Mitochondrial DNA mutations

Associate Professor Dan Mishmar explains the significance of research into mitochondrial DNA and the direction of his work at the Ben-Gurion University of the Negev in Israel.

Mitochondria have their own distinct genome. What accounts for the sequence variations among individuals around the globe?

Unlike the nuclear genome, mitochondrial DNA (mtDNA) resides in multiple copies per cell, ranging from ~100,000 copies in the ovum to ~100 copies in the sperm. Mutations that occur in our body’s cells result in increased intracellular sequence variation; if such mutations occur in the ovum (but not the sperm) they will be transferred to the next generation, and will either be fixed or lost during cell divisions depending on a bottleneck that occurs during the maturation of the ova. Therefore, cells that divide a lot, such as those in the haematopoietic system, may lose much of their intracellular variation as compared to slow-dividing post-mitotic cells, such as neurons and skeletal muscle cells. It is estimated that mtDNA has an order
of magnitude higher mutation rate than the nuclear genome on the evolutionary timescale. To these pieces of evidence, one could add evolutionary forces such as Darwinian selection that shape variation both within the cell and between individuals. We recently exemplified all this through the study of intracellular mtDNA variation in human identical twins.

Can you elucidate your hypothesis surrounding the functionality of haplogroup-defining mutations within the mtDNA transcription/replication regulatory region?

mtDNA is inherited solely through the maternal lineage and therefore genetic variation in the population stems from the accumulation of mutations during the course of evolution. Since mtDNA does not undergo recombination, one cannot assess the functionality of individual mtDNA mutations independent of their linked genetic background. Moreover, there is currently no available technology to mutate the human mtDNA sequence at will in the cell.

Therefore, to assess the functionality of individual mutations we sought an in vitro assay to overcome the aforementioned obstacles. Since many of the evolutionary variants lie within or adjacent to mtDNA promoters, we chose in vitro transcription as an assay to assess the functionality of mtDNA variants. This led us to discover that certain ancient variants in the vicinity of mtDNA promoters affect in vitro transcription, the binding capacity of mitochondrial transcription factor A (TFAM) and the replication efficiency of mtDNA (mtDNA copy number in cells). With this in mind, we asked ourselves whether the mtDNA transcription machinery is confined to the mtDNA promoter or if there are additional undiscovered transcriptional regulatory elements in other human mtDNA regions.

This question led us to screen for alternative transcription regulatory elements throughout the mtDNA sequence in a hypothesis-free manner, which yielded the exciting preliminary identification of novel transcription factors that bind and regulate human mtDNA.

You and your collaborators screened thousands of complete human mtDNA sequences for natural variation in experimentally established protein and RNA-coding genes, and regulatory regions. Have your investigations uncovered new information on mutations?

We recently published a comprehensive analysis of more than 9,800 whole human mtDNA sequences representing all major human populations. In that study, we aimed at identifying the mutations that were retained in the human phylogeny in certain ‘branches’ of the phylogenetic tree, i.e. mutations that define genetic backgrounds. We also identified mutations that independently recurred in distant mtDNA lineages.

Strikingly, we found mutations with a high functional potential both among the large repertoire of lineage-defining and recurrent ‘nodal’ mutations. This raises a question: if those mutations are found in the general population and they are similar to disease-causing mutations, how are we still healthy? The answer is still open, but our hypothesis is that these mutations were likely compensated by changes elsewhere in the genome, including the nucleus. If this is true, certain combinations of mutations involving these functional variants will be beneficial and others will alter susceptibility to diseases. Our preliminary results indicate just that.

What do your team members contribute to your investigations? Does being based at Ben-Gurion University offer advantages in terms of collaboration?

I am blessed by a group of outstanding PhD and MSc students and a very capable technician who not only perform the experiments but contribute intellectually to the design and experimental testing of our hypotheses. For disease association studies, especially in relation to Type 2 diabetes, we have a wonderful ongoing collaboration with the Israeli Diabetes Research Group (IDRG), to which I am most grateful for medical information and DNA samples of hundreds of patients and controls. In the frame of other projects we have excellent local, European and US collaborators.

Ben-Gurion University is a dynamic and relatively young research institute which is eager to provide a vibrant working environment for researchers, lively discussions with colleagues and cutting-edge research facilities. This atmosphere is essential for my work.
INTELLIGENCE

**MtDNA**

**OBJECTIVES**

To provide evidence for the hypothesis that mitochondrial genetic variability and evolutionary dynamics play a role in major evolutionary transitions including the emergence of new species and also in the tendency of humans to develop complex disease phenotypes.

**KEY COLLABORATORS/PARTNERS**

The Israeli Diabetes Research Group (IDRG)

Dr Raz Zarivach; Professor Amir Aharoni; Professor Amos Bouskila; Professor Ofer Ovadia, Ben-Gurion University of the Negev, Israel

Professor Eran Meshorer, Hebrew University, Israel

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**FUNDING**

Israeli Science Foundation

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[www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1000474](http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1000474)

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**ASSOCIATE PROFESSOR DAN MISHMAR**

obtained a BA in Archaeology from Hebrew University in 1992. He was then supervised by Professor Batsheva Kerem whilst completing a PhD in Human Genetics also at Hebrew University. His postdoctoral training mentor was Professor Douglas C Wallace. Since 2004, he has been Principle Investigator (Senior Lecturer) at the Ben-Gurion University of the Negev, Israel, before becoming Associate Professor in 2011.

Mishmar’s work has the potential to shed light on the functioning of the mitochondrion as an organelle vital to life as well as to pick apart the intricacies of its high degree of interdependency with the nucleus genetic variations as being governed by the same evolutionary principles.

Much of their work concerns complex diseases, which are determined by multiple genes in multiple locations as well as by environmental factors, as Mishmar underlines: “Complex diseases could be caused by combinations of variants, each of which contributes only little to the disease phenotype”. This active gene-environment interface draws the forces of natural selection into play. A simple example of the tight relationship between evolution and disease would be the positive selection of efficient energy-producing genotypes in an ancestral age when food was scarce. This same adaptation, upon encountering the abundance of food in the modern world, has led to the rise of obesity and diabetes.

Whilst it could be assumed that evolutionary processes would negatively select disease-causing mutations in mtDNA, there is evidence that in some contexts they could present an evolutionary advantage. The group, hypothesising that recurrent nodal mutations are retained due to positive selection pressures, have investigated the functions of these variants. By looking at the recurrence of mtDNA ancient variants in a different genetic context, they have shown that the genetic landscape in which a mutation occurs can have a strong influence on its effect on the development of mitochondrial disorders such as the legal blindness caused by Leber’s hereditary optic neuropathy (LHON).

**THE MITOCHONDRIAL FUTURE**

Since the functional impact of a genetic variant is likely to be only slight, the team is now searching for experimental methods sensitive enough to determine this. They are also currently working to unravel the mysteries of mtDNA transcription, identifying factors and their binding sites involved in the regulation of this process. In addition, the researchers are pioneering methods of identifying the signatures of natural selection both for variants between individuals and for the intracellular mtDNA variants.

Mishmar’s work has the potential to shed light on the functioning of the mitochondrion as an organelle vital to life, as well as to unpick the intricacies of its high degree of interdependency with the nucleus. Through this new knowledge, it is hoped that further insights will be gleaned into both evolutionary processes and susceptibility to a host of diseases, paving the way towards preventative medicines.