

## Howard Hang: Posttranslational pathogenesis

Howard Hang is cutting through the forest of posttranslational modifications in an attempt to better understand how human pathogens alter this terrain.

Phosphorylation and ubiquitination get most of the attention, but plenty of other posttranslational modifications abound. New Rockefeller University assistant professor Howard Hang believes scientists may be overlooking many of these important decorations because they lack the means to identify them.

As a graduate student, Hang was fascinated by carbohydrate moieties called glycans and the vast array of possibilities they offer. When stuck onto surface proteins, these sugar groups can form slippery, protective cellular barriers. Glycans can also stabilize a protein, change its cellular destination, or alter its interaction partners (1).

Most glycosyltransferases lack convenient consensus target sequences, so Hang sought approaches to identify their substrates. He found small molecule inhibitors for specific glycosyltransferases (2) and synthesized identifiable sugar tags (3, 4). After extending his methods to lipid modifications (5), Hang is now poised to look towards the biology. He believes that human pathogens attempt to outwit their targets—our cells—by manipulating posttranslational modification pathways.

### CALIFORNIA DREAMING

#### *What got you interested in science?*

I actually liked organic chemistry in college (at UC Santa Cruz). That was the first time science really made sense to me, mostly because it involved learning some basic principles that allowed you to build more complex molecules. I liked learning the rules of molecules' interacting with each other rather than remembering what they are. And then it evolved from building molecules to applying them to understanding biology.

#### *So you focused on organic chemistry for your Ph.D. at UC Berkeley?*

Yes. I did my Ph.D. with Carolyn Bertozzi. She's a very creative chemist who is mostly geared towards developing tools to understand glycochemistry. In her lab, I learned about synthesizing organic molecules and asking biological questions with them.

Much like kinases, there are several isoforms of glycosyltransferases with similar catalytic activity but most likely with discrete functions. There were really no tools at the time to evaluate their functions with small molecules. The hope was that we would find selective inhibitors of each of the isoforms that add each individual carbohydrate onto a growing lipid or protein.

We also tried to develop methods to identify the proteins that carry specific types of modifications. Just like for kinases or the ubiquitin field, we asked what proteins were modified with specific glycans.

#### *How did this work drive your scientific career path?*

Over the last few years, I've been generally interested in how posttranslational events affect protein function. But for many modifications, we don't have very good detection methods to identify which proteins are modified.

#### *Why do you think that's been so difficult?*

The problem with these modifications is that they're transient and heterogeneous. They're often at substoichiometric levels. They come on and off at different cellular states. So, abundance is naturally an issue.

Heterogeneity is the other problem. It's not like one modification is on one protein. A given protein can have five of the same modifications on different sites. So that makes things kind of complicated.

#### *What are the biggest problems you've encountered?*

Historically, all modifications have been detected by radioactivity: radioactive ATP, or nucleotide sugars, or fatty acids. The

problem is, if you want to identify a protein selectively that carries those modifications, there's no intrinsic way to do affinity retrieval of radioactively labeled proteins. And often, radioactivity is—while effective—a bit cumbersome and slow.

Even for modifications which we have antibodies to detect selectively, like phosphorylation of histones, for example, they are often context specific. I think the only antibody that's specific for a posttranslational modification is phosphotyrosine, and that's a rare exception that's very general.

So part of my interest is in developing general chemical tools for looking at different types of modifications. We started with glycosylation, and I ventured into protein lipidation, which we're continuing to work on.

### EAST COAST HIP

#### *That interest in protein lipidation was piqued by your postdoctoral work in Hidde Ploegh's lab at MIT. How did you choose his lab?*

That came from the fact that I got interested in host–pathogen interactions. I liked the idea of how viruses and bacteria are able to evade the immune response, how over years of evolution, two organisms coevolved to coexist.

It always seemed to me that nature was much more clever than scientists at manipulating cell biology. And studying that would actually give you some key insight into how cell biology worked.

#### *And how is that linked to posttranslational modifications?*

Since we don't have very many tools to study posttranslational modifications, it's hard to analyze all the cellular effects of an infection. So we often find things that bacteria or viruses do to modulate



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**"I liked learning the rules of molecules' interacting with each other rather than remembering what they are."**

phosphorylation or cytoskeletal rearrangements, just because we have good methods to analyze those pathways.

**What sort of tools have you developed to look at other modifications, and how do they work?**

We've developed reagents for glycosylation and lipidation, and we're starting to think about other types of posttranslational modifications.

We take chemically reactive functional groups and install them onto the substrates that would be used by enzymes that add the modifications. For example, for the lipid work, we modified fatty acids with a chemical reporter, such as an azide or an alkyne group.

Then we take advantage of the fact that those two groups have some unique chemical properties that we can use to convert them into a fluorescent tag or an affinity handle. We essentially install on small molecules, like metabolites, the same thing people do for proteins. But instead of putting on an HA tag, we put a small chemical reporter that allows us to see them selectively.

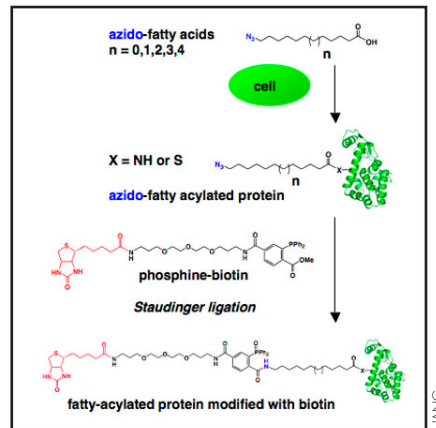
**"It always seemed to me that nature was much more clever than scientists at manipulating cell biology."**

The hope is that the derivitized substrates will be used by the cell's enzymes, which then gives us a signal to follow that's more robust than radioactivity. And it gives us the opportunity to enrich for things that are modified.

**How will you now apply these tools to biology in your new lab at Rockefeller?**

Many pathogens—particularly bacteria—inject enzymes directly into the host cell that modulate signaling pathways, which we have very little data on. Now that we have better methods to look at posttranslational modifications, we can ask, in the presence of these enzymes, are these pathways perturbed?

And we don't have to go in totally blind. For some, we match the chemical tool with the proposed enzymatic activity of a bacterial enzyme or toxin based on bio-



**Fatty-acylated proteins can be detected in cells via metabolic labeling with azido-fatty acids.**

informatics analysis. For example, there's a family of phospholipases from several gram-negative bacteria that we don't have substrates for. Now that we have tools to look at lipidated proteins, we can ask, are there potential substrates of the phospholipases that are lipidated proteins?

**Have you had much experience in the biology of infectious disease?**

In Hidde's lab I got interested in how intracellular bacteria like *Salmonella* avoid degradation by proteases in mammalian cells. We showed that active proteases were excluded from *Salmonella*-containing vacuoles (6). This might be one way bugs manage to survive in host cells.

**Another goal of your lab is to identify antigens that are involved in immune responses?**

When we get infected with or exposed to pathogens, we present antigens to the immune system, and that educates the body on what we've been exposed to. This essentially vaccinates us against a second round of infection.

For many bacterial pathogens, we don't know what the antigens are at the molecular level. This makes it difficult to design effective vaccines. So we are trying to directly purify the antigens from infected cells and characterize them by mass spectrometry.

**What's the long-term goal from that?**

One goal is to identify antigens that are presented to the immune system at dif-

ferent stages of infection, so that you can then identify antigen-specific immune responses in mouse models.

The corollary to that is, once we identify new antigens, they're potential vaccine candidates. You could test whether these specific antigens might be more effective versus what we already use, which are attenuated strains of bacteria for which we don't know the antigens. Hopefully, it will be a more precise method of designing vaccines.

**IF HE CAN MAKE IT THERE...**

**You arrived at Rockefeller University in February of this year. How are you liking it?**

It's a great place to start. I think the big advantage is the freedom of this place. The small but very diverse community encourages me to ask questions about general problems that we don't understand. It gives me the freedom to try different methods and approaches. I don't feel constrained, based on whether I should be a chemist, or a biologist, or whatever. Here, I can just ask scientific questions and not worry about whether my department chair is into that or not.

**So you interact with many colleagues who have different areas of expertise?**

Definitely, just within the small campus itself—from immunologists, to neuroscientists, to other chemical biology people. Rockefeller also has a very rich tradition in bacterial pathogenesis. I think it was ideal for me, from a scientific point of view. And from a personal point of view, I enjoy New York City as well. **JCB**

1. Hang, H.C., and C.R. Bertozzi. 2005. *Bioorg. Med. Chem.* 13:5021–5034.
2. Hang, H.C., et al. 2004. *Chem. Biol.* 11:337–345.
3. Hang, H.C., et al. 2003. *Proc. Natl. Acad. Sci. USA.* 100:14846–14851.
4. Hang, H.C., et al. 2003. *J. Am. Chem. Soc.* 126:6–7.
5. Hang, H.C., et al. 2007. *J. Am. Chem. Soc.* 129:2744–2745.
6. Hang, H.C., et al. 2006. *ACS Chem. Biol.* 1:713–723.