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Assignment of the human gene for liver-type 6-phosphofructokinase isozyme (*PFKL*) to chromosome 21 by using somatic cell hybrids and monoclonal anti-L antibody

(Down syndrome/gene-dosage effect/enzyme-immunoprecipitation using staphylococcal protein A/human gene mapping/glycogenosis type VII)

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ABSTRACT **Human 6-phosphofructokinase (PFK; ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11) is under the control of structural loci that code for muscle (M), liver (L), and platelet (P) subunits, which are variably expressed in different tissues; human diploid fibroblasts and leukocytes express all three genes. Random tetramerization of these subunits produces various isozymes, which can be distinguished from one another by ion exchange chromatography or by subunit-specific monoclonal antibodies. We have examined 17 somatic cell hybrids established between Chinese hamster cells and human diploid fibroblasts or leukocytes for the expression of L-type subunits of human PFK. As electrophoresis does not distinguish between Chinese hamster PFKs and human PFKs, we used an anti-human L-subunit-specific monoclonal antibody, which does not react with Chinese hamster PFKs. The expression of human L subunits in the hybrids was detected by the enzyme-immunoprecipitation technique using staphylococci bearing protein A as an immunoadsorbent. Twelve out of 17 hybrids expressed human L subunits and retained chromosome 21, as determined by chromosome and isozyme marker analysis, whereas 5 did not express human *PFKL* and lacked chromosome 21. The mean erythrocyte PFK of seven individuals with trisomy 21 was found to be elevated (147% of normal). A specific increase in L subunits in trisomic erythrocytes was evident chromatographically by a striking increase in L_4 species (50%; normal 10%) and immunologically by decreased precipitation with anti-M monoclonal antibody (50%; normal 80%). We conclude from these data that *PFKL* is located on chromosome 21 and that the previously noted elevation of erythrocyte PFK activity in individuals with trisomy 21 is due to a gene-dosage effect.**

Phosphofructokinase (PFK; ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11), the key regulatory enzyme of glycolysis, is a tetrameric protein with $M_r \approx 340{,}000$ (1). Inherited deficiency of PFK is manifested in man as a group of clinically heterogeneous syndromes characterized by myopathy or hemolysis or both. The most common form is glycogen storage disease type VII, or Tarui disease, which is characterized by a total lack of muscle PFK but only an ≈50% reduction in erythrocyte (RBC) PFK (2–4). In contrast, increased PFK activity has been found in individuals with trisomy 21 (Down syndrome). In these individuals, the ≈50% increase in RBC PFK has led to the hypothesis that this might be a gene-dosage effect resulting from the presence of a gene for PFK on chromosome 21 (5–10).

Vora *et al.* (2, 11, 12) have recently established the existence of a trigenic isozyme system for human PFK. Three structural loci, *PFKM*, *PFKL*, and *PFKP* encode distinct PFK subunits; i.e., muscle (M), liver (L), and platelet (P). A tissue may express one, two, or all three genes; the products of these genes randomly form tetramers, which can be identified by ion exchange chromatography, or subunit-specific mouse monoclonal antibodies (11, 12). The partial reduction of RBC PFK in a patient with glycogenosis type VII was shown to result from a total deficiency of M-type subunits (2).

The expression of all three PFK genes by human diploid fibroblasts suggested that interspecific somatic cell hybrids may also express these genes (12). In this study, by using anti-human L-subunit-specific monoclonal antibody, we have analyzed a panel of human–Chinese hamster somatic cell hybrids for the expression of human L subunits to establish the chromosomal location of the *PFKL* gene. The human *PFKL* expression segregated concordantly with human chromosome 21, and none other, indicating that *PFKL* locus is on chromosome 21. In addition, we have determined quantitative and qualitative RBC PFK profiles of individuals with trisomy 21 and have shown that the elevation in RBC PFK is due to a specific increase in L-type subunits. These data demonstrate that the previously observed elevation of RBC PFK in individuals with trisomy 21 is a manifestation of a gene-dosage effect.

MATERIALS AND METHODS

Production of Somatic Cell Hybrids. Four sets of Chinese hamster–human somatic cell hybrids were used. Experiment XV clones were derived from 380-6 cells, hypoxanthine phosphoribosyl transferase (EC 2.4.2.8)-deficient derivatives of Chinese hamster lung fibroblast line V79 that were fused in mixed monolayer to human fibroblasts containing a t(1;6) translocation [46,XY,t(1;6)(p3200;p2100)] (13). In experiment XXI, a23 cells, thymidine kinase (EC 2.7.1.75)-deficient derivatives of Chinese hamster lung fibroblasts, were hybridized with human fibroblasts containing a t(11;15) translocation [46,XY,t(11;15)(p11;p12)] (14, 15). In addition, six 380-6-derived hybrid clones that were made with human leukocytes containing a t(X;14) translocation were used. The derivation and properties of these hybrids (XIII-J, M, N, and P and XII-K and L) have been reported (16). Hybrid cells were harvested as described (13) and stored as dried pellets at −80°C for periods of 1 week to 2 years; however, storage time had no apparent influence on PFK activities.

Chromosome Analysis. Hybrid cell chromosomes were analyzed at the subculture at which cells were harvested for enzyme

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Abbreviations: PFK, 6-phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11); RBC, erythrocyte; M, P, and L, muscle, platelet, and liver subunits; SOD1, superoxide dismutase 1.

studies. Chromosome preparations were made in suspension by a standard air-drying technique or by an *in situ* method with cells grown on coverslips overnight. Slides as well as coverslips were subjected to the heat and trypsin/Giemsa banding technique as described (17). Photographic prints of randomly chosen well-banded metaphase spreads were analyzed. These hybrid clones had been studied before, and their human chromosome content was known. The purpose of the present analysis was to establish the relative frequency of each chromosome at the time of the PFK studies. For most hybrids, 15–29 cells were analyzed; for stable hybrids with a rather uniform karyotype, the analysis of 10 cells was considered sufficient.

Assays of PFK Activity. Assays were performed with a Gilford model 250 spectrophotometer at 26°C as described (11); RBCs were purified, and RBC PFK activity was expressed as units per 10^{10} cells as described (12).

Chromatographic Separation of RBC PFK Isozymes. Hemolysates from two individuals, one with trisomy 21 and the other with a 14/21 translocation, were chromatographed on a DEAE-Sephadex A-25 column as described (11, 12).

Production of Anti-M and Anti-L Monoclonal Antibodies. Subunit-specific and species-specific monoclonal antibodies to M and L subunits of human PFK were produced by the lymphocyte hybridoma technique. The details of the immunization protocol, technique of hybridization, screening of the secretory hybrids, production of antibody-rich mouse ascites fluids, and characterization of the secreted antibodies are described elsewhere (18).

Enzyme Immunoprecipitation Assays. Hybrid cell extracts were prepared just prior to precipitation studies by using 0.2–0.3 ml of chilled 50 mM potassium fluoride buffer, pH 7.5/5 mM EDTA/5 mM ammonium sulfate/0.6 mM AMP/3 mM dithiothreitol/0.6 mM fructose 6-phosphate as described (12). Fifty microliters of diluted PFK preparation (0.05–0.1 units/ml) were mixed with 50 μl of the diluted mouse ascites fluid (1:200) containing anti-L (V65-06) antibody; control tubes contained nonimmune mouse serum or buffer. The mixtures were incubated for 30 min at 37°C and then 150 μl of a freshly washed 10% suspension of IgGsorb (staphylococci bearing protein A; The Enzyme Center, Boston, MA) was added.‡ The mixtures were incubated at 4°C for 1 hr with continuous shaking, centrifuged at 4000 × *g* for 10 min and assayed for residual enzyme activity. To take experimental variability into account, only tubes showing >10% precipitation in comparison with their controls were considered to be definitely positive. Each supernatant was tested in duplicate with duplicate controls on two different occasions. In coded blood samples from seven individuals with trisomy 21 and seven normal individuals, RBC PFK was similarly investigated by using both types of monoclonal antibodies.

Other Isoenzyme Markers. Additional isozymes were assayed after electrophoresis as markers for human chromosomes 11, 12, 14, 19, 20, and 21, respectively, by cellogel electrophoresis as reported (20): lactate dehydrogenase A and B (EC 1.1.1.27), nucleoside phosphorylase (EC 2.4.2.1), glucose phosphate isomerase (EC 5.3.1.9), adenosine deaminase (EC 3.5.4.4), and superoxide dismutase 1 (SOD1 1.15.1.1).

RESULTS

Chromosome Content of Chinese Hamster–Human Somatic Cell Hybrids. A panel of Chinese hamster–human hybrids was selected from four independent hybridization experiments that involved two different Chinese hamster cell lines and four different donors. The 17 hybrid clones were derived from at least 13 independent fusion events; some of the lines were recloned to reduce intraclonal heterogeneity. The human chromosome contents were monitored quantitatively at the time of each harvest for enzyme studies. The frequencies of human chromosome 21 at the passage level at which cells were harvested for the PFK studies are given in Table 1. Not only were the hybrid cell metaphase spreads scored for the presence of human chromosomes, but the hamster chromosomes were also examined carefully for the presence of *de novo* structural arrangements. Therefore, the chances for undetected rearrangements involving parts of human chromosomes are considered small. Results of the electrophoretic isozyme marker studies for chromosomes 11, 12, 19, 20, and 21 were consistent with the cytological results.

Subunit and Species Specificities of Monoclonal Antibodies. The biochemical and immunochemical characterization of five monoclonal antibodies to M- and L-type subunits of human PFK is described elsewhere (18). Four of these antibodies (V44-08, V44-09, V47-20, and V65-06) react with L-type PFK from man and a few other vertebrate species but not with PFKs from Chinese hamster. An excess of each of these antibodies precipitates completely not only L_4-type homotetramers but also heterotetramers containing one or more of the L subunits—i.e., L_3M, L_2M_2, and LM_3 (12). The anti-M antibody (V96-26) reacts with M-type PFK from man and monkey but not with PFKs

Table 1. Frequency of human chromosome 21 and expression of human *SOD1* and *PFKL* in hybrid clones

Cell line	Frequency of chromosome 21, copies per cell	Human *SOD1* expression	Immunoprecipitation with anti-L*
Human fibroblast	2.0	+	70
Chinese hamster			
a-23	—	−	0
380-6	—	−	2
Control hybrid†	1.0	+	46
Clone			
XV-D	1.0	+	44
XV-E	0.8	+	43
XXI-F	0.4‡	+	39
XXI-G	1.0	+	34
XV-H	1.1	+	58
XXI-I	0	−	4
XIII-J	0	−	0
XII-K	0.9	ND	57
XII-L	1.0	ND	29
XIII-M	0	ND	6
XIII-N	0	−	0
XXI-O	0.7§	−	22
XIII-P	0	−	5
XXI-R	0.8	+	50
XXI-S	0.8	ND	48
XV-T	0.9	+	44
XV-U	0.9	+	37

Ten to 29 metaphases per clone were analyzed.

* Values given are the percent of enzyme activity precipitated as compared with that of the control under the conditions described in *Materials and Methods*. Each value represents the average of four determinations; values <10% precipitation are considered not significant. ND, not done.

† Chinese hamster–human hybrid (X-7A) contains one copy of each human chromosome.

‡ Long arm of 21 only in t(8p21q).

§ 21q+ (rearranged).

‡ IgGsorb was washed twice with 50 mM Tris·HCl, pH 7.5/150 mM NaCl/5 mM EDTA/0.5% Nonidet P-40/0.02% NaN_3 and then twice with 50 mM Tris·HCl, pH 7.5/150 mM NaCl/5 mM EDTA/0.05% Nonidet P-40/0.02% NaN_3. It was then suspended to the original volume in the latter buffer (19).

from Chinese hamster. Therefore, we expected these antibodies to precipitate interspecific heterotetramers, containing either human L or M subunits.

Detection of Human PFK L Subunits in Hybrid Clones by Enzyme Immunoprecipitation Assay. As shown in Table 1, anti-L antibody (V65-06) precipitated ≈70% of PFK from human parental fibroblasts; however, it showed no reactivity with PFKs from both Chinese hamster parental cell lines (Don/a23 and V79/380-6). Five of 17 hybrids did not express L subunits, as indicated by negligible precipitation values (0–6%), whereas 12 of 17 hybrids exhibited varied precipitation values (22–58%), indicating expression of human L subunits. For each hybrid, the precipitation value was consistent and reproducible on retesting. There was no apparent quantitative difference between fibroblast-derived hybrids (series XV and XXI) and leukocyte-derived hybrids (series XII and XIII) in the expression of human *PFKL*.

Segregation of Human Chromosomes with Human *PFKL* Expression. In the 17 Chinese hamster–human hybrids studied, human chromosome 21 segregated concordantly with the expression of human L subunits without exception (Tables 1 and 2). Two clones were positive for human PFKL but did not contain a normal chromosome 21. In one of them (XXI-F), the long arm of 21 was fused at the centromere with the short arm of human chromosome 8; this clone expressed human SOD1, the enzyme marker of chromosome 21. The other exceptional clone (XXI-O) contained a rearranged chromosome 21 and did not express human SOD1. A number of discordant clones excluded all other human chromosomes as possible sites for human *PKFL*.

Studies of RBC PFK from Individuals with Trisomy 21. *Quantitative assay.* Seven individuals with Down syndrome (six with trisomy 21 and one with a 14/21 translocation) were studied. Purified RBCs from trisomic individuals showed a mean increase of 47% in PFK activity (Table 3).

Table 2. Segregation of *PFKL* expression and human chromosomes in 17 chinese hamster–human hybrid clones

Chromosome	PFK L/chromosome +/+	+/−	−/+	−/−	% discordant clones
1	4	5	1	4	43
2	5	7	0	5	41
3	8	4	2	3	35
4	5	7	1	4	47
5	7	5	1	4	35
6	6	3	1	4	29
7	2	8	3	2	73
8	8	3	1	4	25
9	2	8	1	4	60
10	2	9	1	4	62
11	4	7	3	2	62
12	8	3	1	4	25
13	6	6	0	5	35
14	11	1	2	2	19
15	7	5	1	4	35
16	6	6	1	4	41
17	3	9	0	4	56
18	5	7	1	4	47
19	9	3	1	4	24
20	7	5	1	4	35
21	11	0	0	4	0
22	11	0	2	3	13
X	6	6	2	3	47
Y	5	7	0	5	41

If a chromosome was present in a rearrangement or in frequencies of 0.2 or less, that particular clone is excluded from this table.

Table 3. RBC PFK from individuals with Down syndrome

	PFK activity, units/10^{10} cells	% immunoprecipitation Anti-M	Anti-L
Trisomy 21 (n = 6)	3.37 ± 0.29	50.83 ± 7.63	93.00 ± 1.55
14/21 translocation (n = 1)	3.34	55	89
Control (n = 7)	2.30 ± 0.08	81.00 ± 4.05	89.67 ± 4.16

Data are mean ± SD.

Chromatographic profile. The representative isozymic pattern of trisomic RBC PFK is compared with that of normal RBC PFK in Fig. 1. In contrast to normal RBC PFK, which shows a five-membered set with ≈10% L_4-type isozyme, PFK from trisomic RBC shows an absence of M_4-type isozyme and a striking increase (≈50%) in L_4 species.

Enzyme immunoprecipitation. An excess of anti-M monoclonal antibody precipitated 51% of RBC PFK from trisomic individuals as compared with 81% of PFK from normal RBC. In contrast, an excess of anti-L monoclonal antibody precipitated 90% of RBC PFK from both trisomic and normal individuals.

DISCUSSION

Interspecific somatic cell hybrids preferentially segregating human chromosomes have proved extremely useful for the assignment of a given genetic locus to a specific human chromosome and for the establishment of linkage relationships between human genes expressed in cultured somatic cells. Concordant segregation of syntenic genes and of human chromosomes from these hybrids has led to the assignment of more than 100 gene loci coding for human enzymes to their respective chromosomes (21).

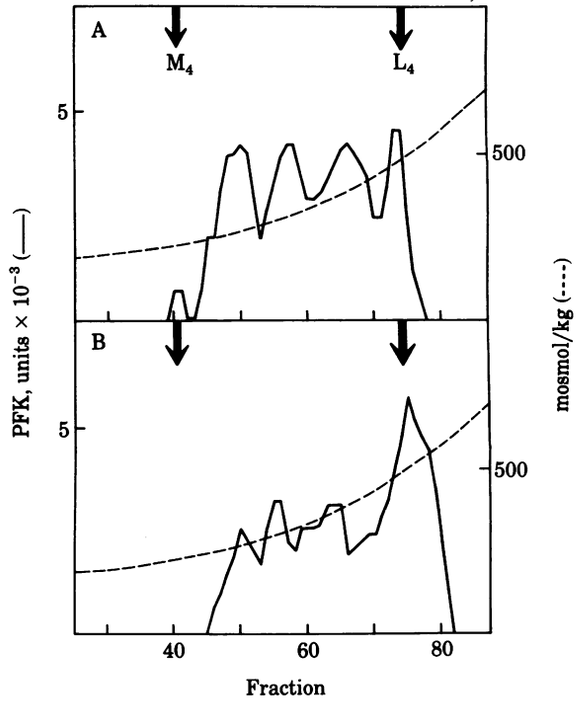

FIG. 1. Comparison of the chromatographic profile of RBC PPK isozymes of trisomy 21 individuals with that of normal individuals. (*A*) Normal RBC PFK, showing entire five-membered set; L_4 comprises ≈10% of the total. (*B*) Trisomic RBC PFK, showing only four isozymes; M_4 is absent and L_4 comprises ≈50% of the total.

The somatic cell genetic approach to mapping genes depends on whether or not the enzyme in question is expressed in tissue culture cells and on whether or not species or allelic differences can be demonstrated. A large number of enzymes of ubiquitous tissue distribution that are involved in basic cell functions (e.g., those of glycolysis) are expressed in cultured cells, while relatively few enzymes associated with a differentiated function are expressed (22). Interspecific differences in homologous enzymes—e.g., electrophoretic, antigenic, or kinetic—are common because their primary structures differ as a result of evolutionary divergence. Therefore, human and rodent enzymes in somatic cell hybrids can usually be distinguished by different electrophoretic mobilities (20, 23, 24). By using the interspecific enzyme differences, 8 out of 11 glycolytic enzymes have been mapped to specific human chromosomes (21). The loci for the glycolytic enzymes that have yet to be assigned are those for phosphofructokinase (EC 2.7.1.11), fructose-bisphosphate aldolase (EC 4.1.2.13), and phosphoglyceromutase (EC 2.7.5.3). A provisional assignment of the gene for the P subunit of PFK (the same as the "F subunit") to chromosome 10 has recently been reported (25).

Recent biochemical, immunochemical, and genetic studies (2, 11, 12) have established the existence of a trigenic isozyme system for human PFK. Human muscle PFK and partially purified liver PFK are distinct homotetramers—M_4 and L_4, respectively—whereas RBC PFK usually consists of an entire five-membered isozyme set composed of M and L subunits—M_4, M_3L, M_2L_2, ML_3, and L_4 (11). The residual RBC PFK from a patient with Tarui disease consisted exclusively of L_4-type isozyme, indicating a complete deficiency of M subunits (2). Platelet PFK consists of three isozymes composed of a distinct subunit "P" in combination with L subunits—P_4, P_3L, and P_2L_2—whereas leukocyte PFKs (both lymphocytes and neutrophils) consist of multiple species composed of all three subunits. Cultured diploid fibroblasts and Epstein–Barr virus-transformed lymphoblastoid cell lines express all three subunits, which results in the formation of more than five (usually 10–12) chromatographically distinguishable species. In contrast, the lymphoblastoid cell line from the M-deficient patient not only exhibits a quantitative PFK deficiency but also consists of only five isozymes composed of P and L subunits, indicating that both normal genes and the genetic defect expressed by somatic cells are also expressed by cultured cell lines (12).

Recently, monoclonal antibodies specific for M- and L-type subunits of human PFK have been produced and characterized (18). All of these mouse antibodies exhibit unique subunit and species specificities. The anti-L (V65-06) and anti-M (V96-26) antibodies used in this study do not react with PFKs from cultured cell lines of BALB/c and Chinese hamster origin, indicating that these may serve as powerful tools in the analysis of man–rodent hybrids for the expression of human *PFKM* and *PFKL* genes.

The feasibility of immunologic discrimination between human and rodent PFKs is of paramount importance, because these could not be separated from each other by electrophoresis. Moreover, it is likely that rodents possess and therefore may express more than one *PFK* gene in cultured cell lines. The multigenic control of rodent PFK is strongly suggested by the presence of distinct isozymes in related species such as the rat (26, 27) and the rabbit (28, 29).

Human fibroblasts are known to express all three subunits. If human–rodent hybrids were to express one, two, or three rodent *PFK* genes as well, theoretically, this could result in the formation of 35, 70, and 126 distinct heteropolymeric isozymic species, respectively (30), which would be impossible to resolve either electrophoretically or chromatographically. However, human- and subunit-specific antibodies may detect the expression of a given human subunit, despite its occurrence in relatively low concentration and in combination with other subunits.

In this study, we used somatic cell hybrids derived from normal human diploid fibroblasts or leukocytes and cultured cell lines from Chinese hamster. The monoclonal anti-human L and M antibodies precipitated 70% and 50%, respectively, of human fibroblast PFK, indicating the expression of these subunits by human fibroblasts (12). Analysis of a panel of 17 independent hybrid lines indicated that only 12 of 17 expressed L subunits (see Table 1). Without exception, human chromosome 21, and none other, segregated concordantly with the expression of human PFK L subunits (see Table 2). Thus, we conclude that the human *PFKL* gene is located on human chromosome 21. The absence of strict correlation between the frequency of chromosome 21 and the percent precipitation of PFK activity in the hybrid clones is probably due to the variable presence of human chromosomes bearing the *PFKM* and *PFKP* genes and possibly also of Chinese hamster chromosomes bearing *PFK* genes. Different hybrids may therefore possess variable amounts of these subunits, which randomly tetramerize with human L subunits. This may result in variable precipitation values, despite identical frequencies of chromosome 21, because anti-L monoclonal antibody precipitates all human L-subunit-containing inter- and intraspecific heterotetramers.

The localization of *PFKL* on chromosome 21 is of interest because, over the last 15 years, the higher activity of PFK found in the RBCs of individuals with trisomy 21 has led to controversy as to whether this represents a gene-dosage effect, indicating control of human PFK by a gene or genes on chromosome 21 (5–10, 31–33). Baikie *et al.* (5) first reported a nearly 50% increase in RBC PFK activity in individuals with trisomy 21 and suggested that this might be a gene-dosage effect. Since then, six additional studies have shown elevated RBC PFK activities (129–175% of normal) in individuals with Down syndrome (6–10, 32). The fact that the activities of other glycolytic enzymes were not increased in RBCs supported the gene-dosage hypothesis (5–7). However, it had been suggested that the increased PFK activity might be related to a relatively young mean cell age (31), as reflected by macrocytosis of trisomic RBCs (31, 34) and by the increased activity of several other RBC enzymes, including glucose-6-P-dehydrogenase (EC 1.1.1.49) and glutamate oxaloacetate transaminase (EC 2.6.1.11) (31, 32). Layzer and Epstein (33) showed that trisomic RBCs are as dense as (and therefore of the same age), if not denser than, normal RBCs and thus disproved the young-RBC hypothesis. Moreover, other investigators have reported glucose-6-phosphate dehydrogenase to be normal (5) or less than normal (9), thus making interpretation of a few reports of increased activity very difficult.

The activity of PFK in other cells of trisomy 21 individuals, leukocytes (10), platelets (8), and cultured fibroblasts (33), have been reported as normal. The normal platelet PFK level was deemed by Doery *et al.* (8) not to be inconsistent with a gene-dosage effect in RBCs. They hypothesized that there may be separate structural loci encoding RBCs and platelet PFKs and that these may be located on different chromosomes. However, they abandoned the hypothesis because identical pH optima were found for these two enzymes, which was interpreted as indicating structural identity. Similarly, Conway and Layzer (10) proposed dual genetic control for human RBC PFK and suggested that one of the two genes (probably *PFKM*) was located on chromosome 21. However, Layzer and Epstein (33) later abandoned this hypothesis because PFK from cultured fibroblasts (known to express M subunits) from trisomy 21 individuals did not exhibit an increased PFK activity.

In light of the gene localization reported here and the data describing subunit structures of PFKs from normal human blood cells and cultured cell lines presented elsewhere (12), it is obvious that elevated PFK activity in trisomy 21 RBCs results from a gene-dosage effect. As the normal contribution of the *PFKL* gene is ≈50% of the total RBC PFK (2, 11), an extra copy of *PFKL* in trisomy 21 should result in an increment of L subunits of ≈25% and in an overall PFK activity level of 125%, as has been reported by Conway and Layzer (10). The reportedly normal PFK activities of trisomic platelets (8), leukocytes (10), and cultured fibroblasts (33) appear to be inconsistent with our subunit structural data, because each of these cell types is known to express L-containing species (12). The failure to find an increment in platelet PFK may be due to the fact that L subunits constitute a minor component of total PFK (≈30%). Therefore, trisomic platelets are expected to exhibit only a minor increment (≈15%) of L subunits, which is within the range of normal platelet PFK activity and therefore may not be detectable. However, it is possible that the isozymic profile of PFK from trisomic platelets may show an increase in L-containing species. In contrast, L subunits constitute a major component of leukocyte and fibroblast PFKs (50–70%), suggesting that trisomic cells are expected to exhibit significantly elevated PFK activity. However, gene-dosage studies on fibroblasts are more difficult because the enzyme activity may be influenced by a number of variables, including passage level, rate of growth, and culture conditions. It is conceivable that the increments may become more evident if PFK activity is expressed on a per cell basis for each of these cell types (12) rather than on a per gram of protein basis (10, 33).

To provide conclusive proof for the gene-dosage effect, we have investigated quantitative and qualitative PFK profiles of RBCs of seven individuals (six with trisomy 21 and one with a 14/21 translocation). We found an average of 50% increment in PFK activity and a striking increase in L_4 species both chromatographically and immunologically (see Table 3 and Fig. 1). An excess of the monoclonal anti-M antibody precipitated only 50% of trisomic RBC PFK, but 80% of PFK from normal individuals, indicating an increase in anti-M-resistant isozymes. The specific increase of L subunits in trisomy 21 RBCs reduces the fraction of active molecules that contain M subunits. The increment in L subunits is not directly evident by immunoprecipitation with anti-L, because this antibody precipitates not only L_4 but also hybrids of L with M and, in normal controls, anti-L already precipitates ≈90% of the total activity.

Thus, our studies provide gene mapping, molecular, and immunologic evidence for gene dosage as the basis for elevated PFK activity in the RBCs of individuals with trisomy 21. Our results also show that the combination of somatic cell hybridization and secretory lymphocyte-hybridoma techniques may prove useful for similar genetic analysis of complex isozyme systems.

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1. Bloxham, D. P. & Lardy, H. A. (1973) in *The Enzymes*, ed. Boyer, P. D. (Academic, New York), pp. 239–278.
2. Vora, S., Corash, L., Engle, W. K., Durham, S., Seaman, C. & Piomelli, S. (1980) *Blood* **55**, 628–635.
3. Tarui, S., Okuno, G., Ikura, Y., Tanaka, T., Suda, M. & Nishikawa, M. (1965) *Biochem. Biophys. Res. Commun.* **19**, 517–523.
4. Layzer, R. B., Rowland, L. P. & Ranney, H. M. (1967) *Arch. Neurol.* **17**, 512–523.
5. Baikie, A. F., Loder, R. B., deGrouchy, G. C. & Pitt, D. B. (1965) *Lancet* **i**, 412–414.
6. Benson, P. S., Linacre, B. & Taylor, A. I. (1968) *Nature (London)* **220**, 1235–1236.
7. Bartels, H., Kruse, K. & Tolksdorf, M. (1968) *Lancet* **i**, 820.
8. Doery, J. C. G., Hirsh, J., Garson, O. M. & deGrouchy, G. C. (1968) *Lancet* **ii**, 894–895.
9. Pantelakis, S. N., Karaklis, A. G., Alexiou, B., Varbas, E. & Valaes, T. (1970) *Am. J. Hum. Genet.* **22**, 184–193.
10. Conway, M. M. & Layzer, R. B. (1970) *Humangenetik* **9**, 185–190.
11. Vora, S., Seaman, C., Durham. S. & Piomelli, S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 62–68.
12. Vora, S. (1981) *Blood* **57**, 724–732.
13. Francke, U. & Pellegrino, M. A. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1147–1151 and 5776.
14. Sanders-Haigh, L., Anderson, W. F. & Francke, U. (1980) *Nature (London)* **283**, 683–686.
15. Francke, U. & Francke, B. (1981) *Somatic Cell Genet.* **7**, 171–191.
16. Francke, U., Busby, N., Shaw, D., Hansen, S. & Brown, M. G. (1976) *Somatic Cell Genet.* **2**, 27–40.
17. Francke, U. & Oliver, N. (1978) *Hum. Genet.* **45**, 137–165.
18. Vora, S., Wims, A. L., Durham, S. & Morrison, S. L. (1981) *Blood* **57**, in press.
19. Kessler, S. W. (1976) *J. Immunol.* **177**, 1482–1490.
20. Meera Khan, P. (1971) *Arch. Biochem. Biophys.* **145**, 470–483.
21. Human Gene Mapping 5 (1979) *Cytogenet. Cell Genet.* **25**, 1–236.
22. Davidson, R. L. (1974) *Annu. Rev. Genet.* **8**, 195–218.
23. Weiss, M. C. & Ephrussi, B. (1966) *Genetics* **54**, 1111–1122.
24. Boone, C. M. & Ruddle, F. H. (1969) *Biochem. Genet.* **3**, 119–136.
25. Weil, D., Cottreau, D., van Cong, N., Rebourcet, R., Foubert, C., Gross, M.-S., Dreyfus, J.-C. & Kahn, A. (1980) *Ann. Hum. Genet.* **44**, 11–16.
26. Tanaka, T., Imamura, K., Ann, T. & Tariuchi, K. (1972) *Gann Monogr. Cancer Res.* **13**, 219–234.
27. Taylor, C. B. & Bew, M. (1970) *Biochem. J.* **119**, 797–799.
28. Tsai, M. Y. & Kemp, R. G. (1973) *J. Biol. Chem.* **248**, 785–792.
29. Tsai, M. Y. & Kemp, R. G. (1974) *J. Biol. Chem.* **249**, 6590–6596.
30. Markert, C. L. (1977) in *Isozymes: Current Topics in Biological and Medical Research* (Liss, New York), Vol. 1, p. 6.
31. Naiman, J. L., Oski, F. A. & Mellman, W. J. (1965) *Lancet* **i**, 821.
32. Rosner, F., Ong, B. H., Paine, R. S. & Mahanand, D. (1965) *N. Engl. J. Med.* **273**, 1355–1361.
33. Layzer, R. B. & Epstein, C. J. (1972) *Am. J. Hum. Genet.* **24**, 533–543.
34. Estham, R. D. & Jancar, J. (1970) *J. Clin. Pathol.* **23**, 296–298.