

Some Lessons from My Work on the Biochemistry of the Ubiquitin System

Published, JBC Papers in Press, December 15, 2008, DOI 10.1074/jbc.X800016200

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When I was asked to write a Reflections article for the *Journal of Biological Chemistry*, I initially hesitated, mainly because I had already written several papers on the story of the discovery of the ubiquitin system (1–4). On second thought, however, it seemed worthwhile to write about it from a different aspect, about what can be learned from this story. I hope that some of the lessons may be of help to students or young investigators in their future work.

I first became interested in biochemistry when I was a medical student at the Hebrew University in Jerusalem. The curriculum of most medical schools includes a considerable amount of basic science, and many medical students are not always happy about it, but I enjoyed my courses in biochemistry so much that I wanted to do some laboratory research. I was very fortunate to have Jacob Mager of the Hebrew University as my first mentor in biochemistry (Fig. 1). Mager was a biochemist of immense knowledge, indeed a walking encyclopedia of biochemical sciences. He was also a very strict and rigorous experimentalist; every experiment had to be done with all possible controls, and every result had to be verified from all possible angles. I owe a lot to him for providing me with a solid and broad background of sound and rigorous biochemistry.

Having completed my medical studies and my Ph.D. thesis with Mager, I went on to do my postdoctoral training with Gordon Tomkins at the University of California in San Francisco (Fig. 2). Here again, I was very fortunate to find another outstanding mentor in science. Gordon was totally different from Mager: he did not care much about controls or experimental detail, but he was richly imaginative. Gordon was always bursting with new ideas, and he was a great inspiration to his postdoctoral fellows and to his colleagues. Thus, I was very lucky to have, at the beginning of my life in science, this blessed mix of two very different mentors. In effect, it is important in science to have vision and new ideas, but then it is equally important to refrain from getting carried away and to test rigorously, with a lot of self-criticism, whether your idea is correct. Having these two mentors taught me to aspire to this combination. So the first lesson from my life in science is the importance of having good mentors (Table 1, Lesson 1). Reading scientific literature is also very important, but one learns how to approach and solve problems in research mainly from daily contact with mentors. I believe that students should choose their mentors very carefully and, if possible, to have different kinds of mentors for graduate and postgraduate training.

Another lesson from my personal experience is that a young scientist should look for a research subject that is important but unique and not yet in the mainstream of current research (Table 1, Lesson 2). It is not a good idea for a young investigator to try to compete with big laboratories on a currently popular subject because the “big guys” will get there before you do! It was during my postdoctoral work with Gordon Tomkins that I became interested in the mechanisms of intracellular protein degradation. At that time, there was a general interest in protein synthesis and its regulation. Very few researchers were investigating the opposite process of protein degradation,



FIGURE 1. Jacob Mager, my first mentor in biochemistry (circa 1970).



FIGURE 2. Gordon Tomkins (circa 1973).

although the importance of protein degradation in the control of enzyme levels had already been recognized (5). Most people in the Tomkins lab were working on the mode by which steroid hormones induce the synthesis of the enzyme tyrosine aminotransferase (TAT), but Gordon agreed that I could work on the opposite process, the degradation of TAT. So good fortune, perhaps combined with some good intuition, led me to work on a subject that I believed was biologically important but not yet interesting to many others, so I did not have to worry much about the competition.

One of the first experiments that I did in the Tomkins lab influenced much of my subsequent work. I found then, quite by accident I must admit, that the degradation of

TABLE 1

Some lessons from my life in science (thus far)

1. It is very important to have good mentors. You learn how to do good science from prolonged interaction with good teachers.
2. Find an important research subject that is not yet interesting to others; otherwise, the "big guys" will get there before you! Do not go with the mainstream.
3. Accidental observations may be the most important ones. Grab your luck!
4. Use whatever experimental approach is most suitable for your objective. It may not necessarily be the latest or "state-of-art" technology. It may even be "old-fashioned" biochemistry!
5. Do not get discouraged by many unsuccessful experiments. To make important contributions, you need patience, persistence, and perseverance.
6. Discoveries are made in work driven by curiosity and excitement. Do not let your "chores" overcome your excitement and fun in science.
7. Never leave bench work, and you shall continue to have a lot of excitement and fun.

TAT in hepatoma cells was blocked by potassium fluoride, an inhibitor of cellular ATP production (6). I should also confess that only after I observed this result did I research the literature and found that Simpson (7) had previously reported that the degradation of labeled proteins in liver slices required cellular energy. Even though my finding was not entirely novel, I was very much impressed by it because it suggested that proteins in cells are degraded by a system that is different from the known proteolytic enzymes. It seemed to me reasonable to assume that in this as-yet-unknown system, energy is utilized for the high selectivity of intracellular protein degradation. Therefore, much of my subsequent work was trying to elucidate how proteins are degraded in cells and why energy is needed for this process. The lesson that may be drawn from this part of the story is that accidental observations may be the most important ones (Table 1, Lesson 3). I stumbled on the energy dependence of protein degradation by accident (combined with ignorance), but then I did not let go. Grab your luck when you get it and never let it pass you by!

After my postdoctoral work, I returned to Israel, set up my laboratory at the Technion (where I have remained ever since), and tried to find out how cellular proteins are degraded. It was clear to me that the only appropriate way to investigate the workings of a completely unknown system was that of "classical" biochemistry. This includes the use of a cell-free system that faithfully reproduces energy-dependent protein degradation *in vitro* and separation of its components by fractionation, followed by purification and characterization of the different enzyme components to understand their mode of action. This is what my laboratory has actually done (1, 4). Much of this work was done during the times when the powerful technologies of molecular biology were emerging. Many biochemists at that time became "converted" to molecular biologists, and

some regarded classical biochemistry as old-fashioned. However, I insisted on using biochemistry because this was the only way to reach the objective of learning how proteins are degraded in cells. Molecular genetics can (and should) be applied mainly when at least part of the basic biochemistry of the system under investigation is already known. For example, it would not have been possible to define the roles of some protein kinases in cell division or signal transduction without any prior knowledge of the biochemistry of protein kinases. Similarly, such an unexpected process as the role of ubiquitin ligation in protein degradation probably could not have been discovered by molecular biology or genetics alone, without any biochemical knowledge. Of course, molecular genetics was vitally important later on for the discovery of the variety of functions of the ubiquitin system in a multitude of cellular processes. The lesson that may be learned is to use whatever experimental approach is most suitable for the research objective, which may not necessarily be the most "fashionable" or "state-of-art" technology (Table 1, Lesson 4). I suspect that "old-fashioned" biochemistry will continue to be needed for many future discoveries because much of the genome encodes proteins of totally unknown function.

As noted, the story of the discovery of the ubiquitin system has already been described (1–4). Briefly, we have fractionated an ATP-dependent cell-free proteolytic system from reticulocytes, initially described by Etlinger and Goldberg (8), and isolated a small heat-stable protein that was essential for the activity of this system (9). Much of this work was done by Aaron Ciechanover, who was then my graduate student. The small protein was later identified by Wilkinson *et al.* (10) as ubiquitin, a protein of previously unknown function. Following its purification, we found that ubiquitin became associated with high molecular weight material in an ATP-dependent process (11). In collaboration with Ernie Rose at the Fox Chase Cancer Center in Philadelphia, we then identified the high molecular weight derivatives as covalent conjugates of ubiquitin with substrate proteins (12). On the basis of these results, we proposed in 1980 that covalent amide linkage of ubiquitin to proteins targets them for degradation (12).

Ernie Rose was the third person, in addition to Mager and Tomkins, who had a great influence on my life in science (Fig. 3). He is well recognized for his work on enzyme mechanisms, a field about which I know very little. He also had a side interest in protein degradation. That was the reason he invited me to his laboratory for a sabbatical year in 1977–1978 and for many subsequent visits. He likes problem solving, and his approach to science is

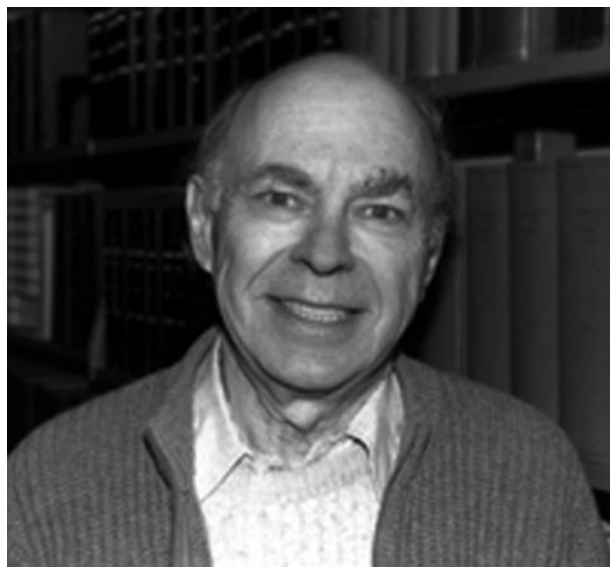


FIGURE 3. Ernie Rose in the library of the Fox Chase Cancer Center in Philadelphia (circa 1992).

analytical. I am more intuitive, so we complemented each other very well. He is also sharply critical, and this got me out of trouble on occasions when my imagination ran away despite my training with Mager. Ernie contributed greatly to the discovery of the of the ubiquitin system by excellent suggestions and important criticisms.

Following the discovery of the ligation of ubiquitin to proteins and the proposal of the ubiquitin tagging hypothesis, my laboratory spent the next decade on the identification of the enzyme components of this pathway. We did this again using the old-fashioned biochemical approaches of fractionation, purification, characterization, and reconstitution. We concentrated mainly on enzymes involved in the ligation of ubiquitin to proteins: the ubiquitin-activating enzyme E1, the ubiquitin carrier protein E2, and the ubiquitin-protein ligase E3 (13). We found that the role of the E3 enzymes is to bind specific protein substrates and suggested that the selectivity of protein degradation is determined mainly by the substrate specificity of different E3 enzymes (1, 14). Since 1990, I have been working on some roles of the ubiquitin system in cell cycle control, again using biochemical approaches and relevant cell-free systems. This resulted in the identification of two ubiquitin-protein ligase complexes: the cyclosome, now also called anaphase-promoting complex or APC/C, involved in the degradation of mitotic cyclins and some other cell cycle regulatory proteins in exit from mitosis (work done in collaboration with Joan Ruderman) (15), and SCF^{Skp2}, which targets the p27 inhibitor of cyclin-dependent kinases for degradation in the G₁-to-S phase transition (work done in collaboration with Michele Pagano) (16, 17). At present, I am trying to use biochemical approaches

to gain insight into the mechanisms by which the mitotic (or spindle assembly) checkpoint system regulates the activity of APC/C (18).

As is evident from this brief description of nearly 40 years of my work on protein degradation, it required a lot of time, patience, and perseverance. Naturally, one writes only about successful experiments, but the young reader should realize that these were always preceded by a much larger number of failed experiments. I often think that if I were not so persistent (or just plain obstinate), I would not have made any important research contributions. So if you believe that your research objective is really important and is experimentally approachable, do not get discouraged by a large number of unsuccessful experiments. If nothing works for many months, try a different approach, but do not abandon your research objective. To make an important contribution, there is a need for much of what may be called the three "Ps": persistence, patience, and perseverance (Table 1, Lesson 5).

Despite the great multitude of unsuccessful experiments, when I look back at my life in science, I realize how much excitement and fun it brought during all these years. I think that this is the right way to do science. If you want to make discoveries, you have to work in a way that excites you. Everyone knows that we have plenty of chores in our scientific life. We must write grant applications, and we have to publish papers to get grants or to get promoted. Although these are duties that we have to do, we should not let these chores become our main occupation. One does not make discoveries when just collecting data to publish papers, which in turn would serve to get more grants, and so on. Discoveries are made in work driven by curiosity and excitement (Table 1, Lesson 6).

My last lesson, Lesson 7, for the young and not-so-young scientists is never to leave bench work. I have always done (and I am still doing and greatly enjoying) bench work, often on a daily basis, and I find it very important for creativity. Testing your ideas yourself will excite you greatly. When I do an experiment myself, there is great anticipation and excitement until the results are obtained. When I see results that are unexpected, then I get even more excited. There is a very intense involvement in research when it is performed with your own hands.

I would like to emphasize, mainly for the young or naïve readers, that not all of my lessons apply to everybody. Max Perutz, a great pioneer in protein crystallography, also loved bench work and was most happy in the laboratory doing experiments. However, he also wrote (19) that "when Crick and Watson lounged around, arguing about problems for which there existed as yet no firm experi-

mental data instead of getting down to the bench and doing experiments, I thought they were wasting their time. However,...they sometimes achieved most when they seemed to be working least... There is more than one way of doing good science." This is certainly correct.

Finally, although it is great to do experiments with your own hands, you cannot accomplish everything by yourself. One needs the help of dedicated research teams, students, and colleagues. I was very fortunate to have highly devoted research teams for many years. In my laboratory at the Technion, my associates Dvora Ganoth, Hanna Heller, Esther Eytan, Sarah Elias, and Clara Segal and my wife, Judith Hershko, all gave me tremendously devoted help for many years, for which I am most grateful. Throughout the years, I was fortunate to have many graduate students (among them, Aaron Ciechanover), too many to list here, many of whom made very important contributions to the discovery of the ubiquitin system, to the basic biochemistry of ubiquitin-mediated protein degradation, or to some of its roles in cell cycle control.

Acknowledgments—I thank Michael Fry and Karl Skorecki for helpful comments on the manuscript. This article was based on a lecture delivered at the Technion Symposium "Marking 60 Years of Science in Israel" on May 19, 2008.

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