6. Outlook

Stable plasmid maintenance in the host cells is the most important process for any bacterial plasmid. There are two well known mechanisms used by various plasmids to control stable plasmid maintenance and inheritance. First is a site-specific recombination system, which ensures resolved recombination of the plasmids with the help of recombinases and others. Second are toxin - antitoxin systems controlling plasmid copy distribution. In this report we analysed the biophysical properties of β recombinase and RelBE TA systems as representative examples of two mechanisms.

The characterization of molecular properties and the observation of molecular behavior present an enormous challenge for biological scientists. Protein folding is a real challenge that nature has presented because each protein knows how to fold, but nobody understands how this is done. Alzheimer's disease, cystic fibrosis, mad cow disease, even many cancers were revealed to be result of protein misfolding. Extensive investigations of many small globular proteins have yielded detailed information concerning thermodynamics of folding. Most proteins are marginally stable, and in many cases, folding can be approximated by a two-state model in which only the native or unfolded protein can be found significant quantities. However, intermediates on the folding pathway have been detected for both small and large polypeptides. This is a basic problem of protein folding which corresponds to the local minima of free energy on a folding pathway. If the long term goal of protein research is the prediction of the tertiary structure of a protein from the primary sequence, then detailed information concerning the existence and structures of even transient intermediates is crucial and generally lacking. Studies based only on small globular proteins may not fully explain the folding and assembly of large multidomain or multisubunit proteins. For these and related reasons, we have studied the folding of *Streptococcus pyogenes* β recombinase; And *Escherichia*

coli and *Methanococcus jannaschii* RelBE system. The data demonstrated that the unfolding of β recombinase occurs by a multistep process that includes a monomeric intermediate, whereas RelBE unfolding is characterized by coupled dissociation-unfolding process.

The general property of autoregulation of transcription in all described addiction systems is that the antitoxin is the main DNA-binding protein of which affinity to promoter DNA is significantly enhanced in the presence of toxin. In our studies, RelB was found to be dimer at high concentrations and this could be an important prerequisite for promoter binding. Therefore; dimerization of antitoxin possibly plays a very significant role in DNA binding. In phd-doc TA system, under physiological conditions unfolded Phd monomers are stabilized by binding to promoter DNA, a process that is accompanied by dimer formation. The additional stabilization of Phd by dimer formation is most probable reason. Antitoxin CcdA, ParD, MazE and RelB (this report), are dimeric proteins in the micromolar concentration range at physiological temperatures. Under these conditions they are also known to bind to DNA. This behavior is not limited to TA systems. RNA-DNA binding proteins such as ROP usually recognize and bind specific sequences of nucleic acids only in their dimeric state, immediately loosing this ability if the 'hinge', *i.e.* the dimer contact, is lost. This is a clear example of molecular switch (the on-off positions corresponding to the dimer-monomer states) that can regulate important functions in living organisms. Another interesting aspect is the structured RelB in our experimental conditions, unlikely to unstructured monomeric Phd antitoxin or a proposal for RelB from *Pyrococcus horikoshii* RelBE crystal structure. This and other findings hint to the randomness of system, a flip between structured and unstructured antitoxin in different TA systems.

Implications

Indepth Studies on these systems already opened up number of application based approaches. β recombinase and their target sites (*six*) are used for the construction of a delivery and clearing system for the generation of food-grade recombinant lactic acid bacterium strains (*201*). Most of the lactic acid bacteria properties like lactose fermentation, citrate transport, protease production, phage resistance mechanisms *etc* are plasmid encoded. In fact, plasmid stability and phage infection are the major sources of disruption in the production fermented products by lactic acid bacteria (*201*). The constructed delivery system contains a heterologous replication origin and antibiotic resistance markers surrounded by two directly oriented *six* sites and a multiple cloning site where passenger DNA could be inserted (*201*).

Biotechnological and medical applications of toxin –antitoxin loci have also been highlighted (*62, 202*). TA loci have been useful for three practical purposes to date: plasmid stabilization, construction of positive selection vectors and active biological containment. TA complexes have also been promoted as useful targets for the development of antibacterial drugs. These antibacterial drugs will harness the property of toxin as active inhibitor of transcription or translation. These new drugs will be designed to directly activate bacterial suicide modules, therefore causing cell death. Designing these new drugs will be facilitated by the knowledge of physical and structural properties of toxins and antitoxins (*202*).

In this report we examined biophysical properties of various proteins important for plasmid maintenance. It might contribute to the deeper understanding of the mechanism of β recombinase, RelBE and other TA systems. However, we believe that even this limited structural and thermodynamic study comprehends the basic physical meaning of the interplay and provides further insights in understanding of system.