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PROFILES AND LEGACIES

Michael Ter-Avanesyan (1949-2018) – Advent of the scientist

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ABSTRACT
This commentary is a tribute to the late colleague, Prof. Michael D. Ter-Avanesyan – prominent contributor into knowledge about prion maintenance and function. The commentary describes his early steps in genetics which brought him into prion research.

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Michael Ter-Avanesyan in the laboratory of physiological genetics (Department of Genetics, Leningrad State University, Russia). Photo of 1971.

It is with great sadness we are trying to cope with the thought that our dear friend Michael D. Ter-Avanesyan, one of the leaders in yeast prion research and long-time Editorial Board member of Prion, had left this world. We knew Michael (or Misha, how all colleagues nicknamed him through his life) from his first appearance at the Department of Genetics 50 years ago in October 1968, and from his very first steps in genetics research. He was then at his third year in St. Petersburg (that time Leningrad) State University (the level of a rising senior in the US universities). This was also his first year in the yeast genetics lab (Laboratory of Physiological Genetics) headed by Sergey G. Inge-Vechtomov, Misha's mentor through undergraduate and Ph.D. programs. Thinking back, we are convinced that the mindset and thinking style combining rigor, discipline, and courage to extrapolate specific findings onto broad biological concepts might

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have been seeded at that time or may be these were intrinsic features of Misha's personality which got a chance to be expressed then for the first time. Many people who knew Misha at these early years promptly recognized that he is a scientist born-to-be. Despite Misha's low voice and seeming shyness, Misha's outstanding potential and great future impact in science were beyond doubts to those near him. After learning about Misha's passing, we thought of what would be the special tribute that we can give to our long-term colleague and dear friend. We decided to bring here several scientific stories from Misha's early research which we closely witnessed – a chain of his three papers and our recollections of ideas and discussion around the topics. Interestingly, this research chain brought Misha to studying translational suppression and later to his seminal findings about yeast prion maintenance.

The first paper [1] was addressing meiotic segregation in tetrads of yeast polyploids. At that time, mating type switching HO-endonuclease gene making creation of yeast polyploids a routine was not cloned yet, because there was neither knowledge about mechanism of mating type switching nor molecular cloning. The only tools were from the state-of-the-art genetics. Yeast polyploids were obtained by rather sophisticated and not understood at that time method of 'illegitimate' mating of non-mating yeast diploids with haploids, followed by the second round of illegitimate mating of triploids with haploids to finally yield tetraploids. Altogether, this was creating a 'dark matter' of strains with suggested levels of ploidy. The goal of that student work was to streamline obtaining of polyploids, which then could be used in multiple studies in the lab. The only way to confirm that yeast cells are polyploid was to follow pattern of allele segregation in meiotic tetrads. Misha built models of segregation patterns accounting for two different modes of meiotic conjugation, bivalent and tetravalent and included recombination between a marker and a centromere into calculations of tetravalent mode as another level of complication. Building models of allele segregation in meiotic tetrads of polyploids even now is a brain damaging exercise for senior students in genetics. Since Misha's work this subject was given to the most advanced students in the Department, when teachers wanted to get gratification from high level of command over genetic analysis demonstrated by their trainees. This was not met with the same enthusiasm by highly evaluated victims of the exam. After building the model, there was a labor of micro-dissecting and analyzing hundreds of yeast meiotic tetrads and rigorous statistical analysis of segregation pattern. As a result, the way of making polyploids by illegitimate mating was confirmed and a fraction of irregular meiotic divisions was estimated. Last but not the least, the creation of polyploid strains in yeast lines used in the lab was streamlined. Interestingly, Misha co-authored this paper with his future spouse, Oksana Nitsai and their marriage happily lasted until his last days.

After graduation in 1971, Misha was offered to take a Ph.D. course in the same Department (the usual career path in Soviet science for talented students at that time). He was given a project within a collective effort of building genetic model of acid phosphatases, enzymes excreted from the yeast cell. In the early years of molecular genetics many labs concentrated on biochemical studies of a single enzyme combined with creating a collection of mutants within this gene. Such *gene-enzyme* models had value at that time because DNA sequencing was not even guessed beyond horizon, but peptide sequencing was available. It is worth noting that the first sequence of short DNA piece was determined by Fred Sherman using his cytochrome C gene-enzyme model based on sequencing short peptides remaining downstream frameshift mutations before new stop codon generated by a frameshift [2].The perceived value of the acid phosphatase model was in easy access to pure enzyme, which was already present in relatively high purity in the growth media. The hope was to deduce molecular nature of some mutations in the gene, which would then facilitate a spectrum of downstream explorations and findings. To get greater quantities of pure enzyme, one would need more enzyme to be produced by cells. At the times before the genetic engineering era, when portable strong promoter cassettes were not even anticipated, the use of polyploid yeast appeared a natural way to increase gene dosage and boost enzyme production. Since polyploids creation by illegitimate mating was streamlined in his first paper, Misha created a series of strains with ploidy 1n, 2n, 3n, 4n and, to his surprise, found that the phosphatase exoenzyme production in tetraploids was the same as in triploids [3]. Then he came up with an insight based on a thought that was as simple as brilliant – protein production inside the cell as well as the total cell mass and cell volume are proportional to gene dosage and to ploidy, but the enzyme excreted to the media should be proportional to the area of cell surface, which is also growing with ploidy, but at a lower rate (second order kinetics) than the cell volume and mass (growing by third order kinetics). Thus, there should be saturation of cell surface with excretable enzyme. Misha did the thing that not many Ph. D. students would do at the time of non-existence of cell sorters and automated microscopes. He measured cell dimensions in several thousand of yeast cells and calculated volumes and surface areas of each.

Calculations were in complete agreement with suggestion of second and third order kinetics for surface area and volume dependences from ploidy, respectively. Misha added an elegant proof to his speculation about cell surface saturation: he measured levels of the excreted enzyme in a series of tetraploids carrying one, two, three and four copies of the wild type phosphatase gene. The result was in complete agreement with the hypothesis. This single-author old paper leaves a reader with feeling of great science, rigid logics, balanced discussion and constrained albeit insightful speculations. One speculation of a broader biological value made by Misha at that time but left beyond the paper's text was about biologically optimal levels of ploidy. Misha noticed that in triploids extracellular enzyme production was still proportional to ploidy and extended this into general thinking about biological effectiveness of ploidy levels. He suggested that triploid level may result in the most efficient balance between intracellular world with the surrounding tissue or with environment. He noted, that plant endosperm is triploid and suggested that evolution set this as a most efficient level for the somatic tissue tasked to nurture next generation of a plant.

The third paper [4] was based on the study that Misha started after his Ph.D. defence. This work stemmed from another genetic tool used at that time to understand the nature of mutations in gene-enzyme models. While DNA sequencing was not available, some mutations could be deduced from the features of translational suppression due to mutations changing tRNA anticodons, thereby enabling translation of full size polypeptide in stop-codon (nonsense) mutants. Interestingly, this utility of tRNA nonsense-suppressors as a tool of molecular genetics also boosted research of translation mechanisms in many labs, including the Inge-Vechtomov lab in the Department of Genetics. Misha and other Department members identified several putative nonsense mutants in the phosphatase gene. Common next step was to pick suppressors with highest efficiency of nonsense read-through based on the level of restoration of enzyme activity, which Misha performed as a next step of gene-enzyme system development. At the same time with the studies of phosphatase model the lab was studying a novel type of translational nonsense-suppressors discovered by Sergey Inge-Vechtomov in 1964 [5,6]. Unlike the tRNA-suppressors that were always dominant, the new suppressors, *sup1* and *sup2*, currently known as *sup45* and *sup35*, respectively, were recessive. Based on the summary of genetic results recessive nonsense-suppressors were suggested to alter protein components of translation machinery participating in termination of polypeptide chain. Because of unknown nature as well as of weak and temperature sensitive cell growth, these recessive suppressors did not carry immediate value for refining the gene-enzyme system. However, Sergey Inge-Vechtomov's results and a plethora of hypotheses about the functions of *sup1* and *sup2* created the sense of potential new findings across the Department, so Misha decided to include recessive suppressors in his study, just because he sensed a chance of extending knowledge. The chance was prompted by his understanding of the value of gene dosage as a tool of genetic research, which developed through his previous experimenting with gene dosage in yeast tetraploids. The question bearing potential mechanistic value was about functional interaction between tRNA suppressors, whose mechanism in translation of nonsense codons was well understood, and the putatively translational recessive suppressors. The obstacle was that any mutation in the recessive suppressor genes was synthetic lethal with dominant tRNA-suppressor mutations. Misha used a gene dosage approach by simply creating double heterozygotes for dominant as well as for recessive suppressors. Recessive suppressors, that were completely inactive in a single heterozygote, boosted suppressor efficiency of dominant suppressors (measured by enzyme activity). This result supported ideas about direct participation of proteins controlled by *SUP45* (*sup1*) and *SUP35* (*sup2*) in termination of polypeptide chain synthesis during translation of mRNAs into proteins. (Now it is well known that these are factors of translation termination and Sup35 is a yeast prion [7–9]). In that very time, end of 1970s-beginning of 1980s, Misha's research focus moved to genetic control of protein biosynthesis, which eventually brought him into the forefront of yeast prion research – the next chapter of his life in science. This chapter was continued in the new place of Misha's work – Cardiology Research Centre, located in Moscow (see V. Kushnirov paper in this issue [10]).

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