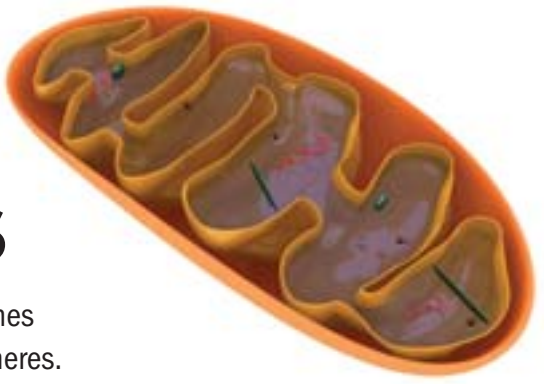
Mitochondrial chromosomes in Bratislava, Slovakia

Answering Age-Old Questions



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From the middle of Europe comes a story of yeast, their chromosomes and how things got cooking; finally leading to the evolution of telomeres.

Most of us know that DNA is tightly organised in structures called chromosomes and, furthermore, that different species have different numbers of chromosomes. But the story doesn't end there. Chromosomes come in many different "flavours", most of which are poorly understood. In this context, a Slovak research group, led by Jozef Nosek, has been cooking up some exquisite questions – and is getting some tasty answers.

How it all started

Once upon a time, in 1984, a new species of yeast was discovered, named *Candida parapsilosis*, which displayed a rather uncommon feature: linear mitochondrial DNA, whereas all other yeasts possess the well-known circular mitochondrial DNA. The "discoverers" were Ladislav Kovac, back then at the Slovak Academy of Sciences in Ivanka pri Dunaji, close to Bratislava, and his French colleagues Jaga Lazowska and Piotr P. Slonimski (*Mol. Gen. Genet.*, 197(3): 420-4). The three suggested that this linear DNA might represent an early version of eukaryotic chromosomes and proposed to use this species for addressing an old question: the "end-replication problem" once formulated by Alexei Olovnikov and James Watson in the early 1970s.

Essentially, because of the way DNA replication works, the enzyme in charge, DNA polymerase, needs a small fragment of DNA to guide it towards replication of each of the DNA strands. On the DNA strand, this small fragment aligns itself ahead of the enzyme – so you can imagine what happens at the end of it, if there is nothing more to come: replication halts a little bit too early to completely include the very outmost bases of the DNA strand. In most eukaryotes this problem is solved by the presence of the repetitive telomere sequences; however, yeast DNA, such as that of *C. parapsilosis* lacks classic telomeres. Thus, the question of how their DNA is "end-replicated" has remained open.

Then Ladislav Kovac became the mentor of a curious young student named Jozef Nosek. Nosek was an exceptional teenager, passionate for books like Paul de Kruif's 'Microbe Hunters' and Alfred Renyi's 'Dialogues on Mathematics'. On his sixteenth birthday, his favourite present was the book his father gave him: James Watson's textbook 'Molecular Biology of the Gene'. It just so happened that when he started university, one of his professors, Vlasta Kovacova, was married to Ladislav Kovac, who at the time was already a well-known researcher. Inevitably, it wasn't long before Kovac and Nosek met and it was during his first visit to Kovac's lab that Nosek became instantly hooked on experiments with yeast.

New strategies

Nosek recalls, "In the summer of 1987, I came to the Kovac lab at the Institute of Animal Physiology, where, together with my friend and colleague Lubomir Tomaska, we developed a strategy for the cloning of terminal DNA fragments of the mitochondrial genome of *C. parapsilosis*. A few years later, our collaboration and passion for biomedical research led us to establish a joint lab, associating researchers and students from the Departments of Biochemistry and Genetics."

Then collaborations started. First, with Hiroshi Fukuhara (Institute Curie, Orsay, France) and Jack D. Griffith (University of North Carolina, Chapel Hill, USA); later on, with Ilona Pfeiffer (University of Szeged, Hungary), B. Franz Lang (University of Montreal, Canada) and Kenneth H. Wolfe (Trinity College Dublin, Ireland).

From structure to evolution

Nosek's lab employs a wide variety of techniques, from the humble gel electrophoresis to whole genome sequencing. Their goal, right from the start, was to study the structure and evolution of mitochondrial chromosomes. First, they characterised the detailed structure of mitochondrial telomeres and made a key discovery: telomerase is not required for their maintenance, suggesting that they not only had a good model system in their hands but also a telomerase-independent system. Then, in a 2000 paper in *Nucleic Acids Research*, they identified extra-chromosomal circles derived exclusively from telomeric DNA (t-circles), which happened to play a key role in mitochondrial telomere maintenance (*Nucleic Acids Res*, 28: 4479-87).

Why is this important? Jozef Nosek gives us the answer, "[...] this replication pathway was identified in yeast and mammalian nuclei lacking the telomerase activity, suggesting that the t-circle-dependent mechanism may represent a universal alternative pathway of telomere maintenance." In other words, their findings not only provided an example of a mechanism of telomere maintenance not requiring telomerase but also a mechanism that might help understand how telomere maintenance pathways in other species evolved.

The diversity within

Another question Jozef Nosek is tackling concerns the diversity found in the genome structure of the yeast *Candida*. For this purpose, his group sequenced the mitochondrial genome of several, closely related species of *Candida* – and they produced some rather unexpected results. While they determined that the species examined shared essentially the same number of genes, they also discovered that these species had major differences in genome size, number of introns, LIRs (long inverted repeats) and genome structure (*Nucleic Acids Res*., 2011 Jan 25, epub ahead of print).

Another line of research by Jozef Nosek *et al.* led to the hypothesis that in some species telomeres might have evolved from selfish DNA elements. According to a textbook definition, "selfish genetic elements are elements that enhance their own transmission relative to their host's genome and, thereby, can be detrimental to the host".

Now for the punch line: they are not only bad, after all!

In a recent article, Fricova *et al.* found that the linear mitochondrial genome of *C. subhashii* ends with invertron-like telomeres (*Microbiology*, 156: 2153-63). These are special types of telomeres commonly found in bacteria and characterised by their long inverted repeats. In addition, at the 5' ends, a protein appeared to be covalently bound to the terminal nucleotides, which in turn seemed to interact with the DNA polymerase to take replication to a successful end. "This discovery supported our hypothesis that telomeres might have evolved from selfish genetic elements that invaded the circular ancestral genome, forced its conversion towards a linear form and, at the same time, provided a solution to the 'end-replication problem'," explains Jozef Nosek.

Research funded by Uncle Sam

Asked about research in Slovakia and, specifically, the funding situation, Nosek replied, "Slovak scientists not only face substantially lower funding than their colleagues in other European countries but also a fast growing bureaucracy associated with the grant administration. A serious problem is the lack of a rigorous peer review system, prevailing mediocracy over meritocracy and nepotism that affects the evaluation of project proposals at the national level."

Like most yeast species, the Nosek group prefers aerobic conditions. Jozef Nosek: second from right, back row.

But what about the European Union? Surely, there must be grants an accomplished Slovakian researcher can apply for? "Yes, there are such grants", says Jozef Nosek, "but getting good reviews doesn't seem to go hand in hand with actually getting funded." For example, he applied for an EU-FP5 grant and received top scores for his proposal – only to finally fall short on something called "partnership and management". Thus, those funds, which more than anything else had been plagued by excessive bureaucracy, never materialised.

So, how is Nosek actually able to perform research in such a broken system? Well, his answer is "collaboration, collaboration, collaboration"! While Jozef Nosek acknowledges that some funding has been obtained from national grant agencies (VEGA and APVV), their most important support comes from international grants. The Howard Hughes Medical Institute (HHMI, USA) has awarded him ten years of support so far, while his colleague, Lubomir Tomaska, has received two Fogarty International Collaboration Awards (FIRCA, USA).

Brain draining

"I do not want to imply that I resigned applying for EU-funded projects but we repeatedly succeeded when applying for grants from the US. And it's mostly this money we built our lab from," Jozef Nosek states. Also, as an added bonus, all the US-based grants had involved considerably less paper work and bureaucracy when compared to the EU-based grant. And this means: more time for research and teaching.

Another problem for Slovakian science is the brain drain. As an understandable consequence of the current situation, young scientists are systematically leaving Slovakia for postdoctoral studies. "However, they are provided with only few opportunities to return and establish their own research teams here," Nosek tells.

What can be done? "To solve these problems, Slovakia has to invest in an active recruitment and repatriation programme for scientists. At the end of the day, they would not only bring back professional experiences as well as contacts to leading foreign scientists and research groups – but they would also 'infect and spread' our labs with the appropriate academic atmosphere and culture from western universities."

Frederick Gruber