Meet our scientists!

When cellular proteins become old and defective, they are degraded. These proteins are first tagged with ubiquitin, a molecule that can be covalently attached to proteins. Specific types of ubiquitination target proteins to proteasomes, cellular structures that degrade proteins. Sayaka Yasuda, a senior scientist in the Protein Metabolism Project, has been studying how proteasome-dependent degradation occurs and has obtained intriguing results. She found that when cells are subjected to certain types of stress, proteasomes congeal into liquid droplet structures in cells, which likely enhances their ability to degrade substrates. Her results are published in "Stress- and ubiquitin-dependent phase separation of the proteasome," Nature, 2020 Feb;578(7794):296-300. She spoke to us about her work.

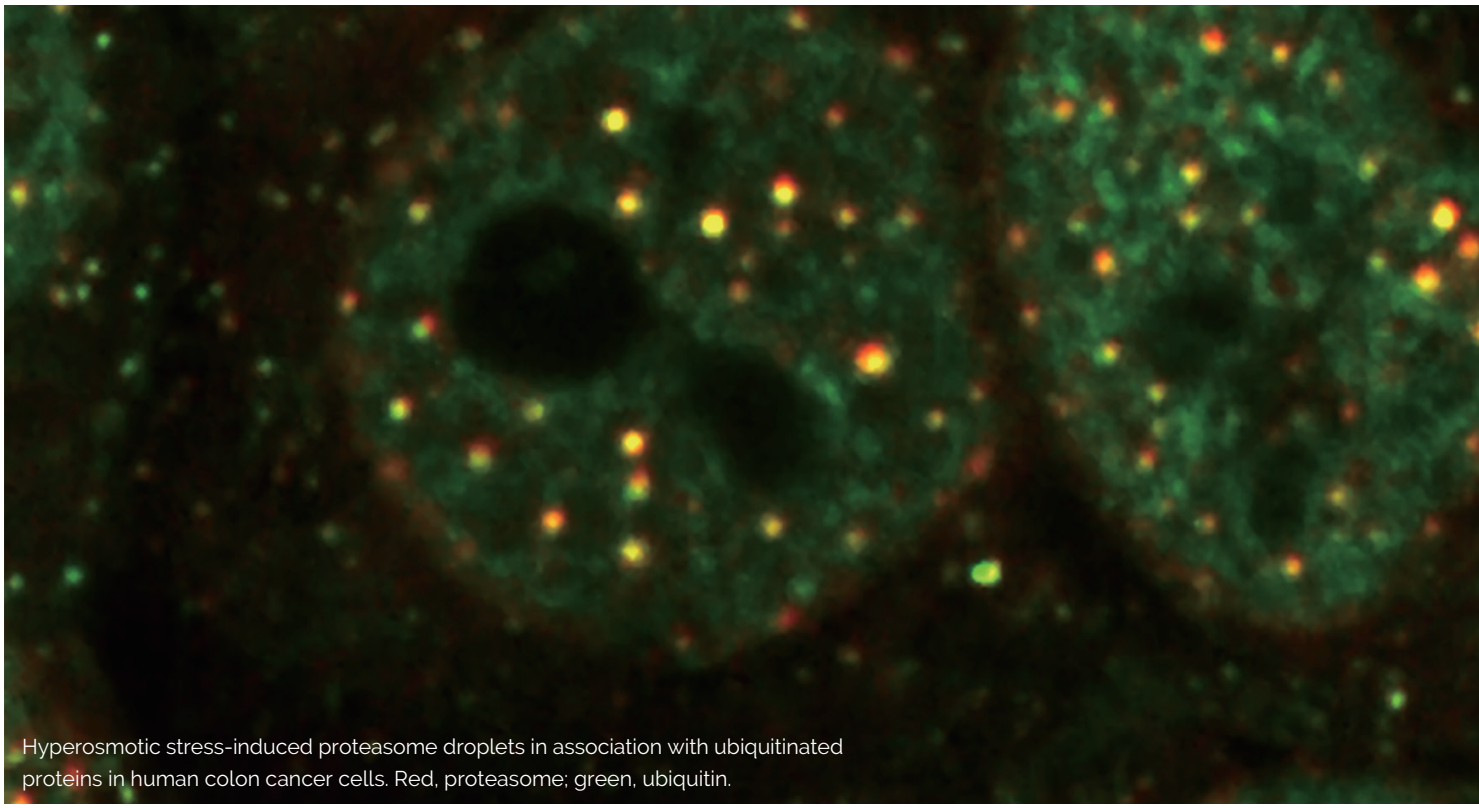
Sayaka YASUDA

How did you start this project?

I first learned imaging techniques when I was in graduate school. When I later joined the Protein Metabolism Project, proteasome dynamics hadn't really been studied before, so I decided to image proteasomes in cells under a microscope in different conditions. Proteasomes are usually found diffusely within a cell, but I found that when cells are stressed, proteasomes congeal into punctate liquid droplet structures. That was the start of this work.

What are liquid droplets and why are they important?

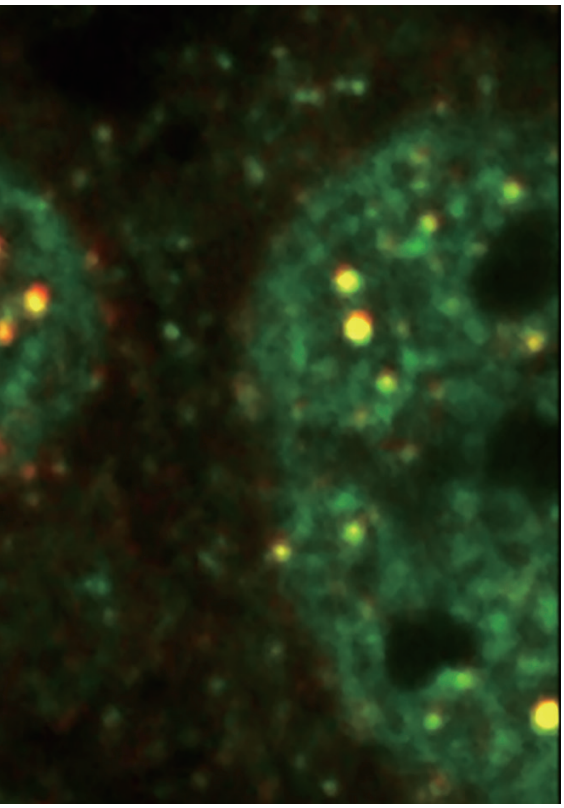
They really look like small droplets of oil in water. Proteasome droplets contain ubiquitinated proteins, RAD23B (a molecule that shuttles ubiquitin to proteasomes and bridges their interaction), proteasomes, and P97 (a molecule required for proteasomal degradation). We think that protein



Hyperosmotic stress-induced proteasome droplets in association with ubiquitinated proteins in human colon cancer cells. Red, proteasome; green, ubiquitin.

degradation occurs in these droplets, and droplets increase the efficiency of proteasomal degradation by bringing all the components and targets for degradation together in a specific location.

Does protein degradation become more important when cells are under stress?
The primary targets that are degraded in our droplets are ribosomal proteins. When cells are subjected to hyperosmotic stress, ribosomal proteins aggregate, and we think that droplets are where these aggregates are degraded. We've shown *in vitro* that ubiquitinated proteins and RAD23B can form droplets on their own. Proteasomes are recruited to droplets, but they aren't necessary for formation. However, if we add proteasome inhibitors once droplets are formed, they don't disperse as readily. Droplets are transient structures that disperse once ubiquitinated proteins are degraded. They last longer than usual if we inhibit degradation and they disperse faster if we accelerate degradation. That's why we think degradation occurs in droplets.



How is droplet formation beneficial to cells?
We thought that droplet formation should increase the likelihood that a cell would survive hyperosmotic stress, so we performed cell death assays. However, our RAD23B knockout lines increase cell death regardless of stress. So, while we think that droplet formation should improve protein degradation and increase cell survival, but we haven't been able to prove that yet. Recently we've identified a sequence in RAD23B that is required for liquid droplet formation. We're planning to put a mutation within this sequence to make mutated RAD23B that is unable to form droplets, and we're planning to use this mutated protein to prove whether there is a significant survival benefit to liquid droplet formation in cells.

What is the significance of your work and how does it differ from previous reports on liquid droplets?
Liquid droplet formation or liquid-liquid phase separation is a subject that is gaining a lot of attention these days. A relatively well-characterized mechanism for liquid phase separation is electrostatic interactions between proteins or RNAs. The idea is that electrostatic interactions cause proteins or RNAs to bind together and form a different phase, separate from other cellular components. Our results are distinct from previous results because we find that interactions between a particular domain of RAD23B and ubiquitin are responsible for phase separation, not just general electrostatic interactions. Since ubiquitination is a regulated post-translational modification, our results suggest that increases in ubiquitination regulate phase separation. This explains how proteasome droplets are initially formed, and also explains how they disperse when ubiquitinated proteins are degraded.

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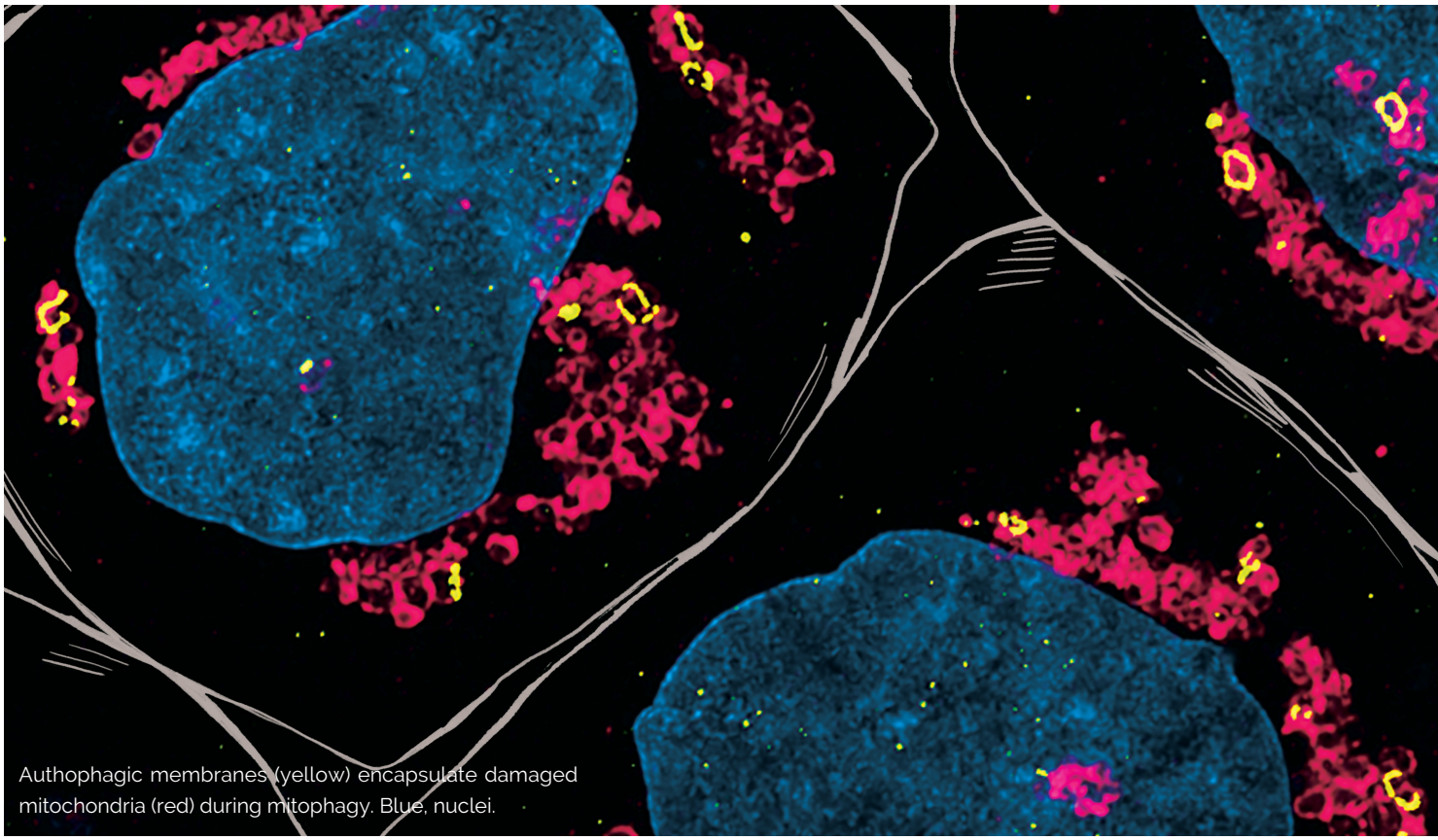


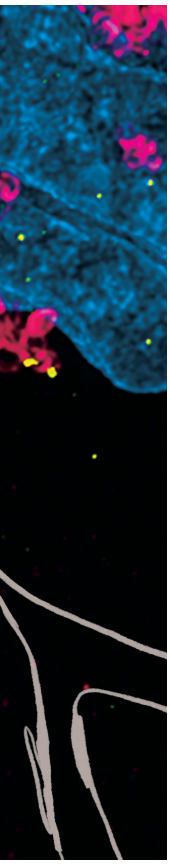
Authophagic membranes (yellow) encapsulate damaged mitochondria (red) during mitophagy. Blue, nuclei.

How are old, damaged proteins and organelles degraded in cells? Defects in degradation cause devastating diseases including Parkinson's disease in humans. Koji Yamano, a senior scientist in the ubiquitin project at TMIMS has been working to understand the mechanisms involved in degradation of defective mitochondria. We spoke to him about his latest research, "Critical role of mitochondrial ubiquitination and the OPTN-ATG9A axis in mitophagy," J Cell Biol 2020 Sep 7.219(9).

Koji YAMANO

Why did you decide to become a research biologist?
As a scientist, we can uncover novel biological mechanisms by using our hands and by using our ideas and creativity. Every day is full of scientific activities with many discussions and many experiments. These kind of things drove me to become a scientist. When I was a high school student, I was very interested in chemistry; I didn't care about biology. But during my Ph.D. studies, I realized that biology is much more important for our health. That's why I decided to become a molecular biologist.

How did you become interested in mitochondrial elimination?
Mitochondria have a membrane potential that it uses to produce ATP. This membrane potential is also important for protein import. Without a membrane potential, mitochondrial matrix proteins cannot go into the mitochondria. This is a fundamental principle of mitochondrial protein import. But in 2008, an interesting paper came out from Richard Youle's group at the NIH in the United States. They found



that a cytosolic protein called Parkin is selectively recruited to damaged mitochondria that don't have a membrane potential and triggers elimination of these damaged mitochondria by autophagy. I'm very interested in this process. How do mitochondria without a membrane potential recruit Parkin? I wanted to know the molecular mechanism of Parkin translocation so I started studying mitochondrial elimination.

What is the relationship between Parkinson's disease and mitochondrial elimination?

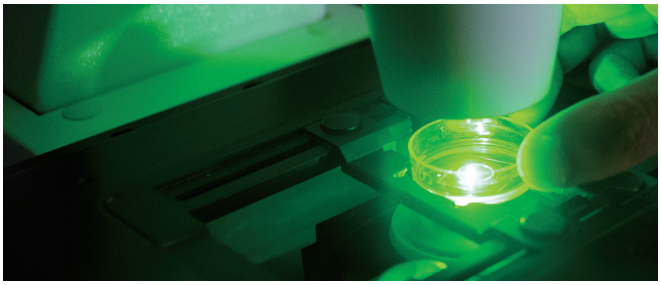
To keep cellular homeostasis, synthesis of new mitochondria is of course important, but the degradation of bad mitochondria is also important. In 2008, Richard Youle's group found that Parkin is essential for mitochondrial elimination. Two years later, in 2010, several groups including ours, independently identified that PINK1 is also essential for elimination and functions upstream of Parkin translocation. Surprisingly, Parkin and PINK1 have both been identified as products of genes mutated in Parkinson's disease. Parkinson's disease is one of the most frequent neurodegenerative diseases. Several papers suggest that accumulation of damaged mitochondria in neuronal cells causes the Parkinson's disease phenotype.

What it the relationship between ubiquitination and mitochondrial elimination?

Parkin is an E3 ubiquitin ligase, which means that Parkin is an enzyme that puts ubiquitin onto substrates. In this case it puts ubiquitin onto proteins on damaged mitochondria. Ubiquitin was primarily known to be important for degradation of individual proteins by targeting them to the proteasome, but recently, we and others found that in some cases, ubiquitination is essential for the autophagy degradation pathway. Proteasomes degrade proteins one by one, but autophagy degrades bigger targets such as protein complexes, aggregates, and even organelles. We call autophagy of mitochondria, mitophagy.

What are the new findings published in your JCB paper?

We and others so far investigated how Parkin and PINK1 work together to put ubiquitin on damaged mitochondria. But we still didn't know how ubiquitin-coated mitochondria are recognized by the autophagy machinery. Autophagy adaptors may be a key to linking ubiquitin to the autophagy machinery. Mammalian cells have five different autophagy adaptors, and all five adaptors contain ubiquitin binding domains and are recruited to the mitochondria. Furthermore all five adaptors contain ATG8 interacting motifs. ATG8 is a part of the autophagic machinery that is covalently attached to autophagic membranes. So many groups thought that autophagy adaptors could act as a bridging molecule, recruiting ATG8 and autophagic membranes to ubiquinated mitochondria. However, only two adaptors, called NDP52 and optineurin, are essential for mitochondrial elimination. We found that optineurin binds to not only ATG8, but also to another autophagy core protein, ATG9, and another group found that NDP52 binds FIP200. ATG9 and FIP200 are also essential autophagy proteins and they are important for the de novo synthesis of autophagic membranes. So now we think that optineurin and NDP52 are recruited to ubiquitinated mitochondria and begin synthesis of autophagic membranes to encapsulate the mitochondria. ATG8 is also important for encapsulation, but we found that ATG9-dependent initiation of *de novo* membrane synthesis is an important earlier step in this process.



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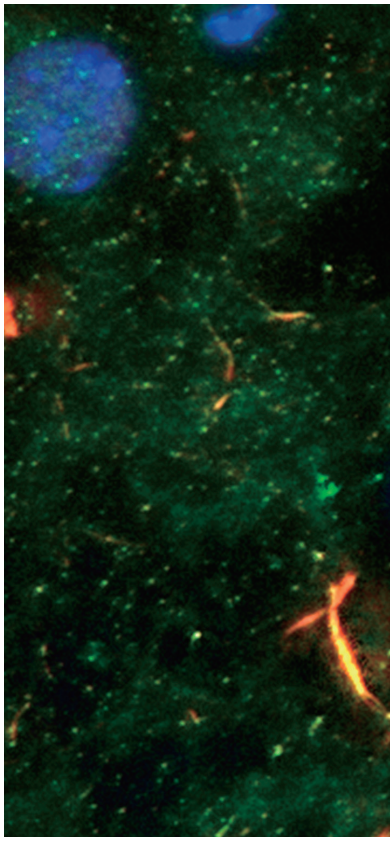


Neurodegenerative diseases are thought to be caused by the accumulation of toxic protein aggregates. For example, aggregates of a protein called α-synuclein cause diseases known as α -synucleinopathies, which include Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. But how do aggregates of one particular protein cause three different diseases with different toxicities and symptoms? Genjiro Suzuki, a senior scientist in the Dementia Research Project has been studying this problem and recently published his work in a paper, "α-synuclein strains that cause distinct pathologies differentially inhibit proteasome," eLife 2020;9:e56825. We spoke to him about his work.

Genjiro SUZUKI

How did you become interested in science?

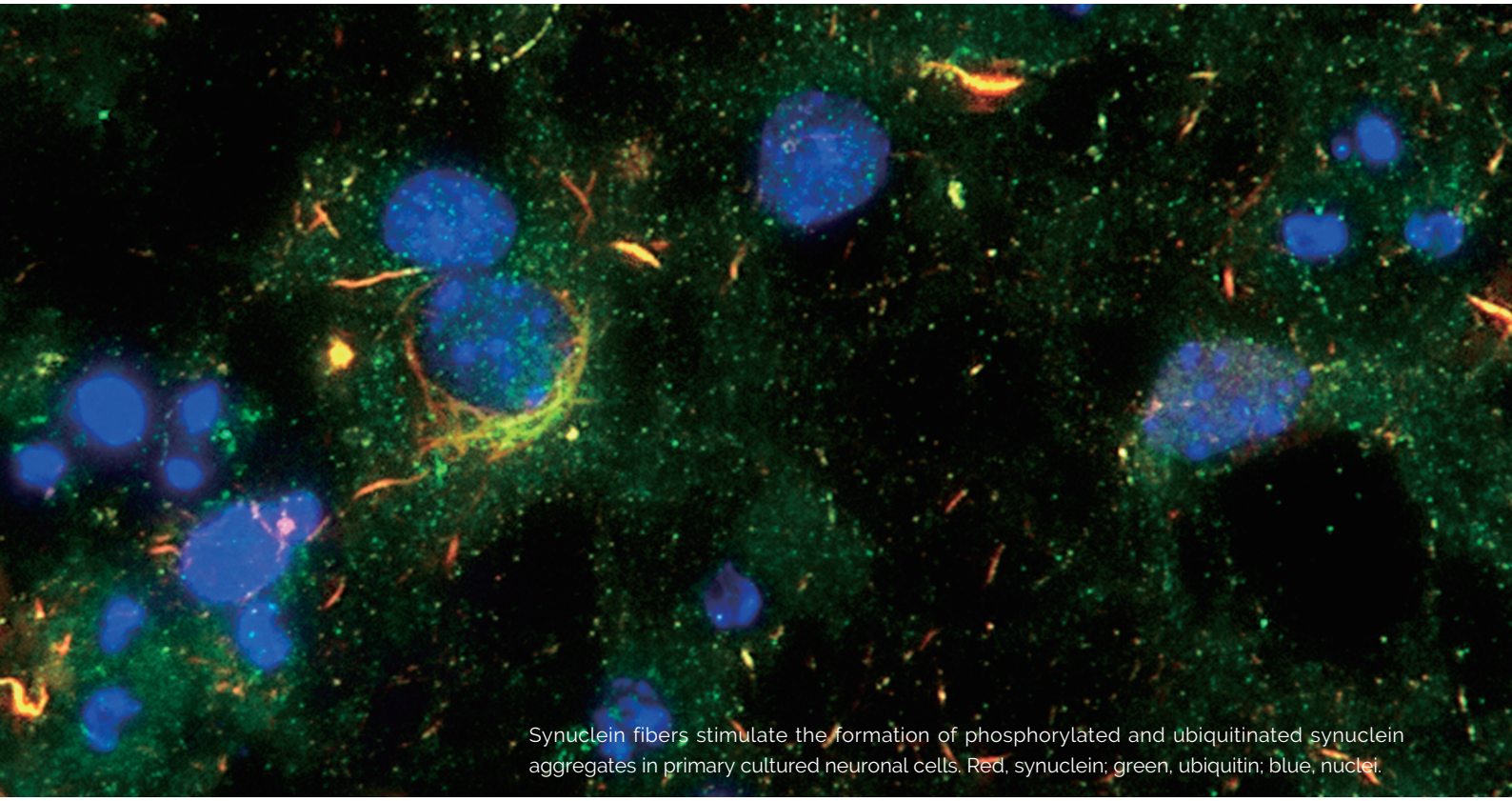
When I was a child, my father bought the science magazine, Newton, each month for me and my brother. This sparked my interest in science and led me to study Biology as an undergraduate at the University of Kyoto and as a graduate student at the University of Tokyo.

What is the relationship between prion protein propagation and neurodegenerative diseases?

Aggregates of proteins such as a-synuclein, tau, and TDP43 are almost always seen in neurodegenerative diseases, and mutations that increase the aggregation of these proteins cause familial forms of these diseases. That means that these neurodegenerative diseases are likely caused by these aggregates, similar to how prion diseases are caused by prion protein aggregates. In neurodegenerative diseases, degeneration doesn't occur immediately throughout the brain, but instead starts at a particular location and spreads in a particular manner. Again, this is similar to the spread of prion protein aggregates. That's why we believe that neurodegenerative disease spread in the brain in a manner similar to prion propagation.

What are prion proteins?

When a protein is made, it folds into a particular conformation that allows it to perform its function. However, in some cases, there are different conformations that a protein can fold into. Prion proteins normally fold in a native conformation that doesn't cause disease, but they can also fold into other harmful conformations. Prion proteins in harmful conformations can bind to other prion proteins in the native conformation and shift to the harmful conformation. This causes the spread of harmful proteins and these proteins get transferred to other cells to spread the disease to other regions of the nervous system.



Synuclein fibers stimulate the formation of phosphorylated and ubiquitinated synuclein aggregates in primary cultured neuronal cells. Red, synuclein; green, ubiquitin; blue, nuclei.

What are the new findings in your eLife paper?

There are at least three different synucleinopathies, Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. This suggests that α-synuclein can fold into at least three different conformations besides the native conformation. In order to test this idea, we made α-synuclein *in vitro* and then aggregated it under different salt conditions to show that different aggregates are formed with different characteristics. Our work shows how aggregation of one protein can cause different diseases with different symptoms and toxicities.

What is the proteasome and how is it related to toxicity of aggregated proteins?

Damaged, or deleterious proteins are tagged by ubiquitin and this ubiquitin tag directs them to the proteasome where they are degraded. However, in many neurodegenerative diseases, we see many ubiquitinated protein aggregates. We believe that cells try, but fail, to degrade these aggregates, so we decided to measure the effects of our aggregates on proteasome activity. We found that our more toxic aggregate abolished proteasome activity while our less toxic aggregate didn't. This shows that one aggregate may be more toxic than the other because of the effect it has on the proteasome and protein degradation.

How do you plan to continue this work?

The α-synuclein aggregates we made *in vitro* are structurally different from aggregates found in disease patients. One of my future plans is to make *in vitro* aggregates that are very close to those found in disease patients. This would allow us to analyze how disease aggregates inhibit proteasome activity. It would also be very useful in screening studies to develop new treatments for these diseases.

Are non-toxic aggregates found in people, and do you think they could be used to treat diseases?

I don't think α-synuclein aggregates have been found in people without neurodegeneration, but there are reports of accumulation of tau aggregates in people without neurodegeneration. So maybe Alzheimer's patients have tau aggregates that are toxic and inhibit the proteasome, while healthy older people can have tau aggregates that aren't toxic and might even function protectively. It would be fascinating if we could make non-toxic protein aggregate seeds that we could use to inhibit the formation of toxic aggregates.