How to identify proteins interacting with cyclic di-nucleotide in bacteria?

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Like corporation uses letters and emails to organize different departments and achieve the profitability, bacterium, a single cellular organism, uses second messengers to coordinate different cellular activities in order to survive in the harsh environment. Second messengers are intracellular signaling molecules that can trigger physiological changes in cell.

In 1987, the first cyclic dinucleotide – cyclic diguanosine monophosphate (c-di-GMP) was identified as second messenger in bacteria. As its name indicates, c-di-GMP is a cycle containing two guanine bases linked by ribose and phosphate. Cyclic-di-GMP is a ubiquitous and versatile second messenger among bacterial species regulating motility-to-sessility transition, biofilm formation, virulence and cell development. Recently, another two cyclic dinucleotides were identified. They are cyclic diadenosine monophosphate (c-di-AMP) and cyclic guanosine monophosphate-adenosine monophosphate (cGAMP). Cyclic-di-AMP is synthesized from two ATPs. Interestingly, unlike the other two cyclic dinucleotides, cGAMP contains more than one type of purine base. It is formed from one ATP and one GTP. Cyclic-di-AMP functions in cell wall homeostasis, osmotic stress, antibiotic resistance and sporulation. Compared with the well-studied c-di-GMP and c-di-AMP, our knowledge about the function of cGAMP is limited. By far, we only know cGAMP plays roles in chemotaxis and intestinal colonization in *Vibrio cholerae* and it regulates excellular electron transfer in *Geobacter*.

Cyclic dinucleotides are produced by cyclases while degraded by phosphodiesterases. The cyclases can be considered as the writer of the messenger. The phosphodiesterases are analogous to the postal clerk who destroy undelivered and redundant mails. The receptors are the recipient of cyclic dinucleotide regulating a wide range of cellular processes.

Currently, what we know about cyclic dinucleotide signaling pathways is merely the tip of iceberg. To facilitate the identification of proteins involved in cyclic dinucleotide signaling pathways, I developed riboswitch-based screening systems to monitor the alteration of cyclic dinucleotide concentration within *Escherichia coli* (*E. coli*) TOP10 cells. Riboswitch is a type of receptors common for all cyclic dinucleotides. Upon binding to the ligand, the expression platform of riboswitch changes its secondary structure and regulates downstream gene expression either at transcription level or at translation level. Riboswitches specific for cyclic dinucleotide(s) upstream of beta-galactosidase encoding *lacZY* genes were integrated into the Tn7 attachment site in TOP10 as a read-out system. Thereby, the changes of intracellular cyclic dinucleotide level can be reflected by alterations in beta-galactosidase activity resulting in color change of bacterial colonies on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) containing plates.

The c-di-GMP specific Vc2 riboswitch-based system is proved to be a suitable tool for the detection of diguanylyl cyclases (DGCs) that synthesize c-di-GMP. The described cGAMP specific 100erfK- and Gm0970-based systems are suitable to detect cGAMP cyclases and phosphodiesterases. In addition, surprisingly, the 100erfK-based system also can detect c-di-GMP cyclases. The 165ydaO riboswitch-based system is only suitable to detect c-di-AMP cyclases.

In summary, these riboswitch-based systems are appropriate tools for monitoring intracellular cyclic dinucleotide concentration and they could be used to detect novel proteins involved in cyclic dinucleotide metabolism *in vivo* and improve our knowledge about cyclic dinucleotide signaling pathways.

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