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The chloroplast DegP2 protease is down regulated in response to pathogen attack

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Introduction

Organisms that perform oxygenic photosynthesis are subjected to photoinhibition of their photosynthetic function when exposed to excessive illumination due to generation of reactive oxygen species (ROS). At ambient temperature photoinhibition occurs mainly at the level of photosystem II (PSII) and involves a reversible inactivation of PSII due to arresting of electron transport within this complex followed by an irreversible damage to the subunits of PSII reaction center (Prasil et al., 1992; Aro et al., 1993). Among the PSII reaction center proteins the D1 protein is the main target of the photooxidative damage. A rapid degradation of the damaged D1 protein and its replacement by a *de novo* synthesized functional copy represents an important repair mechanism crucial for plant survival under light stress conditions (Mattoo et al., 1981; Prasil et al., 1992; Aro et al., 1993).

Recently, we reported the isolation of a novel chloroplast DegP2 protease from *Arabidopsis thaliana*, which is responsible for the primary cleavage of the photodamaged D1 protein and hence catalyzing the key step of in the repair cycle in plants (Haußühl et al., 2001). A functional copy can co-translationally exchange the photodamaged D1 protein only when the damaged D1 protein has been removed from the PSII reaction center by a proteolytic event. The DegP2 protease is a member of a large family of related Deg/Htr serine endopeptidases present in most organisms including bacteria, humans and plants (Adam et al., 2001). In *A. thaliana* DegP2 protease is peripherally associated with the stromal surface of the thylakoid membranes and its level increases under high salt, desiccation and light stress conditions (Haußühl et al., 2001). Interestingly, a strong reduction of DegP2 transcript and protein levels was measured after treatment of leaves with H_2O_2 and after exposure to heat shock (Haußühl et al., 2001). Both treatments were reported to induce ROS and lead to hypersensitive responses (HR) and finally to cell death in plants (Dangl et al., 1996; Mittler and Lam, 1996). Thus, it is very likely that a repression of DegP2 expression and inactivation of the D1 protein repair cycle might be one of the earliest steps of HR. To test this hypothesis we investigated the expression of DegP2 in *A. thaliana* after pathogen attack using the bacteria *Pseudomonas siringae* and the fungus *Peronospora parasitica*. Inoculation of *A. thaliana* leaves with avirulent pathogen induces secondary oxidative bursts in cells that in turn leads to HR (Bi et al., 1995). Our results revealed that DegP2 protease was down regulated as a consequence of

pathogen attack. Application of paraquat, a ROS generating agents, led to repression of DegP2 level confirming that down regulation of this protease is related to ROS formation.

Materials and methods

Arabidopsis thaliana L. were grown a growth chamber on soil at 25°C at a light intensity of 100 µmol/m^2s under short-day (*A. thaliana* cv. Columbia) or long-day (*A. thaliana* cv. Landsberg) conditions. The pathogen experiments were performed as described by Ehlting et al. (1999). For bacterial infections four weeks old *A. thaliana* cv. Columbia were infiltrated 5 min under vacuum with 10 mM $MgCl_2$ (control) or with a suspension of *Pseudomonas syringae* pathovar *tomato DC3000* at OD_{600} = 0.2. For fungal infections ten days old seedlings of *A. thaliana* cv. Landsberg were sprayed with spores of *Peronospora parasitica* pathovar *Noks1* or H_2O as a control. Hormone treatments were performed on fully expanded 4 to 5 weeks old detached leaves of *A. thaliana* cv. Columbia floated on distilled water in the absence (control) or in the presence of 25 µM paraquat (MV, methylviologen) or 5 mM salicylic acid (SA) for 8 h at 25°C and a light intensity of 100 µmol/m^2s. Isolation of total RNA or thylakoid membrane proteins for Northern blot or Western blot analysis, respectiveley, were performed as previously described (Haußühl et al., 2001).

Results and Discussion

Because chloroplasts were shown to be involved in HR (Seo et al., 2000) and generated ROS play a role in programmed cell death after pathogen attack, we tested whether the DegP2 protease expression is influenced by infection of plants with the bacteria *Pseudomonas syringae* pv *tomato* and the fungus *Peronospora parasitica* pv *Noks*. Infiltration of *Arabidopsis* plants with *Pseudomonas syringae* or with $MgCl_2$ (control) caused a transient induction of DegP2 transcripts and starting from 2 to 4 h after treatment (Fig. 1 A). Since this effect was visible in both, the pathogen-treated and mock-treated plants we assumed that this transient induction of DegP2 transcripts might result from the wounding of leaves and is not specifically related to the pathogen attack. With a prolonged incubation time the expression of DegP2 decreased in plants treated with *Pseudomonas syringae*. After 7 h from inoculation of plants with this pathogen the DegP2 transcript level was 3-4-fold lower than that before the inoculation. Incubation of plants for 21 h resulted in 6-7-fold lower expression of DegP2 as compared with the untreated control (Fig. 1A). No DegP2 protein was detectable 21 h after infection (not shown). A similar negative regulation of DegP2 expression was measured after infection of *Arabidopsis* seedlings with spores of the fungus *Peronospora parasitica* (Fig. 1B). After 24 h from infection only traces of DegP2 transcripts were detected in pathogen attacked leaves. In contrast, the DegP2 mRNA level was not significantly changed in control plants. The down-regulation of DegP2 expression one day after infection with *P. parasitica* is concomitant with the expression of defense-related genes like *At4CL1, At4CL2, AtC4H, AtPAL* and *AtPOX* (Ehlting et al., 1999).

To investigate whether the down regulation of DegP2 expression is related to ROS formation *Arabidopsis* leaves were treated with paraquat for 8 h and the DegP2 transcript and proteins levels were assayed by Northern and Western blotting, respectively (Fig. 1 C). Paraquat is widely used oxidative agent, which causes damage to the chloroplast components due to generation of ROS (Varadi et al., 2000). The results revealed that the treatment with paraquat diminish the DegP2 expression at the transcript and protein levels (Fig. 1C). Also the D1 protein of the PSII reaction center was influenced by the paraquat treatment and only traces of this protein were detected in the thylakoid membranes after 8 h of treatment.

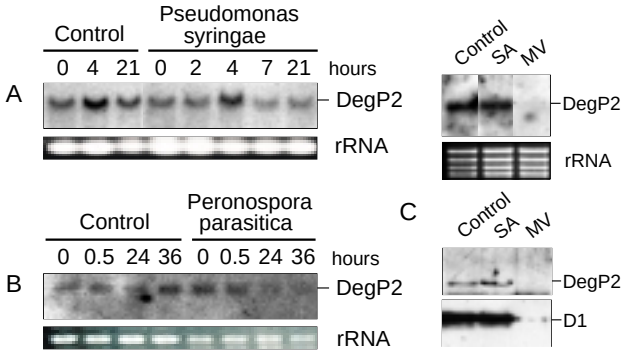

Fig 1. The DegP2 expression is down regulated in response to pathogen attack and treatment with paraquat. (A) Expression of DegP2 in *Arabidopsis* plants infected with the bacteria *Pseudomonas syringae* assayed by Northern blotting. (B) Expression of DegP2 in *Arabidopsis* seedlings infected with spores of the fungus *Peronospora parasitica* assayed by Northern blotting. As a reference, the rRNA pattern in the gel, visualized by staining with ethidium bromide, is shown. (C) Expression of DegP2 in mature leaves treated with 25 μM paraquat (MV) or 5 mM salicylic acid (SA) for 8 hours assayed by Northern (upper panel) and Western (lower panel) blotting.

Since salicylic acid was reported to be involved in signaling of pathogen-induced disease (Alvarez, 2000) we tested whether incubation of leaves with salicylic acid will influence expression of DegP2. The results show (Fig. 1C) that neither DegP2 transcript nor protein levels were effected by 8 h treatment with this compound. Also the prolongation of the incubation time up to 3 days had no effect on DegP2 expression (not shown).

Based on our previous (Haußühl et al., 2001) and current (this paper) data we propose that regulation of DegP2 protease represent an early response to pathogen attack and disturbance of D1 repair cycle due to ROS might be a key step of HR. This working hypothesis is supported by the findings that also the expression of the thylakoid membrane FtsH protease, was found to be reduced during HR after infection of tobacco plants with tobacco mosaic virus (TMV). Furthermore, there was a close correlation between the amount of FtsH protease in transgenic tobacco plants and the size of necrotic lesions induced by the TMV (Seo et al., 2000). Therefore, it was proposed that the loss of certain chloroplast functions might accelerate the HR in plants. The FtsH protease in *Arabidopsis* was shown to be involved in secondary proteolysis of the 23 kDa proteolytic fragment of the D1 protein (Lindahl et al., 2000). Two such fragments, a N-terminal 23 kDa fragment and a C-terminal 10 kDa fragment, are generated (Aro et al., 1993) during the primary cleavage of the photodamaged D1 protein by the DegP2 protease (Haußühl et al., 2001). In conclusion following working model for the role of DegP2 protease in HR is proposed (Fig. 2).

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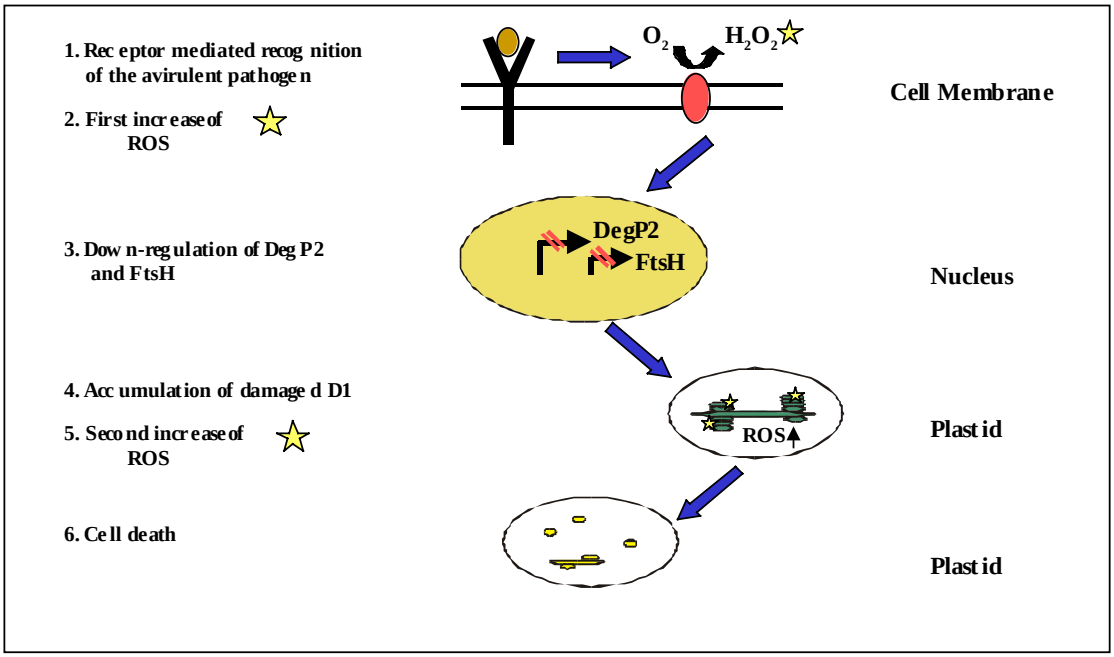

Fig. 2. Working model for the role of DegP2 protease in HR. When the level of D1 protein degrading enzymes, such as DegP2 and FtsH proteases are diminished after pathogen attack, the repair cycle of the damaged D1 protein cannot take place. The inhibition of photosynthesis may then disturb the cell homeostasis leading to HR and cell death.

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