Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 9996–10000, December 1989
Genetics

I/Lyn mouse phosphorylase kinase deficiency: Mutation disrupts expression of the α/α'-subunit mRNAs

(isoforms/X chromosome-linked glycogen-storage disease/chromosome mapping)

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Communicated by Oscar L. Miller, Jr., September 5, 1989

ABSTRACT **A cDNA encoding the α subunit of mouse skeletal muscle phosphorylase kinase was used to compare the expression of α mRNAs in normal and phosphorylase kinase-deficient tissues of the I/Lyn mouse. The results demonstrate that two different molecular weight species of poly(A)$^+$ RNA in normal mouse heart and skeletal muscle hybridize to the α cDNA. These two mRNAs direct the synthesis of α protein and its isoform α' in a cell-free translation system. Thus, α and α' are encoded by two distinct mRNAs. The abundance of both of these mRNAs is reduced dramatically in the phosphorylase kinase-deficient skeletal muscle and heart tissues from the I/Lyn mouse strain. This result indicates that a mechanism common to both α and α' expression is disrupted by the I/Lyn mutation. The I/Lyn deficiency is inherited as an X chromosome trait. By Southern mapping of Chinese hamster–mouse cell hybrids the α gene was localized to the mouse X chromosome, supporting the possibility that the I/Lyn mutation is in the α gene. These results are discussed in terms of a cis or trans mutation influencing the expression of either a single α/α' gene or two genes encoding α and α'.**

Phosphorylase kinase (ATP: phosphorylase-*b* phosphotransferase, EC 2.7.1.38) is a multisubunit enzyme expressed primarily in liver, cardiac, and skeletal muscle tissues, where it functions to couple glycogenolysis to hormonal stimuli and contraction (1, 2). Several human glycogen-storage diseases result from a deficiency in the skeletal muscle, heart, or liver forms of phosphorylase kinase (3–6). These diseases exhibit both autosomal and X chromosome-linked modes of inheritance. Understanding the molecular basis of these disorders is complicated by the multisubunit composition of phosphorylase kinase. It contains two regulatory subunits, designated α and β, that are substrates for the cAMP-dependent protein kinase and mediate the hormonal activation of phosphorylase kinase (7–9). A third regulatory subunit, δ, is known to be calmodulin and mediates the calcium requirement of phosphorylase kinase (10). The catalytic subunit is designated γ (11, 12). In addition to these four subunits there is an isoform of α, designated α', that is expressed in slow-twitch muscle fibers and cardiac tissue (13–15). Although a mutation in any of these phosphorylase kinase structural genes could result in a deficiency, the tissue-specific phenotype of the deficiencies requires that either alternative mechanisms regulate expression or different genes are expressed in the unaffected tissues.

An animal model for the study of phosphorylase kinase deficiencies is the I/Lyn mouse strain (16, 17). In this strain a mutation exhibiting X chromosome inheritance results in <0.2% of normal enzyme activity in the adult mixed-fiber skeletal muscle and ≈6% of normal activity in the heart (17). Liver tissue and neonatal skeletal muscle have normal enzyme activity. The deficiency in the skeletal muscle results from an absence of detectable α, β, and γ subunits (18) and a 50% decrease in calmodulin levels (10, 19). However, the β, γ, and calmodulin genes are not X-linked and, therefore, cannot be the site of the I/Lyn mutation (18, 20). Only the α gene is a potential site of an X-linked mutation in a phosphorylase kinase structural gene. However, an explanation of the I/Lyn phenotype invoking a mutation in the α gene must account for the absence of the α'-containing phosphorylase kinase isoforms as well as the presence of normal liver phosphorylase kinase activity.

In order to investigate the I/Lyn mutation a cDNA that encodes a portion of the mouse skeletal muscle α subunit was isolated.§ We have used this cDNA to show that the α gene is located on the X chromosome and that α mRNA is significantly reduced in I/Lyn skeletal muscle and heart. Furthermore, we have identified two additional mRNA species that are closely related to the α mRNA. One encodes the α' subunit and is reduced significantly in I/Lyn heart and skeletal muscle. The other encodes a high molecular weight protein that has not yet been characterized. In both normal and I/Lyn mouse liver tissue no mRNAs were identified that hybridized with the skeletal muscle α-encoding cDNA. These data demonstrate that the I/Lyn mutation affects α and α' mRNA accumulation. Therefore, we suggest that the α and α' mRNAs are either transcribed from the same gene or, if transcribed from two different genes, share a common regulatory mechanism. We propose that an additional gene may encode a homologous liver α subunit whose mRNA does not hybridize with the skeletal muscle α cDNA.

MATERIALS AND METHODS

Identification of a cDNA Encoding the Mouse Skeletal Muscle α Subunit. A mixture of degenerate oligonucleotides was synthesized (Operon Technologies, San Pablo, CA) that encoded a sequence of 6 amino acids from the published rabbit skeletal muscle α sequence (21).

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          381                          387
      Asp-Glu-Glu-Tyr-Gln-Asn-(Pro)
5'-GAY-GAR-GAR-TAY-CAR-AAY-CC-3'
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This oligonucleotide mixture was end-labeled by reaction with phage T4 polynucleotide kinase and used for plaque hybridization to a cDNA library made from mouse mixed-fiber skeletal muscle RNA in the λgt11 vector with *Eco*RI linkers (12-mers) (22). Plaque hybridization was according to published procedures (23). The DNA insert from the positive phage clone was excised by *Eco*RI restriction enzyme diges-

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Abbreviation: HPRT, hypoxanthine phosphoribosyltransferase.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. M28867).

tion and subcloned into a plasmid vector [pBluescript KS(+), Stratagene] to yield the α cDNA plasmid used in subsequent experiments. The nucleic acid sequence of the insert was obtained by the Sanger dideoxy method (24) using Sequenase (United States Biochemical) and synthetic oligonucleotide primers.

For hybridization to Northern and Southern blots, the α cDNA plasmid was digested with *Eco*RI restriction enzyme, and the insert was isolated by agarose gel electrophoresis and phenol extraction of the gel segment containing the insert. The insert was labeled with [α^{32}P]dCTP by using the random-primed DNA labeling kit (Boehringer Mannheim).

RNA Preparation and Northern Blot Analysis. RNA was isolated from tissues by extraction in guanidine hydrochloride solution and sedimentation through a CsCl cushion (25). This total RNA fraction was purified further by chromatography on oligo(dT)-cellulose (type VII; Pharmacia) to obtain the poly(A)$^+$ RNA fraction. For Northern blot analysis, RNA was electrophoresed in formaldehyde/agarose gels (26) and the fractionated RNA was electroblotted onto modified nylon membranes (Nytran; Schleicher & Schuell). After UV crosslinking (27), the membranes were prehybridized at 42°C in buffer containing 50% formamide, 6× SSC (1× is 150 mM NaCl/15 mM sodium citrate, pH 7), 20 mM NaH_2PO_4, 1 mM EDTA, 0.1% $NaDodSO_4$, 5× Denhardt's solution (1× is 0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin), and 100 μg of salmon sperm DNA per ml. The blots were hybridized in the same buffer except with 2.5× Denhardt's solution and 2–3 × 10^6 cpm of labeled α cDNA per ml. Final washing was in 0.1× SSC/0.1% $NaDodSO_4$ at 58°C.

Hybridization Selection and Cell-Free Translation. The procedure for hybridization selection of mRNA was modified from a published procedure (28). The modification was to use nylon membranes instead of nitrocellulose as a solid support for the cDNA probe. This allowed the α cDNA containing-plasmid (10 μg) to be denatured in 0.4 M NaOH/0.6 M NaCl and blotted directly onto the membrane segments in the same solution. After neutralization in buffer containing 100 mM Tris/HCl (pH 7.8) and 5 mM EDTA, the membranes were baked at 80°C for 1.5 hr. Membranes were hybridized with 250 μg of a total RNA fraction from ICR Swiss White mouse skeletal muscle for 6 hr at 37°C. Selected RNA was eluted by boiling in 250 μl of 1 mM EDTA for 1 min. Twenty percent of the eluted volume was transferred to a separate tube containing 5.0 μg of tRNA, and sodium acetate was added to give 250 mM. This sample was extracted with phenol/chloroform and the RNA was precipitated by the addition of 2.5× volumes of ethanol and held at −20°C until used for Northern analysis. To the remaining 80% of the eluted RNA were added 5 μg of tRNA, Tris/HCl (pH 7.5) to 10 mM, $MgCl_2$ to 2 mM, NaCl to 50 mM, and 1 unit of RNase-free DNase (RQ1 DNase; Promega). This DNase treatment removed any plasmid that may have been eluted from the membrane with the RNA. If not removed, this plasmid could have inhibited the subsequent cell-free translation. After 15 min at room temperature, EDTA was added to 5 mM and sodium acetate to 250 mM. The sample was extracted once with phenol/chloroform and precipitated with 2.5× volumes of ethanol. For cell-free translation the precipitated RNA was resuspended in H_2O and translated in a Promega *in vitro* translation kit following the manufacturer's recommended procedure, using translation-grade [^{35}S]methionine (ICN). After translation a 5-μl aliquot was removed and mixed with 20 μl of $NaDodSO_4$ denaturant and electrophoresed in an 8.0% polyacrylamide gel. The synthesized proteins were visualized by autoradiography after electrophoretic transfer to nylon membranes.

Preparation of Somatic Cell Hybrids. Cell hybrids between Chinese hamster cells (clone E36) deficient in hypoxanthine phosphoribosyltransferase (HPRT$^-$) and mouse spleen cells from BALB/c mice were generated and maintained as described (29). Under the conditions employed, hybrid clones retain all the Chinese hamster chromosomes but randomly segregate mouse chromosomes, resulting in the isolation of Chinese hamster–mouse cell hybrids that contain different numbers and combinations of mouse chromosomes. The mouse chromosome content of the hybrid clones was determined enzymatically and cytogenetically. Each hybrid clone was analyzed for the expression of 25 enzymes representing linkage groups assigned to 16 of the 19 autosomes and the X chromosome as described (30–32). Trypsin–Giemsa banding was used to identify each of the mouse chromosomes (32). The enzymatic, cytogenetic, and Southern blotting analyses were carried out on parallel cultures of each hybrid clone so that all the data were correlated. Individual clones were considered positive for a given chromosome if >15% of the metaphases examined contained the chromosome; the clones were scored negative for that chromosome if <5% of the metaphases contained the chromosome. If chromosomes were retained with a frequency between 5% and 15% the score was considered indeterminant and not included in the segregation analysis of that chromosome.

Isolation of Genomic DNA and Southern Blot Analysis. Genomic DNA from mouse tissues and tissue culture cell lines was prepared by a published procedure (33). Samples of DNA after restriction enzyme digestion were electrophoresed in TBE (89 mM Tris·HCl/89 mM boric acid/2 mM EDTA, pH 8.0)-buffered agarose gels (26) and processed through brief acid depurination before vacuum blotting in alkaline solution to nylon membranes (34). Hybridization of Southern blots was in the same buffer solution as for Northern blots except that the formamide was reduced to 40% and the hybridization buffer contained 10% (wt/vol) dextran sulfate.

Miscellaneous. BALB/c and I/Lyn (I/LnJ) mice were from The Jackson Laboratory. Phosphorylase kinase was purified from rabbit skeletal muscle by standard procedures (7).

RESULTS

To investigate the effect of the I/Lyn mutation on expression of the phosphorylase kinase α-subunit gene, a cDNA encoding the mouse skeletal muscle α subunit was isolated. The nucleic acid sequence of this cDNA and its encoded amino acid sequence are shown in Fig. 1. This cDNA is not full-length but does encode the amino acid sequence of the mouse skeletal muscle α subunit from asparagine-243 to asparagine-579. Over this sequence it has >95% similarity with that of the rabbit α subunit. Almost all of the substitutions are conservative and none of them are near the phosphorylation sites. Although this cDNA is not full-length, it is an effective probe for the identification of α mRNAs.

Fig. 2 shows the results of a Northern blot hybridization using the α cDNA to identify α mRNAs in RNA samples from ICR (normal) and I/Lyn (phosphorylase kinase-deficient) mouse skeletal muscle, heart, and liver tissues. The results demonstrate that in normal skeletal muscle and heart there are two different molecular weight species of RNA that hybridize to the α cDNA probe. The abundance of both species is greatly reduced in the phosphorylase kinase-deficient tissues. The residual amount detectable may result from vascular tissue contained in the skeletal muscle used for preparing the RNA or it may represent a low level of skeletal muscle α mRNAs. In contrast to the very low abundance of α mRNAs, the reported abundance of the γ and calmodulin mRNAs in the I/Lyn skeletal muscle is reduced compared to normal by only about 50% (19, 20). Thus, the major effect of the I/Lyn mutation is on the abundance of α mRNAs. A comparison with the β mRNAs has not been done because

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AATTCACTACTGCCCAGGGCTTCAACATCCAAAGAAGTTGATGCCAGTCTGCTCTCAGTG
243N S L L P R A S T S K E V D A S L L S V
GTCTCTTTCCCAGCCTTTGCTGTAGAGGACAGCCATTTGGTGGAGCTCACCAAACAGGAG
 I S F P A F A V E D S K L V E I T K Q E
 V                       H       L
ATCATCACCAAGCTTCAGGGTCGTTATGGTTGCTGTCGTTTTCTGCGAGATGGATATAAA
 I I T K L Q G R Y G C C R F L R D G Y K
ACTCCTAAAGAGGATCCCCAACGCCTATACTATAACCCAGCTGAGCTGAAGCTATTTGAA
 T P K E D P N R L Y Y E P A E L K L F E
             Q           N
AACATTGAGTGCGAATGGCCATTGTTCTGGACATACTTTATCCTTGATGGGATCTTCAGT
 N I E C E W P L F W T Y F I L D G V F S
                                     I
GGCAACGTAGAACAGGTTCAAGAATATAGAGAGGCTCTTGATGCAGTCCTCATCAAGGGC
 G N A E Q V Q E Y R E A L E A V L I K G
       V                 D
AAAAATGGAGTCCCTCTTCTTCCAGAGCTGTACAGTGTCCCTCCTGACAGGGTTGATGAA
 K N G V P L L P E L Y S V P P D K V D E
                                   R
GAGTATCAAAATCCCCACACTGTGGATCGAGTCCCTATGGGAAAATTGCCTCACATGTGG
 E Y Q N P H T V D R V P M G K L P H M W
GGTCAGTCTCTATACATTTTAGGAAGCTTGATGGCAGAGGGATTTTTAGCTCCTGGAGAA
 G Q S L Y I L G S L M A E G F L A P G E
ATTGATCCCCTGAATCGTAGGTTTTCTACTGTGCCAAAGCCAGATGTGGTGGTTCAAGTC
 I D P L N R R F S T V P K P D V V V Q V
TCCATTCTGGCTGAAACAGAAGAAATCAAGGCCATTTTGAAGGACAAAGGAATTGATGTG
 S I L A E T E E I K A I L K D K G I N V
                                     D
GAGACCATTGCTGAAGTGTACCCCATAAGAGTACAGCCAGCTCGTATTCTCAGCCATATT
 E T I A E V Y P I R V Q P A R I L S H I
TATTCTAGTCTAGGATGCAACAGTAGAATGAAACTCAGTGGACGACCCTACAGGCTCATG
 Y S S L G C N N R M K L S G R P Y R H M
              S                         L
GGTGTGCTTGGAACATCAAAACTTTATGACATTCGCAAAACTATCTTTACTTTCACTCCA
 G V L G T S K L Y D I R K T I F T F T P
CAGTTTATAGACCAGCAACAGTTCTACCTGGCTCTGGACAACCAGATGATAGTAGAAATG
 Q F I D Q Q Q F Y L A L D N K M I V E M
                                   Q
CTCAGAACAGACCTTTCCTACCTCTGTAGCCGCTGGAGGATGACAGGCCAGCCCACGATC
 L R T D L S Y L C S R W R M T G Q P T I
ACTTTCCCTATCTCGCACACCATGCTTGATGAAGATGGAACCAGCTTGAATT
 T F P I S Q T M L D E D G T S L N579
               H
```

FIG. 1. Nucleic acid and amino acid sequence of the mouse skeletal muscle α cDNA. Below the mouse α sequence are shown the amino acid differences with the rabbit α sequence. The mouse α cDNA is not full-length; numbering of its amino acid sequence is from the alignment with the rabbit subunit.

the necessary nucleic acid probe has been prepared only recently (35). However, the β gene is autosomal, and the effect on its mRNA abundance is expected to be similar to that of γ.

The normal and I/Lyn liver RNA samples showed no detectable hybridizing α mRNA. The same result has been reported for the γ mRNAs (20, 22). The absence of detectable α and γ liver mRNAs reflects either a paucity of these mRNAs in liver or that the liver mRNAs do not hybridize to the skeletal muscle cDNAs.

The observation of two α mRNA species and the reduction of both in the phosphorylase kinase-deficient tissues suggests that one of the mRNAs may encode the α' subunit. The origin of the α' subunit has not been identified. This subunit could be encoded on a gene distinct from α, could be encoded on the same gene as α but with an alternatively processed transcript, or could be processed from the α protein. To determine whether one of the mRNAs evident on the Northern blots encoded the α' subunit, the α cDNA was used to purify the two mRNAs by hybridization selection and then the mRNAs were translated in a cell-free system. Translated α and α' proteins were identified by electrophoresis in polyacrylamide gels.

The results of this hybridization-selection cell-free translation assay are shown in Fig. 3. The Northern blot (Fig. 3*A*) shows the mRNAs that were selected from a sample of normal mouse skeletal muscle RNA by hybridization with the α cDNA. In addition to the two mRNAs (labeled 2 and 3 in Fig. 3) that were identified on the Northern blot in Fig. 2,

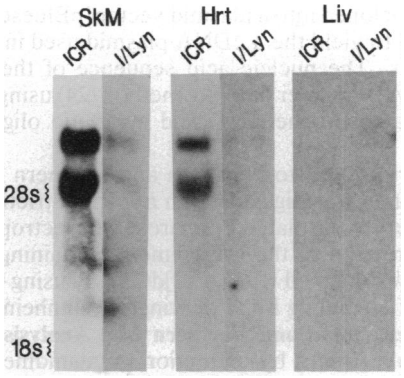

FIG. 2. Analysis of α mRNAs in normal and phosphorylase kinase-deficient tissues. The poly(A)$^+$ RNA fraction from normal (ICR) and mutant (I/Lyn) mixed-fiber skeletal muscle (SkM), heart (Hrt), and liver (Liv) tissues was isolated and processed for Northern blot analysis with the α cDNA. Approximately 5 μg of skeletal muscle RNA, 8 μg of heart RNA, and 12 μg of liver RNA were electrophoresed in the corresponding lanes. The heart, liver, and I/Lyn skeletal muscle blots were autoradiographed $\approx$5 times longer than the ICR skeletal muscle blot. The mobilities of 18S and 28S rRNAs are illustrated.

there are two other bands. The one of lower molecular weight is the α cDNA resulting from the hybridization selection procedure. This band is removed by DNase treatment before translation. The band of higher molecular weight (labeled 1 in Fig. 3) was not evident on the previous Northern blot. It must

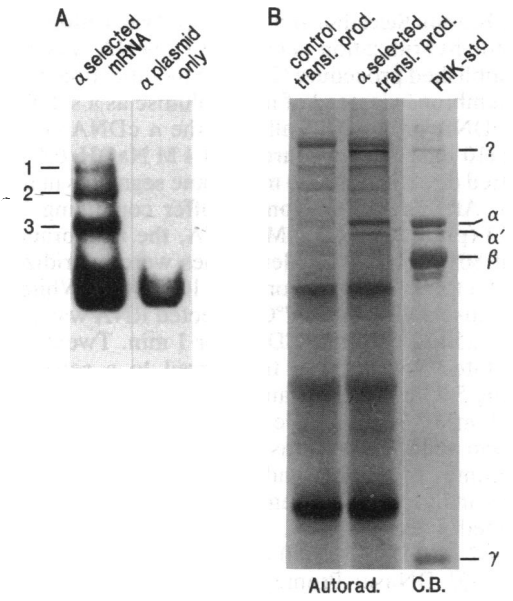

FIG. 3. Hybridization selection with the α cDNA and cell-free translation products of the selected mRNAs. (*A*) The left lane shows a Northern blot of the mRNAs that were selected and hybridize to the α cDNA. In the adjacent lane (α plasmid only) was electrophoresed the plasmid containing the α insert. Its position allows the identification of any plasmid that was eluted from the membrane with the mRNA. The positions of three RNA bands that hybridize to the α cDNA are indicated. (*B*) Autoradiogram of the proteins synthesized by selected mRNAs. The left lane shows the translation products directed by mRNAs selected with a control plasmid not containing the α insert. The pattern of proteins is the same as if no RNA were added. The middle lane shows the proteins that were synthesized by the α-selected mRNAs. Adjacent to this lane a sample of phosphorylase kinase standard (PhK-std) from rabbit muscle was loaded and visualized by Coomassie blue (C.B.) staining. The mobilities of the α, α', β, and γ subunits are indicated as well as an additional protein (?) that comigrates with a protein synthesized by the α-selected mRNAs.

Mouse Chromosome Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X
2		*	*						+			<10%		+	+	+				+
2Ag	+	+	+			+	+	+	+	+		+	+	<10%	+	+	+		+	
13		+		+		+		+		+		+	+		+	+	+		+	+
13Ag		+					+			<10%		+			+		+		+	

FIG. 4. Cell hybrids were made by fusion of Chinese hamster cells (clone E36, HPRT$^-$) with BALB/c mouse spleen cells. Cultures of the resulting cell hybrids 2, 2Ag, 13, and 13Ag were karyotyped, and the complement of mouse chromosomes carried in each is indicated. Plus sign indicates presence of that chromosome. In some of the cell hybrid lines <10% of the cells contain particular chromosomes. Cell line 2 carries a fragment of the mouse chromosomes 2 and 3 (*).

represent a species of RNA that is closely related to α but is not very abundant in the poly(A)$^+$ RNA fraction used for the previous Northern analysis. The products translated by these three mRNAs are shown in Fig. 3*B*. Two proteins are evident that comigrate with the α and α' subunits of a phosphorylase kinase standard. A third protein, of higher molecular weight, also is evident. Presumably this protein arises from the translation of the high molecular weight RNA species that was selected.

To determine whether the chromosome location of the α gene is consistent with the X-linked inheritance of the I/Lyn mutation, the chromosomal location of the α gene was mapped. For this study genomic DNA was isolated from a series of cell hybrids prepared by the fusion of Chinese hamster cells (clone E36, HPRT$^-$) with spleen cells from the BALB/c mouse strain. After passage, cell lines were selected and karyotyped. The individual cell lines contain all the Chinese hamster chromosomes and differing complements of mouse chromosomes. Fig. 4 lists the mouse chromosomes contained in the selected cell hybrid lines EBS-2, -2Ag, -13, and -13Ag. Only cell lines 2 and 13 contain the mouse X chromosome, and no other mouse chromosome is uniquely contained in these cell lines. By comparing restriction enzyme digests of the DNA from these cell lines with those of the parental hamster cell line and BALB/c mouse DNA, restriction fragments can be identified that are specific to the mouse α gene. The results of this comparison using the α cDNA for hybridization to Southern blots are shown in Fig. 5. Restriction enzyme fragments specific to the mouse α gene are indicated by the arrows. All the fragments present in the BALB/c genomic digest are present also in cell lines 2 and 13. The remaining fragments in cell lines 2 and 13 also are present in the other cell lines as well as in the parental hamster cell line and identify the Chinese hamster α gene. These results demonstrate that the α gene is located on the mouse X chromosome, and no other chromosome contains hybridizing sequences.

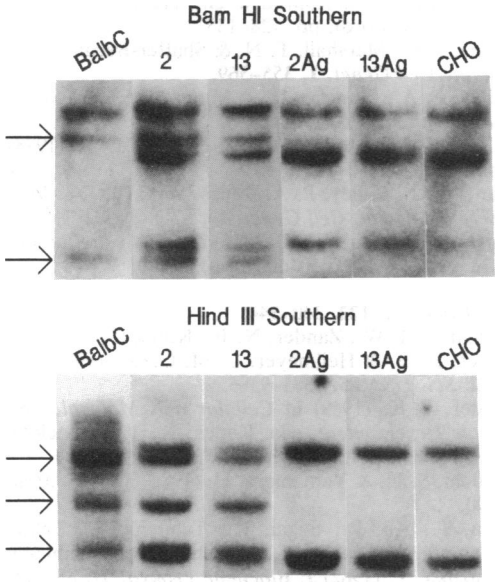

FIG. 5. Genomic DNA was isolated from BALB/c mouse tissue, the parental Chinese hamster ovary (CHO) cell line, and the cell hybrid lines listed in Fig. 4 (2, 2Ag, 13, 13Ag). The DNA was digested separately with the restriction enzymes *Bam*HI and *Hin*dIII. These digests were processed for Southern blot analysis by electrophoresis in agarose gels, transfer to nylon membranes, and hybridization with the α cDNA. The autoradiograms show regions of the Southern blots that have restriction fragments of the α gene that are different between the BALB/c mouse and parental hamster cell line. Restriction fragments marked by an arrow identify the mouse α gene in the Chinese hamster–mouse cell hybrids 2 and 13.

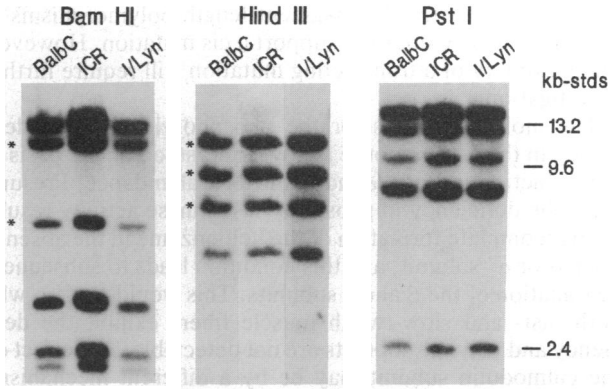

FIG. 6. Genomic DNA from BALB/c, ICR, and I/Lyn mouse strains was isolated and digested with *Bam*HI, *Hin*dIII, or *Pst* I restriction enzyme. The digests were then processed for Southern blot analysis with the α cDNA. The mobilities of λ phage DNA fragments used as molecular size standards [sizes in kilobases (kb)] are shown at right. The α fragments used for mapping the α gene in Fig. 5 are indicated by asterisks.

The results of the Northern and Southern analyses indicate that the I/Lyn α gene could contain the mutation. To identify any deletions, insertions, or inversions indicative of a cis mutation within the I/Lyn α gene, restriction enzyme digests of DNA from I/Lyn, BALB/c, and ICR mice were compared on Southern blots (Fig. 6). No restriction fragment length differences are evident. However, this analysis is limited by the α cDNA used for hybridization. Lacking any of the 5′ promoter region of the α gene, this cDNA may not hybridize to fragments 5′ from the coding sequence where the mutation could reside.

DISCUSSION

The effect of the I/Lyn mutation is a coordinate reduction of both the α and α' mRNAs. This result indicates that these two mRNAs have some mechanism in common that is disrupted by the I/Lyn mutation. This observation and the localization of the α gene to the X chromosome are the basis for suggesting that the α gene is the site of the I/Lyn mutation and that the α' mRNAs are derived from the same gene by the selection of alternative exons. This suggestion explains how a cis mutation could affect the transcription, processing, or stability of both the α and α' mRNAs. An alternative suggestion is that the α and α' mRNAs are transcribed from two different genes on the X chromosome, both of which are affected coordinately by the I/Lyn mutation. This would imply either that these two genes are closely linked and the I/Lyn mutation acts in cis to affect the expression of both genes or that the I/Lyn mutation is in a different gene, which acts in trans to regulate the two α and α' genes. The absence

of detectable restriction fragment length polymorphisms in the I/Lyn α gene does not support a cis mutation. However, the possibility of a trans-acting mutation will require further investigation.

The molecular basis for the phosphorylase kinase deficiency in the I/Lyn mouse is now understood on the basis of the reduction in both α and α' mRNA abundance. Presumably, the deficiency in phosphorylase kinase activity results from incomplete formation of the holoenzyme in the absence of an α or α' subunit, and this condition leads to subsequent degradation of the β and γ subunits. This would explain why both fast- and slow-twitch muscle fibers exhibit the deficiency and why the subunits are not detectable. The effect on the calmodulin subunit may be by a different mechanism, which has been discussed (19, 36). The absence of α and α' mRNAs does not explain why liver and neonatal skeletal muscle have normal phosphorylase kinase activity. This discrepancy could be explained by invoking additional α gene(s) that are specifically expressed in the unaffected tissues. Both the tissue-specific phenotype of the I/Lyn mutation and the absence of a detectable liver α mRNA indicate that the liver α gene differs from that expressed in skeletal muscle and heart. If there is a liver-specific α gene, then we estimate its similarity to the skeletal muscle α probe to be <75%. Otherwise, its mRNA would have been detected on the Northern blots. The alternative possibility to a liver-specific α gene is that the skeletal muscle α gene has a liver-specific promoter sequence. If the I/Lyn mutation were in the skeletal muscle and heart promoter, then expression in liver could be unaffected. In this case the absence of α mRNA in liver would mean that its abundance is normally too low to detect and is significantly lower than that seen in heart. This is unlikely given that the abundance of the phosphorylase kinase protein is approximately equal in liver and heart (37, 38).

The authors thank Dr. Charles Emerson for helpful discussion during the progress of this work. P.K.B. wishes to thank Dr. Philip Cohen for bringing to his attention the fact that the I/Lyn heart tissue also exhibits a phosphorylase kinase deficiency. This was incorrectly stated in a previous publication (22). This work was supported by a grant from the National Institute of Diabetes and Digestive and Kidney Diseases (DK34213), awarded to P.K.B.

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