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GREETINGS AND REMINISCENCES

Dr. Željko Kućan – A Mentor of Great Impact

Dr. Željko Kućan became a part of my scientific education in the fall of 1981 when as a second year Chemistry major at PMF, I was taking a course in Biochemistry. Dr. Kućan was teaching it. He had recently returned to Zagreb after years at Rockefeller University in New York, where he worked, among others, with Fritz Lipmann. The course was covering the usual subjects, various metabolic pathways and the like when a completely new world suddenly opened to us; Dr. Kućan started teaching about recombinant DNA. It was an amazing experience as all of us students observed how his tone of voice changed, and his passion for the significance of the field grew. In retrospect, we were experiencing presentations at the highest level, by a teacher who was deeply attached to and in a way fascinated with the subject. That was the way Dr. Kućan introduced molecular biology to Croatia, by teaching a new generation a whole new discipline of modern biology. Personally, I have shared his vision and passion for molecular biology ever since. I feel very privileged to have been shaped in my scientific education by Dr. Kućan's understanding of the importance of molecular biology.

So, where have this fundamental teacher and his teachings taken my work? Indeed, I left Croatia to learn more about molecular biology, and got my PhD in Madrid. I then went on to the United States, where I have been since 1991. I have cloned genes, characterized translational initiation factors, screened large libraries and identified lead candidates for potential new drug development. I have been a part of many sides of science, from making and analyzing point mutations affecting the functions of proteins to the »big picture« – deciding how to create novel drugs that will help millions of people with diabetes or chemotherapy induced anemia.

I am convinced all this would never have happened, had Dr. Kućan not given me excellent basic scientific training and through his personal accounts taught us the importance of pursuing one's scientific instincts and true beliefs. He taught me the importance of persistence, scientific integrity, and solid scientific judgment, and most importantly, the potential power of a single, creative mind.

Currently I hold a CSO position in a privately owned biotechnology company. Our research focuses on the modulation of cell surface receptors through a site that is not involved in ligand binding. The challenge that we embraced several years ago is whether the insulin receptor (IR) or erythropoietin receptor (EPO-R) can be modulated (activated/deactivated) by a small molecule binding to a site on the extracellular portion of the receptor that is not involved in the binding of the natural hormone, *i.e.,* insulin or erythropoietin (EPO). In other words, the question was how to achieve true hormone mimicry from an activity standpoint, without competing/altering the binding of a natural hormone.

As a student in Dr. Kućan's laboratory, I worked on tRNA molecules and their respective synthetases. Although I left that field many years ago, the principles that I observed and learned at that time had an impact on my scientific thinking and resulted in the collaborative development of what has turned out to be a novel proprietary technology for the modulation (activation/deactivation) of receptor activity. The general idea of protein-protein interactions, and how conformational changes induced in a small (two to three amino acids) functional region can completely alter protein activity was successfully applied to the system of growth factor receptors and their ligands.

Erythropoiesis (red blood cell formation) is regulated by a number of growth factors and cytokines, among which EPO plays an essential role. Its effect on survival, proliferation and differentiation of erythroid progenitor cells depends on the signal emanating from EPO-R. The crystal structure of the erythropoietin receptor (EPO-R) revealed that the receptor exists as a preformed dimer, with a geometry different from that of the erythropoietin-bound receptor, indicating that EPO activates the receptor by changing the conformation of a pre-existing dimer. Binding of EPO triggers a conformational change in the receptor, bringing the two receptor chains closer together, forming a short-distance contact interface between receptor chains. Receptor-ligand interaction and

the assumed geometry of the hormone-receptor complex has served as general guidance in hormone mimicry research. The assumed sequence of events in EPO-R activation by EPO and its size (35 kDa protein) have been major challenges in attempts to design a small molecule mimic of EPO. Many programs failed because their major focus was a replacement of EPO by molecules binding into the same site.

We have taken a different approach. It is based on the idea that once you determine the site on the receptor that undergoes conformational change as a consequence of the hormone binding, it is possible to induce the same conformational change by binding a small molecule to this site, and thus bring the receptors into the proximity required for the activation of signaling events. The approach relies on the concept that an evolutionary conserved, receptor specific, dimerization domain (or protein-protein interaction interface) exists in many, if not most, receptors. Identification of such functional sites on a number of growth factor receptors has led to the development of our platform technology. As for the problem of EPO mimicry, a small molecule was identified that binds to the receptor interface region and activates the EPO-R signaling pathway. It directly causes a conformational change in the receptor interface that is similar to the change that occurs as a consequence of EPO binding to the N-terminal portion of the receptor.

From the scientific standpoint, this is a significant break-through. It is a novel idea of how receptor activity can be modulated. More importantly, from a patient's standpoint, this means that tablets taken orally can be developed to replace drugs currently available only as injectable hormones like insulin or EPO.

Today, I live far away from Zagreb and my scientific work does not involve protein synthesis – but through all these years I have followed the principles and general rules governing biological processes that I was taught by Dr. Kućan. I know that I am just one of the students, friends and colleagues who was inspired and deeply influenced by Dr. Kućan during sharp-witted discussions in the laboratory, and relaxed conversations over a glass of wine. For the years to come I wish him further indefatigable enthusiasm in science and I am most grateful for his fundamental influence on my own scientific life.

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