The role of inorganic polyphosphate in the regulation of *Escherichia coli* CobB deacetylase activity during amino acid starvation

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Inorganic polyphosphate (PolyP) is a ubiquitous polymer of hundreds to thousands of phosphate moieties held together by high-energy phosphoanhydride bonds. In bacteria, it regulates a variety of physiological processes, but the main function was assigned to the stress response and cell survival during advert conditions. The molecular mechanism by which such a simple molecule exerts its diverse functions is likely to be related to its ability to bind to specific proteins, mainly through ionic interactions. However, only a handful of reports have been published so far describing a proteomic analysis of PolyP granules and the relevance of PolyP-protein interactions.

Here, two novel approaches for isolation and identification of proteins interacting with PolyP from *Escherichia coli* are proposed. In the first approach, PolyP immobilized on the magnetic beads was used as a bait to pull-down interacting proteins. The second technique utilized the properties of a recombinant PolyP binding domain from *E. coli* exopolyphosphatase to bind and capture PolyP chains from cells together with interacting proteins.

One of the 21 proteins identified as PolyP interactors was CobB deacetylase. The interaction between PolyP and long isoform of CobB (CobB-L) was confirmed by three independent methods, which suggest that N-terminal extension, absent in short isoform (CobB-S), is responsible for the binding. CobB activity is important for bacterial cells for prompt recovery from stress as one of the proteins that undergo acetylation-dependent inactivation is DnaA, the DNA replication initiator protein. The activity assays show that PolyP binding to either deacetylase or substate inhibits deacetylation reaction and therefore affects DnaA acetylation status. These results add a new context to the pleiotropic effects of PolyP on bacterial cell DNA replication and the control of CobB activity. They also support a novel concept, in which chromosome and PolyP granules serve as alternative scaffolds that may direct proteins to different localization and change their activity for the time of unfavorable conditions.