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In this issue of Molecular Cell, Foit et al. (2009) probe cellular protein folding using a split β-lactamase approach for evolving protein stability in the absence of any requirement for function.

Since the discovery that protein folding in vitro is primarily determined by the chemical information within the polypeptide sequence, much has been learned about the details of the physical chemical forces that mediate protein folding in dilute solutions at moderate temperatures. Unfortunately, we know much less about how the kinetic competition between folding, misfolding, and aggregation is managed in the crowded environment of the cell, where the protein concentration is very high, facilitating aggregation.

It is now becoming apparent that protein folding in vivo is mediated by interactions between the folding polypeptide chain and the components of the cellular proteostasis network (Balch et al., 2008)—including chaperones, folding enzymes, trafficking factors, and degradation machinery—that together influence the stability, concentration, and localization of proteins in the cell. Due to these dynamic interactions, successful protein folding in a cell is dependent on both the kinetic and thermodynamic stability of the protein of interest and the relative capacity of the proteostasis network under a given set of cellular conditions (Wiseman et al., 2007).

Very little is known about why evolution has selected the protein sequences it has from a vast number of possibilities. The interactions between folding polypeptide chains and the proteostasis network (dependent on these sequences) have complicated our understanding of how protein sequences have evolved to optimize their function for cell survival. Protein function in vivo is dictated both by the inherent activity of the folded protein and its intracellular concentration (influenced by folding energetics). The involvement of the proteostasis network (Balch et al., 2008) in defining the population of folded proteins in vivo has required evolution to efficiently optimize the amino acid sequences to take advantage of the proteostasis network, while maintaining sufficient protein activity for function in vivo.

In this issue of Molecular Cell, Foit et al. (2009) have created a very interesting genetic selection-based approach to understand protein stability in the cell. This approach is based on the observation that cleavage of the TEM-1 β-lactamase protein between residues 196 and 197 affords two fragments that are catalytically inactive, unless fused to proteins that interact and thus bring the fragments in proximity, allowing an active β-lactamase enzyme to form. By incorporating a “guest” protein between residues 196 and 197 of β-lactamase (Figure 1A), the extent of cellular guest folding can be quantified by β-lactamase activity, easily discerned by measuring the concentration of a cytotoxic β-lactam substrate that permits growth. They explored the hypothesis that guest proteins exhibiting stable structures in the periplasmic space of Escherichia coli will exhibit maximal β-lactam resistance, while largely unstable proteins would display reduced antibiotic resistance due to degradation of the guest protein by the cellular quality control machinery (Figure 1B). Using over 60 mutants of four structurally distinct guest proteins, they demonstrated a striking correlation between antibiotic resistance and the kinetic and thermodynamic stability of the guest proteins, establishing a robust, generic and quantitative readout of protein stability in vivo. This system complements existing technology to monitor protein folding and stability in vivo, including protein-GFP folding reporters developed by Waldo (Waldo, 2003) and the PROSIDE method developed by Schmid (Sieber et al., 1998), by establishing a quantitative, selectable output to evolve proteins based on their stability in the absence of any requirement for function.

Applying their split β-lactamase approach to explore the potential for evolving protein stability in vivo, Foit et al. (2009) performed error-prone PCR on the gene encoding immunity protein 7 (Im7) and incorporated these constructs into the β-lactamase reporter as the guest protein. Using the resistance to an increasing concentration of the β-lactam penicillin V for selection, they demonstrated the ability to stabilize Im7 through evolving specific mutations in the gene product. The quantitative output (antibiotic resistance) varied over a 7-fold range for a 20 kJ/mol change in stability. Interestingly, the vast majority of mutants that stabilize the protein map to the functional surface of Im7 required for binding its cognate toxin colicin, suggesting that protein evolution has maximized protein stability within the constraint of not disrupting activity. Despite mutations being primarily targeted to the functional face of Im7, a subset of the mutations selected for maintained the function of the protein (colicin inhibition) and demonstrated a significantly higher thermodynamic and kinetic stability, confirming the hypothesis that protein stability can be evolved...
independent of activity. A variety of control experiments revealed that selection for antibiotic resistance resulting in increased thermodynamic stability also increased the expression level of soluble periplasmic proteins in *E. coli*. This demonstrates that the split β-lactamase approach can be used as a practical tool to enhance protein expression levels, implying that the resulting sequences must harbor the ability to interact with the proteostasis network to maximize cellular protein levels. Many proteins apparently exhibit less than maximal thermodynamic and kinetic stability in vivo, suggesting that dynamic protein folding and unfolding may be required for trafficking, function, and turnover.

The development of genetic selection approaches, such as the Bardwell and Radford split β-lactamase approach described in this issue, has opened the door to exploring many aspects of the role of the proteostasis network in the maintenance and evolution of the proteome. The majority of eukaryotic proteins fail to express in *E. coli*, and we argue that this is partly because prokaryotes lack the sophisticated proteostasis network with which these proteins coevolved in eukaryotes. Using a genetic selection approach, the role of specific components of the mammalian proteostasis network on the folding of a given guest protein can now be evaluated, potentially providing significant insights into their roles in maintaining and/or regulating the cellular proteome. Similarly, the genetic approach to monitor protein folding also provides a sensitive measure of the impact of stress responses and transcriptional programs on the stability and integrity of the proteome, revealing the biological mechanisms for protein maintenance and evolution in response to a given cellular stimulus. By taking advantage of known fluorescent substrates of β-lactamase, this split β-lactamase approach should exhibit utility in mammalian cells and animal models to accurately and sensitively report on the extent of guest protein folding in the complex eukaryotic environment.

The split β-lactamase approach, and related folding sensors under development, should provide the necessary quantification to understand how protein sequence and the proteostasis network coevolved to define the cellular proteome. The ability to monitor protein conformational equilibria in vivo, previously a significant obstacle for protein folding studies in vivo, is now possible thanks to the efforts of the Bardwell and Radford groups, and this approach has the potential to answer many of the important biological questions related to the mechanisms by which proteins fold and evolve to optimize their function in a given cellular environment.

**REFERENCES**


