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The Molecular Basis of Mitochondrial Disease: Elucidating the Function of the Mitochondrial Inner Membrane Protein MPV17.

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Mitochondria generate energy for the cell, and to do so requires the protein products of a small piece of DNA (mitochondrial DNA, or mtDNA), which it harbours, as well as many proteins from the nucleus. One nuclear gene called *MPV17* is needed to help maintain mtDNA, we know this because Dr Spinazzola discovered that mutations in *MPV17* are responsible for a human disease which results in mitochondrial DNA depletion syndrome (MDS). The primary research aim of the fellowship was to understand the function of MPV17, thereby aiding the design of rational therapies for MPV17 deficiency and potentially also other forms of MDS. At the inception of the project, neither the function of MPV17, nor the mechanism leading to mtDNA depletion was known.

Before Dr Spinazzola's arrival in Cambridge, the host laboratory had been developing methods of affinity purifying tagged proteins from human mitochondria, and as there was no information as to MPV17's partners it was decided to invest considerable effort in applying the local expertise to the identification of MPV17's interactors. Therefore, Dr Spinazzola expressed a tagged form of MPV17 in human cells. After extensive purification she was able to identify 20 potential binding partners. These included nine proteins found previously in enriched preparations of mitochondrial nucleoprotein complexes; four proteins involved in amino acid metabolism and lipogenesis, and three structural or scaffold proteins.

Encouragingly, the protein partners of MPV17 correlated with the distinct phenotypes of animal and yeast knockout strains reported previously. Moreover, the association of MPV17 with mtDNA binding proteins suggested that the mtDNA depletion seen in patients with *MPV17* gene mutations is related to a close interaction between MPV17 and mtDNA. DBT (dihydrolipoamide branched chain transacylase) was the single most abundant partner of MPV17. It is the E2 subunit of the mammalian branched-chain α-ketoacid dehydrogenase complex (BCKDC), responsible for leucine, isoleucine and valine degradation, and DBT has previously been found enriched in mitochondrial nucleoprotein preparations of amphibians and human cells. BCKD deficiency has severe clinical consequences including often-fatal ketoacidosis, and neurological impairment (Maple syrup disease).

MPV17 mitochondrial DNA maintenance and mitochondrial biogenesis

Dr Spinazzola began the follow up studies by focusing on mtDNA copy number, both because of the loss of mtDNA in patients with MPV17 deficiency and local expertise. Although the precise function of MPV17 in mtDNA maintenance remains to be elucidated, it became clear from her experiments that MPV17 plays an unusual, if not unique, role in mtDNA metabolism, as it accelerated the recovery of mtDNA copy number after drug-induced mtDNA depletion, whereas three other mtDNA binding proteins had the opposite effect. An important conclusion of this finding is that it identifies MPV17 as a factor that could potentially ameliorate mitochondrial diseases associated with mtDNA depletion.

Most proteins when over-expressed either produce a dominant negative effect or are benign. The fact that MPV17 was not only tolerated but had a positive effect on mtDNA copy number suggested it was fully functional and able to exert its natural role(s). Therefore the cells expressing recombinant MPV17

were further investigated to determine the general effect of the protein on mitochondria function. Assays of mitochondrial membrane potential, oxidative phosphorylation (oxygen consumption) and mitochondrial protein synthesis showed that all were increased in response to elevated expression of MPV17. Thus, the combined effects of MPV17 on mitochondrial DNA replication, protein synthesis, oxidative phosphorylation and membrane potential constitute a powerful argument for the protein having the ability to stimulate mitochondrial biogenesis. This is a major new hypothesis as to the function of MPV17, which is being prepared for publication.

The positive effect of high MPV17 expression on mitochondrial protein was an important finding in its own right, particularly when combined with the fact that DBT was the most prominent candidate partner protein of MPV17. This led Dr Spinazzola to hypothesize that amino acid homeostasis in mitochondria is a key regulator of protein synthesis in the organelle. Therefore, she next explored the effects of amino acid starvation on mitochondrial function. Growth of human cells in medium lacking amino acids led to a modest yet consistent increase in respiration, and a marked increase in mitochondrial protein synthesis. In the future, Dr Spinazzola plans to build on these findings, to understand how nutrient availability influences mitochondrial function and in turn how this impacts on the whole animal in normal and disease states.

During the fellowship Dr Spinazzola increasingly worked independently, as highlighted by her establishing collaborations with national and international researchers. While in Cambridge, Dr Spinazzola has gathered one of the largest collections of cell lines of MPV17 deficient patients in the world, from four continents. This collection represents a valuable resource that will be of great value for her future studies. Already she has used them to show that MPV17 deficiency inhibits mitochondrial protein synthesis and reduces respiratory capacity, when mtDNA copy number is normal. Therefore, as with yeasts, the effects on oxidative phosphorylation and mtDNA maintenance of MPV17 (SYM1 in yeasts) deficiency are separable.

Dr Spinazzola has used the fellowship to acquire key technical skills and resources, and to develop as an independent researcher. She has orchestrated far reaching new projects such as the investigation of the other members of the family of MPV17 proteins and the acquisition and breeding of an MPV17 knockout mouse. In order to elucidate the physiological consequences of MPV17 deficiency, Dr Spinazzola has begun to collaborate with experts in metabolism, in Cambridge. To work with mice, Dr Spinazzola had to gain a personal animal license, which required her to study an intensive course and sit five demanding examinations. Unlike many PIs she passed every one at the first attempt. The success of the Marie-Curie fellowship award is attested to by Dr Spinazzola having been made a PI at the Mitochondrial Biology Unit. Thus, she has achieved the primary goal of the fellowship programme, which is to help establish the careers of talented research scientists. The societal benefits are potentially considerable as Dr Spinazzola has identified a factor that could help treat mitochondrial depletion syndrome, and because mitochondria are implicated as contributor to major causes of human ill-health such as neurodegeneration and cancer, the societal and health implications of her work extend far beyond classical mitochondrial disorders.