Amidophosphoribosyltransferase Limits the Rate of Cell Growth-linked de Novo Purine Biosynthesis in the Presence of Constant Capacity of Salvage Purine Biosynthesis*

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Factors controlling relative flux rates of the de novo and salvage pathways of purine nucleotide biosynthesis during animal cell growth are not fully understood. To examine the relative role of each pathway for cell growth, three cell lines including CHO K1 (a wild-type Chinese hamster ovary fibroblast cell line), CHO ade^-A (an auxotrophic cell line deficient of amidophosphoribosyltransferase (ATase), a presumed rate-limiting enzyme of the de novo pathway), and CHO ade^-A transfected with human ATase cDNA (A+hATase) resulting in 30–50% of the ATase activity of CHO K1, were cultured in purine-rich or purine-free media. Based on the enzyme activities of ATase and hypoxanthine phosphoribosyltransferase, the metabolic rate of the de novo and salvage pathways, the rate of cell growth (growth rate) in three cell lines under various culture conditions, and the effect of hypoxanthine infusion on the metabolic rate of the de novo pathway in rat liver, we concluded the following. 1) In A+hATase transfectants, ATase activity limits the rate of the de novo pathway, which is closely linked with the growth rate. 2) Purine nucleotides are synthesized preferentially by the salvage pathway as long as hypoxanthine, the most essential source of purine salvage, can be utilized, which was confirmed in rat liver in vivo by hypoxanthine infusion. The preferential usage of the salvage pathway results in sparing the energy expenditure required for de novo synthesis. 3) The regulatory capacity of the de novo pathway (about 20%) was larger than that of the salvage pathway (about 20%) with constant hypoxanthine phosphoribosyltransferase activity.

Purine nucleotides synthesized via de novo and salvage pathways are indispensable for cell growth through DNA and RNA syntheses and for the ATP energy supply. Interference of purine metabolism has thus been the target of antineoplastic drugs. However, it is unclear which of the two pathways is more important for the supply of purine nucleotides during cell growth. Purine nucleotide synthesis catalyzed by HPRT, the key enzyme of the purine salvage pathway, was reported to be more active than that catalyzed by ATase, the presumed ratelimiting enzyme of the de novo pathway, in many tissues and malignant cells (1, 2). The increased metabolic rate via the de novo pathway and ATase activity are, however, more strongly linked with cell growth and malignant transformation than the metabolic rate via the salvage pathway and HPRT activity (1, 3–7). To interpret this enigma, we investigated the mutual regulation of purine biosynthesis between the de novo and salvage pathways. The cDNA cloning of rat and human ATase (hATase) in our laboratory (8, 9), an ATase-deficient auxotrophic Chinese hamster ovary fibroblast cell line of CHO ade^-A, and CHO ade^-A transfected with hATase cDNA driven by the cytomegalovirus promoter (A+hATase) enabled us to explore the rate-limiting property of ATase and to study the relationship between the two pathways in three cell lines in two purine-free or two purine-rich media relative to their contribution to cell growth. Furthermore, the effect of continuous Hx supplement on the metabolic rate of de novo synthesis was examined in rat liver.

EXPERIMENTAL PROCEDURES

Cell Culture—CHO K1 (wild type), CHO ade^-A (10), which were gifts from Dr. David Patterson (Eleanor Roosevelt Institute for Cancer Research, CO), and A+hATase were cultured at 37 °C in a CO2 incubator in the following media: (I) Ham’s F-12-purine-rich medium containing 30 μM Hx with 10% fetal calf serum (FCS); (II) Ham’s F-12 with 10% FCS treated with 1.25 mg (0.9 unit)/liter xanthine oxidase (XO) from buttermilk (Sigma) at 37 °C overnight, serving as a purine-free medium; (III) RPMI 1640 purine-free medium supplemented with 10% purine-free FCS; (IV) RPMI 1640 with 10% purine-free FCS and 30 μM Hx, serving as a purine-rich medium. In media I and IV, both the de novo and salvage pathways function in CHO K1 and A+hATase, but only the salvage pathway functions in CHO ade^-A. In media II and III, only the de novo pathway functions in CHO K1 and A+hATase, and neither of the two pathways functions in CHO ade^-A. Purine-free FCS was prepared by dialysis against 100-fold volumes of 0.9% NaCl with several exchanges of the dialysis solution at 4 °C for 24 h. The complete removal of Hx in media II and III was confirmed first by the disappearance of the Hx peak in the reversed phase high performance liquid chromatography analysis through a C18 column in 50 mM potassium phosphate buffer (pH 4.6) with a gradient from 0 to 5% of methanol for 20 min at the flow rate of 1.5 ml/min (data not shown), and second by no growth of CHO ade^-A in these media (Table I). Transfection of CHO ade^-A Cells with hATase cDNA—A+hATase cDNA (2.2 kilobase pairs) was inserted into the cloning site downstream of the cytomegalovirus promoter in pBCMGSNeo (14.5 kilobase pairs), which includes the replication origin of bovine papilloma virus (BPV) leading to 10–50 copies per cell in mammalian cells (11, 12). Five micrograms of this plasmid construct purified with the Plasmid Maxi Kit (QIAGEN, Hilden, Germany) were mixed with Transfectam solution (BioSera PRPP, 5-phosphoribosyl 1- pyrophosphate; XO, xanthine oxidase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.)
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Inc., Marlborough, MA) according to the manufacturer’s protocol, and overlaid onto CHO ade−2,8 T10 cells/90 mm tissue culture plate) in 5 ml of Opti-MEM (Life Technologies, Inc.). After the incubation at 37 °C for 6 h in a CO2 incubator, the DNA-containing medium was replaced with 10 ml of Ham’s F-12 with 10% FCS and 500 μg/ml G418 to select the clones. After the recovery of cell functions from plating by 18 h of G418 selection, a high expression of the HPRT transscript was achieved. Several clones of A+ATase were isolated after G418 selection for 7 days. To select clones with high ATase activity, A+ATase was further cultured in Ham’s F-12 + XO medium for 7 days. The culture in this purine-free medium positively selected the clones with high ATase activity associated with a high growth rate. The number of copies in these clones was determined from cell counts during the logarithmic growth phase and its reciprocal was defined as the growth rate.

Measurement of the Growth Rate—In a 35-mm dish, 1 × 106 cells were plated in 2 ml of medium and incubated at 37 °C in a CO2 incubator. After culturing for 24, 48, 72, or 96 h, the cells were counted after trypsinization with 0.1% trypsin and 0.02% EDTA, using an improved Neubauer hemocytometer. The doubling time (hours) of cultured cells was determined from cell counts during the logarithmic growth phase and its reciprocal was defined as the growth rate.

Determination of Metabolic Rates of de Novo and Salvage Pathways—The metabolic rates of de novo and salvage pathways were, respectively, determined by the incorporation of [14C]glycine and [14C]Hx in acid-soluble purines in comparison to the acid-insoluble [14C]Hx by high voltage paper electrophoresis at 800 W for 15 min, and [14C]Hx was administered intravenously. The rat liver was sampled exactly 30 min after the injection by freeze-clamping, and the liver sample pulverized under liquid nitrogen was placed in 4 ml of 2 M perchloric acid. The metabolic rate of the de novo pathway was then determined by the procedures mentioned above. To examine the effect of the continuous supply of Hx on the increased rate of the de novo pathway by glucagon in liver (19), 0.5 mg of glucagon/200 g of body weight/12 h or without Hx of 5.0 μmol/200 g of body weight/12 h was similarly infused, and the metabolic rate of the de novo pathway in liver was determined.

Animal Study—To examine the effect of the continuous supply of Hx on the metabolic rate of the de novo pathway in rat liver in vivo, 5.0 μmol/200 g of body weight/12 h of Hx were continuously infused for 12 h via an intravenous cannula into the jugular vein of 8-week-old Wistar rats, according to the method of previous studies. Thirty minutes before the end of infusion, 185 kBq/44–45 nmol [14C]glycine was administered intravenously. The rat liver was sampled exactly 30 min after the injection by freeze-clamping, and the liver sample pulverized under liquid nitrogen was placed in 4 ml of 2 M perchloric acid. The metabolic rate of the de novo pathway in liver was determined.

Mutual Regulation between Two Purine Biosynthetic Pathways—There were three treatments: (1) Hx alone, (2) glutamine alone, and (3) Hx and glutamine together. The metabolic rate of the de novo pathway by glucagon in liver (19), 0.5 mg of glucagon/200 g of body weight/12 h or without Hx of 5.0 μmol/200 g of body weight/12 h was similarly infused, and the metabolic rate of the de novo pathway in liver was determined.

The stimulative effect of glucagon on the de novo purine synthesis in liver has been reported (19, 20) and is applied to glucagon and insulin therapy for acute liver failure.

Statistical Analysis—All data were presented as means ± S.D. The number of repetitive determinations for each value or point is as follows: six to eight for Table I, three for Table IV, three to four for Tables V and VI and Fig. 3, and two to four for Figs. 1 and 2. For comparison of data, Student’s paired or unpaired t test was used. A probability of less than 0.05 was considered statistically significant.

RESULTS

Effect of Purine Bases on the Growth Rate—To determine the metabolic rate of CHO fibroblasts, ATase-deficient CHO ade−2 cells were cultured in purine-free RPMI medium supplemented with Hx, adenine, or guanine at various final concentrations of up to 50 μM. Of the three purine bases, Hx most effectively increased the growth rate, while no cell growth was observed with guanine as the only source of the salvage pathway. Because Hx at a concentration of more than 30 μM increased the growth rate of CHO ade−2 fibroblasts to the maximal plateau level, and because adenine in addition to 30 μM Hx inversely decreased the growth rate (data not shown), it was considered that ATase activity at the concentration over 30 μM drives the flux through the salvage pathway at its maximum in CHO ade−2 fibroblasts. These observations demonstrated that ATase is the principal source for the salvage pathway in CHO ade−2 fibroblasts.

Characterization of A+ATase—Six clones of A+ATase obtained after G418 selection in purine-free medium were cultured in purine-rich medium. The level of ATase expression in these clones ranged from about 30 to 350% of that in CHO ade−2. The level of ATase expression (plotted on the y axis) strongly correlated with the metabolic rate of the de novo pathway and with the growth rate of CHO ade−2 fibroblasts (Fig. 1), with correlation formulas of y = 0.00047x − 0.53 (r = 0.83) and y = 0.0045x − 3.8 (r = 0.95), respectively. The metabolic rate of the de novo pathway also strongly correlated with the growth rate (r = 0.93, data not shown). It is notable that...

Northern blot Analysis of HPRT—Because the HPRT activity in CHO fibroblasts was nearly constant under all of the experimental conditions in this study (see Table VI), mRNA levels of HPRT were examined by Northern blot analysis using rat HPRT cDNA as a probe. For the preparation of the probe, total RNA was extracted from rat 3Y1 fibroblasts by ISOGEN (Nippon Gene, Osaka, Japan) and reverse-transcribed to cDNA with the BPhV reverse transcriptase first-strand cDNA synthesis kit (Life Sciences, Inc., St. Petersburg, FL). Rat HPRT cDNA in 69 base pairs (a sense primer; 5′-GACCAGTCCGATCGTCCAGCCT-3′ and an antisense primer; 5′-CTTGTATTCCGATTCCGCTGT-3′) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA in 621 base pairs (a sense primer; 5′-TTGCTATGCAGGCATGGAGAC-3′ and an antisense primer; 5′-TTTCTATTGAGGACGTTCCAGG-3′) were amplified by the polymerase chain reaction using the primer pairs shown in parentheses and cloned into a pCR II vector (Invitrogen Corp., San Diego, CA). The base sequence of the cloned polymerase chain reaction products was confirmed to be identical to the reported sequence by the fluorescent automated DNA sequencer (ABI 377, Perkin-Elmer, Division of Applied Biosystems, Foster City, CA). Northern blot analysis was performed by the standard method (14).

Animal Study—To examine the effect of the continuous supply of Hx on the metabolic rate of the de novo pathway in rat liver in vivo, 5.0 μmol/200 g of body weight/12 h of Hx were continuously infused for 12 h via an intravenous cannula into the jugular vein of 8-week-old Wistar rats, according to the method of previous studies. Thirty minutes before the end of infusion, 185 kBq/44–45 nmol [14C]glycine was administered intravenously. The rat liver was sampled exactly 30 min after the injection by freeze-clamping, and the liver sample pulverized under liquid nitrogen was placed in 4 ml of 2 M perchloric acid. The metabolic rate of the de novo pathway was then determined by the procedures mentioned above. To examine the effect of the continuous supply of Hx on the increased rate of the de novo pathway by glucagon in liver (19), 0.5 mg of glucagon/200 g of body weight/12 h or without Hx of 5.0 μmol/200 g of body weight/12 h was similarly infused, and the metabolic rate of the de novo pathway in liver was determined.

The stimulative effect of glucagon on the de novo purine synthesis in liver has been reported (19, 20) and is applied to glucagon and insulin therapy for acute liver failure.
that the doubling time of the A+hATase clone with the highest ATase expression among the 6 transfectants examined was shortened down to 10.5 h, i.e. 9.5 \times 10^{-5} h of growth rate, from the initial 16.8 h for wild-type CHO K1 cells. This correlation does not appear to be saturated even at the highest ATase activity of 350% of that in CHO K1. These results obtained from A+hATase clearly demonstrated that ATase is a rate-limiting enzyme of the de novo pathway. The levels of ATase activity in A+hATase clones closely correlated with their growth rate even under conditions where purine nucleotide synthesis by means of the salvage pathway was available in purine-rich medium (Fig. 1).

The growth rate of A+hATase clones gradually increased during their positive selection in purine-free medium. The A+hATase clone with the lowest ATase activity was cultured in purine-free medium up to passage number 30, where its growth rate reached the maximal plateau. The number of copies of the transgene, the ATase activity, the metabolic rate of the de novo pathway, and the growth rate were determined in Ham’s F-12, a purine-rich medium. The hatched bar represents the range of ATase activity in wild-type CHO K1 cells.

The growth rate of 5.2 \times 10^{-2} h achieved by the participation of both pathways was lower than the additive contribution of the de novo pathway to the salvage pathway was also significantly greater than that of the salvage pathway (4.2 versus 3.4 \times 10^{-2} h). The growth rate of 5.2 \times 10^{-2} h achieved by the participation of both pathways was lower than

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**TABLE I**

| Culture Cells | (I) HamF | (II) HamF+XO | (III) RPMI-XO | (IV) RPMI+X-Hx |
|---------------|----------|--------------|---------------|----------------|
| CHO K1        |          |              |               |                |
|               | 16.8 ± 0.4 (6) | 20.9 ± 0.3 (6) | 31.5 ± 1.5 (6) | 22.8 ± 0.6 (6) |
| CHO ade A     |          |              |               |                |
|               | 26.0 ± 2.5 (6) | 21.2 ± 0.6 (6) | 24.3 ± 1.5 (6) | 20.4 ± 1.1 (6) |
| *A+hATase     |          |              |               |                |
|               | 18.5 ± 0.3 (6) | 21.2 ± 0.6 (6) | 24.3 ± 1.5 (6) | 20.4 ± 1.1 (6) |

* Both de novo and salvage pathways.
* Only de novo pathway.
* Only salvage pathway.
* Neither of the two pathways is functioning.
* (n), sample number;
* p < 0.05; ** p < 0.01. HamF, Ham’s F-12.

**TABLE II**

See Table I for an explanation of symbols and letters. The reciprocal of doubling time was defined as the growth rate.

**Doubling Time and Growth Rate in Three Cell Lines under Four Culture Conditions**—Three cell lines including CHO K1, CHO ade A, and the A+hATase clone stably expressing ATase activity at the same level as CHO K1 were used. The doubling time and the growth rate of the three cell lines of CHO fibroblasts under the various culture conditions tested in this study are summarized in Tables I and II, respectively. In purine-free medium, CHO ade A did not proliferate at all. The growth rate under conditions where both purine biosynthetic pathways were functioning were significantly higher than those under conditions where either the de novo or the salvage pathway was functioning. The difference in the growth rate between the two different conditions was calculated and presented as the increase in the growth rate in Table III. The independent contribution of the de novo pathway in the absence of the salvage pathway (4.2 versus 3.4 \times 10^{-2} h). The additive contribution of the de novo pathway to the salvage pathway was also significantly greater than that of the salvage pathway (4.2 versus 3.4 \times 10^{-2} h). The growth rate of 5.2 \times 10^{-2} h achieved by the participation of both pathways was lower than
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The sum of $7.6 \times 10^{-3}$/h obtained by adding $4.2 \times 10^{-3}$/hr through the \textit{de novo} pathway alone and $3.4 \times 10^{-3}$/hr through the salvage pathway alone. To interpret the mechanism of this nonadditive antagonism, the metabolic rates of both pathways and the enzyme activities of ATase and HPRT, the key enzymes of the \textit{de novo} and the salvage pathways, respectively, were determined.

**Metabolic Rates of de Novo and Salvage Pathways in Three Cell Lines under Four Culture Conditions**—To explain the antagonism between two purine biosynthetic pathways, the metabolic rates of the \textit{de novo} and the salvage pathways were assayed by the incorporation of $[^14]C$glycine and $[^14]C$Hx, respectively (Table IV). When both pathways were functioning, the metabolic rate of the \textit{de novo} pathway was suppressed by $-69$ and $-64\%$ in CHO K1 and $\text{A}+\text{hATase}$, respectively, compared with that when only the \textit{de novo} pathway was functioning. On the other hand, the salvage pathway activity was modestly suppressed through the additive availability of the \textit{de novo} pathway only by $-17$ and $-24\%$ in CHO K1 and $\text{A}+\text{hATase}$, respectively, compared with that when only the salvage pathway was functioning. These results indicate that purine nucleotides are synthesized preferentially via the salvage pathway as long as CHO fibroblasts are allowed to use the salvage pathway. Moreover, the preferential utilization of the salvage pathway with the concomitant suppression of the \textit{de novo} pathway led to a large reserve capacity of \textit{de novo} synthesis. From a comparison between the metabolic rates of CHO K1 cultured in Ham’s F-12 and Ham’s F-12 + Hx, the reserve capacity of \textit{de novo} synthesis of CHO K1 in Ham’s F-12 was estimated at $218\%$ ($0.89 - 0.28/0.28 \times 100$), whereas the reserve capacity of the salvage pathway of CHO K1 in Ham’s F-12, compared with CHO ade $\text{A}$ in Ham’s F-12, was estimated at only $20\%$ ($2349 - 1962/1962 \times 100$).

**ATase and HPRT Activities in Three Cell Lines under Four Culture Conditions**—The enzyme activities of ATase and HPRT under four culture conditions were summarized in Table IV.

**Northern Blot Analysis of HPRT mRNA**—Although little change of HPRT activity was observed in CHO fibroblasts during growth arrest (Table VI), Northern blot analysis of HPRT showed an obvious decrease in growth-arrested CHO fibroblasts. The stability of the HPRT enzyme activity despite its decreased mRNA is probably due to the long half-life of the HPRT protein rather than constant transcriptional activity or mRNA stability.

**Animal Study**—Hx infusion through a cannula into the jugular vein of 8-week-old male Wistar rats for 12 h suppressed the metabolic rate of the \textit{de novo} pathway from $0.19 \pm 0.02$ (n = 4) with saline infusion to $0.09 \pm 0.02$ (n = 4), or $-53\%$. Furthermore, the increased metabolic rate of the \textit{de novo} pathway by $295\%$ by glucagon infusion ($0.56 \pm 0.05, n = 5$) was completely canceled by the concomitant infusion of Hx ($0.17 \pm 0.06, n = 4$). Thus, the preferential utilization of the salvage pathway with the suppressed metabolic rate of the \textit{de novo} pathway observed in CHO fibroblasts was ascertained by the decrease in the metabolic rate of the \textit{de novo} pathway in liver during Hx infusion in this animal study.

**Effect of Purine Bases on the Growth Rate**—Hx, guanine, and adenine incorporated into cells are converted to IMP, GMP, and AMP, respectively, by the catalysts of HPRT and adenine phos-
phosphoribosyltransferase. In cells, IMP is converted to GMP and AMP as required. However, interconversion between GMP and AMP through IMP is often insufficient for a balance of these two purine nucleotides (21). Therefore, generally Hx is the most important source, and HPRT is the most important enzyme for the salvage pathway. Indeed, of the three purine bases, Hx had the greatest effect on the growth rate of CHO ade^-A fibroblasts. Hx at a concentration of more than 30 $\mu$M increased the growth rate of CHO ade^-A fibroblasts to its maximum, but a further addition of adenine decreased the maximal growth rate. In mitogen-stimulated human T cells and a human B lymphoblast cell line of WI-L2, under the inhibition of de novo synthesis with aminopterin, the addition of 30 $\mu$M Hx was reported to restore the growth rate and DNA and protein syntheses to normal levels (22). The inhibitory effect of adenine observed in our study is compatible with the reported inhibitory effect of adenine and adenosine on the growth of WI-L2, although the mechanism is unknown (23).

Characterization of A+hATase—The molecular cloning of mammalian ATase made it possible to test the hypothesis of the rate-limiting property of ATase for de novo synthesis utilizing an ATase-deficient auxotrophic cell line of CHO ade^-A. After the transfection of CHO ade^-A fibroblasts with hATase cDNA, the ATase activity, the metabolic rate of the de novo pathway, and the growth rate were closely correlated for six clones of A+hATase (Fig. 1) and for different passage points of one clone of A+hATase, with an increase in ATase activity along with its passage (Fig. 2). These results for the first time provided molecular evidence that ATase limits the rate of the de novo purine synthetic pathway. ATase was linked with the growth rate even in a purine-rich medium where the salvage pathway was also functioning. It should be noted that the increase in the growth rate was not saturated even with an ATase activity as high as 350% of the level in CHO K1 (Fig. 1).

The doubling time of the A+hATase clone with the highest ATase expression was as short as 10.5 h. Such rapid cell growth was hardly observed even in undifferentiated neoplasms. ATase may function as a progression factor for malignant cells, a transforming factor, or a product of proto-oncogene. The transforming ability of ATase could not, however, be examined in our system because we used already transformed CHO fibroblasts and the BPV vector with the low oncogenic potential (13). Furthermore, the ATase activity, the metabolic rate of the de novo pathway, and the growth rate of A+hATase gradually increased in purine-free medium with an increasing number of copies of the transgene (Fig. 2). The number of copies of the BPV vector was reported to be maintained constant in each transfectant, although it differed for the various transfectants (12, 13). The number of copy of the BPV vector in one transfectant in this study was, however, demonstrated to gradually increase from 10 to 40 per cell during its maintenance through 24 passages in the purine-free medium. The purine-free medium was considered to have served as a positive selective pressure for the high ATase activity associated with the high growth rate.

The Energy-saving Regulation of Metabolic Rates via Two Synthetic Pathways—Although the de novo pathway contributed to the increase in the growth rate more significantly than the salvage pathway, an antagonism between the two pathways was observed when both pathways were functioning (Table III). The determination of the metabolic rates of both pathways under various conditions revealed that the inhibition of the de novo pathway by the salvage pathway is much greater than that of the salvage pathway by the de novo pathway (Table IV). Purine nucleotides were thus shown to be synthesized preferentially via the salvage pathway with the concomitant suppression of de novo synthesis, as long as the salvage pathway can be utilized. This regulation in CHO fibroblasts results in a sparing of the energy expenditure inevitably required for the de novo pathway, in which many ATP molecules are required for purine nucleotide synthesis.

Regulation of ATase and HPRT Activities—ATase expression is regulated by means of the purine repressor in bacteria (24, 25). The purine repressor with a purine corepressor, either Hx or guanine, specifically binds its 16-base pair operator site, and suppresses ATase expression. Through this mechanism, purine
nucleotides in bacteria are synthesized preferentially via the salvage pathway with the concomitant suppression of de novo synthesis, as long as substrates for the salvage pathway are available. To determine whether a repressor-type mechanism is functioning in CHO fibroblasts, the enzyme activities of ATase and HPRT were assayed (Tables V and VI). Because ATase and HPRT are fully activated under the assay conditions in this study, the enzyme activities directly reflect the amount of the corresponding enzymes. Although the metabolic rate of the de novo pathway was distinctly suppressed when both pathways were functioning (Table IV), the ATase activity under this condition was even higher than that when only the de novo pathway was functioning (Table V). The mechanism by which the salvage pathway inhibits the de novo pathway is thus not due to the decrease in ATase expression, unlike the repressor mechanism in bacteria. The suppression of the metabolic rate of the de novo pathway by the salvage pathway probably results from 1) the increased feedback inhibition of ATase by purine nucleotides produced via the salvage pathway and 2) the consumption of PRPP, a common substrate for both pathways and an activator of ATase, through the salvage pathway. The close correlation of ATase expression with the growth rate irrespective of the activity of the salvage pathway (Fig. 3A) suggested that ATase expression is linked with the signal for cell growth rather than the concentrations of PRPP and purine nucleotides.

Because the HPRT activity, unlike the ATase activity, was nearly constant (Table VI), the modest suppression of the salvage pathway by the de novo pathway (Table IV) is not due to a decrease in HPRT expression, but is presumably due to PRPP consumption via the de novo pathway. A large consumption of PRPP by de novo synthesis was suggested by the 4-fold increase in PRPP concentration in murine leukemia cells treated with 6-mercaptopurine and methotrexate to inhibit de novo synthesis (26).

**Regulation of HPRT Activity**—Even after the growth arrest of CHO ade A fibroblasts in purine-free medium for 48 h, HPRT activity did not decrease (Table VI), while the HPRT mRNA level was remarkably decreased (Fig. 4), suggesting that HPRT is a relatively stable protein. In human peripheral lymphocytes, the half-life of the HPRT protein was reported to be more than 48 h, in contrast to an estimated half-life of HPRT mRNA of only 5.1 h (27).

**Animal Study**—Intravenous infusion of Hx to 8-week-old male Wistar rats for 12 h suppressed the metabolic rate of the de novo pathway by ~53% and canceled its increase by glucagon stimulation. Thus, the preferential utilization of the salvage pathway with the suppression of the de novo pathway was observed not only in CHO fibroblasts, but also in rat liver in vivo. A preference for the salvage pathway is probably due to the following factors: 1) In many tissues and cultured cells, the specific activity of HPRT is much higher than that of ATase (1). For example, the ratio of HPRT activity to ATase activity is 6.6 in rat liver (2), or about 10 in CHO fibroblasts (Tables V and VI). 2) The affinity of HPRT to PRPP, a substrate shared by both enzymes, is markedly higher than that of ATase; the $K_m$ of HPRT to PRPP is 4 to 40 $\mu M$ and that of ATase is 400~900 $\mu M$ (1, 2, 7). 3) Purine nucleotides produced via the salvage pathway allosterically inhibit ATase, whereas HPRT is not affected. It was reported that physiological concentrations of Hx strongly suppress de novo synthesis in human bone marrow in vivo (28).

Although the salvage pathway is preferentially utilized, its reserve capacity for purine synthesis during cell growth was small because of the nearly constant activity of HPRT (Table VI). In contrast, the reserve capacity of de novo synthesis for changing its metabolic rate was relatively high, and could be attained through several regulatory mechanisms including the allosteric activation or inhibition of ATase activity (29, 30), the cell cycle-dependent expression of the ATase gene (31), and the relatively short half-life of the ATase protein. Resting human T lymphocytes were reported to meet their metabolic demands via the salvage pathway except during cell growth, while intact de novo synthesis is essential for the proliferation of photo- and glutaminin-stimulated T lymphocytes (32). The large capacity of de novo synthesis has been shown in patients with Lesch-Nyhan’s syndrome (complete deficiency of HPRT) and in cultured cells with this syndrome. Although these patients are affected with severe lesions in the central nervous system, the growth and development of other organs are almost normal due to compensation by de novo synthesis and, in part, the salvage pathway by adenine phosphoribosyltransferase. Fibroblasts from these patients showed a normal PRPP concentration and nucleotide pools were compensated by an increased de novo synthesis (30). Also, the proliferation of T lymphocytes from the patients in response to mitogenic and antigenic stimulation was normal (33). In the leukocytes from these two gouty patients affected with a partial deficiency of HPRT, de novo synthesis was accelerated to more than 13 times that of normal controls (34).

The regulatory factors of ATase gene expression, which is essential for the high capacity control of de novo synthesis, include tissue specificity (1), cell cycle (31), cell density, malignant transformation (1, 2, 6, 7), and differentiation (35). Biochemical and molecular biological studies are underway in our laboratory to investigate the regulatory mechanism of ATase gene expression and its activity.

In conclusion, the following concepts were supported in this study using CHO fibroblasts and rat liver. First, the ATase activity in CHO transfectants with hATase cDNA, which ranges from 30 to 350% of that of wild-type CHO K1, limits the metabolic rate of de novo synthesis and strongly correlates with the growth rate. This was confirmed by a parallel increase in the ATase activity, the metabolic rate of the de novo pathway, and the growth rate of one CHO transfectant with hATase cDNA relative to its passage in purine-free medium. Second, purine nucleotides are synthesized preferentially through the salvage pathway not only in cultured fibroblasts, but also in rat liver in vivo, as long as a source of purine salvage is available, with the concomitant suppression of the de novo pathway. This mechanism results in the sparing of energy expended by de novo synthesis. Last, the complex regulatory mechanisms of the gene expression and enzyme activity of ATase compared with the relatively constant enzyme activity of HPRT provide for a larger reserve capacity of de novo synthesis than of salvage synthesis, and reflect the molecular basis for meeting the metabolic demands required for the increased rate of cell growth.

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