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THE ECTOPIC ATP SYNTHASE. A BRIEF SURVEY. By now we mitochondriacs are getting comfortable with the notion that mitochondrial proteins are also present if only transiently in other compartments of the cell including the cytosol and nucleus. However, the idea that mitochondrial proteins, and more specifically components of oxidative phosphorylation, are present and function at the plasma membrane: sounds like heresy, or is it? THE ATP SYNTHASE, AND PROBABLY OTHER MITOCHONDRIAL INNER MEMBRANE PROTEINS, ARE COMPONENTS OF THE PLASMA MEMBRANE IN MANY CELL TYPES. The evidence for an ATP synthase identical to the mitochondrial form, almost certainly derived from the organelle and shipped to the plasma membrane in caveolae, is increasingly strong. There is also good evidence for the presence of other components of oxidative phosphorylation on the plasma membrane of many cells based on immunofluorescence studies, e.g., Yonelly SK & Capaldi RA. *Mitochondrion* 6: 305-14 (2006) as well as from proteomic studies of lipid rafts isolated from plasma membrane preparations. Kim BW, et al. *Expert. Rev Proteomics* 7: 849-66 (2010). But what are they doing there?

THE ECTOPIC ATP SYNTHASE IS THE HIGH DENSITY LIPOPROTEIN (HDL) RECEPTOR AND CONTROLS CHOLESTEROL MOVEMENT. It came as a great surprise to most when a publication by Martinez, Champagne and colleagues showed that the ectopic ATP synthase on hepatic cells acts as a/the high density lipoprotein (HDL) receptor. More recent work by the same group and others have confirmed that Apo A-1, and in fact the whole HDL particle, binds to the ATP synthase to increase ADP levels in the extracellular medium. This in turn triggers a cascade of events involving purinergic P2Y₁₃ receptors that lead to hepatic HDL endocytosis. The work of this group has been reviewed recently. Vantourout P, Radojkovic C, Lichtenstein L, Pons V, Champagne E & Martinez LO. *World J. of Gastroenterology*. 16: 5925-35 (2010). The site of binding of apo A-1 to the ATP synthase has been partly mapped. It involves the interface of alpha and beta subunits and overlaps the binding site of the inhibitor protein IF1. Radojkovic C., et al. *Arterioscler. Thromb. Vasc. Biol.* 29: 1125-30 (2009). Other evidence that the ectopic ATP synthase is a/the receptor for ApoA-1 has come from a study by Howard, et al. who showed that a monoclonal antibody against the beta subunit of the ATP synthase blocked Apo-A1 binding to adipocytes and prevented its recycling. Howard AD, Verghese PB, Arrese EL & Soulages JL. *Mol. Cell. Biochem.* 348: 155-64 (2011). Further an interesting correlation between cholesterol metabolism and the ectopic ATP synthase comes from a study of infantile neuronal ceroid lipofuscinosis (INCL or Battens disease). A key characteristic of this disease is altered levels of Apo A1 and an altered cholesterol use. Lyly, et al. reported that palmitoyl protein thioesterase 1 (the protein mutated in INCL) interacts directly with the ectopic ATP synthase, and that cells deficient in PPT1 show increased levels of the synthase on the plasma membrane. Lyly, et al. *Human Mol. Genetics* 17: 1406-17 (2008). Surprisingly, this work

was published in 2008 and has not been followed up with further publications.

INVOLVEMENT OF ECTOPIC ATP SYNTHASE IN ALZHEIMERS DISEASE (AD): ANOTHER STORY OF CHOLESTEROL METABOLISM?

In seminal work Schmidt, et al. showed that amyloid precursor protein (APP) and amyloid beta peptide both bind to the ectopic ATP synthase on the surface of neuronal cells via the alpha subunit of the enzyme. They found that transfection of APP deficient neuroblastoma cells with APP resulted in increased translocation of the ATP synthase alpha subunit to the plasma membrane (Schmidt C, Lepscerdize E, Chi SL, Das AM, Pizzo SV, Dityatev A & Schachner M. *Mol. Psychiatry* 13: 953-69. 2008).

More recently, Ortona and colleagues have provided a different line of evidence for a role of the ectopic ATP synthase in the pathogenesis of AD. Specifically, they show that patients with AD have serum auto-antibodies to the ATP synthase (present in 38% of AD patients but not found in age matched healthy patients or ones with Parkinson's disease). Studies with SH-SY5Y cells showed that these auto-antibodies were capable of inhibiting the ATP synthase and causing cell death by apoptosis. Vacirca D., et al. *Neurobiol. Aging*. 2011 (June 29. E-pub ahead of print).

Following on from this work the same group has shown that the auto-antibodies found in the patients with AD increase cellular uptake of HDL, which is, as they point out, a risk factor for the development of AD. Vacirca D, et al. *J. Alzheimers Dis*. 2011 (June 15 Epub ahead of print).

There is now strong evidence that the ectopic ATP synthase is:

- 1) Involved in maintenance of cancer cells as their environment becomes more acid (the Warburg effect)
- 2) Protection of cancer cells from T lymphocyte recognition and destruction
- 3) Helps regulate angiogenesis and acts as the angiostatin receptor.
- 4) Functions in blood pressure control through a subunit of the enzyme, CF6, being released from the plasma membrane and then binding to the enzyme at the F1 alpha/beta interface rather than at its normal site in the stalk region.

These functions will be covered in the next edition of the MitoAlmanac. We will also discuss how levels of the ATP synthase subunit CF6 in plasma may be a diagnostic for heart attack, stroke, diabetes and more. Oh joy, studies of mitochondrial proteins will keep us all busy for a long time yet!

NEW METHODS OF MONITORING MITOCHONDRIAL STRUCTURE AND FUNCTION.

Rodrigues RM, Macko P, Palosaari T & Whelan MP. Toxicology Lett. 2011 July 20. Epub ahead of print. This work describes the use of NADH auto-fluorescence to monitor the morphology of mitochondria inside living cells, allowing structural analysis of these organelles in a non-invasive way. According to the authors the key is to establish the optimal illumination conditions for each cell type that give good visibility of the organelle but do not damage the organelle. The utility of the method is shown through examination of the toxicity of several drugs in human and mouse cells.

Dranka BP et al. Free Radical Biol Med. 2011 Aug 16 Epub ahead of print. In this recent PAPER by Darley-Usmar and colleagues, details of the use of high resolution polarographic and fluorescence methods to monitor mitochondrial functioning in intact cells in response to oxidative stress are described. Studies involved renal, cardiovascular, nervous and tumorigenic cell types.

THE PIONEERS OF MITOCHONRIAL RESEARCH REMEMBERED. 1). DAVID EZRA GREEN

A comprehensive review of Green's career has been published and this provides an indication of the vast number of important studies that came from Green's lab, and equally important, the number of distinguished scientists who trained under him. This biography gives not only the facts but conveys very well the personality of the man:

<http://www.Nap.edu/readingroom.php?book=biomems&page=dgreen.html>

Everyone who worked with David has anecdotes of their time with him. I have three:

1) I arrived to work with Green knowing something about membrane proteins but nothing about mitochondria. It seemed logical on my first morning at work to go into the library next to Green's office and read up on the new topic. I'd been in there about an hour when Green came in and said, "**Rod, we don't read the literature, we make it**" and marched me into a lab to learn how to measure ATP-Pi exchange with what at the time seemed like several millicuries of P-32.

2) My wife and I arrived in Madison recently married and with very little money. David Green loaned us money to buy a few furnishings for our apartment and for reasons not immediately apparent was very keen that we get a telephone (so we could keep in touch with our families in England?) On about the 3rd night after we moved in, and at around 10pm, the phone rang for the first time. It was David; he wanted to discuss an idea with me. Next night at around 11pm we got another phone call, it was David with a new idea. The following night at midnight the phone rang again; it was David, but this time my wife answered. To this day I do not know what she said but he never called me at home again.

3) Green took a daily constitutional from the Enzyme Institute along University Avenue to the Student Union for lunch a distance of about a mile and a half. He usually asked his favorite post-doc of the moment to accompany him. My time came in January, when the snow lay thick and the temperature was below freezing. I soon learned that the intense cold could be endured by stopping for a minute in the entry of each of the several stores along the route that had hot air blowers over the front door, and then running to catch David up. Green was most of all a gentleman, and never made mention of my odd behavior. However, it was not long before I found a package on my desk without attribution; it contained thermal underwear.