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Heterogeneous expression of protein and mRNA in pyruvate dehydrogenase deficiency

(immunoblot/RNA blot/mutations/lactic acidosis)

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ABSTRACT **Deficiency of pyruvate dehydrogenase [pyruvate:lipoamide 2-oxidoreductase (decarboxylating and acceptor-acetylating), EC 1.2.4.1], the first component of the pyruvate dehydrogenase complex, is associated with lactic acidosis and central nervous system dysfunction. Using both specific antibodies to pyruvate dehydrogenase and cDNAs coding for its two α and β subunits, we characterized pyruvate dehydrogenase deficiency in 11 patients. Three different patterns were found on immunologic and RNA blot analyses. (*i*) Seven patients had immunologically detectable crossreactive material for the α and β proteins of pyruvate dehydrogenase. (*ii*) Two patients had no detectable crossreactive protein for either the α or β subunit but had normal amounts of mRNA for both α and β subunits. (*iii*) The remaining two patients also had no detectable crossreactive protein but had diminished amounts of mRNA for the α subunit of pyruvate dehydrogenase only. These results indicate that loss of pyruvate dehydrogenase activity may be associated with either absent or catalytically inactive proteins, and in those cases in which this enzyme is absent, mRNA for one of the subunits may also be missing. When mRNA for one of the subunits is lacking, both protein subunits are absent, suggesting that a mutation affecting the expression of one of the subunit proteins causes the remaining uncomplexed subunit to be unstable. The results show that several different mutations account for the molecular heterogeneity of pyruvate dehydrogenase deficiency.**

The pyruvate dehydrogenase complex (PDHC), a mitochondrial multicomponent enzyme, plays a pivotal role in energy metabolism by catalyzing the oxidative decarboxylation of pyruvate and its conversion to acetyl-CoA. Mammalian PDHC consists of three catalytic proteins: pyruvate dehydrogenase (E_1) [pyruvate:lipoamide 2-oxidoreductase (decarboxylating and acceptor-acetylating), EC 1.2.4.1], dihydrolipoamide acetyltransferase (E_2) (acetyl-CoA:dihydrolipoamide *S*-acetyltransferase, EC 2.3.1.12), and dihydrolipoamide dehydrogenase (E_3) (dihydrolipoamide:NAD^+ oxidoreductase, EC 1.8.1.4). Other proteins of the complex include two regulatory proteins, E_1 kinase (ATP:[pyruvate dehydrogenase (lipoamide)]phosphotransferase, EC 2.7.1.99) and phospho-E_1 phosphatase ([pyruvate dehydrogenase (lipoamide)]-phosphate phosphohydrolase, EC 3.1.3.43), and a protein X of unknown function (1–3). E_1, a thiamine pyrophosphate-dependent enzyme that catalyzes the initial decarboxylation of pyruvate, consists of two nonidentical subunits, α and β, with molecular masses of 41,000 and 36,000 Da, respectively. E_1 is a tetramer ($\alpha_2\beta_2$) and is present in multiple copies in the complex. Phosphorylation of $E_1\alpha$ by E_1 kinase inactivates E_1, whereas dephosphorylation by phospho-E_1 phosphatase restores catalytic activity (1, 2).

PDHC deficient subjects have an enzymatic block that prevents entry of pyruvate into the tricarboxylic acid cycle. Individuals with this genetic disorder have both lactic acidosis and neurologic disability ranging from severe brain stem dysfunction incompatible with life to moderate ataxia with otherwise normal mental development (4–9). Defects involving either the catalytic or regulatory components of PDHC have been described (4, 5, 8). Although in many reported cases identification of the affected component has not been well established, defects of E_1 appear to be most common (4).

Characterization of deficiency states has depended on determination of the activity of PDHC and its components (4, 10, 11). Recently, antibodies raised against PDHC or its components have been employed in immunoassays to identify abnormalities at the protein level (12–16). Several laboratories, including ours, have isolated cDNAs coding for the $E_1\alpha$, $E_1\beta$, E_2, and E_3 components of human PDHC (17–23). A human $E_1\alpha$ cDNA was used to analyze mRNA from three patients with E_1 deficiency (17).

We investigated 11 patients with E_1 deficiency using a comprehensive approach combining measurements of enzyme activity, protein immunoreactivity, and specific mRNAs. Our findings demonstrate significant heterogeneity in the expression of E_1 deficiency.

SUBJECTS AND METHODS

Identification of E_1-Deficient Subjects. E_1-deficient patients were identified by assaying the activity of PDHC, PDHC components, and related enzymes in cultured skin fibroblasts. Fibroblasts were obtained from either patients with lactic acidosis of undetermined etiology or subjects in whom the diagnosis of PDHC deficiency had been previously made by other investigators. Whenever possible, blood samples from these patients and concurrent controls were obtained to

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Abbreviations: PDHC, pyruvate dehydrogenase complex; E_1, pyruvate dehydrogenase; $E_1\alpha$ and $E_1\beta$, subunits α and β of E_1; E_2, dihydrolipoamide acetyltransferase; E_3, dihydrolipoamide dehydrogenase; CRM^+, crossreactive-material positive; CRM^-, crossreactive-material negative.

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assay the activity of PDHC in lymphocytes. In several cases, biopsy or postmortem tissues were available; control specimens were obtained from children with disorders unrelated to PDHC deficiency. These studies were approved by the Institutional Review Board of University Hospitals of Cleveland. The clinical and pathologic characteristics of several of these patients have been published (6, 12, 13, 24, 25).

Measurement of Enzyme Activity. For assay of enzyme activity, skin fibroblasts were grown until confluent and harvested (13). Lymphocytes were isolated from anticoagulated blood by the Ficoll-Paque method; whole tissue fragments were kept frozen at −70°C and then homogenized for assay as described (13). PDHC activity was assayed by decarboxylation of [1-^{14}C]pyruvate in disrupted cells or tissues with the addition of thiamine pyrophosphate, NAD^+, and coenzyme A (11, 13). Each sample and a concurrent control were assayed in quadruplicate in the untreated, dichloroacetate-activated, and fluoride-inactivated states. When tissues were available, PDHC activity was measured after preincubation with added phospho-E_1 phosphatase (provided by T. E. Roche, Kansas State University) (13). The E_1 component of PDHC was assayed in a similar manner except that coenzyme A and NAD^+ were omitted from the assay, and ferricyanide was added (13). Activity of E_2, E_3, pyruvate carboxylase, phospho*enol*pyruvate carboxykinase, and citrate synthase were assayed by described methods (11, 13, 26, 27). Normal ranges of activity for PDHC, its components, and other mitochondrial enzymes in cells or tissues have been established in our laboratory (13, 14). Subjects selected for this study had abnormally low total PDHC and E_1 activities but normal activities of E_2, E_3, and other mitochondrial enzymes.

Immunoblots. Immunoassays, using $NaDodSO_4$/PAGE and electrotransfer (immunoblot), were done on fibroblast specimens as described elsewhere (12). Immunoblots were incubated with specific antibodies for $E_1\alpha$ and $E_1\beta$ (affinity purified as described below) (20) and separately to anti-E_2 and anti-E_3 antibodies. Purified bovine PDHC (Sigma) was included as a standard in these experiments.

Preparation of PDHC Component cDNAs. Isolation, identification, and analysis of cDNAs for $E_1\beta$ and E_3 have been described (20, 22). $E_1\alpha$ cDNA was isolated from a human liver λgt11 library (provided by T. Chandra and S. L. C. Woo, Baylor College of Medicine, Houston, TX). To assist in the identification of $E_1\alpha$ cDNA, $E_1\alpha$ was separated from bovine heart E_1 (provided by L. J. Reed, University of Texas, Austin) (28), and its purity was confirmed by $NaDodSO_4$/PAGE. A sequence of 20 amino acids starting from the amino terminus was derived (29). Two separate 14-mer oligonucleotide mixtures were synthesized based on either the amino acid sequence (Phe-Glu-Ile-Lys-Lys) from the 7th to 11th residues from the N-terminus or the previously reported amino acid sequence around phosphorylation site III (2). To isolate $E_1\alpha$ cDNA, the λgt11 library was screened using anti-E_1 antibody. Affinity-selected antibodies isolated using fusion proteins produced by two of the recombinant phage (20) reacted only with $E_1\alpha$ of purified bovine heart PDHC and human liver extract on immunoblot analysis. Both clones hybridized with the labeled $E_1\alpha$ phosphorylation-site oligonucleotide probe, and the larger of the two clones [1.4 kilobases (kb)] also hybridized with the labeled $E_1\alpha$ N-terminus probe. Nucleotide sequence analysis of the 1.4-kb cDNA revealed an open reading frame that encodes for a polypeptide containing both the 20 N-terminal amino acids and the phosphorylation site III sequence of bovine heart $E_1\alpha$. In addition, the sequence of this clone matches sequences of other recently reported human $E_1\alpha$ cDNAs (17, 19, 21), thereby establishing its identity as a human $E_1\alpha$ cDNA.

RNA Blot Analysis. RNA was isolated with guanidinium isothiocyanate using density-gradient centrifugation with cesium chloride (30). The amount of RNA in each sample was determined by UV absorption at 260 Å, and the samples were stored at −70°C until analyzed. Equal amounts of total RNA from each of the samples were separated by electrophoresis in a formaldehyde/1% agarose gel and transferred to a nylon membrane (GeneScreen, DuPont–New England Nuclear). RNA blot analysis was then performed according to described methods (31) with RNA from fibroblasts of non-PDHC-deficient subjects as controls. ^{32}P-labeled probes were prepared from denatured double-stranded cDNAs using the random priming method (32, 33). For $E_1\alpha$, the 1.4-kb cDNA clone was used as a template, whereas for $E_1\beta$, a mixture of 1.0- and 0.5-kb *Eco*RI fragments from a digest of a 1.5-kb $E_1\beta$ clone were labeled (20). A radiolabeled 1.1-kb fragment containing the 5′ end of the 2.2-kb E_3 cDNA was used as an internal control (22). Membranes were rehybridized with successive cDNA probes after removing previously used radioactive probes (34). The relative amount of specific mRNAs was determined for selected radiographs by laser densitometry (Ultroscan; LKB, Bromma, Sweden).

RESULTS

Nine of 132 individuals with lactic acidosis that we screened fulfilled the criteria for having E_1 deficiency. Two other E_1-deficient subjects were referred to us by other investigators. All 11 had PDHC activity below the normal range in cultured skin fibroblasts; 8 subjects for whom additional samples were available also had low levels of activity in lymphocytes or other tissues (Table 1). All of the subjects' fibroblasts had levels of E_1 activity that were low relative to both the normal range established in our laboratory and to concurrent controls (Table 2). Activities of E_2 and E_3 were normal. Ten subjects had normal activity for three other mitochondrial enzymes—pyruvate carboxylase, phospho*enol*pyruvate carboxykinase, and citrate synthase (Table 1)—indicating satisfactory cell culture conditions or postmortem tissue recovery. One subject, JH, had normal levels of phospho*enol*pyruvate carboxykinase, citrate synthase, E_2, and E_3, with slightly reduced levels of pyruvate carboxylase. However, PDHC and E_1 activity in this individual were well below the range of normal. In three subjects from whom tissues were available (GB, PH, and BK), preincubation with phospho-E_1 phosphatase resulted in no increase of total PDHC activity; PDHC activity did increase in concurrent controls, indicating that lack of phosphatase was not the cause of low PDHC activity in these three cases.

Assay of PDHC components by immunoblotting was performed with fibroblasts from all 11 subjects to determine whether low E_1 activity was associated with lack of protein or presence of abnormal protein. Two patterns emerged from this investigation. Seven patients had immune crossreactive material (CRM^+) present for both $E_1\alpha$ and $E_1\beta$ (Table 2). The other 4 subjects had no or barely detectable crossreactive material (CRM^-) for either $E_1\alpha$ or $E_1\beta$. All of the CRM^- patients had normal levels of crossreactive material for E_2 and E_3, demonstrating adequate loading and transfer of proteins to the membrane (data not shown). Examples of patients who are either CRM^+ or CRM^- are shown in Fig. 1. In our immunoblots, $E_1\alpha$ appeared as a doublet. The separation of $E_1\alpha$ into a doublet may under certain conditions be due to the state of phosphorylation (35). Patient BK (Fig. 1, lane 7) who is CRM^+ had an additional reactive protein band with a molecular mass corresponding to ≈43 kDa as estimated in relation to the mobility of other PDHC components. Other examples of CRM^- immunoblots of either fibroblasts and/or tissue samples from patients JS and EU have been published (12, 13). Subjects who were CRM^+ had

Table 1. PDHC and related enzyme activities in cells and tissues from enzyme-deficient patients

Subject	Sex	Age	Clinical problems‡	Total PDHC activity*†				Fibroblasts		
				Fibroblasts	Lymphocytes	Liver	Muscle	PC*	PEPCK*	CS*
Controls										
Mean ± SD				2.54 ± 0.94	1.83 ± 0.64	2.23 ± 0.78	2.54 ± 1.26	1.2 ± 0.7	4.4 ± 3.1	29 ± 13
Range				(1.1–6.7)	(0.9–3.8)	(1.0–3.7)	(1.0–5.5)	(0.4–3.8)	(0.7–14)	(12–63)
n				(54)	(72)	(11)	(8)	(54)	(51)	(40)
Patients										
LA	F	9 mo§	B, D, R, U	0.53	0.37			0.4	2.5	15
GB	F	2 days§	B, R	0.58		0.57		1.6	3.9	34
CC	F	12 yr	D, S	0.23	0.33			0.9	2.9	45
BK	M	4 mo§	B, R, S, U	0.09	0.13	0.09	0.14	0.7	5.0	16
CP	F	4 yr	D, S	0.05	0.90			0.4	4.1	18
KP	F	17 yr	B, D, S	0.14	0.49			1.3	6.0	16
BW	M	2 days§	B, R	0.39				1.3	5.8	22
PH	M	14 yr	A, S	0.75			0.13	1.8	4.9	21
EU	M	1 yr§	D, L, R, S	0.71	0.16	0.20		2.0	6.6	47
JH	M	8 yr	A, D	0.58				0.3	1.7	14
JS	M	8 yr§	A, D, L, S	0.46				0.4	3.0	19

PC, pyruvate carboxylase; PEPCK, phospho*enol*pyruvate carboxykinase; and CS, citrate synthase.
*Reported in nmol/min per mg of protein.
†PDHC activated with dichloroacetate (cells) and phosphatase (tissues).
‡A, ataxia; B, abnormal anatomical brain development; D, developmental delay; L, autopsy-proven Leigh disease; R, respirator dependent; S, seizure disorder; and U, unexpected sudden death.
§Subject expired at this age.

both $E_1\alpha$ and $E_1\beta$ present, whereas CRM^- individuals always lacked both subunits.

To determine whether absence of immunoreactive $E_1\alpha$ and $E_1\beta$ proteins was associated with quantitative changes of mRNAs for $E_1\alpha$ or $E_1\beta$, blot analysis was performed on total RNA isolated from fibroblasts of the four CRM^- subjects (JS, EU, JH, and PH) and controls. As noted by others (17, 19), there are two different molecular-size species of $E_1\alpha$ mRNA in normal subjects (Fig. 2, *Top*, lanes 2, 4, 6, 8, and 10). The more intense band corresponds to a 1.6-kb species, and the less intense band corresponds to a 3.3-kb species. For CRM^- subjects, two patterns were discernible (summarized in Table 2). Two of the four subjects (EU and PH) had normal levels of both $E_1\alpha$ mRNA species (Fig. 2, *Top*, lanes 1 and 9). The other two CRM^- subjects (JS and JH) had decreased amounts of $E_1\alpha$ mRNA as compared to controls (Fig. 2, *Top*, lanes 3 and 7, respectively). For JS, both the 1.6-kb and 3.3-kb species of $E_1\alpha$ mRNA were present at low levels, whereas in JH, only the 1.6-kb $E_1\alpha$ mRNA could be detected even after prolonged radiographic exposure. These differences were confirmed by densitometry and repeat RNA blots using different concentrations of total RNA (data not shown). All four CRM^- samples had normal levels of $E_1\beta$ mRNA with a size of 1.5 kb, similar to the controls (Fig. 2, *Middle*). RNA blot hybridization of subject BK, who was CRM^+ but had an additional immunoreactive band, showed $E_1\alpha$ and $E_1\beta$ mRNAs of normal size and amount (Fig. 2, *Top* and *Middle*, lane 5). Hybridization with cDNA for E_3 using the same

Table 2. PDHC component activities, immunoreactivity, and mRNA levels in skin fibroblasts

Subject	Component activity			Immunoreactivity		mRNA	
	E_1	E_2	E_3	$E_1\alpha$	$E_1\beta$	$E_1\alpha$	$E_1\beta$
Controls							
Mean ± SD	0.08 ± 0.02	2.9 ± 1.0	39 ± 18	+	+	+	+
Range	(0.06–0.11)	(1.3–5.3)	(15–67)				
n	(12)	(14)	(16)				
Patients							
CRM^+							
LA	0.04	1.7	27	+	+		
GB	0.01	2.5	28	+	+		
CC	0.03	5.7	65	+	+		
BK	0.03	1.6	91	+	+	+	+
CP	0.01	5.6	36	+	+		
KP	0.02	2.6	19	+	+		
BW	0.01	2.8	40	+	+		
CRM^-							
PH	0.04	1.7	24	−	−	+	+
EU*	0.04	2.9	61	−	−	+	+
JH	0.04	3.5	28	−	−	−	+
JS†	0.01	4.6	42	−	−	−	+

Component activity is reported in nmol/min per mg of protein.
*See ref. 13.
†See ref. 12.

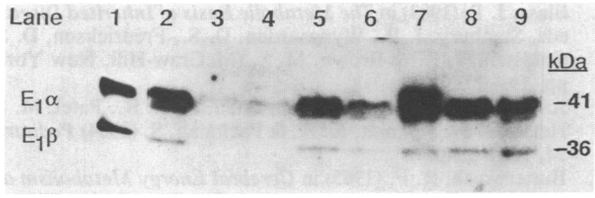

FIG. 1. Immunoblot analysis of E_1 from fibroblasts of E_1-deficient subjects using affinity-purified anti-E_1 antibodies followed by ^{125}I-labeled Protein A. Lanes 2–9 were loaded with 500 μg of total protein per lane. Lane 1 contains a sample of purified bovine PDHC (0.4 μg) used as a standard, and lanes 2, 5, and 8 are different control fibroblast cell lines. Patients GB (lane 6), LA (lane 9), and BK (lane 7) are CRM$^+$, whereas JH (lane 3) and PH (lane 4) are CRM$^-$ for both subunit proteins. The numbers on the right side of the figure indicate the molecular mass of the E_1 subunits.

membrane demonstrated normal levels of E_3 mRNA in all the samples (Fig. 2, *Bottom*), indicating that comparable amounts of RNA from each sample had been transferred to the membrane.

The ages of the eleven E_1-deficient subjects at the time of evaluation ranged from 2 days to 17 years; six subjects have died. The five survivors have significant morbidity ranging from abnormal brain development to ataxia with otherwise normal mental development (Table 1). No correlation was found between clinical severity of disease and levels of activity of PDHC or E_1. Similarly, no association could be found between clinical severity of PDHC deficiency and absence of immunoreactive E_1 subunits or $E_1\alpha$ mRNA. The two patients missing $E_1\alpha$ mRNA differed dramatically in the severity of their symptoms. Patient JH is alive with moderate ataxia, whereas JS died after a more severe course and had pathological evidence of Leigh disease (24).

DISCUSSION

In this series of eleven patients, E_1 deficiency was comprehensively characterized at the activity, protein, and mRNA levels. From the results of immunological and RNA blot analyses using specific antibodies and cDNAs for $E_1\alpha$ and $E_1\beta$, we found three different forms of E_1 deficiency at the molecular level (Table 3). One group of patients had immunologically detectable $E_1\alpha$ and $E_1\beta$ (type I). The second group had negligible amounts of immunoreactive protein for either $E_1\alpha$ or $E_1\beta$ (CRM$^-$), but had normal levels of mRNAs for both subunit proteins (type II). The third group were also CRM$^-$, but in contrast to the second group had diminished levels of mRNA for $E_1\alpha$ (type III).

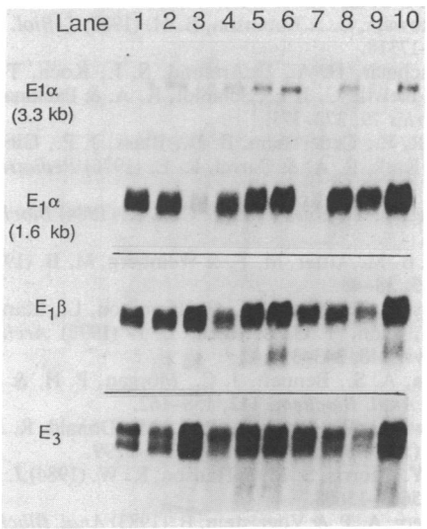

FIG. 2. RNA blot analysis of total RNA from fibroblasts of E_1-deficient subjects. Extraction of total RNA and fractionation by electrophoresis was performed as described. Each lane contains 15 μg of total RNA. The membrane was sequentially hybridized with radiolabeled cDNAs for $E_1\alpha$ (*Top*), $E_1\beta$ (*Middle*), and E_3 (*Bottom*). Lanes 2, 4, 6, 8, and 10 are samples of total RNA isolated from different control human fibroblast cell lines. CRM$^-$ subjects are EU (lane 1), JS (lane 3), JH (lane 7), and PH (lane 9); BK (lane 5) is a CRM$^+$ patient.

The association of low enzymatic activity without evidence of an immunologically detectable abnormality of E_1 has been noted by others (15, 16). Several possible types of mutations might cause low E_1 activity without resulting in any absence of immunoreactive protein. A change in the primary amino acid sequence of either subunit could alter the catalytic activity of this rate-limiting component. Alternatively, defective phospho-E_1 phosphatase could account for the lack of E_1 activity because E_1 would remain in a fully phosphorylated (inactive) form. We were able to exclude this possibility in two CRM$^+$ patients (BK and GB) from whom tissues were available by demonstrating failure of activation upon adding exogenous phospho-E_1 phosphatase. Finally, it is conceivable that the primary defect in these patients does not involve either E_1 subunit and the low activity detected by enzymatic assay of E_1 is incidental. Considering that the E_1 assay measures <5% of total PDHC activity, errors could occur in this measurement because the differences between normal and abnormal values are relatively small (8). We found that this assay is reliable for measurement of E_1 in isolated mitochondria from liver or muscle (13, 14), but the assay in disrupted skin fibroblasts must be interpreted very cautiously. In CRM$^+$ cases in which only fibroblasts are available for analysis, the diagnosis of E_1 deficiency should be supported by evidence of normal E_2 and E_3 activity in addition to low E_1 and total PDHC activity. This approach was followed for all patients in our series.

Our findings differ from those of McKay *et al.* (16), who described altered electrophoretic mobility in three CRM$^+$ patients with E_1 deficiency whose $E_1\alpha$ protein migrated to a position corresponding to a molecular mass of 41 kDa, whereas in other E_1-deficient patients and controls, migration of $E_1\alpha$ corresponded to 43 kDa. In contrast, all seven CRM$^+$ subjects in our series had $E_1\alpha$ that migrated to the same position as bovine $E_1\alpha$ (41 kDa). From one CRM$^+$ patient, BK, an additional band of crossreactive material appeared that corresponded to a molecular mass of ≈43 kDa. The additional band might be due to a mutation that expresses a larger-size protein; this explanation is unlikely because two $E_1\alpha$ bands are present; only one band would be expected if there were an insertional mutation. A more plausible explanation is that the extra band represents a precursor protein that is incompletely processed by the mitochondria.

Of the four patients who were CRM$^-$, both protein subunits were either absent or present in only minimal amounts. The absence of both $E_1\alpha$ and $E_1\beta$ subunit proteins in these CRM$^-$ patients seems to contradict a case described by Wicking *et al.* (15), in which immunoreactive $E_1\alpha$ was absent, but $E_1\beta$ was reported as present. The apparent difference between our findings and those of Wicking *et al.* (15) may depend on detectability of $E_1\beta$. We used an antibody

Table 3. Observed patterns of expression of E_1 deficiency

Type	Cases, no.	E_1 activity	Protein		mRNA	
			α	β	α	β
I	7	−	+	+	+	+
II	2	−	−	−	+	+
III	2	−	−	−	−	+

raised against purified E_1 that reacted well with both $E_1\alpha$ and $E_1\beta$ (Fig. 1) (13, 14), whereas the antibody used by Wicking *et al.* was prepared against the entire complex and was only weakly reactive against $E_1\beta$. Moreover, we noted that the rates of electrotransfer of $E_1\alpha$ and $E_1\beta$ are differentially affected in cell or tissue extracts in contrast to purified PDHC, such that $E_1\beta$ is less efficiently transferred than $E_1\alpha$ (12). These factors could explain why Wicking *et al.* claimed to have been able to detect $E_1\beta$ in normal fibroblasts only after prolonged radiographic exposure. Their technique may not have been sensitive enough to discriminate whether $E_1\beta$ was present in normal or reduced amounts in E_1-deficient cells.

Analysis of mRNAs with probes for both $E_1\alpha$ and $E_1\beta$ demonstrates that there is a subset of CRM^- subjects (EU and PH) who have normal amounts of mRNA for both subunits. In an earlier study, only the presence of $E_1\alpha$ mRNA was tested in CRM^- patients (17). Absence of E_1 proteins in the presence of normal levels of mRNA for $E_1\alpha$ and $E_1\beta$ may be due to a mutation that affects translation or post-translational processing of one of the subunit proteins, resulting secondarily in rapid degradation of the other uncomplexed subunit because tetrameric ($\alpha_2\beta_2$) E_1 cannot be formed. An alternative possibility is that translation of both $E_1\alpha$ and $E_1\beta$ proteins is normal, but some other factor causes their mutual rapid degradation.

Two of the CRM^- patients (JS and JH) had low levels of $E_1\alpha$ mRNA. The presence of normal amounts of $E_1\beta$ mRNA in these patients suggests that the mutation is located on the gene coding for the α subunit. Again, a probable explanation for absence of both protein subunits despite normal levels of $E_1\beta$ mRNA in these cases is that the mutation affecting transcription or posttranscriptional processing of the $E_1\alpha$ gene product results in the absence of both protein subunits due to more rapid degradation of the unaffected subunit.

The heterogenity in expression of E_1 deficiency in our series of patients indicates that several different types of mutations cause E_1 deficiency. Further research is necessary to determine how different mutations lead to these abnormalities and how they relate to the clinical variations among patients with E_1 deficiency. Characterization of these mutations should provide better understanding of the function of E_1 and the pathophysiology of E_1 deficiency.

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