UNDERSTANDING THE MECHANISM OF RNA-PROTEIN INTERACTION INVOLVED IN THE PROTEIN TRANSLOCATION IN ARCHAEA

ABSTRACT

Index no. 203/18/Life Sc./26

Protein translocation is a critical process in the maintenance of cellular life which has been critically addressed in eukaryotes and bacteria. However, little information is available regarding the domain Archaea. The Signal Recognition Particle (SRP) plays an important role in the process of translocation in all three domains of life. It binds the signal peptide at the N-terminus of the nascent polypeptide chain. Together the SRP-ribosome-nascent polypeptide complex binds to the cognate SRP receptor (FtsY) located on the target membrane. Concomitant GTP hydrolysis by SRP and its receptor delivers the polypeptide to the adjacent protein-conducting channel. The archaeal SRP machinery bears high similarity with its eukaryotic counterpart where the proteins SRP54 and SRP19 bind the 7S RNA and together the ribonucleoprotein complex identifies the signal sequence. Previous works in *E. coli* have established a proper biochemical understanding of the SRP-FtsY interaction in bacteria, but nothing has ever been addressed in archaeal machinery. In the present study, we have successfully characterized the components of the SRP pathway in *Sulfolobus acidocaldarius*, a thermoacidophilic archaeon, with various biochemical and biophysical tools. Our findings prove that the GTPase activity of SRP54 is mildly influenced by its association with the SRP RNA. However, fluorescence analyses show that RNA accelerates the association between the nucleotide-bound forms of SRP54 and FtsY. With the help of corresponding mutants of SRP54 that either lack in GTPase or RNA-binding activity, it could be established for the first time in archaea that a successful turnover of GTP hydrolysis, that is crucial for the whole translocation process, is mostly dependent on the SRP54-FtsY interaction in presence of the nucleotide and 7S RNA. Subsequent deletion of multiple domains in FtsY further signified the role of N-terminal acidic domain and alpha-helices in associating the receptor with SRP54 as well as the archaeal plasma membrane. With the help of critical assay designing and specialized techniques such as EMSA, FRET, CD, structural analyses and MD simulation, the exact nature of SRP-FtsY-Membrane interaction in archaea has been investigated here, successfully, for the first time.

Abhrajyoti Ghosh
25/02/2022

Dr. Abhrajyoti Ghosh
Associate Professor
Department of Biochemistry
BOSE INSTITUTE
Block-EN, Plot No-80, Sector-V
Salt Lake City, Kolkata-700 091, INDIA