Cell Death and Differentiation (2001) 8, 207–209

www.nature.com/cdd

News and Views

Insight into age-related macular degeneration: New vision in sight

C Richter*,[1]

[1] Institute of Biochemistry, Swiss Federal Institute of Technology (ETH), Universitätstr. 16, CH-8092 Zurich, Switzerland
* Corresponding author: E-mail: richter@bc.biol.ethz.ch

Age-related macular degeneration (AMD) affects 10–20% of people at an age over 65 and constitutes one of the leading causes of severe visual impairment in the elderly in industrialized nations. Whereas the clinical and the histopathological pictures of AMD are well known, molecular events initiating the disease remain elusive. Accumulation of the autofluorescent age pigment lipofuscin in phagolysosomes of retinal pigment epithelium (RPE) cell constitutes a predicament for the development of the disease. Lipofuscin in the eye harbors two unusual retinoids, the lipophilic cations N-retinyl-N-retinylidene ethanolamine (A2E) and its isoform, *iso*-A2E. The compounds can be synthesized from 2 molecules of retinal and 1 molecule of ethanolamine. Both precursors are present in photoreceptor outer segment membranes, where 11-*cis*-retinal serves as the chromophore of the visual pigment rhodopsin, and phosphatidylethanolamine is an abundant membrane phospholipid. A recent report[1] now shows that A2E at concentrations found in human eyes induces apoptosis in cultures of RPE cells. A2E appears to target directly the function of cytochrome oxidase (COX). These findings give insight into the molecular mechanism underlying A2E's cytotoxicity and suggest strategies to retard or overcome AMD.

A2E and *iso*A2E were first isolated from eyes of old individuals and initially proposed to be bis-Schiff bases.[2] This was later revised[3] to a pyridinium bis-retinoid structure, which was confirmed by H-NMR (for the correct, albeit awkward, term of A2E see).[1 and 3] The idea that A2E may cause apoptosis[1] was based on two considerations. First, mitochondria are important for the execution of apoptosis, as first proposed 8 years ago.[4] They are targets of pro-apoptotic signaling molecules such as ceramides or peroxides, and provide ATP and apoptotic executioners such as cytochrome *c*, caspases, or apoptosis inducing factor (AIF). Second, energized mitochondria have a membrane potential, negative inside, and therefore accumulate lipophilic cations. A classical example is rhodamine, used for staining of mitochondria in living cells. The targeting of mitochondria by lipophilic cations can be devastating, as shown, e.g., with the cation methylphenyl-pyridinium, which causes Parkinson's disease.[5] Suter *et al.*[1] now report that A2E at concentrations found in the human and rat retina induces apoptosis in RPE and other cells. Apoptosis is accompanied by appearance of cytochrome *c* and AIF in the cytoplasm and the nucleus. Biochemical examinations show that A2E specifically targets COX. With both, isolated mitochondria and purified COX, A2E inhibits oxygen consumption. Interestingly, inhibition is stronger in the light than in the dark, the reason for this being unknown. Inhibition is due to detachment of cytochrome *c* from mitochondria, and is reversed by added cytochrome *c* or cardiolipin, a negatively charged phospholipid which facilitates the binding of cytochrome *c* to COX. Mitochondrial electron flow upstream of cytochrome *c* is not altered by A2E. Thus, A2E can act as a pro-apoptotic molecule *via* a mitochondria-related mechanism through site-specific targeting to COX (Figure 1).

Release of cytochrome *c* from mitochondria into the cytosol has two major consequences. One is the interruption of electron flow along the respiratory chain. Single electrons are now transferred by components upstream of the cytochrome *c* binding site to single oxygen molecules, which results in superoxide anion formation, oxidative stress, and damage to mitochondria.[6] The other is the formation of an 'apoptosome' in the cytosol, a pro-apoptotic complex comprising cytochrome *c*, apaf-1, and procaspase-9.[7]

To visualize the mitochondrial localization of A2E in cells Suter *et al.*[1] used cerebellar granule cells (CGC), which are richer in mitochondria than RPE cells. Collapse of the mitochondrial membrane potential resulted in loss of A2E from mitochondria. Others had previously reported the presence of A2E in lysosomes.[8] The reason for this discrepancy is presently not clear. Conceivably A2E after damaging lysosomes is released and subsequently taken up by mitochondria. Alternatively, A2E may initially accumulate in and be retained by mitochondria, which when damaged may be engulfed by lysosomes. In contrast to these presumably physiological steps of intracellular A2E traffic cell death may be mainly due to overloading of mitochondria with the lipophilic cation. The biological relationship between lysosomes, mitochondria, and A2E could be clarified by measuring its distribution after treatment of A2E-exposed cells with mitochondria- and lysosome-specific poisons.

The specificity of A2E's action in isolated mitochondria at the level of cytochrome c/cardiolipin/COX and the predominantly mitochondrial localization in CGC found by Suter *et al.*[1] raises several questions. First, does A2E target mitochondria also in RPE cells? This is very likely because also their mitochondria can be expected to have a membrane potential. Second, can A2E-induced apoptosis in cell culture be prevented by cardiolipin? If yes, this would provide compelling evidence for interference by A2E at the level of cytochrome *c*/COX, and that the release of cytochrome *c* into the cytosol is the trigger for apoptosis. Third, if AMD is a 'mitochondrial disease', i.e., a disease

which is caused by malfunctioning mitochondria, AMD should have a higher prevalence in patients which suffer from already identified mitochondrial diseases such as myopathies or maternally inherited diabetes and deafness (MIDD). There is evidence that this is indeed the case.[9–11] Fourth, what is the contribution of light? The key component of A2E is, of course, retinal, which is liberated from rhodopsin when light strikes the eye, and A2E mediates blue light-induced damage in RPE cells.[12] Does increased visual activity raise the level of A2E in RPE cells? Does light increase the toxicity of A2E *in vivo*, as suggested by the findings with isolated mitochondria and COX?

Loss of RPE cell viability and subsequent progressive degeneration of the central retina through inhibition of mitochondrial function provides rationales for the prevention and possibly even therapy of AMD. The level of A2E is at least 10-fold higher in old compared to young rats[1] or humans.[3] The quality of mitochondria declines with age, as measured by many parameters, and can be improved by antioxidants and micronutrients.[13,14] More importantly in the context of A2E toxicity, the cardiolipin content decreases with age.[15] Methods presently available for the treatment of mitochondrial diseases comprise biochemical and nutritional approaches, particularly the use of general antiox-

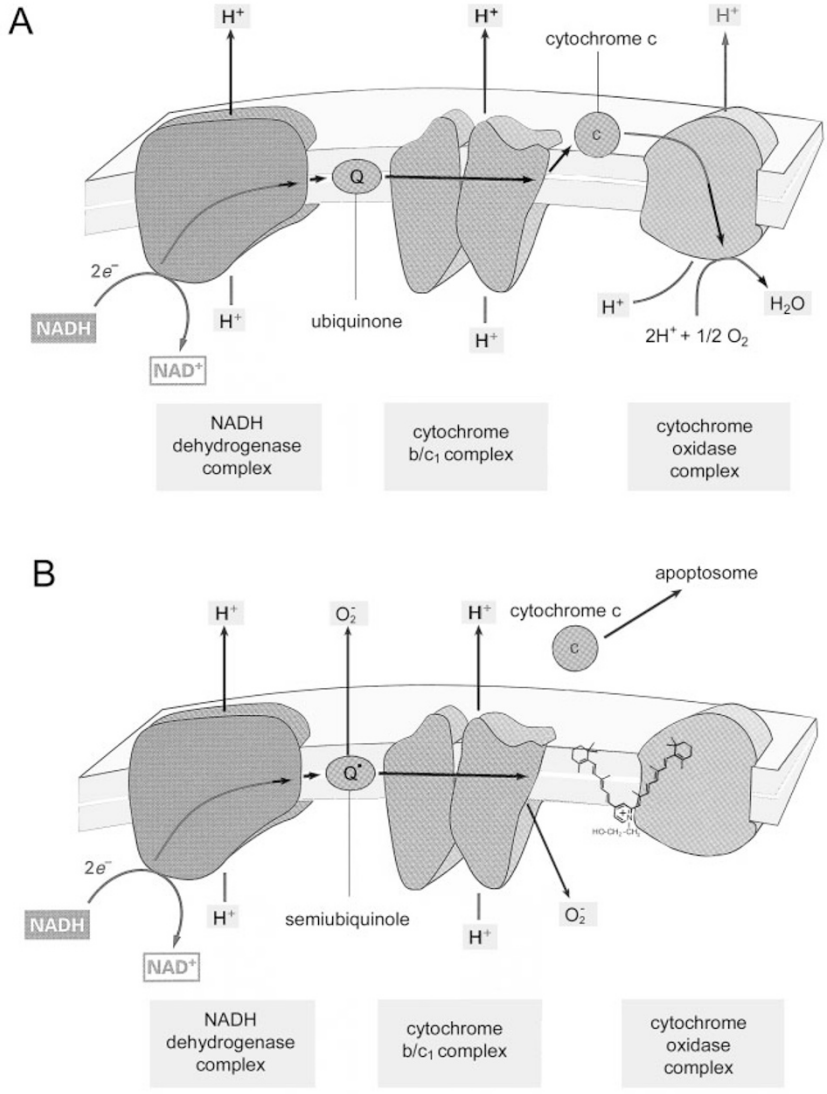

Figure 1 (**A**) The mitochondrial respiratory chain. Electrons provided by NADH flow through the various components and complexes of the respiratory chain, where they are finally transferred to molecular oxygen with the formation of water at the level of cytochrome oxidase. Electron flow is accompanied by extrusion of protons, which results in the formation of a potential (not shown), negative inside, across the membrane. (**B**) The lipophilic cation A2E targets mitochondria in response to the membrane potential. A2E prevents specifically the interaction of cytochrome *c* with cytochrome oxidase, which is mediated by the negatively charged phospholipid cardiolipin (not shown). Detachment of cytochrome *c* from mitochondria has two major consequences. One is the interruption of electron flow through the respiratory chain. Single electrons are now transferred by components upstream of the cytochrome *c* binding site to single oxygen molecules, which results in superoxide anion (O_2^-) formation, oxidative stress, and damage to mitochondria. Whether under these circumstances protons are still being pumped is unclear. The other consequence is the formation of the 'apoptosome' in the cytosol, a pro-apoptotic complex comprising cytochrome *c*, apaf-1, and procaspase-9

idants or specific electron transfer mediators. For example, MIDD is successfully treated with coenzyme Q10,[16] and defects in electron chain components can be bridged with appropriate reductants.[17] Limiting exposure to light may also be useful to control A2E formation and/or its cell toxicity. Another possible strategy is the prevention of A2E formation by, e.g., outcompeting ethanolamine with a secondary amine, or outcompeting retinal with another aldehyde. This somewhat brute-force method may not be useful because of unwanted side-reactions. Gene therapy of mitochondrial DNA defects bears promise[18] but it should be recalled that most of the mitochondrial proteins are not coded for in mitochondrial but in nuclear DNA. Since the level of retinal is decreased by retinal dehydrogenase, stimulation of this enzyme may also be useful to counteract AMD.

Acknowledgement

I thank Drs. Thomas Choinowski and Mattias Schweizer for help while preparing the manuscript.

References

1. Suter M *et al.* (2000) J. Biol. Chem. 275: 39625 – 39630
2. Eldred GE *et al.* (1993) Nature 361: 724 – 726
3. Parish CA *et al.* (1998) Proc. Natl. Acad. Sci. USA 95: 14609 – 14613
4. Richter C (1993) FEBS Lett. 325: 104 – 107
5. Ray SK *et al.* (2000) Ann. N. Y. Acad. Sci. 914: 275 – 283
6. Cadenas E *et al.* (2000) Free Radic. Biol. Med. 29: 222 – 230
7. Li P *et al.* (1997) Cell 91: 479 – 489
8. Sparrow JR *et al.* (1999) Invest. Ophthalmol. Vis. Sci. 40: 2988 – 2995
9. Modi G *et al.* (1992) Br. J. Ophthalmol. 76: 58 – 60
10. Souied EH *et al.* (1998) Am. J. Ophthalmol. 125: 100 – 103
11. Massin P *et al.* (1999) Ophthalmology 106: 1821 – 1827
12. Sparrow JR *et al.* (2000) Invest. Ophthalmol. Vis. Sci. 41: 1981 – 1989
13. Hagen TM *et al.* (1998) Ann. N. Y. Acad. Sci. 854: 214 – 223
14. Shoffner JM *et al.* (1994) Annu. Rev. Nutr. 14: 535 – 568
15. Ames BN *et al.* (1995) Biochim. Biophys. Acta 1271: 165 – 170
16. Suzuki S *et al.* (1998) Diabetologia 41: 584 – 588
17. Eleff S *et al.* (1984) Proc. Natl. Acad. Sci. USA 81: 3529 – 3533
18. Taylor RW *et al.* (1997) 29: 195 – 205