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A conversation with Aaron Ciechanover

Ushma S. Neill

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Conversations with Giants in Medicine

For decades, the attention of the scientific community was focused on the central dogma of biology — the decoding of the genetic information embedded in DNA. Little research was dedicated to how proteins are degraded and removed from cells. Enter onto the scene a young graduate student, Aaron Ciechanover, who with his mentor Avram Hershko, uncovered the complex and elegant ubiquitin proteolytic system. For his discovery, Ciechanover (Figure 1) shared in the 2004 Nobel Prize in Chemistry with Hershko and Irwin Rose. The complete interview, with more stories about being a member of the Pontifical Academy of Sciences, the magic of "A-ha" moments, and the conflict between religion and Darwinism, can be seen on the JCI website, http://www.jci.org/kiosk/cgm. JCI: What was your childhood like? Ciechanover: I was born in Israel in 1947, the year Israel became independent, to parents that had emigrated as children from Poland, escaping the rising anti-Semitism there. I grew up in a modest Jewish conservative home. My mother was an English teacher and my father a lawyer. I remember that our home was cluttered with books. We had wall-to-wall, floor-to-ceiling libraries filled with Jewish and law books, but not so many on science, as I was the only one interested in science. My parents taught me to read and write early on. It was a scholarly […]

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JCI: What kindled your interest in science?

Ciechanover: I cannot pinpoint it, by maybe it was the close vicinity to nature. I used to walk on the slopes of the Carmel Mountain just behind our home, collecting and drawing flowers, plants, lizards, skeletons — so it was an interest in what I would call today 'taxonomic' biology. When I was 11-years-old, my brother went abroad and I asked him to bring me a microscope — I still have it. My first tiny, small microscope! I started to do experiments: peeling onions and putting the thin layers under the microscope to see the cells, immersing them in pure and salted water to expand and shrink the cells. I remember piercing myself with a needle and smearing blood on a cover glass.

JCI: What led you to medical school?

Ciechanover: I was interested in the complexity of the human body and disease mechanisms.

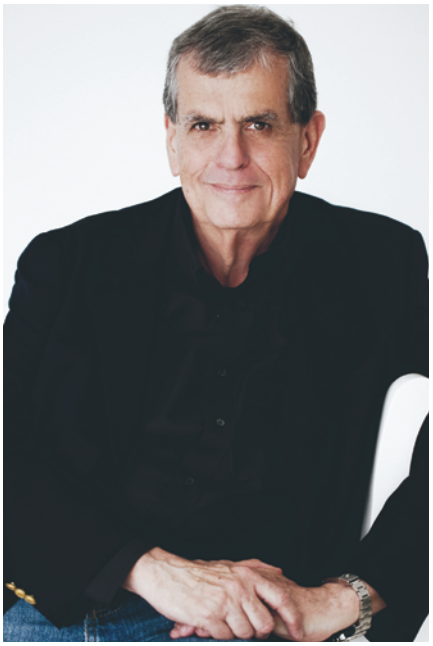

Figure 1
Aaron Ciechanover on May 9, 2013. Image credit: Alena Soboleva.

I joined a program called the "Academic Reserve" where the Israeli military postpones the mandatory national service for students who study professions that are also in need for the Army, as medicine and engineering for example. The first years at the Hebrew University in Jerusalem were exciting because we studied basic sciences such as biochemistry, microbiology, and pathology.

But, when we approached the clinic, I started to feel restless — it was not what I wanted to do — and I decided to take a year off and carry out research in biochemistry; I fell in love with this discipline. I then completed my medical studies and my military service as a combat physician, including serving in the 1973 war. After being discharged, I landed safely in biochemistry.

JCI: It was after this that you first came to Avram Hershko's laboratory?

Ciechanover: Medical students have to submit a small research thesis in order to graduate. This I did with Avram Hershko, who had just returned from his postdoctoral training as a young assistant professor. We kept our ties during my military combat physician service after which I decided to do my PhD with him.

JCI: How did you come across the idea to study protein degradation?

Ciechanover: Avram had started to work on the subject when he was a fellow at UCSF. He had come across earlier — apparently thermodynamically paradoxical data — that protein degradation in both bacteria and mammalian cells require metabolic energy. Our dietary proteins provide us with energy, so it did not make sense to invest energy to degrade them to their low-energy amino acids building blocks. Yet, energy is required for proteolysis via the lysosome that was discovered by Christian de Duve, as the maintenance of its low acidic milieu requires pumping of hydrogen ions. There were, however, signs in the literature that it was not the lysosome that degrades most of intracellular proteins. We hung on those findings and glued them together, initiating a search for a non-lysosomal proteolytic system.

We started by establishing a cell-free system from reticulocytes that degrades a model protein. Shortly after, we realized we had a novel finding — the activity we discovered was not resolved as expected, as a single protease, but rather as two complementing inter-dependent activities. At that point in the summer of 1977, less than a year after I started my studies, Avram went on sabbatical to Philadelphia to work with Irwin [Ernie] Rose, and I stayed behind in Israel.

JCI: How did you hit on using red cells extracts?

Ciechanover: The reticulocyte is a great cell, because it doesn't have lysosomes; along its differentiation to the mature red blood cell, it extensively degrades all its machineries and proteins, leaving behind mostly hemoglobin. The problem was that hemoglobin comprises ~85% of all the cellular proteins, and it was difficult getting rid of it. One of our two complementing activities was resolving along with hemoglobin, but we could not isolate the culprit whatever we did. As a last resort, and while Avram was away, along with a colleague of mine, Michael Fry, we decided to literally boil the "red" extract. The hemoglobin precipitated like mud and the yellowish supernatant had all the activity. While we could not believe it was a protein, it was sensitive to proteases, had a high molecular weight, and was precipitable with ammonium sulfate. The two findings — the two complementing activities and the heat resistance

of one of them — were critically important as they set our future research direction. These embarrassingly simple experiments were published in *BBRC* after they were rejected from *JBC*, teaching us an important lesson, particularly these days — it does not matter where you publish but what you publish.

JCI: Rose provided crucial input, given that he was coming at the problem from a different direction.

Ciechanover: During the summer of '79, on another visit to the Fox Chase Cancer Center, Ernie helped us to solve an important problem. Beforehand we had purified the active heat-stable polypeptide and realized that when incubated in the presence of ATP and the other active fraction, it generated high molecular weight complex with some other protein(s). We thought that the complex could result from a gentle association — for example, a cryptic protease that is activated by our protein. With Ernie's advice, we found that ATP helps to catalyze a stable peptide bond between our protein and endogenous substrates in the crude extract, and hypothesized that this conjugation signals the tagged proteins for degradation. We called our first purified protein APF-1 (ATP-dependent proteolysis Factor-1). Other people at Fox Chase highlighted to us a protein with a similar molecular weight — ubiquitin — that is conjugated to histone H2A in an isopeptide bond. The similarity to APF-1 was striking, and along with Keith Wilkinson and Art Haas at Fox Chase, we found that APF-1 was indeed ubiquitin. This was an important discovery, as not only it did unravel the nature of the chemical bond between APF-1 and its target substrate, but it also explained the mechanism of action of ATP, solving the energy requirement mystery, and enabled the discovery of the conjugating machinery that is made of three enzymes: E1, E2, and E3, that act in concert.

JCI: What motivated you to do your post-doc with Harvey Lodish at MIT?

Ciechanover: Following graduation, though I could stay as a faculty member, I wanted to become independent. I wrote to Harvey who worked on the cleavage of the Poliovirus polyprotein.

Arriving at the laboratory, Harvey suggested I could work on receptor-mediated endocytosis. This was good advice: coming from pure biochemistry, I not only delved into a new field — that of cell biology and the problem of routing of proteins in cells — but, along with Alan Schwartz and Alice Dautry-Varsat, we discovered the cycle of the transferrin receptor and iron delivery into cells. It was an important experience, but then, I gradually slid back to work on ubiquitin.

JCI: Meaning that during that time you were also interacting with Alexander Varshavsky, who drew you back into ubiquitin research.

Ciechanover: Alex had discovered in the literature a temperature-sensitive cell cycle arrest mutant in which ubiquitinated histone H2A disappeared at the high temperature. We discussed and thought either the cells lost their ability to ubiquitinate the histone or gained the ability to rapidly deubiquitinate it. It made more sense that they lost their ability to ubiquitinate, as mutations typically result in a loss of function. Along with Daniel Finley, a graduate student in Alex's laboratory, we ended up discovering a mutation in E1 — the ubiquitin-activating enzyme, the first enzyme in the ubiquitination cascade. Needless to say, the cells were defective also in degradation of short-lived proteins. We were lucky, because if it had been a mutation in the histone E3, it would have taken a long time to discover it. Since it was a cell cycle arrest mutant, we speculated that the ubiquitin system is involved also in cell cycle regulation, which later turned out to be correct. The description of the E1 mutation was another corroboration of our earlier findings that ubiquitination signals proteins for degradation.

I went on to study the requirement for tRNA in the proteolytic process, which I discovered when I was still with Avram, but never pursued. This turned out later to be part of the N-end rule, discovered and studied independently by Alex. Basically, in the second half of my post-doctoral fellowship, I became a 'freelancer' in Harvey's laboratory. He did not know much about ubiquitin, but was gracious to learn it and help me. I owe a lot to the independence that Harvey gave me and to the openness I enjoyed in his laboratory. I remember fondly his advice when I hesitated about returning to Israel. He was all for it, where he knew my family will be most comfortable. He argued that even under less favorable conditions it is mostly the scientist's quality and drive that would be detrimental to his/her success.

JCI: You decided to go back to the same institute as Hershko in Israel.

Ciechanover: To the same institute, yes, but I was completely independent. I brought my own projects and started an independent career, completely away from Avram, though being close and having the ability to discuss problems clearly helped.

JCI: Does ubiquitin still motivate you, 35 years later?

Ciechanover: More than ever! We haven't even begun to understand the complexity of the system. It has almost 2,000 components, approximately 7% of the human genome. The ubiquitin system plays a major role in clearing defective/misfolded proteins. Besides quality control, it removes in a programmed manner important cellular proteins like cell cycle regulators and transcription factors. Importantly, there are drugs already on the market to treat aberrations in the system and now more diseases — inflammatory disorders, neurodegenerative diseases and malignancies — are being tied to defects in the system.

JCI: You've said you did your science because of scientific curiosity, not to win prizes. But surely, sharing in the Nobel Prize with Hershko and Rose must have been fairly sweet.

Ciechanover: You celebrate for one day, and then you celebrate the second day, and the third day you have to decide what you are going to do with yourself. I decided to do two things: first, to continue my research; being in the laboratory is so exciting now, with smart students and fellows, and sophisticated technologies. Second, to leverage my 'status' to highlight two major issues. One is education of children in Israel and worldwide. I talk to them at eye level and they see, wow — it is possible to make a major achievement and still remain a regular human being. We need children falling in love with science at an early age.

The recognition also enabled me to trace my Jewish heritage. I try to build close relations with Jewish communities, mostly small and remote ones. For example, there is a tiny Jewish community in Greece left after the extermination during the holocaust, or a tiny one in Paraguay made mostly of holocaust survivors. I speak in different community activities — sermons in synagogues, for example — and it is fascinating, as I feel I blow wind in their sails. I feel an amazing sense of belonging.

JCI: If you were not a scientist, what do you think you would have done?

Ciechanover: The natural track would have taken me to surgery, and I believe to cardiac or neurosurgery. There is something mystical about these two organs where emotion and reason reside. But the fact that the discovery of the ubiquitin system led to the unraveling of related diseases and development of drugs is kind of a cycle closure for me.

Ushma S. Neill