Fungicide Sensitivity of Multicellular Microorganisms

excerpt from the Dissertation

Structure and Function of poly-HAMP Histidine Kinases

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Abstract

Many organisms employ sensory histidine kinases that monitor environment and cell status, activating adequate adaptational responses. HAMP domains are small, essential signal transmission modules found frequently in histidine kinases or similar signaling proteins of microorganisms and plants. Poly-HAMP histidine kinases combine a large number of HAMP domains into N-terminal arrays, which presumably serve to both sense and transmit signals. These proteins are prevalent in Actinobacteria, Myxobacteria, *Dictyostelium* and fungi. Their HAMP units share a striking 70% sequence identity between different phyla. While fungal homologs are known to be central sensors, integral for cell physiology, prokaryotic homologs were largely uncharacterized. The proteobacterium *Myxococcus xanthus* harbors two homologous hybrid poly-HAMP histidine kinases, MXAN_0712 and MXAN_6735, comprising large N-terminal arrays of 21 and 31 HAMP domains, respectively.

The first part of this thesis consists in the structural characterization of a poly-HAMP array, including solving crystal structures of fragments encompassing the first four and first six HAMP domains of MXAN_0712. Crystallization was achieved using stabilizing GCN4 leucine zippers, fused using a custom-designed vector. The poly-HAMP arrays are shown to be tightly organized in a pattern in which two classes of HAMP, distinct in sequence and structure, alternate along the array. While the two forms rely on opposite patterns of additional conserved hydrophobic core residues, individual HAMPs showed considerable diversity in inter-helical packing, charting the conformational space available to HAMP domains. Highly conserved interactions are found to connect HAMPs into an extended array structure, observed as long needles in electron micrographs of the full-length arrays of both proteins. Modelling the structure of full-length arrays, combined with analysis of sequence conservation, finally gave clues about possible mechanisms of action.

In the second part of this work, the two *M. xanthus* poly-HAMP histidine kinases were used as model systems to study the role of prokaryotic histidine kinases in developmental signaling processes, and the contributions of poly-HAMP domains to kinase function. Deletion mutant phenotypes revealed that the proteins have partially redundant as well as unique functions during growth, single-cell-, and multicellular development. Double in-frame deletion- and double kinase inactivation mutants exhibited a novel phenotype: formation of spore-like cells in nutrient-rich medium; captured via time-lapse microscopy using a newly developed slide setup suitable for long-term imaging at high-resolution. A method for automated quantitative metrics of bacterial cell shapes from light microscopy images was also developed. *M. xanthus* MXAN_0712 and MXAN_6735 were shown to interact via the poly-HAMP array. It was further revealed that removal of several or all HAMP domains of MXAN_0712 strongly affected the cellular response to the known, starvation-independent sporulation inducer glycerol, with one of these mutations exerting a dominant negative effect. Surprisingly, treatment of *M. xanthus* with certain fungicides induced a dramatic change of cell shape into spore-like cells, whose ultrastructure resembled glycerol spores. Fungicide susceptibility also depended on presence of at least one functional poly-HAMP histidine kinase with an intact poly-HAMP array. These results assign both proteins pivotal roles in control of morphological differentiation, as gatekeepers for sporulation and as mediators of glycerol- and fungicide sensitivity; while the interference of fungicides with essential bacterial development processes may have widespread consequences for the functioning of microbial soil ecosystems.

These observations, together with an analysis of the phylogenetic distribution and conservation of poly-HAMP histidine kinases, suggest that they control stress-induced morphological differentiation in many multicellular microorganisms.