Publication

An extended cyclic di-GMP network in the predatory bacterium Bdellovibrio bacteriovorus

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Over the course of the last three decades the role of the second messenger cyclic di-GMP (c-di-GMP) as a master regulator of bacterial physiology was determined. Although the control over c-di-GMP levels via synthesis and breakdown, and the allosteric regulation of c-di-GMP over receptor proteins (effectors) and riboswitches have been extensively studied, relatively few effectors have been identified and most are of unknown functions.The obligate predatory bacterium Bdellovibrio bacteriovorus has a peculiar dimorphic life cycle, in which a phenotypic transition from a free living attack phase (AP) to a sessile, intracellular predatory growth phase (GP) is tightly regulated by specific c-di-GMP diguanylate cyclases. B. bacteriovorus also bears one of the largest complement of defined effectors, almost none of known functions, suggesting that additional proteins may be involved in c-di-GMP signaling.In order to uncover novel c-di-GMP effectors, a c-di-GMP capture-compound mass-spectroscopy (CCMS) experiment was performed on wild type AP and host-independent (HI) mutant cultures, the latter serving as a proxy for wild type GP cells. Eighty four proteins were identified as candidate c-di-GMP binders. Of those, 65 did not include any recognized c-di-GMP binding site and three carried known unorthodox binding sites. Putative functions could be assigned to 59 proteins. These proteins are included in metabolic pathways, regulatory circuits, cell transport and motility, thereby creating a potentially large c-di-GMP network. False candidate effectors may include complex members as well as proteins binding nucleotides or other co-factors that were, respectively, carried over or unspecifically interacted with the capture compound during the pull-down. Sixty two of the 84 candidates were found to specifically bind the c-di-GMP capture compound in AP or in HI cultures, suggesting c-di-GMP control over the whole cell cycle of the bacterium. Highly affine and specific c-di-GMP binding was confirmed using microscale thermophoresis with a hypothetical protein bearing a PilZ domain, an acyl-coA dehydrogenase and a two component system response regulator, indicating that additional c-di-GMP binding candidates may be bona fide novel effectors.; In this study 84 putative c-di-GMP binding proteins were identified in B. bacteriovorus, an obligate predatory bacterium whose lifestyle and reproduction are dependent on c-di-GMP signalling, using a c-di-GMP capture compound precipitation approach. This predicted complement covers metabolic, energy, transport, motility and regulatory pathways, and most of it is phase specific, i.e. 62 candidates bind the capture compound at defined modes of B. bacteriovorus lifestyle. Three of the putative binders further demonstrated specificity and high affinity to c-di-GMP via microscale thermophoresis, lending support for the presence of additional bona fide c-di-GMP effectors amongst the pulled down protein repertoire.
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