Journal of Bioenergetics and Biomembranes, Vol. 28, No. 2, 1996

Importance of Mitochondrial Transmembrane Processes in Human Mitochondriopathies

Marjan Huizing,[1] Vito DePinto,[2] Wim Ruitenbeek,[1] Frans J. M. Trijbels,[1] Lambert P. van den Heuvel,[1] and Udo Wendel[1]

Received May 15, 1995; revised June 20, 1995

In a substantial group of subjects suspected to have a mitochondriopathy no defect in the mitochondrial energy metabolism (pyruvate dehydrogenase complex or respiratory chain complexes) can be demonstrated. At least in some of these subjects it seems justified to consider a defect in one of the proteins which mediate the transport of several ions and substrates across the mitochondrial membranes. Of particular interest are proteins which are directly involved in the process of oxidative phosphorylation, such as the adenine nucleotide translocator (ANT) and the phosphate carrier (PiC). However, defects in transmembrane ion transporters also may induce impaired energy metabolism probably as a result of osmotic disturbances within the mitochondrial matrix. In this respect, the voltage-dependent anion channel (VDAC) and other ion channels have to be taken into consideration. Here we review the still incomplete knowledge of the occurrence of ANT, PiC, VDAC, cation channels, and a few substrate carriers in human tissues, as well as their possible role in pathology.

KEY WORDS: Human; mitochondriopathy; oxidative phosphorylation; energy metabolism; membrane transport; VDAC; phosphate carrier; adenine nucleotide translocator; cation transport.

INTRODUCTION

The major pathway by which eukaryotic organisms produce energy is mitochondrial oxidative phosphorylation. This is a precisely regulated complex metabolic pathway and any defect in the system may severely affect the organism. Several disorders in mitochondrial energy metabolism (MITEM[3]) of humans have been recognized in the last decade (DiMauro, 1993; Shoffner and Wallace, 1994). Defects have been reported in the oxidation of fatty acids (Coates and Tanaka, 1992), the pyruvate dehydrogenase complex, or in one or more of the multisubunit complexes of the respiratory chain (Scholte, 1988; DiMauro, 1993). Abnormalities in the mitochondrial genome have also been reported (DiMauro, 1993; Shoffner and Wallace, 1994). Subjects with a mitochondrial disorder show a diverse clinical spectrum of symptoms, varying from pure myopathy with lactic acidosis to a severe multisystem disease with predominantly central nervous system involvement. Frequently, they show qualitative and quantitative abnormalities of mitochondria in skeletal muscle.

Biochemical investigations in subjects suspected to have a mitochondrial disorder on clinical, morphological, and clinical-chemical grounds are commonly performed on skeletal muscle. They may involve measurement of substrate oxidation or oxygen comsumption by intact mitochondria in order to obtain information about the overall flux through the various parts of the MITEM. In case of impaired flux, various

[1] Department of Pediatrics, University Hospital, Nijmegen, The Netherlands.
[2] Institute of Biochemical and Pharmacological Sciences, University of Catania, Catania, Italy.
[3] Abbreviations used: MITEM, mitochondrial energy metabolism; ANT, adenine nucleotide translocator; PiC, phosphate carrier; VDAC, voltage-dependent anion channel; IMM, inner mitochondrial membrane; OMM, outer mitochondrial membrane.

0145-479X/96/0400-0109$09.50/0

enzyme complexes of the respiratory chain and the pyruvate dehydrogenase complex are measured (Fischer *et al.*, 1986).

Despite this extensive diagnostic approach, in almost a quarter of the patients with evidently reduced substrate oxidation in muscle mitochondria, these abnormalities could not be ascribed to a known defect in one of the mitochondrial enzymes. It was hypothesized that in this category of patients malfunctioning of the transport mechanism across the mitochondrial membranes may cause mitochondrial disorders (Ruitenbeek *et al.*, 1996). Consequently, subjects lacking ANT or VDAC were diagnosed applying immunochemical studies with specific antibodies against these proteins (Bakker *et al.*, 1993; Huizing *et al.*, 1994).

Metabolite transport across the inner mitochondrial membrane (IMM) is mediated by a series of carrier-type proteins, such as ANT, PiC, and different cation transporting proteins. Transport across the outer mitochondrial membrane (OMM) is less well studied, but a regulated channel-forming protein, VDAC, certainly plays a role.

At present there is little knowledge about the different mitochondrial transport systems in human tissues. Here we review facts of the carriers involved in MITEM, such as ANT, PiC, VDAC, and some cation and substrate carriers in human tissues, both at protein and DNA level, as well as the possible role which they may play in pathology.

ADENINE NUCLEOTIDE TRANSLOCATOR (ANT)

ANT is an integral IMM protein of about 30 kDa. It catalyzes the transmembrane 1:1 exchange of cytosolic ADP and matrix ATP in the process of oxidative phosphorylation (Klingenberg, 1981; Brandolin *et al.*, 1993). ANT has a low turnover rate, but a high concentration, so the capacity for ADP and ATP transport across the IMM can easily cope with the energy requirement of the different cell types (Battini *et al.*, 1987; Houldsworth and Attardi, 1988). It has been shown that ANT partly contributes to the control of respiration (Tager *et al.*, 1983; Letellier *et al.*, 1993).

Three human ANT isoforms (ANT1, ANT2, and ANT3) have been identified and sequenced. They are expressed in a tissue-specific manner (Battini *et al.*, 1987; Neckelmann *et al.*, 1987; Houldsworth and Attardi, 1988). In Table I these aspects as well as the chromosomal location of the genes are listed. The expression pattern of the different ANT isoforms seems to be related to their function. ANT1 may permit rapid exchange of ADP and ATP to accommodate the high energy demand associated with contraction of striated muscle fibers (Neckelmann *et al.*, 1987). The ANT2 isoform is mainly associated with smooth muscle cells (Stepien *et al.*, 1992). Furthermore, it is induced in rapidly dividing cells such as fibroblasts, human leukemic cells (Battini *et al.*, 1987), and myoblasts (Stepien *et al.*, 1992). The ANT3 isoform seems to be ubiquitous in humans, expressed in all tissues, mainly in kidney (Torroni *et al.*, 1990).

Recently, a deficiency of ANT in muscle (most probably of the ANT1 isoform) has been reported, demonstrated by immunochemical techniques (Bakker *et al.*, 1993). This patient showed severe myopathy with lactic acidosis. The patient's impaired substrate oxidation in muscle mitochondria could be readily explained by the defective transport protein. The fibroblasts did not show the defect, pointing to a tissue-specific expression.

PHOSPHATE CARRIER (PiC)

The supply of inorganic phosphate (P_i) required for oxidative phosphorylation is maintained by two systems which transport P_i across the IMM. The P_i/dicarboxylate carrier mediates electroneutral P_i:dicarboxylate or P_i:P_i exchange, whereas the PiC mediates the P_i/H^+ symport (or P_i/OH^- antiport) (Stappen and Krämer, 1994). Because the supply of inorganic phosphate for oxidative phosphorylation depends mainly on PiC activity, PiC can play a role in regulation of the MITEM (Ferreira and Pederson, 1993).

PiC is an IMM protein of about 32 kDa (see Table I). A full-length cDNA encoding the precursor of the human heart PiC has been synthesized and characterized (Dolce *et al.*, 1991). The gene encoding human PiC is located on chromosome 12 (Jabs *et al.*, 1994) and is spread over 7.9 kb of DNA, consisting of nine exons (Dolce *et al.*, 1994). Two alternatively spliced mRNAs for PiC (IIIA and IIIB) have been reported, both of which were present in a human heart cDNA library. The alternative splicing mechanism introduces a different region of 13 amino acids into the human carrier protein, the functional consequences of which are not yet understood (Dolce *et al.*, 1994). Expression studies on the bovine heart PiC gene, which is 94% identical to human PiC, showed that the IIIA form has a level of expression in heart and liver > brain and

Table I. Features of Human Mitochondrial Membrane Carriers and Channels

Carrier	Mw	Location	Cloned*	Isoforms	Chromosome	Main expression	Reference
ANT	30 kDa	IMM	+	ANT1	4	Skeletal muscle, heart	Cozens *et al.*, 1989; Li *et al.*, 1989
			+	ANT2	X	Fibroblasts, liver, kidney, brain, heart	Battini *et al.*, 1987
			+	ANT3	?	Ubiquitous	Houldsworth and Attardi, 1988
PiC	32 kDa	IMM	+	IIIA	12	Heart, liver (brain, kidney)[b]	Dolce *et al.*, 1994; Jabs *et al.*, 1994
			+	IIIB	12	Lung (brain, kidney)[b]	Dolce *et al.*, 1994; Jabs *et al.*, 1994
VDAC	35 kDa	OMM	+	HVDAC1	X	Ubiquitous	Blachly-Dyson *et al.*, 1993
			+	HVDAC2	21	?	Blachly-Dyson *et al.*, 1993
			+	HVDAC2′	21	?	Ha *et al.*, 1993
			+	HVDAC3	12	?	Blachly-Dyson *et al.*, 1994
			+	HVDAC4	1	?	Blachly-Dyson *et al.*, 1994
K^+/H^+	82 kDa[b]	IMM	+[b]	?	?	?	Brierley *et al.*, 1994; Garlid, 1994
Na^+/Ca^{2+}	110 kDa[b]	IMM	−	?	?	?	Li *et al.*, 1992; Cox and Matlib, 1993
Na^+/H^+	59 kDa[b]	IMM	−	?	?	?	Brierley *et al.*, 1994; Garlid, 1994
K^+_{ATP}	54 kDa[b]	IMM	+[b]	?	?	?	Beavis *et al.*, 1993; Garlid, 1994
Ca^{2+}	?	IMM	−	?	?	?	Garlid, 1994
Oxoglutarate	32 kDa	IMM	+	?	?	Heart, liver, brain	Palmieri *et al.*, 1993
Carnitine	32.5 kDa	IMM	−	?	?	Ubiquitous	Indiveri *et al.*, 1992
Pyruvate	34 kDa[r]	IMM	−	?	?	Brain[r]	Nalecz *et al.*, 1992
Citrate	30/37kDa[r]	IMM	+[r]	?	?	Liver, brain[r]	Azzi *et al.*, 1993

[b] = reported for bovine; [r] = reported for rat; * = cDNA known; ? = unknown; + = yes; − = no.

kidney > lung. The opposite was found for the IIIB form (Dolce *et al.*, 1994).

Although no PiC deficiency has been described so far, such a defect has to be considered when studying mitochondriopathies.

VOLTAGE-DEPENDENT ANION CHANNEL (VDAC)

A substantial part of the molecular traffic across the OMM is mediated by an abundant pore-forming protein, VDAC (or mitochondrial porin). At low transmembrane voltage the VDAC pore is open for anions such as phosphate, chloride, and adenine nucleotides. At higher transmembrane voltage or in the presence of a VDAC modulating protein, VDAC functions as selective channel for cations and uncharged molecules. These features make VDAC likely to play a regulatory role in MITEM (Mannella, 1992; Benz, 1994; Lui *et al.*, 1994). The NADH concentration (Zizi *et al.*, 1994) and colloidal osmotic pressure (Zimmerberg and Parsegian, 1986) may also function as VDAC regulator by shifting the membrane potential required for opening and closure of the pores. In addition to the OMM, VDAC may be localized in the plasmalemma of certain cells (Thinnes, 1992), although this extramitochondrial localization is still under discussion (Yu *et al.*, 1995).

With respect to human VDAC (HVDAC), four genes encoding different HVDAC isoforms have been reported, which are listed in Table I together with their chromosomal locations. Until now, only HVDAC1 and

HVDAC2 have been shown to be expressed at the protein level, HVDAC1 being the most abundantly expressed (Winkelbach *et al.*, 1994; Yu *et al.*, 1995).

Recently, we could demonstrate VDAC deficiency in a patient with impaired substrate oxidation in muscle mitochondria (Huizing *et al.*, 1994). The patient showed psychomotor retardation, macrosomia, and macrocephaly. The presence of an almost normal VDAC amount in the patient's fibroblasts suggests that VDAC is expressed in a tissue-specific manner.

CATION TRANSLOCATING PROTEINS

The maintenance of volume and ion composition of the mitochondrial matrix within narrow ranges is crucial for proper MITEM. Permeability changes of the IMM or OMM may influence the respiration rate (Halestrap *et al.*, 1992; Brdiczka and Walliman, 1994). H^+, K^+, Na^+, Ca^{2+}, and Mg^{2+} ions have their specific role in mitochondrial physiology and are transported across the IMM by one or more specific carriers. So far no studies on human cation transporters have been reported. A few cation carriers have been isolated and identified from mammalian tissues (see Table I), none of which have been cloned and sequenced yet (Li *et al.*, 1992; Garlid, 1994; Jung and Brierley, 1994).

The mitochondrial volume is controlled by the K^+ cycle, which includes the K^+/H^+ antiporter (82 kDa), the K_{ATP} channel (54 kDa), and a K^+ leak induced by high membrane potential (Garlid, 1994). However, Mg^{2+} also is involved in volume maintenance, besides stimulation of numerous enzymes and transporters, and ligation of different metabolites. Whereas the uptake of Mg^{2+} occurs by a nonspecific diffusion in response to elevated membrane potentials, the efflux of Mg^{2+} may occur in exchange for H^+ (Jung and Brierley, 1994).

Respiratory chain activity generates a H^+ gradient across the IMM which is used for ATP production. In this respect it is known that a lower pH in the mitochondrial matrix resulting from any disturbance of H^+ transport across mitochondrial membranes leads to decreased mitochondrial ATPase activity and thus decreased ATP production (Senior, 1988). Also, various components of the respiratory chain and mitochondrial (cation) transport systems can be inhibited (Hak *et al.*, 1993; Brierley *et al.*, 1994).

Mitochondrial Ca^{2+} plays a special role in muscle metabolism. Besides its common role in activating the citric acid cycle (by activating various dehydrogenases; Gunter, 1994), it is important for the regulation of cytosolic Ca^{2+} concentration in muscle, thereby influencing the contraction–relaxation process (Carafoli *et al.*, 1982). Electrophoretic Ca^{2+} uptake across the IMM proceeds via a Ca^{2+} uniporter, whereas the electroneutral Ca^{2+} release proceeds by a Na^+/Ca^{2+} antiporter (110 kDa). This process is balanced by a Na^+/H^+ antiporter (59 kDa) (Carafoli *et al.*, 1982; Li *et al.*, 1992; Garlid, 1994).

The primary or secondary role of Ca^{2+} in human myopathies is likely underestimated. In diseases such as Duchenne muscular dystrophy, myotonic dystrophy, and polymyositis, muscle fibers contain an elevated Ca^{2+} concentration (Stadhouders, 1981). In some patients with a MITEM disorder calcification of the basal ganglia is reported (Samsom *et al.*, 1994). Accumulation of calcium in cytoplasma and mitochondria can lead to cell death (Engel, 1979; Stadhouders, 1981).

OTHER CARRIERS

Several mitochondrial carriers specific for different substrates are described, which are directly or indirectly involved in MITEM. This group includes carriers for pyruvate, oxoglutarate, citrate, succinate, carnitine, glutamate, and the aspartate/malate shuttle (Tyler and Sutton, 1984). Out of this group, only defects in the human carnitine carrier (Pande and Murthy, 1994) and the aspartate/malate shuttle (Hayes *et al.*, 1987) have been reported. Almost no studies of the other carriers have been reported for human; the characteristics mentioned in Table I have been obtained from animal studies.

DISCUSSION

It is well known that the ATP/ADP ratio, the mitochondrial Pi concentration, as well as the transmembrane proton gradient may significantly contribute to the control of mitochondrial respiration (Tager *et al.*, 1983; Letellier *et al.*, 1993). As a consequence, defects of carriers such as ANT or PiC might be considered to cause mitochondriopathy. This holds also for other mitochondrial carrier protein or ion channels which contribute to strictly maintaining the osmotic homeostasis in the mitochondrial matrix (Li *et al.*, 1992; Cox and Matlib, 1993).

Possibly MITEM is not only influenced by individually functioning transmembrane carriers, but also by complicated structures in which several types of transport proteins and other components are present. In these structures, i.e., contact sites and megachannels, VDAC, ANT, hexokinase, glycerol kinase, and the benzodiazepine receptor are involved (McEnery *et al.*, 1993; Benz, 1994; Brdiczka and Walliman, 1994; Gellerich *et al.*, 1994). These interactions are tissue-specific and dependent on the developmental and metabolic stages of the cells. Their roles in human MITEM are speculative at this time.

At least some carriers show similarities in the amino acid sequence, particularly in the membrane-spanning regions, indicating that they are encoded by a small gene family (Palmieri, 1994). Since most of the carriers occur in tissue-specific isoforms it can be imagined that a defect in one of these carriers may cause a disorder with a tissue-specific nature, a phenomenon not uncommon in mitochondriopathies (Trijbels *et al.*, 1988; DiMauro, 1993).

So far, only few subjects with a specific defect in mitochondrial membrane transport have been reported, among which are ANT and VDAC deficiencies (Bakker *et al.*, 1993; Huizing *et al.*, 1994), a defect in the protein import machinery (Schapira *et al.*, 1990), and a disturbed malate–aspartate shuttle (Hayes *et al.*, 1987). More systematic studies on the different transport proteins may reveal additional disturbances causing mitochondriopathies.

ACKNOWLEDGMENTS

MH is financially supported by the Prinses Beatrix Fonds (grant No. 93-018) and VDP by Thelethon Italy (grant No. 541).

REFERENCES

Azzi, A., Glerum, M., Koller, R., Mertens, W., and Spycher, S. (1993). *J. Bioenerg. Biomembr.* **25**, 515–523.

Bakker, H. D., Scholte, H. R., Van den Bogert, C., Ruitenbeek, W., Jeneson, J. A. L., Wanders, R. J. A., Abeling, N. G. G. M., Dorland, B., Sengers, R. C. A., and Van Gennip, A. H. (1993). *Pediatr. Res.* **33**, 412–417.

Battini, R., Ferrari, S., Kaczmarek, L., Calabretta, B., Chen, S., and Baserga, R. (1987). *J. Biol. Chem.* **9**, 4355–4359.

Beavis, A. D., Lu, Y., and Garlid, K. D. (1993). *J. Biol. Chem.* **268**, 997–1004.

Benz, R. (1994). *Biochim. Biophys. Acta* **1197**, 167–196.

Blachly-Dyson, E., Zambronicz, E. B., Yu, W. H., Adams, V., McCabe, E. R. B., Adelman, J., Colombini, M., and Forte, M. (1993). *J. Biol. Chem.* **268**, 1835–1841.

Blachly-Dyson, E., Baldini, A., Litt, M., McCabe, E. R. B., and Forte, M. (1994). *Genomics* **20**, 62–67.

Brandolin, G., Le Saux, A., Trezeguet, V., Lauquin, G. J. M., and Vignais, P. V. (1993). *J. Bioenerg. Biomembr.* **25**, 459–472.

Brdiczka, D., and Wallimann, T. (1994). *Mol. Cell. Biochem.* **133/134**, 69–83.

Brierley, G. P., Baysal, K., and Jung, D. W. (1994). *J. Bioenerg. Biomembr.* **26**, 519–526.

Carafoli, E., Caroni, P., Chiesi, M., and Famulski, K. (1982). In *Metabolic Compartmentation* (Sies, H., ed.), Academic Press, London, pp. 521–547.

Coates, P. M., and Tanaka, K. (1992). *J. Lipid Res.* **33**, 1099–1110.

Cox, D. A., and Matlib, M. A. (1993). *J. Biol. Chem.* **268**, 938–947.

Cozens, A. L., Runswick, M. J., and Walker, J. E. (1989). *J. Mol. Biol.* **206**, 261–280.

DiMauro, S. (1993). In *The Molecular and Genetic Basis of Neurological Disease* (Rosenburg, R. N., Prusiner, S. B., DiMauro, S., Barchi, L. R., and Kunkel, L. M., eds.), Butterworth-Heinemann, Boston, pp. 665–694.

Dolce, V., Fiermonte, G., Messina, A., and Palmieri, F. (1991). *DNA Sequence* **2**, 133–135.

Dolce, V., Iacobazzi, V., Palmieri, F., and Walker, J. E. (1994). *J. Biol. Chem.* **269**, 10451–10460.

Engel, W. K. (1979). *Arch. Neurol.* **36**, 329–339.

Ferreira, G. C., and Pederson, P. L. (1993). *J. Bioenerg. Biomembr.* **25**, 483–492.

Fischer, J. C., Ruitenbeek, W., Gabreëls, F. J. M., Janssen, A. J. M., Renier, W. O., Sengers, R. C. A., Stadhouders, A. M., Ter Laak, H. J., Trijbels, J. M. F., and Veerkamp, J. H. (1986). *Eur. J. Pediatr.* **144**, 441–444.

Garlid, K. D. (1994). *J. Bioenerg. Biomembr.* **26**, 537–542.

Gellerich, F. N., Kapischke, M., Kunz, W., Neumann, W., Kuznetsov, A., Brdiczka, D., and Nicolay, K. (1994). *Mol. Cell. Biochem.* **133/134**, 85–104.

Gunter, T. E. (1994). *J. Bioenerg. Biomembr.* **26**, 465–469.

Ha, H., Hajek, P., Bedwell, D. M., and Burrows, P. D. (1993). *J. Biol. Chem.* **268**, 12143–12149.

Hak, J. B., Van Beek, J. H. G. M., and Westerhof, N. (1993). *Pflügers Arch.* **423**, 324–329.

Halestrap, A. P., Connern, C. P. and Griffiths, E. J. (1992). In *Molecular Mechanisms of Transport* (Quagliariello, E., and Palmieri, F., eds.), Elsevier, Amsterdam, pp. 259–266.

Hayes, D. J., Taylor, D. J., Bore, P. J., Hilton-Jones, D., Arnold, D. L., Squier, M. V., Gent, A. E., and Radda, G. K. (1987). *J. Neurol. Sci.* **82**, 27–39.

Houldsworth, J., and Attardi, G. (1988). *Proc. Natl. Acad. Sci. USA* **85**, 377–381.

Huizing, M., Ruitenbeek, W., Thinnes, F. P., and DePinto, V. (1994). *Lancet* **344**, 762.

Indiveri, Tonazzi, A., Dierks, T., Krämer, R., and Palmieri, F. (1992). *Biochim. Biophys. Acta* **1140**, 53–58.

Jabs, E. W., Thomas, P. J., Bernstein, M., Coss, C., Ferreira, G. C., and Pedersen, P. L. (1994). *Hum. Genet.* **93**, 600–602.

Jung, D. W., and Brierley, G. P. (1994). *J. Bioenerg. Biomembr.* **26**, 527–535.

Klingenberg, M. (1981). *Nature* **290**, 449–454.

Letellier, T., Malgat, M., and Mazat, J. P. (1993). *Biochim. Biophys. Acta* **1141**, 58–64.

Li, K., Warner, C. K., Hodge, J. A., Minoshima, S., Kudoh, J., Fukuyama, R., Maekawa, M., Shimizu, Y., Shimizu, N., and Wallace, D. C. (1989). *J. Biol. Chem.* **264**, 13998–14004.

Li, W., Shariat-Madar, Z., Powers, M., Sun, X., Lane, R. D., and Garlid, K. D. (1992). *J. Biol. Chem.* **267**, 17983–17989.

Lui, M. Y., Torgrimson, A., and Colombini, M. (1994). *Biochim. Biophys. Acta* **1185**, 203–212.

Mannella, C. A. (1992). *Trends Biochem. Sci.* **17**, 315–320.

McEnery, M. W., Dawson, T. M., Verma, A., Gurley, D., Colombini, M., and Snyder, S. H. (1993). *J. Biol. Chem.* **268**, 23289–23296.

Nalecz, K. A., Kaminska, J., Nalecz, M. J., and Azzi, A. (1992). *Arch. Biochem. Biophys.* **297**, 162–168.

Neckelmann, N., Li, K., Wade, R. P., Shuster, R., and Wallace, D. C. (1987). *Proc. Natl. Acad. Sci. USA* **84**, 7580–7584.

Palmieri, F. (1994). *FEBS Lett.* **346**, 48–54.

Palmieri, F., Bisaccia, F., Capobianco, L., Dolce, V., Fiermonte, G., Iacobazzi, V., and Zara, V. (1993). *J. Bioenerg. Biomembr.* **25**, 493–501.

Pande, S. V., and Murthy, M. S. R. (1994). *Biochim. Biophys. Acta* **1226**, 269–276.

Ruitenbeek, W., Huizing, M., DePinto, V., Thinnes, F. P., Trijbels, J. M. F., Wendel, U., and Sengers, R. C. A. (1996). In *Progress in Cell Research* (Palmieri, F., ed.), Elsevier, Amsterdam, in press.

Samsom, J. F., Barth, P. G., De Vries, J. I. P., Menko, F. H., Ruitenbeek, W., Van Oost, B. A., and Jakobs, C. (1994). *Eur. J. Pediatr.* **153**, 510–516.

Schapira, A. H. V., Cooper, J. M., Morgan-Hughes, J. A., Landon, D. N., and Clark, J. B. (1990). *N. Engl. J. Med.* **323**, 37–42.

Scholte, H. R. (1988). *J. Bioenerg. Biomembr.* **20**, 161–192.

Senior, A. E. (1988). *Physiol. Rev.* **68**, 177–231.

Shoffner, J. M., and Wallace, D. C. (1994). *Annu. Rev. Nutr.* **14**, 535–568.

Stadhouders, A. M. (1981). In *Mitochondria and Muscular Diseases* (Busch, H. F. M., Jennekens, F. G. I., and Scholte, H. R., eds.), Mefar B. V., Beetsterzwaag, Neth., pp. 77–88.

Stappen, R., and Krämer, R. (1994). *J. Biol. Chem.* **269**, 11240–11246.

Stepien, G., Torroni, A., Chung, A. B., Hodge, J. A., and Wallace, D. C. (1992). *J. Biol. Chem.* **267**, 14592–14597.

Tager, J. M., Wanders, R. J. A., Groen, A. K., Kunz, W., Bohnensack, R., Küster, U., Letko, G., Böhme, G., Duszynski, J., and Wojtczak, L. (1983). *FEBS Lett.* **151**, 1–9.

Thinnes, F. P. (1992). *J. Bioenerg. Biomembr.* **24**, 71–75.

Torroni, A., Stepien, G., Hodge, J. A., and Wallace, D. C. (1990). *J. Biol. Chem.* **265**, 20589–20593.

Trijbels, J. M. F., Sengers, R. C. A., Ruitenbeek, W., Fischer, J. C., Bakkeren, J. A. J. M., and Janssen, A. J. M. (1988). *Eur. J. Pediatr.* **148**, 92–97.

Tylor, D. D., and Sutton, C. M. (1984). In *Membrane Structure and Function*. Vol V (Bittar, E. E., ed.), Wiley, New York, pp. 181–270.

Winkelbach, H., Walter, G., Morys-Wortmann, C., Paetzold, G., Hesse, D., Zimmermann, B., Flörke, H., Reymann, S., Stadtmüller, U., Thinnes, F. P., and Hilschmann, N. (1994). *Biochem. Med. Metab. Biol.* **52**, 120–127.

Yu, W. H., Wolfgang, W., and Forte, M. (1995). *J. Biol. Chem.* **270**, 13998–14006.

Zimmerberg, J., and Parsegian, V. A. (1986). *Nature* **323**, 36–39.

Zizi, M., Forte, M., Blachly-Dyson, E., and Colombini, M. (1994). *J. Biol. Chem.* **269**, 1614–1616.