Phytochrome signalling modulates the SA-perceptive pathway in *Arabidopsis*

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Summary

The interaction of phytochrome signalling with the SA signal transduction pathway has been investigated in *Arabidopsis* using single and multiple mutants affected in light perception (phyA and phyB deficient) and light-signal processing (*psi2*, phytochrome signalling). The induction of *PR1* by SA and functional analogues has been found to strictly correlate with the activity of the signalling pathway controlled by both phyA and phyB photoreceptors. In darkness as well as dim light, and independently of a carbohydrate source, SA-induced *PR* gene expression as well as the hypersensitive response to pathogens (HR) are strongly reduced. Moreover, the initiation of HR also exhibits a strict dependence upon both the presence and the amplitude of a phytochrome-elicited signal. The growth of an incompatible strain of bacterial a pathogen (*Pseudomonas syringae* pv. *tomato*) was enhanced in *phyA-phyB* and decreased in *psi2* mutants. While functional chloroplasts were found necessary for the development of an HR, the induction of *PR*s was strictly dependent on light, but independent of functional chloroplasts. Taken together, these data demonstrate that the light-induced signalling pathway interacts with the pathogen/SA-mediated signal transduction route. These results are summarized in a formalism that allows qualitative computer simulation.

Keywords: phytochrome, salicylic acid, cross-talk, PR1, HR.

Introduction

As sedentary organisms, plants have evolved a high plasticity in metabolism and development to cope with the multiple changes of their environment. The various modifications in the biotic and abiotic parameters are perceived by numerous specific plant receptors, and the resulting information is transduced and integrated by a complex signalling apparatus.

For example, in response to the presence of a pathogen, a very sophisticated set of responses is induced by a plant signalling network (Maleck *et al.*, 2000; Reymond and Farmer, 1998; Schenk *et al.*, 2000). The initial recognition events in plant-pathogen interactions are followed by the activation of a syndrome of defense reactions including the synthesis of various proteins (pathogenesis-related proteins, PRs) and phytoalexins, structural cell wall changes, and a localized and active cell death referred to as hypersensitive reaction (HR; Durner *et al.*, 1997; Greenberg *et al.*, 1994). The production of a short burst of active oxygen species rapidly follows the first event in the recognition of a pathogen. This oxidative burst is induced unspecifically in response to both pathogenic and non-pathogenic organisms (Levine *et al.*, 1994). In the case of a race-specific interaction, where the product of a pathogenic avirulence gene (*Avr*) interacts specifically with a plant resistance gene product (R protein), a second much larger production of oxidants leads to the development of an incompatible response characterized by HR cell death (Lamb and Dixon, 1997; Staskawicz *et al.*, 1995).

The salicylic acid (SA) pathway is an important route inserted in the network of defense signalling. The synthesis of PR proteins can be activated by an ectopic treatment with SA (Delaney *et al.*, 1994; Gaffney *et al.*, 1993; Métraux *et al.*, 1990; Uknes *et al.*, 1992; Ward *et al.*, 1991) or functional analogues such as BTH (benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester; Görlach *et al.*, 1996). In transgenic *Arabidopsis* plants expressing a SA-hydroxylase gene of *Pseudomonas putida* (*NahG* gene), SA is degraded to catechol leading to a loss of *PR1* gene

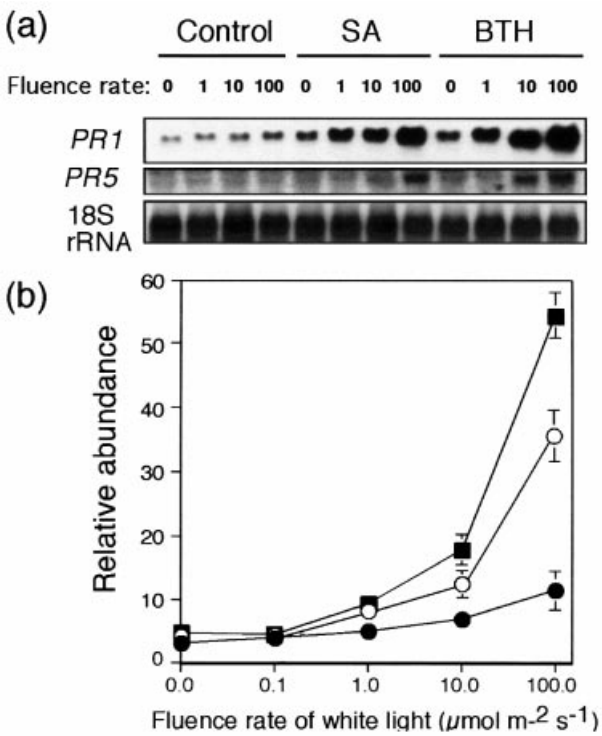

Figure 1. The SA and BTH induction of *PR1* and chlorophyll is light dependent.
WT seedlings grown on a 3%-sucrose-containing medium, and under various fluence rates of white light, were treated with SA (250 μM) or BTH (1 mM). Total RNA was extracted 48 h after treatment and used for gel blot analysis.
(a) Expression of *PR1* and *PR5*. Plants grown on soil under low light intensity (15 μmol m^{-2} sec^{-1}) were treated 3 weeks after germination with SA (250 μM) or BTH (1 mM) in darkness (5 min Dark), and then transferred to various fluence rates. RNA was extracted 48 h after treatment and used for gel blot analysis.
(b) *PR1* expression in 7-day-old seedlings grown on sucrose-containing medium. ●: untreated; ○: treated with SA; ■: treated with BTH.

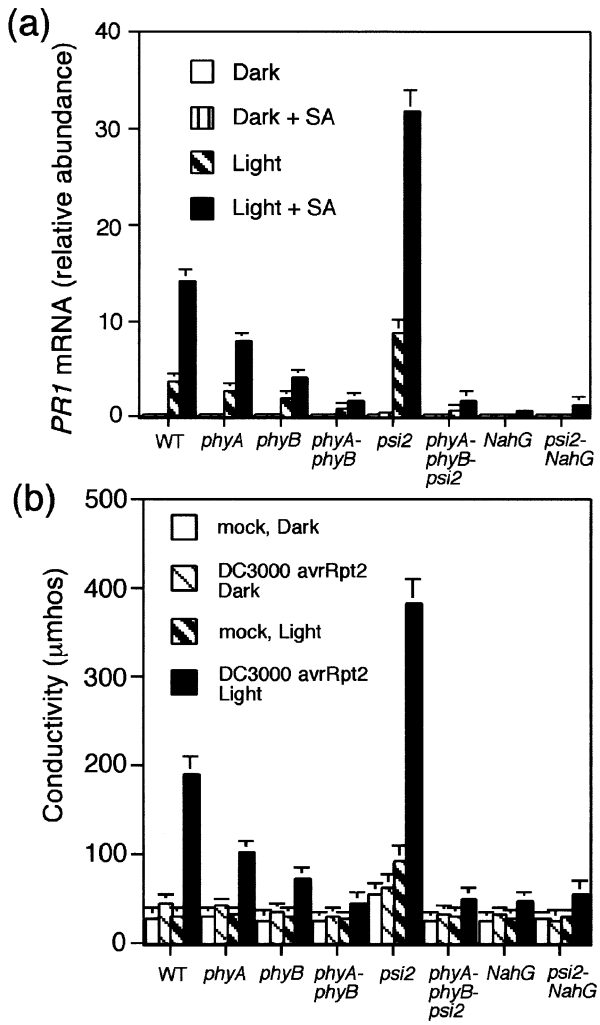

Figure 2. The modulation of defense by light is phytochrome-dependent.
WT, *psi2*, *phyA-phyB*, and triple mutant *phyA-phyB-psi2* grown in darkness or under high white light fluence rate on a 3% sucrose-containing medium. Plants were injected 5 days after germination with 250 μM SA under very low (dark) fluence rate (0.1 μmol m^{-2} sec^{-1}) or under light condition (30 μmol m^{-2} sec^{-1}).
(a) *PR1* expression. Total RNA was extracted 48h after the treatment and used for gel blot analysis.
(b) Electrolyte release in tissue infected by the avirulent *Pseudomonas syringae* pv. *tomato* DC3000 containing the avirulent gene *avrRpt2*. Ion leakage in discs of infected leaves was measured by conductometry.

expression, and a higher susceptibility to virulent pathogens (Delaney *et al.*, 1994). SA has also been implicated in the gene-for-gene defense responses (Delaney *et al.*, 1994).

Recent biochemical and genetic investigations have brought some insights on the nature of the components linking SA to the defense responses. In *Arabidopsis*, several mutants with a defect in the activation of *PR* genes have been isolated, some of them contain mutations that interrupt the signal transduction downstream of SA. For instance, in the *npr1*, *nim1* and *sai1* mutants (respectively: No PR gene expression, Cao *et al.*, 1994; non-inducible immunity, Delaney *et al.*, 1995; SA insensitive, Shah *et al.*, 1997) no activation of *PR* genes occurs in response to application of SA or functional analogues. In consequence, these mutants display a higher susceptibility to infectious agents. The *NPR1/NIM1/SAI1* encodes for a protein containing ankyrin repeats (Cao *et al.*, 1997; Ryals *et al.*, 1997). Upon activation of SAR, NPR1/NIM1/SAI1 accumulates in the nucleus (Kinkema *et al.*, 2000) where it interacts with a basic leucine zipper protein transcription factor (Zhang *et al.*, 1999), a factor binding to sequences of the *PR1* gene promoter.

Other mutants, such as *lsd, cpr*, and *dnd,* constitutively express the *PR* genes (reviewed in Dangl *et al.*, 1996; Dietrich *et al.*, 1997). Most of them show an accumulation of SA together with a high *PR* expression.

Studies with *Arabidopsis* and maize mutants that develop spontaneous HR lesions and transgenic tomato expressing the R gene *Pto,* have suggested that light might

Table 1. Effect of light perception on the expression of *PR* genes and the growth of an avirulent pathogen (*Pseudomonas syringae* pv. *tomato* DC3000 carrying *avrRpt2*)

	Dark (0.1 μmol m^{-2} sec^{-1})		Light (25 μmol m^{-2} sec^{-1})	
Plants:	Bacterial titre[a] (± SD)	*PR1* expression[b] (± SD)	Bacterial titre[a] (± SD)	PR1 expression[b] (± SD)
Wt	7.4 (0.5)	2.1 (0.8)	5.3 (0.8)	16.3 (2.1)
phyA-phyB	7.7 (0.4)	0.8 (0.7)	7.8 (0.6)	3.2 (0.6)
psi2	7.3 (0.7)	2.3 (0.9)	4.1 (0.4)	35.8 (4.2)
phyA-phyB-psi2	7.7 (0.6)	1.3 (0.7)	7.7 (0.4)	3.6 (0.5)
NahG	7.5 (0.5)	0.5 (0.3)	7.9 (0.5)	2.8 (0.4)
NahG-psi2	7.6 (0.8)	0.4 (0.2)	7.8 (0.5)	4.0 (0.5)

[a]Plants were injected with a solution of 0.5 $\times 10^3$ bacteria cm^{-2} and the number of colony-forming units were measured 3 days after injection; data are expressed in Log cfu cm^{-2}.
[b]Relative abundance.

influence the formation of defensive cell death in plants (Dietrich *et al.*, 1994; Tang *et al.*, 1999). In this article, the role of the light signal transduction pathway on the control of SA-dependent HR and *PR* gene expression has been examined.

Results

Light potentiates the response of plants treated with SA and BTH

To estimate the influence of light on perception of SA, the expression of *PR1* was determined in plants grown on medium containing 3% sucrose and exposed to an increasing intensity of continuous white light after treatment with 250 μM SA or of its functional analogue BTH (Figure 1). At low fluence rates of white light the effect of the two inducers on *PR1* expression was almost suppressed. This expression was re-established only at a fluence rate above 5 μmol m^{-2} sec^{-1}, increasing exponentially with higher intensities. The same correlation was observed at higher concentrations of SA and BTH (data not shown).

The modulation of PR1 expression and HR by light is phytochrome-dependent

Since phytochrome A and B are crucial photoreceptors in *Arabidopsis*, we tested their possible involvement in the modulation of the *PR1* expression by light. Plants with different phytochrome activity were grown in darkness and in continuous high fluence rate of white light, and the expression of *PR1* gene was measured in the leaves after treatment with 250 μM SA. While SA induces *PR* expression under high light fluence rates, this response is almost completely absent in darkness (Figure 2a), and the absence of both phytochrome A and B receptors (phyA and phyB) gives rise to a strong reduction in the expression of *PR* genes upon treatment with SA, in a clearly cumulative manner, with a stronger influence of phyB deficiency (Figure 2a). In accordance with these results, the expression of *PR1* in the mutant *psi2* (which is characterized by a hyperactive phytochrome pathway) is amplified in a phytochrome- and SA-dependent manner, since the absence of phyA and phyB, as well as the presence of the bacterial gene *NahG* (encoding for a salicylate hydroxylase that degrades SA; Gaffney *et al.*, 1993) strongly reduce the expression of the *PR1* gene in *psi2*. These results indicate that phytochrome signalling strongly modulates the response of *Arabidopsis* to endogenous SA.

Among the mechanisms of defense, the formation of local HR lesions is often correlated with the induction of *PR* genes (Glazebrook, 1999; Sticher *et al.*, 1997), we therefore tested whether the HR formation in the leaves of *Arabidopsis* was also light-modulated by measuring the amplitude of cell death elicited by the avirulent pathogen *Pseudomonas syringae* pv. *tomato* DC3000 containing the avirulence gene *avrRpt2* in infected leaves of *Arabidopsis*. The degree of HR was measured by conductometry of the electrolytes liberated by leaf discs of *phyA* and *phyB* mutants and double mutants, of plants containing the *psi2* mutation, and of plants expressing the *NahG* gene. Figure 2(b) presents the results of infections in these plants, and shows that the formation of HR is strongly reduced by the absence of phytochrome and amplified, in a SA-dependent manner, in the *psi2* mutant.

The reciprocal interference, i.e. the modulation of the phytochrome signalling by the SA pathway, was examined by testing the influence of SA or BTH on the induction of typical light-controlled gene expressions. The data indicated that, whereas the light perception apparatus potentiates the SA signalling pathway, the reverse effect does not take place (Table S1).

The modulation of PR1 expression by light occurs downstream of SA-synthesis

As a variation in the accumulation of endogenous SA, associated with the activity of the phytochrome pathway, might explain the difference of *PR1* expression in WT, *phyA-phyB,* and *psi2* plants, we determined the SA levels in 3-week-old plants exposed to various light intensities. No significant variations in endogenous SA levels were found between WT, *phyA-phyB,* and *psi2* genotypes, even if a 3-fold increase of the background SA content paralleled the increasing light intensity (Figure 3). Hence the modulation of the SA-controlled *PR1* expression by phytochrome signalling occurs downstream of SA production. In addition, no significant correlation was observed between the chlorophyll concentration and the amplitude of *PR1* expression in these plants (data not shown).

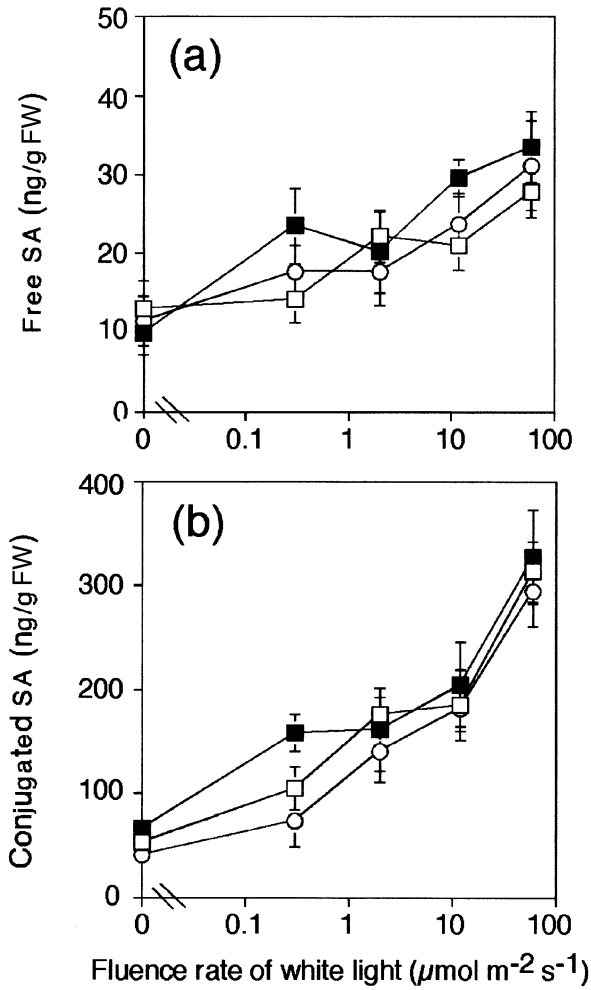

Figure 3. **S**A content is not correlated to phytochrome activity.
Free and conjugated SA content in *phyA-phyB* (○), psi2 (■) and WT (□) was measured 5 days after germination in seedlings exposed to increasing light intensity.

The potentiation of SA signalling by light is independent of protein synthesis

The dependency of the light potentiation effect on protein synthesis was tested in SA-treated plants that received a prior treatment with cycloheximide, an inhibitor of protein synthesis (Figure S1). The light-modulated expression of PR1, 30 min after a BTH exposure, is mostly insensitive to cycloheximide.

The perception of light modulates the resistance against Pseudomonas syringae

High levels of *PR* gene expression are frequently correlated with an increase in plant resistance. Since the activity of the phytochrome pathway has a strong effect on the SA-dependent expression of *PR1*, and therefore could affect the efficiency of the plant defense reaction, we compared the ability of a plant pathogen to grow on leaves of WT plants, or *phyA-phyB* and *psi2* mutants under a moderate fluence rate of white light (25 μmol m^{-2} sec^{-1}). Leaves of the three genotypes were inoculated with the avirulent strain of *P. syringae* pv. *tomato* DC3000 carrying *avrRpt2* (Nawrath and Métraux, 1999) and bacterial growth was scored by counting colony-forming units (Dangl *et al.*, 1992) 3 days following infection. Table 1 shows that the pathogen growth is increased in the double-mutant plants *phyA-phyB* but clearly reduced in the *psi2* mutant hypersensitive to light. These results suggest that light perception may be an important factor affecting the general process of defense; they also confirm the existence of a correlation between the level of *PR1* and the resistance of the plant. In addition, it has been observed that during a compatible interaction, the growth of a virulent strain of *Pseudomonas* is similarly affected by the plant perception of light through phytochrome A and B (data not shown).

Role of chloroplasts in the SA-controlled pathway

The modulation of the SA-perception by phytochrome signalling suggests that one, or many, light-dependent functions, such as the synthesis of sugars, are engaged in the defense process. In etiolated seedlings, neither HR nor *PR* expression, in the presence and absence of a carbon source (3% sucrose in the medium), have been observed (Figure 1 and data not shown). This indicates that SA-dependent defenses do not depend on the carbohydrate production by chloroplasts. To determine the involvement of these organelles in the defense process controlled by SA, we compared the response of green and white areas of variegated mutants of *Arabidopsis*. Such variegated plants were produced by crossing phytochrome-related mutants with mutants presenting a leaf variegation phenotype (as

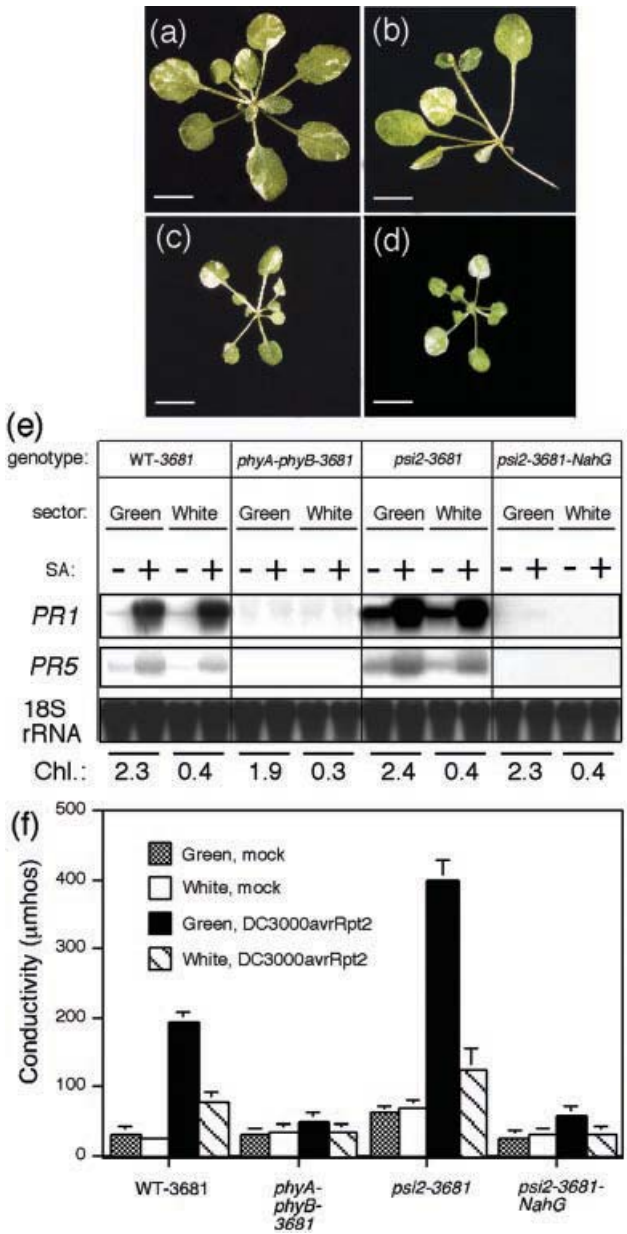

Figure 4. Influence of chloroplasts on the phytochrome-modulated defense responses.

Phytochrome-related mutants were crossed with variegated mutants of *Arabidopsis* and the expression of *PR1* as well as ion leakage induced during an incompatible response were measured in white versus green sectors.

(a) Single mutant 3681-variegated.

(b) Triple mutant 3681-variegated *phyA-phyB*.

(c) Double mutant 3681-variegated *psi2*.

(d) Double mutant 3681-variegated *psi2* containing the *NahG* transgene.

(e) *PR*-expression in green and white tissues of the 3681-variegated mutant, and of the triple mutant 3681-variegated *phyA-phyB* treated with 250 µM SA.

The average chlorophyll content (Chl., bottom of panel (b) of each sector was quantified (expressed in mg chlorophyll per mg protein). Bars: In (a) (b) (c) (d): 5 mm.

described in the seed library of the *Arabidopsis* Stock Center) but no developmental alterations (Figure 4a-d).

The *PR1* expression was scored in green versus white zones in the variegated WT and in variegated *phyA-phyB* and *psi2* as well as *psi2-NahG* (Figure 4e). Under white light exposure, in the presence and absence of SA, no difference in *PR1* expression was observed, with and without treatment with SA, on green and white areas, and in both the single and the triple mutants.

Similarly, the extent of the HR in the same genotypes was quantified by conductometry after injection with the *avrRpt2*-containing *Pseudomonas* strain (Figure 4f). In this case, a low number of functional chloroplasts produces a significant reduction in the release of electrolytes, a reaction that is characteristic for the HR observed during an incompatible interaction.

Discussion

In our experiments, a strict light- and phytochrome-dependency of the *PR* gene expression and HR process during the incompatible response was observed. Since the level of endogenous SA is not affected by light under the conditions where the SA-induced expression of *PR* genes is increased, we conclude that the phytochrome-signalling pathway modulates the plant sensitivity to SA. This is supported by a strong correlation between the activities of the SA- and phytochrome pathways. For instance, in the light-hypersensitive mutant *psi2*, the sensitivity to SA was also increased, whereas plants lacking both phyA and phyB receptors exhibited a strong reduction of their response to SA. These effects show no dependence on the developmental stage, at least during the growth phase, since the SA-related responses in young (7-day-old) seedlings as well as older *Arabidopsis* plants (3-week-old) were similar (or comparable). The light requirement is also independent of the sugar availability, since treatments of plants growing on sucrose-containing medium or on soil produced the same effect.

Because the phytochrome signal precedes and controls the activation of genes related to light, we deduced that light must have a function in the SA-controlled defense. This may occur at various levels, the most evident being the photosynthetic apparatus. We tested this conjecture by examining variegated mutants forming chloroplast-deficient leaf zones near to intact green areas. This variegated phenotype was introduced in various light-response mutant backgrounds. Results of this study indicate that a signal resulting from both the SA- and the phytochrome pathways controls the regulation of the HR induced by an avirulent pathogen. Indeed, whereas the expression of *PR*s is not modified by the reduction of active chloroplasts (in the white sectors of the variegated plants), the characteristic leakage of electrolytes observed during HR is strongly

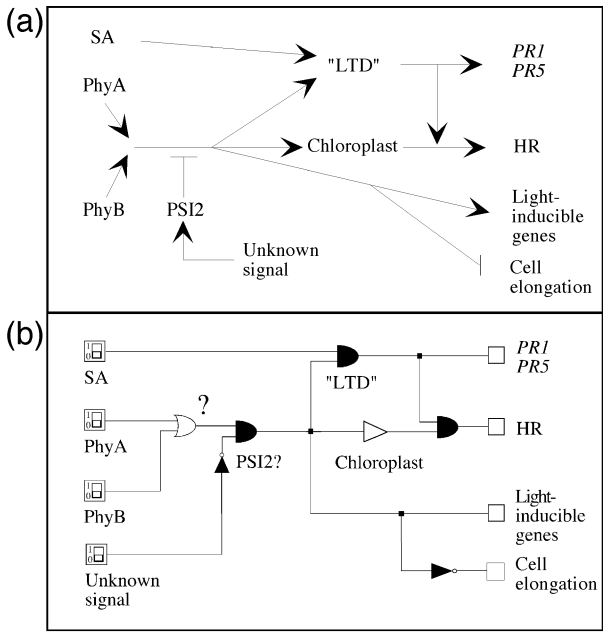

Figure 5. Schematic representation of the modulation of defense by phytochrome using intuitive (a) and Boolean formalism (b).
In (b), the respective inputs are located on the left, outputs are on the right and represent physiological reactions or gene expressions. A signal emitted by phyA and phyB, and regulated by the PSI2 molecule, is integrated with a SA-dependent signal to modulate the expression of *PR1*. The regulation of HR needs the integration of an additional chloroplastic and phytochrome-dependent factor/signal. In (b) the connection from light to defense has been represented by an AND operator (called LTD, for '*l*ight *t*o *d*efense').

reduced in the same white sectors upon interaction with an avirulent *Pseudomonas* strain. This is a clear indication for an implication of a chloroplastic signal/factor in the control of the pathogen-activated pathway leading to HR. To be released, this plastidic signal may depend upon the presence of light or of a fully functional chloroplastic machinery. At least our results indicate that the known involvement of SA in the positive control of HR (Rate *et al.*, 1999; Tenhaken and Rubel, 1997) depends on the association of a chloroplastic and a SA-dependent signal (Figure 5). This operation of integration must include a molecular operator that can be the target of a genetic investigation. A possible reason for the interference of the chloroplast in HR can be the implication of the photosynthetic apparatus as a producer of AOS during pathogen attack, thus, in the presence of light, a chloroplast-dependent defensive pathway might be pre-selected. It is known that uncoupling or inhibition of the photosystem machinery leads to the formation of active oxygen species (AOS), which can affect the oxidoreductive homeostasis in the chloroplast or cytoplasm. These molecules have also been implicated in the process of defense (Jabs *et al.*, 1996; Levine *et al.*, 1994) both as signalling molecules and deleterious species. Superoxide might co-operate with the HR process via lipid peroxidation and ensuing membrane damage (Lamb and Dixon, 1997). Hence, in addition to a possible activation of a plasmalemma-associated NADPH oxidase, an alternative source for superoxide and H_2O_2 might be the plastids. Support for this hypothesis is provided by the observation that a tomato overexpressing the *Pto* resistance gene displays HR lesions upon exposure to high light (Tang *et al.*, 1999), exclusively in the green mesophyll cells, a tissue-specificity that suggests a role for chloroplasts in a particular step of the HR formation. The fact that *PR* gene expression is not affected by a decrease in chloroplast number suggests that light might be involved directly or in connection with the activity of one or several *PR*s but independently of the photosynthetic apparatus.

What molecules might control the phytochrome potentiation of defense in plants? Possible candidates are any light-controlled elements involved in signal transduction downstream of SA perception. Phytochrome signals, by tuning the level of such an element, could directly modify the sensitivity of the SA pathway in function of light intensity. Calcium-controlled steps have been implicated in light (Neuhaus *et al.*, 1993) and defense signalling pathways (Levine *et al.*, 1996). Therefore, any specific modulator of calcium metabolism could be a potential candidate for the inductive signal. Results from experiments with inhibitors of the high-light inducible HR in *psi2* (data not shown) suggest that a calcium-controlled protein, such as a CDPK (Hong *et al.*, 1996), could be involved in the induction of lesions.

It is possible to infer from our data the existence of a specific dark-setting of the SA-dependent defense, since the results suggest that light-independent defense mechanisms (dark-defense) also occur in plants. These mechanisms may compensate for the reduced HR observed under low light exposures. One example is the SA-independent pathway regulating the expression of the defensin gene *PDF1.2* (Figure S2). The specific SA subpathways activated in presence and absence of light will be the object of further experiments and represents a starting conjecture to set out microarray analysis of *PR* gene expression in dark versus light conditions.

As a conceptual framework, we have proposed that the structure of signalling networks is encoded in the genome following a digital language (Genoud and Métraux, 1999). We applied such an approach to scheme the crosstalk of light perception with the SA-pathway (Figure 5b), this permits a more precise description of pathway interactions and the possibility of a direct qualitative simulation on a computer (Genoud *et al.*, 2001).

Materials and methods

Plant growth conditions

Seeds of the various genotypes were routinely soaked and sterilized once with 70% ethanol for 1 min, then with 30% hypochlorite solution containing 0.1% Tween 20 for 10 min, and finally washed with three changes of sterile water, and sown on Murashige and Skoog (JRH Biosciences, Lenexa, KS, USA) solid medium containing 3% sucrose. Plates were kept in the dark at 4°C for 4–5 days before germination. To synchronize seed germination, plates were exposed to 20–30 min white light (25 μmol m^{-2} sec–1) after 3 days imbibition in darkness. Continuous white light of various fluence rates was provided by fluorescent bulbs (GRO-LUX F96T 12/GRO; Sylvania Co., Danvers, MA, USA). The fluence rate was attenuated by covering the plates with a combination of paper filters.

Red and far-red light were supplied by LED light sources (Quantum Devices, Barnfeld, WI, USA). Far-red light was filtered through a Plexiglas layer (model 067894; West Lake Plastics, Lenni, PA, USA).

Northern blot analysis

For RNA extraction, the seedlings were harvested with a sterile microscope glass and the tissues were immediately frozen in liquid nitrogen. RNA was prepared from samples kept at –80°C using the aurintricarboxylic acid (ATA) method (Barnes *et al.*, 1996). RNA gels (20 μg $lane^{-1}$) were blotted onto nylon filters (Stratagene, La Jolla, CA, USA) and hybridized with random-primed probes prepared according to the manufacturer's recommendations (Amersham Biosciences Corp., Piscataway, NJ, USA). Membranes were routinely stripped for reprobing. *PR1* probe and *PR5* probe were *Xho*1-*Eco*RI fragments of the respective coding sequences cloned into pBluescript (Stratagene); the vectors containing *PR* probes were a gift from the laboratory of John Ryals (Novartis, Research Triangle Park, NC, USA). As loading control for total RNA, we used an 18S rDNA fragment cloned from *Arabidopsis* (Takahashi *et al.*, 1995). Hybridization signals were quantified using a Model 400E PhosphorImager (Molecular Dynamics, Sunnydale, CA, USA).

Dosage of SA content

400–600 mg of seedlings was harvested as previously described, weighed in plastic tubes, frozen in liquid nitrogen and kept at –80°C. Extraction and analysis of SA content were performed as described in Mölders *et al.* (1996). Protein concentration was determined with the BCA protein assay reagent from Pierce (Pierce Chemical Co., Rockford, IL, USA).

Chlorophyll content measurement

Leaf chlorophyll content was measured as described by Takahashi *et al.* (1995) and protein concentration was determined with the BCA protein assay reagent kit (Pierce Chemical Co., Rockford, IL, USA).

Treatment with chemicals

Solutions containing SA and BTH in phosphate buffer at pH 7.5 with 0.02% Triton X-100 were sprayed onto plants or injected into leaves with a 1-ml syringe (approximately 10 μl per leaf), in darkness under green safe light or in dim white light at 0.1 μmol m^{-2} sec^{-1}. As a control, wetting powder (Görlach *et al.*, 1996) suspended in the same buffer, was used. An additional cycloheximide solution (300 μM) was injected in dark-adapted plants 45 min before the SA treatment and a subsequent transfer to light; protein inhibition (85%) was measured as described in Ahmad *et al.* (1998). Rose bengal, a specific activator of defensin genes (Penninckx *et al.*, 1996), was applied on leaf as 5 μl droplets of a 20-mM solution (5 droplets per leaf).

Bacterial infection

Plants germinated and grown on plates for 7 days were transplanted to sterilized soil and kept for 15 days under the following conditions: 60% humidity, 30 μmol m^{-2} sec^{-1} white light (9 h L; 18 h D) at 20°C. Bacterial growth and leaf infection were performed as described in Dangl *et al.* (1992) and Davis *et al.* (1991). *P. syringae* pv. *tomato* strain DC3000 (containing the avirulence gene *avrRpt2*) in suspension was injected through stomatal pores into whole leaves, at a concentration of 10^5 cfu ml^{-1} in 10 mM $MgCl_2$ (0.5×10^{-3} bacteria cm^{-2}) by using a 1-ml syringe, without needle. Control plants were injected with the buffer only. After infiltration the plants were transferred to continuous white light, under a transparent plastic foil (during the first 2 days post-infection). Leaf disks were then excised with a 0.5-cm corkborer at indicated times and homogenized in 0.4 ml $MgCl_2$. After various dilutions, homogenates were plated on agar plates containing selective King's B medium. Data were expressed as colony-forming units (cfu) per leaf surface.

Electrolyte leakage measurement

The level of HR was scored in the plants by measuring the release of ions during infection of leaves. Injected plants were incubated for 8 h under medium fluence rate of white light (25 μmol m^{-2} sec^{-1}). Leaves were then removed and discs (0.5 cm diameter) cut in the infected area using a corkborer. The discs were washed 3 times for 5 min in distilled water as described in Mittler *et al.* (1996) before being floated 3 by 3 on 500 μl distilled water in a well of a 24-well plate with shaking for 6 h. The conductance of the solution corresponding to 6 discs (1 ml) was measured for each sample with a conductometer (model E587, Metrohm, Herisau, Switzerland), each data corresponds to the mean of 3 samples.

Multiple mutants and NahG*-containing plants*

Triple mutants *psi2-phyA-phyB* were obtained by crossing a double mutant *phyA-201/phyB-1* with a double mutant *phyA-201/psi2* (Genoud *et al.*, 1998), followed by selection of triple mutant lines through examination of the hypocotyl elongation under high red light fluence (40 μmol m^{-2} sec^{-1}) of the F_2 seedlings; plants with the longest hypocotyl were selected and backcrossed with a *phyB-1* and a *psi2* mutant. Three F_2 lines, whose F_1 generation of a backcross with *phyB-1* presented a long hypocotyl phenotype in high red light, and whose F_1 generation of a backcross with *psi2* mutant presented a *psi2* phenotype, were selected and amplified.

Plants containing the *NahG* transgene of *P. putida* were created by crossing plants with *Arabidopsis* containing the transgene (Gaffney *et al.*, 1993) followed by a PCR selection (presence of *NahG*) of the *psi2* plants in the F_2 and F_3 generations. Five lines of

psi2-NahG were examined for their response to red light. The primers used for amplifying *NahG* gene were: 5′-GAAAAAC-AATAAACTTGGCTTGCG-3′ (F-primer) and 5′-GAAACTCGTAT-AACTCGCCGGTC-3′ (R-primer). The gene product of the right size (1144 bp) was sequenced for authentication.

To obtain mutants with variegation, potentially variegated plants with a nuclear recessive mutation were selected following the seed banks description (Nottingham *Arabidopsis* Stock Center and *Arabidopsis* Biological Resource Center). Nine genotypes were grown and examined for the development of variegation on leaves, namely: CS 3131, CS 3246 (*chm1–2*), CS 3320, CS 3446, CS 3621, CS 3659, CS 3681, N 457, and N 519. The variegated mutants presenting no developmental alterations and displaying transient white areas were selected (CS 3681, CS 3446) and crossed with *psi2 and* with *psi2-NahG* plants. F_2 seedlings presenting *psi2* phenotype and variegated leaves, and variegated *psi2* containing *NahG*, were isolated and amplified. To obtain triple mutants *phyA-phyB* with variegation on leaves, we proceeded as for the creation of the triple mutant *phyA-phyB-psi2,* but replaced the crosses with *psi2* mutant by crosses with the variegated plants CS 3681 and CS 3446. Plants were further selected for the presence of leaf variegations and for a block in both red and far-red light perception, as described above.

Acknowledgements

We thank Leslie Friedrich and John Ryals for *PR* plasmids; Christiane Nawrath and Liliane Sticher for discussions; Dominique Genoud for advice on computer analysis; the Nottingham *Arabidopsis* Stock Center and *Arabidopsis* Biological Resource Center for seeds of variegated plants and EST plasmids. Support from the Swiss National Science Foundation (FN 3100–055662.98) is gratefully acknowledged.

Supplementary Material

The following material is available from http://www.blackwell-science.com/products/journals/suppmat/TPJ/TPJ1338/TPJ1338sm.htm

Figure S1 The light modulated expression of *PR1* in SA-treated plants is independent of protein synthesis. Three-week-old WT plants were pretreated with cycloheximide, injected with 250 μM SA, and exposed to high light fluences for 30 min.

Figure S2 The expression of a defensin gene is not upregulated by light: Expression of *PDF1.2* under increasing light intensity.

Table S1 Effect of red light on the induction of CAB and ChS expression. Five-day-old seedlings grown in darkness were treated with SA or BTH alone, or in conjunction with a pulse of 5 min red light (25 μmol m^{-2} s^{-1}). Each value represents the average response of two sets of 150 seedlings

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