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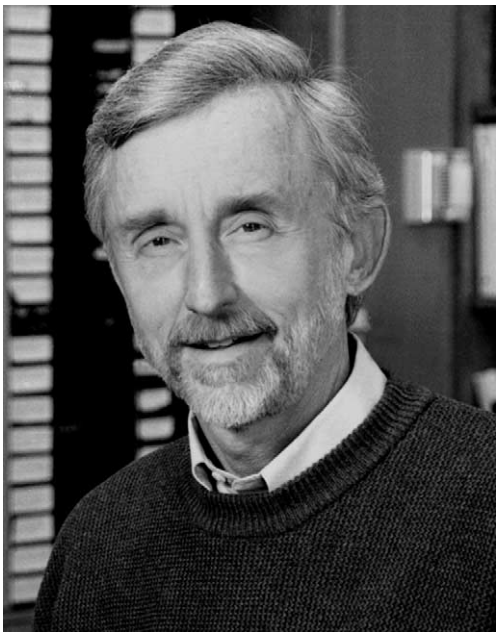
Interview

A conversation with Thomas D. Pollard



A pioneer in the field of cell motility, Thomas Pollard has studied cell movement, since he discovered myosin-I, the first unconventional myosin, as a postdoctoral fellow. Since then, his work has elucidated many of the fundamental mechanisms of actin and myosin function. A National Academy of Sciences member and recipient of many awards, he has served as President of the American Society for Cell Biology, the Biophysical Society, and the Salk Institute. He also chaired the Commission on Life Sciences for the National Research Council of the National Academy of Sciences for 5 years. Dr. Pollard currently leads an active research program as a professor at Yale University. Cell Biology, a textbook by Pollard and Earnshaw, was published earlier this year.

BBRC: You started your training as an MD, but the work you do now is very much rooted to the basic sciences. How did that transition come about?

Pollard: I was a science major at Pomona College, a wonderful liberal arts school in California. They did not offer a major in biochemistry or molecular biology at the time, so I majored in chemistry/zoology. I learned a lot about biology and a lot about chemistry too. Pomona had no biochemistry course in 1963, so I made a deal with a new faculty member, John Penniston, to read Fruton's biochemistry book over the summer for a half course credit. It sparked my interest in biochemistry. I was the first person at Pomona to take a biochemistry course.

For two summers during college, I worked at the University of Southern California Medical School in a phage genetics laboratory, but also, more importantly, one summer with the tissue culture pioneer, Charles Pomerat, who studied cells in culture using time-lapse movies. He loved movies of cells. So he'd gather whomever he could find one or two hours per day to review the latest time-lapse movies of cells crawling around. Viewing those hours of movies got me hooked on studying cell motility.

However, no one knew much about the ultrastructural or molecular basis of these movements, so when I went to Harvard Medical School, I found a lab where I could pursue this research problem. I was fortunate to find Sus Ito, an Anatomy Professor who studied the ultrastructure of the gastro-intestinal tract. His research had nothing to do with cell motility, but he was (and is) exceptionally kind and generous. He gave me bench space, taught me how to do electron microscopy, and helped with my experiments. Working part-time through medical school, I discovered some interesting things about filaments (that turned out to be actin and myosin filaments) in cell-free extracts of amoebas.

I set out to repeat some work reported by Lewis Wolpert's group in England, not knowing that others were skeptical about it, since they could not reproduce it. Fortunately, after many failures, I managed to reproduce their results. We took an extract of amoeba cytoplasm that was made in the cold, added ATP, and sealed a sample in a little chamber on a microscope slide. When it warmed up, the cytoplasm crawled around on the slide outside the living cell. This was absolutely fantastic! You could call people in from the corridor to look at it and they'd ooh and ah about this amazing cell free motility. We discovered that two kinds of filaments were required for movement. The thin filaments, later shown to be actin, assembled during this reaction and interacted with thicker filaments, now known to be myosin. That was the beginning of my interest in actin and myosin as the molecular basis for cell movement.

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When I presented my work on filaments in cell-free extracts at the American Society for Cell Biology meeting in 1969, I had the only talk about filaments and their relationship to cell motility. I spoke in a microtubule session because they didn't know where else to put me. Times have changed. ASCB meetings now have hundreds of posters on this topic.

Two students, Hadashi Hatano with his Professor Fumio Oosawa in Japan and Mark Adelman with his Professor Ed Taylor in Chicago, independently discovered nonmuscle actin and myosin. They were real biochemists who purified actin and myosin from slime mold for the first time; we took a cell biology approach using light and electron microscopy. That's how I got started. I was fortunate enough to be in on the ground floor, and it's been a lot of fun ever since.

BBRC: Obviously, motility was never thought to be a simple question, but was there any concept that there were so many different proteins involved?

Pollard: Very good point. Once we knew about actin and myosin in nonmuscle cells, my assumption was that cell motility might not be much more complicated than muscle contraction. Even today, just a dozen proteins can explain most things about how muscle contracts. And so, the fact that more than 70 families of proteins regulate actin and myosin in nonmuscle cells is one of the biggest surprises to emerge over the last 3 decades. This an order of magnitude more complicated than we thought at the beginning.

My lab has worked on some proteins that turned out to be important, but many other labs have contributed. The growth of the field has been fascinating, starting with less than 5 or 10 of us and growing to many hundreds of people. It has also been amazing to see how far and fast the field has grown since the late 1960s, when work was limited by primitive technology.

BBRC: There's a big difference in the techniques available now. Your group is using genetic analysis, X-ray crystallography—a wide variety of approaches.

Pollard: Yes, things we never dreamed about. One of my jokes is that I'm the last person to purify a protein without running an SDS gel. I purified myosin-I, the first unconventional myosin, when I was a post-doc with Ed Korn at the NIH. I purified myosin-I until the enzyme-specific activity was constant over a peak off the column—the old-fashioned criterion for homogeneity. The last thing that I did was to run a gel when the project was all done. The protein was pure!

We hoped someday to know the primary structures of some of the proteins, which was accomplished in the early 1970s for actin, and in the 1980s, myosin. We didn't actually dream about X-ray crystallography or knowing the atomic structure; that was too far off. Genetics had no impact on actin research in the 1960s, but now we can't conceive of starting a project without being able to clone and sequence genes, knock out genes, and express recombinant proteins, or tag proteins for real-time viewing by fluorescence microscopy.

These everyday methods were still far in the future. Improved laboratory technology has accelerated progress far beyond what the pioneers in the field dreamed about back then.

BBRC: What are the clinical applications of your work?

Pollard: Scientists working on cell motility and muscle were interested in the fundamentals of life and did not anticipate that they were working on proteins that contribute to disease. So it was a surprise that mutations in the genes for most of the proteins in this huge complicated system actually predispose to human diseases. You know some of the examples. Muscular dystrophy is caused by defects in a muscle membrane protein that binds to actin. Hereditary spherocytosis, a very common hereditary blood disease, is caused by mutations in spectrin or other proteins that also interact with actin. Cardiomyopathies have been traced to mutations in essentially all of the proteins found in the heart muscle contractile apparatus. Wiskott–Aldrich syndrome, an immune deficiency, is caused by defects in a protein that activates Arp2/3 complex.

It turns out that, like everyone else in basic biological research, we have been studying disease genes. More than likely variations in each human gene contribute to disease risk, often in combination with variations in other genes.

The second point about disease relates to the potential for using components of the motility system as targets for drugs, raising the question if activating or inhibiting any components of the motility system would be useful for treating human diseases.

Several of the commonly used cancer chemotherapeutic agents target microtubules, like Vinblastine, Vincristine, and Taxol. So there's good precedent for components in cytoskeleton and motility system being reasonable drug targets. However, there are no drugs in clinical use that interact with any of the contractile proteins in the cardiovascular system. All of the drugs used to treat cardiovascular diseases interact with receptors or signaling molecules, rather than contractile proteins.

A biotech company in California called Cytokinetics is active in this area. They have compounds that target microtubule motors entering clinical trials for treating cancer. This is a wonderful example of how basic research, driven by people's curiosity about how things move around in cells, led to a potential new class of cancer drugs. Advances in basic science, even learning how amoebas crawl around, will continue to open up new opportunities for understanding human disease, and perhaps treating it, too.

I have to laugh about this, because a lot of our work over the years has been done on an unpopular experi-

mental organism called *Acanthamoeba*, a little soil amoeba. It's not a near relative of animals, but it's not all that far away. Several times we've discovered something new in this creature, that was dismissed by those in the know as being something peculiar about amoebas and probably not applicable to more interesting cells such as animal cells.

The best example was myosin-I, the first unconventional myosin, that I discovered in Ed Korn's lab at NIH. My first talks about this funny myosin in 1971–1972 were usually in a session on muscle contraction. My many friends in that field were cordial but later admitted they didn't believe a word of it. They were sure that the odd amoeba myosin was a crazy artifact, because it did not have two heads and a long tail that makes filaments, like a respectable muscle myosin. Instead, it had only one head and some other peculiar characteristics.

It took more than 10 years for people to catch on that this was the tip of the proverbial iceberg and that this was the first of the 17 different families of unconventional myosins that many other people, such as Mark Mooseker here at Yale, have discovered. We're still trying to figure out what myosin-I is doing in cells.

The same thing happened with the Arp2/3 complex. My student Laura Machesky did not receive much attention when she discovered Arp2/3 complex in *Acanthamoeba* in 1994, but it has turned into a huge area of research in the last few years.

I suppose that people don't believe strongly enough in evolution, so they sometimes resist novel findings discovered in some distant part of the phylogenetic tree—at least distant from where they are looking.

BBRC: I wanted to touch on your service roles as President of American Society for Cell Biology, President of Biophysical Society, President of the Salk Institute.... How do you balance that with your research?

Pollard: I have enjoyed helping these organizations to be successful. I like to see organizations thrive. But it's necessary for some members to volunteer time to provide leadership to make success possible.

BBRC: You've been involved in several political organizations as well—the Congressional Liaison Committee and the Commission on Life Sciences at the National Research Council.

Pollard: Simply providing some leadership can often amplify your impact. Let's take the Congressional Liaison Committee, for example. This grassroots effort is organized by the Joint Steering Committee for Public Policy, a coordinating group from several scientific societies: American Society for Cell Biology, American Society for Biochemistry and Molecular Biology, Genetics Society of America, and the Society for Neuroscience. These societies have about 40,000 members. Harold Varmus is the current Chair, following Marc Kirschner and Eric Lander.

Initially, the JSC emphasized activities in Washington DC. We have a former Congressman who represents us in Washington and supports the Congressional Biomedical Research Caucus. My focus with the JSC has been to involve bench scientists in advocacy, since we should be able to amplify our impact by getting many scientists involved. We started by simply inviting scientists to participate in advocacy by writing letters to their elected officials. However, in contrast to actin filaments, scientists don't self-assemble into political groups very readily. So we learned that it is important to provide professional staff to help the scientists to be good advocates. We started by hiring a staff member on an experimental basis for one year. They doubled the number of scientists participating in Pennsylvania, our target state, and then they went on to North Carolina and Illinois. Subsequently, we raised money in California to hire another staff person for the West Coast. Now, we have more than 3000 bench scientists communicating with Members of Congress.

BBRC: And the role of the staff is to help scientists to educate policy makers?

Pollard: Their role is to help mobilize the scientists. It is usually gratifying to visit your Member of Congress in the local district office and explain what you're doing in your lab. Invariably, politicians are pleased to learn about research in their district. However, the chances of a biochemist or a cell biologist making such a visit under their own power are small. A staff person can call a CLC member and say, "We're going to go visit your Member of congress. What's a good day for you? I will set up the appointment, and I will come along to explain how the visit will go." With that kind of help, scientists will visit their Members of Congress.

BBRC: Is the CLC effective?

Pollard: Yes, though we'd like to do even better. We have gotten many scientists to visit their Member of congress and to write letters on various issues: often about funding for research, but sometimes on policy issues, such as the human cloning bills being considered by the Senate.

This is an example of how a small effort on my part can get something significant done. I'm pleased to work in a community where an individual can get others excited about a good idea, such that the idea eventually has a life of its own. Volunteer activities often allow the participants to amplify their impact by working with a group such as a scientific society.

BBRC: You've been here at Yale a year now. Is it different from some of the other institutions with which you've been involved?

Pollard: The essential features of all my institutions are the same. That is, Harvard Medical School, Johns Hopkins Medical School, Salk Institute, and Yale are all high powered basic research institutions. Salk doesn't have an education program per se, but is linked with the

University of California in San Diego. They are all interesting, exciting places.

One of the pluses of leadership at the Salk Institute was the opportunity to meet interesting people who supported the institute, in gratitude to Jonas Salk for his polio vaccine. Many people in my generation remember the advent of the Salk vaccine during the polio epidemic of the 1940s and 1950s. We were afraid of public swimming pools until the polio vaccines came along. Brady Metheny, a journalist covering science in Washington, DC, recalled those times in an obituary: "Thank you, Dr. Salk for giving summer back to the American children." That's the way I remembered it too.

I enjoy the academic community at Yale, the first time that I've been based on an arts and sciences campus. During my years at medical schools, I missed the full range of the academic enterprise.

We love New Haven and live on the shore of Long Island Sound. Poor New Haven has a bad reputation but everyone who comes here discovers that it is a great little city. Tonight, we will hear the Metropolitan Opera on the New Haven Green, just one of the many examples of how culture is alive here.

BBRC: Certainly different institutions or regions have stronger ties to industry.

Pollard: The biotech industry is much further developed in California than Baltimore or New Haven. More than 100 biotech companies are located within a couple of miles of the Salk Institute. Many people take biotech jobs after they complete their training in an academic lab. This is still developing in Baltimore and Connecticut. There's much to be done, so I'm sure places like New Haven have a future in the biotech industry.

Many regions are trying to start up science parks, but it will be tough to make this work outside of areas with big basic research and clinical research operations. Even New Haven, with a huge research enterprise at Yale, will have to struggle compared with San Francisco or Boston, which have much larger academic sectors.

BBRC: What's next in the field?

Pollard: It has been an unbelievable thrill to get from looking at cells in a microscope to the atomic structures and the detailed chemistry of some of the molecules contributing to cell motility. I used to tell students and post-docs that few of the most obvious experiments in the field had ever been done. This is not true anymore. A lot of key experiments have been done, but that doesn't mean we're out of things for people to do in the future.

For example, some questions have been avoided simply because they were too hard to work on. Those questions provide interesting things to do in the future. For example, we're putting a huge effort into studying cytokinesis. We've worked on it off and on for years, but this essential biological activity was difficult to study, because there is no biochemical assay for cytokinesis. Experiments must still be done on live cells, so biochemical analysis is largely missing. To get to the biochemistry, genetically tractable model organisms are required to make an inventory of the molecular components for cytokinesis.

We, and about a dozen other labs, are using the fission yeast *Schizosaccharomyces pombe* to study cytokinesis. Fission yeast appear to divide like animal and protozoan cells rather than like plants. Thanks to excellent genetics and cytology, more than 50 genes have been implicated in cytokinesis. The first of these were found by Paul Nurse when he screened for cell cycle defects in the 1970s. He found, Cdc2, the famous mitotic kinase, but he also found a number of genes required for cytokinesis including Cdc3 (the actin-binding protein profilin), Cdc4 (a myosin light chain), Cdc8 (tropomyosin), and Cdc12 (a mitotic formin). The field is now poised for rapid progress in learning how cells determine the site of cleavage and the time that the ring of actin and myosin contracts, so off we go.

Hopefully, we'll be able to do some biochemistry on cytokinesis, with the idea of getting to the level of detail that we now have on cellular actin filament assembly. We'd like to know the atomic structures of the key proteins, the rate and equilibrium constants for the reactions, and the concentration of the proteins in the cell. We can hope for a quantitative biochemical model for cytokinesis, but we and everyone else are just getting started.

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