20 Aging as a Cardiolipin Disease That Can Be Treated

As background for our final statement, we return full circle to the Global Burden of Disease Study that states that a majority of patients in the future will require treatment for age-dependent diseases, especially ailments of an aging brain and nervous system (see Section I). Currently, many of these diseases are untreatable, and in the case of aging, the Food and Drug Administration (FDA) of the United States has yet to approve a single drug treatment. Indeed, according to the FDA, aging is not a disease to be cured. Thus, we are faced with a quandary since results of the Global Burden of Disease Study imply the opposite—many ailments of aging are diseases that need to be cured and will dominate healthcare in the twenty-first century. In this chapter we go a step further and evaluate evidence showing that age-dependent diseases and aging itself share much in common and might be treated by common drugs. That is, the molecular pathologies associated with aging and age-dependent diseases seem to converge on the same point. According to this unified concept, advances in understanding or treatments for age-dependent disease such as Parkinson's disease will likely apply to other ailments of aging and vice versa.

20.1 WORKING DEFINITION OF CARDIOLIPIN DISEASES

Mitochondria-targeted antioxidants are a specialized class of antioxidants designed to protect mitochondria against oxidative damage. So far this class of drugs has had only limited success in treatment of human diseases, but a new generation of mitochondria-targeted antioxidants is sparking excitement in the field of neurodegeneration and age-dependent diseases. For a review of mitochondria-targeted antioxidants used previously in human drug trials, see Smith and Murphy (2011), and for a review of the linkage of mitochondrial bioenergetics and Alzheimer's disease, see Calkins and colleagues (2012). Recently Ji and colleagues (2012) reported a critical test of the cardiolipin hypothesis of age-dependent diseases with implications for aging. These important data have two major consequences. First, these data provide the most compelling evidence yet that cardiolipin oxidation triggers neurodegeneration in rodent models. A causal relationship between cardiolipin and neurodegeneration was established using a mitochondria-targeted antioxidant that blocks oxidation of cardiolipin while preventing neurodegeneration. The second contribution is that these data illuminate the potential of mitochondria-targeted antioxidants in treatment of many human diseases. These data also suggest that many

catastrophic and untreatable human diseases might be defined in terms of a cluster of diseases with cardiolipin as the trigger—cardiolipin diseases. A second class of antioxidants targeting membranes in general, but especially mitochondrial phospholipids (including cardiolipin), has been shown in rodent models to protect parkinsonian neurons against oxidative death.

20.2 DEFINITIVE PROOF THAT DOUBLE BONDS OF POLYUNSATURATED MEMBRANE PHOSPHOLIPIDS CAN CAUSE OXIDATIVE DEATH OF CELLS

In Silicon Valley, California, a start-up company called Retrotope, Inc. is pioneering a new class of membrane-targeted drugs to treat neurodegenerative diseases (Hill et al., 2011, 2012; Shchepinov et al., 2011). A specific bioactive fatty acid derivative called deuterated linoleic acid (D-18:2) is found in small amounts in nature, but Retrotope, Inc. uses a chemically pure, deuterated derivative of D-18:2 in which D replaces a specific, facile hydrogen along the chain. By substituting deuterium in place of hydrogen on a specific location on the unsaturated fatty acid chain, Retrotope scientists are able to prevent oxidative damage from triggering a deadly chain reaction. These researchers initially used a yeast model to show for the first time that double bonds of 18:2 trigger oxidative death of yeast cells.

Yeast cells whose membranes are enriched with polyunsaturated fatty acids (PUFAs) such as 18:2 replacing monounsaturated fatty acids (MUFAs) such as 18:1 (Walenga and Lands, 1975) are known to be sensitized to killing by oxidative stress (Do et al., 1996). Lethality might be caused by increased rates of peroxidation of PUFAs triggering catastrophic oxidative damage to membranes and other cellular constituents. The previous correlative evidence supporting this model in PUFA-enriched yeast cells (which normally don't produce polyunsaturated fatty acids) is as follows:

- O_2 is required for killing of 18:2-enriched cells.
- Copper, a known catalyst for membrane peroxidation, greatly enhances cellular death.
- 18:3-enriched cells are more sensitive to copper-mediated killing than 18:2, with the trend 18:1 < 18:2 < 18:3 in increasing sensitivity.
- Reactive oxygen species (ROS) levels are elevated in PUFA-enriched cells.

These data are consistent with a mechanism in which peroxidation of PUFAs causes cellular death. Do and coworkers (1996) also reported that ubiquinone-deficient mutants of *Saccharomyces cerevisiae* are especially sensitive to PUFAs. This hypersensitivity of yeast to a genetic block in ubiquinone synthesis (Do et al., 1996) was initially explained by a role of reduced ubiquinone acting directly as an antioxidant. However, a possible alternative mechanism has now emerged. Solution of the structure of complex 1 of the respiratory chain (see Chapters 11 and 16) provides new insight into how ubiquinone-deficient mutants of yeast might become hypersensitive to killing by dietary PUFAs. According to the flavin site mechanism

of complex 1 developed by Pryde and Hirst (2011), any event that raises or lowers the mitochondrial NAD^+ pool may effect ROS production, which is closely linked to oxidative damage of mitochondrial membranes. Thus, a deficiency of ubiquinone is expected to decrease electron flow along the electron transport system of yeast mitochondria and increase NADH levels, resulting in hyperreduction of the high-energy electron site of complex 1. The net effect is formation of excessive amounts of ROS by complex 1, ultimately lethally damaging membranes. Death of a PUFA-enriched yeast cell might be the result of both an oxidative chain reaction propagated by PUFAs replacing MUFAs in the inner mitochondrial membranes and the loss of coenzyme Q acting as an antioxidant.

Recently scientists associated with Retrotope applied knowledge of the hallmark chemistry involving initiation and propagation of membrane peroxidation reactions to show definitively that PUFAs trigger lethal oxidative stress in yeast and how deuterated PUFAs incorporated into membrane phospholipids protect cells against oxidative death (Hill et al., 2011, 2012). These authors show that yeast cells enriched with polyunsaturated fatty acids deuterated at bis-allylic sites (Figure 20.1a) are protected against oxidative stress because of the isotope effect (Figure 20.1b) (Hill et al., 2011, 2012). The chemical rationale for the isotope effect on PUFA peroxidation is that the rate of reaction involving C-H bond cleavage is typically five to ten times faster than the corresponding C-D bond cleavage, due to the two-fold difference in the masses of H versus D. Deuterium effects on enzymatic lipoxidation reactions serve as a model to show that hydrogen abstraction is usually the rate-limiting step. For example, the reaction of soybean lipoxygenase-1 with its substrate, linoleic acid (18:2), displays very large kinetic isotope effects, predicting similar effects in membranes (Glickman and Klinman, 1995). The data of Hill and colleagues (2011, 2012) are a milestone in biochemistry of membranes since they demonstrate a causal relationship between oxidation of double bonds of PUFAs and cellular death. A model developed by Retrotope to explain how PUFAs trigger a lethal chain reaction is shown in Figure 20.2. The above models also explain how deuteration prevents both chemical peroxidation and enzymatic lipoxidation of cardiolipin.

20.3 DEUTERATED 18:2 (D-18:2) PROTECTS NEURONS AGAINST OXIDATIVE DEATH IN A MOUSE MODEL OF PARKINSON'S DISEASE

Isotope protection of yeast against polyunsaturation-mediated oxidative killing suggests a new approach to preventing ROS-induced membrane damage during aging and age-dependent diseases (Shchepinov, 2007; Shchepinov et al., 2011). For example, Parkinson's disease is thought to be caused in part by oxidative damage of mitochondrial membranes generating catastrophic oxidative damage in parkinsonian cells of the brain. Shchepinov and coworkers (2011) have tested this hypothesis with the result that deuteration provides isotopic protection against oxidative damage and provides partial protection against nigrostriatal injury in a mouse model of Parkinson's disease. The neurotoxin MPTP was used as a positive control in these experiments, where, as expected, this potent parkinsonian chemical, like paraquat,

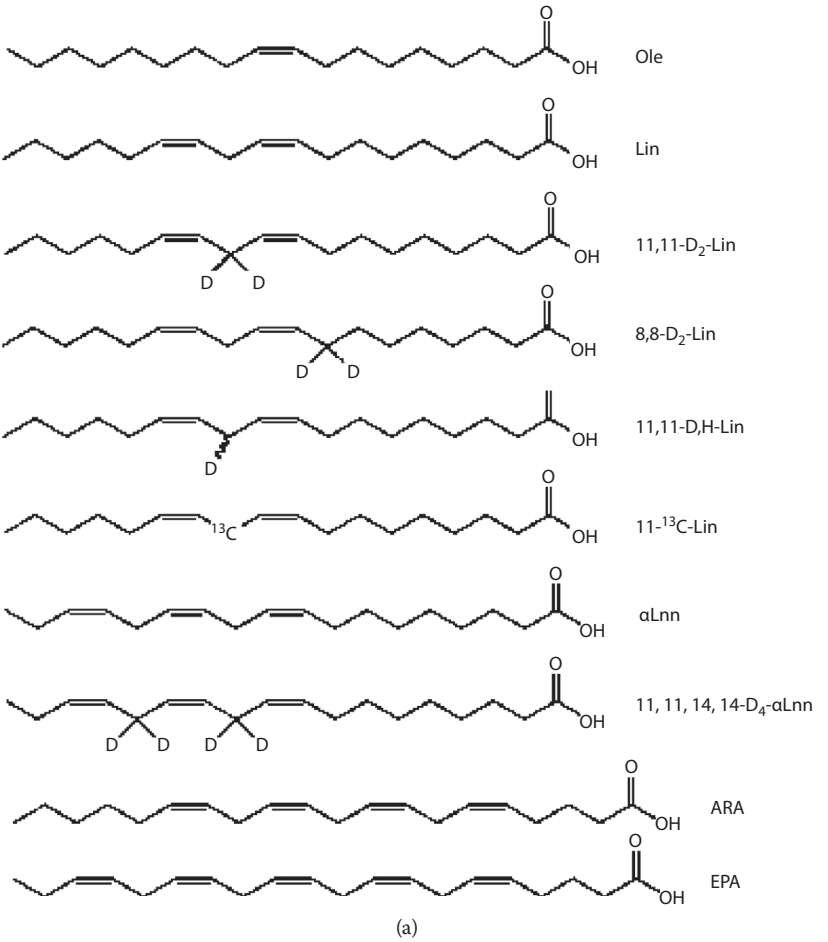
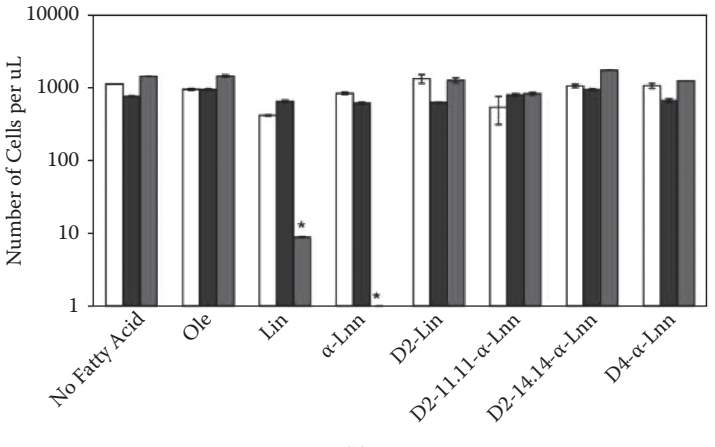


FIGURE 20.1 A battery of deuterium isotopes of fatty acids are being tested for protection of membranes against oxidative damage. (a) Structures of fatty acids used or being considered for deuterium reinforcement studies in yeast, mice, and eventually humans. Ole, oleic acid (18:1, cis-9-octadecenoic acid); Lin, linoleic acid (18:2, cis,cis-9,12-octadecenoic acid); 11,11-D_2-Lin (11,11-D_2-18:2; 11,11-D_2-cis,cis-9,12-octadecenoic acid); 8,8-D_2-Lin (8,8-D_2-18:2; 8,8-D_2-cis,cis-9,12-octadecenoic acid); 11,11-D,H-Lin (11,11-D,H-18:2; 11,11-D,H-cis,cis-9,12-octadecenoic acid); 11-^{13}C-Lin (11-^{13}C-18:2; 11-^{13}C-cis,cis-9,12-octadecenoic acid); αLnn, linolenic acid (18:3, cis,cis,cis-9,12,15-octadecenoic acid); 11,11,14,14-D_4-αLnn (D_4-18:3; 11,11,14,14-D_4-cis,cis,cis-9,12,15-octadecenoic acid); ARA, arachidonic acid; EPA, eicosapentaenoic acid. (From Hill et al., *Free Radic. Biol. Med.* 53:893–906, 2012. Copyright © 2012. Reprinted with permission from Elsevier.) *(continued)*

accelerates death of parkinsonian neurons (see Chapter 3). In the substantia nigra the number of nigral dopaminergic neurons following MPTP exposure in deuterium (D)-PUFA-fed mice is 79.5 percent of the control versus 58.5 percent of the control in (H)-PUFA-fed mice. Biochemical studies of dopamine levels in brain tissue of D-PUFA- versus H-PUFA-fed mice show significant protection by D-PUFA



(b)

FIGURE 20.1 ***(continued)*** A battery of deuterium isotopes of fatty acids are being tested for protection of membranes against oxidative damage. (b) Hill and coworkers (2011) reported a definitive experiment showing that oxidative damage to double bonds of polyunsaturated membranes kills yeast cells. Yeast cells (i.e., coq3 mutants) normally killed by alpha-linolenic acid or linoleic acid become remarkably resistant to toxicity when these membrane building blocks are deuterated. This effect is attributed to the isotope effect of deuterium replacing the facile hydrogen at the double bond, blocking fatty acid peroxidation. The three strains in each set are: wild type (first in panel), atp2 (second), and coq3 (third). (From Hill et al., *Free Radic. Biol. Med.* 50:130–38, 2011. Copyright © 2011. Reprinted with permission from Elsevier.)

of dopamine biosynthesis capacity. Retrotope researchers conclude that dietary D-PUFA partially protects against nigrostriatal damage from oxidative injury elicited by MPTP in mice. Thus, protection of PUFAs against peroxidation by introducing deuterium at the site of double bonds appears to be a universal mechanism explained by the isotope effect. From a fundamental perspective, these data show that oxidative stress can originate at the level of double bonds of unsaturated membrane fatty acid chains. These data have important implications in understanding and treating cardiolipin diseases. Note that the above data do not show directly that cardiolipin is being protected by the deuterium effect. However, in combining data from Retrotope with data discussed next, it seems likely that some of the deuterated fatty acid is incorporated into cardiolipin and protects it against oxidation.

20.4 NEW GENERATION OF ANTIOXIDANTS TARGETING MITOCHONDRIAL MEMBRANES SUPPRESS OXIDATIVE DAMAGE AND IMPROVE MITOCHONDRIAL FUNCTION IN A MOUSE MODEL OF HUNTINGTON'S DISEASE (HD)

XJB-5-131 represents a powerful new generation of mitochondria-targeted antioxidants that suppress oxidative damage to mitochondria (Xun et al., 2012). This bifunctional antioxidant features a mitochondrial membrane-targeting moiety conjugated to a

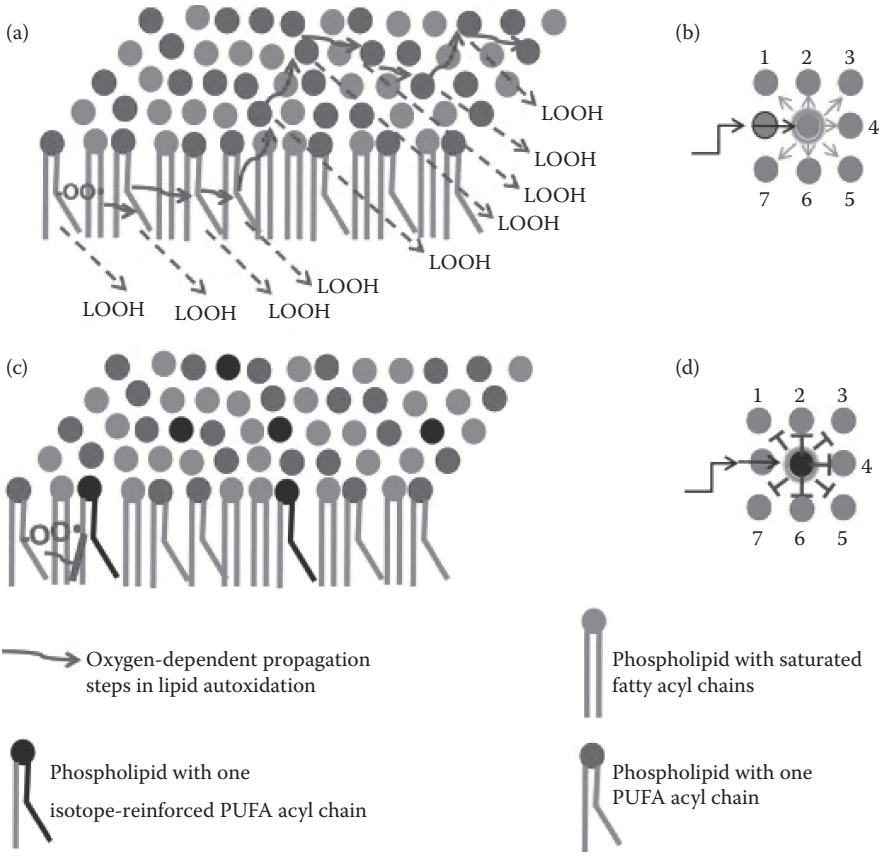


FIGURE 20.2 See color insert. Model to explain how deuterium-reinforced PUFAs limit the chain reaction of membrane lipid peroxidation. (a) A theoretical chain reaction is depicted in a membrane where a single initiation event producing a lipid peroxyl radical (denoted by –OO•) starts a chain reaction of lipid autoxidation that in the presence of O_2 may continue indefinitely (red arrows) and produce many molecules of lipid peroxides. Susceptible phospholipid molecules containing a PUFA acyl chain are designated by a kinked blue line and a red dot. (b) Propagation of PUFA autoxidation can progress by interaction with any neighboring PUFAs. (c) The presence of 20 percent isotope-reinforced PUFA (denoted by a black kinked line and a black dot) inhibits (or slows) chain propagation. (d) Propagation is inhibited for PUFAs neighboring the D-PUFA. (From Hill et al., *Free Radic. Biol. Med.* 53:893–906, 2012. Copyright © 2012. Reprinted with permission from Elsevier.)

radical scavenger. The membrane-targeting portion of the molecule is a modification of a peptide segment of the antibiotic gramicidin S that localizes to the mitochondrial membrane. XJB-5-131 might be more efficient than previous mitochondria-targeted antioxidants because its delivery to mitochondria does not depend on the potential gradient across the inner membrane. Instead, localization into the mitochondrial bilayer is directed by the lipophilic peptide segment. As a result, a wide spectrum of mitochondria, including energy-uncoupled mitochondria exhibiting

lower membrane potential, is targeted. A second advantage is that the efficacy of a membrane-localized antioxidant may be greater than that of earlier generations of mitochondria-targeted antioxidants that accumulate in the mitochondrial matrix.

Xun and colleagues (2012) show that XJB-5-131 enables suppression of motor decline, inhibits weight loss, reduces mtDNA damage, maintains mtDNA copy number, improves mitochondrial function, and enhances neuronal survival in a mouse model of HD. These data imply that specific targeting of this synthetic antioxidant to mitochondrial membranes has beneficial effects at both the cellular and whole animal level. These findings set the stage for testing of efficacy in a broader set of mitochondrial-mediated diseases and premature aging phenotypes. Xun and coworkers (2012) do not deal with the question of whether a specific molecular species of phospholipid in mitochondrial membranes is being protected by XJB-5-131. However, this topic is covered in a separate paper by Ji and colleagues (2012), discussed next.

20.5 CARDIOLIPIN OXIDATION MEDIATES NEURON DEATH DURING TRAUMATIC BRAIN INJURY IN RATS, AND MITOCHONDRIA-TARGETED ANTIOXIDANT XJB-5-131 PROTECTS CARDIOLIPIN AND PREVENTS APOPTOSIS

A News and Views article by Chan and Di Paolo (2012) in the journal *Nature Neuroscience* describes an important study by Ji and colleagues (2012) concerning the role of cardiolipin as a trigger point for traumatic brain injury (TBI). The general public in the United States has recently been exposed to news reports on TBI as the likely cause of dementia in popular sports figures, especially in professional football and other contact sports, including boxing and soccer. Chan and Di Paolo (2012) point out that in Europe and North America alone, about 3 to 4 million cases of TBI occur, with a fatality rate of 3 to 6 percent. The hidden cost may turn out to surpass that of initial treatment because TBI likely resets and shortens the normal pacemaker of the brain span, resulting in premature onset, by about a decade or two, of dementia. That is, following trauma, TBI is like a ticking time bomb in the brain, giving no visible symptoms or changes that can yet be detected before classic symptoms of neurodegeneration are diagnosed. There is an alarming similarity between TBI-induced events and the effect of aging on brain biochemistry. Following TBI, various forms of neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, can emerge early. Even substance abuse and psychiatric disorders can be linked to TBI (Chan and Di Paolo, 2012). Figure 20.3 provides an overview of the molecular pathology associated with TBI that can result from blows or jolts to the head from falls, contact sports, motor vehicle accidents, assaults, and during warfare. Figure 20.3 shows that the primary injury sets in motion a complex cascade(s) of molecular and cellular events that can kill neurons and cause dementia. Figure 20.3 is based on data by Ji and colleagues (2012). The data of these authors support a causal relationship between oxidative damage to cardiolipin and TBI. In essence, CL enriched in the inner mitochondrial membrane acts as a tipping point determining the life or death of neurons after TBI

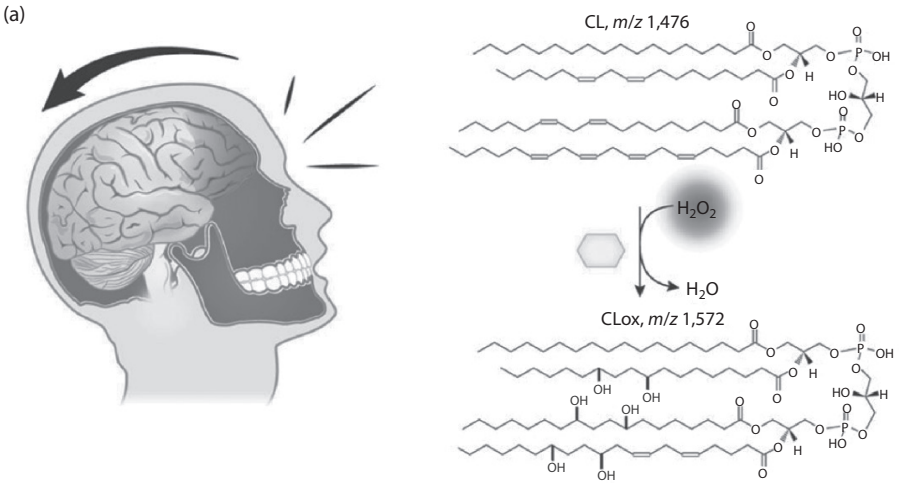


FIGURE 20.3 See color insert. Cardiolipin (CL) as trigger molecule in the molecular pathology of traumatic brain injury (TBI) and discovery of mitochondria-targeted antioxidants protecting mitochondria. (a) Inflammation following TBI causes oxidation of highly unsaturated molecular species of cardiolipin, resulting in dysfunctional derivatives designated CLox. *(continued)*

and suggest a new therapeutic avenue. Once again, the many years that may elapse between the initial TBI and visible pathological events show that the TBI-induced cascade has earmarks of an age-related disease or aging itself.

Ji and coworkers (2012) provide the best evidence yet that cardiolipin, enriched in and being the signature lipid in the inner mitochondrial membrane (Figure 20.3a), is a key mediator of the cascade linking TBI to neuron death. As discussed in previous chapters, the four acyl chains of human CL designated as $(18:2)_4$-CL are characteristic of human mitochondrial membranes, with more highly unsaturated chains being relatively rare. In contrast, CL from many organisms, including rodents, contains polyunsaturated chains ranging from 20:4 (arachidonic acid) to 22:6 (DHA). These data complicate extrapolation from rodents to man. That is, the inner mitochondrial membrane of mice or rats is far more readily oxidized than that of humans, or conversely, human mitochondrial membranes are more saturated and perhaps better shielded against oxidative damage than those of rodents.

The cascade triggering cardiolipin diseases is currently being unraveled at the molecular level. Cytochrome c is part of this cascade and normally behaves as a critical electron carrier shuttling electrons from complex 3 to complex 4 of the mitochondrial electron transport chain. Cardiolipin associates with cytochrome c via negative charges on the CL head group and through hydrophobic forces, effectively keeping cytochrome c anchored to the outer leaflet of the inner membrane. Oxidatively damaged CL has been proposed to trigger the mitochondrial switch from ATP generation to apoptosis initiation. Oxidized CL (CLox) can be generated chemically by peroxidation or by a reaction catalyzed by oxidatively damaged cytochrome c, at which point this electron carrier switches to being a fatty acid peroxidase (Abe

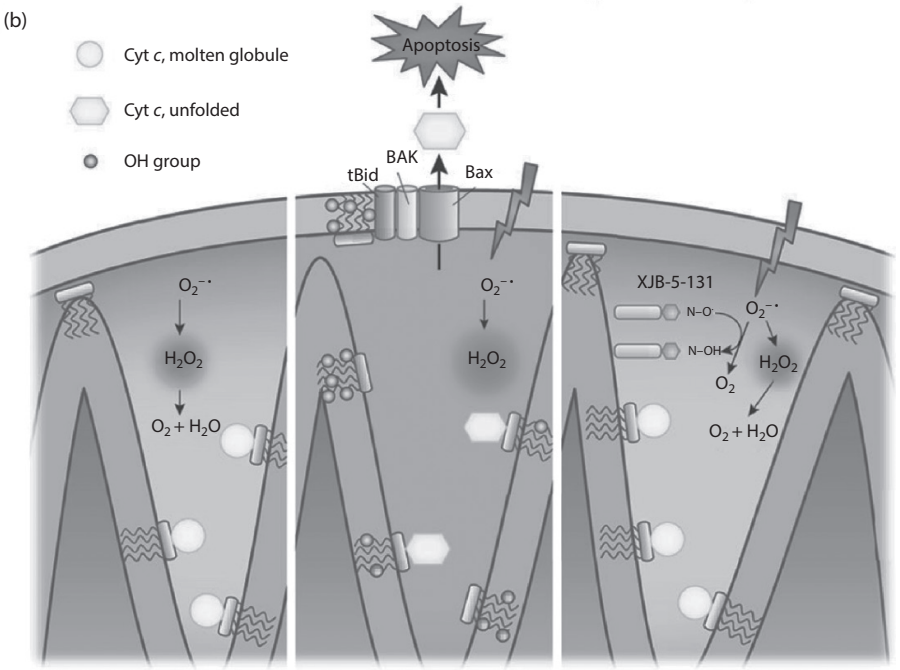


FIGURE 20.3 ***(continued)*** **See color insert.** Cardiolipin (CL) as trigger molecule in the molecular pathology of traumatic brain injury (TBI) and discovery of mitochondria-targeted antioxidants protecting mitochondria. (b) Left panel shows reactive oxygen species such as H_2O_2 being detoxified and maintained at levels below a critical threshold for initiating a chain reaction. In the middle panel TBI overwhelms defenses against reactive oxygen species, unleashing an oxidative chain reaction propagated by oxidatively damaged CL. Oxidative membrane damage is so severe that membrane-bound cytochrome c is damaged and converted to its peroxidase derivative, further damaging the mitochondrial membrane. Cytochrome c escapes to the cytoplasm, triggering apoptosis. Right panel shows the antioxidant XJB-5-131 being targeted to the inner mitochondrial membrane. This membrane-targeted antioxidant intercepts sufficient numbers of superoxide radical ($O_2^{-\cdot}$), allowing superoxide dismutase to form H_2O_2, which is degraded by catalase yielding O_2 and H_2O. Thus, with the help of the membrane-targeted antioxidant, an oxidative chain reaction leading to cellular death is prevented. Note that the mechanism shown in the right panel can be generalized to cover oxidative stresses generated by aging, neurodegeneration, cancer, chronic inflammation, and mitochondrial diseases as well as TBI. (From Chan and Di Paolo, *Nat. Neurosci.*15:1325–27, 2012. Copyright © 2012. Reprinted by permission from Macmillan Publishers Ltd.)

et al., 2011; Patriarca et al., 2012). Fatty acid peroxidases can be highly active, to the point of producing CLox faster than cellular defensive mechanisms can detoxify this dysfunctional phospholipid. Accumulation of CLox results in translocation of more of this defective lipid from the inner to the outer membrane, where its presence recruits the pro-apoptotic Bcl-2-related proteins tBid, BAK, and Bax, opening mitochondrial transition pores. At the same time, oxidation of cardiolipin weakens the association between cytochrome c and CL, releasing cytochrome c from the

inner membrane, permitting escape from the mitochondrion through transition pores of the outer membrane. The presence of cytochrome c in the cytoplasm triggers the apoptosis cascade through sequential interaction with apoptosis protease activator protein-1 (Apaf-1) and caspases 9 and 3. This picture of CL's contribution to apoptosis discussed above served as background for the groundbreaking research by Ji and colleagues (2012), who are focused on curing cardiolipin diseases.

Ji and colleagues (2012) used advanced lipidomics methodology (Samhan-Arias et al., 2012) to show that CL was subject to oxidation, resulting in the generation of 150 different degradation products, including chain-shortened derivatives. In contrast, conventional phospholipids present in the same membrane, including phosphatidylcholine and phosphatidylethanolamine, showed little damage, consistent with the view that CL in mitochondria of rodents has extraordinary sensitivity to oxidation. The amount of CLox increased about twenty-fold, while a similar amount of native CL as substrate for oxidation was consumed. The data confirm that acute brain injury caused by TBI activates CL oxidation. These data open a new window for screening drugs that, after passing across the blood-brain barrier, are able to target brain mitochondria, prevent cardiolipin oxidation, and relieve disease symptoms (Figure 20.3b, right).

Above all, the paper of Ji and colleagues (2012) highlights the role of oxidative damage of cardiolipin in triggering apoptosis and the importance of mitochondria-targeted antioxidants in blocking oxidation of CL. However, this paper does not cover the important linkage between energy stress and aging. Their data do provide clues that energy stress might be involved. TBI-induced peroxidation of CL results in 150 different oxidative products, some of which likely remain in the membrane as dysfunctional lipids. As discussed in Chapters 8 and 13 and in previous books (Valentine and Valentine, 2009, 2013), data generated using chemically defined lipid vesicles and from yeast show clearly that asymmetrical phospholipids such as chain-shortened derivatives of CL oxidation can act directly as potent energy uncouplers of proton gradients. Yeast, for example, naturally produces chain-shortened, saturated phospholipids during anaerobic growth for incorporation and fluidization of its membranes. These phospholipids feature a range of short acyl chains, from C-8 to C-12, paired with longer 18:0 chains in phospholipids, and when incorporated into mitochondrial membranes, they act as uncouplers of proton electrochemical gradients. Also, chemically defined lipid vesicles containing phospholipids formed with acyl chains of C-12 fatty acids are highly permeable to protons, in contrast to lipid vesicles formed from 18:0 and other long-chain fatty acids. Recent data suggest that truncated or chain-shortened membrane phospholipids are major oxidation products formed during TBI-induced peroxidation of CL. We propose that these chain-shortened phospholipids of CL act as energy uncouplers in human mitochondria, supporting the view that oxidative stress and energy stress act in synergy. Both of these powerful stresses might be prevented by membrane-targeted antioxidants.

20.6 SUMMARY

In the 1950s Denham Harman visualized the chemical power of oxidative chain reactions occurring in polyunsaturated membranes as a cause of aging. Later in his

career, Harman applied free radical theory in his attempt to understand the role of oxidative chemistry as a possible cause of Alzheimer's disease. Now more than half a century later, two independent research groups report that targeting antioxidants to polyunsaturated mitochondrial membranes of rodents prevents neuron death, with major implications not only for preventing neurodegeneration, but also for treating harmful effects of aging and age-related diseases, including cancer.

Obviously, there are many hurdles to be overcome before discoveries related to protecting cardiolipin in rodent models can be applied in human medicine. Recall that each day billions of human cells, especially epithelial cells such as those lining the colon, naturally senesce to be replaced by a new layer of cells. If senescing cells are not dispatched cleanly, colon polyps can arise and eventually cause colon cancer. An increasing amount of data show that cardiolipin acts as a natural assassin for dispatching lingering colon cells. Thus, the risks of cardiolipin discussed above become a benefit in preventing colon cancer. This benefit might be lost if peroxidation is hindered by drugs such as the antioxidants described here. However, the simple addition of fish oil in the diet might ensure that cardiolipin maintains its potency as a natural oxidative killer of senescing colon cells (see Valentine and Valentine, 2009, for overview and references). The blood-brain barrier represents another major hurdle in developing mitochondria-targeted antioxidants for treating neurodegeneration, by limiting entry of drugs. Interestingly, both deuterated fatty acids and the experimental antioxidant used by Ji and colleagues (2012) exhibit hydrophobic properties facilitating their passage across the blood-brain barrier. Another concern is that the highly unsaturated molecular species of cardiolipin of rodents is atypical when compared to more saturated species of CL found in mitochondrial membranes of humans. A model developed by Hill and coworkers (2012) (Figure 20.2) shows how human $(18:2)_4$-CL might ignite an oxidative chain reaction, spreading damage to the cell. Data by Roginsky (2010) support the model proposed by Hill and colleagues (2012) and show that the rate of peroxidation of $(18:2)_4$-CL is about double that of the methyl ester of 18:2. Also, the rate of $(18:2)_4$-CL oxidation and subsequent pathological effects would be expected to skyrocket after cytochrome c is converted to its peroxidase form in oxidatively damaged mitochondrial membranes.

It is now clear that cardiolipin contributes at least two essential benefits to the cell. The first involves bioenergetic gain, and the second is beneficial pruning of cells during growth and development. In a strange twist of nature, the benefits of cardiolipin are balanced against a potentially lethal risk in which the beneficial role of CL in pruning cells is hijacked, resulting in disease pathologies—cardiolipin diseases. It is safe to say that many secrets of cardiolipin structure and function remain to be discovered.

Ending on a positive note, the availability of several classes of promising mitochondria-targeted antioxidants represents a major milestone toward understanding and treatment of age-dependent diseases and ailments of aging. Ji and colleagues (2012) suggest that windows for drug intervention in the case of TBI might be split, first at the acute phase and later during the long incubation period leading to premature dementia. This opens up the option that one class of targeted antioxidants might be effective when administered immediately after TBI, but only for a short time period. In contrast, a separate membrane antioxidant might be developed when

prolonged treatment is required. Ultimately, a cocktail of mitochondria-targeted antioxidants might be developed against a wide range of currently untreatable diseases. We suggest that cardiolipin-targeted antioxidants show enough promise, underpinned by solid science, to be considered a backup to the widely acclaimed human trials involving humanized antibodies that remove beta-amyloid plaque from the brain. Data are expected in the next five years from current human trials testing the amyloid hypothesis. Many neuroscientists are eagerly anticipating the results of these trials, the results of which will determine whether new research and treatment strategies might be needed.

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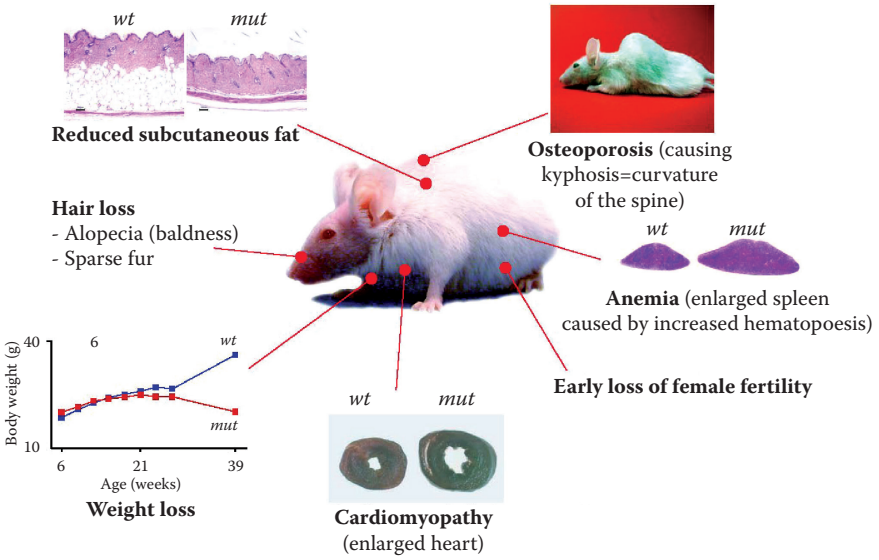
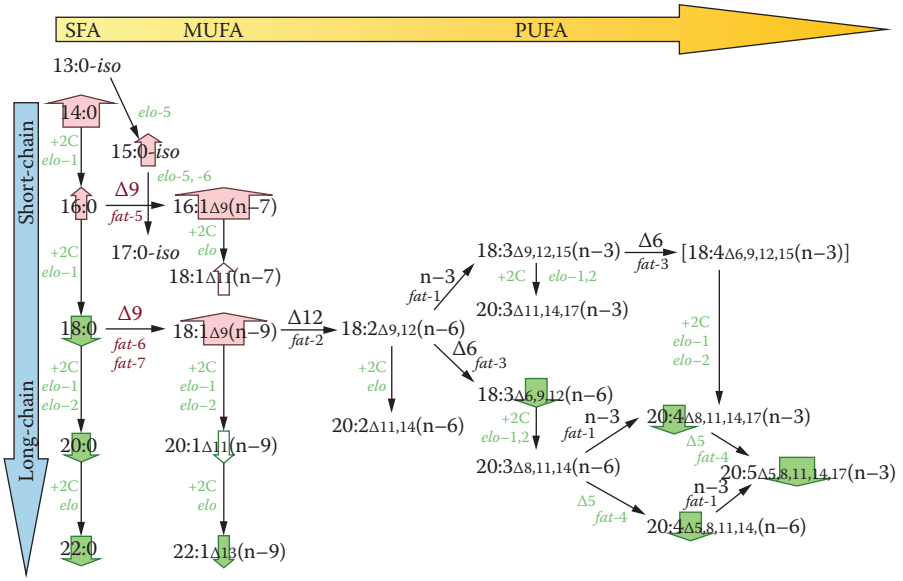


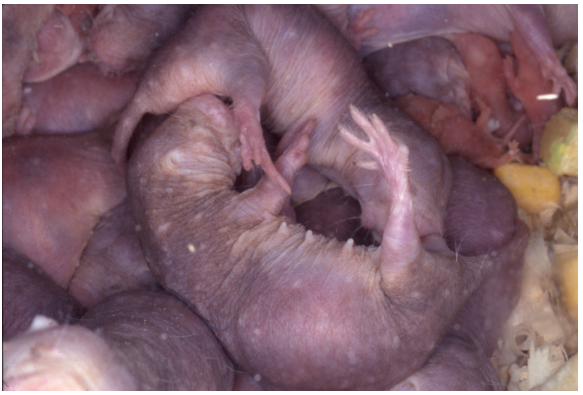
COLOR FIGURE 1.1 Aging phenotypes in mtDNA mutator mice. The mtDNA mutator mouse is genetically engineered to express a proofreading-deficient version of the mtDNA polymerase. This "mutator gene" leads to a three- to five-fold increase in somatic point mutations of mitochondrial DNA, an occurrence of a linear deleted mtDNA molecule, a progressive respiratory chain dysfunction, an expression of a variety of premature aging phenotypes, and a shortened life span. Humans harboring a naturally occurring mutator gene display numerous pathological phenotypes, as discussed in Chapters 8 and 9. (Reprinted from Edgar et al., *Cell Metab.* 10:131–38, 2009. Copyright © 2009. With permission from Elsevier.)

(a)

COLOR FIGURE 2.1 Paraquat catalyzes formation of reactive oxygen species (ROS), which can kill plants and humans and cause Parkinson's disease. (a) Photo of a corn leaf with lesions bleached by tiny droplets of paraquat drifting on the wind following spraying of a nearby weed field. (Photo used with permission from Dr. Kevin Bradley at the University of Missouri.)

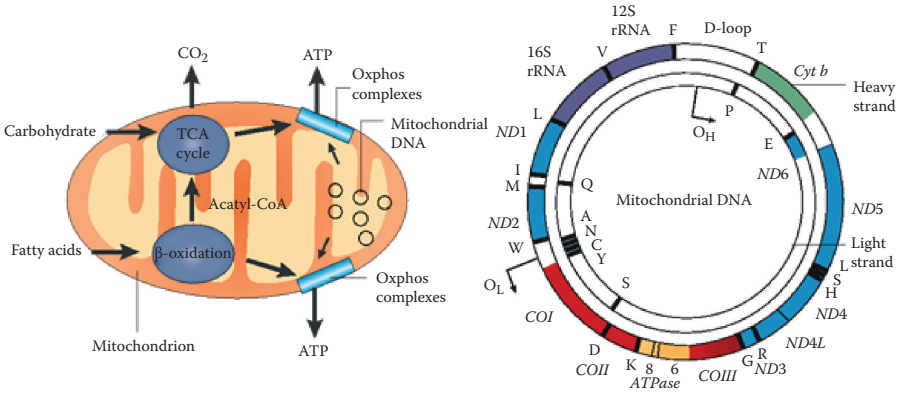


COLOR FIGURE 4.2 Regulatory patterns of biosynthesis of EPA and other unsaturated fatty acids linked to aging. See Shmookler Reis and colleagues (2011) for a detailed explanation of regulatory patterns of fatty acid synthesis in *C. elegans*. Three points are highlighted here: (1) Black arrows show the overall pathway of membrane fatty acid biosynthesis leading to EPA. (2) Enzyme activities (+2C for elongases; Δn, n-3, or n-6 for desaturases) and their genes are shown beside each arrow. (3) Bold (dark) font over the fatty acid classes indicates up- or down-regulation with increasing life span. The width of the arrow corresponds to the strength of the correlation to longevity. (Shmookler Reis et al., *Aging (Albany NY)* 3:125–47, 2011. Copyright © 2011 Shmookler Reis et al.)

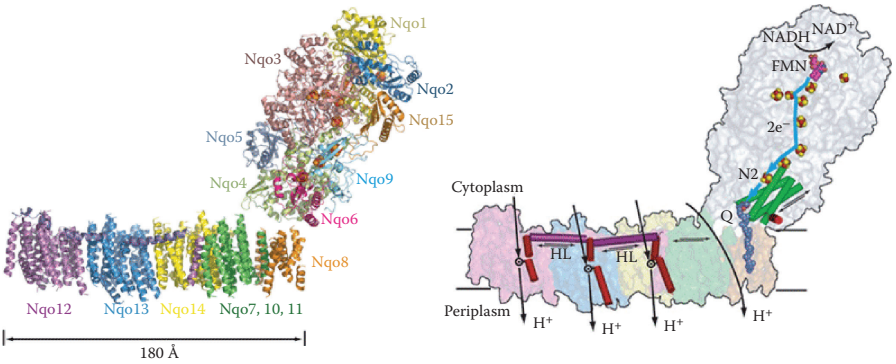


COLOR FIGURE 6.2 Naked mole rat queen shown with one of her several consorts and other members of her colony. Like queens of social insects, including ants, a single naked mole rat queen gives birth to all individuals in her colony. This mouse-sized rodent outlives mice by almost ten times. A combination of diet, genes, and a unique underground environment is proposed to account for the cancer-free life and extraordinary longevity of this South African rodent. (From van der Horst et al., *BMC Evol. Biol.* 11:351, 2011.)

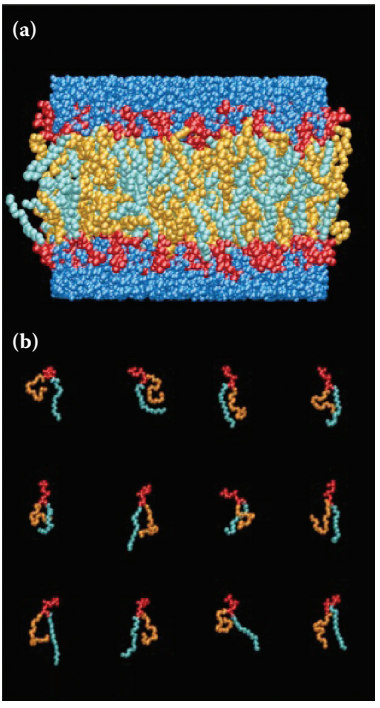
COLOR FIGURE 7.1 Grandparents and great grandparents emerged late in human evolution. Recent archeological data suggest that prior to 30,000 years ago life spans were too short to achieve grandparenthood. Genetic, sociological, and environmental changes that might have enabled a spike in human longevity remain a mystery. From a genetic perspective, it is now known that mutations in one or a small number of genes or DNA regulatory elements are able to enhance longevity of animals, raising the possibility that longevity genes are important in the evolution of human life span. The photo shows four generations in the family of Carla Valentine, MD: Carla with daughters Stella (held), Isabella, and Sienna, and Carla's mother and grandmother. (Courtesy of David L. Valentine, PhD.)



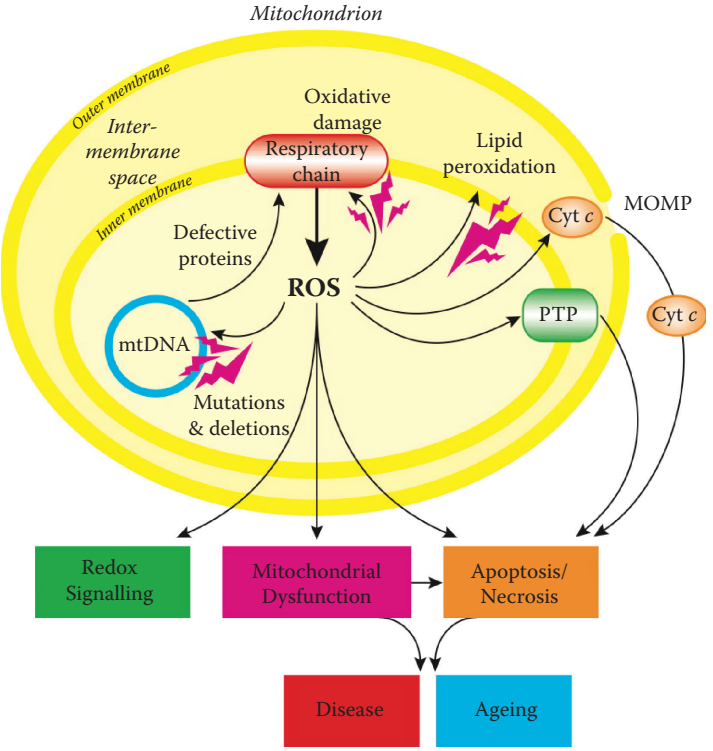
COLOR FIGURE 8.1 Human mitochondrial DNA (mtDNA) is a small, circular double helix encoding several essential energy-producing genes. The genes that encode the subunits of complex 1 (ND1–6 and ND4L) are shown in blue; the terminal complex, cytochrome c oxidase (COI-COIII), is shown in red; cytochrome b of complex 3 is shown in green; and the subunits of ATP synthase (ATPase 6 and 8) are shown in yellow. RNA genes are also listed (purple and black slashes). (From Taylor and Turnbull, *Nat. Rev. Genet.* 6:389–402, 2005. Copyright © 2005. Reprinted by permission from Macmillan Publishers Ltd.)



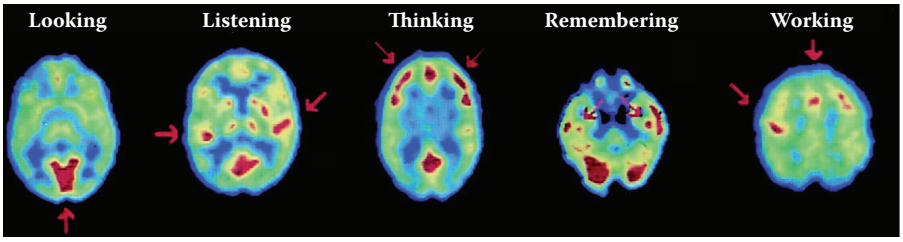
COLOR FIGURE 9.1 Structure of chair-shaped complex 1 showing numerous subunits (left) and topology of electron flow (right). Note that electrons flow from NADH as donor through iron-sulfur centers located in the long arm of the enzyme extending into the mitochondrial matrix. The proton-pumping portion of the enzyme is embedded in the membrane. High-energy electrons flowing from NADH to ubiquinone lose energy while energizing the efflux of protons. Complex 1, composed of forty-five different subunits, is by far the most complicated member of the electron transport chain and is a major target of mutations occurring in mtDNA. Complex 1 is the largest enzyme whose structure has been solved, and this achievement opens a new window for understanding membranes' contribution to longevity. (From Efremov et al., *Nature* 465:441–45, 2010. Copyright © 2010. Reprinted by permission from Macmillan Publishers Ltd.)



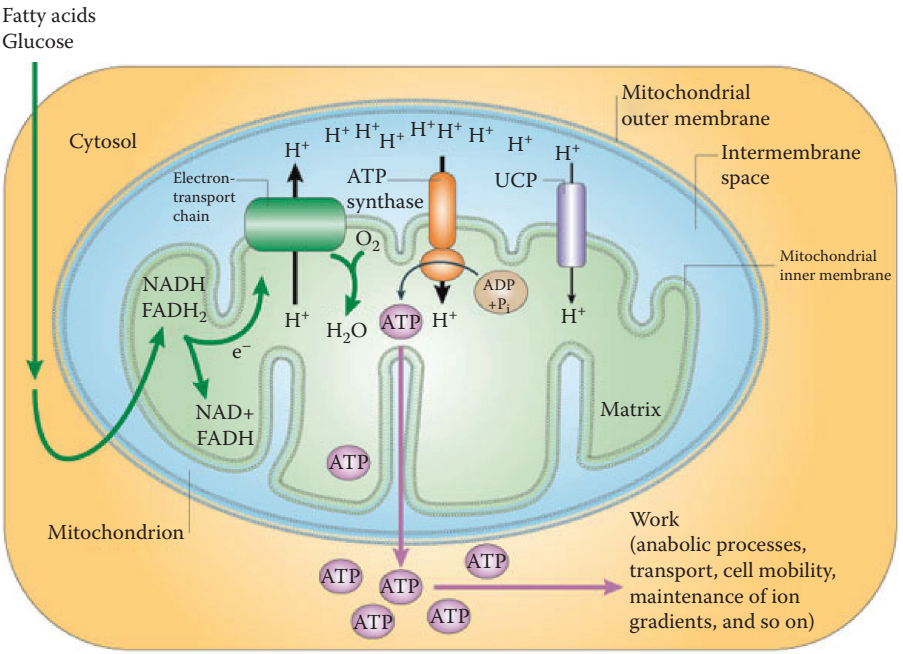
COLOR FIGURE 10.1 Extraordinary conformational dynamics of DHA. (a) DHA-enriched membrane. (b) Dynamic conformations of DHA phospholipids. (Images courtesy of Scott Feller, and generated by Matthew B. Roark, both of Wabash College.)



COLOR FIGURE 12.1 Overview of major cellular targets of oxidative damage. This diagram shows that electron leaks from the respiratory chain of mitochondria form ROS, which damage membrane lipids, generate defective proteins, and act as signaling molecules for opening permeability transition pore (PTP) and releasing membrane-bound cytochrome C (cytC). (From Murphy, *Biochem. J.* 417:1–13, 2009. Copyright © 2009, The Biochemical Society. Reproduced with permission.)

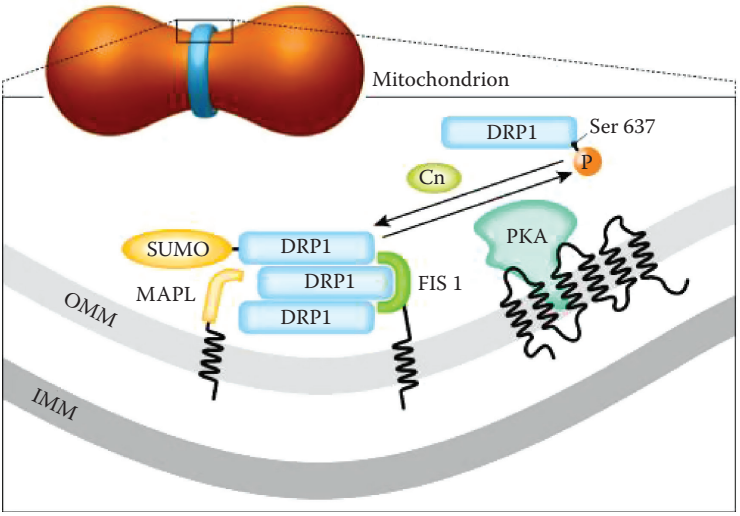


COLOR FIGURE 15.1 Positron emission tomography (PET) shows that metabolic activity and respiration in different regions of the brain are activated by different mental tasks. Since glucose consumed as a major energy source must be tightly coupled to lactate utilization by mitochondria, these data suggest that oxygen consumption and levels are also variable. Although a robust circulatory system feeds large amounts of oxygen to the brain, vast numbers of neurons and astrocytes use much of the oxygen, with the overall effect being a differential lowering of oxygen levels throughout the brain. (From Phelps, *Proc. Natl. Acad. Sci. USA* 97:9226–33, 2000. Copyright © 2000, National Academy of Sciences, U.S.A.)

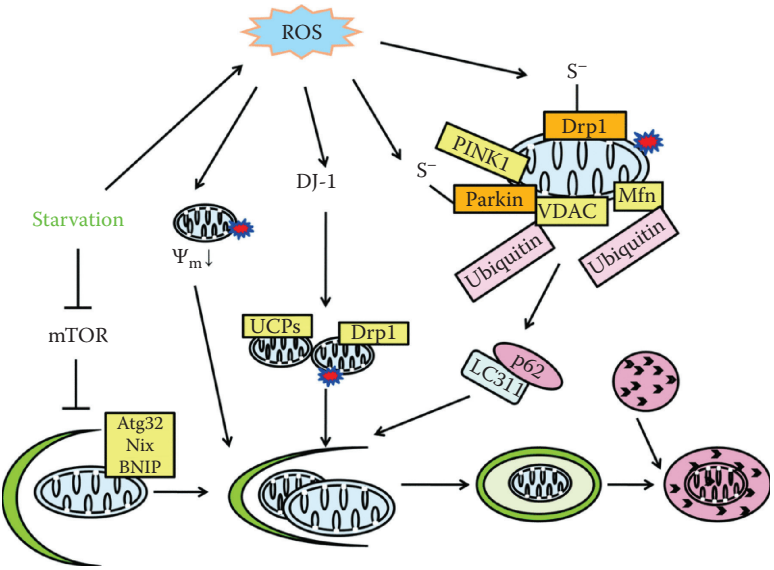


(a)

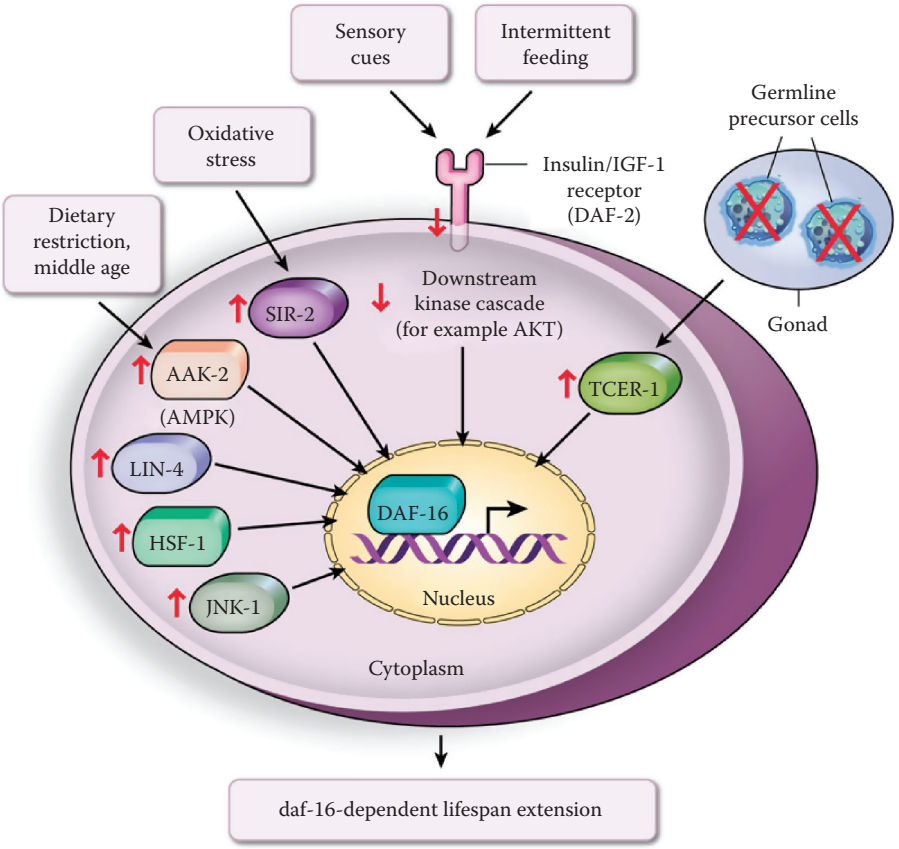
COLOR FIGURE 16.1 UCPs 2–5 dissipate energy held in proton gradients and help protect the polyunsaturated fatty acid (PUFA)-enriched inner mitochondrial membrane against an oxidative chain reaction. (a) Diagram integrates mitochondrial bioenergetics and shows that UCPs act to uncouple the proton electrochemical gradients of mitochondria. See text for details. (From Krauss et al., *Nat. Rev. Mol. Cell. Biol.* 6:248–61, 2005. Copyright © 2005. Reprinted by permission from Macmillan Publishers Ltd.)



COLOR FIGURE 17.1 Diagram showing mechanism of formation of a fission "collar" generated by dynamic trafficking of dynamin-like protein DRP1 to the site of fission. A putative network regulating DRP1 accumulation and assembly is shown, in which DRP1 translocation is controlled by calcineurin-mediated dephosphorylation of Ser 637. Mitochondrial PKA (phosphokinase) then rephosphorylates the same site, pushing DRP1 away from the organelle. MAPL-mediated SUMOylation stabilizes DRP1 on mitochondria and might prevent its retranslocation to the cytoplasm. FIS1, fission 1; IMM, inner mitochondrial membrane; MAPL, mitochondrial-anchored protein ligase; OMM, outer mitochondrial membrane; PKA, protein kinase A; SUMO, small ubiquitin-like modifier. [Reprinted by permission from Macmillan Publishers Ltd: *EMBO Rep.* 10:694-6. Scorrano, L. and D. Liu. The SUMO arena goes mitochondrial with MAPL, © 2009.]

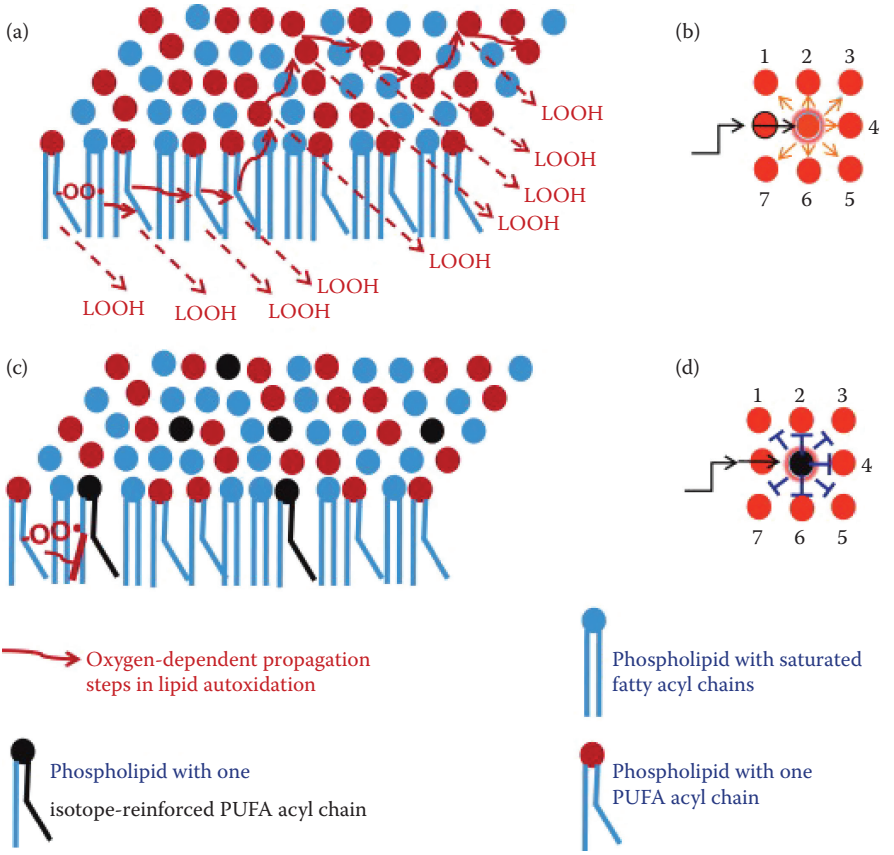


COLOR FIGURE 18.2 Oxidative stress can activate mitophagy. An elaborate global signaling network allows mammalian cells to tag and eliminate defective or unwanted mitochondria by the process of mitophagy. General signals include oxidative stress and starvation; specific signals include signaling proteins or modification of mitochondrial proteins. In yeast, Atg32 is specific for mitophagy and acts by targeting mitochondria to the autophagosome. In mammalian cells, Nix is involved in mitochondrial clearance during maturation of erythrocytes. During energy stress, when ATP levels drop, AMPK is activated and phosphorylates ULK1 and ULK2 (both Atg1 homologs) in turn activate mitophagy and general autophagy. Parkinson's disease genes encoding a-synuclein, parkin, PINK1, and DJ-1 are all involved in mitophagy. A decrease in mitochondrial membrane potential (ψ_m) can be induced by ROS and by targeting a-synuclein to the mitochondria. A depression in mitochondrial membrane potential serves as a signal for mitophagy. In addition to a decrease in membrane potential, mitochondrial fission is another signal for mitophagy. PINK1 facilitates parkin targeting to the mitochondria and ubiquitinates the mitochondrial outer membrane VDAC. Ubiquitinated VDAC can be recognized by p62 to initiate mitophagy. DJ-1 senses oxidative stress and serves as a parallel pathway to maintain mitochondrial membrane potential and preserve mitochondria from fragmentation. Many of the regulators of mitophagy can be modulated by ROS. For example, a-synuclein is nitrated and, as a consequence, increases aggregation propensity. Parkin can be sulfonated and S-nitrosated. Drp-1 S-nitrosation is also involved in regulation of mitochondrial fission and associated induction of mitophagy. (From Lee et al., *Biochem. J.* 441:523–40, 2012. Copyright © 2012, The Biochemical Society. Reproduced with permission.)

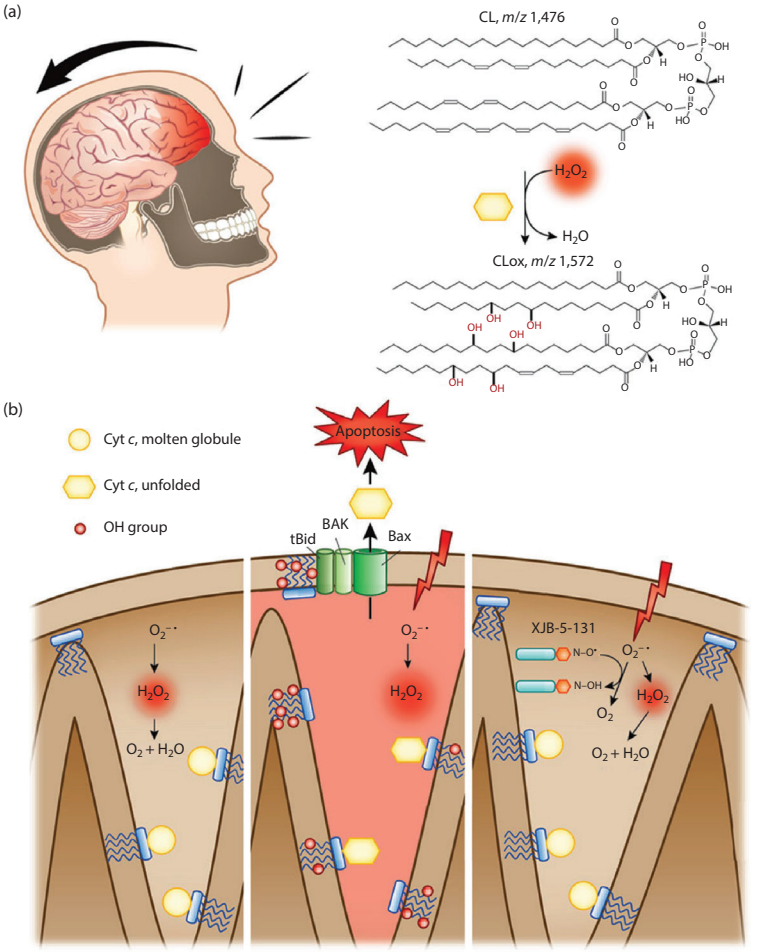


(a)

FIGURE 19.1 DAF-16 (FOXO-like transcription factor of *C. elegans*) regulates stress responses in *C. elegans*. (a) Overexpressing DAF-16, SIR-2 (sirtuin), HSF-1 (heat shock transcription elongation factor), LIN-4 (developmental-tuning micro RNA), AAK-2 (a subunit of AMP kinase), JNK-1 (JUN kinase), or the transcription elongation factor TCER-1 extends life span. Inhibiting the DAF-2 insulin/IGF1 receptor or components of its downstream kinase cascade also extends life span. In each case the extension of life span is DAF-16 dependent. (From Kenyon, *Nature* 464:504–12, 2010. Copyright © 2010. Reprinted by permission from Macmillan Publishers Ltd.) (b) Multiple FOXO transcription factors govern stress tolerance in mammals. (From Greer and Brunet, *Oncogene* 24:7410–25, 2005. Copyright © 2005. Reprinted by permission from Macmillan Publishers Ltd.)



COLOR FIGURE 20.2 Model to explain how deuterium-reinforced PUFAs limit the chain reaction of membrane lipid peroxidation. (a) A theoretical chain reaction is depicted in a membrane where a single initiation event producing a lipid peroxyl radical (denoted by –OO•) starts a chain reaction of lipid autoxidation that in the presence of O_2 may continue indefinitely (red arrows) and produce many molecules of lipid peroxides. Susceptible phospholipid molecules containing a PUFA acyl chain are designated by a kinked blue line and a red dot. (b) Propagation of PUFA autoxidation can progress by interaction with any neighboring PUFAs. (c) The presence of 20 percent isotope-reinforced PUFA (denoted by a black kinked line and a black dot) inhibits (or slows) chain propagation. (d) Propagation is inhibited for PUFAs neighboring the D-PUFA. (From Hill et al., *Free Radic. Biol. Med.* 53:893–906, 2012. Copyright © 2012. Reprinted with permission from Elsevier.)



COLOR FIGURE 20.3 Cardiolipin (CL) as trigger molecule in the molecular pathology of traumatic brain injury (TBI) and discovery of mitochondria-targeted antioxidants protecting mitochondria. (a) Inflammation following TBI causes oxidation of highly unsaturated molecular species of cardiolipin, resulting in dysfunctional derivatives designated CLox. (b) Left panel shows reactive oxygen species such as H_2O_2 being detoxified and maintained at levels below a critical threshold for initiating a chain reaction. In the middle panel TBI overwhelms defenses against reactive oxygen species, unleashing an oxidative chain reaction propagated by oxidatively damaged CL. Oxidative membrane damage is so severe that membrane-bound cytochrome c is damaged and converted to its peroxidase derivative, further damaging the mitochondrial membrane. Cytochrome c escapes to the cytoplasm, triggering apoptosis. Right panel shows the antioxidant XJB-5-131 being targeted to the inner mitochondrial membrane. This membrane-targeted antioxidant intercepts sufficient numbers of superoxide radical ($O_2^{-\bullet}$), allowing superoxide dismutase to form H_2O_2, which is degraded by catalase yielding O_2 and H_2O. Thus, with the help of the membrane-targeted antioxidant, an oxidative chain reaction leading to cellular death is prevented. Note that the mechanism shown in the right panel can be generalized to cover oxidative stresses generated by aging, neurodegeneration, cancer, chronic inflammation, and mitochondrial diseases as well as TBI. (From Chan and Di Paolo, *Nat. Neurosci.*15:1325–27, 2012. Copyright © 2012. Reprinted by permission from Macmillan Publishers Ltd.)