Getting where you want to go

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ABSTRACT **In 1956, referring to the emerging application of electron microscopy to the study of eukaryotic cells, Keith R. Porter wrote, "For those of us who are fortunate to be part of this new development, these are days of great interest and opportunity." Those early days left us a rich legacy of knowledge on the internal organization of eukaryotic cells that provides a framework for current research on cell structure and function. In this vein, my long-time quest has been to understand how proteins and organelles travel through the cytoplasm to reach their respective destinations within the cell. This research has led us to elucidate various mechanisms of protein sorting and organelle transport and how defects in these mechanisms cause human disease.**

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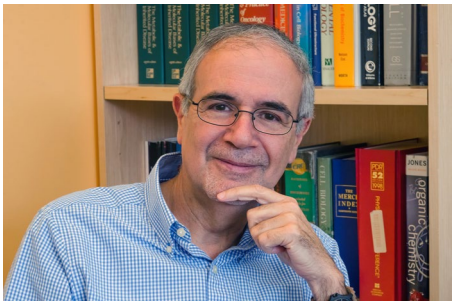
I am deeply honored to have been selected to present the 2022 Keith R. Porter Lecture, particularly because Porter's discoveries on the internal organization of the eukaryotic cell have served as the foundation for much of my work. The primary focus of my research is the intracellular transport of integral membrane proteins, which involves several organelles described by Porter, including the endoplasmic reticulum, coated vesicles, and microtubules.



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I first became interested in integral membrane proteins as an undergraduate student at the University of Buenos Aires, Argentina, when our class discussed the article "The fluid mosaic model of the structure of cell membranes" by Jon Singer and Garth Nicolson (Singer and Nicolson, 1972). I was fascinated by how, through careful analysis of emerging evidence, the authors overturned previous models of membrane structure and proposed a more dynamic view of integral membrane proteins. I subsequently delved into the properties of integral membrane proteins through my research on prolactin and growth hormone receptors as a PhD student in the laboratory of Alejandro Paladini, also at the University of Buenos Aires. It was at that time that I first learned of the National Institutes of Health (NIH) in Bethesda, Maryland, a place that I pictured in my mind as some sort of science Shangri-La. Little did I know that this place would become my scientific home for the next four decades.

After completing my PhD studies, I had the good fortune (which happens to be the meaning of my Italian surname in English) to obtain a postdoctoral fellowship to continue my career at the NIH and, even more luckily, to work in the laboratory of Rick Klausner on the relationship between the assembly and transport of newly synthesized subunits of the T-cell antigen receptor, a multiprotein complex that had just been discovered. We found that subunit assembly was required for export of the complex from the endoplasmic reticulum (Bonifacino *et al.*, 1989). Furthermore, with another postdoctoral fellow in the laboratory, Jennifer Lippincott-Schwartz, we discovered that unassembled subunits were degraded by a novel nonlysosomal pathway (Lippincott-Schwartz *et al.*, 1988) that would later become known as "ER-associated degradation" (ERAD) (McCracken and Brodsky, 1996). We also mapped the assembly and degradation determinants to the transmembrane domains of the subunits (Bonifacino *et al.*, 1990b) and established the general principle that colocalization of assembly and degradation determinants ensures that only fully assembled complexes survive in the cells (Bonifacino *et al.*, 1990a).

Toward the end of my postdoctoral training, I traveled to Boston to present a poster on T-cell receptor assembly and degradation at

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Abbreviations used: AP, adaptor protein; BORC, BLOC-one-related complex; ERAD, ER-associated degradation; GGA, Golgi-localized, gamma-ear containing, ADP-ribosylation factor binding; HOPS, homotypic fusion and protein sorting; HPS, Hermansky-Pudlak syndrome.

the 1991 annual meeting of the American Society for Cell Biology (ASCB). Rick was scheduled to chair and speak about this same topic at a plenary symposium on protein degradation the first day of the meeting at 8:00 am. However, Rick fell ill the night before and asked me to step in for him. The next morning, after briefly announcing the substitution, I proceeded to give the talk using Rick's slides. Later, I was told that a late arriver was overheard saying, "I didn't know Klausner spoke with an accent!" The talk was well received and served as a baptism of fire for all of my subsequent talks in front of large audiences.

When the time came to become independent, I was again lucky to be offered a tenure-track position at the NIH. Because the position was in the same branch as Rick's, I decided to switch topics. I chose to study the signal-mediated mechanisms by which integral membrane proteins are sorted to different compartments of the endomembrane system including endosomes, lysosomes, lysosome-related organelles, and distinct domains of the plasma membrane. In essence, I wanted to know how sorting signals encoded within the sequence of integral membrane proteins were recognized by the cellular machinery. When learning of my plans, two senior colleagues told me that it was a poor choice because other important laboratories had long been working on that very problem without success. For me, however, it just seemed like a fundamental question that needed to be addressed.

With my first postdoctoral fellows, I focused on identifying the recognition protein(s) for a type of sorting signal containing a critical tyrosine residue found in the tail of some endocytic receptors (Lazarovits and Roth, 1988; Lobel *et al.*, 1989; McGraw and Maxfield, 1990). There were many false starts that painfully reminded me of my senior colleagues' comments. But then, a postdoctoral fellow in the laboratory, Hiroshi Ohno, in a collaboration with the laboratory of Tommy Kirchhausen, discovered that such signals are recognized by the μ1 and μ2 subunits of adaptor protein (AP) complexes AP-1 and AP-2, respectively (Ohno *et al.*, 1995). These complexes are components of clathrin coats like those that had first been visualized by Tom Roth and Keith Porter as bristles decorating the surface of pits and vesicles in mosquito oocytes (Roth and Porter, 1964). Hiroshi's discovery was the beginning of an exciting time in the laboratory, as we went on to find how various types of sorting signals are recognized by AP-1 and AP-2, and by newly discovered adaptors including AP-3, AP-4, and the GGA proteins (Dell'Angelica *et al.*, 1997, 1999a; Puertollano *et al.*, 2001; Janvier *et al.*, 2003). The latter adaptors are components of other protein coats that were independently described in our laboratory and in the laboratories of Scottie Robinson, Rick Kahn, Scott Emr, Greg Payne, and others (Cowles *et al.*, 1997; Simpson *et al.*, 1997; Hirst *et al.*, 1999; Boman *et al.*, 2000).

In the course of these studies, we added another dimension to our work: the relationship between protein transport and disease. This line of research started when another postdoctoral fellow, Esteban Dell'Angelica, in a collaboration with the laboratory of Bill Gahl, found that mutations in the β3A subunit of AP-3 are the cause of the pigmentation and bleeding disorder Hermansky–Pudlak syndrome (HPS) type 2 (Dell'Angelica *et al.*, 1999b). This was the first of many diseases now known to be caused by mutations in components of protein coats, for which we coined the term "coatopathies" (Dell'Angelica and Bonifacino, 2019). The application of fundamental research to the elucidation of disease mechanisms thus became another focus of my career.

In later work, we became interested in how organelles are coupled to microtubule motors. A key discovery by postdoctoral fellow Jing Pu was a multiprotein complex named BORC that regulates the coupling of lysosomes to kinesin-1 and -3 motor proteins (Pu *et al.*, 2015), and to the tethering complex HOPS (Jia *et al.*, 2017). Jing and other members of the laboratory also described the mechanisms of BORC function and demonstrated the critical importance of this complex in various cellular processes, including cell adhesion and migration, autophagy, nutrient signaling, and maintenance of axonal health. As in the preceding studies, we found that mutations in BORC subunits cause human diseases, particularly affecting the development of the central nervous system. Our current efforts are aimed at investigating how defects in protein and organelle transport alter axonal integrity and function, a common cause of many neurodevelopmental and neurodegenerative disorders.

Looking back at what we were able to accomplish at the interface of cell biology and disease, I cannot emphasize enough the importance of being at the NIH. Wonderful colleagues, interactions with both basic and clinical investigators, the atmosphere of intellectual freedom, and stable funding have all contributed to making this type of research possible. A special expression of gratitude goes to my institute, the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), which has funded our projects for all these years. NICHD was established 60 years ago during President John F. Kennedy's administration, partly in response to the strong advocacy efforts of his sister, Eunice Kennedy Shriver, after whom the institute is now named. The founding mission of the NICHD was to combat intellectual and developmental disabilities, a goal that our laboratory is happy to share.

An additional boon of working at the NIH is to be surrounded by amazing scientists from all over the world who collaborate in pursuit of common goals. I often think of the scientific community as a model of international cooperation, which, if universally applied, could contribute to the solution of some of the world's most urgent problems, including hunger, disease, war, climate change, environmental degradation, and the extinction of species.

I wish to end with a simple piece of advice to anyone considering a career in science: follow your dreams, your passions. Imagine where you would like to be, what you would like to achieve, and go for it. You will meet challenges and failure along the way, but with perseverance, faith in yourself, and good mentors you will eventually get where you want to go – just like the proteins find their way through the intricacies of the endomembrane system to their final destinations within the cell.

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