Review: Mitochondrial Medicine – Molecular Pathology of Defective Oxidative Phosphorylation

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Abstract. Different tissues display distinct sensitivities to defective mitochondrial oxidative phosphorylation (OXPHOS). Tissues highly dependent on oxygen such as the cardiac muscle, skeletal and smooth muscle, the central and peripheral nervous system, the kidney, and the insulin-producing pancreatic β-cell are especially susceptible to defective OXPHOS. There is evidence that defective OXPHOS plays an important role in atherogenesis, in the pathogenesis of Alzheimer's disease, Parkinson's disease, diabetes, and aging. Defective OXPHOS may be caused by abnormal mitochondrial biosynthesis due to inherited or acquired mutations in the nuclear (n) or mitochondrial (mt) deoxyribonucleic acid (DNA). For instance, the presence of a mutation of the mtDNA in the pancreatic β-cell impairs adenosine triphosphate (ATP) generation and insulin synthesis. The nuclear genome controls mitochondrial biosynthesis, but mtDNA has a much higher mutation rate than nDNA because it lacks histones and is exposed to the radical oxygen species (ROS) generated by the electron transport chain, and the mtDNA repair system is limited. Defective OXPHOS may be caused by insufficient fuel supply, by defective electron transport chain enzymes (Complexes I - IV), lack of the electron carrier coenzyme Q10, lack of oxygen due to ischemia or anemia, or excessive membrane leakage, resulting in insufficient mitochondrial inner membrane potential for ATP synthesis by the F_0F_1-ATPase. Human tissues can counteract OXPHOS defects by stimulating mitochondrial biosynthesis; however, above a certain threshold the lack of ATP causes cell death. Many agents affect OXPHOS. Several nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit or uncouple OXPHOS and induce the 'topical' phase of gastrointestinal ulcer formation. Uncoupled mitochondria reduce cell viability. The *Helicobacter pylori* induces uncoupling. The uncoupling that opens the membrane pores can activate apoptosis. Cholic acid in experimental atherogenic diets inhibits Complex IV, cocaine inhibits Complex I, the poliovirus inhibits Complex II, ceramide inhibits Complex III, azide, cyanide, chloroform, and methamphetamine inhibit Complex IV. Ethanol abuse and antiviral nucleoside analogue therapy inhibit mtDNA replication. By contrast, melatonin stimulates Complexes I and IV and *Gingko biloba* stimulates Complexes I and III. Oral Q10 supplementation is effective in treating cardiomyopathies and in restoring plasma levels reduced by the statin type of cholesterol-lowering drugs. *(received 15 September 2000; accepted 30 October 2000)*

Keywords: mitochondria, oxidative phosphorylation, apoptosis, atherosclerosis, diabetes mellitus, gastrointestinal disease, Alzheimer's disease, Parkinson's disease, *Helicobacter pylori*, ethanol, statin, melatonin, cancer.

Introduction

Historical perspective, In 1956 Harman introduced the free radical theory of aging suggesting that over time, free radicals cause damage to macromolecules (DNA, lipids, proteins) [1]. Mitochondrial medicine was initiated six years later with the report on Luft's syndrome, a mitochondrial functional disorder involving loose coupling and a greatly elevated basic metabolic rate despite normal thyroid metabolism [2].

In 1963, threadlike structures that could be digested by DNAase were discovered in mitochondria of chick embryos [3,4]. The following year, mtDNA was isolated from purified yeast mitochondria. Phosphory-

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lating submitochondrial particles were first isolated from yeast in 1966 [5].

In 1970, the first respiratory chain deficiency without elevated basal oxygen consumption that was due to a reduced activity of a specific enzyme, cytochrome b, was identified. The symptoms included dementia, cerebellar ataxia, and proximal muscle weakness [6]. Next came reports on a defect of pyruvate dehydrogenase [7] and two years later the identification of reduced cytochrome oxidase c in Menkes' syndrome [8]. Another year later, myopathies due to a defect in muscle carnitine [9] and defects in carnitine palmitoyl transferase were reported [10].

In 1981, the first complete (Cambridge) sequence of mitochondrial DNA (mtDNA) was published [11]. In 1984, Miquel and Fleming emphasized that the mitochondrion is the major site of oxidative damage and that oxidative damage to mitochondria plays an important role in aging [12]. The first neurodegenerative disease associated with a mutation of mtDNA was described in 1988 [13].

The following year Linnane et al expanded on the mitochondrial theory of aging: somatic accumulation of mitochondrial genome mutations during life is a major cause of human aging and degenerative disease [14]. Their theory was based upon the following observations: (a) high frequency of gene mutation in mitochondrial DNA, (b) the small size of the mitochondrial genome, (c) lack of effective repair mechanism for mtDNA, and (d) somatic segregation of individual mtDNA during eukaryotic cell division.

Mitochondrial DNA damage may be a biomarker of oxygen damage over time. Mutations in mtDNA, whether inborn or acquired, accelerate mitochondrial dysfunction that induces further damage, and additional mutations and deletions [15].

Presently, it is realized that a gradual decline in ATP generation with age induces loss of stamina, memory, vision, and hearing and contributes to aging-related diseases such as cardiovascular disease and diabetes [16]. In addition, many exogenous agents, even therapeutic ones such as acidic nonsteroidal anti-inflammatory drugs (NSAIDs), can inhibit the mitochondrial energy system [17] and cause disease at any age.

Oxidative phosphorylation. An efficient mitochondrial oxidative phosphorylation (OXPHOS) system is essential since it provides most of the adenosine triphosphate (ATP), the chemical energy required for a cell's metabolism [18]. Therefore, each step of the mitochondrial energy conversion system must function efficiently for cell survival. Auspiciously, it is a redundant system, since each cell carries up to a few thousand mitochondria. Furthermore, when a cell with a few defective mitochondria divides, one daughter cell might receive the defective mitochondria and perish, while the other daughter cell is restored to normal. However, in post-mitotic cells this option is not available. It has been estimated that over 90% of mitochondria are contained in postmitotic cells [19].

Because of differences in the threshold values of different tissues, only some tissues may be affected and exhibit pathology as a result of an inherited mitochondrial DNA mutation, even if that mutation is present in all tissues [20]. Similarly, exogenous substances that affect OXPHOS may affect different tissues differently.

Classification of Defects

Structural defects. 1. Inherited. Primary mitochondrial diseases are caused by inherited defects in the mitochondrial structure, which lead to defective function of the mitochondria. They involve oxidative phosphorylation or other mitochondrial pathways like the urea cycle and fatty acid oxidation [21]. They cause a wide range of diverse clinical manifestations; such as ataxia, cardiomyopathy, dementia, epilepsy, myopathy, polyneuropathy, and retinal pigment anomaly [22,23].

2. Acquired. *(a) Ischemia and deletion*. In ischemic heart disease, a 40-45-fold increase in the common (4,799 bp) mtDNA deletion in the myocardium has been reported. Transcripts of mtDNA-coded oxidative phosphorylation enzyme subunits were increased, pointing to an association between ischemia and OXPHOS deficiency, enhanced ROS generation and mtDNA deletions [24]. Furthermore, animal models point to ischemia as the primary event. For instance, myocardial ischemia produced by ameroid constriction of canine coronary arteries resulted in the development of myocardial mtDNA deletions similar to deletions observed in human ischemic hearts [25].

(b) Organ preservation. Prevention of serious impairment of oxidative phosphorylation from ischemia reperfusion injury is critical to transplantation and open heart surgery. It has been suggested that lipid peroxidation plays a role in the deterioration in

mitochondrial oxidative phosphorylation observed in the isolated rat heart after 8-12 hr of hypothermic ischemia [26].

Functional defects. This intricate energy conversion system is susceptible to malfunction by many causes occurring either alone or in various combinations. A few causes are illustrated in Figure 1. Many NSAIDs that inhibit oxidative phosphorylation inhibit the enzyme activities rather than their synthesis.

1. Inadequate fuel supply. The generation of the mitochondrial membrane potential requires an adequate supply of metabolites to Complexes I and II of the electron transport chain. For instance, aspirin inhibits β-oxidation and the delivery of metabolites to the electron transport chain [27] and reduces the mitochondrial fuel supply and energy flux. It has been implied that chronic inhibition of β-oxidation causes hepatic steatosis by choking the respiratory chain and thus increasing the production of reactive oxygen species. These effects might lead to lipid peroxidation that causes steatohepatitis and the formation of Mallory bodies, necrosis, and fibrosis [28].

2. Dysfunction of the electron transport chain. Electrons from the metabolites must be efficiently carried via coenzyme-Q10 (Q10) to Complex III, and then via cytochrome c and Complex IV to oxygen. An ample supply of oxygen as electron acceptor must be provided. Uninhibited, effective proton pumping by Complexes I, III, and IV must deposit the released energy as an electrical charge across the mitochondrial inner membrane. Salicylate and indomethacin can inhibit the electron transport chain. The electron transport may be reduced or blocked by an inadequate supply of oxygen. Blood flow reduction due to atherosclerosis, reduction in oxygen carrying capacity due to anemia or smoking, or a reduction in the oxygen partial pressure at high altitude or reduced airplane cabin pressure can all impair the function of the electron transport chain. Furthermore, defective ATP generation may be due to lack of substrates, essential cofactors, or may be caused by inefficient use of oxygen [29]. A virus [30,31] may induce OXPHOS dysfunction. Conditions of oxidative stress, chronic alcohol abuse, a large number of drugs, or toxin can also lead to a decline of mitochondrial function [21].

3. Abnormal coupling and uncoupling. The inner mitochondrial membrane potential serves as a convertible energy currency of the cell [32] (Figure 1).

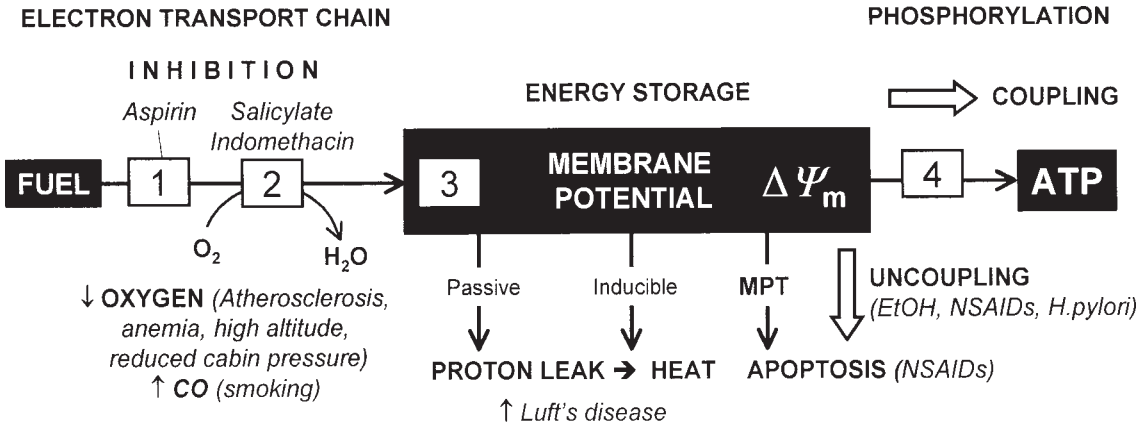

Fig. 1. Illustration shows defective mitochondrial oxidative phosphorylation (OXPHOS). Numbers in brackets refer to numbers in square boxes. Defective OXPHOS impairs the electron flow from metabolites (fuel) [1] through the electron transport chain [2] (enzyme Complexes I - IV) to oxygen. The generation of the inner mitochondrial membrane potential ($\Delta\Psi_m$) [3] is lowered and phosphorylation [4] reduced, leading to insufficient generation of adenosine triphosphate (ATP) by F_0F_1-ATPase (OXPHOS complex V) for proper cellular function. Inhibition of any of the enzyme complexes of oxidative phosphorylation (for example by an acid NSAID) lowers $\Delta\Psi_m$ generation. Ischemia caused by atherosclerosis or anemia reduces respiration. Inhibition of phosphorylation or loss of potential without ATP generation by passive or induced membrane proton leaks causes uncoupling of oxidative phosphorylation. During the mitochondrial permeability transition (MPT) mitochondrial pores open, causing membrane depolarization, loss of membrane potential, cessation of oxidative phosphorylation, and initiation of apoptosis. The text in italics indicates exogenous sources of mitochondrial dysfunction.

Therefore, the majority of the stored energy must be efficiently coupled to Complex V to phosphorylate adenosine diphosphate (ADP) to adenosine triphosphate (ATP). Uncoupling in the form of inhibition of phosphorylation must be limited. Uncoupling in the form of losses due to passive membrane permeability, altered membrane lipids, or uncoupling proteins must be controlled. The amount of heat generated when some hydrogen ions passively leak back across the membrane must be restricted, and the heat released by active channeling of protons back through the membrane by uncoupling proteins (UCP) must be efficiently regulated. Induction of the membrane permeability transition (MPT) by NSAIDs (eg, diclofenac sodium,mefenamic acid [33]), or by toxic endogenous or exogenous agents, must be limited.

As an example of membrane leakage, in Luft's disease the loose coupling causes increased mitochondrial respiration but reduced phosphorylation with low ATP generation [34]. The excess metabolic turnover produces heat instead of ATP, resulting in a steady elevation of body temperature. Muscle fibers show evidence of attempts to compensate for loose coupling in the form of aggregates of large mitochondria full of cristae [35], the primary location of OXPHOS enzymes. Compensatory increase of mitochondrial synthesis may occur in the presence of other causes of defective oxidative phosphorylation [36,37].

Damage and repair. 1. Reactive oxygen metabolites. The reactive oxygen metabolites (ROM) generated during mitochondrial respiration can damage membranes, DNA, and mitochondrial oxidative phosphorylation enzymes, causing a vicious cycle of declining mitochondrial function [38]. For instance, in the cultured endothelial cell and the vascular smooth muscle cell reactive species preferentially damage mtDNA compared with the transcriptionally inactive nuclear β-globin gene. The mitochondrial mRNA transcripts, protein synthesis, and ATP synthesis decreased. Smooth muscle cells suffered less impairment than the endothelial cells [39].

Diseases of oxidative phosphorylation often occur together with impaired β-oxidation. Reduced flux through the respiratory chain increases the NADH/NAD^+ ratio. Beta-oxidation is inhibited and produces secondary carnitine deficiency. Generation of reactive oxygen species increases and α-tocopherol is depleted probably because of consumption due to lipid peroxidation [40].

2. Antioxidants. Experimentally, the oxidative stress can be enhanced by gene inactivation of the antioxidant enzyme glutathione peroxidase-1 (Gpx1) in the mutant mouse. It is normally highly expressed in the mouse liver. The presence of the mutated Gpx1-gene causes a significant increase in hydrogen peroxide release by the liver mitochondria. Moreover, it markedly degrades the efficiency of mitochondrial oxidative phosphorylation as evidenced by a significant reduction in the respiratory control ratio [41].

Mitochondrial Dysfunction in Major Diseases

Cardiovascular diseases. 1. Early suggestions of OXPHOS involvement in atherogenesis. Atherosclerosis is the major cause of ischemic heart disease. From the 1960s, Whereat emphasized that abnormalities of mitochondrial oxidative phosphorylation might play an essential role in atherogenesis [42,43]. He suggested that anoxia or carbon monoxide or "other mitochondrial electron transport chain inhibitors that contaminate our hazardous environment" could initiate atherosclerosis [44].

Moreover, hypoxia and mitochondrial dysfunction might help explain the cause of lipid accumulation in the atheromatous plaque. For instance, local arterial hypoxia might increase endothelial permeability. This would in part explain the lipid insudation theory that increased permeability promotes mural insudation of plasma lipid. Experiments show that reduced oxygen tension or the presence of carbon monoxide accelerates the development of atherosclerosis in the rabbit fed a high fat diet. In humans, the increase in blood carbon monoxide in tobacco smokers might be the link between tobacco smoke and atherosclerosis [44].

As an alternative to this insudation hypothesis it has been postulated that hypoxia enhances mural lipid accumulation in atherosclerosis through fatty acid synthesis by mitochondria in the aorta. Whereas normally fatty acid synthesis in the aorta is minimal, a block in the respiratory chain, for instance by hypoxia, impairs the oxidation of NADH and causes NADH accumulation that initiates mitochondrial fatty acid synthesis [44,45].

Involvement of defective oxidative phosphorylation in atherogenesis is supported by experiments showing lack of mitochondrial ATPase and succinate dehydrogenase activity in cultured aortic vacuolated smooth muscle cells from the atherosclerosis-susceptible pigeon [46]. Furthermore, there is evidence that reduced intimal mitochondrial energy generation at lesion sites contributes to lesion formation in susceptible compared with the resistant pigeon [45].

2. Inflammation and mitochondrial dysfunction. *(a) Cyclooxygenase induction*. Recent experimental evidence, particularly using gene knockout animal models, not only corroborates the idea that dysfunction of oxidative phosphorylation plays an important part in atherogenesis but also points to a close association between inflammatory components in the atherosclerotic lesion and mitochondrial dysfunction. In an inflammatory environment oxidized lipids are generated by the activity of lipoxygenases and cyclooxygenases. Mitochondria release radical oxygen species that deplete the cellular content of reduced glutathione. Oxidized fatty acids and oxidized forms of cholesterol accumulate. These alterations are associated with apoptosis and necrosis as seen in the necrotic core of the advanced atherosclerotic lesion [47].

The expression of cyclooxygenase-2 (COX-2) and other pro-inflammatory mediators has been demonstrated in macrophages, smooth muscle cells, and endothelial cells of small vessels in the atherosclerotic plaque [48]. Others described finding an immunoreactive 70-kDa COX-1 protein and a smaller, 50-kDa COX-2 protein. The COX-2 isoform was located together with macrophages primarily at the perimeter of the lipid core and the shoulder area of the atheroma and secondarily in the microvascular endothelium of the lesion. By comparison, the normal artery only expresses the constitutive COX-1 isoform [49].

(b) Prostaglandin synthesis. COX-2 converts arachidonic acid to prostaglandin-E2 (PGE2), which induces interleukin-6 (IL-6) [50]. PGE2 inhibits cholesterol esterification in vitro, and might do the same in the arterial wall [51]. PGE2 extracted from lesions of rabbits rendered atherosclerotic by supplementing their diet with 1% cholesterol inhibited cholesterol esterification in vitro [51]. The levels of PGE2 and IL-6 in atheromas vary greatly but are significantly different from normal mural levels. For instance, atherosclerotic aneurysms of the abdominal aorta of 13 patients contained on average 29 times more PGE2 and almost 8 times more IL-6, compared to 16 normal abdominal aortas [52].

IL-6 induces haptoglobin (Hp) and both contribute to inflammation and angiogenesis [50]. Haptoglobin binds hemoglobin [53] and serves as an important antioxidant. Its angiogenic activity was first demonstrated in 1993 [54]. Both the in vitro Matrigel model of angiogenesis and in vivo models showed that purified haptoglobin stimulated angiogenesis [54]. It was suggested that haptoglobin might be important for angiogenesis as part of tissue repair in chronic inflammatory conditions [54]. A marked increase in the haptoglobin was detected in protein extracts from human aortic fibro-fatty lesions but not in aortic intima from patients without atherosclerosis [55]. Haptoglobin enhances cholesterol crystallization in the bile [56], but a similar mechanism has not yet been demonstrated for the cholesterol crystal formation in the atherosclerotic lesion.

(c) The need for cholic acid in atherogenic diets. High fat diets that are commonly used to induce atherosclerotic lesions in animal models of atherogenesis contain cholic acid [57]. It induces nitric oxide (NO) synthesis in endothelial cells and reduces apoA-I [58]. Dihydrocholic acid induces COX-2 [59]. Whereas high-fat, high-cholesterol diets without cholic acid resemble human diets more closely and elevate LDL and HDL, they do not induce atherosclerosis in animals that are not genetically prone to develop atherosclerosis. Addition of cholic acid elevates LDL but lowers HDL and overcomes the resistance to the development of atherosclerosis. Findings obtained in the mouse model of diet-induced atherosclerosis partly explain the reduction in the plasma levels of HDL. The addition of cholic acid to the diet induces the expression of apoA-I regulatory protein-1 and decreases the expression of apoA-I [60].

(d) Inhibition of electron transport by interferon-gamma. $CD4^+$ and $CD8^+$ T-cells in the atherosclerotic lesion of the apoE null mouse secrete interferon-gamma (INF-γ). Targeted disruption of the IFN-γ receptor in such mice reduces lesion size, cellularity, and lipid content and indicates that IFN-γ contributes to lesion formation [61]. In vitro discoveries corroborate these observations. IFN-γ induced the inducible nitric oxide

(NO) synthase (iNOS) of the endotoxin-activated J-774 macrophage cell. The NO inhibited the mitochondrial respiration of coincubated L-929 fibroblasts [62]. It competed with oxygen at Complex IV of cultured L-929 fibroblasts and reversibly inhibited Complex IV. Others have shown that Trolox, a lipid-soluble vitamin E analogue, but not ascorbate, can protect against NO-induced damage of cytochrome oxidase, suggesting that the damage is mediated through lipid peroxidation [63] (Figure 2). Gene knockout and in vitro studies indicate that the nitric oxide secreting, activated macrophage in the atherosclerotic lesion might very likely inhibit respiration, not only of its own mitochondria, but also of mitochondria in other lesion cells, such as the smooth muscle cell [62].

A direct molecular connection between inflammation and Complex I deficiency has also been proposed based upon the study of inflammatory myopathies. A complete antisense homology was detected between the 5'UTR of the mRNA for the INF-γ-inducible precursor protein (IP-30) and the 3'UTR of the NDUFV1-mRNA that codes for a 51 kDa nuclear subunit of Complex I [64]. However, while plausible, it is presently not known whether this direct INF-γ-link to a deficiency of mitochondrial oxidative phosphorylation operates in the atherosclerotic lesion as well.

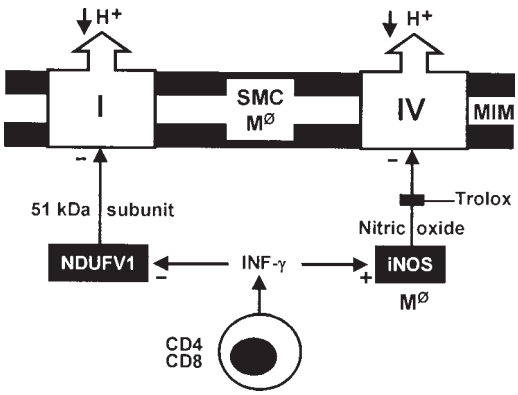

Fig. 2. Links between inflammation and inhibition of electron transport and proton-pumping Complexes I and IV in atherogenesis. The illustration is based upon in vitro findings that interferon-gamma (INF-γ) released by CD4 and CD8 cells induces the inducible nitric oxide synthase (iNOS) in the activated macrophage (MØ]. Nitric oxide inhibits Complex IV. Secondly, INF-γ inhibits expression of the nuclear gene NDUFV1 that codes for a subunit of Complex I. MIM: mitochondrial inner membrane; SMC: smooth muscle cell.

3. Infectious agents. *Chlamydia pneumoniae* infection can damage mitochondria; they become swollen and their cristae become fragmented [65]. *Chlamidia,* other bacteria and several types of viruses have repeatedly been detected in the human atherosclerotic lesion, suggesting that some infectious agents initiate atherosclerosis [66]. Moreover, infection with cytomegalovirus (CMV) is significantly associated with coronary heart disease, particularly in patients with diabetes [67]. And in the New Zealand White rabbit fed a 2% atherogenic diet the intravenous inoculation with the Herpesvirus type-4 (BHV-4) accelerated the development of atherosclerotic lesions [68]. However, experiments with germ-free apoE(-/-) mice showed that a bacterial or viral infection is not a sine qua non for the development of atherosclerosis [69].

4. Observations in gene knockout and transgenic mice. *(a) The apoE knockout mouse*. The apoE(-/-) mouse develops massive atherosclerosis on a normal diet. Surprisingly, the presence of just a small amount of systemic apoE, less than 2% of the wild type, prevents lesion development. When transgenic apoE-knockout mice that had been engineered to express apoE strictly in the adrenal gland were fed an atherogenic diet, it was found that such low levels of systemic apoE blocked atherosclerotic lesion development in the aorta even in the presence of hypercholesterolemia [70]. Four weeks after bone marrow transplantation to generate macrophages expressing various forms of apoE in the murine apoE null mouse, the macrophages expressing murine apoE significantly reduced the size of atherosclerotic lesions and lowered the serum cholesterol level. However, the macrophages expressing human apoE3-Leiden or apoE2 did not curtail lesion size [71].

Infection with the murine gamma-herpesvirus-68 (MHV-68) accelerated atheroma formation in infected apoE(-/-) mice compared with control mice. [72]. By contrast, addition of oral Q10 to a high-fat diet given to uninfected apoE null mice significantly reduced the size of atherosclerotic lesions in the aorta [73].

(b) The LDL-receptor knockout mouse. The LDL-receptor knockout mouse, LDLR(-/-), develops atherosclerotic lesions of the thoracic and abdominal aorta after 12 weeks on a diet not supplemented with cholic acid. Two groups of mice were fed either a high-fat diet or a high-fat diet supplemented with sodium cholate. Similar lesions developed in both groups demonstrating that the receptor defect overcomes the resistance to develop atherosclerosis on a high-fat diet not supplemented with cholic acid [74].

Overexpression of LPL can protect against atherosclerosis in the LDL-receptor (LDLR) deficient mouse. At the end of an 8-week atherogenic diet the LDLR(-/-) mouse with transgenic overexpression of human LPL had 18-fold smaller lesion areas, compared with the LDLR(-/-) mouse not possessing the LPL transgene. It was suggested that the LPL-induced reduction in plasma levels of remnant lipoproteins caused the reduction in lesion area in the mice that overexpressed LPL [75].

In ovariectomized LDLR(-/-) mice, aortic lesion size was reduced by physiologic amounts of exogenous 17β-estradiol. The effect was independent of alterations of the cholesterol concentration in the plasma [75].

(c) Lipoprotein lipase overexpression. Observations in the muscle-specific LPL transgenic mouse suggest that the lipoprotein lipase activity might be a rate-limiting step in the triglyceride-derived supply of free fatty acids to mitochondria and that LPL expression participates in the regulation of the biogenesis of mitochondria. The overexpression of LPL in cardiac and skeletal muscle increased the uptake of free fatty acids and induced muscle fiber degeneration, glycogen storage, and marked mitochondrial proliferation [77].

5. Defective fuel supply. *(a) Defect in the cellular uptake of fuel*. As noted above, the apoE knockout mouse develops severe atherosclerosis. LDL from aortic atherosclerotic lesions (A-LDL) is chemically modified and is not taken up as much as normal LDL by rabbit aortic smooth muscle cells (SMC) in vitro. Both lipoproteins down-regulate the cell surface LDL receptor and both failed to induce cellular cholesterol-ester accumulation in the cell [78]. However, incubation of human arterial SMC with human chylomicron remnants increases cell cholesterol and suppresses LDL-receptor activity [79].

The LDL-receptor down-regulation can also be bypassed in vitro by covalent linkage of N,N-dimethyl-1,3-propanediamine (DMPA) to LDL, leading to vast uptake and extensive accumulation of intracellular lipid inclusions containing liquid crystals of cholesteryl esters. These in vitro features resemble the alterations that are found in smooth muscle cells in vivo during atherogenesis [80]. HIPDM (N-N-N'-trimethyl-N'-(2-hydroxy-3-methyl-5-iodobenzyl)-1,3 propanediamine) is structurally similar to DMPA. It accumulated preferentially in mitochondria when given intravenously to rabbits, and its distribution was similar to succinate cytochrome c reductase. It remained in mitochondria for at least five hours after injection [81].

(b) Intracellular defect in fuel transport and delivery to mitochondria. A study of human atherosclerotic plaques material removed percutaneously showed many smooth muscle cells, mainly of the intermediate phenotype, having abundant perinuclear fat and glycogen deposits and many mitochondria [36]. The increase in the number of mitochondria in the atherosclerotic lesion indicates enhanced synthesis in an attempt to compensate for dysfunction of the mitochondrial bioenergetics system. The perinuclear lipid accumulation is similar to that in Reye's syndrome. There is evidence suggesting that the mitochondrial permeability transition (MPT) is involved in mitochondrial injury of the liver in this rare disorder. It is strongly associated with viral infection and concomitant aspirin ingestion and appears primarily [82] but not always [83] in childhood.

6. Defective oxidative phosphorylation in atherogenesis. *(a) Inherited structural defects*. Direct evidence of a deficiency of respiratory enzymes in atherogenesis was provided by two cases of metabolic disease with premature atherosclerosis considered of mitochondrial origin. The premature atherosclerosis was diagnosed in two brothers whose parents and two sisters were free of symptoms. The brothers, who died prematurely in their third decade of life, also suffered from diabetes mellitus, sensorineural deafness, photomyoclonic epilepsy, and progressive renal failure. The mitochondrial abnormalities consisted of partial deficiencies of Complexes III and IV. The enzyme defect could be ascertained in the kidney and in fibroblasts, but not in muscle. Whether the mutation was located in the mtDNA or the nDNA coding for

mitochondrial enzyme subunits was not resolved [84].

(b) Acquired structural defects. Somatic mtDNA damage accumulates with age, degrades oxidative phosphorylation and accelerates aging. In the normal human heart the 'common' 4,977 nucleotide pair (nt) deletion (mtDNA4,977) and the mtDNA7,436 and mtDNA10,422 deletions accumulate with age above 40. In atherosclerosis of the coronary arteries this somatic damage is significantly accelerated. In human hearts with coronary artery disease (CHD), the 'common' deletion is up to over 200 times more frequent than in age-matched control hearts free of CHD [85].

The 'common' deletion abolishes the mitochondrial synthesis of subunits for Complexes I, IV, and V. Significantly higher levels of this deletion were detected by the polymerase chain reaction (PCR) in subjects aged 73-95 compared with subjects aged 60-72 years old in mtDNA extracted from smooth muscle cells of aortic atherosclerotic lesions from 18 surgical and 9 autopsy cases. These findings support the theory that defective oxidative phosphorylation contributes to the degenerative cell phenotype and atherosclerosis during aging [86].

(c) Reperfusion injury. Cardiac mitochondria isolated after reperfusion are structurally abnormal, carry large amounts of ionized calcium, and generate a disproportionate quantity of oxygen free radicals that cause deletions of mtDNA. The oxidative phosphorylation is irreversibly damaged [87]. Myocardial recovery after ischemia and reperfusion of isolated perfused hearts from rats was not improved by prior Q10 supplementation and myocardial Q10 content was unaffected [88].

In the dog model of reperfusion, the PGE2 level in vivo in the great cardiac vein and ex vivo in heart mitochondria changed significantly after 20 minutes of left artery occlusion. Pretreatment with indomethacin inhibited the PGE2 increase [89] suggesting cyclooxygenase-2 induction or activation by the reperfusion. When isolated mitochondria were exposed to PGE2 in the presence of ionized calcium in vitro, proton conductivity increased, leading to uncoupling, inhibition of respiration, and lowering of ATP synthesis [90].

(d) Inhibition of the electron transport chain results in intracellular lipid accumulation. The essential role of Q10 in the electron transport system has been demonstrated using chloroquine, a Q10 analog. Chloroquine reversibly inhibits electron transport and cell growth [91]. In an in vitro model for lipid accumulation in atherosclerosis, smooth muscle cells from pig aortas were incubated with LDL, chloroquine, or both. LDL supplemented cells showed normal morphology save for a few cells containing large lipid droplets. By comparison, chloroquine was toxic to the cells, which developed large autophagic vacuoles. LDL and chloroquine together induced autophagic vacuoles, large lipid droplets, and a marked increase in esterified cholesterol [92]. Treating the remnant cells with chloroquine (30 mM) increased the content of cholesterol by 90% and cholesterol ester by 370% [79].

(e) Cellular regulation of intracellular cholesterol synthesis. It has been postulated that the protective effect of estrogen against heart disease is due to its tissue-specific regulation of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the main regulator of the isoprenoid metabolic pathway. It converts 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) to mevalonate. Its promoter region harbors a sequence element that differs from the consensus sequence of the estrogen-responsive element (ERE) by only one mismatch in each half of the palindrome. It is unresponsive to estrogen in the liver but responds to estrogen in other tissues that require enhanced cell proliferation [93]. Lipoprotein-deficient serum from women using oral contraceptives induced HMG-CoA reductase in the cultured human aortic smooth muscle cell [94]. ATP inhibits HMG-CoA reductase [95,96].

7. Speculations; filling gaps in present knowledge. This review of atherosclerosis analyzes and correlates present experimental evidence of defective oxidative phosphorylation in its etiology. A few suggestions are introduced here to fill the gaps in our understanding, as present experimental data are insufficient to establish a unified theory of the pathophysiology involved.

(a) Reduced binding of liposomes to mitochondria reduces fuel transfer. The apoE null mouse develops atherosclerotic lesions even when fed a diet free of cholic acid supplementation. The common explanation is that the lack of apoE inhibits removal of lipoproteins by the liver, leading to accumulation of atherogenic lipoproteins in the circulation. ApoE binds to the cellular surface low-density lipoprotein (LDL)

receptor, also known as the apo B,E(LDL) receptor, and the LDL-related protein (LRP). In addition, it binds to a 59-kDA intracellular apoE binding protein identified to contain the α- and β-subunits of F_1-ATPase. It was detected in the canine and human liver cell [97].

As an alternative explanation of intracellular lipid accumulation, I suggest that the intracellular lipid-transport system utilizes liposomes that bind via their surface apoE to associate with mitochondria. This idea is derived from the above reports of intracellular apoE-binding proteins that are imported into the mitochondrion. A lack of liposome binding via apoE to the mitochondrial protein in the apoE-null mouse would lead to a defect in the lipid fuel for mitochondrial energy conversion and to intracellular lipid accumulation. Similarly, in humans, such reduced binding by apoproteins like apoε4 might contribute not only to atherogenesis, but also to other diseases such as Alzheimer's disease.

(b) Lack of LPL. Furthermore, one might speculate that intracellular LPL induces increased release of fatty acids from mitochondrial-bound liposomes for oxidative phosphorylation and thus protects intimal cells and reduces the lesion areas in the LDL-receptor null mouse. A defect in LPL synthesis would therefore not only interfere with intravascular but also with intracellular lipolysis (Figure 3).

(c) Defective oxidative phosphorylation. Reduced ATP production due to defective OXPHOS reduces inhibition of HMG-CoA-reductase activity, and increases synthesis of Q10 to enhance OXPHOS, but simultaneously increases cholesterol synthesis.

The gene for COX-2 is located at chromosome region 1q25, the same region as the gene for cytosolic phospholipase (cPLA2), and it has been suggested that the two genes are co-regulated [98]. cPLA synthesizes arachidonic acid from membrane phospholipids. Arachidonic acid selectively inhibits Complexes I and III and significantly elevates mitochondrial hydrogen

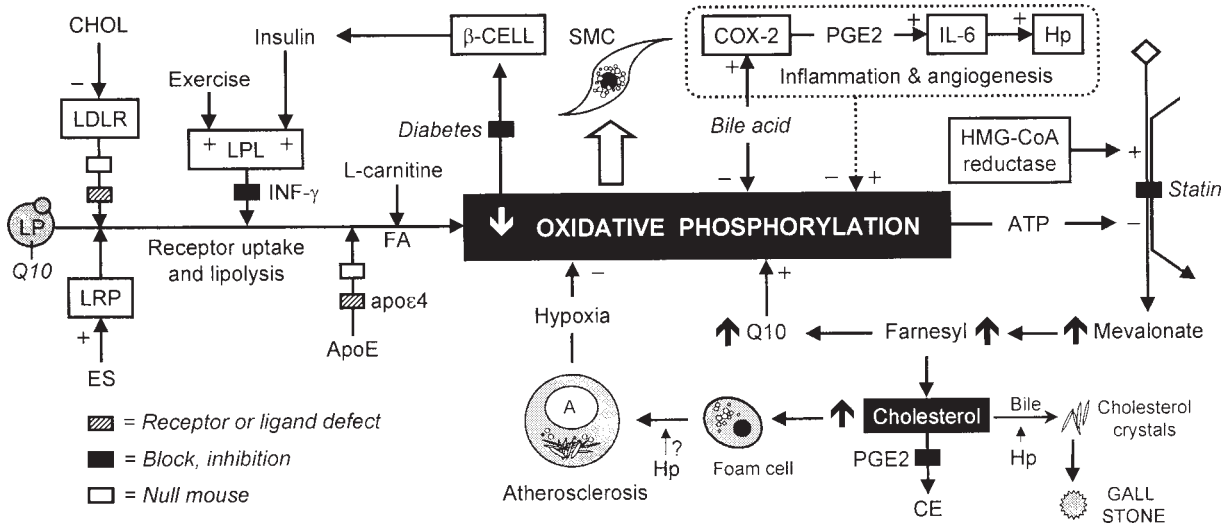

Fig. 3. Illustration showing evidence-based and hypothetical role of dysfunctional oxidative phosphorylation (OXPHOS) in atherogenesis. A combination of inflammation (COX-2 induction) and dysfunction of OXPHOS causes the atherosclerotic lesion. The latter induces HMG-CoA reductase and increases the synthesis of Q10 as well as cholesterol as they share the same pathway via mevalonate and farnesyl. Hypercholesterolemic diets used to induce experimental atherosclerosis contain bile acids, which inhibit OXPHOS and induce COX-2. Abbreviations: LP: lipoprotein; LDLR: low-density lipoprotein receptor; LRP: low-density lipoprotein receptor related protein; LPL: lipoprotein lipase; CHOL: cholesterol; FA: fatty acid; apoE: apolipoprotein E; SMC: smooth muscle cell; A: atherosclerotic artery, cross section view; COX-2: cyclooxygenase-2; PGE2: prostaglandin E2; IL-6: interleukin-6; Hp: haptoglobin; CoQ10: coenzyme Q10 (ubiquinone), CE: cholesteryl ester. For further explanation see text.

peroxide production. Unsaturated acid inhibits more than saturated acid [99]. These findings suggest another link between inflammation and OXPHOS.

The co-localization of HIPDM with succinate cytochrome c reductase suggests that the enzyme might be inhibited by HIPDM, causing accumulation of intracellular lipids, reduced ATP synthesis, less inhibition of HMG-CoA reductase, increased Q10 synthesis and simultaneous increase in cellular cholesterol synthesis.

(d) Combined defects. The detrimental effects of reduced fuel supply and inhibition of oxidative phosphorylation on cellular energy metabolism is demonstrated by Reye's syndrome. Aspirin has been shown to inhibit β-oxidation and thus the fuel supply, and certain viruses induce the MPT. Additional damage could be induced by salicylate, a metabolite of aspirin. Applying the same reasoning to atherogenesis, it appears reasonable to assume that the inhibition of the availability of lipid fuel to mitochondria combined with drug- or virus-induced direct inhibition of oxidative phosphorylation would significantly reduce ATP generation and be highly detrimental for cell survival. For instance, as noted above, the Herpesvirus accelerates experimental atherosclerosis. A way for estrogen to protect intimal cells would be the increased synthesis of Q10 by stimulation of HMG-CoA-reductase. The findings that ovariectomized rabbits given estrogen do not develop atherosclerosis even in the presence of a high blood cholesterol level suggest that the atherogenic defect is mural, and that local cellular regulation is most important in atherogenesis. This is also supported by the results showing that when Q10 is blocked by chloroquine, the low ATP level induces an increase in receptor uptake.

8. Cardiomyopathy. Several mtDNA mutations and reduced respiratory enzymes activities have been associated with hypertrophic cardiomyopathy. For instance, deficiencies in respiratory chain enzyme activities were detected in half of a series of 32 endocardial biopsies. The activity of either Complex I, IV, or both, was significantly reduced relative to the combined activities of Complexes II and III [100].

Diabetes. Mitochondrial DNA with large deletions has been detected in patients with diabetes associated with deafness [101]. Diabetes causes impairment of glucose uptake into cells. It is an independent risk factor for atherosclerosis [102]. The pancreatic β-cell depleted of mtDNA does not secrete insulin [103]. Beta cells with mtDNA mutations show a defective mitochondrial inner membrane potential and induce diabetes. Insulin resistance is closely related to the regulation by uncoupling proteins and other energy regulators [103]. The high-glucose environment induces glycation of proteins. The concurrent ROS-generation induces apoptosis in vascular cells involved in complications of diabetes mellitus [103].

Maternally inherited diabetes and deafness (MIDD) is a rare disease found only in 1% to 2% of individuals with diabetes. Enzyme activities of less than five percent of the tolerance levels of Complexes I, I+III, and IV have been detected in skeletal muscle biopsies. Modification of diet and increased exercise has provided only temporary improvement [104].

In the rat model of diabetes induced by streptozotocin (STZ) the mitochondrial oxidative phosphorylation is significantly reduced. However, the ATP generation can be completely restored by physical training even if the plasma glucose or insulin levels remain essentially unaltered [105]. Others have reported that endurance exercise training increased oxidative capacity of the muscle and doubled the number of mitochondria in rat muscle [106]. Reduced oxygen consumption per mitochondrion might lead to less oxygen radical generation per mitochondrion with less damage to individual mitochondria [107].

IL-1β is an acknowledged mediator of dysfunction of the pancreatic β-cell in Type I diabetes mellitus. Differential-mRNA display reveals that IL-1β induces ANT1 expression in cultured, purified rat β-cells [108], suggesting an altered ANT isoform expression.

There is in vitro evidence that sulphonylureas might impair mitochondrial function. For instance, tolbutamide can depolarize the mitochondrial potential of the cultured pancreatic β-cell [109].

Gastrointestinal disease. 1. The "topical" effect in ulcer formation. *(a) Nonsteroidal anti-inflammatory drugs*: Local inference with mucosal mitochondrial oxidative phosphorylation by nonsteroidal anti-inflammatory drugs (NSAIDs) is pivotal in the development of gastrointestinal ulcers in patients treated with them.

Indeed it has been postulated that this "topical" phase is a sine qua non of NSAID-induced gastrointestinal damage [110].

Like bile acids, nonsteroidal anti-inflammatory drugs can inhibit oxidative phosphorylation [111]. Lipophilic NSAIDs are readily transported through the cell wall and are enriched within the mitochondrion [112]. Whereas the anti-inflammatory effects of NSAIDs are due to inhibition of COX-2, the concurrent inhibition of COX-1 by non-selective NSAIDs and their interference with mitochondrial oxidative phosphorylation can cause serious gastrointestinal side effects. NSAIDs such as indomethacin are very effective analgesic and antiphlogistic agents and are widely used, resulting in a large number of patients that experience side effects of the treatment. For instance, it has been estimated that NSAID-induced hemorrhage and ulcerations cause up to 20,000 deaths yearly in the United States [17].

Indomethacin uncouples oxidative phosphorylation at micromolar concentrations and inhibits respiration at higher concentration in vitro [113]. Oral administration of indomethacin in the rat significantly lowered ex vivo jejunal ATP and α-tocopherol levels [114]. Electron microscopic examination of the mucosa revealed that it caused dose-dependent mitochondrial changes comparable to those inducible in vivo by the classical mitochondrial uncoupler dinitrophenol [113]. Other NSAIDs such as nimesulide, meloxicam, and piroxicam also uncouple mitochondria and stimulate respiration in vitro [115]. They stimulate basal and uncoupled respiration in mitochondria incubated in the presence of either glutamate plus malate or succinate [115]. Diclofenac, but not naproxen, also slightly inhibits ATPase activity. Furthermore, nimesulide and diclofenac, but not naproxen, reduce ATP synthesis by blocking the activity of the adenine nucleotide translocase [115].

Diphenylamine is a shared structure of several NSAIDs that are able to uncouple mitochondrial oxidative phosphorylation. For instance, diphenylamine, mefenamic acid, and diclofenac produce mitochondrial swelling of rat liver mitochondria obtained from freshly isolated hepatocytes and decrease cellular ATP content, mainly through uncoupling of the mitochondrial oxidative phosphorylation [116]. An important finding was that fructose, an effective glycolytic substrate, provided partial protection against the ATP depletion and cell injury induced by these compounds [117].

Direct evidence that uncoupling of oxidative phosphorylation is important in the pathogenesis of NSAID-induced gastrointestinal side effects was obtained from studies of intestinal damage in rats caused by parenteral administration of either aspirin, the uncoupling agent 2,4-dinitrophenol, or indomethacin. Aspirin given orally inhibited intestinal mucosal cyclooxygenase without causing a topical effect: it increased mucosal permeability and reduced mucosal prostanoid levels but did not alter mitochondrial morphology. In these experiments, aspirin caused neither significant inflammation nor ulcer formation. However, the mitochondrial uncoupler 2,4-dinitrophenol increased intestinal permeability, but had no effect on intestinal prostanoid levels. In contrast, the two agents administered together induced alterations similar to those induced by oral indomethacin administration such as altered mitochondrial morphology, increased permeability, decreased prostanoid levels and the formation of intestinal ulcers [118].

Five hours after application directly to the human gastric mucosa, aspirin induces dilatation of mitochondria and the endoplasmic reticulum, rupturing of apical membranes, intercellular edema, and widening of capillary fenestrae [119]. These findings resulted from a controlled clinical trial involving 5 healthy volunteers as probands and 5 other healthy subjects as controls. All volunteers were free of *Helicobacter pylori* (*H. pylori*) infection (a significant point, vide infra). It has also been suggested that aspirin at relative concentrations similar to human antipyretic and anti-inflammatory pharmacological doses can uncouple oxidative phosphorylation of rat mitochondria in vivo and in vitro [120]. However, these in vivo experiments might not have differentiated between the effect of aspirin and its salicylate metabolite.

Importantly, even if an agent itself does not affect oxidative phosphorylation, one of its metabolites might do so. As an example, salicylate, a main metabolite of aspirin, but not aspirin itself, uncouples oxidative phosphorylation. A close correlation between the uncoupling activity of salicylate and its congeners and

their anti-inflammatory potency had been noted almost forty years ago [112]. Acetylsalicylic acid in the low millimolar range both uncoupled and inhibited oxidative phosphorylation of rat renal cortex mitochondria in vitro; in comparison, the NSAID dipyrone only uncoupled OXPHOS [121]. Other investigators reported that salicylate induced the mitochondrial permeability transition (MPT), depleted the mitochondrial inner membrane potential and uncoupled oxidative phosphorylation [27].

Different NSAIDs differ in their effect on mitochondria, and their effects on mitochondria may be concentration dependent. A number of NSAIDs and acidic prodrugs stimulate mitochondrial respiration in vitro and uncouple mitochondria. Modification or removal of the ionizable group can eliminate the adverse effect on mitochondria. For instance, dimeroflurbiprofen, a modified flurbiprofen, non-acidic prodrugs such as nabumetone, and non-acidic selective COX-2 inhibitors do not cause in vitro uncoupling [122].

(b) Helicobacter pylori. NSAIDs that do not induce the "topical" phase of gastrointestinal damage, such as the selective COX-2 inhibitors celecoxib and rofecoxib, cause fewer gastric ulcers than indomethacin in animal experiments and are better tolerated in clinical trials [123]. However, infection with the gastrophilic Gram-negative *H. pylori* might increase the risk of ulcer formation in patients receiving such NSAID therapy because the vacuolating cytotoxin (VacA) released by the bacterium might induce the "topical" effect. Support for this assumption has evolved from in vitro observations: vacuolating cytotoxin prepared from *H. pylori* decreases ATP levels and increases the number of cultured gastric cells in the G_0/G_1 phase. The cytotoxin decreases the potential $\Delta\Psi_m$ across the mitochondrial inner membranes of the cultured gastric AZ-521 epithelial cell [31].

(c) Non-acidic and COX-2 selective NSAIDs. One might presume that non-acidic NSAIDs such as nabumetone and NSAIDs that have no enterohepatic circulation do not induce a 'topical" phase. Nevertheless, it has been reported that nabumetone can inhibit mitochondrial respiration. It limited the mitochondrial inner membrane potential and reduced ATP synthesis via specific inhibition of Complex I of the respiratory chain [115]. Nabumetone reduced ATP synthesis via specific inhibition of Complex I of the respiratory chain of cultured cells, and in the whole heart nabumetone reduced the oxygen uptake [115, 113]. Moreover, cultured cells and the whole heart exposed to nabumetone showed a reduction in oxygen uptake indicating in vivo inhibition of the respiratory chain [115]. Nonetheless, clinical studies indicate that the COX-2 selective inhibitor celecoxib, which does not affect oxidative phosphorylation, and nabumetone are somewhat similar in their rate of ulcer induction. Both drugs show about a 4-fold reduction in ulcer complications vs comparator NSAIDs. [124,125].

(d) The effect of DMSO. In many comparison animal studies of the gastrointestinal effects of various NSAIDs, eg indomethacin versus celecoxib, the drugs are diluted in dimethyl sulphoxide (DMSO). When DMSO is used as a vehicle for suspension of lipophilic NSAIDs in animal or clinical studies it might alter the effect of the drugs on oxidative phosphorylation. An important, sometimes overlooked, variable in studies of NSAIDs is the effect of DMSO itself. As an example, isolated rat kidney mitochondria exposed in vitro to cyclosporin A with and without DMSO were minimally affected. However, when exposed to DMSO alone there was an increase of about 20% in spontaneous ATP generation [126]. The NSAID imidazole causes hyperpolarization of the mitochondrial membrane in cultured mouse erythroleukemia cells exposed to DMSO as opposed to membrane depolarization observed with DMSO alone [127]. In addition, the vehicle can affect different drugs differently [128]. It has been suggested that DMSO itself may be an anti-inflammatory agent [129]. DMS, a known metabolite of DMSO, but not DMSO, produces mitochondrial uncoupling in vitro [130]. It might explain the observation that DMSO administration to rats induces structural alterations in mitochondrial membranes leading to enhancement of cytochrome oxidase activity in liver mitochondria [131].

2. Restoration of apoptosis in neoplasia. The early observation that lipophilic NSAIDs are readily transported through the cell wall and are enriched within the mitochondrion [112] has attracted much recent attention. This has happened because acidic NSAIDs can initiate apoptosis in many tumors, for instance colorectal neoplasms. The fact that some NSAIDs

exhibit significant anti-tumor effects was first detected in epidemiological studies and later verified in animal studies and in clinical trials. A major part of this effect is due to the NSAID-induced inhibition of cyclo-oxygenase-2 (COX-2) in the tumor, and selective COX-2 inhibitors have been shown to prevent or inhibit the growth of certain tumors [123]. In comparison, a significant part of tumor growth inhibition by non-selective, acidic NSAIDs is due to the NSAID-induced depolarization of the mitochondrial inner membrane potential that brings about the MPT and restores apoptosis [50].

Ethanol-induced disease. Ethanol depresses the oxidative phosphorylation of hepatic mitochondria from rats fed ethanol chronically [132]. Such mitochondria display a reduced capacity to incorporate ^{35}S-methionine into mitochondrial polypeptide gene products in vitro. The ethanol exposure reduces the steady-state concentration of every mitochondrial gene product. However, below a threshold, ethanol enhances mitochondrial DNA replication, for instance in the ethanol-treated embryo [133].

The rat liver mitochondrion exposed to ethanol in vitro exhibits malfunction of the transporter of glutathione (GSH). As a result, the mitochondrial matrix is depleted of GSH that normally metabolizes hydrogen peroxide. The mitochondrion becomes more susceptible to alcohol-induced oxidative stress. However, the effect can be prevented by supplementation with S-adenosyl-L-methionine [134].

Alcoholics may show abnormalities of mitochondria even after cessation of ethanol intake [135]. The investigation of oxidative phosphorylation of mice that were fed ethanol might suggest an explanation. The studies revealed that the GSH levels were reduced and the extent of oxidation of mitochondria DNA was significantly increased. These alterations combined with the lack of an effective mtDNA repair system might result in permanent damage to the mitochondria of alcoholic subjects [135].

Mitochondria from rats fed ethanol chronically show a reduction of total phospholipids (except for cardiolipin) in the inner membrane, alteration in the cytochrome content, and a decline of the ability to produce ATP [136]. It was also demonstrated that chronic ethanol consumption caused a marked decrease in ATPase activity in the rat liver mitochondrion [137]. In addition, it has been found that chronic ingestion of ethanol significantly reduced the concentration of two mtDNA-encoded polypeptides, subunits 8 and 6, of the F_0F_1-ATPase in the rat liver. In contrast, ethanol consumption had neither an effect on the nuclear encoded subunits of F_0F_1-ATPase nor on the nuclear-encoded adenine nucleotide transporter [138].

It has been suggested that a myocardial metabolite of ethanol, fatty acid ethyl ester (FAEE), might be a link between chronic ethanol abuse and myocardial dysfunction. In the rabbit, FAEE binds to myocardial mitochondria in vivo and in vitro and can be hydrolyzed to fatty acid, an uncoupler of oxidative phosphorylation [139].

Ethanol at a concentration of 1 mM and 10 mM lightly reduces cell viability of cultured rat hepatocytes and increases the combined ROS generation at Complexes I and III about 70% and 150% respectively [140]. Whereas ethanol consumption significantly increases ROS generation by the rat hepatocytes in such in vitro studies, the loss of hepatocyte viability is mostly correlated with a decrease in the cellular ATP level [141]. Moreover, the amount of dietary fat (high-fat or low-fat diet) has no significant effect on these ethanol-induced alterations [141]. Remarkably, supplementation with fructose [140] or pretreatment with the alcohol dehydrogenase inhibitor 4-methylpyrazole (4-MP) [142] prevented in vitro ethanol-induced cytotoxicity. In a rat model of the fetal alcohol syndrome, ethanol exposure in utero reduces perinatal activities of Complexes II and IV and depresses respiration of mitochondria from fetal hearts [143].

Neurological disorders. 1. Primary and secondary defects of OXPHOS. Recent evidence suggests that defective oxidative phosphorylation contributes to neurodegenerative disorders. Primary defects in the electron transport chain have been detected in Alzheimer's disease (AD) and Parkinson's disease (PD). In comparison, in Friedreich's ataxia and Huntington's disease, mutations of the nuclear DNA, probably induced by free radicals, result in low levels of aconitase that cause secondary defects in oxidative phosphorylation. Lack of ATP that lowers the threshold to undergo apoptosis may be a central mechanism in the pathogenesis of these neurodegenerative diseases [144].

2. Alzheimer's disease. *(a) The effect of aging and oxidative damage*. Until recently the association of any mitochondrial defect with the etiology and pathogenesis of Alzheimer's disease had been doubtful [145]. However, a few reports suggest that oxidative stress and mitochondrial dysfunction play a significant role in the pathogenesis [146]. It is probable that the age-related impairment of mitochondrial function induced by oxygen reactive metabolites might predispose or cause diseases such as Alzheimer's disease [147]. Importantly, it has been demonstrated that brains from AD-patients show more nicking and fragmentation of both the nuclear and mitochondrial DNA. In addition, the amount of mtDNA was reduced and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-OH-dG) was detected by immunostaining indicating oxidative damage. These alterations might predispose to further neuronal damage by exposure to other endogenous or environmental factors [147].

(b) The effect of apoE4. The role of mitochondrial dysfunction might depend on the presence of apoE4 [148]. The apoε4 allele is associated with increased H_2O_2-induced lipid peroxidation in the frontal cortex of patients with Alzheimer's disease [148]. Clinically, the presence of the ε4 allele of the apoE gene increases the risk of dementia on average 5-fold and is independent of its effect on plasma lipids and atherogenesis [149].

In AD patients who carried the ε4 allele the clinical dementia rating (CDR) score correlated with diminished activity of the mitochondrial enzyme α-ketoglutarate dehydrogenase. By contrast, in AD patients without the ε4 allele the CDR correlated significantly better with the densities of neuritic plaques and tangles [150]. Reduced levels of pyruvate dehydrogenase and cytochrome oxidase have also been detected in AD brain tissue [146].

(c) Amyloid precursor protein. Transfection of the gene for amyloid precursor protein (βAPP) into cultured normal human muscle fibers caused overexpression of the protein and induced structural abnormalities of mitochondria and reduced cytochrome-c oxidase activity. Abnormal accumulation of βAPP and similar mitochondrial abnormalities with reduced activity of this enzyme complex have been observed in the brain of patients with Alzheimer's disease and in patients with inclusion-body myositis suggesting that βAPP is an important factor in the pathogenesis of the two diseases [151].

3. Parkinson's disease. Mitochondrial DNA with large 5kb deletions, eliminating genes encoding protein subunits essential for mitochondrial ATP synthesis, have been found in the striatum of patients with Parkinson's disease. However, such deletions were also found in aged subjects without the disease [152]. It has been suggested that the significant increase of Complex IV defects found in neurons of the substantia nigra are most likely due to accelerated aging [153]. Reduced activity of Complex I has been implicated in both idiopathic Parkinson's disease and Parkinsonism induced by the mitochondrial permeability transition [154]. In vitro, the apoptosis-inducing neurotoxin N-methyl-4-phenylpyridinium (MPP^+) inhibits Complex I, depletes ATP, and induces release of cytochrome c by opening the membrane pores [155].

Reduced availability of coenzyme Q10 may also play a role in the etiology of Parkinson's disease. In the mouse model, oral therapy with Q10 protected against the detrimental effects of PMTP on the dopaminergic system of the substantia nigra [156].

Neoplasia. There is evidence that somatic mutations of the mitochondrial genome may be involved in the etiology of cancer and that aggregation of non-somatic mutants may play a role in their progression. An analysis using 2-dimensional gene scanning of mtDNA from 21 papillary carcinomas of the thyroid gland revealed 3 different somatic mutations in 5 of the tumors, which mainly affected genes encoding enzyme Complex I of the respiratory chain [157].

A reduced activity of Complex IV and V, the latter due to a decrease of the F_1-β moiety, was detected in mitochondria from hepatocellular carcinoma [158]. The F_0 moiety of the F_0F_1-ATPase of the rat liver mitochondrion contains a distinct binding site for diethylstilbestrol (DES), and at low concentrations, it is a potent F_0F_1-ATPase inhibitor [159]. DES is associated with the development of clear cell adenocarcinoma of the vagina and cervix in women exposed to it in utero.

Neoplastic transformation can be associated with markedly altered expression of both the nuclear and mitochondrial DNA encoded oxidative phosphorylation genes [160]. For instance, an analysis of human

diploid fibroblasts and their SV 40-transformed counterparts revealed that the mtDNA number declined. However, the mRNA levels for the mtDNA-encoded 12 S rRNAs, ND2, ATPase6+8, COIII, ND5+6, and cytochrome b (CYTB) genes and the mRNAs for the nuclear-encoded ATP-synthase-β were increased. In addition, the adenine nucleotide translocator (ANT) isoform 1 and 2 genes were markedly induced. A different study found that the ATPase activity in mitochondrial particles from Zajdela hepatoma and Yoshida sarcoma was substantially lower than the control mitochondrial particles isolated from rat heart and rat liver [161]. Tumor mitochondrial particles contained 2 to 3 times more ATPase inhibitor than particles from control mitochondria.

A number of findings support a role for oxidative stress and defective oxidative phosphorylation in carcinogenesis. For instance, biochemical alterations in biopsy tissue from 59 patients with gastric cancer were compared with tumor-free, adjacent mucosa. All tumors exhibited a significant impairment of function as evidenced by reduction in tetrazolium dye staining and of the antioxidant enzyme superoxide dismutase (SOD), and glutathione S-transferase (GST) consistent with oxidative stress. Positive staining with *H. pylori* antibody significantly decreased the reduction in tetrazolium staining [162].

Gastric cancer is associated with defective mitochondrial function in the tumor and the adjacent tumor-free mucosa [162] and *H. pylori* infection might be involved in the pathogenesis of gastric cancer: 10% to 20% of infected cases develop ulcers and many develop atrophic gastritis [163].

Mitochondrial dysfunction of Complexes I and IV has been observed in HIV-1-negative children whose mothers had been administered a prophylactic combination of the nucleoside analogue zidovudine and lamivudine or zidovudine alone during pregnancy to prevent mother-to-child HIV-1 transmission [164]. It has been suggested that AZT-induced oxidative damage in nuclear DNA of fetal tissues might be the reason why it can be a perinatal carcinogen. For example, treatment of CD-1 Swiss pregnant mice and the pregnant patas monkey (*Erythrocebus patas*) with AZT induces a significant increase in 8-oxo-2'-deoxyguanosine (8-oxo-dG) in fetal tissues such as the liver and kidney [165,166].

It has been suggested that fragments of mitochondrial DNA released during mtDNA damage and incorporated into nDNA might cause cancer [167]. Recent experimental evidence supports this hypothesis: it was demonstrated that in the mouse and rat mitochondrial-DNA-like inserts were much more abundant in tumors than in normal tissue [168].

Molecular Pathology

Mitochondrial synthesis and turnover. 1. Nuclear DNA. The biosynthesis of mitochondria is controlled by the nucleus. Nuclear DNA codes for most mitochondrial proteins needed for the synthesis of mitochondria and encodes most of the estimated 1000 proteins required for proper OXPHOS function [169] (Figure 4). Remarkably, mitochondria in the ρ^0-cell lack mtDNA but the cell still synthesizes mitochondria in such cells, showing that nuclear DNA alone controls mitochondrial synthesis.

The nuclear genes that are phylogenetically of mitochondrial origin code for the many proteins that are imported into the mitochondria. For instance, the import of the F_1-β subunit of the F_1 moiety of F_0F_1-ATPase requires both an intact membrane potential and ATP [170]. Nuclear coded proteins are imported into mitochondria typically after proteolytic elimination of their leader peptides and require an intact mitochondrial membrane potential and ATP [171].

2. Regulation and coordination of mitochondrial biosynthesis. By stimulating mitochondrial biogenesis, human tissues seek to counteract defective oxidative phosphorylation, which might be caused by a mtDNA mutation [172]. The exact molecular mechanism that regulates the coordinated mitochondrial and nuclear gene expression for the complete synthesis of mitochondria is uncertain [173]. Some evidence suggests that the control region of the F_0F_1-ATPase genes coordinate the expression of the 2 genomes depending on the energy demands of the cells, especially in muscle [174].

Experiments using the transgenic fruit fly *Drosophila megaloblaster* have provided evidence that the ubiquitous DNA binding transcription factor Sp1 regulates the expression of nuclear genes involved in mitochondrial biogenesis [175]. In these experiments, promoter regions for the mitochondria transcription

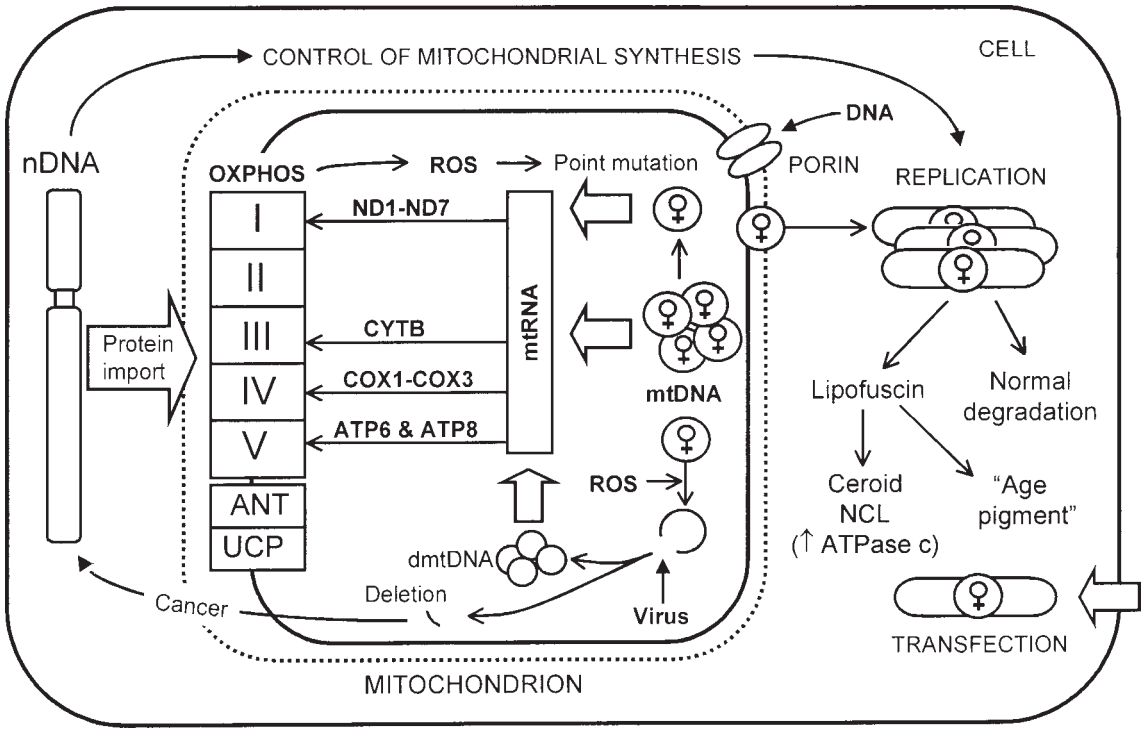

Fig. 4. The synthesis of mitochondria is controlled by the nucleus. An individual mitochondrion may have up to ten copies of mtDNA in its matrix compartment. Most significantly, the vital proton pumping subunits of the respiratory complexes (I, III, IV) are all coded by the mtDNA. In addition, mtDNA encodes the two proton-conductive subunits of complex V, the F_0F_1-ATPase. In contrast, the respiratory chain enzyme complex II is entirely coded by nDNA and does not pump protons across the inner mitochondrial membrane. The circular, bacterial-plasmid-like mtDNA is densely packed and lacks introns and an effective repair system. Located in the matrix, it is exposed to radical oxygen species (ROS) generated during mitochondrial respiration. ROS can cause point mutations in mtDNA leading to deletions and release of mtDNA fragments, some of which are incorporated into nDNA. It has been proposed that such fragments might cause cancer. Extra-mitochondrial DNA can enter mitochondria via porin, a possible entry point for introducing therapeutic DNA. Transfection of old or uncoupled mitochondria reduces cell viability in vitro. NCL = neuronal ceroid lipofuscinosis.

factor (mtTFA), cytochrome c1, adenine nucleotide translocator 2, and F_1-ATPase β subunit were transfected into the *Drosophila* cell lines. All 4 promoters harbor multiple, proximal Sp1-activating elements that account for half or more of transcription activation by Sp1, regulating both positive and negative expression of the nuclear genes that code for OXPHOS subunits.

A study of human tissues containing 12% or less non-mutant mtDNA revealed increased transcript levels of a variety of oxidative phosphorylation and related bioenergetic genes. The patients suffered either from myopathies, encephalopathy, lactic acidosis, stroke-like episodes (MELAS), or other pathogenic mtDNA mutations. The level of mtDNA transcripts was increased. Nuclear OXPHOS gene transcripts were also increased including the ATP synthase β subunit, the heart-muscle isoform of the adenine nucleotide translocator, and subunits for Complex I. Besides, ancillary nuclear gene transcripts were increased including muscle mitochondrial creatine phosphokinase, hexokinase I, the E1α subunit of pyruvate dehydrogenase, muscle glycogen phosphorylase, and phosphofructokinase [172]. There is evidence that transcription of nuclear-encoded molecules that support mtDNA replication is unaltered even by substantial variations in mtDNA levels [176].

3. Mitochondrial DNA. *(a) Inhibition of mtDNA replication.* Azidothymidine (3'-azido-3'-deoxythy-

midine, AZT, zidovudine) and other antiviral nucleoside analogue drugs inhibit mitochondrial DNA replication [177]. They are toxic to muscle mitochondria. AZT is a DNA chain terminator, which inhibits the mitochondrial γ-DNA polymerase that is required for mitochondrial DNA replication [178, 179]. Long-term treatment with AZT induces delayed and sometimes severe mitochondrial toxicity [180]. For instance, Southern blotting of muscle biopsy specimens of AZT-treated patients with myopathy revealed an up to two-thirds reduction in mitochondrial DNA compared with normal adult controls. However, cessation of the treatment reversed the depletion of mtDNA [181].

It has been argued that either infection with the human immunodeficiency virus (HIV) or azidothymidine treatment can cause myopathy [182, 179]. One study postulated that HIV infection rather than the AZT (ZDV) caused the myopathies in the majority of the patients [183]. However, several other studies indicate that typical mitochondrial abnormalities are observed only in biopsies of AZT-treated patients but not in mitochondria from the non-treated HIV positive patients [182, 179]. In one such study, the abnormal mitochondria contained para-crystalline inclusions and were found in "ragged-red" fibers of the biopsy specimen [182].

Azidothymidine has also been employed to develop a rat model of the mitochondrial energy decline associated with overt mitochondrial diseases and the aging process [184]. Treatment of the rats with AZT induced a decline in soleus muscle function in vivo and ex vivo and decreased bioenergetic capacity of heart sub-mitochondrial particles. When such particles were prepared from heart mitochondria of young and aged rats treated with AZT, the mitochondrial particles derived from the aged rats were less able to maintain the membrane potential, compared to those prepared from the young rats. Remarkably, treatment with Q10 improved soleus muscle function in vivo and significantly improved cardiac mitochondrial membrane potential capacity in vitro [184].

Damage to mtDNA by oxygen radicals is the primary cause of mitochondrial myopathy with AZT therapy. A 4-week period of administration of low doses of AZT to mice caused the mouse liver mitochondria to convert one quarter of the total deoxyguanosine (dG) to 8-OH-dG, indicating ROS damage. It was suggested that lack of a mitochondrial DNA-repairing system enhanced the damage to mtDNA caused by AZT. The hypothesis was that the combined effect resulted in impaired mitochondrial respiratory chain function causing oxygen radicals that were responsible for 8-OH-dG formation [185].

Azidothymidine reduces the activities of Complexes I and III of the mitochondrial respiratory chain. After supplementation with AZT of human muscle cells in vitro their mitochondria were enlarged and contained electron-dense deposits in the matrix and abnormal cristae. The respiratory control ratio was decreased indicating uncoupling [186]. Rats treated with AZT showed increased serum lactate and glucose levels and 100-fold elevation of creatine kinase. The highest tissue concentration of AZT was found in the skeletal muscle and the heart [186]. Other findings indicate that inhibition of electron transport chain enzyme complexes is tissue-specific. In vitro, AZT inhibited Complex I in mitochondria isolated from liver, skeletal muscle, and brain. In addition, in this study it inhibited Complex II of mitochondria isolated from the muscle [187].

4. Abnormal mitochondrial degradation. Lipofuscin, the "wear-and-tear" aging pigment found in the heart and other organs of older individuals, probably develops from dead mitochondria. With increasing age, the yellow brown pigment accumulates around the nucleus of the postmitotic cell. In vitro experiments using isolated mitochondria suggest that lipofuscin can develop from mitochondria by lipid peroxidation: the conversion could be prevented by addition of an antioxidant to the incubation medium [188]. Furthermore, electron microscopy findings of old myocardium have identified mitochondrial membrane fragments in lipofuscin granules. The fragments have been tentatively identified as remnants of mitochondrial cristae, supporting the notion that the lipofuscin is derived from dead mitochondria in the postmitotic cell [189]. In vitro exposure to 40% ambient oxygen or inhibition of proteases with the thiol protease inhibitor leupeptin caused accumulation of fluorescent material consistent with ceroid and lipofuscin within the secondary lysosome of cultured AG-1518 human fibroblasts. The findings suggest that ceroid and lipofuscin form through peroxidative damage of autophagocytosed material [190,191].

Mitochondrial energy conversion. 1. The membrane. *(a) Conductivity*. Membrane conductivity depends upon the type of lipid in the membrane as well as the type and concentration of membrane protein [192] (Figure 5). The composition of fatty acids of the membrane phospholipids affects the permeability of the membrane [193]. The effect of dietary lipids and mitochondrial function are of particular interest, since it has been found that mitochondrial enzyme activities are affected both by alterations of mitochondrial membrane lipids as well as mitochondrial enzymes.

Examples of important membrane dysfunctions are an increase in the intrinsic rate of proton leakage across the inner mitochondrial membrane, a decrease in membrane fluidity, or a decrease in cardiolipin, the protein that supports the function of many inner membrane enzymes. Serious alterations of these functions may be caused by the synthesis of defective mitochondrial lipids or proteins, by a decrease in the synthesis of lipids or mitochondrial proteins or the synthesis of faulty proteins, or by increased turnover of mitochondrial lipids or proteins [194].

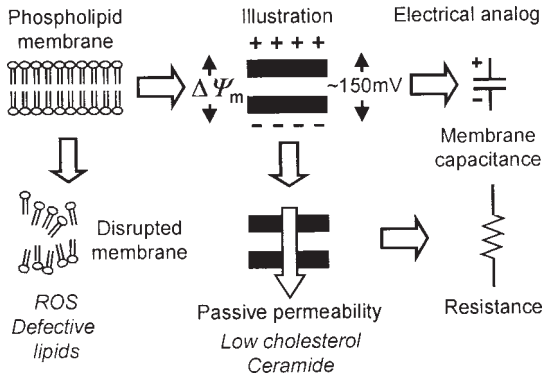

Fig. 5. Illustration showing causes of dysfunction of the inner mitochondrial membrane. Its bilipid structure with the hydrophobic center and hydrophilic outsides provides capacitance that permits it to be charged with an electric potential. The interaction between membrane lipids and proteins has been studied using alternating current at 1 Hz and 1KHz to investigate in vitro both the resistance and capacitance of a lipid bilayer membrane made from either phosphatidylinositol or oxidized cholesterol. Such measurements have shown that membrane conductivity depends upon the type of lipid in the membrane as well as the type and concentration of membrane proteins. For details see text.

(b) Hyperpolarization. Hyperpolarization of the inner mitochondrial lipid-bilayer membrane leads to a large, non-linear increase in proton permeability [195], similar to the proton permeability of pure phospholipid bilayers. In vitro findings suggest that the lipid-dependent proton leak accounts for only about 5% of the total mitochondrial proton leak [196]. In addition, the proton leak is significantly modified by the presence of proteins embedded in the mitochondrial membrane [197]. Membrane hyperpolarization through ATP hydrolysis by F_0F_1-ATPase enhances generation of reactive oxygen species. For instance, isoprenoid farnesol induces ATP hydrolysis in mitochondria in cells of *Saccharomyces cerevisiae*. It inhibits oxygen consumption and induces the proton pumping function of F_0F_1-ATPase, which results in mitochondrial inner membrane hyperpolarization. The F_0F_1-ATPase inhibitor oligomycin and the F_1-ATPase inhibitor sodium azide abolish the effect [198].

(c) Cardiolipin. Cardiolipin is synthesized in mitochondria and is localized almost exclusively within the inner mitochondrial membrane. It is a phospholipid (disphospatidylglycerol). Cardiolipin interacts with membrane-bound proteins to orient and activate them. It affects matrix proteins, mitochondrial membrane receptors, and leader peptides. Cardiolipins, especially those with high linoleic acid (18:2) content, strongly bind many mitochondrial carrier proteins and oxidative phosphorylation enzymes. They exhibit an especially high affinity for cytochrome oxidase. Whereas cardiolipins are not absolutely essential for activation of this enzyme complex in vitro, maximal activities of cytochrome oxidase are only obtained when cardiolipins are present [199,200].

In vitro, saturated cardiolipins form membrane bilayers while unsaturated cardiolipins form nonlamellar phases. Cardiolipins are capable of participating in proton conductive pathways along bilipid membranes when embedded in an ordered bilipid matrix containing phosphatidyl choline and phosphatidylethanolamine, such as the inner membrane of the mitochondrion [201].

Anti-cardiolipin antibodies are independent risk factors for atherosclerotic vascular disease [202]. Infection with the herpes simplex virus (HSV) can markedly inhibit the synthesis of cardiolipins while leaving the synthesis of other phospholipids relatively

unaffected [203]. Extracts of diesel exhaust particles decrease mitochondrial cardiolipin, induce uncoupling, lower the membrane potential and ATP levels, and induce apoptosis [204].

2. The electron transport chain (Figure 6). *(a) The enzyme complexes of the mitochondrial inner membrane.* Most of Complexes I and III and up to 4 copies of Complex IV are arranged together in supramolecular structures referred to as respirasomes [205] or supracomplexes [206]. Complex I (NADH:CoQ-oxidoreductase) is a pyridine nucleotide transhydrogenase and a proton pump. It couples the transfer of hydride between $NADP^+$ and NAD^+ to proton translocation across the mitochondrial membrane [207]. Complex IV, cytochrome c oxidase, is the third respiratory chain proton pump. It catalyzes the reduction of oxygen to water. Its active site consists of a heme group with a binuclear center of a copper ion [208].

Isolated Complex I deficiency is one of the most common defects of the respiratory chain [209]. Most cases occur in children. Interestingly, a majority of these defects are caused by mutations in nuclear DNA. As an example, a muscle biopsy from a 10-year-old female with arthrogryposis multiplex congenita (Guérin-Stern syndrome) and mild myopathy revealed only half the normal specific activity of Complex I. The defect was in part compensated for by an increased number of mitochondria [37].

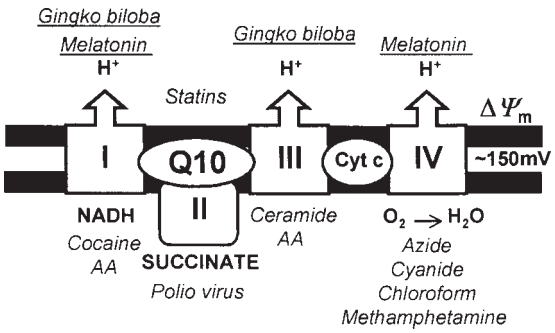

Fig. 6. Schematic illustration of the energy flow in the electron transport chain. The proton-pumping complexes I, III, and IV generate the inner mitochondrial membrane potential, $\Delta\Psi_m$, typically measuring about 150 mV. Coenzyme Q10 (Q10) accepts electrons from complexes I and II and transfers them to complex III. Cytochrome c transfers electrons from complex III to complex IV where the electrons are transferred to oxygen. The mammalian respiratory chain enzymes are not randomly distributed in the mitochondrial inner membrane but arranged in supramolecular clusters referred to as supramolecular structures or respirasomes. Listed in Italics are agents that inhibit (below membrane) or stimulate (above membrane). AA: arachidonic acid. For details see text.

Human cocaine abuse is associated with heart and liver toxicity. In vitro exposure of neonatal rat cardiomyocytes to cocaine induced slight leakage of lactate dehydrogenase and significantly inhibited glutamate/malate-mediated respiration of isolated mitochondria, suggesting inhibition of Complex I [210]. Norcocaine, norcocaine nitroxide, and N-hydroxynorcocaine, the N-oxidative metabolites of cocaine, but not cocaine, deplete ATP of isolated mouse liver mitochondria in vitro. Norcocaine could completely inhibit mitochondrial respiration [211].

A mutation at base pair 3,243 in MELAS disrupts transcription of a termination sequence located with the tRNA (Leu) [UUR] gene leading to the synthesis of an abnormal 16S ribosomal RNA. It causes defective translation of ND1-7, the mtDNA-encoded subunits of respiratory Complex I, and alters its affinity for the NADH substrate and reduces the mitochondrial membrane potential. Increased mitochondrial NADH might partially compensate for the defect but requires an intact membrane potential for transport of hydrogen from cytosolic NADH into the mitochondrion. Interestingly, a 5-month course of oral nicotinamide administered to a patient with this MELAS mutation resulted in a 50% fall in blood lactate plus pyruvate concentration [212].

(b) Coenzyme Q10 - the electron carrier. Coenzyme Q10 (Q10), also known as ubiquinone-10, is an endogenously synthesized vitamin-like lipid (Figure 7), an antioxidant, and an essential electron carrier in the mitochondrial respiratory chain [213]. Orally administered it is absorbed to a significant degree. As an example, supplementation in a randomized crossover study by Q10 at concentration of 30 mg/day resulted in significant increases in serum Q10 levels whether mixed with the food or administered as a capsule [214].

In the blood, Q10 is carried in the circulation in LDL particles. Inhibition of LDL uptake by a receptor

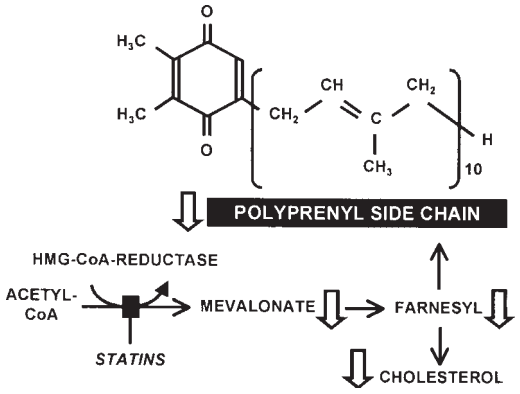

Fig. 7. This illustration shows the chemical structure of Coenzyme (Co) Q10 (ubiquinone) and part of the synthetic pathway. The polyisoprenoid lateral chain of CoQ10 originates from acetyl-CoA that is converted by 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase to mevalonate, which is then converted to farnesyl. Next, farnesyl is converted either to cholesterol or CoQ10. Administration of a statin-type cholesterol-lowering druginhibits HMG-CoA reductase and reduces the synthesis of cholesterol and ubiquinone. For details see text.

or ligand defect therefore might make a cell more dependent upon intracellular synthesis of Q10, since Q10 is essential for proper mitochondrial electron chain transport.

The daily uptake of Q10 differs depending upon the type of food in the diet. For instance, the daily uptake from the average Danish diet has been estimated at only 3.5 mg of Q10 per day, and was mainly derived from meat and poultry. A recent study of a group of Greenland Eskimos showed that their serum Q10 levels were significantly higher than the Danish population. These Eskimos live in a most remote area of Greenland and have a low prevalence of ischemic heart disease. It is possible that their high serum levels of Q10 are due to the high Q10 levels in their diet, which is derived primarily from sea mammals and fish [215].

(c) Coenzyme Q10 - structure and synthesis. Coenzyme Q10 regulates oxidative phosphorylation and prevents lipid peroxidation [216]. Its polyisoprenoid lateral chain originates from acetyl-CoA via mevalonate and isopentenylpyrophosphate sharing its biosynthetic pathway with cholesterol [216]. Mevalonate is converted to farnesyl, which is converted to either cholesterol or Q10 [216]. Because of the shared pathway from acetyl-CoA via farnesyl, the administration of a statin-type of cholesterol-lowering drug that inhibits HMG-CoA reductase reduces the synthesis not only of cholesterol, but also of Q10, and lowers the blood level of Q10 [216].

The results from studies on the regulation of expression and activity of HMG-CoA-reductase, a 97-kDa-protein [217], and the rate-limiting enzyme of the cholesterol biosynthetic pathway are conflicting. As examples, one study found that ATP and insulin stimulate rat hepatic microsomes HMG-CoA-reductase activity, and the activity was 4-fold higher at night compared to daytime activity [218]. However, others report that ATP inhibits [95] or inactivates [96] the enzyme. ATP at physiological concentration causes swift and irrevocable inactivation of the enzyme activity in the cultured digitonin-permeabilized ovary cell of the Chinese hamster [217].

(d) Coenzyme Q10 - inhibition by statins. The statin types of cholesterol-lowering drugs that inhibit HMG-CoA reductase also reduce synthesis of ubiquinone and lower the blood level of Q10 [216,219] (Figure 8). The serum Q10 level is also decreased by low-density lipoprotein (LDL) apheresis, and in patients with non-insulin dependent diabetes mellitus (NIDDM) and normal cholesterol levels, but elevated in diabetic patients with hypercholesterolemia [220]. When a group of NIDDM patients with hypercholesterolemia were treated with daily doses of 20 mg of simvastatin, their Q10 blood levels declined significantly. In contrast, Q10 blood levels in a similar group of 30 patients supplemented with 100 mg Q10 per day increased significantly [216] (Figure 8).

Statin therapy has been reported to induce toxic myopathy that might be related to dysfunction of mitochondrial oxidative phosphorylation. Indirect evidence in support of this hypothesis was obtained in clinical studies in a group of 60 hypercholesterolemic patients, of which 40 were treated with statins and the rest served as controls. Compared with the control group, the statin-therapy group showed a significant increase of the blood lactate/pyruvate ratio. The Q10 serum levels in the statin group fell to 0.75 compared with 0.95 mg/L in the untreated patients [219].

Experiments with brief ischemia and reperfusion in dog myocardium suggest that lipophilic but not hydrophilic HMG-CoA-reductase inhibitors enter

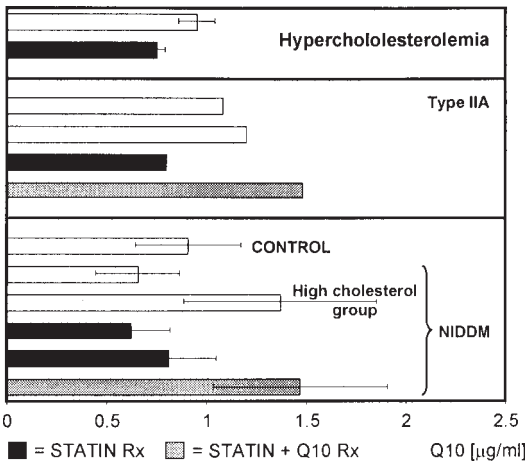

Fig. 8. Serum (top and bottom) and blood (middle) Q10 levels of patients with hypercholesterolemia (top and middle) and NIDDM (bottom). Statin therapy lowers Q10 levels. However, oral Q10 supplementation restores blood Q10 levels. Data from the following references: De Pinieux et al [219] (top), Bargossi et al [216] (middle), and Miyake et al [220] (bottom).

mitochondria, inhibit Q10 biosynthesis and thereby mitochondrial electron transport, and depress ATP generation [221]. In the rat model, administration of the water-soluble pravastatin to rats age 35 and 55 weeks significantly accelerated the age-related decline in the activity of Complex I of mitochondria from the diaphragm and the psoas major muscles. In comparison, pravastatin had no significant effect on mitochondria from the rat heart and liver, which did not show any age-related diminution of respiratory function up to age 55 weeks. Based upon these findings it was suggested that proper diaphragmatic function should be carefully considered when prescribing pravastatin for the elderly patient [222].

In contrast, others found evidence of simvastatin and pravastatin inhibition of oxidative phosphorylation in the rat heart after experimentally induced ischemia. The ATP production per unit oxygen in rat heart mitochondria ex vivo was decreased after one hour of prior in vivo ischemia. Pretreatment of the rats by statins enhanced the decline in ATP synthesis. The lipid-soluble simvastatin caused a greater decline than the water-soluble pravastatin [223]. The effect on myocardial Q10 levels was not determined.

However, in a series of similar experiments, but using only 30 minutes of ischemia, pretreatment with the lipid-soluble simvastatin, but not with pravastatin, significantly reduced the myocardial level of Q10. The ex vivo ADP/O ratio with succinate was significantly reduced in mitochondria only from the simvastatin-treated, and not from the pravastatin-treated rats. Thus the decrease of myocardial Q10 levels induced by cholesterol-lowering therapy with a lipid-soluble, but not a water-soluble, statin might cause worsening of heart mitochondrial respiration during ischemia [224].

A significant, progressive deficiency in blood Q10 levels was reported in patients infected with HIV, with ARC, and with AIDS. Treatment with Q10 led to appreciable and sometimes marked clinical improvement [225]. After interventional cardiac procedures, old patients show inferior recovery compared to young patients. In the rat model of stress induced by rapid electrical pacing of isolated working hearts, pre-stress work performance of senescent hearts was inferior to that of young hearts. Remarkably, pretreatment with Q10 abolished the difference between the young and old rat hearts [226].

In rabbits, simvastatin induced mitochondrial swelling, autophagic vacuoles, and muscle fiber necrosis in all 6 rabbits, pravastatin (300 mg) in 2, and pravastatin (100 mg) in none of the rabbits. However, surprisingly, in spite of the decrease in muscle Q10 content, more so in the pravastatin group, and the mitochondrial swelling observed particularly in the simvastatin group, the mitochondrial respiratory chain enzymes were normal in all groups in this study [227].

(e) Coenzyme Q10 - regulation of Q10 synthesis. A study of cultured human skin fibroblasts failed to detect any feedback regulation by Q10 of 3-hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) reductase. Whereas supplementation of the cells with Q10 in the medium increased the cellular content of Q10 about 10-fold, it did not suppress HMG-CoA reductase nor the incorporation rate of ^{14}C- acetate into Q10 [228].

This would appear to rule out any involvement of Q10 in the regulation of HMG-CoA. However, if regulation of HMG-CoA occurs via ATP, an alternate interpretation is possible. OXPHOS might have been fully saturated with Q10 due to an ample endogenous supply of Q10. The ATP supply would then have been adequate before supplementation, and there might have

been no reason for a compensatory increase in the expression of the reductase to enhance further Q10 synthesis, even after the cellular uptake of a significant amount of exogenous Q10.

(e) Other inhibitors. The poliovirus can significantly alter mitochondrial function as demonstrated by inhibition of Complex II of cultured COS-1 and T47D cells [30]. Ceramide mediates the effects of tumor necrosis factor-alpha (TNF-γ). It might be involved in TNF-γ induced apoptosis since exposure to N-acetylsphingosine, a C2-ceramide analog, rapidly inhibits Complex III in isolated mitochondria [229]. In the rat model, methamphetamine or 3,4-methylene-dioxymethamphetamine inhibits expression of Complex IV as evidenced by a rapid decrease in cytochrome c oxidase staining in the substantia nigra, the nucleus accumbens, and the striatum [230].

A sufficient supply of iron is necessary to prevent dysfunction of iron-containing OXPHOS enzymes. ^{31}P magnetic resonance spectra of the gastrocnemius muscle of iron-deficient Wistar rats revealed slow recovery of intracellular phosphate and phospho-creatine concentration after exercise. The alteration in the muscle bioenergetics persisted beyond treatment with iron and complete correction of the anemia suggesting impaired oxidative phosphorylation [231].

(f) Aging. Neither Sprague-Dawley rats nor C57/B17 mice lived longer on a diet supplemented daily with 10mg/kg of Q10 compared to control animals not receiving Q10 supplementation. In the rat, plasma and liver Q10 levels were significantly elevated by the supplementation. However, tissue levels of Q10 in the heart, brain, or kidney were unaffected [232].

3. Phosphorylation. *(a) Complex V - the F_0F_1-ATPase.* The final step of the mitochondrial energy conversion is the phosphorylation of ADP (Figure 9). Complex V, the F_0F_1-ATPase, provides approximately 95% of the adenosine triphosphate (ATP) produced by the cell [18]. It has been estimated that 3 protons are used by F_0F_1-ATPase for each ATP molecule that is synthesized [233]. Complex V is inhibited by phenothiazines and similar compounds [234], and by di-(2-ethylhexyl)phthalate (DEHP), a plasticizer [235]. The anesthetic n-butanol and tetracaine can inhibit ATPase activity. Irradiation with ultraviolet light results in a conformational change of amino acid residues in the active site of ATPase and inhibition of ATPase activity in both membrane-bound and soluble F_1 in vitro [236]. Succinate may partially protect against these ultraviolet light-induced effects [236].

The F_0 proton-conductive part of F_0F_1-ATPase conducts protons through the mitochondrial inner membrane into the matrix. The membrane energy released is used by the F_1 moiety to convert ADT to ATP. The phosphorylation step responds rapidly to alterations in the cellular energy demand by adjusting the rate of the intramolecular rotation of F_0F_1 [174].

Mutations of a gene coding for an OXPHOS enzyme subunit may alter the sensitivity of the enzyme to inhibitors. As an example, the Sprague-Dawley and the BHE/Cdb rat differ in their mtDNA sequence for a subunit of Complex V. Base substitutions in the ATPase 6 gene result in the substitution of aspartate for asparagine at position 101 and the substitution of leucine for serine at position 129. As a consequence, isolated mitochondria from the rat with the base substitution are more sensitive to oligomycin inhibition of OXPHOS compared with mitochondria isolated from the Sprague-Dawley rat. These results are consistent with results from human fibroblasts having a mutated ATPase-6 gene [237].

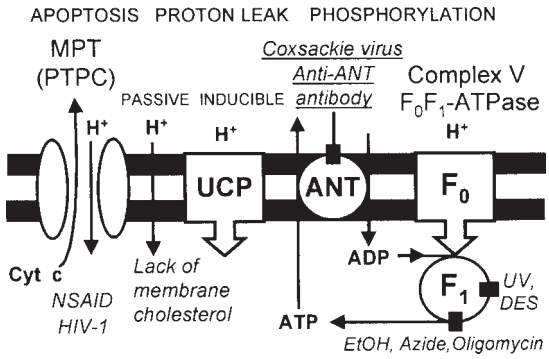

Fig. 9. A schematic illustration of phosphorylation, uncoupling protein (UCP), and the mitochondrial permeability transition pore complex (PTPC). Complex V, the F_0F_1-ATPase, utilizes the membrane potential to phosphorylate adenosine diphosphate (ADP) to adenosine triphosphate (ATP) and provides most of the ATP produced by the cell. The adenine nucleotide transporter (ANT) exchanges the ADP and ATP across the membrane. Agents that interfere with normal functions are indicated in italics. For details see text.

(b) The adenine nucleotide transporter (ANT). The transmembrane transport of adenine nucleotides by ANT can be inhibited by morphine [238]. Specific antibodies against ANT can limit oxidative phosphorylation by inhibiting transmembrane nucleotide transport. Recent findings suggest that a virus might induce cardiac mitochondrial dysfunction via an antibody-mediated attenuation of the capacity of ANT to exchange nucleotides. First, immunizing guinea pigs with ANT disturbs heart mitochondria and reduces heart function. Second, infecting mice with the Coxsackie B3 virus induces specific anti-ANT antibody production in over 70% of the mice. Perfused, isolated hearts from the animals that produced the anti-ANT antibody showed significantly reduced mitochondrial oxidative phosphorylation and over 50% reduction in left ventricular pressure [239]. Bile acids reduce ANT in vitro [240].

The carboxyl end of the HIV-1 viral protein R binds to purified ANT and rapidly dissipates the membrane potential of isolated mitochondria and induces apoptosis in cultured cells. Addition of Bcl-2, which inhibits the opening of the permeability transition complex pore, prevents the apoptosis [241].

(c) Regulation of oxidative phosphorylation. Oxidative phosphorylation is controlled differently in different tissues. In muscle and heart, control of respiration is the primary control, whereas in brain, liver, and kidney, control is through regulation of the ATP synthase and the phosphate carrier [242].

One theory of the regulation of oxidative phosphorylation is that breakdown products of ATP diffuse freely to the mitochondria to stimulate OXPHOS. However, calcium entry into the mito-chondria cannot explain the first fast phase of oxidative phosphorylation activation, and it has been proposed that delay of the energy-related signal in the cytoplasm dominates the response time of OXPHOS [243].

Thyroid hormone enhances oxidative phosphorylation as shown by thyroidectomy in the rat model of hypothyroidism. Removal of the thyroid reduces ATPase activity by over 30% in the liver mitochondria [244]. In the rat, thyroid hormone up-regulates the expression of selected nuclear genes that encode OXPHOS complex sub-units [245]. Even subunits of the same enzyme complex may be differentially regulated by thyroid hormone. For instance, the mRNA for cytochrome c1 is increased as much as 20-50-fold by thyroid hormone. However, other nuclear OXPHOS genes such as the F_1-ATPase β-subunit and the core protein 1 of Complex III do not respond to thyroid hormone [245]. Mitochondria from thyroxin-treated rats incubated with succinate as substrate showed an ex vivo increase in the respiration rate of almost 50%, and there was a 10% increase in the membrane potential compared with mitochondria from normal, non-treated rats [246].

In hyperthyroidism, triiodothyronine induces increased ATP consumption in the heart. However, relatively more membrane energy is used for heat production and less directed toward ATP production and muscle contraction [247].

Anabolic-androgenic steroids can affect respiratory chain enzymes. For instance, there was a significant decrease in the activities of Complexes I, III, and IV in rats treated with either fluoxymesterone, methyl-androstanolone, or stanozolol [248]. Others have reported that glucocorticoids such as hydrocortisone, prednisolone, dexamethasone, and triamcinolone, inhibit Complex IV [249].

In the animal model the effect of cannabinoids is different for single use compared with prolonged use. A single dose (10 mg/kg) of δ(9)-tetrahydrocannabinol increased both oxidative phosphorylation of rat brain mitochondria and cerebral lipoperoxidation ex vivo. When the same dose was administered twice daily for 4.5 days, it resulted in uncoupling of brain oxidative phosphorylation and induced neuronal damage [250].

4. Uncoupling. *(a) Effects on cell survival.* Mitochondrial transfer experiments have demonstrated that damaged mitochondria can reduce the viability of a cell [251]. For instance, 20% of young human fibroblasts injected with isolated mitochondria from old rats exhibited signs of degeneration after a few days [252]. In contrast, only 5% of cells microinjected with fresh mitochondrial preparations from young rats showed signs of degeneration. Young mitochondria have a high respiratory control ratio: The energy released during mitochondrial respiration is tightly coupled to phosphorylation of ADP. However, if young mitochondria are uncoupled by exposure to an uncoupler such as 2,4-dinitrophenol, the synthesis of ATP is reduced. Remarkably, young mitochondria that are partially uncoupled induce changes in the recipient

cells similar to those induced by old mitochondria [252]. In such mitochondrial transfer experiments, the proportion of dead cells is dependent on the state of uncoupling of the injected mitochondria. On the other hand, supplying the recipient cells with a substrate that is easily metabolized protects the cells. For instance, supplementation with D(-)-beta-hydroxybutyrate sodium salt in a dose-dependent manner prevents degeneration induced by micro-injection of uncoupled mitochondria.

Fatty acids cause non-shivering thermogenesis in larger mammals by inducing uncoupling proton flow across the inner mitochondrial membrane, only above a threshold membrane potential of 125 mV. [253].

The branched-chain phytanic acid, 3,7,11,15-tetramethylhexadecanoic acid, which accumulates throughout the body in Refsum disease, increases the mobility of inner membrane phospholipids, alters the conformational state and mobility of transmembrane proteins, and induces uncoupling in vitro [254].

(b) Passive proton leak. Transmembrane outward pumping of protons from the mitochondrial matrix to the intermembranous space and inward transmembrane proton leak results in a futile proton cycle. It dissipates the mitochondrial redox energy, which consumes approximately 15% of the standard metabolic rate of working muscle and liver in vivo [255,256]. The proton leak is a basic component present in all mitochondria.

Hyperthermia can increase proton conductance of the mitochondrial inner membrane and degrade oxidative phosphorylation. The resulting proton leak is caused by alteration of the membrane order. An in vitro study of intact female rat mitochondria using fluorescent measurements to determine membrane phospholipid polarization revealed that the transition occurred between 40 and 43°C [257].

In addition, an augmentative component present in some mitochondria induces a significant flow of protons across the mitochondrial inner membranes into the mitochondrial matrix. Some suggest that in the rat resting hepatocyte almost one-third of its resting oxygen consumption is dissipated as heat due to proton leaks [258]. The proton leak in rat tissues varies depending upon the tissue examined [259,255].

(c) Active proton leak. Uncoupling proteins regulate the dissipation of the membrane potential formed through respiration. Instead of being used for ATP synthesis the mitochondrial membrane energy is converted to heat [260]. The heat production not only protects against cold environments but also regulates the mitochondrial energy balance [261]. Through the regulation of uncoupling [262], mitochondria can adjust their metabolism to the supply of substrates and the cellular ATP requirement, while minimizing ROS production by lowering the membrane potential [103].

The first uncoupling protein (UCP) that was discovered is a classical example of an augmentative component. It is a 32-kDa membrane-protein that is specifically induced in rat brown adipose tissue [263]. It was later renamed UCP1, as additional protein homologues, namely UCP2, widely expressed in rodent and human tissues [263], and UCP3 were identified. UCP1 uncouples mitochondria of brown adipose tissue [264]. Purine nucleotides on the cytosolic side of UCP1 inhibit its proton conductance. Fatty acids increase UCP1-induced energy dissipation [265] and regulate the mitochondrial energy system by tuning the degree of coupling of oxidative phosphorylation [266]. Retinoids induce UCP1 transcription in transgenic mice [263].

In the mouse, LPS, IL-1β, and TNF induce the expression of UCP2 mRNA. However, the effect is tissue dependent and apparently differently regulated. For instance, LPS strongly induces the UCP2 expression in muscle and liver, but prior administration of indomethacin inhibits expression only in liver [267].

The UCP3 gene is expressed in rodent skeletal muscle and brown adipose tissue and might also play a role in the mitochondrial degradation of fatty acids [261]. Two UCP3 RNA transcripts produce 2 isoforms, one long, full length (UCP3L) and one short, truncated (UCP3S) isoform, that are highly expressed in skeletal muscle. Both strongly impair the mitochondrial coupling efficiency and increase thermogenesis, with the short isoform being the most active [268]. A mutation of the UCP3 gene that increases the proportion of the short isoform slows its insertion into the mitochondrial inner membrane, decreases fat oxidation, and enhances the susceptibility to obesity [269].

The absence of UCP-1 in the UCP1-ablated mouse leads to high expression levels of UCP2 and UCP3 and induces low cold tolerance. Interestingly, the mice

do not become obese, and in these experiments, UCP2 and UCP3 were not associated with any inherent uncoupling or with an increased basal metabolism [264]. In comparison, a 24-hour starvation period in the regular rat increased UCP2 and UCP3 in the skeletal muscle more that 4-fold and doubled the UPC3 protein levels while mitochondrial proton conductance remained constant [270].

The adenine nucleotide translocator (ANT) and the voltage-dependent anion channel (VDAC) are part of the permeability transition pore complex [241]. During a mitochondrial permeability transition (MPT), the complex opens the high conductance pore that increases membrane permeability to solutes of molecular mass up to 1.5 kDa [27]. The mitochondria swell, the membrane depolarizes and its potential dissipates; oxidative phosphorylation is uncoupled.

The onset of a mitochondrial permeability transition can be observed with confocal fluorescence microscopy by using red fluorescing tetramethylrhodamine methylester as a membrane potential-indicating fluorophore in combination with cytoplasmic calcein [271]. As pores open, green-fluorescing calcein moves into mitochondria. There is a simultaneous release of the red dye. Remarkably, cyclosporin A blocks the opening of the pores and prevents cell death. In vitro, salicylate induces the mitochondrial permeability transition. It kills cultured rat hepatocytes at concentrations of 0.3-5 mM in a concentration-dependent manner [27,272].

Similarly, other compounds, such as 3-mercaptopropionic and 4-pentenoic acids, adipic, benzoic, isovaleric, and valproic acids, and Neem oil that have been implicated in the pathogenesis of Reye's syndrome can induce the onset of the mitochondrial permeability transition in freshly isolated hepatic mitochondria in vitro. Surprisingly, the induction of the MPT in these experiments did not substantially reduce the membrane potential. This might be due to a rapid increase in respiration that temporarily maintains the membrane potential [273]. Apparently, an intact, rapidly responding OXPHOS regulatory system might maintain the inner membrane potential temporarily, even in the presence of some degree of MPT. Such a mechanism might be very important to cell survival, since these findings suggest that uncoupling alone does not always induce complete depolarization. It has also been suggested that onset of MPT might be involved in chemical toxicity and Jamaican vomiting sickness which, like Reye's syndrome, are characterized by hyper-ammonemia, hypoglycemia, microvesicular steatosis, and encephalopathy [273].

Low temperatures markedly decrease the resting respiration due to membrane leak; however, the respiration due to intrinsic proton pump uncoupling increases [274]. In rat liver mitochondria, the glycoside antibiotic sporaviridin uncouples oxidative phosphorylation. It increases the permeability of the inner membrane of the mitochondria [275]. Regulating the proton leak may rapidly permit mitochondria to channel energy flux to or from ATP synthesis [193].

5. Functional equivalency diagram. The function of the oxidative phosphorylation system can be simplified by reference to a schematic diagram where the function of the electron transport chain is compared to that of a fuel cell (Figure 10). The input to the fuel cell consists of the food metabolites and oxygen; the energy released charges a capacitor (the membrane). The energy stored temporarily in the capacitor is either coupled to an ATP converter or uncoupled due to inhibition of the converter or due to heat loss caused by fixed or variable discharge of the capacitor. The mitochondrial permeability transition (MPT) is analogous to a switch that rapidly discharges the capacitor. If the switch is closed only momentarily the fuel cell might rapidly respond and recharge the capacitor. However, if the fuel cell is damaged, complete discharge might occur.

Mitochondrial Genetics

The genome. 1. Phylogenesis. Mitochondrial DNA sequence data suggest that animal and fungal mitochondrial DNA share a common ancestor. However, the fungal mitochondrial genomes contain a large number of introns, which are absent in human mtDNA [276]. As a result, a deletion of part of the human mitochondrial genome results in the loss of a coding region resulting in defective mitochondrial protein synthesis of one or more of 13 oxidative phosphorylation enzyme subunits. According to the classical serial endosymbiosis hypothesis, mitochondria are derived from the capture of an α-proteobacterial endosymbiont by a nucleus-containing eukaryotic host

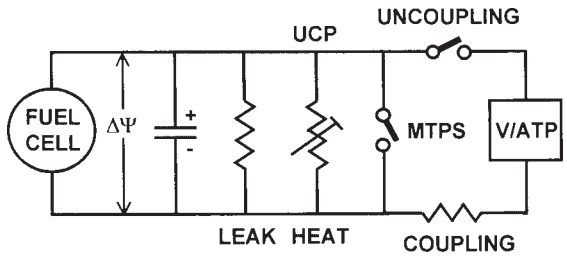

Fig. 10. Simplified schematic diagram of oxidative phosphorylation. The electron transport chain is compared to a fuel cell with an input side consisting of food metabolites and oxygen (not shown) and an output side that charges a capacitor (the membrane) up to about 150 mV. The voltage is coupled either to (a) an ATP converter or (b) dissipated as heat. Uncoupling is the reduction of ATP generation due to either (a) inhibition of the converter or (b) fixed or variable heat loss. Activation of the mitochondrial permeability transition (MPT) is compared to a switch that rapidly discharges the capacitor. When closure of the MPT switch starts to discharge the capacitor, a fully operational fuel cell might rapidly respond to prevent complete discharge. However, if the fuel cell response is inadequate, complete discharge follows (apoptosis).

[277]. The ancient anaerobic host, the archaebacterium, engulfed the respiring symbiont [278], a proteobacterium, which evolved into the mitochondrion. An analysis of genomic signatures indicates that the progenitors of animal mitochondria (and various primitive eukaryotes lacking mitochondria) were fusion microbes composed of a *Clostridium*-like eubacterium and a Sulfolobus-like archaebacterium [279].

The energy-converting metabolism, originally bound to the plasma membrane, was relocated to the intracellular space. This endosymbiosis led to the transfer of most genes from the symbiont to the nuclear genome of the host [280]. Evolution might have favored the transfer of bacterial DNA to the more protected environment of the nuclear genome to prevent defective mitochondria from flourishing to the detriment of an organism, particularly in its syncytial tissue [281].

A few genes essential to OXPHOS remained in the mitochondrion during phylogenesis. Some of these genes code for the proton-pumping subunits of electron transport chain enzyme complexes. As hydrophobic proteins they could not be synthesized in the cytoplasm and imported into mitochondria. Many additional bacterial genes present in the nuclear genome may have originated from bacteria that were simply taken up and processed as food [282].

Some sequence data suggest that the formation of the mitochondrion occurred simultaneously with the formation of the nuclear genome of the eukaryotic cell [277]. Phylogenetic DNA sequence comparisons suggest that extensive lateral gene transfer occurred between early bacteria, archaea, and ancestral eukaryotes, resulting in "chimeric" genomes containing genes from multiple sources [283].

2. The structure. The Cambridge sequence was mainly derived from a single human placenta with a few regions coming from HeLa cell mtDNA and bovine mtDNA, which were used to establish the base for several ambiguous nucleotides. The Cambridge sequence consists of 16,569 base pairs encoding hydrophobic protein subunits of the mitochondrial electron-transport system and ATPase: seven subunits of complex I, apocytochochrome b of Complex III, three subunits of Complex IV, and two subunits of ATPase (Complex V). The remaining mtDNA genes specify 2 mitochondrial ribosomal RNA (RNA), 22 organelle-specific transfer RNA (tRNA) and genes regulating transcription and replication (D-loop) [11]. All other mitochondrial proteins are coded for by the nuclear genome.

There are 10^3 to 10^5 circular mitochondrial DNA molecules in the human cell [284]. Heteroplasmy is the simultaneous presence of both normal and mutated mtDNA [20].

3. The high mutation rate of mtDNA. The stability of the mitochondrial genome and its efficiently regulated expression is essential for maintaining the membrane potential and a functional oxidative phosphorylation pathway [285]. However, the rate of mutation of mtDNA, caused, for instance, by radical oxygen metabolites produced by the respiratory chain, is 10 to 20 times higher than the mutation rate of nuclear DNA. Besides, the mtDNA lacks effective repair and contains no histones.

Mutations of the mitochondrial genome are the most important causes of known inherited and

acquired genetic OXPHOS deficiency [145]. A comparison of the evolutionary rate of the nDNA-encoded β subunit with that of the mtDNA encoded ATPase 6- and 8-subunits, 7 other mtDNA OXPHOS genes, and a number of nuclear genes revealed significant differences in their synonymous substitution rate. The rate for the ATPase6 and 8 genes was 12 times greater than the rate of the nuclear gene for the β subunit. Even more remarkable was the finding that the mutation rate of the average mtDNA gene was 17-fold that of the nuclear β subunit gene. These high substitution mutation rates and strong selective constraints of mammalian mtDNA proteins explain why mtDNA mutations result in a dispropor-tionately large number of human hereditary diseases of OXPHOS [286].

Propagation. 1. Mitochondria of the male gamete. The fate of mitochondrial DNA differs significantly in male and female gametes. Analysis of the entire mitochondrial genome in sperm donors revealed that most of the spermatozoan mitochondria from patients with oligoasthenospermia had multiple mtDNA deletions. Surprisingly, multiple deletions were found in normal sperm as well [287].

The Darwinian competition to fertilize the ovum should favor the sperm with the most efficient OXPHOS and therefore the highest motility. There is evidence that factors reducing the mitochondrial energy production are responsible for some cases of male infertility. For instance, there is a direct correlation between sperm mitochondria respiratory chain enzyme activities and sperm motility. Some findings argue that particular cases of asthenozoospermia are caused by defective mitochondrial energy production. For instance, the activities of Complexes I, II, and IV in sperm samples from asthenozoospermic subjects were significantly lower compared with those from the control individuals [288]. The sperm motility depended primarily on the mitochondrial volume and therefore the total energy available generated by OXPHOS in the sperm midpiece.

Flow cytometry findings using the cationic dye JC-1 to measure the sperm mitochondrial membrane potential corroborate a correlation between the potential and sperm motility [289]. Moreover, cytometry revealed a correlation between mitochondrial uncoupling and immature spermatozoa. The degree of uncoupling also correlated with spermatozoa defective for nuclear maturity. A higher percentage of immature spermatozoa was found in men from barren couples compared to donors of proven fertility.

In a patient with inherited mitochondrial disease caused by reduced activity of Complexes I and IV the sperm motility was reduced compared to normal control [290]. Remarkably, in vitro supplementation of the medium with pyruvate that enters the respiratory chain at Complex I and succinate that enters at Complex II tripled the sperm motility compared with only 12% motility when supplemented with glucose alone. Ultrastructural changes of the mitochondria were found not only in the spermatozoa but in spermatids as well, suggesting that the abnormalities were due to a primary mitochondrial defect rather than to secondary degeneration of the spermatozoa.

Paternal mitochondrial DNA has not been detected in human somatic cells of the offspring, at least not at the limits of detection of present technology. It is not known how the paternal mitochondria are eliminated from the human morula; however, the process in humans might be analogous to the mechanism in the mouse, which rejects the sperm-derived mitochondria at the 4- to 8-cell stage [291].

2. Mitochondria and the female gamete. Delayed motherhood is characterized by a higher risk of conceiving an offspring suffering from a mitochondrial DNA disorders [292]. And while the mitochondria from oocytes collected from twelve women showed few mtDNA rearrangements [287], there was considerable mutational heterogeneity in the individual oocyte donor. However, oocytes have an efficient mtDNA repair system, which is basically independent of maternal age [292]. It protects and perpetuates the maternal mitochondrial genome.

In addition, the process of oogenesis, follicle formation, and loss of the less fit purifies the female germ-line mitochondrial DNA [293]. This culling revives the mitochondrial genome as it passes, by way of the oocyte cytoplasm, from one generation to the next. This process provides the bottleneck that refines the haploid mitochondrial genome in the oocyte and maintains the integrity of maternal mitochondrial inheritance.

Cloning. The loss of non-oocyte mitochondria has also been detected in mammalian cloning that fused a somatic cell by electroporation to an enucleated oocyte from the same animal. It might be expected that the cloned progeny should contain mtDNA from both the donor and recipient, resulting in heteroplasmy. However, somatic cells in cloned sheep contain only the female germ-line mitochondrion [292]. How the donor cell mitochondria disappear just like sperm mitochondria is unknown. Possibly, contrary to the somatic cell mitochondria, oocyte mitochondria might carry some kind of recognition signal sequence that protects them from expulsion or rapid degradation.

As the first cloned sheep showed advanced biological age, it was suggested that this might be due to free radical-induced cellular damage to their inherited somatic mitochondria [294]. However, the premature aging of the cloned sheep is probably related to their shortened telomeres. Remarkably, the opposite was observed in cloned cows, where the telomeres in several cases were elongated, suggesting that cloning can also result in a prolonged life span.

Genetic diseases. 1. The problem with limited diversity. An important point in inherited mitochondrial diseases is the fact that populations with limited genetic diversity are at risk for diseases that are rare elsewhere. A case in point is the so-called "Finnish disease heritage" [295]. An analysis of mitochondrial mutations that have accumulated revealed that only a small number of men and women contributed to the genetic lineage present in the Finnish population. The mitochondrial genes examined were those of the mtDNA nucleotide positions that evolve slowly in the mitochondrial control region. For the nuclear genes, the Y-chromosomal haplotype population of the peroxisome proliferator-activated receptor gamma (PPARγ)-coactivator-1 (PGC-1) was analyzed [295]. PGC-1 coordinates the expression of both mitochondrial and nuclear encoded OXPHOS enzyme subunits and the uncoupling protein [103].

2. Heteroplasmy. In diseases caused by inherited mutations of mtDNA, the link between genotype and phenotype varies so that the same mtDNA mutation may give rise to a variety of phenotypes. Moreover, the same phenotype may be seen with different mtDNA mutations [145]. Differing points of view have been introduced to explain this phenomenon. For instance, it has been proposed that sporadic stem cell mutation during embryogenesis or mitotic segregation might result in different degrees of heteroplasmy in various tissues [20]. Heteroplasmy can also result from acquired mutations of mtDNA.

3. Mutations and deletions. A defect in the germline mtDNA affects all mtDNA plasmids in all mitochondria of all somatic cells and explains why inherited mtDNA diseases can be very serious. Mitochondrial DNA mutations causing overt mitochondrial diseases are characterized by a decline in mitochondrial respiratory function [184]. This has been demonstrated in cases of progressive kidney disease or maternally inherited diabetes and deafness, both caused by a single point mutation of the mitochondrial tRNA. Cybrid cells were prepared by inserting donor mitochondria into ρ^0 cells, which lack mtDNA. The mitochondria were derived from fibroblasts of a patient carrying an A to G transition at nucleotide position 3,243 of the mtDNA (Figure 11). The heteroplasmy in the cybrid cells varied from none to 100%. The cybrid cells containing predominantly mutant mtDNA showed marked defects in mitochondrial morphology, poor respiratory chain Complex I and IV activities, and lactic acidosis [296].

Similarly, cybrid clones were used to demonstrate mitochondrial respiratory chain dysfunction due to a single point mtDNA mutation in a case of familial hypertrophic ventricular cardiomyopathy [297]. A fibroblast cell line derived from the patient carried a T9997C (Figure 11) mutation of the mtDNA gene encoding tRNA glycine. The cybrid clones were obtained by fusion of ρ0 osteosarcoma cells to enucleated patient skin fibroblasts. The clones having high levels of heteroplasmy of mutant mtDNA showed mainly Complex I and cytochrome c oxidase deficiency. An elevated lactate/pyruvate (L/P) ratio corroborated the presence of respiratory chain deficiency.

More than 100 primary defects in the mitochondrial genome have been associated with encephalomyopathies and the majority has been linked to defective oxidative phosphorylation [298]. For instance, several mtDNA mutations cause rare encephalomyopathies such as Leigh and Leigh-like syndromes, fatal infantile lactic acidosis, neonatal cardiomyopathy with lactic acidosis, and macrocephaly

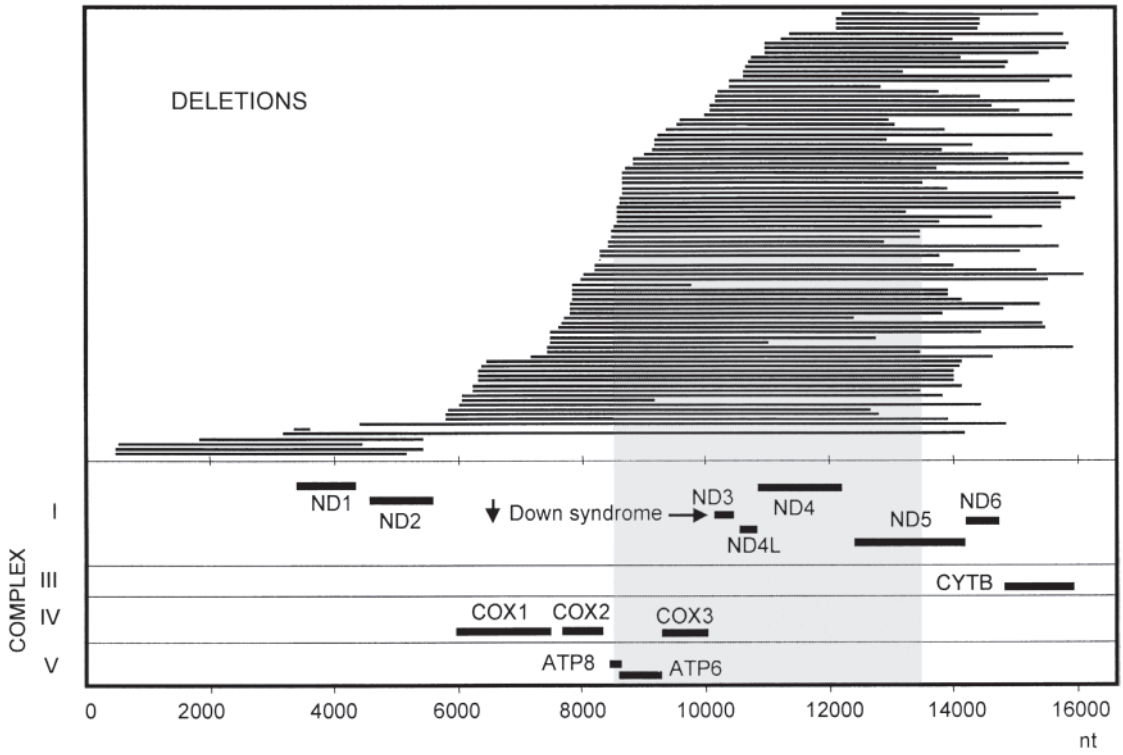

Fig. 11. Mitochondrial deletions (top) of the mitochondrial genome (bottom), which are illustrated in nucleotides (nt) in linear form along the x-axis. For clarity, the mitochondrial genes coding for subunits of oxidative phosphorylation (OXPHOS) enzyme Complexes I, III, IV, and V are shown in separate lanes. Genes that are partially or completely deleted by the 'common' deletion are highlighted. The map was prepared from numerical data downloaded from "MITOMAP: A Human Mitochondrial Genome Database. Center for Molecular Medicine, Emory University, Atlanta, GA, USA, http://www.gen.emory. edu / mitomap.html, 1999".

with progressive leukodystrophy [209]. Knowledge of the normal mtDNA variation in a specific human population is essential to understand the pathophysiology of diseases caused by an mtDNA aberration [299].

By comparison, mutated nDNA genes encode mitochondrial OXPHOS proteins that cause Friedreich's ataxia and hereditary spastic paraplegia [145]. In the case of amyotrophic lateral sclerosis and Huntington's disease, the defect of oxidative phosphorylation is secondary to events induced by a mutation in a nuclear gene encoding a non-mitochondrial protein [145].

Therapy

Several approaches have been used to treat mitochondrial diseases, ranging from dietary measures to administration of redox agents, vitamins, coenzymes, and enzyme activator [212].

Antioxidants limit lipid peroxidation and decrease prostaglandin synthesis [300]. The risk of oxygen-derived free radical damage can be significantly reduced by improved diet, particularly fruits, vegetables, and grains, by reasonable exercise, and by reduced alcohol consumption [301].

Q10. Therapeutic use of Q10 can benefit patients with cardiomyopathy. Determination of Q10 tissue levels in myocardial biopsies from a series of 45 patients with cardiomyopathy using high performance liquid chromatography (HPLC) showed significantly lower levels of the coenzyme in the cases of more advanced than in the milder cases of heart failure. Oral supplementation with 100 mg of Q10 daily benefited

nearly two-thirds of the patients. Those with dilated cardiomyopathy showed the most clinical improvement [29].

Administration of Q10 significantly reverses exercise-induced abnormalities of oxidative phosphorylation of the brain of patients with known mitochondrial enzyme defects. Magnetic resonance spectroscopy studies of such patients show that exercise increases the level of ADP and inorganic phosphate and reduces the level of phosphocreatine in the brain. Treatment with Q10 significantly shortens the rate of brain phosphocreatine recovery after the exercise and corroborates in vitro observations that Q10 concentration in the inner mitochondrial membrane regulates the efficiency of oxidative phosphorylation [302].

The liver mitochondria of the diet-induced atherosclerotic rabbit showed a significant increase in hydroperoxides and a serious drop in the content of Q10. Virgin olive oil or vitamin E-stabilized fish oil, but not sunflower oil, added to the diet in part reversed the diet-induced alterations [303]. Coenzyme Q10 (3 mg/kg/day) administered to rabbits fed an atherogenic diet significantly reduced aortic cholesterol and aortic as well as coronary artery plaque size [304]. When applied to the epidermis, Q10 penetrates into the viable layers. Weak photon emission measurements indicate that it reduces the level of epidermal oxidation. It prevents photoaging, suppresses the expression of collagenase, and reduces the wrinkle depth [305].

An adequate supply of vitamin B6 is essential for the endogenous synthesis of the quinone nucleus of coenzyme Q10 (Q10) from tyrosine. It should therefore be recommended that patients treated with Q10 should receive concurrent supplementation with B6 to enhance endogenous synthesis of Q10 [306].

Melatonin. A pineal hormone melatonin, N-acetyl-5-methoxytryptamine [307], lowers plasma cholesterol levels in genetically hypercholesterolemic rats. It also reduces the fatty changes in their liver [308]. Most importantly, melatonin concentrates in mitochondria. In rats fed a 1% cholesterol + 0.5% cholic acid diet daily, treatment with ip injections (4 mg) of the antioxidant melatonin for periods of up to 4 weeks lowered the diet-induced increases in plasma levels of VLDL and LDL. It decreased plasma HDL and diminished diet-induced fatty change of the liver [307].

Melatonin enhances the activity of Complexes I and IV and prevents ruthenium-red-induced reduction in the activities of these complexes in vivo, suggesting a therapeutic use of melatonin in drug-induced mitochondrial damage [309]. Melatonin can also attenuate ethanol-induced mitochondrial DNA depletion, which occurs after an alcohol binge [310].

However, the lack of purity of commercial melatonin preparations limits their use. For instance, 6 impurities detected in 3 commercial melatonin preparations are structural analogs of contaminants found in the dietary supplement L-tryptophan. These contaminants were implicated as etiologic agents in the eosinophilia-myalgia syndrome epidemic that occurred a few years ago [311].

Carnitine. Carnitine is essential for long-chain fatty acid oxidation and for shuttling accumulated acyl groups out of the mitochondria [312]. In the chronic alcohol-fed rat, oral L-carnitine by itself, but more so if administered with oral Q10, protects against alcohol-induced hepatic lipid infiltration [313].

In humans, therapy with carnitine ameliorates the symptoms of claudication of peripheral arterial disease. It restores skeletal muscle function of patients on dialysis who suffer from dialysis-induced reduction of muscle carnitine. However, while carnitine supplementation improves exercise performance in certain disease states, any benefit in healthy individuals to support the high metabolic demands of heavy exercise is uncertain [312].

Gingko biloba. By scavenging the superoxide anion, the *Ginkgo biloba* Egb761 extract provides post-ischemic protection against re-oxygenation injury to rat liver mitochondria in vitro [314]. Besides, treatment with Egb761 extract in part prevents the development of peroxide formation in the mitochondrion of the old rat [315]. Extract of *Ginkgo biloba* is anti-ischemic mainly due to the presence of bilobalide in the terpenoid fraction. Bilobalide delays hypoxia-induced decrease in ATP content of endothelial cells in vitro [316]. It increases the activity of Complex I, protects Complexes I and III activities and delays the onset of ischemia-induced damage. It permits respiratory activity and ATP regeneration by mitochondria under ischemic conditions as long as

some oxygen is present [317]. These insights were obtained using mitochondria isolated from rats treated with bilobalide (2-8 mg/kg). There was a dose-dependent increase in the respiratory control ratio, by reason of lower oxygen consumption during state 4. Bilobalide reduced the sensitivity of oxygen consumption to inhibition of Complex I by amytal or Complex III by myxothiazol or antimycin A. In Alzheimer's disease, therapy with recombinant human apoε3/ε3, apoe3/e4, dehydroepiandrosterone (DHEA), or *Ginkgo biloba* extract (EGb 761) protected against the lipid peroxidation [148].

Dietary fats. Consumption of fish oil lowers plasma triacylglycerol levels. There is evidence that the effect is due to increased mitochondrial fatty acid oxidation. As an example, in rat liver parenchymal cells and in purified mitochondria, eicosapentaenoic acid (EPA) increases mitochondrial fatty acid oxidation. It also increases carnitine palmitoyltransferase-I [318]. Supplementation of the diet with virgin olive oil to enrich the mitochondrial membranes with mono-unsaturated fatty acids and Q10 prevents free radical damage to heart mitochondria of male rats [319].

Genetic engineering. Genetic material can enter mitochondria in vivo under both physiological and pathological conditions. In vitro, double-stranded DNA crosses lipid bilayers doped with isolated mitochondrial porin that serves as a voltage-dependent anion channel. DNA crossing requires application of an electrical field to the membrane and is blocked by addition of anti-porin antibody [320]. This discovery opens up the possibility of mitochondrial genetic engineering by introducing new DNA into the mitochondrion to repair mtDNA defects. Besides, it might be possible to introduce DNA sequences that might bestow resistance to adult-onset diseases by preventing obesity and atherosclerosis or premature cognitive decline. For instance, a mitochondrial genotype, mt5178A, was recently identified in Japanese centenarians, suggesting that some mtDNA sequences might be more resistant to mutation than others [321].

To cure mitochondrial diseases due to mutated mtDNA, it has been proposed to introduce anti-mtDNA sequence-specific molecules into the mitochondrion, selectively inhibiting replication of mutated genomes and restoring health by permitting the propagation of only the wild-type mitochondrial DNA [322].

Conclusions

Mitochondrial medicine began with the description of Luft's syndrome in 1962. The field developed rapidly after the complete sequence of mitochondrial DNA was determined in 1981 and the methods of molecular biology were applied to study the role of the mitochondrion in the etiology of human diseases.

This unique organelle developed from endocytosed bacteria. Most of the bacterial genes ended up in the host cell nucleus that controls the biosynthesis of the present day mitochondrion. Only 37 genes remained as maternally-inherited mitochondrial DNA. A few of these genes code for the lipophilic, proton-pumping subunits of Complexes I, III, and IV of the electron transport chain, which establish the mitochondrial transmembrane potential that powers the synthesis of ATP. Hence, the expression of both genomes is essential for the proper biosynthesis and function of the mitochondrial oxidative phosphorylation system.

Populations with limited genetic diversity have high risk for rare mitochondrial diseases. Mitochondrial DNA has a much higher mutation rate than nuclear DNA, because it lacks histones and is exposed to radical oxygen species (ROS) generated by the electron transport chain, and the mitochondrial DNA repair system is limited. ROS-induced deletion of fragments of mtDNA promotes premature aging, and migration of the deleted fragments into the nuclear genome has been linked to carcinogenesis. When the amount of mtDNA with mutation or deletion in a cell reaches a tissue-dependent threshold, the cellular metabolism becomes critical and the cell undergoes necrosis.

In specialized tissues, mitochondria perform a number of diverse functions, but the common function in all mitochondria is the generation of ATP. The electron transport chain carries electrons from metabolites, which acts as a fuel, to oxygen, and stores the extracted energy as a membrane potential that is used as needed for ATP or heat generation. However, the oxidative phosphorylation system is vulnerable to structural and functional damage. For instance, a temporary lack of oxygen caused by ischemia and

reperfusion increases the generation of ROS. The membrane energy stores can be depleted by excessive membrane leakage due to damage by ROS or due to inappropriate membrane lipid content. Inhibition of enzymes by endogenous or exogenous compounds or lack of or inhibition of the electron carrier Q10, ANT, or other transporters can reduce ATP generation.

Acidic NSAIDs inhibit or uncouple oxidative phosphorylation and induce the "topical phase" of gastrointestinal ulcer formation. In vitro, vacuolating cytotoxin prepared from *H. pylori* decreases the mitochondrial inner membrane potential of the cultured gastric epithelial cell. If the bacterium causes uncoupling in vivo as well, it might induce the "topical phase," leading to ulcer formation even when a selective COX-2 inhibitor is administered. However, it has not yet been shown that *H. pylori* uncouples mitochondria in vivo. Aspirin inhibits β-oxidation, but not electron transport. However, the aspirin metabolite salicylate decouples mitochondria by inducing the mitochondrial permeability transition. Cocaine inhibits Complex I, the poliovirus inhibits Complex II, ceramide inhibits Complex III, and azide, cyanide, chloroform, and methamphetamine inhibit Complex IV. By contrast, melatonin stimulates Complexes I and IV and *Gingko biloba* stimulates Complexes I and III.

Up to a certain limit, a cell has the ability to counteract reduced synthesis of ATP by synthesizing more mitochondria, as is the case with moderate ethanol use. However, abuse of ethanol or therapy with AZT leads to inhibition of mtDNA replication.

Defective oxidative phosphorylation contributes to atherogenesis, diabetes, Alzheimer's disease, Parkinson's disease, and aging. In classical animal models of atherogenesis, cholic acid added to the diet promotes lesion formation. Cholic acid, like INF-γ, induces the formation of nitric oxide, which inhibits Complex IV. ApoE-null and LDL-receptor-null mice develop atherosclerosis on a diet not supplemented with cholic acid. In the latter case, overexpression of LPL reduces atherosclerosis.

In humans, the presence of apoE4 increases the risk of both atherosclerosis and Alzheimer's disease. A common feature is the reduced availability of high energy fuel to the oxidative phosphorylation system because of lack of (a) cellular uptake or (b) possibly the binding of apoE4 on intracellular liposome to the imported, apoE-binding part of Complex V. However, the second option has not been demonstrated experimentally.

Oral therapy with α-tocopherol and Q10 helps prevent atherosclerosis by protecting the LDL particle, the major carrier of Q10 in the plasma, from peroxidation. Lipoprotein uptake and intracellular synthesis provide Q10 for regulation of oxidative phosphorylation where Q10 is essential as it transfers electrons from Complexes I and II to Complex III. Importantly, statins that inhibit HMG-CoA-reductase lower plasma levels of cholesterol and Q10 because Q10 shares part of its isoprenyl side-chain synthesis pathway with cholesterol. Oral therapy with Q10 normalizes its plasma levels and as therapy can also improve cardiac function in cardiomyopathies.

Much useful knowledge has been revealed by recent research on oxidative phosphorylation. A few therapeutic alternatives are available. Avoiding substances that damage mitochondria and supplementation with compounds that protect mitochondrial structure and function are presently most important. There is a need to develop genetic engineering methods to repair or replace damaged mitochondrial DNA.

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