THE FACULTY OF MEDICINE
Harvard University

Alfred (Fred) L. Goldberg

Photograph courtesy of Gretchen Ertl

Fred Goldberg, a leader of the field of protein turnover for a remarkable 50 years, died peacefully in his Brookline home on April 18, 2023 at the age of 80. He had been in a struggle against lymphoma for several years. Fred's dedication to the study of protein turnover and more specifically the proteasome was legendary, and his impact on our understanding of the field was so pervasive that it is difficult to imagine how it might have evolved were it not for his contributions. Fred was the husband of hematologist Joan Helpern Goldberg, and the father of jazz pianist Aaron Goldberg and software engineer Julie Goldberg.

The trajectory of Fred's career was unlikely in many respects, firstly in that he was heedless enough to focus on this problem at all. Reports of protein turnover followed quickly upon the introduction of isotopic labelling methods in the 1940s, but the field was poorly regarded when Fred decided to tackle the problem. For example, Nobelist Jacques Monod had published not only that protein turnover was at best negligible in *E. coli*, but that prior work demonstrating the process in eukaryotic experimental systems was so flawed as to be dismissed out of hand. So the reputation of the field was below zero in many circles. Although Monod is beyond criticism, his logic in this case was highly flawed in that he generalized freely from the relative stability of ß-galactosidase to proteins in general, whereas we now know that protein turnover rates in nature up to 10^4, reflecting diverse regulation. Adding to this complexity was the discoveryin the 50s by deDuve of protease-rich organelles called lysosomes, which were anticipated to be reposonsible for what little protein degradation might be occurring in eukaryotic cells.

Fred managed to join Jim Watson's laboratory as an undergraduate at Harvard, there was perhaps no better place to explore unsolved issues surrounding the central dogma of molecular biology, that DNA goes to RNA goes to protein. Not long afterwards, the atmosphere in Cambridge would be charged by the

In tribute to their dedicated efforts to science and medicine, deceased members of the Harvard Faculty of Medicine (those at the rank of full or emeritus professor) receive a review of their life and contributions with a complete reflection, ***a Memorial Minute****.*

pioneering work on gene regulation by Walter Gilbert and Mark Ptashne, who discovered and isolated the lac and λ repressors, respectively. At this place and time it must have seemed incongruous for a Watson-trained researcher to abandon central dogma work and turn to the fledgling field of protein turnover.

Fred left Watson's laboratory, and after a years stay as a Churchill Fellow in Cambridge UK, enrolled at Harvard Medical School, but struggled to sustain interest in his coursework. After two years he baffled his parents in Rhode Island by suspending his medical studies and focusing solely on research. But he never actually withdrew from the medical program, and later relished his imaginary dual existence as perpetual student and tenured faculty. At this point Fred began to study protein turnover, and among the most important aspects of his early work was the establishment of what were to become canonical model systems in the field¬: muscle, reticulocytes, and *E. coli.*

In his early studies on *E. coli*, Fred found that structurally abnormal proteins were major targets of degradation pathways. We now refer to this as quality control degradation, and it is currently recognized as one of the most complex and important aspects of the ubiquitin-proteasome system. Decades later, as the etiological agents of various neurodegenerative diseases were progressively identified, and their pathological forms found to be misfolded and moreover ubiquitinated, Fred's advocacy of quality control degradation was vindicated, and the field shifted in his direction.

Turning to skeletal muscle in order to study protein turnover, Fred established a central paradigm of muscle physiology – that the size of the muscle can be altered in different settings by manipulating the ratio of protein synthesis and protein catabolism. In sequential single-author papers in the late 1960s he documented that in settings of skeletal muscle hypertrophy – where the muscle is induced to become larger due to increased load on the muscle – protein turnover decreases while protein synthesis increases. These findings established skeletal muscle atrophy and hypertrophy as model systems to understand mechanisms of protein catabolism and anabolism.

Unlike the skeletal muscle cell, the reticulocyte – the immediate precursor of the red blood cell – undergoes proteome-wide protein turnover as a differentiative program. Fred and his trainee Joe Etlinger established reticulocyte lysates as a faithful in vitro system that could allow the dissection of protein degradation pathways. This system quickly led to the discovery of protein ubiquitination by Hershko and Ciechanover, which was foundational for the field. Regulated protein turnover would then be seen as governed by ubiquitin ligases, and it gradually dawned on the field that there were in fact not one but many hundreds of these enzymes, each functioning as a specificity factor.

Fred is best known for his work on the proteasome, which his group and that of Marty Rechsteiner discovered independently during the 80s. Fred's work demonstrated how this large protease used ATP to promote the degradation of ubiquitylated proteins and defined many features of this large protein complex.

The physiological roles of the proteasome, which were to prove so interesting, were still completely unclear. These insights emerged to a large degree from the discovery of proteasome inhibitors in the early 1990's, which Fred spearheaded. The initiative evolved not just from the increasing refinement of proteasome biochemistry but also from Fred's longstanding muscle project, and hinged on a string of lucky breaks. At the time too little was known about the proteasome to predict what putative applications might work out – even the mechanism of peptide bond cleavage was a mystery at the time. Adding

to these liabilities, it seemed clear that if they were ever found, such inhibitors would be pretty toxic. Nonetheless, adequate capital was raised by 1993 to form a small company in Cambridge with this objective. It was named MyoGenics since the projected clinical application was to suppress the muscle wasting seen in patients with various diseases. Later Tom Maniatis joined the board, bringing his interest in NF-kB regulation, and with revised objectives the company was renamed ProScript. Once you put your mind to it, it wasn't so difficult to find proteasome inhibitors–it didn't require large-scale screening or vast DNA-encoded libraries. We now know that many microorganisms produce them. By the time the company was formed, proteasome inhibitors were already in the literature, but mostly misassigned as inhibitors of other proteases. That is how MG101 was found, which led to the research compound MG132, and eventually the FDA-approved PS-341.

Even with good inhibitors in hand, ProScript was running out of gas when the National Cancer Institute was induced to check them for anti-cancer activity, and multiple myeloma scored as a hit. There were still few takers among the investment community, but the company was saved by being acquired by another small biotech, LeukoCyte. The project was still not out the woods though, because LeukoCyte itself was quickly acquired by Millennium, where proteasome inhibitors were regarded as more of a liability in the deal. But through the persistence of Julian Adams, formerly CSO at ProScript, Millennium was coaxed to bring PS-341 forward, and it proved to revolutionize the treatment of multiple myeloma. It was approved by the FDA in 2003 as Bortezomib (a.k.a. Velcade). Toxicity proved to be less of a problem than anticipated because interestingly the inhibitors are therapeutically effective at doses where inhibition is far from complete. Another disease now treated by proteasome inhibition is light chain amyloidosis. The inhibitors do not act on amyloid but attenuate its formation by killing the transformed cells that secrete the amyloidogenic light chain. To date hundreds of thousands of patients have been treated with proteasome inhibitors, likely nearing one million.

Even to his scientific "competitors," Fred was a generous scientist. He often exchanged ideas and data before they were published. He wanted to test his ideas through discussion, and he was happy to hear conflicting or complicating results. He was all science but rarely strung together more than a few sentences without stopping for some quip that just struck him or an ironic aside. If you thought journal club would wrap up at the hour, think again. During the pandemic his physical isolation was extreme, as he was immunocompromised, but even when hospitalized he managed to reliably zoom into journal club and lectures. He would often explain the newest details of his treatments, which were indeed often experimental, no differently than if an interesting new paper had just appeared.

At least at Harvard Medical School, Fred had no equal in the composition of light verse. They came out like limericks but he never seemed to observe the rules of limerick construction. Say a departmental chair stepped down, Fred would have a poem for it, whipped out of his pocket late in the evening when the crowd had loosened up, somehow working in bawdy passages however dry the actual subject matter. At Fred's funeral, his children Julia and Aaron paid tribute to him with their own verse, referring to Fred's many poems about his medical problems:

So let's spare you the science encomiums
Fred's output more polished than chromium
I'll save that for rhyming chatGPT
My aim now is his humanity.

Ever more generous in facing the end

Fred turned his brave gift of verse to legend
Mary was right that she'd birthed a bard
Those 500 papers they weren't near as hard.
Rhyme was his sword and shield, with pure wit he
Battled and found grace. No self-pity.

Respectively submitted,

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