PROTEINS AS INFECTIOUS AGENTS AND IDENTIFICATION OF PRION-LIKE SELF-PROPAGATING PROTEINS IN EXTRATERRESTRIAL SAMPLES. C. Soto[1] and R. Diaz-Espinoza[1], [1]Department of Neurology, University of Texas Medical School at Houston. 6431 Fannin Street, Houston, TX. E-mail, Claudio.Soto@uth.tmc.edu.

Introduction: The discovery that proteins can behave like micro-organisms to transmit disease is a significant milestone in biology. The unorthodox prion hypothesis was proposed decades ago to explain the surprising transmission mechanisms of a group of rare diseases known as transmissible spongiform encephalopathies, or prion diseases (1;2). The prion hypothesis states that the infectious agent in prion diseases is composed exclusively of a misfolded form of the prion protein, which replicates in infected individuals by transforming the normal version of the prion protein into more of the misfolded isoform. This hypothesis remained controversial for decades (3), but recent studies have settled all doubts by demonstrating that infectious material can be generated *in vitro,* in the absence of genetic material, by replication of the protein misfolding process (4;5). The infectious protein (called prion) exhibits the typical characteristics of bona-fide infectious agents (3), namely: exponential multiplication in an appropriate host, transmission between individuals by various routes including food-borne and blood-borne, titration by infectivity bioassays, resistance to biological clearance mechanisms, penetration of biological membrane barriers, "mutation" by structural changes forming diverse strains, and transmission controlled by species barriers. Despite that prions fulfill the Koch's postulates for infectious agents, it remains surprising that a single protein possesses the complexity and flexibility required to act like living micro-organisms that transmit disease.

Recent exciting research has led not only to the end of the skepticism that proteins can transmit disease but also to expanding the concept that infectious proteins might be at the root of some of the most prevalent human diseases. The transformation of a natively folded protein into a misfolded, toxic form that causes tissue damage and disease is not a mechanism exclusive to prion diseases. Misfolded protein aggregates are implicated in more than 20 human diseases, collectively called protein misfolding disorders (PMDs), including highly prevalent and insidious illnesses such as Alzheimer's disease, Parkinson's disease, and type 2 diabetes (6;7). Although the proteins implicated in each of these pathologies and the clinical manifestations of the diseases differ, the molecular mechanism of protein misfolding is strikingly similar.

The notion that protein misfolding and aggregation is associated with a small family of proteins has also dramatically changed in recent years. The pioneering work of Dobson and colleagues has demonstrated that many (if not all) proteins can form β-sheet intermolecular interactions to adopt an amyloid-like conformation under appropriate conditions (7). Strikingly, the structures formed and the underlying mechanism of misfolding and aggregation are similar to those for PMDs. These findings suggest that any protein has the potential to form misfolded aggregates that might replicate by the prion principle. Taken together, these results form the basis of a general biological principle whereby protein function and activity might be regulated by folding changes that can be rapidly propagated from protein to protein.

Few years ago we developed a technology termed Protein Misfolding Cyclic Amplification (PMCA) to efficiently reproduce prion replication in the test tube. The PMCA technology has been applied to convert large amounts of the normal prion protein into the abnormal form by incubating it with minute amounts of misfolded infectious prion protein. The system consists on cycles of accelerated prion replication to reach an exponential increase in the conversion. These findings mark the first time in which the folding and biochemical properties of a protein have been cyclically amplified in a manner conceptually analogous to the amplification of DNA by PCR. PMCA has contributed enormously to understand the underlying biology of prions, to identify other factors that may be implicated in prion protein conversion, and to discover novel drugs for prion diseases (8). In addition, PMCA has enormous potential in allowing current diagnostic tools to detect prion disease during the pre-symptomatic period and perhaps in living individuals, because it can multiply the number of prions facilitating their detection. Indeed, PMCA enables more than 3 billion fold increase on sensitivity for PrP detection and the possibility to detect as little as a single molecule of the misfolded protein (9). This level of sensitivity allowed us to detect for the first time prions in the blood and urine of sick as well as pre-symptomatic animals (10-12). These findings have had a major impact in various fields including the diagnosis of prion disease, blood banks safety, meat industry, environmental detection of prions, etc (8). More recently we have adapted the PMCA technology to amplify the processes of misfolding and aggregation of other proteins implicated in PMDs as

well as to identify new proteins with the ability to self-propagate their conformations.

Taken into account the facts that proteins can behave like infectious agents to transmit disease, that many disease-associated and non-disease associated proteins can self-propagate using the prion principle and that many (if not all) proteins can adopt a misfolded aggregated form it becomes highly relevant to search for new forms of self-propagating proteins. We believe that the PMCA technology provides a promising tool to identify novel infectious proteins in many samples, including extra-terrestrial materials.

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