Overexpression of the Normal Phosphoribosylpyrophosphate Synthetase 1 Isoform Underlies Catalytic Superactivity of Human Phosphoribosylpyrophosphate Synthetase*

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To define the enzymatic and genetic basis of X-linked phosphoribosylpyrophosphate synthetase (PRS) catalytic superactivity, we measured concentrations of X-linked PRS1 and PRS2 isoforms in cultured fibroblasts and lymphoblasts by immunoblotting after separation by polyacrylamide-urea isoelectric focusing. PRS1 comprised >80% of measurable PRS isoforms in all fibroblast strains but PRS1 concentrations in cells from six affected males exceeded those in normal cells by 2–6-fold. PRS absolute specific activities (activity per mg of PRS isoforms) were comparable in all fibroblast strains and in purified recombinant normal PRS1, confirming selectively increased levels of PRS1 isoform as the enzymatic basis of PRS catalytic superactivity. Cloning, sequencing, and expression of normal subject- and patient-derived PRS cDNAs predicted normal translated region sequences for both PRS isoforms and revealed no differences in catalytic properties of recombinant PRS1. Normal and patient PRPS1 transcribed but untranslated DNA sequences were also identical. Northern blot analysis showed selective increase in relative concentrations of PRS1 transcripts in patient fibroblasts. In PRS catalytic superactivity, overexpression of the normal PRS1 isoform thus appears to result from an altered pretranslational mechanism of PRPS1 expression. In lymphoblasts, however, expression of this alteration is attenuated, explaining the absence of phenotypic expression of PRS catalytic superactivity in these cells.

Supercactivity of phosphoribosylpyrophosphate (PRPP) \( ^{1} \) synthetase (EC 2.7.6.1; PRS) is an X-linked human disorder (1) characterized by purine nucleotide and uric acid overproduction (2–4), gout (2, 5), and, in some families, neurodevelopmental impairment (5–8). Accelerated production of the purine-regulatory substrate PRPP provides a unitary pathogenetic mechanism for excessive synthesis of purine nucleotides and, ultimately, uric acid in affected individuals (3, 4, 9). Nevertheless, diversity is apparent in the aberrant enzymatic properties resulting in PRS superactivity in different families (3, 5, 8). Defective allosteric regulation of PRS activity by purine nucleotide inhibitors (such as ADP and GDP) and by the activator P,

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1 The abbreviations used are: PRPP, 5-phosphoribosyl 1-pyrophosphate; PRS, phosphoribosylpyrophosphate synthetase(s); IEF, isoelectric focusing; PCR, polymerase chain reaction; bp, base pair.
PRS Activity and Isoform Analyses—PRS activities were determined by a two-step procedure previously described in detail (20). Stocks of purified recombinant human PRS1 and PRS2 have identified differences between these structurally similar isoforms (95% amino acid sequence identity) with respect to substrate and activator affinities, purine nucleotide inhibitor responsiveness, pH optima, specific catalytic activities, and stability of activity upon dilution (13). In the current studies, PRS assays were carried out (except where specifically stated) at pH 7.5 in the presence of saturating substrate concentrations (500 μM MgATP; 350 μM ribose-5-phosphate), 5.0 mM MgCl₂, and 32.0 mM P₃. Dilution of recombinant PRS1 or PRS2 was made in a stabilizing buffer at pH 7.5 containing 50 mM Tris-HCl, 0.3 mM ATP, 6.0 mM MgCl₂, 1.0 mM sodium phosphate, and 1 mM MgCl₂ bovine serum albumin. Activities of PRS are expressed in units, where 1 unit is defined as 1 μmol of PRP formed per min at 37°C.

A difference in pI's of human PRS1 and PRS2 (8) was exploited in the development of an isoelectric focusing (IEF)-immunoblotting procedure for separation and quantitation of the isoforms in human cell extracts. Supernatant layers of fibroblast and lymphoblast extracts (5–150 × 10⁶ fibroblasts and from 5–10 × 10⁶ lymphoblasts) were washed in dilution buffer, immersed in ECL Western blot detection reagent (Amersham Life Science Inc.), and exposed to x-ray film for 2–5 min before development of the film. The intensity of bands utilized appropriately oriented primer sequences (Table I) from the poly(A) tract of PRS1 cDNA beginning at nucleotide position 1952.

### Table I

| Primer type and no. | Oligodeoxynucleotide 5′-3′ sequence | Priming site | Strand |
|--------------------|-------------------------------------|--------------|--------|
| PRPS1–3            | GTCGACTCATCTGAGGTTCCCTC            | –1167 to –1142 | +      |
| PRPS1–5            | GGGTTACCTTTGGCATTAAAC              | –1020 to –999  | +      |
| 3595               | CAGGGTATGACCTCTTCTGATC             | 862 to 885    | +      |
| R-13               | GCAGAGACTATGACAGA                  | 1993 to 1975  | +      |

Amplification

3597

GCTCCCTGGTGGCTGAAATTT

119 to 99

3598

TTGCAAGAGGCCATCAGAG

887 to 905

PRPS1–25

TGATACAGATCCCAAAATCACAG

1967 to 1944

Amplification and sequencing

Sequence

PRPS1–6

TAACGTGTCGCGGAGGTTG

–202 to –181

PRPS1–7

TGCCGACATCTTCTACATCC

–104 to –122

PRPS1–18

AGGAGGACCCCATGTTGGACC

1120 to 1139

PRPS1–19

AGGGACCCCATGTTGGACC

1335 to 1354

PRPS1–20

CCCTTTCGATTGTGTGACC

1359 to 1616

PRPS1–22

GTCGACGCAGATTACTCA

1139 to 1120

PRPS1–23

GCTGTTGTCGATGCTGCTTTGA

1793 to 1815

### Notes

1. Priming sites are numbered with respect to the A base of the translation initiation AUG codon and follow human PRS1 cDNA and genomic DNA sequence previously reported (14, 28), except that primers PRPS1–3, PRPS1–8, PRPS1–25, and R-11 incorporate previously unreported genomic DNA sequences.

2. +, sense strand; –, antisense strand.
PRS activities and isoform concentrations in normal and patient fibroblast extracts

| Cell strain | PRS activitya | Isoform content* | PRS absolute specific activity |
|-------------|---------------|------------------|-------------------------------|
|             | milliunits/mg protein | µg/mg protein | units/mg PRS isoforms |
| Normal      |               |                  |                              |
| WO          | 7.95          | 0.28             | 0.02                         | 26.5 |
| LEO         | 6.68          | 0.21             | 0.05                         | 25.7 |
| SEL         | 8.99          | 0.33             | 0.04                         | 24.3 |
| DT          | 12.25         | 0.36             | 0.05                         | 29.9 |
| ON          | 8.38          | 0.25             | 0.04                         | 28.9 |
| Patient     |               |                  |                              |
| TB          | 43.98         | 1.88             | 0.10                         | 22.2 |
| SS          | 35.39         | 1.46             | 0.04                         | 23.6 |
| RA          | 17.99         | 0.61             | 0.03                         | 28.1 |
| ZB          | 27.32         | 0.98             | 0.05                         | 26.5 |
| AD          | 28.59         | 1.12             | 0.04                         | 24.6 |
| TC          | 40.12         | 1.48             | 0.08                         | 25.7 |

PRS activities and isoform concentrations in normal and patient lymphoblast extracts

| Cell strain | PRS activitya | Isoform content* | PRS absolute specific activity |
|-------------|---------------|------------------|-------------------------------|
|             | milliunits/mg protein | µg/mg protein | units/mg PRS isoforms |
| Normal      |               |                  |                              |
| LIV         | 8.08          | 0.22             | 0.16                         | 20.7 |
| L5          | 8.83          | 0.24             | 0.18                         | 21.0 |
| Patient     |               |                  |                              |
| TB          | 12.11         | 0.41             | 0.16                         | 21.2 |
| AD          | 13.94         | 0.49             | 0.19                         | 20.5 |

* All values represent means of at least three determinations, with agreement among measurements within 15%.

PRS1 and PRS2 Transcript Levels—Steady state levels of PRS1 and PRS2 transcripts were estimated by Northern blot analysis after electrophoresis (4 h at 150 V) of samples of fibroblast and lymphoblast total RNA on 1% agarose-formaldehyde denaturing gels and transfer of RNA to nitrocellulose membranes (26). After prehybridization for 4 h at 42 °C, membranes were hybridized at 42 °C for 18 h with oligo-32P-labeled human PRS1 cDNA (2.3 kilobase pairs) or PRS2 cDNA (2.7 kilobase pairs) (or with both probes together) and with a human glyceraldehyde-3-phosphate dehydrogenase cDNA probe (1.8 kilobase pairs). Specific radioactivities of labeled PRS1 and PRS2 cDNA were identical when used to probe a single filter or duplicate filters. Blots were then washed at suitable stringency (26), and radioactivities in the regions of the membrane corresponding to PRS and control transcripts were quantified on a PhosphorImager (Molecular Dynamics) (12 h) before exposure 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 glyceraldehyde-3-phosphate dehydrogenase transcript level measured in that sample.

RESULTS

PRS Activities in Cell Extracts—PRS activities in extracts of fibroblasts derived from six individuals with purine nucleotide uric acid overproduction and previously described PRS catalytic superactivity (16, 17, 19) exceeded the mean value of PRS activity in cell extracts from five normal individuals by 2.0–5.0-fold (Table II). Each patient-derived strain exhibited a PRS activity in cell extracts from five normal individuals by catalytic superactivity (16, 17, 19) exceeded the mean value of fibroblasts derived from six individuals with purine nucleotide parallel experiments (16, 17, 19) defining the class of PRS activities and isoform concentrations in normal and patient lymphoblast extracts.* 

| Cell strain | PRS activitya | Isoform content* | PRS absolute specific activity |
|-------------|---------------|------------------|-------------------------------|
|             | milliunits/mg protein | µg/mg protein | units/mg PRS isoforms |
| Normal      |               |                  |                              |
| LIV         | 8.08          | 0.22             | 0.16                         | 20.7 |
| L5          | 8.83          | 0.24             | 0.18                         | 21.0 |
| Patient     |               |                  |                              |
| TB          | 12.11         | 0.41             | 0.16                         | 21.2 |
| AD          | 13.94         | 0.49             | 0.19                         | 20.5 |

* All values represent means of at least four determinations, with agreement among measurements within 10%.

IEF gel electrophoresis. In preliminary studies, samples containing known amounts of highly purified recombinant normal human PRS1 and PRS2 (13) were separated and immunoblotted, and the bands corresponding to the respective isoforms were measured on a computing densitometer. These studies confirmed quantitative entry of the proteins into the gel as well as the range of the linear relationship between the amounts of each isoform applied and total densities of the immunospecific bands (Fig. 1). Both recombinant isoforms consistently appeared as doublet bands in this system, with additional bands (comprising up to 15% of total density) appearing when larger amounts of recombinant PRS1 were applied (Fig. 2). The sums of the densities of the bands were used in estimating quantities of the respective isoform. In initial measurements of cellular PRS isoform contents, equivalent quantities of protein from the supernatant layers of fibroblast and lymphoblast extracts were applied to the IEF gel along with a series of purified recombinant PRS1 and PRS2 standards. In subsequent fibroblast studies, appropriate dilutions of individual extracts were made (in extraction buffer containing 1 mg/ml bovine serum albumin) to permit the respective band densities to fall in the linear range of the standard curves. (Curves resulting from serial dilutions of extract samples and recombinant PRS isoform standards were nearly identical.) Bands identified in samples of all cell extracts corresponded in mobilities to those observed with purified recombinant normal PRS1 and PRS2.

A representative IEF-immunoblot analysis of fibroblast PRS isoforms is shown in Fig. 2A. Extracts of normal and patient fibroblasts contained substantially higher concentrations of PRS1 than PRS2 (Table II). Fibroblast PRS1 concentrations in extracts derived from patients with PRS catalytic superactivity consistently exceeded those from normal individuals, and PRS2 concentrations in normal and patient fibroblast extracts were comparable. For each normal and patient cell strain, fibroblast PRS activity corresponded closely with total PRS isoform concentration, so that calculated absolute specific activities of PRS isoforms (milliunits/mg PRS isoforms) were quite similar in patient and normal fibroblast extracts (Table II). These calculated specific activities also agreed closely with the reported specific activity of highly purified recombinant normal human PRS1 (25.1 units/mg protein (13)). These findings provide strong evidence that PRS superactivity in fibroblasts from these patients results from a selective increase in levels of the PRS1 isoform and do not support the idea (20) that PRS1 in the cells of these patients display increased activity per immunoreactive enzyme molecule.

For each lymphoblast line, PRS2 isoform concentration (Fig. 2B) comprised a greater proportion of total PRS isoform concentration than in fibroblasts (Table II), permitting more accurate quantitation of PRS2 levels in cell extracts when samples of equivalent protein content were tested. Normal and patient lymphoblast lines contained comparable PRS2 isoform
accompanied each measurement of isoform content in cell extracts. Similar standard panel utilizing purified recombinant PRS isoforms was applied to a polyacrylamide-urea IEF gel, subjected to electrophoresis, and transferred to a polyvinylidene difluoride membrane, all as described under "Experimental Procedures." A similar standard panel utilizing purified recombinant PRS isoforms accompanied each measurement of isoform content in cell extracts.

concentrations in each of four determinations carried out in extracts of cells harvested over a range of log phase culture growth from 0.5–1.5 × 10⁶ cells/ml. PRS1 isoform concentrations in these extracts differed, however, with consistent increases, averaging 1.7- and 2.0-fold, respectively, in extracts of cells derived from patients TB and AD (Table III). When PRS absolute specific activities were calculated from the results of PRS activity and isoform assays, values for normal, TB, and AD PRS were similar, approaching those of purified recombinant normal PRS1. As is the case for fibroblasts, the increments in PRS activities in TB and AD lymphoblasts, although of lesser magnitude, are explainable by selective increases in PRS1 isoform concentrations in these cells.

PRS1 and PRS2 cDNA Sequences and Expression of Recombinant PRS1 Isoforms—The 954-bp translated regions of PRS1 and PRS2 cDNA derived from fibroblast and lymphoblast total RNAs of five normal individuals and four patients with catalytic superactivity (TB, SS, AD, ZB) were sequenced directly from PCR pools. The sequences of both PRS1 and PRS2 cDNA derived from patients were identical with those of the respective normal PRS1 cDNA (14, 15). This finding contrasts with the demonstration, by means of the identical reverse transcription-PCR amplification and sequencing strategies used here, of single base substitutions in the PRS1 cDNA derived from patients with allosteric regulatory defects in PRS (8, 10).

Because the results of PRS isoform analyses suggested that selectively increased PRPS1 gene expression provides the basis of PRS catalytic superactivity, normal and patient PRS1 cDNA were cloned into pSPRBS, and both the plasmid and recombinant derivatives were used to transform E. coli BL21 (DE3/pLysS). During induction with isopropyl-1-thio-β-D-galactopyranoside, recombinant normal and patient PRS1 cDNA were expressed, resulting in high levels of PRS activity in bacterial cell extracts and the appearance of a 34.5-kDa band on SDS-polyacrylamide gel electrophoresis, identified as human PRS1 by immunoblot analysis (8). Recombinant human PRS1 comprised 4–10% of total bacterial cell protein in the supernatant fraction of bacterial cell lysates. Based on enzyme activities and on the proportion of total bacterial lysate protein represented by recombinant PRS1 isoforms, provisional estimates of the absolute specific activities of recombinant normal and patient PRS1 were made, indicating comparable values. When recombinant normal and patient (TB and AD) PRS1 were purified to >98% homogeneity, the specific activities of each of the purified recombinant enzymes were nearly identical (recombinant normal PRS1, 23.4–26.7 units/mg protein; TB, 27.2 units/mg; AD, 24.1 units/mg). Together, these studies provide evidence against the view that catalytic superactivity of PRS results from mutation in the protein-encoding regions of PRPS1 and favor an alternative possibility, increased expression of the normal PRPS1 gene product.

PRS1 Transcript Sequence—Segments of the PRPS1 gene including the 5'-transcribed but not translated region and the 3'-untranslated portion of the last exon (exon 7) were amplified by PCR from normal and patient fibroblast genomic DNA and sequenced. Identical sequences corresponding to those previously reported (28) were confirmed for the gene segment inclusive of both previously demonstrated transcription initiation sites and extending to the initiation codon (−141 to −1) in two
normal and five patient genomic DNA preparations. Similarly, the 997-bp terminal portions of exon 7 and the adjacent 3’-DNA, extending from the translation termination codon (955–957) to 33 bp beyond the polyadenylation signal sequence (1928–1933), were identical in the two normal and two patient (TB and AD) fibroblast genomic DNAs studied. In conjunction with the normal translated region sequence demonstrated in PRS1 cDNA from patients with PRS catalytic superactivity, these findings support the view that increased expression of the PRS1 isoform in this disorder is unlikely to result from primary alteration in the 2.1-kilobase portion of the 2.3-kilobase mature PRS1 transcript encoded by the PRPS1 gene.

PRS Transcript Levels—Northern blot analyses of PRS transcripts in total RNAs extracted from cultured cells of normal individuals and patients with PRS catalytic superactivity are shown in Fig. 3. The relative abundance of 2.7-kilobase PRS2 transcripts is substantially greater in lymphoblasts than in fibroblasts, but within each cell type normal and patient cells contain comparable steady state levels of this transcript relative to glyceraldehyde-3-phosphate dehydrogenase mRNA (Table IV). In contrast, relative levels of 2.3-kilobase PRS1 transcripts in patient fibroblasts exceeded those in normal fibroblasts by 1.7–6.6-fold (Table IV). In addition, a nearly coordinate relationship between the increase in relative PRS1 mRNA abundance and the relative increment in PRS1 isoform content and enzyme activity was demonstrable in each patient fibroblast strain. More modest but similarly selective increases in PRS1 transcript levels were also found in lymphoblasts from patients TB and AD (Table IV). As was the case in fibroblasts, relative increases in PRS1 transcript levels in these cell lines correlated well with relative increases in PRS1 isoform concentrations.

DISCUSSION

These studies establish the enzymatic basis of human X-linked PRS catalytic superactivity as selective overexpression of the PRS1 isoform. In six fibroblast strains from unrelated individuals in whom PRS catalytic superactivity is associated with uric acid overproduction and accelerated fibroblast PRPP and purine nucleotide synthesis, the concentrations of PRS1 (but not PRS2) substantially exceeded those in normal fibroblast strains. Moreover, in all normal and patient fibroblast strains, PRS activities and total immunoreactive PRS isoform concentrations were closely related, so that calculated PRS absolute specific activities were comparable and were, in fact, very similar to the specific activities of highly purified recombinant normal PRS1 measured here and previously reported (13). Three inferences may be drawn from these findings: (a) the immunoblotting procedure appears to provide quantitative or nearly quantitative measurements of PRS isoforms in cell extracts; (b) with PRS1 constituting the major portion of total PRS isoform content in fibroblasts, the nearly uniform absolute specific activities calculated for PRS in normal and patient cells indicates that PRS catalytic superactivity must derive from increased concentrations of PRS1 with normal or near normal maximal reaction velocities rather than, as previously suggested (20), from PRS1 with increased catalytic activity per immunoreactive molecule; and (c) the comparable values of PRS absolute specific activities calculated for fibroblast strains and measured for recombinant normal PRS1 make it unlikely that the molecular basis for PRS superactivity involves a discrepancy between normal and patient PRS1 in binding to an effector of PRS activity, such as the inhibitory PRS-associated protein described in rat liver (29).

The enzymatic basis of both PRS catalytic superactivity and PRS superactivity associated with defective allosteric regulation of enzyme activity is overexpression of the PRS1 isoform, but the genetic defects underlying these classes of inherited enzyme overactivity are distinct. Defects in purine nucleotide inhibition and P activation of PRS1 are the consequences of single amino acid substitutions reflecting point mutations in the translated sequence of PRPS1 (8, 10). In contrast, the allosteric properties of PRSs in cells of patients with catalytic superactivity are normal, and the translated sequences of PRS1 and PRS2 cDNA derived from fibroblasts of affected

**TABLE IV**

| Cell source | Relative transcript level* |
|-------------|----------------------------|
|             | PRS1/GAPDH | PRS2/GAPDH | PRS1/PRS2 |
| Fibroblasts  |             |             |           |
| Normal      |             |             |           |
| WO          | 2.9         | 0.5         | 5.8       |
| LEO         | 2.8         | 0.5         | 5.6       |
| SEL         | 2.4         | 0.4         | 4.8       |
| DT          | 2.0         | 0.6         | 3.3       |
| ON          | 2.2         | 0.4         | 5.5       |
| Patient     | 16.3        | 0.5         | 32.6      |
| TB          | 14.7        | 0.6         | 24.5      |
| SS          | 4.3         | 0.4         | 10.8      |
| RA          | 6.4         | 0.4         | 16.0      |
| ZB          | 7.7         | 0.5         | 15.4      |
| Lymphoblasts|             |             |           |
| Normal      |             |             |           |
| LIV         | 3.6         | 5.6         | 0.6       |
| LIS         | 3.6         | 4.4         | 0.8       |
| Patient     | 6.3         | 4.9         | 1.3       |
| TB          | 7.4         | 5.7         | 1.3       |

* Values given are means of three determinations of the ratios of densities in the respective bands measured on a PhosphorImager screen during 16 h of exposure. Agreement among measurements was within 20%.

**Fig. 3.** Northern blot analysis of fibroblast (A) and lymphoblast (B) total cellular RNA. Samples of RNA (approximately 10 μg per lane) were applied to denaturing gels, and electrophoresis, RNA transfer to nitrocellulose filters, prehybridization, hybridization, and washing of filters were carried out as described under “Experimental Procedures.” Specific radioactivities of PRS1 and PRS2 cDNA probes were 2 × 10⁶ cpm/μg. Washed filters were exposed to a PhosphorImager screen for 16 h and then to x-ray film for 40 h at –70 °C. A: lane 1, ZB; lane 2, TB; lane 3, LEO; lane 4, SS; lane 5, DT. B: lane 1, LIV; lane 2, TB, lane 3, AD; lane 4, LIS.
individuals are also normal. In addition, both crude and highly purified preparations of recombinant PRS1 expressed in E. coli transformed with PRS1 cDNA derived from these patients display normal catalytic and allosteric properties, in distinction to the aberrant allosteric properties identified in recombinant PRS1 expressed from the PRS1 cDNA of patients with point mutations in the PRS1 cDNA coding sequence (8). These data are consistent with the view that excessive expression of PRS1 activity in the fibroblasts of patients with PRS catalytic superactivity results from increased concentrations of the normal PRS1 isoform.

The level of regulation of PRPS1 expression altered in inherited PRS1 superactivity remains to be defined. We have shown evidence, however, that the sequence of the mature 2.3-kilobase PRS1 transcript is identical in normal fibroblasts and in fibroblasts from two of the affected individuals. Moreover, by Northern blot analysis, selective increases in the PRS1 transcript were demonstrable in the fibroblast total RNA of the five patient strains tested, and overall there was a close relationship between relative increases in levels of PRS1 transcript and in PRS1 activity and isoform contents. The results of these studies thus support the contention that a pretranslational defect (or defects) in regulation of PRPS1 expression underlies PRS catalytic superactivity. Whether such a defect acts through increases in rates of PRPS1 transcription or through altered PRS1 transcript processing or stability and whether, in fact, PRS1 catalytic superactivity reflects mutations in the PRS1 locus or, alternatively, in a gene modifying PRPS1 expression is an issue requiring additional studies aimed at defining the regulation of expression of this gene.

Prior studies of PRS transcript levels (30, 31) have established organ- and cell type-specific differences in the expression of PRPS genes. The current measurements of X-linked PRS transcripts and isoforms extend these observations to a substantially greater relative contribution of PRPS2 to total PRPS gene expression in normal lymphoblasts than in normal fibroblasts. Not only do PRS2 transcript levels exceed those of PRS1 transcript in normal lymphoblasts but also PRS2 constitutes 40% of total PRS isoforms in these cells, compared with <20% in normal fibroblasts. Of greater interest in the current context, however, is the apparent attenuation of selective overexpression of PRS1 in the lymphoblasts of patients (TB and AD) whose PRS superactivity was substantially greater (4- and 3-fold, respectively) in fibroblasts. Although consistent but low level overexpression of PRS1 was detectable in the lymphoblasts of these patients (1.4- and 1.6-fold, respectively), as reported by Losman et al. (21), rates of PRPP and purine nucleotide synthesis were normal. It seems likely that the normal metabolic phenotype of the lymphoblasts from these patients reflects the low level of overexpression of PRS1 in these cells (compared with fibroblasts), as a consequence of which increased PRPP availability sufficient to activate the pathway of purine synthesis denovo to an abnormal rate is not achieved. PRS catalytic superactivity may thus be an example of a gene-regulatory defect in which cell-specific differences in phenotypic expression reflect variation among cell types in mechanisms modulating aberrant gene expression.

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