The Pollard lab at Salk: moving the leading edge forward

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Abstract

This essay will review the years that the Pollard lab was at the Salk Institute in the last half of the 1990s. It was a highly productive time both in research and in training. For me personally, it shaped my career for the better in ways I am still discovering.

Keywords Arp2/3 complex · WASp

Similar to many who have worked with Tom, it is safe to say that the experience has made me the scientist I am today. The lessons I learned from Tom, both as a post-doc and subsequently, have been vital to my career. Many of these lessons were taught by example, and some were only realized after having my own lab.

I might be one of the few people to have worked with Tom exclusively at the Salk Institute, arriving in his lab in the summer of 1997 and leaving in the summer of 2001. Two things drew me to Tom's lab: Arp2/3 complex and the argon plasma generator. The former is somewhat self-explanatory, so I will explain the latter. When I was looking at possible post-doc mentors, one of Tom's papers particularly caught my eye. It was a methods paper for studying myosin I motility on supported lipid bilayers (Zot and Pollard 1993). They described how to clean the cover glass as follows: "the preferred method is to purchase an ion plasma generator and connect it to a vacuum pump and an Ar source. Alternatively, we have found that a microwave oven can be modified to produce an Ar plasma. To modify the microwave, drill two holes in the side of the inner chamber ..." and it goes on in detail. The ingenuity and audacity made a great impression on me.

I got to Salk about 5 months after the lab had moved from Hopkins. Enrique de la Cruz had just left for his post-doc, which was too bad for me but his spirit lingered. Dyche Mullins and Laurent Blanchoin were post-docs; Magdalena Bezanilla, Wei-Lih Lee, Kirsi Turbedsky, and Jia Lu were

grad students; and the inimitable Don Kaiser was a research assistant. Our lab was on the main floor (technically the third floor) of the South Building, together with the labs of Joe Noel and Senyon Choe as part of the "Structural Biology Laboratories" (SBL). We ate lunch in the heat of the sun facing the Pacific almost every day, watching the hang gliders go back and forth along the cliff. Leslie Orgel and Francis Crick would eat lunch out there frequently, and I remember us debating whether to present Dr. Orgel with Don's hypothesis that the pre-biotic earth was, in essence, a gigantic PCR machine, heating and cooling daily several hundred billion times. I believe we eventually did, and I believe he was unimpressed.

This was a period of intense productivity in the lab. Dyche's work on Arp2/3 complex erupted into the dendritic nucleation model in 1998. Laurent was learning fundamental things about ADF/cofilin (actophorin in *Acanthamoeba*) that were to set the stage for our current understanding of this important protein. In addition, Laurent was contributing to multiple other projects, discovering that actin filaments were Arp2/3 complex activators, developing a microscopy assay for dendritic nucleation and de-branching that was a precursor to the TIRF systems, conducting a quenched-flow study on ATP hydrolysis by actin, and a number of other collaborative projects. My work addressed the mechanism of Arp2/3 complex activation by WASp, and of WASp regulation by Cdc42 and phosphoinositides. Another post-doc, Jean-Baptiste Marchand, and I worked together on a kinetic study of Arp2/3 complex activation, and Tom very graciously placed me as the last author on that work. Another big success was solving the crystal structure of Arp2/3 complex. I purified the protein, Jean-Baptiste set up crystal trays and got crystals almost immediately, then Kirsi and Bob Robinson (in Senyon's lab) took

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care of the structure-solving (I will point out Tom's direct contribution later). Magdalena was establishing the *S. pombe* system and investigating cytokinesis, learning from Susan Forsburg. Lee was continuing the lab's work on myosin I with some nice biochemical findings, then took advantage of the *pombe* system to show Arp2/3 complex activation by myosin I in this system. Jia Lu did a careful mutagenesis study of *pombe* profilin. Kurt Amann started his post-doc towards the end of my time, and got TIRF going. David Kovar showed up only a few weeks before I left the lab, and I remember that we drank beer in the parking lot at Qualcomm Stadium while waiting for the traffic to clear after a Padres game. David, of course, went on to do great things with Tom at Yale.

We had a couple of very fruitful collaborations with other area scientists at that time. Work between our lab and Dorit Hanein's lab resulted in EM structure of Arp2/3 complex at filament branch points. Another collaboration with Dave Sept in Andrew McCammon's lab resulted in important insights on actin polymerization kinetics. Dave became a valued fixture at our lab meetings, and of course has remained a colleague and friend to many of us ever since.

Tom's "hands on/hands off" approach

I grew to appreciate many things about Tom during my time in the lab. I was amazed at Tom's foresight in starting the *pombe* system, which was to pay off for the next two decades. Another thing I appreciated was Tom's "blue collar" attitude, which definitely applied to himself as well. For example, during the Arp2/3 complex structure work, Tom took a very active role in threading the chains into the density. He spent many hours in the computer room getting that done. Perhaps, the most important thing was his ability to keep the lab protected from whatever else was going on. Obviously, as President of the Salk, there was a lot going on. None of this impacted us at that time. There must have been many good days and many bad days in terms of administration, but Tom was always Tom to us, every day.

An extremely useful practice of Tom's was to involve lab members in the lab's major decisions. It was a time of rapid technical change in science—mainly through digitization. When I started in the lab, we had a 35-mm camera attached to one microscope and a SIT camera/VCR attached to the other. By the time I left, we had gone digital. I learned a lot from Tom through the process of buying the Olympus microscope. He carefully evaluated every component, discussing with us about what we really needed. Tom involved many of us in this process, to the benefit of the lab and to us as trainees. He did the same thing on his R01 renewals, and I learned a fantastic amount from the process.

One thing I learned early about Tom was that he was very much involved in the lab's day-to-day research. In fact, I

learned this on my very first day, when he said "Dyche's doing an amoeba prep today, you should do it with him." A short while later, I was elbow-deep in amoeba juice.

Despite his administrative duties, Tom was in the lab every day. He wanted to be involved in the details, and contribute when appropriate. Lab meeting was something that was sacred on his schedule. If he was in town, or anywhere on the West Coast actually, we would have lab meeting. I remember a couple of times him calling in to say he was on his way from the airport and would be in the conference room in 30 min.

At lab meeting, I slowly started to appreciate Tom as some sort of non-canonical Zen master. He wanted people to take a middle path, neither too high nor too low. If somebody was doing a lot of chest-thumping, Tom had the ability to bring them down to size pretty quickly. However, if somebody seemed down about their results, Tom would try to bolster them. I distinctly remember a lab meeting where a depressed lab member showed a sad SDS-PAGE gel of a protein that would not purify. Tom's comment was "wow, those markers ran really great!" This tendency trying to find the good in things and people is something I have seen again and again from Tom over the years.

No matter how busy he was, Tom would come through the lab every day, and would want an up-date on each person's project. He would want it concise. It took me a while to understand this, but I understand now as a PI. The advice one would get might not be what you wanted to hear, but would almost always be right on the mark. I remember showing him a Coomassie-stained gel of Arp2/3 complex, hard-won from human neutrophils and requiring about 24 h of continuous work. Tom's remark was "Great job! Test it for nucleation ... today." Advice like this is something I have grown to appreciate. Frequently, I feel that I disappoint my students/post-docs by only briefly celebrating the current accomplishment, and leaping quickly to future plans. However, that purified Arp2/3 complex was falling apart at an unknown rate, and testing it early was important.

While being immersed in the details, Tom would also leave you alone to tinker. For me, this was crucial. I did a large number of hair-brained things, some being productive and some decidedly not. As one example, we had a mammalian culture room that was used by pretty much nobody but me. In that room, I messed around with a myriad of cells. Don Kaiser also hung his biking shorts in there to dry. Tom tolerated this (the cells, not the shorts), despite zero resulting publications. The things I learned there enabled me to do that sort of work in my own lab, which is now a mainstay for me.

Another example of Tom's hands off attitude was his tolerance of my somewhat circuitous search for a good source of mammalian Arp2/3 complex. My main protein source eventually became bovine thymus (rich in Arp2/3 complex and fine after freezing), but I tried a couple of other things *en route*. First, suspecting that white blood cells would have lots of complex, and wanting the

material to be fresh and abundant, I used cow blood. The only major slaughter house in Southern California was right smack dab in the middle of L.A. I made the 2.5-h drive up there, handed a 5-gal bucket (containing some EDTA) along with 20 bucks to a guy, and received a full bucket. I then drove back to La Jolla stirring the blood. If I had gotten into an accident, it would have been tough to explain the copious blood drenching my Honda Civic. It turned out that cow blood was not convenient, because the RBCs are not very dense (and difficult to separate from WBC). Two trips to L.A. were required to figure that out. So, the second source was six units of very fresh *Homo sapiens* blood from a nearby hospital, which I walked back to the lab in a brown paper bag. Despite the low amount of starting material, I isolated about a milligram of Arp2/3 complex from that source (Higgs et al. 1999). After a couple rounds of that, I got more sensible and went to cow thymus. A number of features of this purification have stood the test of time.

Tom's continued influence on me as an independent investigator

I remember once, while I was still a post-doc, Tom told me “being a mentor is a lifetime commitment.” I have certainly required my share of commitment after leaving the lab, and Tom has never let me down. In fact, he has been crucial to my career on several occasions.

One occasion was within a year of leaving the lab. I had embarked on an ambitious project to study Arp2/3 complex activity in lymphocytes, following up an observation by others that Wiscott-Aldrich Syndrome patients lacked microvilli on their lymphocytes. My little lab (two of us) developed some effective techniques in a short period of time (pay-off for all those un-published experiments in Tom's culture room) but ended up finding out that the microvillar phenotype could not be reproduced. Even so, Tom read my lousy R01 and made substantive comments (although nothing could save it). Also during that period, despite having a questionable future, I obtained a Pew fellowship. Tom's letter undoubtedly had a lot to do with that. Much more recently, I had some uncertainties as to career path and asked Tom's advice. I had a very useful half-hour phone conversation with him on a snowy Sunday afternoon, which helped me figure things out.

There have been numerous additional situations when I have been wondering about something, and think “Tom would know.” I then send Tom a loquacious email. Invariably, he replies quickly with a short but information-packed email. Problem solved. On one occasion, when

Pinar Gurel and I were puzzled about some bizarre effects of INF2 on actin polymerization, we took a road trip to Yale to get input from Tom and Enrique. Within 5 min, we had the information we needed, and also got some excellent New Haven pizza. I am not sure an email would have sufficed, so I really appreciated their time.

Many lessons from Tom were learned by example. For example, my lab meetings and journal clubs are based on his template, because it is an extremely effective way of doing things. I cannot imagine spending a whole hour going over one article in a journal club. Here is a partial list of other lessons learned.

- 1) Stick with a research subject. All the things that have happened in Tom's lab have developed from a long-term commitment to two projects (essentially, actin and myosin). I have tried to follow that in my lab, and it has paid off. We started working on FMNL formins in 2002 and INF2 in 2004, and they have taken us places we never would have dreamed.
- 2) Have a good ground game. Those are my words, not Tom's, but I learned this from Tom. For those who do not know, this is a football reference, meaning that you should not try to throw a long pass for a touchdown every play. Run the ball too, taking a few yards at a time. While Tom has had plenty of landmark discoveries, he has always kept up a steady publication record in solid journals. I have done that in my lab, and it has served me very well. We have recently thrown the ball downfield a little more, but we continue to focus on getting things published rather than battling for a “higher” journal. I think it makes for better science.
- 3) Finish what you started. Tom rarely leaves data hanging. He figures out how to wrap it up and get it out there. I cannot say that I am perfect in this respect, having several unfinished works at the moment. But I try. I was talking with Tom a few years ago, saying that it drives me crazy when people leave the lab with manuscripts un-finished. His response: “I still have a folder of yours. You can finish things up whenever you want.”
- 4) Bring it back to the cell. Late in my post-doc, Tom gave a talk about cytokinesis in fission yeast, perhaps one of his first. After the talk, somebody came up to him and said “wow, Tom, you're becoming a cell biologist.” Tom's answer was that he had always been a cell biologist! While Tom's reputation is one of rigorous quantitative biochemistry/biophysics, I think what separates him from others is that he knows which parameters are important to quantify, and he knows that from the cell biology. I remember him getting frustrated with people extending the precision of a measurement, when the number we already had was fine for cellular understanding. At the end of the day, the cell tells you what is important.

- 5) Keep going forward. I saw Tom in good times and bad during my post-doc, and the one constant was that Tom kept showing up. I will never forget the morning he announced that he was stepping down as President of Salk: we had a lab meeting the hour before that. I have tried to learn from him to brush off set-backs, squabbles, political shenanigans, and downright unreasonable behavior by others. Just stay on your path and good things will happen.

I am extremely grateful to have Tom as a mentor.

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Compliance with ethical standards

Conflict of interest Henry N. Higgs declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by the author.

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