Dankesrede

von

Prof. Dr. Frederick W. Alt

anlässlich der Verleihung

des Paul Ehrlich- und Ludwig Darmstaedter-Preises

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Anrede,

I was stunned when Thomas Boehm informed me that I would be a recipient of the 2023 Paul Ehrlich and Ludwig Darmstaedter prize. I am humbled to be included on a list with the distinguished past recipients. I am particularly honored to share the prize with David Schatz, whose remarkable work has been foundational for our field. I thank the committee for selecting us, given many others who have deeply contributed to our understanding of factors and mechanisms that diversify antigen receptor repertoires.

I learned about Paul Ehrlich's pioneering work early in my training. But, I more fully appreciated the immense brilliance and versatility of his contributions to medical science, along with his impressive personal qualities, while reading about him more recently. I have worked in three areas deeply impacted by Paul Ehrlich, namely chemotherapy, immunology, and cancer research. Work on the latter two has intersected throughout my career.

My parents both died from cancer when I was in elementary school. Although I was a cancer orphan, I was fortunate to be raised by my sister and brother-in-law with their 6 kids. I benefited immensely from family support and support of close friends. It was during that childhood period that I became determined to become a cancer researcher.

When I went to Stanford University for PhD studies in 1971, I took a pharmacology course in which the late Robert Schimke discussed cancer cell resistance to methotrexate, a potent cancer chemotherapeutic. While powerful, methotrexate is often limited by emergence of cancer cells resistant to its activity. Inspired by his lecture, I asked Dr. Schimke to take me into his lab to study methotrexate-resistance. While he had not previously worked on this subject, he was deeply interested. He accepted me and purchased equipment to support my work.

After 5 years, our studies suggested the then heretical idea that methotrexate-resistance might derive from cancer cells that had generated many copies of the cellular target gene. In those early days, testing this hypothesis was challenging. However, I developed a new technique that allowed me to isolate a tiny bit of radioactive DNA encoding the suspected methotrexate-resistance gene. With that DNA, we discovered gene amplification, a process that leads to 100s of extra gene copies in drug-resistant cancer cells.

In 1976, Susumu Tonegawa published his Nobel prize winning discovery that developing lymphocytes intentionally undergo genomic rearrangements, known as V(D)J recombination, to generate diverse antibody repertoires. When I joined David Baltimore’s lab for postdoctoral studies, I intended to work on cancer viruses. But, Baltimore was preparing to enter the

immunology field and he presented compelling opportunities for me to address mechanisms of programmed V(D)J rearrangements in lymphocytes that fit well with my background in studying genomic rearrangements in cancer cells.

Baltimore and Naomi Rosenberg developed leukemia-virus transformed progenitor B lymphocyte cell lines. Our studies of these lines revealed them to undergo V(D)J recombination in culture. Based on a V(D)J recombination data-set I generated from these lines, David Baltimore and I, over a dinner and a late working night at his home, generated a model for the V(D)J recombination mechanism, which Dr. Tonegawa kindly presented in a subsequent review as the Alt-Baltimore model.

The first postulate of the model was that V(D)J recombination is initiated by an endonuclease enzyme that cuts at specific V, D, J recognition sites. Many labs, including mine, tried in vain to identify this key endonuclease. But, David Schatz, working as a student in the Baltimore lab, did a remarkable set of experiments that allowed him to transfer this activity into non-lymphoid cells and then identify the RAG proteins that generated the activity. With those amazing experiments, Schatz opened up the V(D)J recombination field.

The second postulate of the model was that V(D)J recombination-generated DNA double-strand break ends are processed to increase diversity through non-templated nucleotide additions, we termed N-regions, by the terminal transferase enzyme. By eliminating this enzyme in mice, my lab and the Mathis/Benoit lab, proved that it is indeed a key V(D)J recombinase component that immensely diversifies antigen receptor repertoires.

Finally, our model proposed that V(D)J recombination-generated ends are processed and joined by an unknown double-strand break end-joining pathway. By screening a panel of DNA repair-deficient cells, my postdoctoral fellow, Guillermo Taccioli, identified mutants that could not join RAG-generated DNA ends. On this basis, with collaborators Penny Jeggo and Steven Jackson, we identified three major components of a novel non-homologous end-joining (NHEJ) pathway, now recognized as a major mammalian cell repair pathway of double-strand breaks. Additional key components were identified by others, notably by Jean-Pierre de-Villartay and Alain Fischer, based on mutations they discovered in human immunodeficient patients. Beyond providing indispensable V(D)J recombinase components, we found that non-homologous end-joining also suppresses genomic instability that leads to oncogenic translocations and amplifications.

For decades we puzzled over how RAG identifies substrate gene segments embedded over long genomic distances across antigen receptor loci. An important insight came from David Schatz, who found that RAG does its work while sitting at one antigen receptor locus location, termed a recombination center. Conventional wisdom was that distant substrate sequences would find RAG through a random diffusion process. We reasoned such a random process would be inefficient and dangerous, as it could allow V(D)J recombination to attack other parts of the genome. To definitively address this mechanism, we developed new technologies that allowed visualization of paths of RAG activity across chromatin domains. What we observed was fascinating. From its recombination center base, RAG linearly explored large, well-defined antigen receptor domains to look for V(D)J recombination targets; but it stopped searching at the end of these domains.

I quickly called David Schatz to get his feedback and advice. We ultimately discovered that RAG harnesses a general genomic organization process, discovered by others, termed chromatin loop extrusion to scan chromatin for substrates. Loop extrusion, like a conveyer belt, presents RAG with distant chromatin containing substrates. This process allows RAG to linearly scan segments of chromatin in antigen receptor loci that are more than one million DNA bases long. The process is terminated by impediments at the end of the locus that prevent RAG from acting on other genomic domains. We also found that a milder impediment activity near the substrates slows scanning at the recombination center to better present substrates to RAG, explaining the chromatin accessibility mechanism, discovered by my student George Yancopoulos and I decades earlier.

I am indebted to my exceptional mentors and many spectacular students and postdocs, including my first postdoctoral trainee Michael Reth who won the Ehrlich prize nearly a decade ago.

I also wish to acknowledge work done by many to elucidate the B cell antigen receptor diversification processes of class switch recombination and somatic hypermutation. Much of that work was facilitated by discovery of the AID diversification enzyme by Tasuku Honjo in collaboration with Anne Durandy and Alain Fischer, and by AID mechanistic work of the late Michael Neuberger.

Paul Ehrlich was known for his inspirational talent in unraveling and visualizing complex processes. I guess he would have enjoyed the amazing and intricate beauty of antigen receptor diversification.