AUTHOR COMMENTARIES - 2009

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Krzysztof Palczewski

Featured Paper Interview

According to Essential Science Indicators℠ *from Thomson Reuters, the paper ranked at #5 in the field of Biology & Biochemistry is the August 2000* Science *paper by Krzysztof Palczewski and his team, "Crystal structure of rhodopsin: a G protein-coupled receptor," (289[5480]: 739-45, 4 August 2000), with 2,467 cites up to June 30th of this year.*

Dr. Palczewski's record in the database includes 171 papers, the majority of which are classified in Biology & Biochemistry, cited a total of 8,609 times. He is the John H. Hord Professor and Chair of the Department of Pharmacology at the Case Western Reserve University School of Medicine in Cleveland, OH.

In the interview below,* ScienceWatch.com *correspondent Gary Taubes talks with Dr. Palczewski about this paper and his subsequent work with rhodopsin.

SW: What were you working on in the years leading up to your landmark 2000 publication in *Science* on the crystal structure of rhodopsin?

We were always interested in molecular detail of vision, namely the understanding of proteins, protein interactions, and signal transduction involved in eyesight at a structural level. We'd been working for many years on different proteins of visual signal transduction. It wasn't until a very talented post-doc from Japan came to my lab—Tetsuji Okada—that we put a lot of resources into studying rhodopsin, which is the key protein of visual signal transduction. He came from Japan with several ideas and a significant understanding of rhodopsin. We put this together with resources in the laboratory to crystallize rhodopsin and identify its crystal structure.

SW: Considering rhodopsin's key role in visual processing, why hadn't this been done before?

It had been tried by several laboratories. But the prevailing understanding of membrane protein crystallography was that these are very unstable proteins. They do not crystallize well, they don't form a proper crystal lattice, and they're just too difficult to handle. And all this is absolutely true. Because of these difficulties, it's still the case that only a very small number of integral membrane proteins have been crystallized.

Once you know how these proteins behave in different detergents, they're not that different than soluble proteins. I think this was a major breakthrough at that time: realizing that some of these G protein-

coupled receptors (GPCRs) are stable enough for crystallization. Since then, there have been more efforts to crystallize other proteins. Today five or six other GPCRs have been crystallized, most in the last year.

So I think the original reason rhodopsin hadn't been crystallized was a combination of a lack of faith that it could be accomplished, and the difficulty in obtaining the quality and quantity of highly purified rhodopsin that was needed. With a little bit of luck and a tremendous amount of work—there were many collaborators on that 2000 *Science* paper—all this was possible.

"Our work on vision now extends to retinal diseases and to other signaling processes in the retina."

SW: Was there one particular obstacle you had to overcome to get rhodopsin crystallized and elucidate the structure?

Yes, it was the purification. The technique for doing that was invented by Tetsuji Okada. It was critical to obtain high-purity material. After our work was published, three groups came out with different protocols for purification that were also successful, so it wasn't absolutely necessary to follow ours. So why did we get there first? I think it was the combination of a very careful production, isolation, and purification of the protein and a very careful analysis of the crystals that led to that success.

SW: What is it that makes rhodopsin itself such an important receptor, so much so that your paper has been averaging a few hundred citations a year for almost a decade?

Rhodopsin is part of this family of receptors called G protein coupled receptors—or "GPCRs," as I've mentioned. There are about 800 members of the same family and they're very critical for virtually all physiological functions. They're involved in sensory transduction, for example, in smell and taste as well as vision, and also in many other biological processes. Virtually all such processes are modulated by this class of receptors.

A cell has to communicate with the external world; it has to receive clues about what the neighbors are doing and it has to receive long-distance signals from the brain and elsewhere, and the mechanisms by which those signals are transmitted from the outside of the cell to the inside requires some sort of receptor. Most of these receptors, although by no means all, are GPCRs.

So there are hundreds of other GPCRs and a great need in the pharmaceutical industry to understand their structures for rational drug design. Today about 50% of all drugs on the market influence receptors of signaling proteins. That stimulates a tremendous interest from a pharmaceutical perspective. I think the work on rhodopsin from my lab and from others really opened the door to the belief that it's possible to crystallize these receptors. It also allowed us to begin understanding the function of these receptors at atomic resolution rather than at just a crude biophysical level.

SW: So only five or six of the 800 have been crystallized and most of those in the past year. That doesn't seem like a lot. Why isn't that number significantly larger?

Yes, it's not a lot. The problem is that each of these other membrane proteins exists in very small quantities in nature and they require very special handling. We need to know the properties of each of these particular receptors very, very well in order to be successful. The next step will be to develop a method that is truly generic and can be applied to all GPCRs. It will have to start with the expression, purification, and crystallization. That's an effort of ours as well—to get all three of these steps lined up in a very generic way.

I think if we can do that we'll advance the field as much and as rapidly as we did with the rhodopsin structure alone nine years ago—if we can come back and say, here's the method, take your receptor of interest, and it will work! And I think it's worthwhile putting a lot of energy to make just that happen. Every time we get another structure, it's a wonderful accomplishment. But the true goal will be a method that is robust and applicable to all GPCRs, not just one.

SW: What's the major problem that has to be solved to make this a reality?

The main roadblock that still prohibits fast resolution of these structures is expression of the protein, and that's going to be a problem until more effort is given to cell biology. We've made a lot of progress in the past 10 years making the construct to drive expression rapidly, but not the folding machinery to provide the platform for proper folding of these GPCRs. This is still a major problem.

When a GPCR is synthesized in the cell, it's post-translationally modified and transported from the cell's endoplasmic reticulum to the plasma membrane where proof-reading machinery checks to see if it's properly folded. Without this process, you get a protein that's incorrectly folded. Then you end up purifying a protein that's in the wrong conformation for crystallization. So separation of properly folded and improperly folded protein is a big issue. We've made a lot of progress since 2000, but this problem still has to be solved.

SW: Are you making progress understanding these receptors even without having the structures?

"Rhodopsin is part of this family of receptors called G protein coupled receptors... There are about 800 members of the same family and they're very critical for virtually all physiological functions."

Well, this year, for example, we had a paper in *Molecular Pharmacology* (Mustafi D, Palczewski K, "Topology of class A G protein-coupled receptors: Insights gained from crystal structures of rhodopsins, adrenergic and adenosine receptors," 75[1]: 1-12, January 2009) where we showed that all those structures are really very homologous to each other, so we are making some progress. We also stress the differences, which account for the specific Actions of these proteins.

But the overall topology, the overall design, like that of a house, is very similar. Between all these GPCRs, some 800 sequences, we can say with some confidence that the overall topology, the three-dimensional structure is going to be very similar. Although obviously each will have some subtle changes to react specifically with one hormone and not another.

SW: We talked seven years ago and at the time you said you had the structure of rhodopsin but not the structure of the form it takes in the cell membranes. Did you ever get that structure figured out?

In order to do that we needed different technologies than just crystallography. But we did it. In 2003, we published the membrane organization of rhodopsin in *Nature* (Fotiadis D, *et al*., Atomic-force microscopy: Rhodopsin dimmers in native disc membranes," 421[6919]: 127-8, 9 January 2003) and the *Journal of Biological Chemistry* (Liang Y, *et al*., "Organization of the G protein-coupled receptors rhodopsin and opsin in native membranes," 278[24]: 21655-62, 13 June 2003).

Those results showed that rhodopsin exists as a dimeric structure. There are two receptors that form a unit. And this appears to be the prevailing finding shown by many other methods as well—all GPCRs may function as a dimer, as a couple. That work, which was done in collaboration with Andreas Engel, is also very highly cited, although not as much as the 2000 *Science* article.

SW: How much do you still work on vision and how much on G protein-coupled receptors in general?

We have an active research program in both. Our work on vision now extends to retinal diseases and to other signaling processes in the retina. That's a large fraction of my lab's interest. In 2006, for instance, we published an article in *PNAS* on the photo-activated structure of rhodopsin (Salom D, *et al.,* "Crystal structure of a photoactivated deprotonated intermediate of rhodopsin," 103[44]: 16123-8, 31 October 2006). This is what rhodopsin looks like when it's activated by light. It took six years to generate those crystals.

So I would say my lab is now working on three areas—one is retinal processes and diseases and another is the rhodopsin structure and its interactions with other proteins. The third is our work on other GPCRs as well.

SW: Has there been any element of serendipity in your research—some accomplishment which came about because you just got lucky?

In my case, I can you tell I've just been extremely lucky to be given the opportunity to work in science in this country. I cannot stress enough how US science is open to immigrants and how much those of us who have come from another country appreciate that and how much we want to contribute. The support we receive—in terms of positions, grants, training—is really phenomenal!

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Krzysztof Palczewski's current most-cited paper in *Essential Science Indicators*, with 2,467 cites:

Palczewski K, *et al.*, "Crystal structure of rhodopsin: a G protein-coupled receptor," *Science* 289 (5480): 739-45, 4 August 2000. 2,467 cites. Source: *Essential Science Indicators* from Thomson Reuters.

Additional Information:

Read a classic *Science Watch*® interview with Krzysztof Palczewski.

KEYWORDS: RHODOPSIN, CRYSTAL STRUCTURE, G PROTEIN-COUPLED RECEPTOR, GPCR, PROTEIN INTERACTIONS, VISUAL SIGNAL TRANSDUCTION, INTEGRAL MEMBRANE PROTEINS, PURIFICATION TECHNIQUE, SENSORY TRANSDUCTION, RATIONAL DRUG DESIGN, FOLDING MACHINERY, MEMBRANE ORGANIZATION, RETINAL PROCESSES, RETINAL DISEASES.

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