoside triphosphates, molecules that are undoubtedly abundant in the cytoplasm of intact cells (data not shown). (iii) We note that the NP-40 nuclear isolation technique is capable of detecting concentration gradients where they exist. Rapaport (20) described conditions under which nu-

FIG. 6. Whole-cell (A) and nuclear (B) dNTP pools from synchronized CHO cells. Results shown here represent averages from three separate synchronizations.

FIG. 7. Whole-cell and nuclear levels of CDP reductase activity throughout the cell cycle of CHO cells.

clear ATP pools nearly doubled with respect to control values. (iv) The stability of our measured dNTP pools with respect to time of detergent treatment and washing means that if dNTP leakage is substantial within the treatment period, then there must be two distinct pools of each dNTP within the nucleus: a loosely bound pool (depleted within 10 s) and a tightly bound pool (stable for at least 30 s). While this is not impossible, Occam's razor plus the cytoplasmic localization of ribonucleotide reductase argue that most of the dNTP content lost during the NP-40 treatment does indeed represent cytoplasmic pools.

Another potential artifact was eliminated by scanning electron microscopy. Examination of a large number of nuclei showed no evidence of whole cells remaining unperturbed on the plates after treatment with the NP-40-containing nuclear isolation buffer, indicating (along with the marker enzyme data) that the pools isolated did not represent whole-cell contamination of nuclear preparations.

The results from our experiments have given us information not only on the relative distribution of dNTPs within the cell but also the nuclear concentrations of these metabolites. Estimation of the nuclear dNTP concentrations in synchronized CHO cells showed that there is an adequate supply of dNTPs to support DNA replication. By using the values determined by Skoog and Bjursell (25) for the volume of CHO cell nuclei during S phase, we calculate that the nuclear dNTP concentrations during S phase are the following: dCTP, 213 μ M; dATP, 104 μ M; dTTP, 88 μ M; and dGTP, 10 μ M. The K_m values for dNTPs for DNA polymer-

ase α are approximately 10 μ M each (9). Three of the four dNTPs have concentrations well above the K_m , while the fourth, dGTP, has a nuclear concentration quite close to the K_m . It is possible, then, that dGTP plays a role in regulating DNA replication through substrate limitation.

The distinctive distribution of the four dNTPs between nucleus and cytoplasm in HeLa cells, in addition to the relatively small CHO cell S-phase nuclear dCTP pool expansion compared with the corresponding whole-cell expansion, indicates that dNTPs are present at different concentrations in the two cellular compartments. However, the fact that HeLa nuclear dNTP values remained a constant proportion of the whole-cell levels at several different densities and the fact that three of the four dNTPs in synchronized CHO cells were distributed according to volume indicate that some mixing occurs between the two compartments. From our results, we hypothesize that compartmentation of dNTPs between nucleus and cytoplasm may be the result of equilibrium concentrations established by the interactions of enzymes that are differentially distributed between the two compartments. Our model is that dNTPs are freely permeable between the two compartments but that some of the dNTP-metabolizing enzymes are located exclusively in one compartment or the other. For example, ribonucleotide reductase seems clearly now to be a cytoplasmic enzyme, whereas DNA polymerase α is located exclusively in the nucleus (1). Other dNTP-metabolizing enzymes have not yet been clearly assigned to one compartment or the other, but work in this direction continues. Our hypothesis could explain why the dNTP concentrations are different between the two compartments, even though the dNTPs should move freely through nuclear pores. The high increase in cellular dCTP concentration during S phase, which may well result from the absence of dCMP deaminase in these cells, is not seen in the corresponding S-phase nuclear dCTP pool. The difficulty of synchronizing other cell lines by methods that do not perturb dNTP pools has kept us from extending these results. In any case, the primary site for dNTP synthesis is the cytoplasm, since that is where rNDP reductase is located. Therefore, the substrate channeling model for mammalian DNA replication (22, 23) requires modification.

In our CHO-K1 cell line, what prevents a large nuclear accumulation of dCTP? One might speculate upon the possible existence of nucleotide-catabolizing activities in the nucleus, which prevents a large imbalance of dNTPs within the nucleus. Newman and Miller (15) have proposed that in some CHO cell lines, a dCTPase, a dCMP deaminase, and cytidine kinase are transiently induced in response to UV irradiation. Although enzyme assay data were not presented, it is intriguing to consider that a dCTPase-like activity could be stimulated by or responsive to a very large dCTP pool. Jackson (12) and, more recently, Nicander and Reichard (17) have found that a large proportion of the dTTP in mammalian cells is derived from CDP via dCMP deaminase. Therefore, some mechanism for dephosphorylation of dCDP must exist. Experiments to search for this type of activity are currently under way. It is unlikely that dUTPase cleaves dCTP, as all previous work with mammalian dUTPase indicates a stringent specificity (4).

Although others have seen large increases in dNTP pools during S phase (14, 18), the accumulations we noted, with the exception of that of dCTP, largely mirror the increase in cell size. Although during S phase, the dNTPs are produced at greater rates than in G1 phase, their turnover rates for DNA replication increase simultaneously. In most metabolic pathways, one would not predict an accumulation of metab-

olites, and, in fact, the concentrations of most metabolites are tightly regulated. Recent work by Nicander and Reichard (17) shows that a substantial quantity of deoxyribonucleotides, once synthesized, are broken down and excreted by the cell. Therefore, actual dNTP concentrations may be regulated closely, and transient overproduction of dNTPs could trigger their degradation and subsequent excretion from the cell.

In summary, compartmentation of the dNTPs may be a kinetic phenomenon involving metabolite turnover rather than an active concentration of dNTPs into the nucleus. In future experiments, the emphasis will be on examining actual levels and rates of equilibration of dNTPs between the nucleus and cytoplasm.

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