Structure and function of tomato disease resistance proteins

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Chapter 1

Introduction and outline

Like all higher organisms, plants are continually attacked by a large variety of potentially pathogenic organisms. Due to their immobile, and at first sight irresponsive nature, plants appear to be sitting ducks for malevolent pathogens. Nevertheless, plants are surprisingly effective in protecting themselves from invaders, and disease is a relatively rare occurrence. To reply to the broad range of pathogens lurking in the ecosystem, plants evolved a sophisticated defence system.

Introduction

Plants evolved a large number of proteins that trigger inducible defence responses upon pathogen perception (Jones and Dangl, 2006). These proteins include the Pathogen Recognition Receptors (PRRs), that sense Pathogen-Associated Molecular Patterns (PAMPs) that are often highly conserved molecules, shared between classes of pathogenic micro-organisms (Zipfel and Felix, 2005). The most well-studied group of proteins recognising potential pathogens are the Resistance (R) proteins that respond to microbial molecules that are generally not conserved between species or isolates of a species (Martin et al., 2003; van Ooijen et al., 2007). These pathogen-derived factors are referred to as Avirulence (AVR) proteins because their presence in a pathogen causes them to be avirulent on plants containing the cognate R protein. The reason that *AVR* genes withstood evolution is most likely that they contribute towards virulence on plants lacking the R protein (Grant et al., 2006; Kamoun, 2006; Bent and Mackey, 2007). Resistance mediated by R proteins is in most cases accompanied by programmed cell death at the infection site, a response aimed at limiting further pathogen proliferation. This localized cell death is called the Hypersensitive Response (HR).

Investigations into disease resistance started when in the 1840s a mysterious phenomenon wiped out most of Irelands primary food source potato; a period referred to as the “Irish potato famine”, or “great hunger”. Pioneer plant pathology research revealed that the devastating effect on the potato plants was the work of a member of the illustrious genus of oomycete plant pathogens *Phytophthora*. This genus is named after the Greek words *phyton* (plant) and *phthora* (destruction). Potato belongs to the plant family *Solanaceae*, in English called the nightshade family. To date, the solanaceous plant species potato and tomato are, together with thale cress (*Arabidopsis thaliana*), still leading model systems to study plant defense against possibly pathogenic organisms. Most of the R proteins that have been cloned over the past 15 years, originate from nightshade species (Chapter 2). The results presented in this thesis focus on the tomato R proteins I-2 and Mi-1. The *I-2* gene confers resistance to Avr2-containing isolates of the soil-borne fungal pathogen *Fusarium oxysporum* f.sp. *lycopersici*, that causes wilt disease on plants lacking *I-2* (Ori et al., 1997; Simons et al., 1998). *Mi-1* was identified as an *R* gene that confers resistance to several nematode species of the genus *Meloidogyne* (Milligan et al., 1998; Vos et al., 1998). Since these nematodes induce feeding structures in the tomato roots, that swell and produce galls, they are named root-knot nematodes (Williamson and Kumar, 2006). Later, this *R* gene was shown to be effective against infestations by the insect species whitefly (*Bemisia tabaci*) and potato aphid (*Macrosiphum euphorbiae*) as well (Vos et al., 1998; Nombela et al., 2003).

Outline of this thesis

In **chapter two**, a detailed description of our current knowledge of solanaceous R proteins and their modular domain structure is provided. Furthermore, this chapter deals with the intramolecular protein interactions required for R protein repression and activation. Another important aspect of R protein signalling that is discussed in this chapter, is the interaction with other proteins, in order to form large multi-protein complexes that are jointly able to induce immune responses to arrest pathogen proliferation.

Most R proteins contain a central NB-ARC domain and a C-terminal leucine-rich repeat (LRR) domain (Martin et al., 2003). The NB-ARC domain is a functional ATPase domain, and its nucleotide-binding state is proposed to regulate the activity of the R protein (Tameling et al., 2002; Tameling et al., 2006). A highly conserved methionine-histidine-aspartate (MHD) motif (Hammond-Kosack and Jones, 1997) is present at the carboxy-terminus of the NB-ARC domain. An extensive mutational analysis of this MHD motif is provided for R proteins I-2 and Mi-1 in the **chapter three**. Several novel autoactivating mutations were identified, indicating an important regulatory role for the MHD motif in the control of R protein activity. To explain this effect, a three-dimensional model of the NB-ARC domain of I-2 was built, based on the APAF-1 template structure (Riedl et al., 2005). The structural position of the MHD motif residues indicated that they coordinate the bound nucleotide and control subdomain interactions within the NB-ARC domain. The presented 3D model provides a framework for the formulation of hypotheses on how mutations in the NB-ARC exert their effects.

Since genetic experiments pointed towards dynamic interactions between the LRR domain and the N-terminus of Mi-1 (Hwang et al., 2000; Hwang and Williamson, 2003), we analysed the intramolecular interaction between these parts. In **chapter four**, we show that these domains do functionally transcomplement; known autoactivating LRR domain swaps were found to induce HR upon co-expression *in trans*. Likewise, some autoactivating mutants in the NB-ARC domain transcomplemented to induce HR, but others surprisingly did not, suggesting differences in the molecular mechanisms conferring autoactivity. Furthermore, we show that dissociation of the LRR is not required to release its negative regulation, as in all combinations of CC-NB-ARC and LRR domains tested a physical interaction was observed.

Not only intra- but also intermolecular interactions have been analysed, in order to determine the composition of R protein complexes. In **chapter five**, we focus on these interacting proteins, most of which are regarded to be R protein chaperones (Shirasu and Schulze-Lefert, 2003). Through yeast two-hybrid analyses we identified HSP17 as a member of the I-2 protein complex. Silencing of HSP17 was found to

disturb the ability of autoactivating R protein variants to induce HR, indicating the functional involvement of this protein in defence signalling. Furthermore, the effect of HSP17 on R protein expression levels was analysed.
Finally, the results presented in this thesis, and opportunities for future research are discussed in relation to current knowledge in **chapter six**.

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