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Mitochondrial ATP synthase subunit c stored in hereditary ceroid-lipofuscinosis contains trimethyl-lysine

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The subunit c protein of mitochondrial ATP synthase accumulates in lysosomal storage bodies of numerous tissues in human subjects with certain forms of ceroid-lipofuscinosis, a degenerative hereditary disease. Subunit c appears to constitute a major fraction of the total storage-body protein. Lysosomal accumulation of subunit c has also been reported in putative animal models (dogs, sheep and mice) for ceroid-lipofuscinosis. In humans with the juvenile form of the disease, hydrolysates of total storage-body protein have been found to contain significant amounts of ε-*N*-trimethyl-lysine (TML). TML is also abundant in storage-body protein hydrolysates from affected dogs and sheep. These findings suggested that one or both of the two lysine residues of subunit c might be methylated in the stored form of the protein. The normal subunit c protein from mitochondria does not appear to be methylated. In a putative canine model for human juvenile ceroid-lipofuscinosis, residue 43 of the storage-body subunit c was previously found to be TML. In the present study, subunit c was isolated from the storage bodies of humans with juvenile ceroid-lipofuscinosis, and from sheep and mice with apparently analogous diseases. In all three species, partial amino acid sequence analysis of the stored subunit c indicated that the protein contained TML at residue 43. These findings strongly suggest that specific methylation of lysine residue 43 of mitochondrial ATP synthase plays a central role in the lysosomal storage of this protein.

INTRODUCTION

The hereditary ceroid-lipofuscinoses are a group of inherited lysosomal storage diseases characterized by the intracellular deposition of large numbers of autofluorescent inclusions in many tissues. Clinical symptoms in human subjects with these diseases include visual loss, seizures, psychomotor and cognitive deterioration, and early death [1–3]. The ceroid-lipofuscinoses that become evident during childhood have been divided into three major forms, commonly designated as infantile, late infantile and juvenile types [1–3]. Each of these forms of ceroid-lipofuscinosis is genetically distinct [4–11]. In the late infantile and juvenile forms, the storage material contains large amounts of the subunit c protein of mitochondrial ATP synthase [12,13]. Animal models for juvenile ceroid-lipofuscinosis have been identified in sheep [14,15], dogs [16,17] and recently in mice [18]. In all three putative animal models of the disease, there is also specific storage of the subunit c protein [13,18–21]. The metabolic basis for the accumulation of this protein is currently not known.

Amino acid compositional analysis of total storage-body proteins from affected dogs and sheep, and from humans with the juvenile form of ceroid-lipofuscinosis, revealed that they are associated with substantial amounts of the modified amino acid ε-*N*-trimethyl-lysine (TML) [20,22–24]. This indicates that, at least in some forms of ceroid-lipofuscinosis, there is specific storage of a protein or proteins containing this modified amino acid. In the canine model, the TML of the storage-body protein was found to be residue 43 of the subunit c [20]. Analyses were conducted to determine whether the same lysine residue of subunit c is trimethylated in affected humans, sheep and mice.

MATERIALS AND METHODS

Tissue sources

Brain tissue was obtained *post mortem* from three human donors who were diagnosed to have the juvenile form of ceroid-lipofuscinosis. The tissues were obtained as soon after death as possible, and were stored frozen until used. Tissue donors died between the ages of 15 and 30 years. Tissues from sheep with hereditary ceroid-lipofuscinosis were obtained at necropsy from a colony established and maintained in New Zealand [14,15]. The disease in sheep has been well characterized [14]. On the basis of a number of biochemical parameters, the disease in sheep appears to be analogous to the juvenile form of the disease in humans [13,22]. Tissues were also obtained from mice with a hereditary disorder first described as motor neuron degeneration [25], but recently proposed as a model of ceroid-lipofuscinosis [18,21]. Like the other affected species, the mice accumulate large numbers of lysosomal storage bodies containing subunit c [21]. The mouse tissues were dissected out at necropsy from animals 6 months of age or older and were frozen at −70 °C until used. The presence of cellular inclusions characteristic of ceroid-lipofuscinosis in all tissues was assessed using fluorescence and electron microscopy [18,22,23,26].

Storage-body isolation

Storage bodies were isolated from sheep tissues using techniques described previously [27]. These techniques were modified somewhat for storage-body isolation from human and mouse tissues. Brain cerebral cortex grey matter from affected human donors

Abbreviations used: TML, ε-*N*-trimethyl-lysine; PVDF, poly(vinylidene difluoride); PTH, phenylthiohydantoin; PVP, polyvinylpyrollidone; OPA, *o*-phthalaldehyde.

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and liver samples from affected mice were thawed, minced and homogenized in ice-cold 0.4 mM Tris/HCl, pH 7.4. Approx. 1.5 g of tissue was homogenized in 25 ml of buffer. The homogenates were filtered through glass wool and then sonicated on ice for 1 min with a Branson model 185 sonifier set for an output of 30 W. The samples were then centrifuged in a swinging-bucket rotor for 20 min at 1300 $g_{av.}$. All centrifugation steps were carried out at 4 °C. The supernatants were discarded and the pellets were each suspended in 2 ml of water and sonicated for 1 min as described above. Each of the resulting suspensions was layered on to a two-step sucrose gradient consisting of sucrose solutions in water with densities of 1.131 and 1.181 g/ml. The gradients were subjected to centrifugation in a swinging-bucket rotor for 3 h at 10800 $g_{av.}$. Material at the interface between the two layers of sucrose was collected, diluted with water and then pelleted by centrifugation for 20 min at 10800 $g_{av.}$. Finally, the pellets were washed once with water.

The purities of the storage-body preparations were assessed by electron microscopy. Suspensions of the isolated storage bodies were added to a mixed aldehyde fixative [28] at room temperature, and were pelleted by centrifugation at 6000 $g_{av.}$ for 3 min. The pellets were fixed for at least 2 h at room temperature with gentle agitation. The samples were then washed, fixed with OsO_4, dehydrated and embedded in plastic as described previously [28]. Ultrathin sections of the embedded samples were cut, stained with lead citrate and uranyl acetate, and were examined in a JEOL 1200EX electron microscope.

Subunit c extraction from storage bodies

The isolated storage bodies from each tissue were subjected to extraction using a method previously found to yield a fraction from sheep storage bodies that is highly enriched in the mitochondrial subunit c protein [19]. Aliquots of the storage bodies suspended in water were vortex mixed and then sonicated in 20 vol. of chloroform/methanol (2:1, v/v) containing 100 mM ammonium acetate. After addition of 1 part of water to 20 parts of the chloroform/methanol extract, the solutions were sonicated again and then allowed to stand at 4 °C until the aqueous and organic phases separated. The samples were then subjected to centrifugation at 1000 *g* for 10 min, which caused insoluble material to collect at the interface between the organic and aqueous phases. The lower organic phases were transferred to new vials and then each was diluted with an equal volume of chloroform and evaporated under argon to less than 10% of the starting volume. Subunit c was precipitated from the latter solution by the addition of at least 20 vol. of diethyl ether and incubation at −70 °C for a minimum of 1 h. The precipitate, consisting primarily of the subunit c protein, was washed several times with methanol to remove any remaining ether.

Gel electrophoresis

The subunit c fractions obtained from the storage bodies were subjected to SDS/PAGE to assess their protein compositions. SDS/PAGE was performed as described previously for similar fractions obtained from storage bodies isolated from dogs with hereditary ceroid-lipofuscinosis [20]. After fixation, the gels were stained with silver nitrate [20], as the subunit c protein stains poorly with Coomassie Blue [29].

Amino acid composition and sequence analyses

The amino acid compositions of the crude storage-body protein preparations were previously reported for human and sheep samples [22,23]. Similar analyses were carried out on samples from the putative mouse model. In addition, amino acid composition analyses were performed on hydrolysates of the subunit c fractions from the storage bodies of all three species. Isolated storage bodies and the subunit c extracts derived from them were lyophilized, weighed and then suspended in 6 M HCl at a concentration of approx. 2 mg/ml. The samples were then purged with argon, tightly capped and incubated at 110–115 °C for 24 h. The amino acid compositions of the resulting digests were determined with a Beckman model 6300 amino acid analyser. Quantification was based on the analysis of amino acid standards, including TML. The identity of TML was confirmed as described previously [22].

The storage-body extracts expected to be enriched in the subunit c protein were subjected to N-terminal amino acid sequence analyses. An aliquot of each extract was solubilized by incubation in 1% SDS at room temperature for 60 min at a protein concentration of approx. 2 μg/ml. The resulting solution was subjected to centrifugation at 10000 *g* for 15 min, and 100 μl of the supernatant was applied to a ProSpin poly(vinylidene difluoride) (PVDF) (Applied BioSystems) membrane. The proteins that bound to the membranes were then subjected to amino acid sequence analysis with an Applied Biosystems 470 gas-phase sequencer equipped with a model 120 phenylthiohydantoin (PTH) analyser. The analyses of the samples from sheep and mice were terminated after the first 10 residues had been identified, whereas as much of the N-terminal sequence as possible was determined for the human protein.

Once the identity of the extracted protein had been confirmed by N-terminal sequence analysis, additional analyses were undertaken to determine whether the lysine residue at position 43 of the stored subunit c protein was methylated. For some internal sequence analyses, the protein was acylated to block sequencing from the N-terminus and also to prevent subsequent cleavage by trypsin at lysine residue 7, but leaving the trypsin-cleavage site at arginine residue 38 unmodified. Additional aliquots of the subunit c preparations were subjected to the same procedures for sequence analysis except that the acylation step was omitted. For acylation, approx. 500 μg of the ether-precipitated protein was dissolved in chloroform/methanol (2:1, v/v). To 10 vol. of this solution was added 1 vol. of pyridine, followed by the addition of 1 mg of acetic acid *N*-hydroxysuccinimide ester in 250 μl of chloroform/methanol (2:1, v/v). The resulting mixture was incubated at 37 °C for 2 h and at room temperature for at least 7 h. After acylation, the solvent was removed by evaporation *in vacuo*. Dried acylated or native samples were then solubilized in buffer containing 0.5% SDS, 0.125 mM EDTA and 2.5 mM Tris/HCl, pH 8. After solubilization, the samples were each diluted with 1.5 parts of water, and an aliquot was applied to a ProSpin PVDF membrane. The membrane was then washed with water, dried and then wetted with 20% methanol. To block any potential residual protein-binding sites, the membrane was incubated in 0.5% polyvinylpyrollidone (PVP)-40 in 100 mM acetic acid for 30 min at 37 °C. Excess PVP-40 was washed from the membrane with three brief rinses in deionized water, followed by two washes in 50 mM Tris/HCl/5 mM $CaCl_2$, pH 8.10 (Tris buffer). The membrane was then suspended in 400 μl of the Tris buffer, followed by the addition of 6 μg of sequencing-grade trypsin (Sigma, St. Louis, MO, U.S.A.). Trypsin digestion was carried out for approximately 20 h at 37 °C, with two more 6 μg additions of trypsin being made after 4 and 6 h of incubation. Finally, the membrane was washed three times with the Tris buffer and twice with water, and the protein fragments remaining bound to the membrane were then subjected to amino acid sequence analysis.

Between the first and second cycles of Edman degradation, the membrane was treated with *o*-phthalaldehyde (OPA) or fluorescamine to block any sequences arising from the N-terminus or from fragments generated by cleavage of the protein at any sites other than the trypsin-cleavage site at the carboxyl side of arginine residue 38 [30]. Residue 40 of mitochondrial subunit c is proline, which is the only amino acid that does not react with either OPA or fluorescamine. Excess OPA or fluorescamine was then washed from the membrane, and sequence analysis was continued for at least seven additional residues.

RESULTS

Buoyant-density centrifugation of tissue homogenates yielded preparations that consisted of almost pure storage bodies, as indicated by electron microscopic examination (Figure 1). As was reported previously, the ultrastructural appearances of the storage bodies varied somewhat between tissues and species [31].

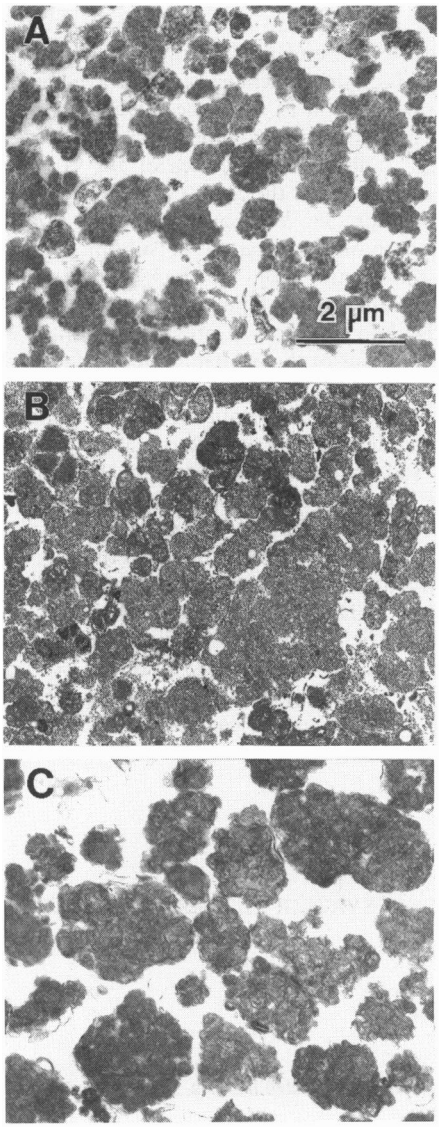

Figure 1 Electron micrographs of storage-body fractions from the brain of a human donor with juvenile ceroid-lipofuscinosis (A), and from the livers of affected sheep (B) and mice (C)

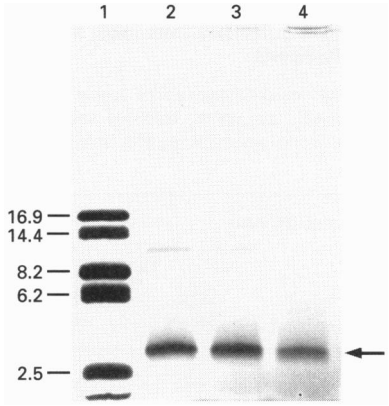

Figure 2 SDS/PAGE analysis of storage-body proteins isolated from tissues of humans, sheep and mice with hereditary ceroid-lipofuscinosis

The storage bodies were isolated from human brain and from sheep and mouse liver as described in the Materials and methods section. Aliquots of the isolated storage bodies were subjected to chloroform/methanol extraction, and subunit c protein was precipitated from these extracts with ether. The extracted and precipitated proteins were solubilized with SDS and analysed by SDS/PAGE. Lane 1, molecular-mass standards [approx. 100 ng each of CNBr-cleavage products of horse heart myoglobin with molecular masses (in kDa) given on the left]; lane 2, protein from human brain storage bodies; lane 3, protein from sheep liver storage bodies; lane 4, protein from mouse liver storage bodies. Approx. 150 ng of total protein was applied to each of lanes 2, 3 and 4. The band indicated with an arrow has the same mobility as mitochondrial subunit c protein [19].

Chloroform/methanol extraction of the storage bodies and ether precipitation from the extracts yielded almost pure subunit c protein, as assessed by SDS/PAGE (Figure 2). Amino acid composition analyses of HCl digests of the total storage-body proteins have been reported previously for human and sheep tissues [22,23]. In both cases, TML was present in significant amounts. The subunit c fractions isolated from the human and sheep storage bodies were enriched in TML. In both cases, the amino acid compositions were close to those predicted from the known amino acid sequence of bovine mitochondrial subunit c, with the notable exception that the lysine contents were lower than that of the mitochondrial protein, and TML and lysine were present in approximately equimolar amounts (Table 1). This suggested that one of the two lysine residues in the storage-body form of subunit c was trimethylated.

As with the other species, the mouse total storage-body protein contained a significant amount of TML (Table 1). The molar ratio of TML to lysine in the total mouse storage-body protein was 1 to 3.7. The amino acid composition of the total storage-body protein from mice differed significantly from the theoretical composition predicted for pure subunit c (Table 1). This reflects the fact that, although the storage bodies are rich in subunit c, a number of higher-molecular-mass proteins are also present in these cellular inclusions [21]. The subunit c fraction from the mouse storage bodies was enriched in TML; the molar ratio of TML to lysine in this fraction was 1.2 to 1. As with the human and sheep protein, this suggests that one of the two lysine residues in the storage-body subunit c protein is trimethylated. Except for an apparent methylation of one of its lysine residues, the amino acid composition of subunit c from the mouse storage bodies was close to that predicted from the known amino acid sequence of bovine mitochondrial subunit c (Table 1). Minor differences between the observed amino acid composition and that predicted from the sequence of the bovine protein probably reflect relative differences in the recoveries of the individual

Table 1 Amino acid compositions of total storage-body protein from mice and storage-body subunit c fractions from mice, sheep and humans with hereditary ceroid-lipofuscinosis

The values for the human amino acid composition are the range of values obtained from analysis of samples from three different tissue donors. Theoretical values for subunit c are those predicted on the basis of the reported amino acid sequence for the bovine mitochondrial protein [32,33].

	Composition (mol/100 mol)				
Amino acid	Mouse storage bodies	Mouse subunit c	Sheep subunit c	Human subunit c	Theoretical
Asx	6.5	4.0	3.5	3.9–4.7	4.0
Thr	4.6	4.1	4.0	3.8–5.6	4.0
Ser	5.4	5.5	5.0	5.5–7.9	6.7
Glx	6.6	4.4	3.9	4.0–5.0	4.0
Pro	6.3	3.5	2.0	0.8–2.7	1.3
Gly	10.6	13.6	13.2	12.0–15.5	14.7
Ala	11.6	15.3	15.7	14.9–17.0	17.3
Cys	0.6	0.4	1.2	0.7–1.0	1.3
Val	7.3	6.7	7.3	5.0–7.9	5.3
Met	2.8	2.8	3.4	2.9–3.1	4.0
Ile	6.5	8.9	9.2	8.3–8.8	9.3
Leu	11.2	13.1	13.7	10.5–14.2	12.0
Tyr	3.0	2.9	2.9	2.1–3.7	2.7
Phe	6.3	8.6	9.5	8.4–9.4	9.3
His	2.1	0.3	0.2	0.0–0.6	0.0
Lys	4.4	1.8	1.7	1.5–1.9	2.7
Arg	3.0	1.9	1.7	1.4–2.2	1.3
TML	1.2	2.2	1.9	1.7–2.4	0.0

amino acids after acid hydrolysis. In addition to lysine and TML, the mouse subunit c fraction differed significantly from the theoretical values in its apparent contents of proline, cysteine and methionine (Table 1). The observed amounts of cysteine and methionine were lower than predicted, probably because these amino acids are partially degraded during acid hydrolysis. Cysteic acid and methionine sulphoxide were detected in the hydrolysates. The amounts of these oxidation products were included in the values given for cysteine and methionine respectively in Table 1. Some cysteine and methionine were apparently lost as the result of further degradation. The basis for the higher than predicted apparent proline content of the mouse storage-body subunit c fraction (Table 1) is not clear. It is possible that the hydrolysates of the mouse subunit c preparations contain an unidentified component that is coeluted with proline from the amino acid analyser. This would produce an anomalously high value for proline. A potential candidate for such a component is a fluorophore bound to the storage-body protein [23].

Partial amino acid sequence analyses were performed on the subunit c fractions of storage bodies from human, sheep and mouse tissues. The sequence of the first 40 residues of the human storage-body protein was identical with that previously reported for the bovine mitochondrial subunit c protein [32,33] (Figure 3). N-Terminal sequence analysis was carried out for only 10 residues for the protein isolated from sheep and mouse storage bodies. In both cases, the amino acid sequence of this part of the protein was also identical with that of the bovine mitochondrial subunit c protein [32,33] (Figure 3). These findings are consistent with previously reported sequence analyses of the major storage-body protein from affected humans, sheep and mice [19,21,34]. The N-terminal sequence analyses indicated that the lysine residue at position 7 was not methylated in the protein from any of the species examined. On the basis of the amino acid composition data, it therefore appeared likely that the second lysine residue at position 43 was trimethylated in all cases. Further sequence analyses were carried out to determine whether this was indeed the case. Because we were unable to obtain reliable sequence data beyond 40 residues, the protein had to be cleaved before sequence analysis of that portion of the molecule containing residue 43. The normal mitochondrial subunit c protein contains trypsin-cleavage sites at the carboxyl sides of the lysine residues at positions 7 and 43, and at the carboxyl side of the single arginine residue at position 38. We have previously shown that trimethylation of lysine residue 43 of subunit c blocks trypsin cleavage at this residue [20]. To block trypsin cleavage at the other lysine residue, some aliquots of the protein were acylated. Acylation also had the effect of preventing Edman degradation from the N-terminus of the protein. After acylation, the protein was cleaved with trypsin, and the products were subjected to partial sequence analysis without further purification. The first cycle of Edman degradation produced a single PTH-amino acid: asparagine. Subsequent cycles began to show multiple PTH-amino acids. However, if the protein was treated with OPA or fluorescamine between the first and second cycles, a single clear sequence emerged. This sequence matched that of residues 39–48 of bovine mitochondrial subunit c except that the PTH derivative of TML and no PTH-lysine or PTH-acetyl-lysine was present on the fifth cycle. The amino acid obtained on the fifth cycle of Edman degradation corresponds to residue 43 of the subunit c protein (Figure 3). In aliquots of the subunit c proteins that were not acylated before trypsin cleavage, the first cycle of Edman

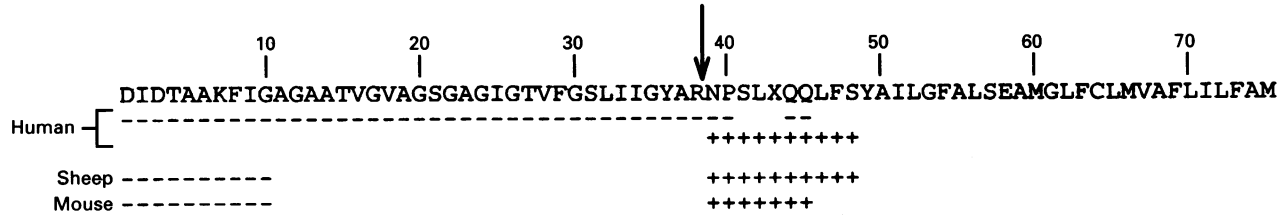

Figure 3 Comparison between amino acid sequences of chloroform-extracted storage-body proteins from affected humans, sheep and mice and the reported amino acid sequence of the mitochondrial subunit c protein

The amino acid sequence shown is that reported for bovine mitochondrial ATP synthase subunit c [32]. Residue 43, indicated by X, was reported to be lysine in the normal mitochondrial protein. The vertical arrow above the sequence indicates the trypsin-cleavage site remaining after acylation of the protein. The dashed lines below the sequence indicate the sequences determined from the whole unmodified proteins isolated from storage bodies. The + marks indicate the sequences determined after acylation and cleavage of the storage-body proteins with trypsin. In the sequences obtained from the storage-body proteins from all three species X was identified as TML by the chromatographic retention of its PTH derivative on the amino acid sequencer.

degradation yielded the PTH derivatives of aspartate, phenylalanine and asparagine, as expected from trypsin cleavage at the carboxyl sides of lysine residue 7 and arginine residue 38. The subsequent sequences were identical with those obtained from the acylated samples. Thus the sequence analyses confirmed that the lysine at position 43 is trimethylated in the storage-body protein from all three species examined.

DISCUSSION

Subunit c of mitochondrial ATP synthase has been shown to be a major constituent of the lysosomal storage material that accumulates in tissues of humans with two forms of hereditary ceroid-lipofuscinosis [12,34,35]. This protein is also present in large amounts in the storage bodies from sheep, dogs and mice with inherited diseases that are apparently analogous to the human juvenile form of ceroid-lipofuscinosis [18–20]. The genetic and biochemical bases for the specific lysosomal storage of this one particular protein are currently unknown.

Subunit c is coded for on two nuclear genes, and is synthesized in the cytosol as larger precursor proteins [36]. Leader peptides are cleaved from the precursor proteins on import of subunit c into the mitochondria [36]. Neither the structural genes for subunit c nor the rates of transcription of these genes appear to be altered in hereditary ceroid-lipofuscinosis [37]. The protein that accumulates in the storage bodies has the same N-terminal sequence as the normal mitochondrial protein. This indicates that the form of subunit c that accumulates in the storage bodies has been cleaved from the precursor proteins at the same amino acid residue as the mitochondrial form. As this cleavage is coincident with import of subunit c into the mitochondria, it is likely that the protein that accumulates in the lysosomes has passed through the mitochondria. Thus the accumulation of subunit c in the hereditary ceroid-lipofuscinoses probably results from a defect in the processing of the protein once it has entered the mitochondria. Indeed, recent evidence suggests that turnover of mitochondrial subunit c is slowed down in one form of ceroid-lipofuscinosis [38].

The discovery of a specific methylation of one lysine residue in the storage-body form of subunit c may provide a key to the elucidation of the biochemical defect responsible for the storage of this protein in the juvenile type of the disease. The normal mitochondrial protein does not appear to contain TML, nor apparently does the subunit c protein that accumulates in at least some humans with the late-infantile form of ceroid-lipofuscinosis [39]. It is possible that the specific methylation of lysine residue 43 of subunit c occurs as a result of genetically altered substrate specificity for a mitochondrial protein methyltransferase [40], such that subunit c is methylated instead of, or in addition to, the normal target protein. The abnormally methylated subunit c may then accumulate in lysosomes as a consequence of the covalent modification. Although the latter mechanism is possible, its likelihood is diminished by the fact that the disease displays a recessive pattern of expression. Carriers that are heterozygous for an abnormal protein methyltransferase would be expected to synthesize some of the methylated form of subunit c, and thus to show some disease symptoms, although with a lower severity or later onset than in homozygous individuals. However, gross disease symptoms have not been reported in carriers.

There is an alternative hypothesis that may explain the specific lysosomal storage of TML-containing subunit c in the juvenile-type ceroid-lipofuscinoses. Carnitine is a compound that plays an important role in mitochondrial fatty acid uptake and metabolism [41,42]. It is synthesized from free TML through a series of four enzymic steps [43]. Apparently mammals cannot trimethylate free lysine, but only lysine residues within proteins [44,45]. TML-containing proteins are then degraded through the actions of proteases to release free TML which can be used for carnitine biosynthesis. It is possible that mitochondrial subunit c is normally trimethylated at lysine residue 43, and that it serves as a major precursor for carnitine biosynthesis. If this is the case, lysine methylation may act as a signal for the protein to be degraded by a specific protease, particularly as the methylated form of subunit c does not appear to be present in appreciable amounts in normal mitochondrial membranes. A defect in either such a hypothetical protease or in one of the enzymes in the carnitine-biosynthetic pathway could explain the observed accumulation of TML-containing subunit c. If conversion of TML into carnitine is impaired, a build-up of TML in cells could inhibit degradation of TML-containing subunit c, perhaps through competitive binding to the TML-recognition site of the proposed protease. Evidence from the canine model for juvenile ceroid-lipofuscinosis is consistent with this hypothesis. Plasma carnitine levels in affected animals were only one-third of those in normal dogs [46]. Plasma carnitine levels were also significantly lower than normal in asymptomatic heterozygous carriers. The latter observation indicates that the reduction in carnitine levels is directly related to the genetic and metabolic defect, and is not secondary to the disease pathology. Preliminary data indicate that plasma carnitine levels are also low in human subjects with juvenile ceroid-lipofuscinosis. Thus it is quite possible that juvenile ceroid-lipofuscinosis and the diseases in the animal models result from a defect or defects in the carnitine-biosynthetic pathway.

The finding of the same amino acid modification in the stored subunit c in humans and in three animal models is significant because it validates the animal models with respect to this biochemical marker for the disease. This is quite important, as it will be much easier to identify the specific defect underlying the disease using animal models than it will be if research is restricted to the use of human material. The animal models will also be useful in evaluating potential treatments for the disease.

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