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Structure and function of tomato disease resistance proteins

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Summary

Race-specific disease resistance in plants is mediated by Resistance (R) proteins that recognise pathogen-derived molecules and subsequently initiate defence responses such as the Hypersensitive Response (HR). The results presented in this thesis pivot on the tomato R proteins I-2 and Mi-1, that mediate resistance against *Fusarium* wilt and root-knot nematodes, respectively.

Most R proteins contain a central nucleotide-binding NB-ARC domain and a C-terminal leucine-rich repeat (LRR) domain (Chapter 2). The NB-ARC domain is a functional ATPase module, and its nucleotide-binding state has been proposed to control the R protein activation state (Takken *et al.*, 2006; Tameling *et al.*, 2006). In this model, the ATP-bound state represents the activated conformation that initiates HR, whereas the ADP-bound state corresponds to the resting state. Three subdomains can be discerned in the NB-ARC domain: NB, ARC1 and ARC2. Each of them carries between three and five conserved sequence motifs (Albrecht and Takken, 2006). One very highly conserved motif at the carboxy-terminus of the ARC2 subdomain is the methionine-histidine-aspartate (MHD) motif (Chapter 3). We have performed an extensive mutational analysis of this MHD motif in the R proteins I-2 and Mi-1. Mutation of the histidine and aspartate residues resulted in several novel autoactivating and loss-of-function mutations. Autoactivation is defined as induction of HR in the absence of the pathogen-derived stimulus. Combination of these MHD mutations with existing autoactivating hydrolysis mutants in the NB subdomain revealed that the autoactivation phenotypes are neither additive, nor synergetic. This result indicates that the MHD motif represents an important element negatively regulating R protein activity.

To reveal the structural basis underlying the function of the MHD motif, a three-dimensional model of the NB-ARC domain of I-2 was built using the structurally related human protein APAF-1 as a template. Based on this model, we propose that in the inactive state, the MHD motif coordinates the bound ADP molecule and thereby controls the physical interactions between the NB and ARC2 subdomains. In this way, the MHD motif fulfils both functions of the sensor II motif, that is found in the related AAA+ protein family, but is absent in R proteins and APAF-1. The 3D model was additionally applied as a framework for the formulation of hypotheses on how previously identified mutations in the NB-ARC exert their effects, and to identify residues that are important for I-2 structure and function.

Next, the effect of specific point mutations in the Mi-1 NB and ARC2 subdomains on intramolecular interactions between the LRR domain and its N-terminus was investigated (Chapter 4). Autoactivating mutations in the NB subdomain trans-complemented and triggered HR upon co-expression of the N-terminus and the LRR domain of Mi-1. These data show that these parts of the R protein can functionally

complement when expressed *in trans*. Likewise, known autoactivating chimaeric LRR domain swaps using an Mi-1 paralogue were found to induce HR upon expression *in trans*. However, point mutations in the MHD motif that induced strong autoactivation in the full-length Mi-1 protein did not lead to HR upon co-expression. All analysed combinations of Mi-1 CC-NB-ARC and LRR domains physically engage in the same protein complex, indicating that total dissociation of the LRR is not required for activation of Mi-1.

According to the general assumption, R proteins function in protein complexes. To discern constituents of such a complex we applied yeast two-hybrid analyses, and identified RSI2 as part of the I-2 protein complex (Chapter 5). This protein is a member of the HSP20/ α -crystallin small heat-shock proteins. Small heat shock proteins are evolutionary conserved across kingdoms and are involved in establishing proper protein folding and stability. The yeast two-hybrid interaction was confirmed by pull-down experiments from plant protein lysates. HR signalling by autoactivating variants of I-2 and Mi-1 was strongly compromised upon silencing of RSI2, indicating its functional involvement in R protein-mediated HR signalling. Furthermore, the accumulation of I-2 protein *in planta* was heavily reduced in RSI2-silenced plants, indicating its role in maintaining I-2 protein stability.

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