A novel capture compound for the identification and analysis of cyclic di-GMP binding proteins

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The second messenger cyclic di-GMP is a near-ubiquitous signaling molecule that globally alters bacterial cell physiology to promote biofilm formation and community behavior. Much progress was made in recent years towards the identification and characterization of diguanylate cyclases and phosphodiersterases, enzymes involved in the synthesis and degradation of this signaling compound. In contrast, our knowledge of the nature and mechanistic details of c-di-GMP effector proteins lags behind, primarily because effective tools for their specific enrichment and rapid analysis are missing. In this report we demonstrate that a novel tri-functional c-di-GMP-specific Capture Compound (cdG-CC) can be effectively used to identify and validate c-di-GMP binding proteins. The cdG-CC was able to specifically and efficiently pull down bona fide c-di-GMP effector proteins. Furthermore, in combination with mass spectrometry (CCMS), this technology robustly identified a substantial fraction of the known c-di-GMP signaling components directly from cell extracts of different model organisms. Finally, we applied the CCMS technique to profile c-di-GMP binding proteins of Pseudomonas aeruginosa and Salmonella enterica serovar typhimurium. Our studies establish CCMS as a powerful and versatile tool to identify and analyze components of the cellular c-di-GMP pathway in a wide range of different organisms.
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