Michael Rape: the biology of destruction

Michael Rape says that ubiquitination's diversity and adaptability makes it an ideal entry point for understanding vast swaths of biology.

Protein degradation is irreversible, which makes it an attractive option for cells regulating crucial decisions. But for a biologist it sounds rather simplistically binary and dreary—the protein is either destroyed or not destroyed. Michael Rape, a new faculty member at the University of California, Berkeley, CA, has found that ubiquitin-mediated regulation and proteolysis is anything but dull.

Originally a biochemical oddity, ubiquitination hit center stage when ubiquitin-mediated cyclin destruction by the anaphase-promoting complex (APC) was found to drive mitotic exit.

"Tremendous progress has been made, [but] a lot of fundamental questions in the [ubiquitin] field still haven't been answered."

By the time Rape started his Ph.D. at the Max Planck (Martinsried, Germany), work on ubiquitin had branched out into discoveries of non-destructive regulation. Rape's colleagues in Stefan Jentsch's lab had found that a ubiquitinated transcription factor called SPT23 could be clipped, but not destroyed, by the proteasome (1). SPT23's dimeric partner sheltered the clipped protein, preventing complete destruction.

But the partner's continued grip stopped the nibbled protein from entering the nucleus. Rape found that the Cdc48 complex came along to extract the shortened SPT23 from its dimeric complex, thus releasing it to enter the nucleus (2). For other extracted proteins, Cdc48 directs them along a pathway leading to the protein-chewing proteasome (3).

Rape moved to Marc Kirschner's lab at Harvard Medical School (Boston, MA), where he figured out how the cell cycle gets restarted after mitosis. Once the APC has completely destroyed cyclin, it turns on itself to chew up one of its own components (4). The resulting inactivity of APC explains why Rape's G1 extracts failed to destroy cyclin A, and how cyclin levels are able to recover, only to activate the APC to continue the cycle.

Rape subsequently explored how APC substrates are destroyed in a defined order. Those that disappear early get multiple ubiquitins from APC in one shot, whereas the slow customers wander away for cycles of deubiquitination in between ubiquitin additions (5).

He now hopes to screen for ubiquitination substrates using expression cloning. Past attempts featured simple cDNA libraries, in which regulatory cDNAs are underrepresented. A Unigene library (with each gene represented exactly once) now allows Rape to test smaller pools of cDNAs for their effects on protein degradation in extracts. In a recent interview, he discussed this and other work he hopes to conduct at Berkeley.

BEGINNINGS

How did you first get interested in science?

My great uncle had a very old pharmacy with a little lab in the back. When I was ten or eleven, I had my own lab in the basement doing chemistry experiments. I was born in an area where we had trouble with pollution—we had a lot of problems with SO_2 in the air—so I tried to make SO_2 in the lab. I gassed some plants and looked how they tried to survive.

What attracted you to Stefan Jentsch's lab for your Ph.D.?

I liked the combination that he developed between genetics—finding new components—and understanding a mechanism.

Why did you think that degradation would be an interesting area to study?

In 1999, it was not like now where there is a big ubiquitin paper every week. I had the feeling that it was not completely solved yet—there were a lot of things to do. It turned out that ubiquitin was a very fruitful area, not only for learning a lot of different techniques and a lot of different approaches but also, in the end, how many different processes it controls. I really saw this when I looked for a postdoc position. I was more interested in questions of proliferation and differentiation, but their regulation brought me back very quickly to the ubiquitin.

Michael Rape

You've described Cdc48 as a ubiquitin-selective desegregase. When does the cell need such a function?

You need this if you want to extract only one subunit out of a complex. Cdc48 can get the subunit out and then attract proteins that deubiquitinate it or can channel it to the proteasome. It's a really crucial, central activity. It shows the power of ubiquitin as a cellular modification.

A FRUITFUL SIDETRACK

What was your initial aim in your postdoc?

I didn't like the cell cycle too much because it seemed to be so complex with so many different groups competing in a relatively small area. I was more interested in the kinds of decisions that cells have to make during differentiation. These are very fundamental decisions and there are a lot of different layers of regulation.

Text and Interview by William A. Wells
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"The fun about ubiquitin is that it can communicate a lot of information."

Various people had demonstrated anaphase-promoting complex (APC) activity in postmitotic brain cells, but nobody knew the pathway that the APC was regulating. My idea was to try to get an entry point into neuronal differentiation by finding the pathway regulated by the APC. I started with a degradation screen to find the important players. But my work turned out to go in a different direction.

When we tested human extracts with known substrates, the APC degraded some substrates like securin but we could never get other substrates like cyclin A to be degraded. We decided that before going on and screening this whole library, we actually should understand why the APC fails to degrade a couple of well-known substrates.

That very quickly brought us back to the regulation of the APC, which we realized wasn't understood as well as people thought at the time. I kind of ended up back in the ubiquitin field as more a matter of chance than what I really intended to do when I looked for a postdoc.

Did the screen ever get done?

At the end of my postdoc, I turned back to this kind of question. We now have a system where the APC can degrade substrates very, very efficiently. I am now in the process of rerunning the screen and getting new substrates. With the new conditions, in vitro expression cloning is a very powerful technique. We have more active extracts and much better negative controls, which allows a much more straightforward identification of substrates.

What else are you doing?

We are using in vivo sensors to measure the localization of degradation. We can test different mutations of the target sites, and couple this with an RNAi screen to identify regulators, seeing if we get more or less degradation or different localization of degradation.

We want to do RNAi to identify ubiquitin ligases that control the entry to differentiation. I'm also studying how deubiquitination enzymes regulate the APC. I think the deubiquitination enzymes are probably just as interesting as ubiquitin ligases.

THE PROMISE OF UBIQUITIN

The ubiquitin field is now far more crowded. Is there still growth potential?

There is huge potential. Although tremendous progress has been made, a lot of fundamental questions in the field still haven't been answered. We have only begun to understand how important the chain length is, and how chains of different topologies are made and recognized. On both sides—the mechanistic questions and the substrates—there is a lot of potential.

After so long, why have these questions not been answered?

Ubiquitination is not a simple or trivial reaction—it involves at least three different enzymatic activities. There is a lot of enzyme-specific regulation, so it has been hard to get paradigms for the whole field.

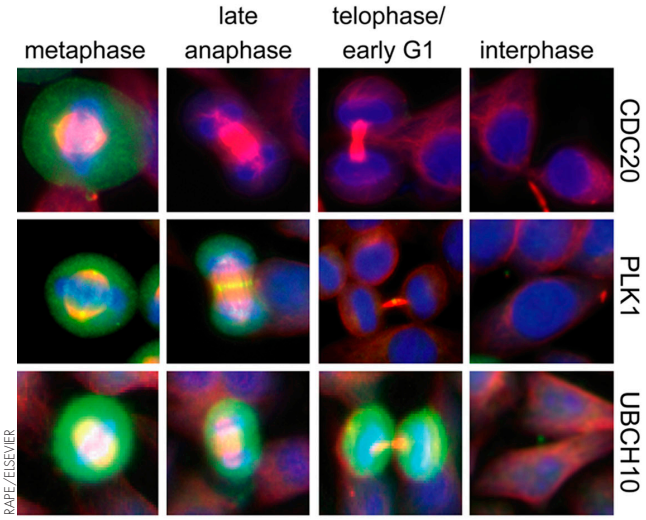
You always have two sides to it: the mechanism aspect on one side and the process that is controlled on the other side. Even though a lot of people are interested in the process that is controlled on the other side, substrate identification has been extremely difficult. It took 15 years from the identification of Rad6 and the fact that ubiquitination was important for DNA repair to find probably the most important substrate in this process which turned out to be PCNA. It's not trivial.

With all this diversity, how do you focus your studies?

One of my very strong interests is the mechanism of the ubiquitination, and how this mechanism is used to achieve a certain type of regulation. An example of this is how the APC orchestrates progression through mitosis using a mechanism akin to kinetic proofreading.

So does ubiquitin do every kind of regulation that you can ever imagine?

The fun about ubiquitin—this is something I really like about it—is that it can communicate a lot of information. You have monoubiquitination versus multiubiquitination, there are different chains with different topologies, and all of them have different binding proteins. The whole system is reversible so you can add and remove. Really it's something that can contain a lot of information.



APC substrates (green) get chewed up at various different times.

We have probably around 1,000 ubiquitin ligases in the genome, we have 100 different deubiquitinating enzymes in the genome, and there are so many proteins that recognize ubiquitinated sites—still there are novel ubiquitin-binding domains that are discovered on a regular basis. It's not a surprise it can control so many processes. JCB

1. Hoppe, T., et al. 2000. *Cell*. 102:577–586.
2. Rape, M., et al. 2001. *Cell*. 107:667–677.
3. Richly, H., et al. 2005. *Cell*. 120:73–84.
4. Rape, M., and M.W. Kirschner. 2004. *Nature*. 432:588–595.
5. Rape, M., et al. 2006. *Cell*. 124:89–103.