as environmental sensors that regulate protein phase behavior. This suggests that prion-like domains are stress-adaptive devices that facilitate the adaptation in unstable environments.

M124

Tardigrade intrinsically disordered proteins mediate desiccation tolerance.

T.C. Boothby[1], S. Piszkiewicz[1], A. Mehta[1], A.H. Brozena[2], H. Tapia[3], D.E. Koshland[3], A.S. Holehouse[4], R.V. Pappu[4], B. Goldstein[5], G.J. Pielak[1];
[1]Chemistry, UNC, Chapel Hill, NC, [2]Chemical Engineering, NC State, Raleigh, NC, [3]Molecular and Cell Biology, UC Berkeley, Berkeley, CA, [4]Biomedical Engineering, Washington University in St. Louis, St. Louis, MO, [5]Biology, UNC, Chapel Hill, NC

Tardigrades (water bears) are a phylum of tiny, extremotolerant animals renowned for their ability to survive desiccation, freezing, boiling temperatures, intense irradiation, and even prolonged exposure to the vacuum of outer space. The functional mediators, and their mechanisms of protection, used by tardigrades to survive these extremes have not been fully elucidated. Tardigrades possess at least three families of novel intrinsically disordered proteins (IDPs). We found that members of one of these IDP gene families are upregulated during, and are required for tardigrades to survive, desiccation. Additionally, these proteins increase the desiccation tolerance of heterologous systems (yeast and bacteria), stabilize protein structure, preserve enzyme function during desiccation and form gels. Gelation increases their beta-sheet content. Structural simulations, bioinformatics analyses, and empirical biochemical experiments indicate that these proteins, while disordered, are comprised of three discrete regions, two terminal regions bridged by an extended linker that together resemble a dumbbell. The terminal regions are ‘sticky’ and display beta-sheet propensity. Based on experimental evidence and simulations, we propose that this dumbbell-like confirmation inhibits intraprotein interactions, but facilitates gelation via interprotein interactions. Upon desiccation, these gels vitrify forming glass-like solids, and the vitrification of these proteins appears mechanistically essential as disruption of this vitreous state correlates with a loss of their protective capabilities. Our results identify the first functional mediators of tardigrade desiccation tolerance, and reveal a potential mechanism of action. More broadly, our findings provide insight into how changes in the conformation and function of disordered proteins can be influenced by environmental factors. These studies provide a platform for pursuing novel methods for stabilizing sensitive biomedical material and engineering stress tolerant crops.

M125

Characterization of p97 mutations causing multisystem proteinopathy support a gain-of-function model for pathology.

E.E. Blythe[1], A. Martin[2], R.J. Deshaies[1];
[1]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, [2]Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA

Valosin-containing protein (VCP, or p97) is an ATPase essential in numerous protein quality control (PQC) pathways, such as ER-associated degradation. p97 functions as a segregase, extracting ubiquitylated proteins from membranes or complexes so they can be degraded by the proteasome. However, the complexity of native p97 PQC substrates has stymied the detailed biochemical study of this function. Previously, to address this problem, we developed an in vitro p97 substrate based on an ubiquitin fusion degradation (UFD) pathway substrate, Ub-GFP, and showed that the unfolding of this substrate by p97 is dependent upon extensive substrate ubiquitylation, the p97 adaptors NPLOC4-

UFD1L, and ATP hydrolysis. Here, we make use of this system, employing an updated version of this substrate, to explore how mutations in p97 that cause multisystem proteinopathy (MSP) affect substrate processing. Previous studies have shown that MSP mutants have higher basal ATP rates than wild type yet cause deficiencies in many p97-dependent pathways, creating controversy as to whether these dominantly inherited mutations cause disease through a gain-of-function or a loss-of-function. We have now analyzed seven distinct MSP mutants, all of which showed modestly improved unfolding of our model substrate over wild type p97, providing evidence that the increased ATPase activity leads to a gain-of-function. Furthermore, we showed evidence that p97 inhibitors may restore proper p97 function to MSP mutants, suggesting a potential treatment strategy for p97 diseases.

M126

Protein arginylation targets alpha synuclein, facilitates normal brain health, and prevents neurodegeneration.

J. Wang[1], X. Han[2], N.A. Leu[1], S. Sterling[1], S. Kurosaka[1], M.E. Fina[1], V.M. Lee[3], D.W. Dong[1,4], J. Yates iii[2], A.S. Kashina[1];
[1]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, [2]The Scripps Research Institute, La Jolla, CA, [3]Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, [4]Institute for Biomedical Informatics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

Alpha synuclein (α-syn) is a central player in neurodegeneration, but the mechanisms triggering its pathology are not fully understood. Here we found that α-syn is a highly efficient substrate for arginyltransferase ATE1 and is arginylated in vivo by a novel mid-chain mechanism that targets the acidic side chains of E46 and E83. Lack of arginylation leads to increased α-syn aggregation and causes the formation of larger pathological aggregates in neurons, accompanied by impairments in its ability to be cleared via normal degradation pathways. In the mouse brain, lack of arginylation leads to an increase in α-syn's insoluble fraction, accompanied by behavioral changes characteristic for neurodegenerative pathology. Our data show that lack of arginylation in the brain leads to neurodegeneration, and suggests that α-syn arginylation can be a previously unknown factor that facilitates normal α-syn folding and function in vivo.

M127

Poly (ADP-ribose) modulates phase separation of the ALS-associated protein TDP-43.

L. McGurk[1], E. Gomes[2], L. Guo[2], J. Mojsilovic-Petrovic[3], V. Tran[1], R.G. Kalb[3], J. Shorter[2], N.M. Bonini[1];
[1]Department of Biology, University of Pennsylvania, Philadelphia, PA, [2]Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA, [3]Department of Neurology, Children's Hospital of Philadelphia, Philadelphia, PA

Stress granules are membraneless organelles that form by liquid to liquid phase separation of RNA-protein complexes. In amyotrophic lateral sclerosis and frontal temporal dementia, pathological protein aggregates of phosphorylated TDP-43 are thought to arise from persistent stress granules, however, the nucleation of this process is unknown. In *Drosophila*, we identified poly (ADP-ribosylation) as a potent genetic modifier of TDP-43-associated toxicity. We establish that polymers of poly (ADP-ribose), PAR, nucleates liquid-liquid phase separation of TDP-43 *in vitro*. We show that disease-associated protein fragments of TDP-43, which lack the PAR-binding region, have impaired phase-separation dynamics and fail to respond to PAR *in vitro*. In mammalian cells, TDP-43 undergoes stress-induced phase separation and we uncover that localization to stress granules is dependent upon PAR binding. The disease-