Phosphoribosylpyrophosphate (PRPP) synthetase (PRS) superactivity is an X-linked disorder characterized by goit with overproduction of purine nucleotides and uric acid. Study of the two X-linked PRS isoforms (PRS1 and PRS2) in cells from certain affected individuals has shown selectively increased concentrations of structurally normal PRS1 transcript and isoform, suggesting that this form of the disorder involves pretranslational dysregulation of PRS1 expression and might be more appropriately termed overactivity of normal PRS. We applied Southern and Northern blot analyses and slot blotting of nuclear runoffs to delineate the process underlying aberrant PRS1 transcript expression in fibroblasts and lymphoblasts from patients with overactivity of normal PRS. Neither PRS1 amplification nor altered stability or processing of PRS1 mRNA was identified, but PRS1 transcription was increased relative to GAPDH (3- to 4-fold normal in fibroblasts; 1.9- to 2.4-fold in lymphoblasts) and PRS2. Nearly coordinate relative increases in each process mediating transfer of genetic information from PRS1 transcription to maximal PRS1 isoform expression in patient fibroblasts further supported the idea that accelerated PRS1 transcription is the major aberration leading to PRS1 overexpression. In addition, modulated relative increases in PRS activities at suboptimal Pi concentration and in rates of PRPP and nucleotide synthesis in intact patient fibroblasts indicate that despite an intact allosteric mechanism of regulation of PRS activity, PRS1 transcription is a major determinant of PRPP and purine synthesis. The genetic basis of disordered PRS1 transcription remains unresolved; normal- and patient-derived PRS1s share nucleotide sequence identity at least 850 base pairs 5′ to the consensus transcription initiation site.

Phosphoribosylpyrophosphate (PRP) is a substrate in the synthesis of virtually all nucleotides (1) as well as an important regulator of rates of the de novo pathways of purine and pyrimidine nucleotide synthesis (2–4). PRPP synthesis from GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PRS, phosphoribosylpyrophosphate synthetase; bp, base pair(s); GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Accelerated Transcription of PRPS1 in X-Linked Overactivity of Normal Human Phosphoribosylpyrophosphate Synthetase*

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Phosphoribosylpyrophosphate (PRPP) synthetase (PRS) superactivity is an X-linked disorder characterized by goit with overproduction of purine nucleotides and uric acid. Study of the two X-linked PRS isoforms (PRS1 and PRS2) in cells from certain affected individuals has shown selectively increased concentrations of structurally normal PRS1 transcript and isoform, suggesting that this form of the disorder involves pretranslational dysregulation of PRS1 expression and might be more appropriately termed overactivity of normal PRS. We applied Southern and Northern blot analyses and slot blotting of nuclear runoffs to delineate the process underlying aberrant PRS1 transcript expression in fibroblasts and lymphoblasts from patients with overactivity of normal PRS. Neither PRS1 amplification nor altered stability or processing of PRS1 mRNA was identified, but PRS1 transcription was increased relative to GAPDH (3- to 4-fold normal in fibroblasts; 1.9- to 2.4-fold in lymphoblasts) and PRS2. Nearly coordinate relative increases in each process mediating transfer of genetic information from PRS1 transcription to maximal PRS1 isoform expression in patient fibroblasts further supported the idea that accelerated PRS1 transcription is the major aberration leading to PRS1 overexpression. In addition, modulated relative increases in PRS activities at suboptimal Pi concentration and in rates of PRPP and purine nucleotide synthesis in intact patient fibroblasts indicate that despite an intact allosteric mechanism of regulation of PRS activity, PRS1 transcription is a major determinant of PRPP and purine synthesis. The genetic basis of disordered PRS1 transcription remains unresolved; normal- and patient-derived PRS1s share nucleotide sequence identity at least 850 base pairs 5′ to the consensus transcription initiation site.

Phosphoribosylpyrophosphate (PRP) is a substrate in the synthesis of virtually all nucleotides (1) as well as an important regulator of rates of the de novo pathways of purine and pyrimidine nucleotide synthesis (2–4). PRPP synthesis from MgATP and ribose-5-phosphate is catalyzed in mammalian cells by a family of PRPP synthetase (PRS; EC2.7.6.1) isoforms in reactions requiring Mg2+ and Pi, as activators and subject to inhibition by purine, pyrimidine, and pyridine nucleotides (5–8). Of the three highly homologous human PRS isoforms identified to date, PRS1 and PRS2 are expressed in all tissues (9, 10) and are encoded by genes (PRPS1 and PRPS2) that map, respectively, to the long and the short arms of the X chromosome (11, 12). PRS3 expression is detectable only in the testes and is encoded autosomally (9, 13).

Superactivity of PRS is an X chromosome-linked human disorder (14) characterized by PRPP, purine nucleotide, and uric acid overproduction (15–17), gout (15, 18), and, in some affected families, neurodevelopmental impairment (18–21). The kinetic mechanisms underlying inherited PRS superactivity are diverse and include defective allosteric regulation of PRS1 activity (regulatory defects) (15–19, 21, 22), increased apparent affinity of PRS for the substrate ribose-5-phosphate (23), and increased activity of the normal PRS1 isoform (formerly called catalytic superactivity) (24–27). Study of the genetic and mechanistic bases of the heterogeneous kinetic alterations associated with PRS superactivity has shed light on the manner in which the synthesis of PRPP is regulated (3, 16, 21). In the case of regulatory defects, for example, patient-derived PRS1 cDNAs bear point mutations encoding recombinant mutant PRS1s with altered allosteric properties (resistance to noncompetitive purine nucleotide inhibition and increased sensitivity to Pi activation) characteristic of those of PRS in cells from the respective affected individual (21). This finding provides evidence that allosteric control of PRS1 activity is important in regulating PRPP synthesis in human cells.

In contrast, overexpression of normal PRS1 transcript as well as PRS1 isoform has been demonstrated in cells from patients with overactivity of normal PRS (24). The association of increased PRS1 transcript level with increased PRS1 isoform content and enzyme activity suggests a pretranslational defect in the expression of PRPS1 in this type of inherited PRS superactivity (24). In studies aimed at further defining the process responsible for overexpression of normal PRS1 transcript and isoform, we have found selective acceleration of PRPS1 transcription in this disorder as well as evidence that in fibroblasts from affected individuals the rate of transcription of PRPS1 serves as a major determinant of PRPP and purine nucleotide production rates despite intact allosteric regulation of PRS activity.

EXPERIMENTAL PROCEDURES

Cell Lines—Fibroblast strains initiated from skin biopsies obtained from five normal individuals and three unrelated males with overexpression of normal PRS (25, 26) were propagated in monolayer in Eagle's minimal essential medium containing 10% fetal bovine serum, 2 mM l-glutamine, nonessential amino acids, penicillin (100 units/ml) and streptomycin (100 μg/ml). B lymphoblast lines, derived as described (28) from two normal individuals and two of the affected males, were propagated in RPMI 1640 medium containing 10% fetal bovine

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7482
Table I
Oligonucleotides used for polymerase chain reaction amplification of 5' promoter and 5'-untranslated regions of PRPS1 and for sequencing amplified and cloned PRPS1 DNAs

| Primer type and No. | Oligodeoxynucleotide 5'-3' sequence<sup>a</sup> | Priming site<sup>b</sup> | Strand<sup>c</sup> |
|---------------------|---------------------------------------------|-----------------|-----------------|
| Amplification PRPS1–3 | -1167 to -1142 | + | + |
| Amplification and sequencing PRPS1–5 | -1020 to -999 | + | + |
| 3597 | 119 to 99 | + | + |
| Sequencing PRPS1–1 | ACTACCTTGTCCAGCAAC | -757 to -739 | + |
| PRPS1–2 | GTGCTTGAGACCAATATGTT | -739 to -757 | - |
| PRPS1–4 | GAAACATGCAACTCTGGG | -535 to -517 | + |
| PRPS1–6 | -292 to -181 | + | + |
| PRPS1–7 | -104 to -122 | + | + |
| PRPS1–8 | CTAGCGGGAATCAACCAGT | -403 to -422 | - | - |

<sup>a</sup> Except where indicated, oligodeoxynucleotide sequences have been reported (24).

<sup>b</sup> Priming sites are numbered with respect to the A base of the translation initiation ATG codon and follow human PRS.

<sup>c</sup> +, sense strand; −, antisense strand.

Established for the 997-bp 3'-untranslated segment of the transcribed region of normal and patient cell-derived PRPS1 DNAs (24).

PRS1 and PRS2 Transcripts Levels—steady state levels of PRS1 and PRS2 transcripts were estimated by Northern blot analysis (30) after electrophoresis and transfer of cellular total RNA samples to nitrocellulose filters, prehybridization, and hybridization, all as described previously (24). Probes for hybridization were oligo[<sup>32</sup>P]-labeled human PRS1 cDNA (2.3 kilobases), PRS2 cDNA (2.7 kilobases), and glyceroldehyde-3-phosphate (GAPDH) cDNA (1.8 kilobases). After washing at suitable stringency, radioactivities in the regions of the membrane corresponding to PRS, and control transcripts were quantitated on a PhosphorImager (Molecular Dynamics) for 12 h before exposure of the membrane to x-ray film for 24–72 h at −70 °C. Values for PRS1 and PRS2 transcript levels in a cultured cell total RNA sample are expressed relative to the GAPDH transcript level measured in that sample.

To estimate PRS transcript stability, relative PRS mRNA levels were determined in lymphoblasts incubated with the RNA polymerase II inhibitor, actinomycin D. Identical cultures of each lymphoblast line (20 × 10<sup>6</sup> cells in 20 ml of growth medium) were incubated at 37 °C for 0 to 24 h after the addition of actinomycin D (final concentration, 5 μg/ml). At appropriate times, cultures were harvested by centrifugation, and cells were washed twice in ice-cold serum-free medium before extraction of RNA (30). Northern blot analysis was carried out as described above, except that the nitrocellulose filters were probed with a labeled cDNA probe (1.4 kilobases) for 18 S ribosomal RNA as well as labeled PRS cDNA probes. Values for PRS transcript levels are expressed relative to that of the 18 S ribosomal RNA level measured in the respective sample.

DNA Preparation and Filter Hybridization—normal male and patient fibroblast genomic DNAs were digested with BamHI and HindIII. After electrophoresis of equal amounts of DNA in 0.8% agarose gels, DNAs were transferred to GeneScreen nylon membrane filters (NEN Research Products) under alkaline conditions (33). Blots were hybridized with oligo[<sup>32</sup>P]-labeled PRS1 cDNA probes (12), including a full-length (2.3 kilobase) PRS1 cDNA and a HindIII fragment containing the 5' 450 bp of PRS1 cDNA. Conditions of hybridization, washing, and exposure of filters to x-ray film were as described (12).

Rates of PRS Gene Transcription—transcription rates were estimated in fibroblasts and lymphoblasts by nuclear runoff analysis (34). Nascent RNA transcripts were [<sup>32</sup>P]UTP-labeled in and isolated from fibroblast (7 to 9 × 10<sup>6</sup> cells/assay) and lymphoblast (1.8 to 2.2 × 10<sup>8</sup> cells/assay) nuclei prepared as described (30, 34). RNAs in each preparation were hybridized to linearized plasmid pGEM3Z or to pGEM3Z-borne PRS1, PRS2, and GAPDH cDNAs immobilized by slot blotting onto a nitrocellulose filter (30). Binding of label to pGEM3Z without a cDNA insert served as background control, and a 28 S ribosomal RNA DNA probe (35) was applied to the filter to bind labeled ribosomal RNA and thus reduce nonspecific binding elsewhere on the filter. After hybridization and washing at suitable stringencies, label corresponding to each slot was quantitated on a PhosphorImager, and the filter was exposed to x-ray film for 48 to 72 h at −70 °C for densitometric quantitation. Rates of PRPS1 and PRPS2 transcription are expressed relative to transcription of GAPDH, determined on the same filter.
Fig. 1. Sequence of normal human PRPS1 extending 1161 bp 5′ to the transcription initiation ATG codon (+1). Numbering is relative to the A of the ATG codon (+1). Transcription initiation sites identified by primer extension and S1 nuclease protection assays (32) are indicated by arrowheads. The 5′ end of the human PRS1 cDNA (122) (cloned from normal human B lymphoblasts) is denoted by the horizontal arrow. With exception of a single base (C) addition at −410, the sequence from −457 to +3 is as previously published (32). An identical sequence was found in the interval from −967 (vertical arrow) to +3 when 3 normal and 3 patient fibroblast-derived PRPS1 DNAs were amplified and sequenced.

RESULTS

PRPS1 Genomic DNA Sequencing—The 1161-bp sequence of normal human PRPS1 preceding the translation initiation ATG triplet is shown in Fig. 1.2 Sequence identity between normal- and patient-derived PRPS1 genomic DNA was confirmed for the 5′-transcribed but untranslated region of the gene from the 3 affected and 3 normal individuals, supporting the contention (24) that PRS1 transcript structure is normal in hemizygous males with overactivity of normal PRS1. In addition, the sequence of polymerase chain reaction-amplified PRPS1 DNA corresponding to the 850 bp immediately 5′ to the consensus transcription initiation site (nucleotides −117 to −115, Fig. 1; Ref. 32) was identical regardless of whether normal- or patient-derived genomic DNA served as template.

Genomic DNA Hybridization—Southern blots of restriction enzyme-digested patient and normal male genomic DNAs showed identical patterns and intensities of hybridizing bands when filters were probed with either full-length normal PRPS1 cDNA (Fig. 2) or a HindIII fragment containing only the 5′-450 bp of normal PRS1 cDNA. These findings exclude gene amplification as a likely basis for PRPS1 overexpression in this form of PRS superactivity.

PRS Transcript Levels and Stability—Northern blot analysis confirmed (24) increased concentrations of PRS1 mRNA in PRS1 fibroblasts but not patient PRS1 fibroblasts, and to a lesser degree, lymphoblasts when expressed relative to levels of endogenous GAPDH transcript (Table II)3 or 18S ribosomal RNA (Fig. 3) in the same extract. Nevertheless, rates of decrement of PRS1 mRNA (Fig. 3, A and B) and of PRS2 mRNA (Fig. 3, A and C) (relative to those of 18S ribosomal RNA) from normal and patient lymphoblasts during incubation with actinomycin D were virtually indistinguishable. PRS1 transcript half-lives were 10.8 ± 1.4 h and 11.1 ± 0.9 h (mean ± S.D. of 3 determinations in each cell line), respectively, in normal and patient cells. Corresponding mean half-lives for PRS2 transcripts were 13.1 and 12.2 h. In all instances, PRS1 and PRS2 transcripts were detectable only as single hybridizing bands at 2.3 and 2.7 kilobases respectively, and no additional hybridizing bands suggestive of immature or alternatively processed PRS transcripts were observed.

Rates of PRPS Gene Transcription—Slot-blot analyses of specific RNAs labeled in nuclei isolated from cultured normal and patient cells showed consistent differences with respect to relative rates of labeling of PRS1 mRNA (Fig. 4; Table III). In fibroblasts from 3 affected males, rates of PRS1 transcript

2 The only sequence in the data base with significant relatedness to the sequence in Fig. 1 is the sequence gb/M31078 for rat PRPS1, exon 1, that shows 94% identity in the region corresponding to −149 to −94 in human PRPS1.

3 Normal and patient cell strains are designated by initials of donors in Figs. 3 and 4 and Tables II and III.
X-linked Overactivity of Normal Human PRPP Synthetase

TABLE II
Relationships between accelerated PRPS1 transcription and PRS activity, PRPP generation, and rates of purine synthesis in human fibroblasts with oeractivity of normal PRS

All values are derived from the means of at least three separate determinations in each fibroblast strain and lymphoblast line. Means for the five normal fibroblast strains and the two normal lymphoblast lines were averaged and are shown. Each normal value was assigned a relative value of 1.0 to which the corresponding relative mean values measured in patient cells are compared. Relative values are in parentheses.

| Cell source | PRPS1 relative transcript rate (PRPS1/GAPDH) | PRS relative transcription level (PRS/GAPDH) | Total PRS isoforms (PRPS1 + PRPS2) | PRS activity at 32 mM Pi (μg/mg of protein) | PRPP generation (milliunits/mg of protein) | Rate of purine synthesis de novo (nmol/h/10^6 cells) |
|-------------|---------------------------------------------|---------------------------------------------|-----------------------------------|---------------------------------------------|---------------------------------------------|--------------------------------------------------|
| Fibroblasts |                                             |                                             |                                   |                                             |                                             |                                                  |
| Normal      | 5.4 (1.0)                                   | 2.5 (1.0)                                   | 0.31 (1.0)                        | 8.11 (1.0)                                  | 2.27 (1.0)                                  | 1.94 (1.0)                                       |
| Patient     |                                             |                                             |                                   |                                             |                                             |                                                  |
| TB          | (4.5)                                       | (5.9)                                       | (6.0)                             | (4.9)                                       | (2.9)                                       | (2.0)                                            |
| SS          | (3.3)                                       | (4.7)                                       | (4.5)                             | (3.9)                                       | (2.6)                                       | (1.9)                                            |
| AD          | (3.0)                                       | (3.4)                                       | (3.5)                             | (3.3)                                       | (2.1)                                       | (1.7)                                            |
| Lymphoblasts|                                             |                                             |                                   |                                             |                                             |                                                  |
| Normal      | 9.5 (1.0)                                   | 3.6 (1.0)                                   | 0.39 (1.0)                        | 8.68 (1.0)                                  | 1.78 (1.0)                                  | 0.32 (1.0)                                       |
| Patient     |                                             |                                             |                                   |                                             |                                             |                                                  |
| TB          | (1.9)                                       | (1.8)                                       | (1.4)                             | (1.1)                                       | (0.9)                                       | (1.1)                                            |
| AD          | (2.4)                                       | (2.0)                                       | (1.7)                             | (1.2)                                       | (1.2)                                       | (1.1)                                            |

DISCUSSION

The current studies provide evidence for a selectively increased rate of transcription of PRPS1 as the pretranslational aberration underlying increased expression of the normal PRPS1 isoform in inherited PRS overactivity. In each of the fibroblast strains and lymphoblast lines cultured from affected individuals, transcription of PRPS1 was consistently greater relative to that of GAPDH than was the case in corresponding normal cells. Although the relative increases in PRPS1 transcription rates were greater in patient fibroblasts than lymphoblasts, study of the latter cell type permitted a more accurate demonstration of the selectivity of accelerated PRPS1 transcription. That is, relative rates of PRPS2 transcription in normal cells were substantially greater in lymphoblasts than fibroblasts (24). In fact, as previously noted (24, 28), the small increases in maximal PRS activities measured in lymphoblast extracts from affected patients appear insufficient to drive excessive production of PRPP or purine nucleotides in intact cells.

Labeling relative to those of GAPDH transcript labeling were 3- to 4-fold greater than in cells from 5 normal individuals (Table II: Fig. 4A). In lymphoblasts, the corresponding increases in relative labeling of PRS1 transcript were 1.9- to 2.4-fold (Table II: Fig. 4B). Relative rates of labeling of PRS2 mRNA were indistinguishable in normal and patient cells, a point more readily apparent in lymphoblasts, which have substantially higher relative rates of PRS2 transcription than fibroblasts (Table III). In conjunction with the preceding studies excluding PRPS1 gene amplification or altered PRS1 transcript structure or stability, these findings suggest that increased expression of PRPS1 in overactivity of normal PRS1 results at least in major part from selective acceleration of PRPS1 gene transcription.

PRPS1 Gene Transcription, PRS Expression, and Purine Nucleotide Synthesis—If accelerated transcription of PRPS1 is the major determinant of PRS overactivity in fibroblasts and lymphoblasts from affected patients, correspondence between rates of PRPS1 transcription and PRS1 mRNA levels, PRS1 isoform concentrations, PRS enzyme activities, and rates of PRPP and purine nucleotide synthesis should be demonstrable. Such correspondence would support the view that PRPS1 transcription rate can determine expression of PRS1 activity even when allosteric regulation of enzyme activity is intact. To assess these relationships, we compared relative rates of PRPS1 transcription, relative PRS1 mRNA concentrations, PRS isoform concentrations, and PRS activities in fibroblast strains and lymphoblast lines derived from normal individuals and affected patients. In addition, PRPP generation and rates of purine synthesis de novo were determined in these cells.

Differences between normal and patient fibroblasts with respect to PRS transcription rates, PRS mRNA and isoform levels, and maximal PRS enzyme activities are presented in Table II, where values for individual patient-derived strains are expressed relative to the respective mean values for the group of 5 normal fibroblast strains. For each patient-derived cell strain, nearly coordinate increases are apparent in all processes relating PRPS1 transcription to PRS activity measured at 1.0 mM Pi, a concentration of Pi at which allosteric inhibition of PRS activity by endogenous purine nucleotides is minimal (16). As is also shown in Table II, intact patient-derived fibroblasts synthesize PRPP and purine nucleotides at increased relative rates, which are, however, more modest than the increased relative rates of PRPS1 gene transcription or the increased relative levels of PRS1 transcript or isoform, or maximal PRS activities. Relative increases in PRPP and purine nucleotide synthesis, in fact, more closely parallel relative increases in PRS activities measured at 1.0 mM Pi, a concentration closer to that in intact fibroblasts (36) and at which allosteric inhibition by endogenous nucleotides is potent (16). The modulated relative increases of PRPP and purine nucleotide synthesis in patient cells thus appears to reflect both the operation of allosteric inhibition of PRS activity and the higher intracellular concentrations of purine nucleotide inhibitors in intact patient fibroblasts (16).

Similar relationships are detectable but less immediately apparent in lymphoblast lines (Table II) than in fibroblast strains, first, because of smaller differences in all measurements comparing patient and normal lymphoblasts, and, second, because the contribution of PRPS2 to total PRS isoform content and PRS activity is substantially greater in lymphoblasts than fibroblasts (24). In fact, as previously noted (24, 28), the small increases in maximal PRS activities measured in lymphoblast extracts from affected patients appear insufficient to drive excessive production of PRPP or purine nucleotides in intact cells.
level of transcription and that an inherited increase in \( \text{PRPS1} \) transcription rate provides the basis for the increase in the concentration of the normal PRS1 isoform in cells of affected individuals.

The genetic basis of inherited acceleration of \( \text{PRPS1} \) transcription remains to be determined. Sequence identity of patient and normal \( \text{PRPS1} \) DNAs in the 850-bp region 5' to the consensus transcription initiation site (32) excludes mutation in the gene promoter and immediate 5' flanking sequence, for which examples of transcriptional dysregulation have been established, as in the thalassemias (37–39) and hereditary persistence of fetal hemoglobin (40, 41). Among alternative possibilities to explain accelerated \( \text{PRPS1} \) transcription are mutations in a more remote promoter element, either in contiguity with the immediate 5'-flanking sequence (42) or even substantially distant (43); in a cis-acting element within or adjacent to the \( \text{PRPS1} \) gene, such as an intrinsic enhancer or suppressor (44) or a 3'-flanking DNA sequence (45); or in a trans-acting gene influencing the regulation of \( \text{PRPS1} \) transcription. In any case, X chromosome-linked transmission of PRS catalytic superactivity (14) favors the view that the primary defect altering \( \text{PRPS1} \) transcription is itself X-linked. In addition to extended \( \text{PRPS1} \) 5'-flanking region sequencing, functional analysis of the \( \text{PRPS1} \) promoter and adjacent 5'-flanking DNA, comparing \( \text{PRPS1} \) promoter-plasmid construct expression in normal and patient cells, should prove helpful in distinguishing among these possibilities.

Prior studies (3, 16) comparing mechanisms of purine nucleotide overproduction in fibroblasts from individuals with PRS superactivity (either catalytic or regulatory defects in PRS1) and severe deficiency of hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8) have confirmed that the rate of the pathway of purine synthesis \textit{de novo} is controlled at the sequential PRS and amidophosphoribosyltransferase (EC
share the biochemical hallmarks of PRS superactivity, increased rates of PRPP and purine synthesis and increased intracellular purine nucleotide concentrations, defect-specific differences in the intracellular control of PRPP and purine nucleotide synthesis are apparent (16).

In the case of PRS regulatory superactivity, where PRS activity in cell extracts or purified enzyme preparations are resistant to purine nucleotide inhibition, accelerated rates of intracellular PRPP and purine nucleotide synthesis are refractory to inhibition by exogenous purine base precursors of purine nucleotides or by endogenous increases in purine nucleotide concentrations (16). Thus, in cells bearing PRS1s with any of an array of point mutations (21), in vitro defects in allosteric regulation of PRS1 activities are paralleled by dysregulation of PRPP and purine synthesis. In contrast, allosteric regulation of PRS activity is normal in enzyme preparations from fibroblasts with overactivity of normal PRS, and suppression of PRPP and purine nucleotide synthesis in response to purine base addition is intact in the corresponding cells (16). Nevertheless, these cells express increased rates of PRPP and purine nucleotide synthesis.

The apparent paradox of increased rates of PRPP and purine nucleotide synthesis in fibroblasts with overactivity of normal PRS catalytic superactivity despite increased purine nucleotide inhibitor pools and normal allosteric regulation of PRS activity (16) is best resolved by the view that the increased concentration of the normal PRS1 isoform (24) results in a rate of PRPP synthesis sufficient to activate amidophosphoribosyltransferase despite coexisting increased levels of inhibitory purine nucleotides (16). Consistent with this formulation are the substantially more modest increases in rates of PRPP and purine nucleotide synthesis in fibroblasts with intact allosteric regulation than in cells in which mutations in PRPS1 impair this control mechanism (16). Thus, although intact allosteric inhibition apparently modulates expression of PRPP and purine overproduction in fibroblasts with excessive PRS1 isoform, this regulatory mechanism is insufficient to overcome the excessive expression of enzyme activity resulting from acceleration of PRPS1 transcription. The current studies provide, then, an example of a circumstance in which transcription of PRPS1 is a major determinant of PRPP and purine nucleotide synthetic rates.

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