Building up degradation

By Lev Osherovich, Senior Writer

A trio of papers makes a case for purging neurotoxic misfolded proteins from neurons by stimulating two protein degradation processes: autophagy and proteasome-based proteolysis.[1–3] The findings could apply to neurodegenerative disorders such as Parkinson's disease and Huntington's disease, in which accumulation of misfolded intracellular proteins is a common feature.

Autophagy involves the engulfment of cytoplasm into membrane-bound compartments called autophagosomes, which deposit their cargo into acidic, protein-dissolving lysosomes. Proteasome-based proteolysis results in selective degradation of proteins marked by the covalent addition of ubiquitin. Both processes are part of the normal cellular protein-recycling machinery.

Previous cell culture and mouse studies have suggested that defects in protein turnover exacerbate the toxic effects of misfolded proteins thought to underlie PD and HD.[4]

Although the precise cause of toxicity by misfolded proteins in neurodegenerative disease is a contentious subject, coming up with a way to get rid of those proteins could render the debate moot.

"There are a number of ways that it might be beneficial to stimulate protein clearance," said Steven Finkbeiner, senior investigator and associate director of the **Gladstone Institute of Neurological Disease** and professor of neurology and physiology at the **University of California, San Francisco**. "If you believe that protein misfolding causes disease by producing a toxic form of the protein, stimulating autophagy can clear away this aggregated protein. If you believe that misfolded proteins cause disease by dysregulating the folding of other proteins, clearing the cell of misfolded protein" could also solve that problem.

Finkbeiner led a team that reported the generation of autophagy-activating compounds in the *Proceedings of the National Academy of Sciences*.

The rationale for increasing protein turnover is "to reduce levels of disease-causing proteins," said Daniel Finley, professor of cell biology at **Harvard Medical School**. Finley, along with Randall King, associate professor of cell biology, was a lead author of a paper in *Nature* that described the generation of proteasome-activating compounds.[1]

The findings in the two papers suggest that the accumulation of misfolded proteins can interfere with proteasome and autophagosome activity and that augmenting these processes potentially can prevent neurotoxicity (*see* **Figure 1, "Boosting protein degradation to treat neurodegenerative disease"**).

Eat it

A specific case for stimulating autophagy to treat PD comes from a third team at the **University of Cambridge**. That group found that α-synuclein (SNCA), which forms intracellular aggregates in PD, interferes with the formation of autophagosomes and blocks the normal turnover of cellular proteins.

A team led by David Rubinsztein, professor of molecular neurogenetics at Cambridge, overexpressed α-synuclein in a variety of human cell lines and found that the protein prevented normal formation and activity of autophagosomes.[3]

Cells overexpressing α-synuclein had lower levels of processing and maturation of a natural autophagosome-associated marker protein than vector-treated controls, indicating that the misfolded protein clogged the autophagosome.

Microscopy showed that α-synuclein overexpression also blocked the assembly of autophagosome components.

Rubinsztein told *SciBX* that excess α-synuclein seems to specifically block a large-scale autophagic structure called the macroautophagosome, which ordinarily digests large intracellular debris.

Although previous findings by other researchers have suggested that α-synuclein could interfere with protein folding and degradation, "we showed that α-synuclein affects macroautophagy, which has a big effect on these cells," arresting the disposal of cellular proteins and even mitochondria that have been used up, said Rubinsztein.

Rubinsztein suspects his team's cell culture studies may mirror what happens in PD, which can be caused by mutations or duplications of the α-*synuclein* gene.

"We've done experiments to show that blocking protein degradation leads to degeneration of dopaminergic neurons," the kind most affected by PD, said Rubinsztein.

Rubinsztein's findings, which were reported in *The Journal of Cell Biology*, provide a mechanistic insight into a 2009 study by a team led by Eliezer Masliah, professor of neurosciences at the **University of California, San Diego**.[5] Masliah's team found that in mice with PD caused by α-synuclein overexpression, the immunomodulatory compound rapamycin stimulated autophagy and decreased PD pathology compared with no treatment.

Rapamycin, which antagonizes mammalian target of rapamycin (mTOR; FRAP; RAFT1), turns on a variety of metabolic and stress response pathways, including kinases that upregulate autophagosome activity.

Brian Spencer, project scientist at UCSD and a coauthor of Masliah's paper, said that whereas the findings in the 2009 study argue that upregulating autophagy can clear misfolded α-synuclein, Rubinsztein's studies suggest that too much α-synuclein can overwhelm the autophagy system.

"Excess α-synuclein could be raising the threshold for activation" of a normal response to cellular debris, said Spencer.

According to Spencer, raising levels of autophagy components may counteract the autophagy-inhibiting effect of excess α-synuclein. A key challenge, he said, will be to induce autophagy without perturbing other cellular systems.

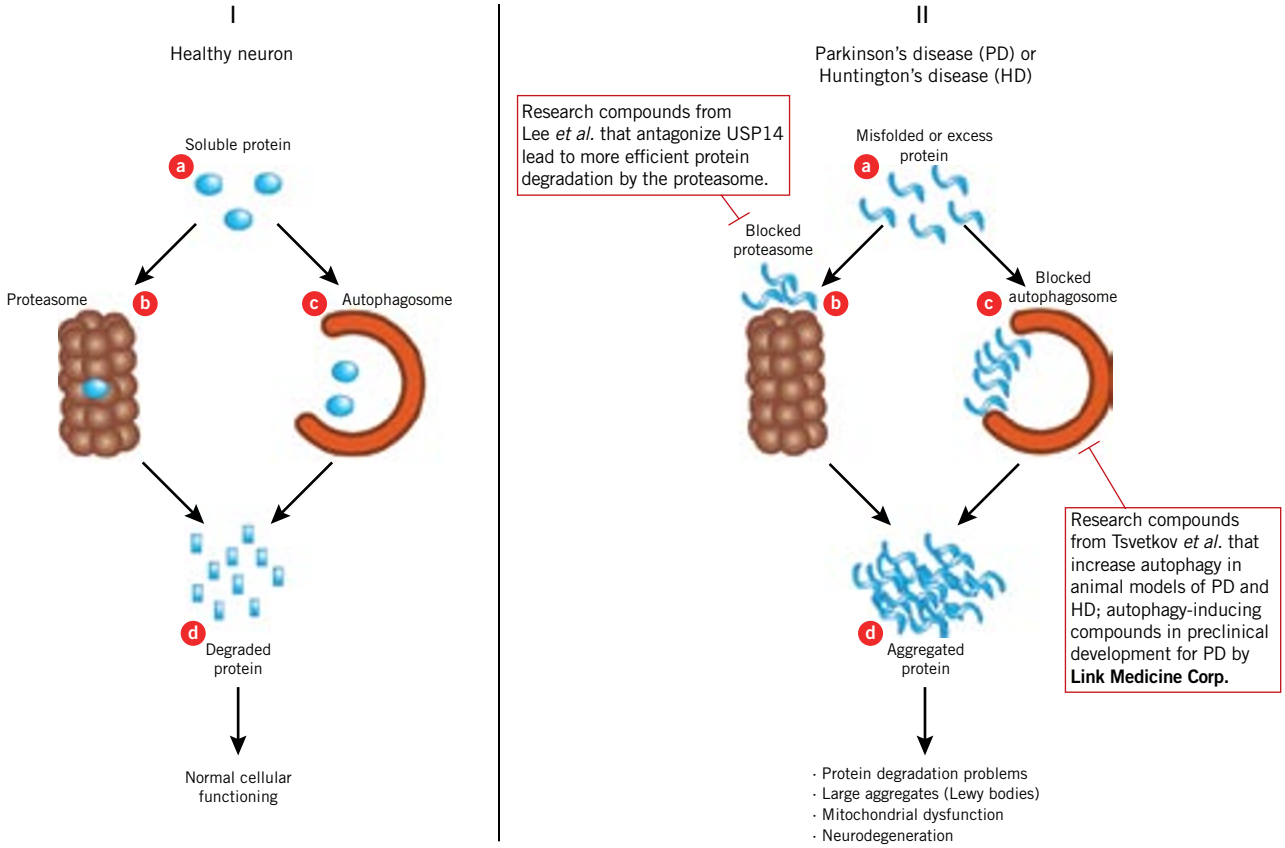

Figure 1. Boosting protein degradation to treat neurodegenerative disease. Several recent papers suggest that increasing intracellular protein degradation could be useful for preventing neurodegenerative diseases like Parkinson's disease or Huntington's disease.

In healthy neurons, soluble α-synuclein (SCNA) or huntingtin (HTT) protein (**I[a]**) undergo constant proteolytic turnover by proteasomes (**I[b]**) and autophagosomes (**I[c]**), leading to degraded protein (**I[d]**).

In PD, excess levels of wild-type α-synuclein or a disease-associated, degradation-resistant mutant form of the protein (**II[a]**) interfere with proteasome activity (**II[b]**) and autophagy (**II[c]**). As a result, α-synuclein accumulates into large intracellular aggregates called Lewy bodies (**II[d]**), normal protein degradation and mitochondrial function become compromised and neurons degenerate. In HD, aggregates of polyglutamine-expanded HTT behave similarly.

Lee *et al.* have identified a small molecule antagonist of USP14 (ubiquitin specific peptidase 14, tRNA-guanine transglycosylase; TGT), an enzyme that removes ubiquitin from proteasome-bound proteins. In cell culture, inhibiting USP14 accelerates proteasomal degradation of aggregated polyglutamine-expanded proteins related to HTT.

Tsvetkov *et al.* have found small molecules that increase autophagy and are testing these compounds in animal models of neurodegenerative disease including PD and HD. **Link Medicine Corp.** has autophagy-inducing compounds in preclinical development for PD, HD and other neurodegenerative diseases.

"Rapamycin affects the master switch for turning on autophagy, but it doesn't readily cross the blood brain barrier" and has a range of undesirable effects like immunosuppression, said Spencer. "We are trying to find a more targeted approach that doesn't induce the side effects of rapamycin."

Rubinsztein has filed for a patent on stimulating autophagy to treat PD and other neurodegenerative disorders. The patent is available for licensing through **Cambridge Enterprise Ltd.**, a subsidiary of the University of Cambridge.

Compound interest

In a hunt for potential HD therapeutics, Finkbeiner's team may have found just the kind of compound that Spencer described. The group identified a class of N10-substituted phenoxazine compounds that stimulated autophagic processing of the same marker—a fragment of microtubule-associated protein 1 light chain 3α (LC3)—used in Rubinsztein's study.

In cultured neurons, the best compound, named 10-NCP, induced formation of autophagosomes and lowered the accumulation of a transgenic form of polyglutamine-expanded huntingtin (HTT) protein compared with no treatment. Misfolding of HTT is thought to underlie HD.

Finkbeiner thinks that 10-NCP works by stimulating macroautophagy and is thus likely to be relevant to other neurodegenerative diseases.

"This study was geared toward HD, but we have seen similar effects [with the compound] in cell models of amyotrophic lateral sclerosis and PD caused by α-synuclein, so our suspicion is that this could work in a range of diseases," he said.

Although the specific target of 10-NCP is not yet known, its mechanism is likely distinct from that of rapamycin because 10-NCP induced autophagy without affecting mTOR activity.

Finkbeiner's study "is encouraging and suggests that one could stimulate autophagy in neurons without touching the mTOR pathway," said Peter Lansbury, CSO of **Link Medicine Corp.** "That's an incredibly important element because that pathway is something that you don't want to hit chronically."

"What's yet to be determined—and will require getting much more potent compounds—is figuring out how exactly the compounds work," added Lansbury.

"It would be nice to take one or two of these leads and do a detailed structure-activity relationship study. Once you have a more potent compound, you might be able to use it to fish out the target" using a reactive radiolabeled version of the molecule.

Link has a preclinical program to identify autophagy modulators to treat neurodegenerative diseases including PD, HD and Alzheimer's disease (AD). The company's lead compound is LNK-754, a farnesyl transferase inhibitor in Phase I testing for mild AD.

Finkbeiner said his team is already developing derivatives of 10-NCP for eventual clinical testing but did not disclose a time line for launching a Phase I trial.

> **"The unresolved issue is how the autophagy and proteasome pathways talk to each other."**
>
> ***—Peter Lansbury, Link Medicine Corp.***

"We now have analogs that have more potency and less toxicity" than 10-NCP, said Finkbeiner. "We also have a new high throughput assay for autophagy that we're using for lead optimization."

Finkbeiner has applied for a patent on 10-NCP derivates to treat neurodegenerative disease. The patent is available for licensing or partnering through the **Taube-Koret Center for Huntington's Disease Research**, a venture philanthropy–backed translational research institute at the Gladstone Institute that Finkbeiner directs.

Open wide

Finley and King's team at Harvard Medical School has opted for a different tactic to boost protein degradation. Instead of focusing on autophagy, the group is antagonizing a protein that acts as a brake on proteasome-mediated protein degradation.

In human cell culture, the Harvard team showed that USP14 (ubiquitin specific peptidase 14, tRNA-guanine transglycosylase; TGT) antagonizes proteasomal degradation of several proteins associated with neurodegenerative disease.[1]

USP14 ordinarily removes ubiquitin from proteins about to enter the proteasome. Previous work in yeast suggested that because ubiquitin promotes proteasomal degradation, removal of ubiquitin by USP14 rescues proteins from destruction.[6]

Thus, Finley and King reasoned that inactivating USP14 would make ubiquitinated proteins more susceptible to degradation.

Indeed, the Harvard team found that disrupting USP14 increased the degradation of misfolded proteins compared with that in wild-type controls, including degradation of a polyQ-expanded protein that causes spinocerebellar ataxia, an HD-like disorder.

The researchers identified a small molecule, dubbed IU1, that reversibly inhibited USP14 but had no effect on related deubiquitinating enzymes *in vitro*.

In a cell culture assay of proteasome activity, IU1 led to rapid degradation of neurodegenerative disease–associated proteins compared with vehicle.

Finley's approach "has the potential to be a very potent mechanism for reducing toxic protein loads," said Peter Reinhart, CEO of **Proteostasis Therapeutics Inc.** "The challenge is to demonstrate that physiologically relevant protein aggregates are actually being degraded" as a result of inhibiting USP14.

Although Finley's cell culture–based protein degradation assay "is a good start, showing that this mechanism can degrade disease-relevant proteins *in vivo* would be better," said Reinhart.

Proteostasis has preclinical programs to identify small molecule modulators of protein folding and degradation to treat a range of neurodegenerative diseases.

Finley's next step is to test IU1 in cellular and animal models of neurodegenerative disease to see whether stimulating degradation of misfolded proteins actually has a therapeutic effect.

He added that PD could be a good starting point because a relatively common form of hereditary PD is caused by a mutation in a gene encoding a ubiquitinating enzyme. That protein, parkin (PARK2), acts counter to USP14. Thus, inhibiting USP14 could counteract PARK2 mutations, Finley said.

One potential downside to targeting USP14 is that the enzyme likely affects the degradation of a broad range of proteins, not just ones associated with neurodegeneration.

Unlike other ubiquitin-specific proteases, USP14 sits directly on the proteasome and is thought to remove ubiquitin from all arriving ubiquitinated proteins, said Rémi Delansorne, CEO of oncology company **Hybrigenics S.A.**

"The fact that USP14 is associated with the proteasome may make it less selective, affecting the degradation of all proteins," said Delansorne. "Other proteins of this class have specific ligands."

Hybrigenics has a discovery-stage cancer program to inhibit human USP7 (ubiquitin specific peptidase 7, herpes virus–associated), a homolog of USP14 that influences the balance between tumor protein p53 (TP53; p53) and Mdm2 p53 binding protein homolog (MDM2; HDM2) levels.

The company's lead compound is inecalcitol, a vitamin D analog that has completed Phase IIa testing for hormone-refractory prostate cancer.

Delansorne suspects that independently of IU1's prospects in neurodegenerative disease, the compound could be useful in oncology. "I have no doubt that some tumors might be influenced by enhancing proteasome activity," he said.

Link's Lansbury said the relative importance of autophagy and proteasome-mediated degradation in cleaning up neurodegeneration-linked proteins isn't yet clear, and it's possible that the approaches taken by the three teams are complementary.

"The unresolved issue is how the autophagy and proteasome pathways talk to each other," said Lansbury.

Finley has filed for a patent on IU1, which is available for licensing. "We are not entirely lacking for suitors" seeking rights to the compound, he told *SciBX*.

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REFERENCES

1. Lee, B.-H. *et al. Nature*; published online Sept. 9, 2010; doi:10.1038/nature09299
   Contact: Daniel Finley, Harvard Medical School, Boston, Mass. e-mail: daniel_finley@hms.harvard.edu
2. Tsvetkov, A.S. *et al. Proc. Natl. Acad. Sci. USA*; published online Sept. 6, 2010; doi:10.1073/pnas.1004498107
   Contact: Steven Finkbeiner, University of California, San Francisco, Calif. e-mail: sfinkbeiner@gladstone.ucsf.edu
3. Winslow, A.R. *et al. J. Cell Biol.*; published online Sept. 20, 2010; doi:10.1083/jcb.201003122
   Contact: David Rubinsztein, University of Cambridge, Cambridge, U.K. e-mail: dcr1000@hermes.cam.ac.uk
4. Cuervo, A.M. *et al. Science* **305**, 1292–1295 (2004)
5. Spencer, B. *et al. J. Neurosci.* **29**, 13578–13588 (2009)
6. Hanna, J. *et al. Cell* **127**, 99–111 (2006)

COMPANIES AND INSTITUTIONS MENTIONED

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