Quantification of DNA Synthesis From Different Pathways in Cultured Human Fibroblasts and Myocytes

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We have quantified DNA synthetic rates from different pathways in cultured cells using a new stable isotope technique. Human fibroblasts and myocytes were grown in culture media supplemented with [U-$^{13}C_6$]glucose and [^{15}N]glycine. The cells were sampled daily from day 1 to day 5. A portion of the cells harvested at day 5 was subcultured for an additional 3 passages to reach isotopic plateau. In both cell types total DNA fractional synthetic rate (FSR) was found to agree closely with the rate of cell proliferation determined by cell counting (FSR = 0.94% · h^{-1} *v* 0.92% · h^{-1} for DNA synthesis and cell count, respectively, in myocytes and 0.85% · h^{-1} *v* 0.91% · h^{-1} for DNA synthesis and cell count, respectively, in fibroblasts). In fibroblasts the deoxyribonucleoside salvage pathway accounted for over 70% of total DNA synthesis. In myocytes the deoxyribonucleoside salvage pathway was minimal, whereas the de novo base synthesis pathway accounted for almost 80% of total DNA synthesis. We conclude that the contributions of various pathways to DNA synthesis are highly dependent on cell type. This new stable isotope technique can be modified for application in in vivo studies.

CELL DIVISION and protein synthesis are different, but potentially related, cellular processes. Thus, an increase in cell division without any change in protein synthesis per cell will result in an overall increase in protein synthesis. On the other hand, an overall increase of protein synthesis may come from a stimulation of the synthetic process in the absence of a change in the rate of cell division. Thus, it may be necessary to differentiate protein synthesis from cell division to understand the mechanisms responsible for regulation of changes in tissue protein content in various circumstances. To do so, a method is needed to quantify protein synthesis and cell division simultaneously. There are different techniques available to quantify protein synthesis in vivo and in vitro, but cell division in vivo cannot be determined directly. Because unscheduled DNA synthesis rarely occurs, cell division can be reflected by DNA synthesis. Therefore, measurement of DNA turnover can serve as a direct reflection of cell division.

DNA is comprised of deoxyribonucleotides that can either be synthesized de novo or produced from salvaged deoxyribonucleosides (Fig 1). Deoxyribonucleotides can be synthesized de novo from phosphoribosylpyrophosphate (PRPP) via a de novo base synthesis pathway in which atoms from glycine, glutamine, and aspartate are incorporated.[1] Deoxyribonucleotides can also be synthesized by combining PRPP with salvaged bases.[1] Thus, there are 3 basic pathways by which deoxyribonucleotides, and ultimately DNA, can be synthesized (Fig 1). It is desirable to not only measure total DNA synthesis, but to differentiate the individual pathways for DNA synthesis as well. In some pathophysiologic states, these pathways may be altered in different directions. Thus, in human fibroblasts with a deficiency in hypoxanthine-guanine phosphoribosyltransferase (the enzyme involved in the salvage base pathway), the incorporation of nucleotides from salvaged base was inhibited, but the de novo base synthesis pathway was simultaneously stimulated.[2] Of perhaps more general importance, ingestion of nucleic acids potentially provides precursors for nucleotide synthesis via the salvage pathway,[3] but activation of this pathway may suppress the de novo synthesis pathway.[4] Depending on the nutritional circumstance, the balance between these 2 responses determines if nucleic acid ingestion affects growth rate.[5] No previously described method allows the quantification of all the various pathways of DNA synthesis. Thus, it was our goal to develop a method that not only enables simultaneous quantification of total DNA, but also the relative contributions of the pathways of DNA synthesis. Further, we wanted to develop a method that could be applied in human subjects.

Determination of DNA synthesis using the tracer incorporation technique involves measuring the rate of incorporation of a tracer into the product (DNA) over time and dividing by the enrichment of the precursor. Determination of the enrichment of the true precursor for incorporation of tracer into DNA has been a problem.[6] The true precursor enrichment in a tracer incorporation study is defined by the ultimate plateau in product enrichment.[7] One approach has been to use ^2H_2O water as a precursor, thereby overcoming uncertainty about precursor enrichment because an isotopic equilibrium can be achieved in the entire water (ie, precursor) pool. However, it may require 2 days or more for sufficient tracer to be incorporated to accurately measure incorporation into DNA by mass spectrometry. Alternatively, some precursors, such as labeled glucose, can be directly incorporated rapidly enough to measure product enrichment in a matter of hours. However, with this approach, the precursor enrichment is uncertain. Previous experiments have consistently found a discrepancy between precursor enrichment in culture media and DNA plateau product enrichment.[6,8] Further, the extent of discrepancy has been found to be variable, depending on the cell type.[6] Thus, in the context of developing a method to quantify the various pathways of DNA synthesis, we have evaluated the possible factors contributing to the discrepancy. We have focused on 2 cell types of physiologic importance. Fibroblasts play a central role in skin protein

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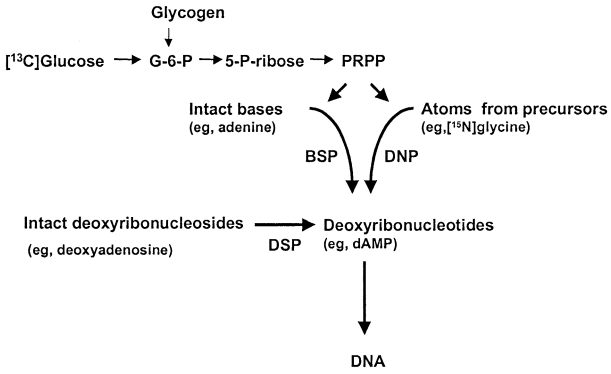

Fig 1. Pathways of DNA synthesis. BSP, base salvage pathway; DNP, de novo base synthesis pathway; DSP, deoxyribonucleoside salvage pathway.

metabolism and wound healing, and myocytes are a model for skeletal muscle. We have used stable isotopes of [U-$^{13}C_6$]glucose and [^{15}N]glycine to quantify the rate of DNA synthesis from different pathways.

MATERIALS AND METHODS

Cell Culture

We used a human breast fibroblast cell line (CRL-1947, American Type Cell Culture [ATCC], Manassas, VA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 40 μg/mL gentamycin sulfate as antibiotics, and 10% fetal bovine serum (containing various growth factors and free nucleosides) for optimal cell growth. The cells were seeded at a density of 1×10^6 cells/dish and were incubated at 37°C in a humidified atmosphere of ambient air with 5% CO_2. The culture media was changed every 48 hours. Cells were grown for 6 passages to obtain a sufficient numbers of cells to perform the experiment. Starting at day 0, cells were grown in DMEM supplemented with stable isotope tracers (Cambridge Isotope Lab, Andover, MA) of [^{15}N]glycine (67.5%) and [U-$^{13}C_6$]glucose (21.0%). (Numbers in parentheses are ratios of labeled to unlabeled substrates.) Cells were grown under these conditions to maintain exponential growth. Cells and culture media were sampled daily from day 0 to day 5. Cells were detached from culture dishes with 0.25% trypsin in EDTA for 10 minutes at 37°C, followed by centrifugation (1,500 rpm $\times$ 10 minutes). Cell numbers were counted with a hemocytometer and viability was assessed by trypan blue. As cell growth characteristics changes after reaching confluence, all of the cells in culture dishes were harvested at day 5 prior to confluence. A portion of the cells harvested at day 5 was subcultured for additional 3 passages to day 20 (5 days per passage) using the same isotope tracer enriched culture media. In this way, all of the new cells were grown in the same media with consistent growth characteristics to reach isotopic enrichment. All of the cell samples were stored at -80°C.

A human breast muscle cell line (CRC–2061, ATCC) was cultured in RPMI 1640 media (ATCC) supplemented with 10% fetal bovine serum and 40 μg/mL gentamycin sulfate. Myocytes were cultured in the media containing [^{15}N]glycine (90.7%) and [U-$^{13}C_6$]glucose (16.5%). The culture protocol was the same as in fibroblasts.

Analytical Methods

The DNA from cultured fibroblasts and myocytes was isolated by phenol-chloroform-isoamyl alcohol extraction. The isolated DNA was enzymatically hydrolyzed to deoxyribonucleosides using DNase I, Nuclease P1, phosphodiesterase, and alkaline phosphatase (Sigma, St Louis, MO).[9] The trimethylsilyl (TMS) derivatives of deoxyribonucleosides were made using bis (trimethylsilyl) trifluoroacetamide: pyridine (2:1) at 100°C for 1 hour. The enrichment of deoxyadenosine (dA) was measured by gas chromatography-mass spectrometry (GC-MS, HP 6890 GC and 5973 MS; Hewlett-Packard, Roseville, CA), using HP-5 capillary column (30 m $\times$ 0.25 mm, 0.25 μm film-thickness, Hewlett-Packard). The column temperature started at 150°C, increased to 300°C at 6°C/minute and was held for 5 minutes at 300°C. The dA peak was eluted at 20.5 minutes, identified by comparison to dA standard mass spectrum and retention time. The abundance of ions was monitored at m/z 467 and 468 (from [^{15}N]glycine) and ions at m/z 467 and 472 (from [U-$^{13}C_6$]glucose). Enrichment is expressed as the tracer/tracee ratio, which is the equivalent to the term "specific activity" when a radioloabeled tracer is used. Detailed description of the calculation of tracer/tracee ratio when selected ion monitoring is used is given in Rosenblatt et al.[10]

Amino acid concentrations in culture media were measured by high-performance liquid chromatography (HPLC) (Waters 1960; Waters, Milford, MA). Intracellular free amino acids were isolated as previously described.[11] Briefly, cultured cells were homogenized in 5% perchloric acid 3 times at 4°C. The pooled supernatant was used to measure intracellular free amino acid enrichment. Amino acids from cell supernatant and culture media were isolated using a cation exchange column (Dowex AG 50W-X8; Bio-Rad, Hercules, CA) and dried in a rotary vacuum evaporator. The isolated amino acids were derivatized with N-methyl-N-(t-butyldimethylsilyl) trifluoroacetamide at 100°C for 1 hour for [^{15}N]glycine enrichment measurements by GC-MS. The abundance of ions was monitored at m/z 160 and 161 for glycine.

Glucose concentration in culture media was measured with a 2300 STAT analyzer (Yellow Spring Instrument, Yellow Spring, OH). Glucose enrichment in culture media was measured following the procedure as described previously.[12] Pentacetate derivatization was used to measure the enrichment of [U-$^{13}C_6$]glucose. The abundance of ions was monitored at m/z 331 and 337.

Calculations

Fractional synthetic rates (FSR) for DNA were calculated as

$$\text{FSR} = \frac{E_{t2} - E_{t1}}{E_p \cdot (t_2 - t_1)}$$

where E_{t2}-E_{t1} is the change in product DNA enrichment between time points t_2 and t_1, and E_p is the average precursor enrichment over the time (t_2 to t_1). The linear change of product enrichment (ie, from day 1 to day 3) was used to calculate FSR. Various values were used for precursor enrichment, as described below.

The tracer precursor for calculation of the total rate of synthesis is defined by the plateau in product enrichment.[13] If [U-$^{13}C_6$]glucose is the tracer, the total rate of DNA synthesis should be accurately calculated by dividing the change in DNA-bound dA enrichment over time by the plateau enrichment in DNA-bound dA. If intracellular [U-$^{13}C_6$]glucose enrichment is used as the precursor (E_P), then the calculated FSR will include both the de novo base synthesis pathway and the base salvage pathway, but not the salvaged deoxyribonucleoside pathway (Fig 1). This is because both pathways involve incorporation of PRPP, which is entirely derived from glucose (Fig 1). However, intracellular glucose concentration is too low to measure enrichment. Consequently, we have calculated DNA synthesis using extracellular [U-$^{13}C_6$] glucose enrichment as an approximation of the true precursor for the calculation of DNA synthesis from the base salvage and de novo base synthesis pathway. This approximation is reasonable, since glycogen breakdown should be minimal and there is no evidence that gluconeogenesis occurs in these cells. Therefore, there

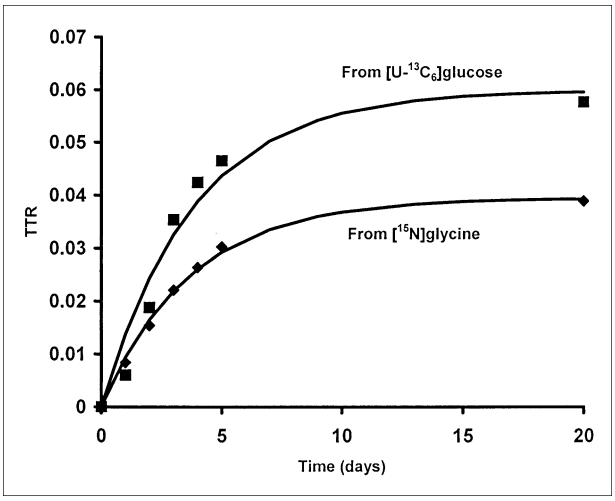

Fig 2. DNA-bound deoxyadenosine enrichments in fibroblasts. TTR, tracer/tracee ratio.

should be little dilution of labeled glucose as it enters the cell. Because the glucose tracer allows calculation of the total rate of synthesis and the de novo and salvaged base pathways, the contribution of the salvaged deoxyribonucleoside pathway to total synthesis can be calculated by deduction.

In contrast to the situation with glucose, [^{15}N]glycine will be incorporated into dA only via the de novo base synthesis pathway (Fig 1). Therefore, it is possible to distinguish the contributions of different pathways using [^{15}N]glycine (or [^{15}N]glutamine) in combination with [U-$^{13}C_6$]glucose. The contribution of the de novo synthesis pathway can be calculated using the intracellular glycine enrichment as precursor. Any dilution of the intracellular precursor reflected by the final product plateau enrichment would have come from the salvaged base pathway. Therefore, the difference in FSR, between the value calculated using media or intracellular [U-$^{13}C_6$]glucose enrichment precursor versus that calculated using the intracellular glycine enrichment as precursor, represents the contribution of the base salvage pathway (Fig 1), assuming the total is greater than the base salvage pathway.

Thus, the calculation of the 3 pathways contributing to DNA synthesis can be calculated as follows:

DNP (de novo base synthesis pathway) = glycine FSR using intracellular glycine as precursor; BSP (base salvage pathway) = glucose FSR using media glucose as precursor – DNP; DSP (deoxyribonucleoside salvage pathway) = glucose FSR using glucose plateau as precursor - glucose FSR using media glucose as precursor.

The fractional rate of cell proliferation by cell counting was calculated as the ratio of the increase of cell numbers within each day to the cell numbers at the beginning of the corresponding day.

RESULTS

The concentration and enrichments of amino acids and glucose were constant in culture media from day 1 to day 5 in both fibroblasts and myocytes. The intracellular free amino acid enrichments in cultured cells were also constant from day 1 to day 5.

Fibroblast Culture

Cell proliferation by cell counting. The average cell numbers increased from1.2 million cells/dish at day 0 to 3.7 million cells/dish at day 5. There was a linear increase from day 0 to day 3, and the net FSR was 0.91%/h. By day 5, 33% of the cells were derived from initial unlabeled cells (old cells). After 3 subsequent passages, the percentage of old cells dropped to 1.2% at day 20. Because 98.8% of the cells at day 20 were newly synthesized cells from labeled media, it is reasonable to assume that isotopic equilibrium in the product was achieved at day 20. This assumption was supported by the data (see below).

DNA synthesis by tracer method. The enrichments of DNA-bound dA from [U-$^{13}C_6$]glucose (m+5) and from [^{15}N]glycine (m+1) are shown in Fig 2. The dA enrichment (tracer/tracee ratio, TTR) from [U-$^{13}C_6$]glucose increased from zero at day 0 to 0.0354 at day 3. The plateau TTR value of dA at day 20 was 0.0577. The calculated total DNA FSR using the plateau TTR as E_P was 0.85% $\cdot$ h^{-1} (Table 1). This value compared favorably to the FSR determined directly by cell count (0.91% $\cdot$ h^{-1}). When the media glucose enrichment was used as E_P, the calculated value for DNA synthesis was 0.23% $\cdot$ h^{-1}. This value represents the sum of the rates of the base salvage pathway and the de novo base synthesis pathway (Fig 1). Thus, the rate of deoxynucleoside salvage pathway, which is the difference of total DNA rate and the sum, was 0.85 to 0.23 = 0.62 % $\cdot$ h^{-1}. The dA enrichment from [^{15}N]glycine also increased linearly from day 0 to day 3, reaching a TTR of 0.0221 at day 3. At day 5, the dA enrichment was close to the value of dA at day 20 (0.038), confirming that an isotopic plateau was achieved in the product at day 20. The DNA FSR from de novo base synthesis pathway calculated using the intracellular glycine enrichment as precursor, was 0.11% $\cdot$ h^{-1}. The base salvage pathway was 0.23 to 0.11 = 0.12% $\cdot$ h^{-1}.

Myocyte Culture

Cell proliferation by cell counting. The average myocyte numbers increased from 0.8 million cells/dish at the outset to 2.5 million cells/dish after 5 days of cell culture. The fractional cell growth was 0.92% $\cdot$ h^{-1}. By day 5, 32% of the cells were derived from initial unlabeled media (old cells). At day 20, 99.0% of the cells were newly synthesized myocytes. Thus, the product enrichment at day 20 was equal to the true precursor enrichment for calculation of total DNA FSR.

DNA synthesis by tracer method The enrichments of dA from [U-$^{13}C_6$]glucose and from [^{15}N]glycine are shown in Fig 3. The enrichment of dA from [U-$^{13}C_6$]glucose increased linearly from day 0 to day 3. At day 5, the dA enrichment (0.137) was close to plateau enrichment at day 20 (0.146). The DNA FSR calculated using the [U-$^{13}C_6$]glucose product plateau

Table 1. Fibroblast DNA FSR Comparison to Cell Count Using Different Precursor Enrichments

Precursor	E_p (TTR)	$E_{t2} - E_{t1}$* (TTR)	FSR (% $\cdot$ h^{-1})
[U-$^{13}C_6$]glucose			
Product plateau	0.0577	0.0354	0.85
Media glucose	0.210	0.0354	0.23
[^{15}N]glycine			
Intracellular free pool	0.292	0.0221	0.11
Cell count			0.91

Abbreviations: E_p, precursor enrichment; E_{t1}, product enrichment at time ti; TTR, tracer/tracee ratio.

*$t_2 - t_1$ = 72 hours.

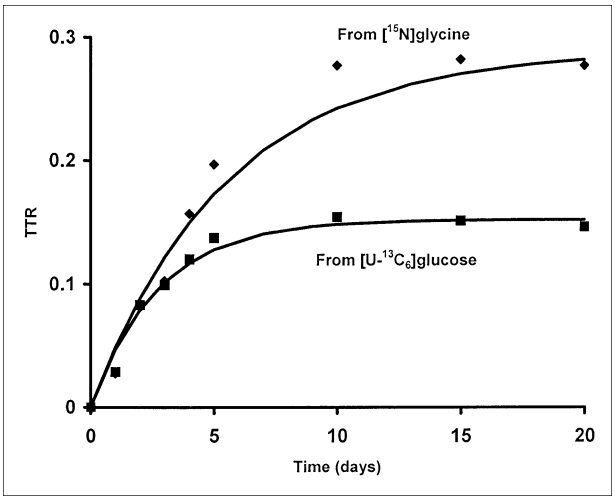

Fig 3. DNA-bound deoxyadenosine enrichments in myocytes.

value as E_P was 0.94% · h^{-1}. If the media [U-$^{13}C_6$]glucose was used as E_P, the calculated FSR (0.83% · h^{-1}) was close to that determined when the product plateau value was used (Table 2), meaning that the combination of the base salvage pathway and de novo base synthesis pathway accounted for most of total DNA synthesis. When the intracellular glycine enrichment was used as E_P to calculate the de novo base synthesis pathway, the rate was 0.74% · h^{-1}. Because the sum of the base salvage pathway and the de novo base synthesis pathway was close to the total rate of DNA synthesis, only about 10% of total DNA synthesis was via the deoxyribonucleoside salvage pathway, which was similar to the contribution of the base salvage pathway, which was found to be 0.09% · h^{-1}.

DISCUSSION

This study was designed to quantify the rates of DNA synthesis in isolated fibroblasts and myocytes grown in culture. Our goal was to develop methods potentially applicable to in vivo studies in human subjects. We assumed that the true rate of DNA synthesis was reflected by the rate of cell proliferation determined directly by cell counting. The tracer method of measuring total DNA synthesis was consistent with the rate of cell proliferation. Fibroblasts and myocytes had different characteristics regarding the pathways of deoxyribonucleotide synthesis. Whereas, the deoxyribonucleoside salvage pathway was an insignificant percentage of the total rate of DNA synthesis in myocytes, it was the major pathway of total DNA synthesis in fibroblasts. In myocytes the rate of de novo base synthesized nucleotides, measured by means of the [^{15}N]glycine tracer, yielded values within 20% of the total rate of DNA synthesis, whereas in fibroblasts only 13% of DNA synthesis was via the de novo base synthesis pathway.

Dynamic measurements of DNA synthesis traditionally involve administration of [^{3}H]thymidine or bromodeoxyuridine (BrdUrd). Whereas both of these tracers can provide clinically useful data regarding the rate of cell division, a qualitative index of DNA synthesis from the deoxyribonucleoside salvage pathway. It is then assumed that synthesis via this pathway is directly related tot the total rate of DNA synthesis. Distinction of individual pathways of synthesis is not possible with these tracers. Further, possible cellular toxicity and DNA damage induced by BrdUrd and [^{3}H]thymidine[14-16] exclude the use of these methods in vivo, particularly in human subjects. In addition, because deoxyuridine is not a subunit of DNA, the extent of cellular uptake of the tracer may be variable and BrdUrd may be discriminated in the DNA synthetic process from naturally-occurring deoxyribonucleosides. Further, DNA repair may occur via this pathway, which does not represent net DNA synthesis and would therefore not necessarily correspond to the rate of cell division. These problems, at least partially, explain the low labeling efficiency of BrdUrd[14,17] and discrepancy in the results of lymphocyte kinetics where BrdUrd or [^{3}H]thymidine was used.[16,18-20] Our stable isotope method avoids these potential pitfalls, and in addition uses stable isotopically-labeled tracers that are naturally occurring, and are not discriminated in the synthetic process (ie, agreement between DNA synthesis and cell division. Further, stable isotopes present no health risk for in vivo studies and can therefore be used for human in vivo studies.

Stable isotope techniques using [6,6-$^2H_2$]glucose, [U-$^{13}C_6$]glucose, and ^2H_2O have been developed to quantify the total DNA synthesis rates.[6,21] These methods have the advantage that they are not toxic. However, the information regarding individual synthetic pathways remains unknown. Because DNA synthesis involves multiple pathways, it is important to understand the mechanism of DNA synthesis in individual pathways, as well as the overall regulation. For this purpose, we have developed our new stable isotope technique to quantify not only the total DNA synthesis rate, but also the rates of individual DNA synthetic pathways.

To validate our new technique, we used cell proliferation rates measured by cell counting as a standard with which to compare the total DNA synthesis rates quantified by the tracer technique. The 2 measurements agreed in both myocytes and fibroblasts. It was not possible to validate the quantitation of the individual pathways, as no independent approach is available. Nonetheless, the validity of the calculation of the independent pathways is supported by the validation of the determination of the total rate of synthesis. Further, the differences in the relative rates of the individual pathways in the different cell types were calculated using the same technique, so that these comparisons were reasonable.

Dissecting and quantifying individual DNA synthetic pathways is clinically relevant. DNA synthesis is a complex process

Table 2. Myocyte DNA FSR Comparison to Cell Count Using Different Precursor Enrichments

Precursor	E_p (TTR)	$E_{t2} - E_{t1}$* (TTR)	FSR (% · h^{-1})
[U-$^{13}C_6$]glucose			
Product plateau	0.146	0.099	0.94
Media glucose	0.165	0.099	0.83
[^{15}N]glycine			
Intracellular free pool	0.191	0.102	0.74
Cell count			0.92

*$t_2 - t_1$ = 72 hours.

and impairment of one pathway can result in detrimental effects. The Lesch-Nyhan syndrome, characterized by hyperuricemia, choreoathetosis, spasticity, mental retardation, and self-mutilation, results from the inhibition of the purine salvage enzyme hypoxanthine phosphoribosyl-transferase (the enzyme catalyzing metabolic salvage of the purine bases with PRPP).[22] While a lack of this enzyme activity has been reported in the extracts of red cells, skin fibroblasts and biopsy brain tissues from Lesch-Nyhan patients, the incorporation of [^{14}C]glycine into urinary uric acid was found to be greatly increased (indicating possible stimulation in de novo base synthesis pathway).[23] Thus, impairment of one pathway may or may not be reflected in the total DNA synthesis rate. In addition, quantitative assessment of different synthetic pathways is essential to evaluate outcomes of clinical treatments and nutritional supplement (such as nucleosides). In this study, for the first time, we quantified dynamic rates of DNA synthesis from individual pathways. We found that the contributions of the deoxynucleoside salvage and purine base salvage pathways to total DNA synthesis vary greatly in different cell types, even though the total synthesis rates are similar.

In theory, product enrichment at isotopic steady state reflects the true precursor enrichment.[13] Although only limited studies have been performed using [U-$^{13}C_6$]glucose to quantify DNA synthesis, product DNA enrichment at plateau has been consistently found to be significantly lower than that of media glucose enrichment[6,8] (and the present report). At present, there is no clear explanation of this discrepancy. Gluconeogenesis within cells is a possible source of dilution. However, Macallan et al[6] have shown that gluconeogenesis was not an important factor in dilution, as there was much less dilution (72.5% of media enrichment) in gluconeogenic heptocytes than that in lymphocytes (52.5% of the media enrichment). The isotopic exchange from [U-$^{13}C_6$]glucose metabolism via glycolysis and nonoxidative pentose pathway may be another possibility, which would give rise to intracellular ribose molecules containing 1, 2, 3 or 4 ^{13}C (ie, m+1-m+4) [$^{13}C_5$]ribose (m+5). But, this, again, was not likely an important factor in our experiment. When culture media was 50% enriched with [U-$^{13}C_6$]glucose, Nissim et al[8] reported that, at isotopic plateau, the intracellular ribose m+5 enrichment was 46% and product DNA m+5 was 35%. The enrichment drop from media to intracellular ribose (50% to 46%) includes the dilution from gluconeogenesis, isotopic exchange, and other possible sources (ie, glycogenlysis). Because this drop is small compared with the drop from media to DNA product (50% to 35%), it is likely that the isotopic exchange effect on the dilution is trivial.

Our results provide an explanation for the discrepancy between the media glucose enrichment and the product plateau in enrichment. The deoxyribonucleoside salvage pathway appears to be the primary contributor to the dilution. In the present study, the contribution from nucleoside salvage pathway toward total DNA synthesis was 73% ([0.85 to 0.23]/0.85) in fibroblasts and 12% ([0.94 to 0.83]/0.94) in myocytes. Correspondingly, the dilution factor of dA (DNA enrichment)/$E_{\text{media glucose}}$ was 27% (0.0577/0.21) in fibroblasts and 88% (0.146/0.165) in myocytes. These data indicate that the dilution is primarily from the deoxyribonucleoside salvage pathway. Consistent with this interpretation, Nissim et al[8] reported a larger dilution between intracelluar ribose enrichment to product (46% to 35%) as compared with the dilution from media to intracelluar ribose (50% to 46%) in myelomonocytes.

In this study, [^{15}N]glycine was used to label the base moiety of deoxyadenosine to quantify DNA synthesis from the de novo base synthesis pathway. It is possible that the enrichment of m+1 in the base moiety includes recycling of the ^{13}C labels in [U-$^{13}C_6$]glucose via glycolysis and the TCA cycle, in which case de novo base synthesis pathway would be overestimated. If it is true, the labeling loss from [U-$^{13}C_6$]glucose metabolism would also result in an underestimation of the total DNA synthesis rate. However, in this study, we found that the total DNA synthesis rates quantified by [U-$^{13}C_6$]glucose agreed closely with the rates of cell proliferation in both myocytes and fibroblasts. Similar agreement has also been reported in hepatocytes and lymphocytes[6] and in myelomonocytes.[8] Therefore, the underestimation of the total DNA synthesis rate due to the [U-$^{13}C_6$]glucose labeling loss is negligible, indicating that possible overestimation of the de novo base pathway synthesis is unlikely.

In vitro cell culture is useful for testing certain assumptions of tracer methodology because the cells can be cultured for sufficient time to achieve a true isotopic plateau in the product, thereby revealing the true precursor enrichment. The results showed that if sufficient labeling time is allowed, the total DNA synthesis rate can be accurately determined with [U-$^{13}C_6$]glucose. However, using the product plateau enrichment as the precursor may not be practical for in vivo studies, particularly when quantifying DNA turnover in muscle, because many days of tracer infusion would be required. However, because the deoxyribonucleoside salvage pathway is only of minor importance in myocytes, the extracellular glucose enrichment provided a good approximation of the true precursor enrichment for calculation of total rate of DNA synthesis. Thus, for in vivo measurement of muscle total DNA synthesis, it would be reasonable to use the plasma glucose enrichment as precursor. In contrast, the same approach for measurement of total in vivo DNA synthesis rate in fibroblasts would likely result in a significant underestimation of the true value. Based on the in vivo data, the measured value for FSR via the de novo base synthesis pathway and the base salvage pathway would have to be divided by a correction factor of 0.27 to approximate the total FSR, assuming a linear relationship between the rate of total DNA synthesis and synthesis via the 2 pathways deriving from PRPP. Another potential problem with in vivo studies stems from our observation that our results indicate that the rates of DNA synthesis via various pathways is dependent on cell types. Therefore in vivo application of the method requires isolation of specific cell types before analysis or the assumption that the pooling of cell times in a tissue sample (eg, skin) provides interpretable data. The extent to which this represents a problem is tissue-specific.

In summary, we have developed a new stable isotope technique to quantify the DNA synthetic rate and the relative contribution of individual pathway. In myocytes and fibro-

blasts, the DNA synthetic rates quantified by tracer technique are consistent with the rates of cell proliferation. The relative contribution from individual pathway varies greatly between these 2 cell types. This newly developed stable isotope technique may be useful in determining mechanisms involved in the regulation of cell division in circumstances, such as wound healing, as well as uncontrolled cell proliferation in various cancers and human immunodeficiency virus (HIV).

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