Designing a Fluorescent Precursor to Test the Partial Hairpin Insertion Model of Tat Protein Transport

Dattagupta, Antara

Protein transporters, located in the bacterial cell membranes, are responsible for moving proteins produced inside bacteria to outside of the cell where they operate. The Tat (twin-arginine translocation) pathway studied here is responsible for the transport of fully-folded and -assembled proteins across the normally impermeable cell membrane. The Tat pathway is found in bacteria causing human disease such as Salmonella typhimurium (Dysenteries and Food Poisoning) and Mycobacterium tuberculosis (TB). The Tat pathway is not only essential for protein transport, but is also important in many processes such as energy metabolism, formation of cell envelope and bacterial pathogeneses. It is imperative to have a good understanding of the protein transport mechanisms by the Tat pathway to disrupt its functioning and thus, stop bacterial infections through design of new drugs. The protein transport mechanism in the Tat pathway is poorly understood in part because of the lack of availability of high resolution structural information. Consequently, relatively little is known about the precise sequence of the translocation. In this project I have investigated a proposed Tat transport mechanism called ‘Partial Hairpin Insertion Model’ using the bacteria E. coli as the host cell. This model was discovered using a specific cargo protein named pre-SufI. The purpose of my project is to determine whether the ‘Partial Hairpin Insertion Model’ is more general and applicable to other cargo proteins. I generated a fluorescent Tat precursor using a protein called mCherry to observe its configurations via fluorescence resonance energy transfer (FRET) and verify the partial hairpin topology.

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