Tau-er of Power

By Kenneth S. Kosik, M.D.

Editor's Note: Tau protein helps nerve cells in the brain maintain their function and structure. When tau turns toxic, replicates, and spreads, neurons misfire and die. If neuroscientists can pinpoint the reasons for toxicity, identify what our author calls "a staggering number of possible modified tau states," and find a way to block tau's movement from cell to cell, then progress can be made in fighting any number of neurological disorders linked to this protein, including frontotemporal dementia, chronic traumatic encephalopathy (CTE), and Alzheimer's disease.

Mention any disease and a few questions immediately come to mind. Chief among them: Who is vulnerable and how does it occur? If it's an infectious disease, it may spread through the air or by touch. But the presiding dogma for most of modern biomedical history tells us that the transmissible agents contain nucleic acid and that replication is inextricably bound to DNA or RNA. As information vehicles, these molecules power the dual aspects of evolution: constancy and change. The constancy of faithful replication allows the inheritance of traits that allow life forms (including classical disease culprits) to survive. Through change, genes assure adaptability to a complex environment. These properties confer pathogenicity by enabling them to prey upon infectious agents and adapt to their hosts.

But a parallel universe of disease transmission ignores these rules. This is the world of prions. In this world, the agent of transmission is a protein and the information lies in the vast shape of the space within which proteins fold. How big is that space? To get a handle on the size of chemical space, the chemistry blogger Derek Lowe quoted Douglas Adams in *The Hitchhiker's Guide to the Galaxy*: "Space is big. Really big. You just won't believe how vastly, hugely, mind-boggling big it is." The immense variety within stretches of ATGCs (the chemicals adenine, cytosine, guanine, and thymine) that build the language of the genetic code pales beside the alphabet of the periodic table with its spelling and grammatical rules of chemical bond formation and compound stability.

Tau, a Protean Protein

Tau is a normal protein used in neurons to shape a dynamic system of tracks that traffic goods to various destinations inside the cell. Tau is one small fraction of the bewilderingly large space within which all proteins fold. But like a fractal image, zooming in on tau opens up a chemical space full of molecular crevices and passageways that mirror the far greater universe from which tau is but one tiny part. Like the early sea god, Proteus, tau can take many forms. It was said that Proteus had the ability to foretell the future, but would change his shape to avoid doing so. Indeed, some forms of tau are harbingers of a future with neurodegeneration (more on this point later). The chemical space that tau occupies in the nervous system must first be catalogued according to the six different molecular isoforms of the tau protein, which many different chemical processes can modify. These six isoforms each have slightly different sequences with small stretches of amino acids either left in or out.

Even a cursory quantitative knowledge of the staggering number of possible modified tau states does not exist. Beyond these molecular states lies a far more extensive terrain of folding patterns, termed conformations (think of multiple ways to a crumple a piece of paper). And the only constraint on these conformations is the time they dwell in any one of them. Some shapes are stable only for a few milliseconds, and how readily the protein can assume a particular shape among all possible shapes is called the kinetic landscape.

Within the enormous realm of protein shapes, those that pertain to tau are little studied and poorly understood. Tau is in a unique class of proteins called intrinsically disordered proteins (IDPs). In contrast to enzymes, for example, which adopt a precise three-dimensional structure to facilitate catalysis, IDPs lack a unique three-dimensional structure and do not exhibit any stable secondary structure in the free form. They can adopt a wide variety of extended and compact conformations that facilitate many vital physiological functions by folding after they bind to targets. As with other IDPs, enzymes act on tau proteins twice as often as on other proteins and can alter their binding properties. Among these enzymes are a category called kinases that add a phosphate to a protein. IDPs are, on average, substrates of twice as many kinases as structured proteins.[1] Tau is particularly singled out as a substrate of multiple kinases, and some investigators believe that the multiple phosphates which decorate tau contribute to its tendency toward misfolding.

IDPs use their lack of structure to their advantage. The protean shapes that these proteins can assume provide a larger interaction surface area than globular proteins of a similar length. The variety of shapes exposes short linear peptide motifs that serve as molecular recognition features, and thereby allow IDPs to scaffold and interact with numerous other proteins. It enables diverse post-translational modifications that facilitate regulation of their function and stability in a cell; and by folding upon binding, IDPs can interact with their targets with relatively high specificity and low affinity. These features are ideal for recognizing partners to interact with and for coordinating regulatory events in space and time.[2] However, these properties require that cells assiduously monitor these proteins because they are potentially dangerous and capable of inflicting damage to cells by binding to each other.

Indeed, cells tightly regulate IDPs throughout, from transcript synthesis to protein degradation. Among the means that cells can use to adjust the levels of proteins is by the process of post-

transcriptional regulation through microRNAs, which have been credited with helping to maintain tau homeostasis.[3] MicroRNAs target specific messenger RNAs, which encode proteins and fine-tune the amount of protein that gets translated from the messenger RNA.

Tau Aggregation

Paradoxically, simple over-expression of tau in a variety of cell types, including neurons in laboratory tissue culture, does not result in the replication and spread of tau (aggregation), even with high expression levels. More often tau overexpression induces the rampant assembly of a structure called microtubules, which are the cell's railroad tracks that ship cargo to different locations in the cell. Normally, tau promotes the assembly of microtubules with the goal of building a protrusion from the cell that will become an axon. These long cylindrical structures use their microtubule railroads to carry cargo over the vast distances that axons traverse, such as the axons that travel from the lower spinal cord all the way to the muscles of the big toe. In the brain, axons connect the two hemispheres and are key elements of brain circuitry. When tau is overexpressed in nonneural cells, the out-of-control microtubule assembly results in numerous microtubule bundles spiraling around the perimeter of the cell, which is unable to form a protrusion in this foreign environment. Curiously, when tau is expressed in a type of cell called Sf9, taken from the ovary of an insect, it acquires many of the modifications seen in neurofibrillary tangles. With tau, these cells extrude a single, very long process that resembles an axon in its shape, but has none of the electrical conduction properties of an axon.[4] Thus, tau makes an axon ghost in these cells. One conclusion from these studies is that simply lowering tau levels across the board is not the most strategic way to approach therapeutics for diseases termed tauopathies. Nor is simply overexpressing tau the way to make aggregates.

Some proteins can misfold into shapes called prions (as noted above) that have the unusual property of inducing other copies of the same protein to misfold similarly. The prion guides similar conformations in additional copies of the same protein. The prototypical prion, known as PrP, is responsible for several human diseases: Creutzfeldt-Jakob disease, Gerstmann–Sträussler–Scheinker syndrome, fatal familial insomnia, kuru, and bovine spongiform encephalopathy (also known as mad cow disease). Each of these diseases has very different clinical presentations, and they are distinguished from other infectious diseases mainly by their

mode of transmission. Given that PrP causes all of these conditions and others found in nonhuman species, the idea that prion strains with distinct phenotypes exist has gained traction and experimental validation. This view suggests that a subset of PrP shapes is transmissible. Depending on the particular folding of a strain, a specific phenotype or species predisposition arises. Faithfully propagating strains, therefore, is a prerequisite for clinically defined presentations, and spread through the brain via specific anatomical routes.

What has been peculiar since the discovery of PrP is that only one human prion is known. Certainly other proteins must have kinetic troughs into which a protein can fall, get stuck, and spread their conformation to others. But only in yeast was a similar phenomenon clearly observed, and remarkably the prion state of the yeast protein confers a selective advantage.[5] For many years, investigators nibbled at the concept of prions to explain numerous neurodegenerative diseases in which a misfolded protein aggregates and remains trapped inside the cell. Among these conditions are the tau aggregates in the tauopathies, synuclein aggregates in Parkinson's disease, huntingtin aggregates in Huntington's disease, TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis, and transthyretin in familial amyloidotic neuropathy. Prions conceptually unify the neurodegenerative diseases, which otherwise lack a fundamental disease mechanism akin to a virus or a malignant cell or an autoimmune process in other disease categories. At the core of neurodegeneration lies an unknown process as mysterious as aging.

Protein aggregates are puzzling entities that lie on the wrong side of what in other contexts we call protein-protein interactions. Normal cellular function requires that proteins bind to each other as pairs or somewhat larger complexes. On the other hand, when tau aggregates, it grows massively, with molecular weights in the millions, and may capture other proteins within the aggregate.[6] In many cases, including tau inclusions, some feature of tau that is predisposed to self-assemble triggers recognition by a class of enzymes called ubiquitin ligases, which mark the protein for degradation. These enzymes enable a set of reactions that attach a chain of ubiquitin peptides to a protein. The ubiquitins tell the cell that the protein to which they are attached is trash and should be thrown away in the cell's trash can (called the proteasome). However, for unknown reasons, when ubiquitins attach to tau, it does not get degraded in the proteasome. Instead, it gets stuck in the cell as an aggregate. Maybe the size of the aggregate does not fit into the opening of proteasome, which is shaped like a barrel into which proteins

enter for destruction inside.

The Spreading of Tau

In addition to the ability to evade the cellular surveillance systems that rid the cell of damaged proteins, prions have the even more insidious property of spreading to contiguous cells. Postmortem brain extracts from humans who had died with various tauopathies were injected into the hippocampus and cerebral cortex of mice and could be propagated between mouse brains.[7] The passage of a particular tau conformer appeared to faithfully replicate the pathology specific to each one of three clinically distinct types of tauopathies.

Faithful replication of tau aggregates was also demonstrated at the cellular level. Strains differed with respect to the morphology, size, and subcellular localization of the aggregates as well as their sedimentation profile, seeding capacity, protease digestion patterns, and toxicity.[8] Whether these features provide reliable "bar codes" for diagnosis will be known in the next few years.

Mice engineered to express a pathogenic human tau transgene in the entorhinal cortex, a highly vulnerable region involved in the sense of smell where tau pathology often begins, can spread to neuroanatomically connected regions.[9,10] Furthermore, mouse tau was bound up in the aggregates, suggesting that the pathological human tau induced normal mouse tau to misfold. Among the cells into which tau spread were dentate granule cells that are separated from the entorhinal cortical cells by a synapse. Whether spread occurs transsynaptically remains a fascinating, open question.

These findings and related tissue culture system studies point to four areas for deeper investigation: (a) how tau exits through the cell's membrane; (b) whether the existence of tau in an extracellular compartment offers a rationale for removing transmissible tau with an antibody; (c) how tau passes into the membrane of into a neighboring cell; and (d) what potential way stations—such as microglia—could interface with tau during its spread.[11]

Tau Seeding

So let's return to the myriad shapes tau can assume. Among these shapes are a few that expose some sticky surfaces normally kept folded and concealed within the protein. When this happens, tau can bind to another tau protein in a process called "seeding." As more and more tau proteins join the pack, eventually the aggregate becomes quite large and often appears like a fibril. Proteins, such as tau, that can wiggle and squirm in numerous ways are intrinsically disordered proteins (IDPs), and they have generated great interest in the scientific community. By changing their shape rapidly, they present different surfaces to other proteins and engage in a variety of binding interactions for their normal function.

Normally tau transitions on and off microtubules by folding in different ways and, in so doing, can stabilize and elongate the microtubule. However, during these on-off transitions, there are vulnerable moments with the potential for misfolding. Or, while tau is being synthesized from its mRNA, inopportune moments might allow tau to fold in a way that permits binding to another tau and seed the growth of a tau aggregate.

An aggregation- prone motif observed in tau is called a steric zipper, in which a pair of sheetlike sequences is held together by the interlinking of small projections.[12] Researchers have designed inhibitors that slow tau fibrillation by targeting a set of six amino acids in tau involved in this interaction.[13] Many unlikely events have to occur together for tau to form an aggregate: It must drop into a rare conformation or shape, it must remain in that shape for sufficient time to seed assembly of other tau proteins, and other tau proteins must be sufficiently close to serve as substrates upon which misfolded tau can template its nefarious shape.

Any mistakes in folding are monitored by classes of proteins called chaperones and co-chaperones. These proteins can refold a protein correctly or, if irreversibly damaged, direct the protein to the proteasome for complete degradation. The tethering of one such complex called BAG2/Hsp70 to the microtubule may provide a protective capture function for misfolded tau.[14]

Misfolded conformations may occur rarely, but in the presence of a tau mutation or traumatic brain injury or beta-amyloid deposition among other precipitants, tau is more likely to assume an aggregation-prone conformation. *In vitro,* polyanions (molecules or chemicals with negative

charges) are capable of inducing tau self-assembly. Similarly, in living cells, contact with charged membrane phosphates or RNA may predispose tau toward an aggregation-prone conformation.[15] A compelling biophysical mechanism that may initiate the misfolding is the elimination of water that normally surrounds each tau molecule, and thereby makes tau sticky and prone to the aggregation into threads.[16] Once an oligomer or fibril is formed, it can seed subsequent reactions within the cell and enter neighboring cells through massive vesicles, termed macropinosomes.[17] Thus, when tau forms an aggregate, it also appears capable of transmissibility from cell to cell.

Patterns of tau spread constitute a neuroanatomical network, and these networks are associated with clinical features.[18] Given the described thinking concerning tau strains, the patterns of spread may arise from the specificity of a particular tau strain for a specific network. These selectively vulnerable patterns can be predicted by a diffusion mechanism modeled by a graph theoretic analysis using tractography data.[19] Neural network compromise may begin long before there is neuropathological evidence of disease in the form of misfolded tau aggregates. A study that recorded from neocortical pyramidal cells in a mouse model of tauopathy found numerous significant physiologic alterations when only a fraction of the neurons showed pathological tau.[20] Membrane potential oscillations were slower during slow-wave sleep and under anesthesia. Firing rates were reduced with longer latencies and interspike intervals. These changes reduce the activity of the neocortical network and suggest that conduction and synaptic transmission deficits may be among the earliest changes induced by tau spread at a resolution below light microscopy.

The interest level in tau among scientists has had the kinds of peaks and valleys that one might compare to the stock market. And like the last few years of the stock market, investment and the trajectory of growth have risen considerably. As we gain a deeper understanding of the molecule, as well as the ability to image tau pathology in the living human brain, we stand on the threshold of treatments.

Bio

Kenneth S. Kosik, M.D., is the Harriman Professor of Neuroscience Research and co-director of the Neuroscience Research Institute at the University of California, Santa Barbara. Kosik's work with early-onset familial Alzheimer's disease at Columbia University was the basis for a novel

prevention trial to treat Alzheimer's disease. His was one of several groups that discovered tau protein in the Alzheimer's neurofibrillary tangle and followed up with many studies on the biology and pathobiology of tau. Kosik received a B.A. and M.A. in English literature from Case Western Reserve University in 1972 and an M.D. from the Medical College of Pennsylvania in 1976. He served as a resident in neurology at Tufts-New England Medical Center and was chief resident in 1980. Since 1980, he has held a series of academic appointments at the Harvard Medical School and achieved the rank of full professor there in 1996. He also held appointments at McLean Hospital, Brigham and Women's Hospital, Massachusetts General Hospital, and the Dana-Farber Cancer Institute. In 2004, he accepted the appointment at UC-Santa Barbara.

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