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OBITUARY

In memoriam
Vera Gamulin (1948–2006)

Vera Gamulin

Dr Vera Gamulin passed away on October 12, 2006 in Zagreb, just a few days after returning from the HDBMB congress, organized on the occasion of the thirtieth anniversary of the Croatian Society for Biochemistry and Molecular Biology. During all that time Vera was its active member.

Dr Gamulin was a distinguished Croatian biologist with major research interest in molecular evolution. We will remember her as an extraordinary person, internationally recognized scientist, eminent intellectual, and excellent teacher and organizer, who was actively present in various forms of scientific and academic life.

Vera Gamulin was born on December 27, 1948 in Jelsa (Hvar), where she finished elementary school. She completed grammar school in Split, and graduated in biology from the Faculty of Science, University of Zagreb (1972). Three years later she won her M.Sc. degree (Dr Branko Brdar, supervisor) and than Ph.D. 1980 (Professor Željko Kućan, supervisor), both from the University of Zagreb. Vera did her postdoctoral research from 1980–1982 with professor Dieter Söll at the Yale University, where she returned as visiting scientist in 1986. She was an employee of the Ruđer Bošković Institute (IRB) during her whole career. She joined the Institute in 1972 as research assistant and in 1998 she was appointed senior research scientist. From 1987 Dr Gamulin served as head of the Laboratory for Biosynthesis, which was later renamed into the Laboratory of Molecular Genetics. In 2002 she was elected full professor at the Faculty of Science, University of Zagreb, where she taught graduate courses for many years. She served as head of Molecular and Cellular Biology program at the Graduate School of Biology (Faculty of Science, University of Zagreb) from 2002 to 2006.

Dr. Gamulin's research has been published in about 80 articles in the field of molecular biology, with most important contribution to molecular evolution. I will classify her extensive work into four major fields: 1) structure and expression of transfer RNA genes; 2) biotechnology and applied studies; 3) the genes of *Streptomyces*; 4) phylogeny of sponges. Our research sites overlapped several times: in Kućan's laboratory at IRB in mid seventies, at Yale in 1986, and again at IRB, when she generously opened her lab for me when I tried to anchor in Croatia, after returning from USA in 1993, and attempted to establish my own laboratory.

Transfer RNA. – After completing her master thesis, which dealt with the studies of the carcinogenic action of benzacridines by affecting RNA synthesis, she joined Professor Kućan's lab and started to work on transfer RNAs and tRNA-interacting proteins, an essential set of molecules ensuring the faithful translation of genetic information. This topic directed her to Yale University, where she was offered a postdoctoral position (1980–1982) by Professor Dieter Söll (and where she returned again as a visiting scientist). In Söll's lab, Vera was for the first time exposed to gene cloning, sequencing, and most importantly to the gene structure analysis, which remained her main research interest during the whole career.

Her study of nonsense suppression in *Schizosaccharomyces pombe* was invaluable for detailed molecular understanding of the aminoacylation process. In order to learn more about arrangement of transcriptional units and the expression of tRNA genes in yeast, she sequenced and analyzed several intron-containing genes. The analysis of

S. pombe genes published in 1985 was her first contribution to molecular evolution.

Cloning, sequencing, biotechnological application. – Returning from Yale, Vera brought to Zagreb her fascination with the gene technology, which she immediately applied in her newly established laboratory, at the Ruđer Bošković Institute. During her short-term occupation with primary structure variability of satellite DNA from the mealworm *Tenebrio molitor*, Vera and her younger collaborators (D. Ugarković and M. Plohl), established the first improvised »sequencing laboratory« and published in 1989 the first DNA sequence generated in Croatia. In parallel, she taught the collaborators how to use computer software for DNA sequence analysis. The combination of experimental and computational approach became a standard practice in Vera's further work. The fact that IRB afforded a sequencing service (in 2004), the first one in Croatia, was her achievement.

Dr Gamulin was certainly among the first Croatian scientist who realized the power of recombinant DNA technology. With colleagues from Pliva, at that time the largest domestic pharmaceutical, chemical and cosmetic industry, she worked on the construction of the first recombinant expression vector, named pZG1 (published in 1988), which enabled gene transfer between *Strepomyces* and *Escherichia coli*. The biotechnological importance of that pioneering work was noteworthy, since it was aimed to explore the genes involved in oxytetracycline biosynthetic pathway. Although the first recombinant plasmid pZG1 suffered from instability, it was later stabilized by intentionally introduced structural rearrangements (pZG5 and pZG6; 1991).

Next, jointly with W. Müller, she realized that sponges are suitable model organism for biomonitoring programmes, because, through the expression of certain genes, they can »sense« the concentration of cadmium in the environment (2000).

Much later, Vera also expressed an interest for applicable research. In collaboration with her young colleague Marko Premzl, she studied the genes encoding prion proteins in cattle, with special emphasis on the gene structure among the modern and rare cattle breeds in Croatia. Quite recently, she was involved in studies of genetic diversity of the Turopolje pig breed.

Streptomyces *tRNA, rRNA and protein genes: nucleotide structure and analysis.* – *Streptomyces*, industrially important bacteria, are the organisms Vera Gamulin used to explore, together with her Croatian team, for many years.

Streptomyces are Gram-positive mycelial soil bacteria of high GC content (around 73 %), a complex life cycle and with relatively large genome (three times that of *E. coli*). When Vera entered the field, very little was known about tRNA genes and their genomic organization in these organisms. She was especially interested in doing genetic studies of an oxytetracyclyne producing strain *Streptomyces rimosus* R6. Relying on her knowledge and experience with the analysis of tRNA genes, she identified, jointly with M. Plohl, a cluster of five tRNA genes lacking CCA termini (1990). The sequence and the analysis of another small RNA gene, encoding 5SrRNA, followed (1991). Since ribosomal RNA genes exist in all living cells and are evolutionary very conserved, they were useful for phylogenetic studies. Having in hands the sequence of several ribosomal RNA operons in *S. rimosus*, Gamulin *et al.* performed comparative analysis of the promoter regions (2001).

Another aspect of *Streptomyces* related research performed by Gamulin and coworkers was focused on recA gene/protein. RecA is a multifunctional protein that plays a central role in the process of homologous recombination and recombinational DNA repair. It is amongst the most conserved bacterial proteins and is universally distributed within the bacterial kingdom, with only a few exceptions. In a series of papers (1997–2005) they described the primary structure analysis and the expression of *Streptomyces rimosus* R6 gene in *E. coli* and in a streptomycetes. Transcriptional analysis of the *recA* gene in *S. rimosus* revealed a new type of promoter.

Sponges. – Dr Gamulin certainly earned the largest international recognition for her research devoted to molecular biology of sponges (Porifera). Specifically, her studies were concerned with uncovering and exploring the unexpected evolutionary relationships between Porifera and other Metazoa. Vera was introduced into the field by Professor Werner Müller from University of Mainz, with whom she started her long term collaboration and friendship in late eighties. They jointly wrote 45 research papers and three book chapters. For their scientific contribution to molecular biology of sponges, the Adriatic marine sponges in particular, Gamulin and Müller shared the award given by the Croatian Academy of Sciences and Arts, in 1996.

Vera was simply fascinated with sponges, because of, as she used to say, their simplicity and sophistication. By exploring numerous sponge genes, Vera got involved in different aspects of sponge biology. Sponges are the lowest multicellular eukaryotic organisms and the most primitive multicellular animal phylum. By performing DNA, RNA and protein sequence based phylogenetic studies, Vera significantly contributed to the clarification of early relationships among sponge species and resolving controversial phylogenetic placement of sponge taxa. The importance of phylogenetic studies of sponges for the classification is emphasized by the fact that these organisms have very few morphological characteristics. After comparing 42 phylogenetically conserved proteins, Müller and Gamulin concluded that sponge proteins are more similar to those of *Homo sapiens* than to *Caenorhabditis elegans*. The sponges were proposed therefore as the reference animals in molecular evolutionary studies of Metazoa.

Due to the relatively low specialization, and concomitantly the high differentiation and dedifferentiation potency of their cells, the sponge cell system has proven to be a useful model to study the mechanism of cell-cell adhesion at molecular levels. By analyzing a number of genes from a marine sponge *Geodia cydonium*, Gamulin and collaborators showed that during the initial phase of cell-cell contact the main cell adhesion molecule, the aggregation factor (AF), causes a rapid stimulation of the phosphatidylinositol pathway, resulting in an activation of protein kinase C and subsequent phosphorylation of topoisomerase II. As one consequence of these processes, the cells undergo a phase of high DNA synthesis. However, at later stages, the AF loses its mitogenic activity; this function is then taken over by the matrix lectin. During this switch, the lectin receptor associates in the plasma membrane with the *ras* oncogene product, whose regulated expression and phosphorylation was one of the first Vera's sponge related tasks in early nineties. Importantly, together with members of Müller's group, she noticed that S-type lectins occurring in sponges, could be considered as the ancestor for vertebrate S-type lectins. This, and Vera's other work, especially on receptor tyrosine kinase of class II family, led to the conclusion that cell adhesion receptors and nuclear receptors are highly conserved from the lowest Metazoa (marine sponges) to vertebrates. These data supported the view that the kingdom of Animalia is of monophyletic origin. By identifying a homeobox-like gene in the marine sponge *Geodia cydonium*, Gamulin *et al.* presented another molecular evidence for the presence of a developmental gene in the lowest animals.

The occurrence of ubiquitin in sponges also suggests that sponges (Porifera) have evolved from the same ancestor as other Metazoa. Ubiquitin is a small protein (76 amino acids), abundantly present in all eukaryotes, which plays a major role in the normal breakdown of needless proteins and in the cell response to stress. In the cytoplasm, ubiquitin is involved in nonlysosomal and ATP-consuming proteolysis. Ubiquitin protein sequences are extremely well conserved; sponge ubiquitin, as expected, is almost identical to other known animal ubiquitins, differing in only one amino acid from human ubiquitin. Due to the extreme evolutionary conservation of ubiquitin amino acid sequence the protein is apparently not suitable for phylogenetic studies. However, because of the different codon usage, gene sequences can diverge considerably without changes in the protein structure. Therefore, polyubiquitin genes are good models for study of the mutational events which occurred in the living word during ubiquitin evolution. The results of Müller and Gamulin revealed that the sponge ubiquitin gene branched off first from the multicellular organisms. They also showed that the expression of the polyubiquitin gene is directly or indirectly regulated by the AF (from signal transduction pathway) and suggested that ubiquitination might be a process which controls the function of the membrane-associated lectin-binding protein during matrix-cell adhesion.

One large group of proteins that have grown in step with the increasing complexity of multicellular organisms is the family of protein tyrosine kinases (PTKs). Over a hundred PTKs are encoded in the human genome. PTKs play a major role in intracellular communication and transduction of extracellular signals across the plasma membrane into the interior of the cells. They are generally divided into receptor PTKs and nonreceptor PTKs. Members of both classes of PTKs are present in sponges and were the subject of Vera's investigation. For example, Gamulin and collaborators identified in marine sponge *Suberites domuncula* a Bruton tyrosine kinase-like protein, BtkSD, which does not occur in *C. elegans*, but only in insects and higher animal taxa. This finding implies that the BTK/TEC genes are of a very ancient origin. Porifera also encode several genes for src-type kinases (not present in *C. elegans* and *Drosophila melanogaster*) and it is very likely that several src genes/proteins were already present in the genome of Urmetazoa, the hypothetical metazoan ancestor. The position of the introns in *src* genes favor the view that ancient genes were not »in pieces«, and the introns were introduced gradually during the (recent) evolution of these enzymes. Vera Gamulin was strong supporter of »introns-late theory«. She discussed the question of loss or gain of introns during evolution in several papers. It seems that sponge genes present a number of experimental indications in favor of the introns-late theory. Based on the work of Vera Gamulin and others it became evident that genes from lower Metazoa, like corals and sponges, best represent ancestral genes structure, and in addition, their proteins are more similar to vertebrate/mammalian homologs than are the homologous proteins from model invertebrates *C. elegans* or *D. melanogaster.* In many cases these ancestral genes do not comprise introns, suggesting that most introns were inserted into the genes of higher animals after splitting off the sponge taxa from other metazoan organisms (over 600 million years ago) (late introns). The last contribution of Vera Gamulin to exploring of sponge genomes, recently completed and communicated by her collaborator Dr Helena Ćetković, was the discovery of a large family of Ras-like small GTP-ases, which also characterize those interesting ancient organisms as true and sophisticated animals, as Vera used to teach us in her papers, lectures and private discussions.

In 2003 Vera Gamulin recieved the Republic of Croatia Annual Science Award for distinguished achievement in exploring the cellular genomes.

With all her activity she added much to domestic and international molecular biology as a dominant figure in the field for several decades. For her strong, warm and

peculiar personality she will be remembered by friends and colleagues. As our mutual friend Krunoslav Pisk said in his talk given at the ceremony after Vera passed away, she was demanding scientist, tough and stubborn collaborator, but extremely soft and careful host at her home. I very much agree with this description. I had a privilege to participate in both parts of her life for more than thirty years. The best moments we shared were our private discussions about science or academic life, over a glass of wine.

Ivana Weygand-Đurašević