BIOGRAPHICAL MEMOIRS

Alfred Lewis Goldberg

September 3, 1942–April 18, 2023
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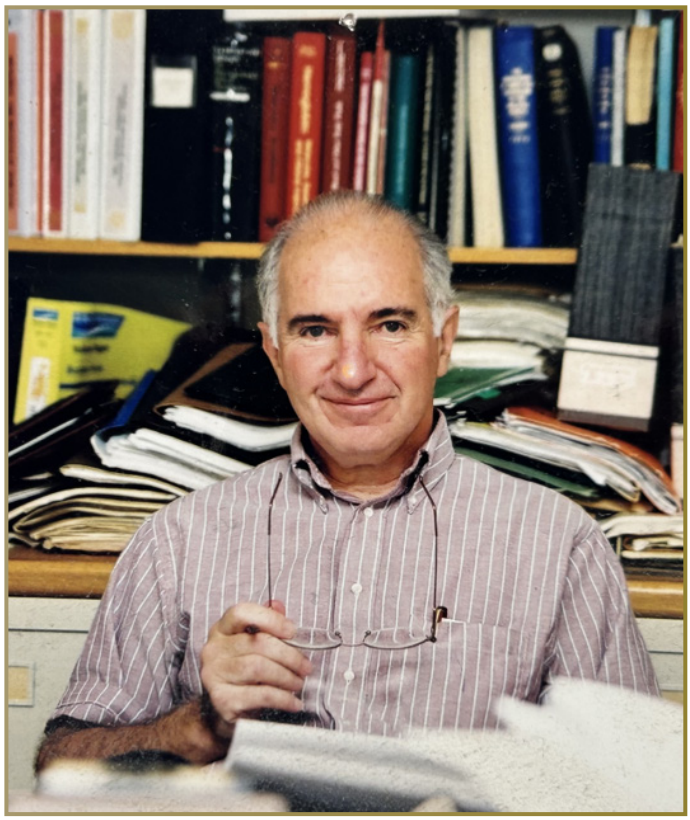
A Biographical Memoir by Aaron Ciechanover



Alfred "Fred" Lewis Goldberg was unquestionably the founding father of regulated intracellular proteolysis as we know it today. At a time when the scientific community focused on protein translation and anabolism, Fred turned his focus to the opposite and neglected area of protein catabolism.[1,2] In doing so, he almost single-handedly discovered the principles and many of the mechanisms as to how cellular proteins are destroyed in a regulated manner. He brought a passion to this fundamental biological problem that knew no boundaries, and he worked on this subject to the last minutes of his life. His last published study appeared in August 2023, four months after he passed away.[3]

Fred was born on September 3, 1942, in Providence, Rhode Island. In 1963, he graduated with a bachelor's degree in biochemical sciences from Harvard College, where part of his research was carried out under James Watson. Following one year as a Churchill Scholar in physiology at Cambridge University (1963–64), Fred started his medical studies at Harvard. After two years (1964–66), he realized that he was much more interested in understanding the mechanisms that underlie the pathogenesis of diseases rather than treating them. Consequently, he transferred to Harvard Medical School's graduate program and completed his Ph.D. in physiology in 1968 under the supervision of H. Maurice Goodman. It was during his graduate studies that Fred became interested in muscle pathophysiology, and in particular in the fate of proteins and amino acids.[4] He then joined the Harvard Medical School Faculty, achieving the rank of full professor at the young age of thirty-five, and spent his entire professional career at this august institution.

Given that many proteases can indiscriminately cleave most proteins, one of the central mysteries about intracellular proteolysis was how such a non-specific process didn't harm the function of cells or even kill them. The first clue to solving this problem came from the observation that protein degradation in both mammalian[5] and bacterial[6] cells required metabolic energy in the form of ATP hydrolysis. This was surprising because hydrolysis of peptide bonds should be exergonic instead of endergonic. Although the mechanism that underlay the involvement of ATP was not known at the time, Fred realized that whenever metabolic control was needed, cells paid with a single currency, which was energy. His assumption was that the expenditure of energy endowed the system with controls that prevented non-specific destruction. In two illuminating

review articles, Fred summarized all that was known about intracellular proteolysis at the time (mid-1970s), including highlighting the major and yet unsolved problems of why this process required energy and how it had substrate specificity.[7,8] One important piece of data was eye catching. The first table in his second review article demonstrated that the half-life of different cellular proteins spanned two orders of magnitude; and in fact, we now know that these differences can be even greater! Another point was that the degradation of many proteins was dependent upon changing pathophysiological conditions, such as starvation, denervation, infection, or various endocrine cues. The two articles made it clear that intracellular proteolysis was far from what the scientific community had thought was a simple and non-interesting cleavage of peptide bonds. Rather, it was likely to be a complex, highly specific, and directional process that was coordinated with protein synthesis to maintain the steady state of the proteome and its quality, and dynamically regulated to cope with such events as cellular stress. Fred made a wise decision to unravel the secrets behind this process. He first turned to bacteria, assuming that the mechanism in these organisms would be simpler than in mammalian cells. In a long series of methodical studies, he described and characterized an entire array of bacterial proteases, including the Lon gene product capR that was ATP-dependent.[9,10] Having an ATP-dependent protease in hand, he meticulously dissected its structure, function, and, importantly, its mechanism of action. These discoveries proved extremely useful when Fred turned his attention to deciphering the corresponding mammalian mechanisms. To solve the problem of specificity in mammalian cells, Fred investigated the different characteristics of protein substrates and tried to correlate them with their rate of degradation. Thus, one attempt was to relate the physical and chemical characteristics of proteins, such as their iso-electric points, to their rate of degradation.[11] Another attempt was to relate unique sequences within proteins that made them susceptible or resistant to specific degradation.[12] Fred then realized that even if proteins exposed characteristics that rendered them susceptible to recognition by the proteolytic system, it was critically important to identify and dissect mechanistically the system that mediated the process. One major breakthrough was the discovery of an ATP-dependent proteolytic activity against abnormal proteins in reticulocyte lysate.[12] Here, Fred brought down two important birds in a single shot: he identified both a non-lysosomal and an energy-dependent proteolytic system that degraded a subset of specific proteins. As evidence for the system being non-lysosomal, Fred demonstrated that the reticulocyte extracts were active optimally at a slightly basic pH, the opposite of lysosomal proteases, which are most active at an acidic pH. Consistent with this, the reticulocytes had expelled most if not all of their lysosomes. The fact that the newly described system was non-lysosomal also made logical sense because it had substrate specificity. In contrast, the mechanism by which the lysosome degrades intracellular proteins is autophagic in nature and non-selective. This is because autophagic vacuoles, such as those generated in micro-autophagy, engulf droplets of the cytosol, whereas all the cytosolic proteins are represented and degraded by lysosomal proteases in a non-discriminatory manner. Using lysosomal inhibitors, Poole and his colleagues showed a bit later that this is indeed the case even in cultured nucleated cells that have a complete cohort of functional lysosomes.[14] These two independent lines of evidence laid by Fred and Poole were important, as they pointed to a novel and as yet undiscovered proteolytic system in the cytosol. Equally important, Fred's experiments showed that this cytosolic system required metabolic energy. As lysosomal degradation also required energy to maintain the acidic pH in the lysosomal lumen, it was important to elucidate the reason that Fred's soluble cytosolic system also required energy. Fred's previous studies on bacterial proteases led him to hypothesize that there were mammalian proteases whose activity required ATP. As he would discover, his hypothesis turned out to be correct, and Fred would name and provide detailed mechanistic insights into the 26S proteasome.[15] Martin Rechsteiner and colleagues were apparently studying the same enzyme.[16] This discovery solved half of the puzzle. In parallel and at about the same time, Aaron Ciechanover and Avram Hershko were also working on degradation of proteins in reticulocytes, and purified from it the ubiquitin system.[17] This was the second half of the puzzle because the conjugation of poly-ubiquitin to proteins required ATP. These two puzzle pieces fit together as polyubiquitin-conjugated proteins that were degraded by the 26S proteosome. Together these discoveries explained both the energy requirement for protein hydrolysis and how cells could employ a cytosolic proteolytic system without wanton destruction of the proteome: The ubiquitin system uses ATP to selectively mark proteins for destruction, and the 26S proteosome uses ATP to thread substrates into an internal catalytic chamber whose active sites are otherwise not accessible to cellular proteins. These discoveries stimulated flourishing new areas of biological research into regulated intracellular proteolysis that have revealed that this process is involved in almost every fundamental cellular process. These efforts placed protein degradation in a seat of honor, side-by-side with protein synthesis, and shed light on the regulation of processes such as cell cycle, protein quality control, differentiation, antigen presentation, and signal transduction and protein quality control, as well as providing therapeutic targets for drug development.

Fred continued his magnificent studies concentrating on the proteasome. He showed that the proteasome and the upstream ubiquitination machinery are involved in muscle wasting. It should be emphasized that massive muscle wasting

that involves degradation of myofibrillar proteins occurs in numerous pathologic states. These states include chronic infection, cancer cachexia, renal insufficiency, denervation from spinal cord injuries, and the long-term immobilization that accompanies lower limb and pelvic fractures, among others. Patients with these conditions constitute a major challenge for rehabilitative medicine, and their treatment is long, interdisciplinary, and expensive. Fred was attracted to this problem and devoted significant effort to dissect its underlying mechanisms. He thought that such a problem, which was based on his studies, should be regarded as an important example for dysregulated protein degradation and therefore epitomize the bridge between the bench and the bedside. He approached this problem using different experimental models, and his discovery of the involvement of the proteasome and then the ubiquitination machinery in the process helped him paint for us a marvelous deep and broad landscape of its pathogenesis.[18,19]

At that time, Fred came up with a courageous idea that inhibition of the proteasome might slow down, at least partially, the wasting process and ease the treatment and possibly the recovery of patients with muscle wasting. It was courageous because one would have expected that inhibition of the proteasome would have unacceptable deleterious side effects, but this turned out not to be the case. Along with other colleagues, Fred was instrumental in establishing a biotech company, MyoGenics, with the aim to develop proteasome inhibitors for the prevention of muscle degeneration and to treat other conditions. At the end of the day the company did not develop drugs for muscle wasting, but their inhibitors have served the scientific community successfully in an endless number of studies, expanding the role of the proteasome to basically every fundamental cellular process.[20] Moreover, the inhibitors developed by the company (later part of Millennium, Inc.) became highly efficient drugs for treating the blood malignancy multiple myeloma (MM). MM is a B cell malignancy characterized by a monoclonal expansion of an antibody-secreting plasma cell. The drug acts most probably by inhibiting proteasome-dependent degradation of defective antibody molecules, such as ones that are misfolded and produced at some level during synthesis. Upon treatment with a proteasome inhibitor, these abnormal proteins accumulate, causing endoplasmic reticulum (ER) stress, which elicits the unfolded protein response (UPR), and subsequent cell death. The proteasome inhibitor was a new and unique class of drug with a novel mechanism and therefore could be administered along with other chemotherapeutic agents. Its introduction revolutionized the clinical course of the disease, extending life span and quality of life in a significant manner. It was a true transformation.[21] I assume that Fred had regarded this achievement as the most important in his career,

an achievement that made true his dream to draw a direct linkage between the laboratory bench and the patient bed, and that has benefitted and will continue to benefit the lives of myriads worldwide.

Fred took his proteasome studies another step forward and deep into immunology. Along with Kenneth Rock, he showed that the proteasome is involved in processing of antigens for presentation on class I MHC molecules, and that the process is sensitive to proteasome inhibitors with implications for treatment of immune disorders.[22,23] A deeper dive into this important process revealed that the proteasome processes the target protein to antigenic peptides, but often leaves an N-terminal extension that has to be cleaved in order for the antigenic peptide to fit well into the MHC class I molecule's peptide-binding site. This trimming is catalyzed by an ER metalloprotease discovered and characterized by Fred and Ken, who named it ER-associated protease, ERAP.[24]

As is apparent, Fred can be justifiably crowned as the father of modern regulated intracellular proteolysis. He was there when nobody believed the process to be important or complex and over the ensuing almost six decades contributed a multitude of key discoveries that have helped establish the field as a mainstream platform in modern cell biology and physiology. His journey was rich with major contributions, from shedding light on the mechanisms that underlie the involvement of intracellular proteolysis in basic cellular processes all the way to their translation to successful drugs. His revolutionary studies were recognized by many learned societies, among them the National Academy of Medicine (elected 2009) and National Academy of Sciences (elected

2015), and the American Physiological Society. He received numerous prizes, among them the Warren Alpert Prize for the development of Velcade (Bortezomib®) for treatment of multiple myeloma and the Passano Award for medical research. The list of honorary degrees he received and distinguished (named) lectures he delivered is miles long.

Fred passed away on April 18, 2023, after a long battle with lymphoma. He will be sorely missed by his family and by all of us who walk along the path he paved. Fred is survived by his wife, Joan Helpern Goldberg, a hematologist, and their two children, Aaron Goldberg, a renowned jazz pianist, and Julie B. Goldberg, a software engineer.

References

1 Goldberg, A. L. 1969. Protein turnover in skeletal muscle I: Protein catabolism during work-induced hypertrophy and growth induced with growth hormone. *J. Biol. Chem.* 244:3217-3222.

2 Goldberg, A. L. 1971. Effects of protease inhibitors on protein breakdown and enzyme induction in starving *E. coli*. *Nature New Biol.* 234:51-52.

3 Lee D., et al. 2023. Molecular mechanism for activation of the 26S proteasome by ZFAND5. *Mol. Cell* 83:P2959-2975.E7.

4 Goldberg, A. L., and H. M. Goodman. 1969. Effects of disuse and denervation on amino acid transport by skeletal muscle. *Am. J. Physiol.* 216:1116-1119.

5 Simpson, M. V. 1953. The release of labeled amino acids from the proteins of rat liver. *J. Biol. Chem.* 201:143-154.

6 Mandelstam, J. 1958. Turnover of protein in growing and non-growing populations of *Escherichia coli*. *Biochem. J.* 69:110-119.

7 Goldberg, A. L., and J. F. Dice. 1974. Intracellular protein degradation in mammalian and bacterial cells. *Ann. Rev. Biochem.* 43:835-869.

8 Goldberg, A. L., and A. C. St. John. 1976. Intracellular protein degradation in mammalian and bacterial cells. Part II. *Ann. Rev. Biochem.* 45:747-803.

9 Swamy, K. H. S., and A. L. Goldberg. 1981. *E. coli* contains eight soluble proteolytic activities one, being ATP dependent. *Nature* 292:652-654.

10 Chung, C.H., and A. L. Goldberg. 1981. The product of the lon (capR) gene in *Escherichia coli* is the ATP-dependent protease, protease La. *Proc. Natl. Acad. Sci. U.S.A.* 78:4931-4935.

11 Dice, J. F., E. J. Hess, and A. L. Goldberg. 1979. Studies on the relationship between the degradative rates of proteins in vivo and their isoelectric points. *Biochem. J.* 178:305-312.

12 Wing, S. S., et al. 1991. Proteins containing peptide sequences related to Lys-Phe-Glu-Arg-Gln are selectively depleted in liver and heart, but not skeletal muscle, of fasted rats. *Biochem. J.* 275:165-169.

13 Etlinger, J., and A. L. Goldberg. 1977. A soluble, ATP-dependent proteolytic system responsible for the degradation of abnormal proteins in reticulocytes. *Proc. Natl. Acad. Sci. U.S.A.* 74:54-58.

14 Poole, B., S. Ohkuma, and M. J. Warburton. 1978. In: *Protein Turnover and Lysosome Function,* eds. H. L. Segal and D. J. Doyle, pp. 43–58. Cambridge, Mass.: Academic Press.

15 Tanaka, K., et al. 1986. A high molecular weight protease in the cytosol of rat liver. I. Purification, enzymological properties, and tissue distribution. *J. Biol. Chem.* 261:15197-15203.

16 Hough, R., G. Pratt, and M. Rechsteiner. 1986. Ubiquitin-lysozyme conjugates. Identification and characterization of an ATP-dependent protease from rabbit reticulocyte lysates. *J. Biol. Chem.* 261:2400-2408.

17 Ciechanover, A., Y. Hod, and A. Hershko. 1978. A heat-stable polypeptide component of an ATP-dependent proteolytic system from reticulocytes. *Biochem. Biophys. Res. Commun.* 81:1100-1105.

18 Mitch, W., and A. L. Goldberg. 1996. Mechanisms of muscle wasting: the role of the ubiquitin-proteasome pathway. *N. Engl. J. Med.* 335:1897-1905.

19 Gomes, M., et al. 2001. Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. *Proc. Natl. Acad. Sci. U.S.A.* 98(25):14440-14445.

20 Kisselev, A. F., and A. L. Goldberg. 2001. Proteasome inhibitors: from research tools to drug candidates. *Chem. Biol.* 8:739-758.

21 Richardson, P. G., et al. 2003. A phase 2 study of bortezomib in relapsed, refractory myeloma. *N. Engl. J. Med.* 348:2609-2617.

22 Goldberg, A. L., and K. L. Rock. 1992. Proteolysis, proteasomes and antigen presentation. *Nature* 357:375-379.

23 Rock, K. L., et al. 1994. Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC-class I molecules. *Cell* 78:761-771.

24 Craiu, A., et al. 1997. Two distinct proteolytic processes in the generation of a major histocompatibility complex class I-presented peptide. *Proc. Natl. Acad. Sci. U.S.A.* 94:10850-10855.