Uncovering the Allosteric Pathway Regulating Alternate Ribosome Binding by the Translation Factor BipA

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Abstract

BipA is a highly conserved translational GTPase that functions in a variety of cellular processes including fitness, pathogenicity and acclimation to adverse growth conditions. Structural and biochemical studies indicate that GTP and pGpp compete for binding to BipA to promote differential association of BipA to either the 70S or 30S ribosomal species1. Exactly how guanine nucleotide binding to BipA prompts a change in the association of this protein with the ribosome is not understood.

Guided by crystallographic models and amino acid conservation across the BipA family, site-directed mutagenesis was utilized to disrupt crucial interactions within the protein that stabilize inter-domain contacts. These include the interface between domains I and V as well as the surface between domains III, V, and the C-terminal domain. Steady state kinetics, isothermal titration calorimetry, ribosome binding assays and structural analysis were carried out to assess the impact of these substitutions on the biochemical properties of the protein. The intrinsic GTPase activity of all of the proteins was increased in comparison to wild-type BipA and several proteins did not exhibit ribosome-stimulated GTPase hydrolysis. This suggests either a disruption in the signaling pathway or an inability to form a complex with the ribosome. In vivo ribosome binding assays confirmed all but one BipA protein had lost their ability to bind to the ribosome. From these data, as well as accompanying computational modeling, we can propose a pathway of communication between the guanine nucleotide binding site and the C-terminal domain of the protein, previously shown to be required for ribosome binding. Our model also accounts for reverse flow of information required for the cellular role of the protein. Mapping the allosteric network within BipA that influences its differential ribosome binding may expose a unique bacterial target that can be exploited for the development of antimicrobial therapies.

PISA Analysis Identified Two Interacting Surfaces in BipA

Isothermal Titration Calorimetry

Preliminary isothermal titration calorimetry experiments to examine guanine nucleotide binding by the various BipA proteins. Measurements were carried out at 25 °C in phosphate buffer (5 mM K2HPO4, 200 mM NaCl and 5 mM MgCl2, pH 7.5) at 15 °C using a Microcal ITC.

These data show that BipA Y466A has the same affinity for guanine nucleotides as wild-type protein where the F111A has a lessened propensity to bind GTP than BipA.

Ribosome Association

Ribosome association of the BipA proteins. In brief, S. enterica SB300A cells with a plasmid encoding one of the His-tagged constructs were grown in MOPS medium at 37 °C to mid-log and induced with 0.2% arabinose. Two hours after induction, the cells were harvested, lysed and applied to sucrose gradient. Fractions were TCA precipitated and analyzed by 12.5% SDS-PAGE and Western blotting.

BipA Y466A, L565A/L569A, and E570A remain at the top of the gradient and so do not bind any ribosomal species. This suggests that these residues may be required for interdomain communication between the CTD and the GTPase domain. Interestingly, F111A associates with the 30S ribosome particles.

Conclusion

We have shown there is an allosteric pathway across the length of the protein involving residues in the nucleotide binding site, a central cavity and a small helix in the CTD. We are carrying out solution dynamics studies which suggest this helix is a critical component acting as a torsion spring to allow the CTD to pivot into a position amenable to ribosome binding.

References


Bar graph comparing the intrinsic and ribosome stimulated rates of hydrolysis for the BipA substituted proteins. Steady state kinetics parameters were measured using the malachite green-aminonmonium molybdate assay. The kcat values were determined by fitting the Michaelis-Menten equation using nonlinear regression algorithms furnished with GraphPad Prism (GraphPad Software, Inc.).

Residues in Contact Across the Domain I-V Interface

Central Helix Provides the Contact Surface Between Domains III, V and the CTD

Hydrophobic amino acids L565 and L569, and E570 are the pivotal players centrally positioned in domain III, V and CTD surface. These residues are also highly conserved in BipA.

Alanine substitutions were introduced into each of these sites and their GTPase, guanine nucleotide and ribosome binding properties assessed.

Steady State Kinetics

To begin to decipher the allosteric pathways governing GTPase activity and ribosome binding, we used PISA to analyze the protein-protein interactions observed in the Salmonella enterica apo BipA crystal structure solved to 2.4 Å resolution1. We identified two major interacting surfaces, one that lies between the GTPase domain (Domain I) and Domain V, and another surface that is composed of residues from Domains III, V and the CTD.

N-terminal

GTPase Domain

β Barrel

GT Domain

Nucleotide Binding Site

~573 Å²

Domain III

CTD

Domain V

~1038 Å²

Domain V

Domain III

Domain V

Domain III

Domain V

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