AUTHOR COMMENTARIES - 2009

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Daniel Klionsky

Featured *Science Watch*® Newsletter Interview

"Autophagy" literally means "self-eating," which seems like a particularly gruesome brand of cannibalism. As it turns out, however, we all do it. Just wait long enough between meals and we start to consume our own fat tissue, and if we wait even longer, we start to live off our muscle as well. Researchers have known for half a century that cells do it, too. They surround defective proteins with double-membraned vesicles and then drag them off to be broken down and the parts reused, but for many, this phenomenon was thought to be a mechanism for clearing away garbage and little more.

All this changed in the last decade, as defects in autophagy were linked to a host of diseases from cancer and neurodegeneration to microbial infection and even aging. Among the leaders of this autophagy revolution is the University of Michigan cell biologist Daniel Klionsky, who, according to a recent extraction from the Thomson Reuters *Hot Papers Database*, has coauthored five highly cited reports on the topic in the last two years. One of these, a *Nature* review from February 2008, "Autophagy fights disease through cellular self-digestion" (with Noboru Mizushima, Beth Levine, and Ana Maria Cuervo) has been cited nearly 150 times in the year and half since then—more than 100 times in the last six months alone. Klionsky's 2000 review in *Science,* "Autophagy as a regulated pathway of cellular degradation," co-authored with his post-doctoral advisor Scott Emr (link: article# 2) of Caltech (now at Cornell), has been cited over 600 times (see adjoining table, paper #1). It's one of over 20 Klionsky papers published in the last decade that have garnered more than 100 citations each.

Klionsky, 50, received his bachelor's degree, magna cum laude, from UCLA in 1980, and went on to Stanford where he obtained his Ph.D. in biological sciences in 1986. He spent the next four years as a post-doctoral fellow in Emr's lab at Caltech until moving, in1990, to the University of California, Davis, where he eventually became a full professor of microbiology. In 2000, he left California for the University of Michigan, where he now holds joint appointments in the Life Sciences Institute, the Department of Molecular, Cellular and Developmental Biology, and the Medical School.

Klionsky spoke to *Science Watch* from his office in Ann Arbor.

SW: What prompted your research on autophagy, considering that it wasn't exactly mainstream science when you began in the early 1990s?

When I was starting my career at UC Davis, I had a new post-doc who came from a laboratory in Spain that had cloned a particular gene encoding a vacuolar enzyme; this was before the yeast genome was sequenced. I had been interested in this gene when I was a post-doc studying vacuolar protein targeting

but had never pursued it. For a new faculty member, it was an easy project to begin with, but it turned out that the gene product didn't go to the vacuole by the normal pathway.

SW: What's the normal pathway?

At that time, we thought that all proteins going to the vacuole first enter the endoplasmic reticulum and then go from there to other locations inside vesicles that bud off from those organelles—first to the Golgi complex, then usually to the endosome, and then to the vacuole. This is part of the secretory pathway, which is also used to get proteins out of the cell. The protein we were looking at is called aminopeptidase I, and it turned out that it didn't go through the secretory pathway. So we figured we were looking at a new pathway to the vacuole that no one had ever shown before. We got mutants, because we were working in yeast—I'm still working in yeast—and it's easy to do genetics. And one of the things we did is compare our mutants with other mutants that are already published in the literature to see if they're novel. What I found was that these mutants overlapped almost 100% with mutants from two other labs—one in Germany and one in Japan—that were looking at autophagy.

SW: Is 100% overlap good or bad?

I was shocked and, I have to say, not very happy about it, for two reasons: one, it obviously meant that I was not alone in looking at this set of mutants; and, two, at that time autophagy was pretty much considered a garbage pathway. The thought was that aberrantly folded or nonfunctional proteins were taken to the vacuole or lysosome to be degraded—sort of garbage-recycling pathways. Who cares about that? Garbage pickup is important, but it is not what I planned to study—my interest was in protein targeting. So at that point I'm studying autophagy and thinking, my goodness, this is terrible. This was the mid-1990s. None of the genes had been cloned at the time. Autophagy itself had been studied in mammalian cells since the 1950s, but not a single gene had been identified, because it is relatively difficult to do genetics in that system. As far as the molecular aspects then, this whole field was going nowhere, which was not exciting to someone who does a lot of molecular genetics.

Highly Cited Papers by Daniel J. Klionsky and Colleagues, Published Since 1999
(Ranked by total citations)

Rank	Papers	Cites
1	D.J. Klionsky, S.D. Emr, "Autophagy as a regulated pathway of cellular degradation," *Science*, 290(5497): 1717-21, 2000.	629
2	B. Levine, D.J. Klionsky, "Development by self-digestion: Molecular mechanisms and biological functions of autophagy," *Develop. Cell*, 6(4): 463-77, 2004.	603
3	T. Shintani, D.J. Klionsky, "Autophagy in health and disease: A double-edged sword," *Science*, 306(5698): 990-5, 2004.	403
4	D.J. Klionsky, *et al.*, "A unified nomenclature for yeast autophagy-related genes," *Develop. Cell*, 5(4): 539-45, 2003.	293
5	D.J. Klionsky, Y. Ohsumi, "Vacuolar import of proteins and organelles from the cytoplasm," *Ann. Rev. Cell Develop. Bio.*, 15: 1-32, 1999.	239

SOURCE: Thomson Reuters *Web of Science*®

SW: So what did you do?

Well, all three labs—the German lab, the Japanese lab, and mine—started to clone the genes, and it was pretty amazing how rapidly it developed. Literally in just over ten years the field exploded, even in yeast, with the identification of 32 genes in this pathway and the discovery, in effect, of a pathway that was almost completely unknown, at least in terms of the molecular aspects. Because of the timing of when we did this work—before the entire genome of yeast was sequenced—this may be one of the last examples where several large-scale screens revealed an entirely unknown molecular pathway. In 1997, the first gene was published for autophagy in yeast. That was by our collaborator/competitor, Yoshinori Ohsumi. Two years later Beth Levine published the first paper showing a connection between autophagy and disease—in this case, cancer.

SW: How is cancer connected to autophagy, and how did this one discovery affect the evolution of the field?

Mice that have a mutation in one of the autophagy genes show a much higher rate of spontaneous tumor formation. And that, of course, attracted considerable attention. People in the field were thrilled to see this sort of connection between autophagy and human disease. Since then, we've continued to see

this amazing networking of connections. Autophagy is involved in the elimination of certain invasive bacteria and viruses from host cells; it's involved in protection against certain types of neurodegeneration; it plays a major role in cellular remodeling in response to various environmental changes or stresses; it is induced by starvation, by low oxygen, and by reactive oxygen species. Because of the unique membrane dynamics of this process, the cell has the capacity, through autophagy, to actually sequester essentially any-sized cargo, which means that this is the only process in which you can enwrap an entire organelle in a membrane and deliver it to the lysosome or vacuole for degradation.

SW: Do you understand the mechanism linking autophagy to cancer, or is it still a black box?

It's not so clear what's going on there. One possibility is based on work from Eileen White's lab. Autophagy is basically a protective mechanism for cells, one that's induced if cells are stressed. If autophagy is defective and then the cell is stressed, it's possible that there's now selective pressure for hypermutation. So, many of the cells may die, but those cell mutants that survive may undergo a change that allows them to survive the stress, but they may also undergo a change that causes them to become cancerous.

SW: How did the research evolve after Levine's discovery and the genetic work?

Once the genes started to come out from the yeast work, people who were working in these various other fields started to ask if it's possible that this pathway is playing a role in what they were studying. For example, what happens if we either induce this process or block it genetically or with a chemical inhibitor? So there was this explosion because of all the other fields now looking at autophagy connections, especially with regard to human disease.

SW: You've noted in your papers that autophagy is turned on during starvation. What's the connection there?

The idea there is simple. It's similar to what happens during starvation on an organismal level. If you fast for long enough, your body first starts to break down your fat reserves and then your protein reserves. If you're not taking in any nutrients, you have to keep the essential processes going. The same thing happens on a cellular level. If the cell is not getting enough food for a certain threshold of time, it needs to cannibalize parts of itself; it takes up cytoplasm non-selectively and delivers it to the vacuole or lysosome, where it can be broken down and the products released back into the cytosol to be reused for new synthesis. The cell can make essential proteins by reusing parts of itself and thereby survive the starvation condition. During starvation most new synthesis is shut down, but the cell will make things that are essential, and autophagy provides it with the resources to do that.

SW: So the cell is effectively eating what it can afford to eat of itself until more nutrients come along from outside?

Exactly. In yeast, if we starve wild-type cells for a month or so, they can do pretty well. If they're autophagy-defective, they'll die within one or two days. That's a huge difference in life span.

SW: Can you describe the link between autophagy and neurodegeneration, and perhaps what the mechanisms are?

Many people are now working with autophagy in neurodegeneration, either looking for additional ways to regulate the process or to understand how it's functioning, for example, in neurons. It appears that autophagy may be protecting neuronal cells from the buildup of certain proteins that become toxic. It's become clear that in some cases autophagy is definitely protecting us from neurodegeneration. It's not crazy to imagine that in some not-too-distant future we'll be able to turn on autophagy in a neuron-specific manner. This might be beneficial, particularly in people who are genetically predisposed to a neurodegenerative disease. It might, at least, delay the onset of symptoms.

SW: What do you think are the big questions in autophagy research that still need to be answered?

You can ask that question at two levels. One is the basic-science level. There we have questions such as what is the membrane source for forming the sequestering vesicle, called an autophagosome? No one knows for sure where that membrane comes from and how it actually forms. This is a major question in the field. Another one is how this process is regulated. You don't want to have too little autophagy, but you can imagine that too much is also a problem. If you eat too much of yourself, you'll probably die. So it has to be regulated pretty tightly, and it's not known in detail how that happens. We now know 32 proteins that are involved primarily or exclusively in autophagy, but for most of them the functions have not been characterized.

At the other, clinical, level there are huge questions to be answered. A lot of people are now trying to see if they can manipulate autophagy as an anti-cancer treatment. But here's the problem: it cuts both ways. In other words, autophagy is generally a cytoprotective mechanism—meaning that it's cell protective. But that's true not just for healthy cells that need it to survive starvation, but also for cancer cells. So it appears that some type of cancer cells induce autophagy to help them survive. In these cases, we may want to shut off autophagy to promote the death of the cancer cells during treatment. In other cases, we might want to induce autophagy to push cancer cells over the edge and kill them during treatment.

The problem is that cancer is so heterogeneous—even within an individual, the cancer cells are not uniform—so we don't know when one method might work and when the other might. At the moment a lot of that work is being done empirically. If we treat with a given anticancer drug and then with another drug that induces autophagy, is the combined effect beneficial or not?

"There was this explosion because of all the other fields now looking at autophagy connections, especially with regard to human disease," says Daniel Klionsky of the University of Michigan, Ann Arbor.

SW: What questions are you focusing on in your own research?

One of the main things we're focusing on is this question of regulation. This has been largely a black box. People have known for many years, for example, that TOR, a kinase, is a negative regulator of autophagy. All we know, however, is that it's involved in regulation. Other than that, there are huge unknowns. We're doing a lot of work on that. We'd like to be able to reconstitute the autophagy process—to really determine what happens step by step. There's no system right now to do the whole thing in a test tube, but we think we can at least reconstitute the individual steps to determine exactly what proteins are needed at each step and what those proteins are doing. Right now there are just many unanswered questions in terms of the mechanisms of this process.

We've also recently started on a new area, studying a subset of autophagy called *mitophagy*: selective mitochondrial degradation. Autophagy can be nonselective, which is what we've been talking about so far, but it can also be selective. If you have damaged mitochondria, for instance, those can be selectively targeted by an autophagic-like mechanism. This is what we call mitophagy, and it's very interesting because a lot of diseases are associated with mitochondrial dysfunction, and it's starting to become clear that this mitophagy is involved with the disease process. Parkinson disease is an example; a defect in mitophagy is apparently part of the problem. So we've carried out screens for mutants defective in mitophagy. A lot of those overlap with genes already discovered for general autophagy, but some appear to be specific to mitophagy.

SW: After starting your career in a field that no one seemed particularly interested in at the time, how does it feel to be at the forefront of a field that's now extremely competitive and evolving at a furious rate?

Well, at one level, of course, it's great. One of the nice things in a practical sense is that although the field has exploded, very few people have come into the yeast field of autophagy. So there's a tremendous increase in almost every other organism you care to mention, and I can look at that work and appreciate it and benefit from it, but I don't have to worry about direct competition with my own laboratory.

One of my goals now is to build the community of autophagy researchers. I'm editor-in-chief of the journal *Autophagy*, and we've been continually trying to establish guidelines for the field and the research. In 2003 we came out with a unified nomenclature for autophagy in yeast in a paper published in *Developmental Cell*. We're now trying to standardize that in other organisms and plan to publish that paper in *Autophagy*. We published a paper last year with over 200 authors on guidelines for monitoring autophagy. What do you need in a paper to say, yes, this is autophagy occurring? What's adequate? What would or should convince a reviewer? Because, not surprisingly, in such a new field, with new researchers coming in all the time, they're not sure about these crucial details. So we set down these guidelines. We also established a reagent forum at the journal site, to give people a place to list their experiences with different reagents so that other researchers can save time and money by using reagents that others have already tested. We're now about to launch an online protocol database. So I guess I'm a nut for organization.

Related Information:

- According to our Special Topics analysis of Autophagy research over the past decade, the work of Daniel Klionsky ranks in the top 20 authors at #3 by total cites, #2 by papers, and #9 by cites per paper, as well as several papers in the 10- and 2-year top 20 lists for the topic..
- *ScienceWatch.com* talks with *Autophagy's* Editor-in-Chief, Dr. Daniel Klionsky, about the journal's history and citation record.

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back to top

2009 : September 2009 - Author Commentaries : Daniel Klionsky

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