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Interview

A conversation with Saul Roseman

BBRC: Your early work was with anticoagulants. Was that the real beginning of your interest in science?

Roseman: No, the beginning was at City College, or even earlier (probably when I read "Microbe Hunters"). I dont know how I got into CCNY. Im sure I didnt have the necessary average, but somehow or other I was admitted. The students were the cream of the crop—very bright, and also very competitive. The teachers there were really unusual. They were dedicated teachers, but they were also dedicated researchers. They would teach, I think it was something like 16 h a week, including teaching labs. And then they would hop on the subway and go to various places, perhaps to Cornell, or Columbia PNAS, or the Veterans Hospital in Brooklyn, to do their research. So these were very dedicated scientists.

One of them, Abraham Mazur, was my special inspiration, as he was to many, many City College students who went on in Biochemistry. Actually, I took two majors, Chemistry and Biology. But Biochemistry was a section of the Chemistry department. It consisted of about four people. Benjamin Harrow was head of the Biochemistry section, and at that time he wrote a classic textbook, and he and Mazur did the next edition. But anyway, Abe Mazur was an absolutely incredible guy and I felt that I had to work for him.

I started working in the bacteriology lab to earn money by washing Petri dishes. I remember that. I had to scrape out a lot of Petri dishes; that stank. Hudson River cultures. But then I was lucky, I got a job with Abe Mazur, washing his glassware, and the glassware of another professor in the department, Ernst Borek, who was also an accomplished, talented scientist.

Abe challenged me all the time. But he also excited me, and to my mind this is the most important quality one must have to be a successful scientist. He would give me some little project to do while I was washing glassware and that little project became the most important thing in my life—what could be more important than doing that analysis? I cannot even remember what it was. He truly stimulated his students, and there have been many CCNY alumni who have said this. So he was a very special guy. When I graduated, he got me a job at Columbia College of Physicians and Surgeons, in the Biochemistry Department. I quickly learned all about hierarchy and class and caste, which didnt exist at City College, but it sure did exist at Columbia. If you were a technician, you didnt talk to the chairman of the department. So, I had my fill of that in about two or three months, and I went out to Wisconsin to graduate school—took my 300 bucks, which was about all I had—to try to do what I could.

I should tell you about my adviser in the Biochemistry Department at Wisconsin, Karl Paul Link. He was the most interesting character at the University of Wisconsin, and I probably got my screwball characteristics from him. I will illustrate his personality with one example. I went home and married my childhood sweetheart. When I came back and brought her up to the lab to introduce her to KP, he was very angry at me because I had not asked his permission to get married. And he said something like this to my new bride: "Dont expect him to be home. Because he wont be. Hell be here working." So, life was different then. But it was very good training. Karl Paul Link had the highest standards of excellence, and so, I think, did all the students that trained in his lab (Clint Ballou, for example).

After my Ph.D., I went to work with Albert Dorfman in the Department of Pediatrics of the University of Chicago School of Medicine. Al was an unusual scien-

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tist. He had an MD and also a Ph.D. in biochemistry. He was virtually unique because he was excellent both as a clinician and as a biochemist, a truly difficult accomplishment.

BBRC: Your training obviously affected your approach to science.

Roseman: My basic philosophy is very simple. We understand very little about the living cell. Theres an argument about how many genes there are in a human genome. It keeps changing. I dont know where it is now, I think 40,000. But even if it is 100,000, we have actually defined the functions of perhaps 5000 gene products or less. By this, I mean the proteins, enzymes, and so on, and what they do. And I dont mean by sequence analogy. So that means that we know the functions of somewhere in the neighborhood of 15% of all of the reactions taking place in the cell. Worse, even after we learn all about the reactions, we have to deal with the more complex problem of regulation. It is clear from present research that there are many modes of regulation, that they probably intersect, and untangling all of this will be like dealing with a bowl of spaghetti and trying to separate it into single strands, and then try to recombine it as it was originally.

So the bottom line is that when you deal with intact cells or with cell extracts, so much is unknown, that your there is only a slim chance that you will be correct in predicting the results of a given experiment. Thats why I tell students that a single *Escherichia coli* cell is smarter than all of us sitting around the table combined. That does not preclude doing experiments. It simply means to expect the unexpected. And thats why Im such a strong believer in serendipity, which was also the major guide of one of my heros, Fritz Lipmann. Practically everything thats good that happened in our lab has been an accident.

Of course, a lot of people abhor this idea of research, and I suppose for some of the molecular biologists its all very simple. You have a gene, you know the sequence, and therefore you know the function. But thats certainly not true. There are many genes where the sequence is insufficient to determine what the real function is. We have cloned genes and studied the enzymes and the enzymes had entirely different functions than predicted by the sequence homologies and sequence identities. The sequence homologies can apply, but they can also be very wrong.

BBRC: You have at least three major projects going on in your lab that are very different from each other. How do you keep them all going?

Roseman: They all arose from a common ancestor: carbohydrates. I became interested in these compounds when I was a grad student working on the metabolism of 4-hydroxycoumarin, the parent compound of the anticoagulant Dicumarol (now called coumadin, I think). The compound was excreted into the urine of dogs as the glucuronide, or glucuronic acid derivative. I had to isolate the derivative and prove the structure by synthesis, and so I got into the complicated world of carbohydrates. I should add that KPL was initially a carbohydrate chemist.

In Albert Dorfmans lab, we were working on hyaluronic acid, which has glucosamine and glucuronic acid moieties, using isotopes and whole cells. When I left his lab, and went to the University of Michigan, I started working with enzymes and cell free extracts. This took me heavily into glucosamine metabolism, that led us to the sialic acids, work which gave us totally unexpected results. By this time, we were into the enzymatic synthesis of the complex carbohydrates that occur on the surfaces of cells.

Perhaps I should back up and tell you that in those days, biochemists generally tended to ignore complex carbohydrates. They were too complicated and nobody knew anything about their function. So, if you didnt mind working in an area with only a handful of other labs, it was really a fun thing to do. During all of our work, there was a persistent, nagging question. What are the functions of this myriad of complicated molecules? We finally turned to this question when I moved to Hopkins from Michigan.

It is my firm belief, or more correctly I should call it intuition, that these substances are the keys to information that is passed between cells. In other words, they comprise a language. There is no way to account for the fact that you have 10^{15} connections in the brain, *specific* connections, and you have at the most, 10^5 genes. How do you go from 10^5 to 10^{15}? With carbohydrates, it is relatively simple to get into these astronomical numbers without invoking too many enzymes. I think that this is how cells recognize one another, for example. So this is one of the projects that we have been working on since we moved here. It is by far the most difficult problem that this lab has ever studied. The problem is that the recognition molecules on cell surfaces are present in such infinitesimal quantities. I think we must have purified one of the factors from chicken liver several million-fold. Perhaps it is finally getting to the point where we can do something with it.

The second of the three projects that you mentioned was discovered just before we left Michigan. While studying sialic acid metabolism, we discovered what turned out to be a sugar transport system in bacteria, the PTS (phosphotransferase system). It is a really interesting system, involving a series of sequential phosphotransfer reactions between proteins, with the sugar phosphorylation taking place as it is translocated across the membrane.

The third project is really going back to my roots, glucosamine metabolism. We are working on the

catabolism of the second most abundant organic substance in nature, a polymer of *N*-acetylglucosamine called chitin. Enormous quantities of chitin are produced annually in marine waters. The estimate is 10^{11} tons. If it was not catabolized and returned to the C and N cycles in the biosphere, the earth and the oceans would consist of nothing but chitin. In fact, marine sediments contain only traces of chitin, and this is because of the action of marine bacteria. We are studying this complex series of steps and processes with one of these organisms, *Vibrio cholerae*. There are somewhere between 50 and 100 genes involved. But the steps are really fascinating.

So, as you can see, there is a common thread in what we do.

BBRC: What are the big challenges?

Roseman: The challenges vary with the project. The most difficult concerns intercellular communication and the complex carbohydrates. As I said, this was virtually just an intuitive guess, but there is a small body of evidence, that is slowly increasing, that this idea may be correct. If the problem we are working on finally comes to fruition, then at least well have one system where we will know what the cells do when they come into contact with these molecules. Its going to be very complicated. In a way, I look at it like electron transport. When people first worked on the electron transport system they would isolate an enzyme and study it. After a long time, the pieces obtained by many such studies fell into place. And I think were at the point now where they were at the beginning of their work.

BBRC: What are the techniques you are using?

Roseman: We are not really bound to a specific technique in the sense that we will use any technique that will solve the problem, and if it is a specialized technique such as fluorescence spectroscopy or NMR, we have managed to establish collaborations with the appropriate expert. If there is one philosophy that permeates this lab it is quantitation and kinetics. In other words, measure the biological phenomenon quantitatively or develop an assay to do it, and, study the phenomenon as a function of time. It is by studying the kinetics that we have made some really interesting and potentially important observations.

The other key parameter that we always keep pushing is biological relevance. This often comes down to understanding the specificity of the system. For instance, if two hepatocytes bind or adhere to one another, is this a specific phenomenon which mimics what takes place in vivo, or a non-specific phenomenon which only occurs in vitro, and is therefore irrelevant for our purposes.

BBRC: We havent talked much about the bacterial sugar transport system.

Roseman: That was serendipity again, really. Its true. Theres a book published on the phosphotransferase system [1] based on a symposium at the Pasteur Institute. The first chapter is called sialic acid, serendipity, and sugar transport. The article describes how one of my first postdocs, Don Comb, discovered *N*-acetylmannosamine and the correct structure of neuraminic acid, the parent compound of the sialic acids. In later work, we found an ATP-dependent specific kinase in animal tissues for *N*-acetylmannosamine. While looking for this enzyme in bacteria, we found not an ATP- but a PEP-dependent "kinase." Anyway, its an amazingly complicated system. Years ago people thought that sugar transport might be really simple. If a sugar is going to go across a membrane, and you want to trap the sugar in a cell, youve got two ways to do it: One is that it comes in and it gets phosphorylated thats what happens in our red cells. But a short cut would be, if it is phosphorylated on the way in. People originally thought, and it was actually proposed, that hexokinase or perhaps glucokinase was in the membrane doing this job. But it is catalyzed by the PTS. To phosphorylate a sugar, the number of proteins involved can be from three to five or even six different proteins, all have to interact, and the driving force is PEP. One of the proteins is the sugar-specific receptor in the membrane. The sugar is translocated across the membrane and simultaneously phosphorylated. Why in the world should it be so complicated? Since it has long been known that when bacteria on simple media grow on sugars, the rate of cell growth is determined by the rate of sugar transport. Thus, the transport process must be stringently regulated and coupled to metabolism and cell growth. We really do not know the molecular mechanisms involved in these processes. Furthermore, we know that the PTS regulates the expression of other proteins in the cell, such as other sugar transporters and catabolic enzymes. So perhaps this explains the complexity of the PTS.

Finally, I want to come back to what is most important to me of all the things that I have said. Excitement. It is only because research is so exciting that I have managed to survive the agonies of grant writing, dealing with referees, university administrators, increasing loads of red tape, and all the other cumulative burdens and frustrations that one encounters in trying to do research in that ivory tower called academia. I still remember the Saturday afternoon at the University of Michigan, about 45 years ago, when the needle on the Beckmann spectrophotometer kept moving which meant that I had discovered a new enzyme (in glucosamine metabolism). I guess that I will keep doing this until such results are no longer any fun, but I really dont anticipate that since I am at least as stubborn as the administrators.

Reference

[1] S. Roseman, Sialic acid, serendipity, and sugar transport: discovery of the bacterial phosphotransferase system, FEMS Microbiol. Rev. 63 (1989) 3–12.

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