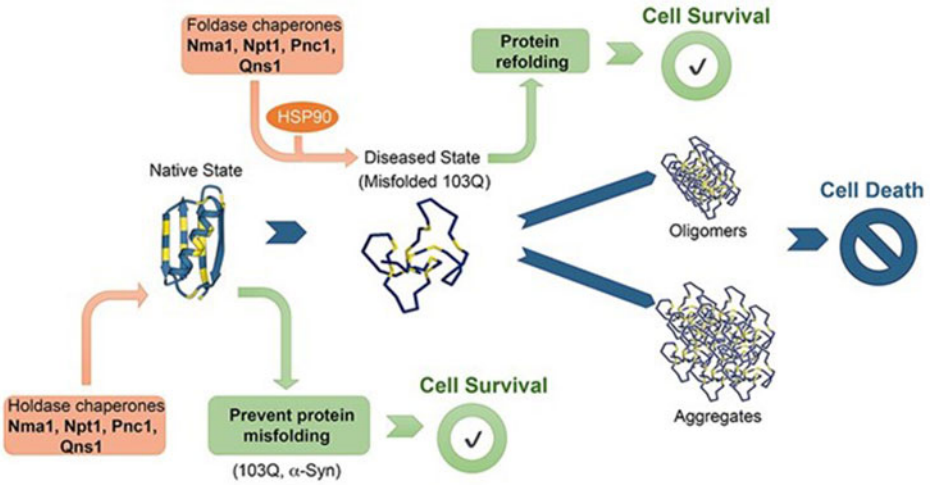
Staff Writer
July 30, 2021

Yeast Studies Help Identify Targets for Fighting Neurodegeneration

Salvage NAD⁺ biosynthetic pathway enzymes moonlight as molecular chaperones to protect against proteotoxicity and repair damage.

An overload of proteotoxins is complicit in neurodegenerative diseases and brain function decline in aging. Now, basic research in yeast screens is blazing a trail for human studies of “repair crews” that protect against these rogue proteins deposited in brain neurons.



Model of proteotoxicity suppression by salvage NAD+ biosynthetic proteins.

Antonio Barrientos, Ph.D., professor of neurology, and

biochemistry and molecular biology at the Miller School of Medicine, is principal investigator on a study published in *Human Molecular Genetics* in May, which identified three new enzymes that work to prevent the insidious protein build-up and misfolding that characterizes this degeneration. These enzymes even assist in their unfolding and proper refolding. His research is supported by grants from The Army Research Office (ARO) and a Merit Award from the Veterans Administration (VA).

These newly discovered enzymes – Npt1, Pnc1, and Qns1 are in the same nicotinamide adenine dinucleotide (NAD+) biosynthetic salvage pathway as Nma1/NMNAT (mononucleotide adenylyltransferase), which coordinates cellular energy metabolism and acts as a neuroprotector.

“These three additional proteins normally function as enzymes in the cellular synthesis of NAD+, but under proteotoxic stress, they are recruited to moonlight as protein chaperones,” Dr. Barrientos said. “In healthy young people, the protein degradation pathways and their expression are sufficient to prevent neuronal deterioration. However, as we age, or in diseases like Huntington’s disease or Parkinson’s disease, these defenses become insufficient. They can’t keep up with the rate of proteotoxin accumulation.”

Probing Neuronal Decline

Dr. Barrientos used yeast models because the mechanisms of proteotoxicity in yeast are similar to those in human neurons.

“When human proteins are expressed in yeast, the yeast cells stop dividing and eventually die, due to associated dysfunction in the mitochondria and several other cellular

compartments," he explained.

To protect against this proteotoxicity, yeast and neurons recruit several anti-stress mechanisms, including NAD+ enzymes, but there is a point at which they become overwhelmed. "This is truly the nature of neurodegenerative disorders," he says.

Discovering New Allies

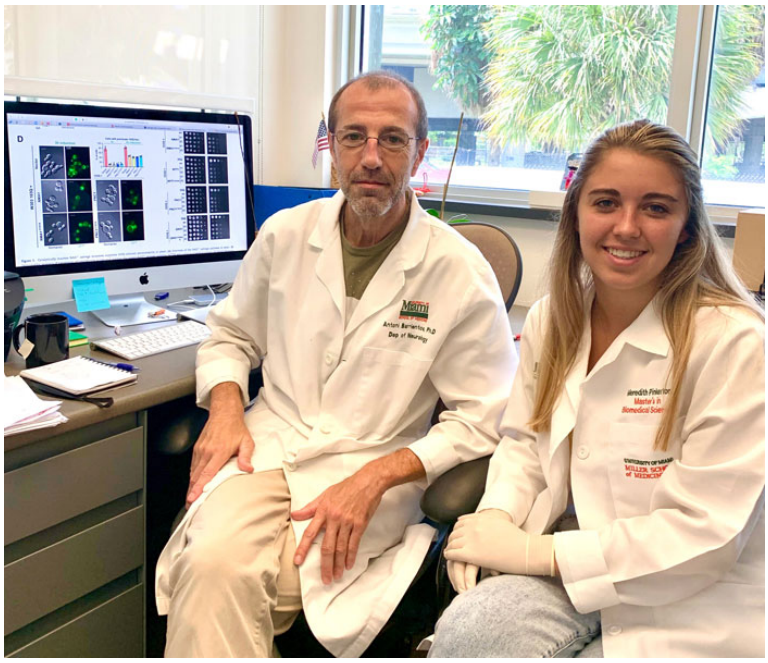
The prelude to this work began years ago, when Dr. Grace Zhai, associate professor of molecular and cellular pharmacology, demonstrated a neuroprotective role for NMNAT in flies. Given NMNAT's structural similarity with other components of the pathway, Dr. Barrientos hypothesized that additional enzymes could impact the neurodegeneration process or aid in its prevention.

Prior evidence showed that:

1. In addition to heat shock proteins, salvage NAD+ enzyme NMNAT acts as a stress-response protein, serving as a chaperone for neuronal maintenance and protection. This was shown in fly and mouse models of neurodegenerative disorders.
2. Overexpression of NMNAT/Nma1, an enzyme in the NAD+ biosynthetic salvage pathway, efficiently suppresses proteotoxicities in yeast, fly and mouse models.

For this study, Dr. Barrientos and his team, including first author, Meredith Pinkerton, a graduate student in the neuroscience program, expressed either human huntingtin or alpha-synuclein in yeast models of Huntington's disease and Parkinson's disease, respectively. "When we examined the action of the NAD+ biosynthetic proteins, we discovered these

three newfound allies in the NAD+ pathway," Dr. Barrientos explained.



Antonio Barrientos, Ph.D., with Meredith Pinkerton.

They found that these proteins were able to maintain proteostasis in the yeast models, independent of their catalytic activity. Further, they found that they do not require cellular protein quality control systems, such as autophagy or mitophagy, to help perform this function.

They hypothesize that these enzymes were acting as molecular chaperones.

"When testing the chaperone activity of these proteins, we identified holdase and foldase activities of sufficient magnitude to suggest a highly significant, if not primary, role in protecting proteins under proteotoxic stress," Pinkerton said.

New Modeling Underway

The team has now extended their modeling to human neuronal stem cells.

“We are now expressing the same protectors in human neuronal models, and finding similar results,” Dr. Barrientos said.

In collaboration with investigators at Johns Hopkins, they are pursuing this line of investigation in mice that have been modified to develop HD.

“We are devising a way to inject these proteins into the brain striatum of mice to see if protection is evident in a living model system,” he said.

Hope for a Therapy

Dr. Barrientos’ ultimate hope is to see these natural minesweepers harnessed to prevent, remove, unfold or refold proteins, maintaining or restoring brain neurons to healthy states.

“Matching our findings in the human neuron and mouse models are essential steps we need to take,” he said. “If this is reproducible in a mouse model, we may be on the path toward a drug that can be injected or taken orally to bolster the volume of these proteins or promote their recruitment to the site of damage.

“The next question is, how can we activate the expression of these chaperone proteins in a human patient pharmacologically or nutritionally? We are currently working to generate molecules that could translate into such pharmacologic therapies.”

Staff Writer
July 30, 2021

Content Type article