SPY-ing into Protein Stability

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Production of human proteins in bacteria has always been an arduous task, as has also been realized by biochemists while working with proteins. We expect our protein to be secreted into surrounding medium instead its seen as insoluble inclusion body in precipitated form inside the bacteria or is found glued to the cell wall of bacteria and may sometimes be, unfortunately seen as lumps at the bottom of test tubes.

Keeping this problem in mind, the primary goal of protein engineering, rational drug design and biopharmaceutical production is the development, production and storage of stable proteins with full functionality. In in vivo systems, chaperones are the proteins responsible for folding of proteins into their biologically active conformations and preventing their aggregation. So, the key here is to search for a molecule; which can stabilize the correctly folded structure or a drug that can disrupt pathways leading to protein misfolding.

This has really come true. A research team led by Prof. James Bardwell, University of Michigan spearheaded the discovery of molecular chaperone protein Spy in *E. coli*. When *E. coli* was challenged to stabilize a very unstable

periplasmic mutant protein Im7, it massively overproduced another periplasmic protein Spy thereby, increasing the steady state levels of Im7 by 700 folds [1, 2]. In vitro studies demonstrate that Spy, a 15.9 KDa protein, is an effective ATP-independent chaperone that causes suppression of protein aggregation. Unlike many other ATP independent chaperones, e.g. DnaK and GroEL systems, it also helps in protein refolding [3]. This study is supported by the effects of Spy in preventing *Malate dehydrogenase* aggregation due to denaturation by urea. Similar results have been seen in prevention of tannin-mediated inactivation of *E. coli* membrane protein by Spy where it protects the membrane proteins from tannic acid-induced activity loss, and interestingly it worked as a chaperone even in substoichiometric quantities, suggesting its high efficiency. Its expression is under the control of Bae and Cpx periplasmic stress response systems which are induced by a variety of stress conditions known to cause protein unfolding and aggregation, e.g. heat, urea, ethanol, butanol, all of which use different mechanisms to unfold proteins [4, 5]. This suggests that Spy has a general affinity for a wide range of different protein-unfolding intermediates. This general affinity to shield the aggregation-sensitive regions of a variety of protein-unfolding intermediates has been attributed to its dimer formation with a novel α helix and absence of globular core which allows higher flexibility.

These properties of Spy can thus be exploited to manufacture proteins to be used in medicines, industry as well as research. Spy thus, appears to be one of the very few chaperones that actively support protein refolding in absence of any obvious energy source and other regulatory proteins suggesting that Spy uses a mechanism to control substrate binding and release that is different from any of the previously characterized chaperones; although how it accomplishes it, is a prospective area of future research.

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