6 Abstract

While the principal mechanism of the cytokinin signaling pathway has been elucidated, redundancy in the system has made it difficult to understand which of the many components of the two-component system (TCS) interact to control the downstream biological processes. To further investigate the functions of TCS proteins, this study placed them in a comprehensive network of protein interactions.

The yeast two-hybrid was used for a matrix assay in which 20 TCS-*Baits* and 25 TCS-*Preys* were investigated for interactions in a grid pattern. Among the 500 combinations 43 new and 25 published interactions were identified. Proteins of the same family showed an identical interaction pattern explaining the functional redundancy of the TCS proteins on molecular level. Putative homo- and heterodimerization among the cytokinin receptors were indicated by the interactions of their cytoplasmic parts (AHK2-AHK2, AHK3-AHK2 and AHK3-AHK4). Two cases of interactions of B-type response regulators (ARR14-ARR14, ARR14-ARR2), which are transcription factors and a direct interaction of a receptor with a response regulator (AHK2-ARR14) indicated putative other mechanisms for cytokinin signaling. To evaluate the quality of the data, 42 interactions were tested by *in vitro* binding experiments and 38 interactions were confirmed. Analysis of AHP5 mutant proteins showed that activation of this protein by phosphorylation at the conserved histidine is not required for its interactions. Additional analysis of AHK2 showed that the histidine kinase domain is required for homodimerization and the receiver domain for interaction with AHP5 and ARR14. Dividing the HPt domain of AHP5 into two parts resulted in loss of interaction. Analysis of ARR14, a B-type response regulator, revealed that the complete protein is required for interaction with AHP5 and AHK2. The isolated output domain of ARR14 is required for self interaction and for interaction with ARR2. At the same time this domain had a transcription activation activity. ARR4, which is an A-type ARR, required the complete protein for interaction.

Because of the functional redundancy within the TCS it was thought that signal specificity is regulated mainly through interactions of the TCS proteins with other proteins. For this, 17 TCS-proteins were investigated for interactions by yeast two-hybrid cDNA-library screens. Screens were optimized by colony hybridization and increasing the number of transformands and using another prey vector. The TCS network included 140 physical interactions of which some were known and others new. The cytokinin receptors interacted mostly with other proteins but less with AHPs, whereas B-type and A-type ARRs interacted mostly with AHPs but less with other proteins. An important novel interaction identified in this study was AHK2-

PI4Kβ1 indicating a cross talk between the cytokinin- and phosphatidylinositol signaling pathway. Additional analysis, in which PI4Kβ1 was tested for interactions with all TCS-proteins, revealed that the interaction AHK2- PI4Kβ1 is specific and that the receiver domain of AHK2 is required for this interaction.

As a beginn for the analysis of multi proteincomplexes and analysis of further protein protein interactions the affinity chromatography and mass spectrometry was used. The cytoplasmic part of AHK3 was used exemplary. This analysis revealed a putative homodimerization of AHK3 and an interaction with a protein of the dehydrin family.

Identified interactions in this work give new insights into putative functions of TCS-proteins and indicate that interactions with other proteins are also important in order to understand the cytokinin signalling pathway.