Expression of Uroporphyrinogen Decarboxylase or Coproporphyrinogen Oxidase Antisense RNA in Tobacco Induces Pathogen Defense Responses Conferring Increased Resistance to Tobacco Mosaic Virus*

Hans-Peter Mock‡**, Werner Heller†, Antonio Molina***, Birgit Neubohn‡, Heinrich Sandermann, Jr.†, and Bernhard Grimm‡

From the ‡Institut für Pflanzenzüchtungskunde and Kulturpflanzenforschung, Correnstrasse 3, D-06466 Gatersleben, the †Institut für Biochemische Pflanzenpathologie, GSF-Forschungszentrum für Umwelt und Gesundheit, D-85764 Oberschleißheim, Germany, and ||Biotechnology and Genomics Center, Novartis Crop Protection, Inc., Research Triangle Park, North Carolina 27709-2257

Transgenic tobacco plants with reduced activity of either uroporphyrinogen decarboxylase or coproporphyrinogen oxidase, two enzymes of the tetrapyrrole biosynthetic pathway, are characterized by the accumulation of photosensitizing tetrapyrrole intermediates, antioxidative responses, and necrotic leaf lesions. In this study we report on cellular responses in uroporphyrinogen decarboxylase and coproporphyrinogen oxidase antisense plants, normally associated with pathogen defense. These plants accumulate the highly fluorescent coumarin scopolin in their leaves. They also display increased pathogenesis-related protein expression and higher levels of free and conjugated salicylic acid. Upon tobacco mosaic virus inoculation, the plants with leaf lesions and high levels of PR-1 mRNA expression show reduced accumulation of virus RNA relative to wild-type controls. This result is indicative of an increased resistance to tobacco mosaic virus. We conclude that porphyrinogenesis as a result of deregulated tetrapyrrole synthesis induces a set of defense responses that resemble the hypersensitive reaction observed after pathogen attack.

All organisms contain tetrapyrroles that play important roles in the transfer of energy, redox sensing, or catalysis. Chlorophylls are the most abundant tetrapyrroles in plants and are involved in the harvesting of light and its subsequent conversion to chemical energy. In response to varying environmental conditions the coordinated synthesis and stoichiometric assembly of the components of the photosynthetic complexes ensure an adjusted electron flow and avoid exacerbated oxidant production (1). In addition to the regulatory mechanisms, plants have evolved effective protection systems against photooxidative stress consisting of enzyme and low molecular weight antioxidants (2).

Genetic or chemical impairment of chlorophyll or heme biosynthesis results in the accumulation of photosensitizing tetrapyrroles, e.g. by feeding of the precursor 5-aminolevulinate or by application of diphenyl ether herbicides acting on protoporphyrinogen oxidase (3). Formation of reactive oxygen species such as singlet oxygen is essential for the photodynamic action of tetrapyrroles (3). Expression of antisense or additional sense genes encoding enzymes of the tetrapyrrole metabolism leads to modified enzymic activities and, in consequence, to an imbalance of the substrate flow. Transgenic tobacco plants with reduced uroporphyrinogen decarboxylase (UROD)1 or coproporphyrinogen oxidase (CPO) were characterized by light-independent formation of leaf lesions and by stunted growth (4, 5). Reduced activity of either of the two enzymes may increase the level of porphyrinogen substrates up to 500-fold in transformants relative to control plants. These compounds, predominately uroporphyrinogen or coproporphyrinogen in UROD or CPO antisense plants, respectively, can be photooxidized, thus triggering photodynamic cellular destruction. Cellular stress responses observed upon accumulation of these tetrapyrroles included increased steady state levels of antioxidant mRNAs and increased activity of enzymes involved in stress defense. In particular, higher activities of superoxide dismutase in younger leaves and of ascorbate peroxidase in older leaves were observed (6). In addition to the plastidic superoxide dismutase isomorph, mitochondrial and cytosolic isomorphs contributed to the increased total enzyme activity suggesting that tetrapyrroles initially synthesized in the plastids may spread to other compartments where they induce local antioxidative defense responses (6). However, limitation of the detoxifying system was indicated by a decrease in the total content and a higher percentage of the oxidized form of ascorbate, reduced content of glutathione, and the loss of tocopherol (4, 6).

In addition to the well established roles of these protective components, phenolic constituents also contribute to the antioxidative capacity of cells (7, 8). Different phenylpropanoids are formed in response to various stresses such as wounding, high light, UV radiation, or pathogen attack. These molecules often exert antimicrobial and UV protecting or signaling functions (9). We have continued to examine the protective responses to oxidative stress following the accumulation of tetrapyrroles in UROD and CPO antisense plants with the...
The chemical synthesis of scopolin was essentially according to Ref. 13 with the following modifications. A mixture of 38.4 mg of scopolin (0.2 mmol), 90.4 mg of acetobromoglucoside (0.22 mmol), 46.4 mg of silver(I) oxide (0.2 mmol), 56 mg of drierite (calsaltate, 0.4 mmol), and 1 mg of 1,4-diazabicyclo[2,2,2]octane in 1 ml of dry pyridine were mixed in an Eppendorf tube and stirred overnight in the dark. After centrifugation the supernatant was separated and the solvent removed under a stream of nitrogen. The residue was dissolved in 0.5 ml of trichloromethane and applied to a preparative silica gel plate purified twice before use with trichloromethane/methanol 1:1 (v/v) containing 2% (v/v) acetic acid. The plate was developed with trichloromethane/glacial acetic acid/water, 70:28:2 (v/v/v), and air-dried. The band at Rf = 0.8—0.9 ahead of the residual scopolin was scraped off, the scopolin tetracacetate eluted with trichloromethane/methanol, 8:2 (v/v), and the solvent removed in vacuo.

The crude scopolin tetracetate was treated with 5 ml of 0.5 M barium hydroxide at 80 °C for 15 min, and the reaction mixture was then cooled to room temperature and brought to pH 7 with 2 M sulfuric acid. The barium sulfate formed was precipitated by centrifugation, the supernatant separated, and the solvent removed in vacuo. A residue was obtained. Recrystallization with 7% glacial acetic acid and 1 mg of 1,4-diazabicyclo[2,2,2]octane in 1 ml of dry pyridine were added to the residue. The preparations were finally resuspended in 20% methanol prior to HPLC analysis which was performed using the HPLC system described above with fluorescence detection. The mobile phase consisted of solvent A and solvent B containing 80% methanol in solvent A (solvent A) and 80% methanol (solvent B1) and a gradient from 0 to 30% B1 over 30 min. Elution was performed at a flow rate of 1 ml min⁻¹ with a hyperbolic gradient from 10% solvent B to 45% B in 25 min using curve 5 offered by the Millenium software. The column was then washed with 100% solvent B and equilibrated. Salicylic acid eluted at 16.4 min. Recovery rates for each individual experiment were determined by spiking parallel samples with appropriate amounts of authentic SA.

Fluorescence Microscopy—Hand-cut cross-sections of leaves were mounted in water and viewed under a Zeiss Axiosvert microscope with a Fluor 20 × 0.75 UV filter. The filter combination used was as follows: excitation BP 365, DBS 395; emission LP 397. Photos were taken using a Kodak Elite 400 slide film.

Western Blot Analysis—Protein extraction, SDS-polyacrylamide gel electrophoresis, and immunoblotting were performed as described previously (4). Protein was quantified according to Ref. 15 using bovine serum albumin as a standard. Antibodies raised against tobacco PR-1c, -2, -Q, and -S were a gift of Drs. P. Geoffroy and B. Fritig (Université Louis Pasteur, Strasbourg, France). The immunospecificity of the antiserum has been described (see Ref. 16 and references therein).

TMV Inoculation and RNA Analysis—Samsun NN tobacco plants, a local lesion host for TMV, UROD, and CPO antisense plants were grown under controlled conditions favoring development of necrotic lesions (6). Ten-week-old plants were inoculated with a suspension of the Flavum
strain of TMV (0.5 mg/liter) in a solution of 10 mM sodium phosphate (pH 7) containing carborundum as described previously (17). In parallel, other plants were mock-inoculated with buffer and carborundum only. At least two leaves per plant and five plants per transgenic line or wild-type were used for inoculation. Leaves were harvested directly before and 5 days after TMV inoculation, and total RNA was isolated by phenol/chloroform extraction followed by lithium chloride precipitation (18). Total RNA (7.5 μg) was subjected to electrophoresis on formaldehyde/agarose gels and blotted to Hybond-N membranes (Amersham, Braunschweig, Germany) as described (19). Equal loading of samples was confirmed by including 40 μg/ml ethidium bromide in the sample loading buffer allowing visualization of RNA under UV light. 32P-Labeled probes were synthesized using the random primers DNA-labeling system (Life Technologies, Inc.). The CDA probes for tobacco PR-1 and reverse-transcribed TMV-RNA have been previously described (20). Hybridization and washing were carried out at 65 °C as described (21). Relative amounts of TMV transcripts were quantified using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) according to the manufacturer’s instructions.

### RESULTS

A Highly Fluorescent Phenolic Compound Accumulates in Leaves of UROD and CPO Antisense Plants—Fig. 1 shows chromatograms obtained from methanolic extracts of leaf 9 of a UROD antisense plant. The main peak observed by UV detection at 280 nm (24.6 min) (Fig. 1A) was identified as chlorogenic acid (CGA) by co-chromatography with authentic CGA, combined with diode array and electrospray mass spectrometry (ES-MS) as described under “Experimental Procedures.” In leaf extracts of transgenic plants the chromatograms recorded by using fluorescence detection showed a dominant peak (24.4 min) eluting very close to CGA (24.9 min) (Fig. 1B). This peak corresponds to the small signal observed at 24.1 min in the UV chromatogram (Fig. 1A, see arrow). The insert in Fig. 1A shows the diode array spectrum of this peak. This compound was absent in UV chromatograms in extracts of wild-type plants (data not shown), and only a small signal was found by fluorescence detection (Fig. 1C). Comparing the integrated peak areas obtained from control and transformed plants on a fresh weight basis, the content of this compound was increased (up to 23-fold) in leaves of the transformants (see Table I below). The content of other constituents remained unchanged or differed only slightly in leaves of control and transformed plants.

Isolation and Identification of the Fluorescent Compound, Scopolin—Based on the strong fluorescence (22) and on the UV spectrum (see inset, Fig. 1A (23)), we tentatively identified the unknown substance as a coumarin. The metabolite was isolated from necrotic leaves of UROD antisense plants by HPLC as described under “Experimental Procedures.” It was identified as scopolin based on HPLC analysis of a β-glucosidase digest, mass spectrometry, and comparison with authentic standards. The isolated compound was incubated with or without β-glucosidase and separated by HPLC (Fig. 2). After glucosidase digestion the fluorescent peak at 24 min completely disappeared, and one new peak of approximately the same fluorescence intensity became apparent. The retention time of this product was identical to that of scopolitin, the aglycon of scopolin, as demonstrated by cochromatography with authentic scopolitin (Fig. 2B). The product released by β-glucosidase also displayed the same diode array spectrum as scopolitin (data not shown). Isoscopolitin clearly differed in its elution time (28.9 min) under the same chromatographic conditions and showed a much lower ratio of peak area to fluorescence by UV detection (data not shown). Analysis of the isolated metabolite by electrospray-mass spectrometry (ES-MS) gave a mono-isotopic mass of 354.1 identical with the value of 354.1 calculated for scopolin, C_{16}H_{18}O_{9}. The collision-induced fragmentation of the protonated molecular ion produced one intense fragment ion at m/z 193.0 (Fig. 3) resulting from the loss of the glucose moiety. The appearance of this ion and a simultaneous reduction in intensity of the protonated molecular ion at 355.1 was observed when the temperature of the drying gas was elevated (data not shown). The chemical structure was further confirmed by chemical synthesis of scopolin from scopolitin and acetobromoglucose. The product obtained after alkaline hydrolysis of the tetra-acetyl intermediate was identical to the compound isolated from necrotic leaves of UROD antisense plants when analyzed by the methods described. In addition, both preparations gave an identical pattern of product ions when the molecular ions at m/z 193 were further fragmented (data not shown). The content of scopolin in leaves of transformants and controls was quantified using the chemically synthesized compound for calibration (Table I).

Scopolin Accumulates in Necrotic Leaf Areas of UROD and CPO Antisense Plants—The content of scopolin was quantified...
The structural formula of scopolin.

B represents the mean calibration. Results from a typical experiment are shown. Each value was described for Fig. 1. Chemically synthesized scopolin was used for in Fig. 1 with fluorescence detection.

Around this tissue, a strong deep-blue fluorescence surrounded the area devoid of lesions. The deep-blue fluorescence indicated the accumulation of scopolin in the vacuoles. Non-necrotic sections of necrotized leaves from UROD antisense plants (Fig. 5B) displayed similar intensity of the red fluorescence, whereas the deep-blue fluorescence was considerably lower, consistent with the quantitative analysis of scopolin (Fig. 4). Fluorescence analysis of necrotic and non-necrotic leaf sections of CPO antisense plants gave similar results (data not shown).

Expression of Pathogenesis-related (PR) Proteins Is Strongly Induced in UROD and CPO Antisense Plants—We were next interested in determining if porphyric plants showed other defense reactions which are normally observed after pathogen attack. TMV infection of tobacco induces the expression of PR proteins including β-glucanases (class two of tobacco PR proteins), chitinases (e.g. PR Q), as well as other proteins of unknown function (24). Western blot analyses of PR proteins representing these different classes showed stronger accumulation of all PR proteins in older leaves of transformants relative to control plants (Fig. 6A). A similar pattern was obtained for CPO antisense plants (data not shown). We also compared the amount of PR proteins in necrotic and non-necrotic areas of the same leaf from UROD and CPO antisense plants, respectively (Fig. 6B). In both transformants PR proteins mainly accumulated in the leaf area with necrotic lesions and were found only to a minor extent in the area devoid of lesions.

UROD and CPO Antisense Plants Accumulate Elevated Levels of Free and Conjugated Salicylic Acid (SA)—Leaf lesion formation in tobacco infected with TMV is accompanied by increased levels of SA (25). We determined the levels of free and conjugated SA in leaves of UROD and CPO antisense and control plants (Table II). The contents of free SA was increased in all leaves of transformants relative to controls but was more enhanced in UROD antisense plants (up to 5-fold) compared with CPO antisense plants. The amount of conjugated SA was severalfold higher in leaves of both transformants, relative to wild-type, and the increase was more pronounced (up to 25-fold) for CPO antisense plants. We next determined if SA was uniformly distributed in leaves or accumulated preferentially in tissue adjacent to the necrotic lesions as was observed after TMV infection (25). In both UROD and CPO antisense plants free SA was equally distributed between necrotic and non-necrotic leaf areas, whereas conjugated SA preferentially accu-

**Table I**

| Leaf   | WT (nmol/g fresh weight) | URODAS (nmol/g fresh weight) | CPOAS (nmol/g fresh weight) |
|--------|--------------------------|------------------------------|------------------------------|
| Leaf 5 | 5.56 ± 0.57              | 82.72 ± 1.56                 | 28.09 ± 0.25                 |
| Leaf 7 | 2.78 ± 0.33              | 64.12 ± 0.82                 | 7.94 ± 0.32                  |
| Leaf 9 | 1.97 ± 0.41              | 45.95 ± 0.49                 | 13.92 ± 0.42                 |
| Leaf 11| 1.47 ± 0.08              | 34.67 ± 0.16                 | 18.76 ± 0.44                 |

**Fig. 2.** HPLC analysis of isolated scopolin incubated with β-glucosidase. Scopolin was isolated from methanolic leaf extracts of UROD antisense plants as described under "Experimental Procedures." Incubations in acetate buffer (pH 5) alone or with the addition of β-glucosidase were performed at 37 °C for 30 min. Assays were then brought to 80% methanol. HPLC analysis was performed as described in Fig. 1 with fluorescence detection. A, buffer control. Inset shows the structural formula of scopolin. B, β-glucosidase digests. Aliquots of the sample obtained after digestion with β-glucosidase were spiked with buffer (shaded area) and authentic scopolin (solid line). Inset shows the structural formula of scopolin.

**Fig. 3.** ES-MS/MS spectrum of scopolin isolated from methanolic leaf extracts of UROD antisense plants. The ES-MS showed a (M + H)+ ion peak at m/z = 355 consistent with the monoisotopic mass of 354.1 as calculated for scopolin. The protonated molecular ion at m/z 355 was isolated in the ion trap and was fragmented resulting in the appearance of one prominent product ion at m/z = 193.0 indicating the loss of the glucose moiety.

The ES-MS/MS spectrum of scopolin isolated from methanolic leaf extracts of UROD antisense plants. The ES-MS showed a (M + H)+ ion peak at m/z = 355 consistent with the monoisotopic mass of 354.1 as calculated for scopolin. The protonated molecular ion at m/z 355 was isolated in the ion trap and was fragmented resulting in the appearance of one prominent product ion at m/z = 193.0 indicating the loss of the glucose moiety.
mulated in leaf tissue with necrotic spots (Table III). For comparison, in the TMV inoculation experiment (see Fig. 7) the amount of free and conjugated SA in Samsun NN controls 5 days after inoculation was $3.6 \mu g/g$ fresh weight and $25 \mu g/g$ fresh weight, respectively.

**Tobacco Mosaic Virus (TMV) Infection Is Reduced in UROD and CPO Antisense Plants—** It has previously been shown that TMV infection of local lesion hosts leads to a hypersensitive reaction and to increased resistance against subsequent infections with either TMV as well as other viruses (26), fungi, and bacteria (27, 28). The development of the hypersensitive reaction and systemic acquired resistance (29) tightly correlates with increased PR protein expression and SA levels (17). We next investigated if the strong induction of PR proteins and SA formation would result in resistance to TMV in UROD and CPO antisense plants. Fig. 7 shows an inoculation experiment with TMV of wild-type tobacco and several transgenic lines of UROD and CPO antisense plants (4, 5). Leaves were harvested before TMV inoculation to analyze PR-1 gene expression levels. As expected from the Western blot analysis (Fig. 6), necrotic UROD (2L and 15L) and CPO (3L) antisense plants had high levels of PR-1 mRNA (Fig. 7A). Wild-type controls (WT) and

### Table II

|          | SA, free |          |          |          | SA, conjugated |          |          |          |
|----------|----------|----------|----------|----------|---------------|----------|----------|----------|
|          | µg/g fresh weight | µg/g fresh weight |          |          |          |          |          |          |
| WT       | 0.278 ± 0.031 0.078 ± 0.012 0.069 ± 0.009 0.058 ± 0.008 | 1.18 ± 0.09 2.54 ± 0.23 1.00 ± 0.10 1.02 ± 0.07 |          |          |          |          |          |          |
| URODAS   | 0.632 ± 0.042 0.439 ± 0.028 0.397 ± 0.041 0.519 ± 0.035 | 4.73 ± 0.26 6.24 ± 0.45 8.23 ± 0.55 6.39 ± 0.51 |          |          |          |          |          |          |
| CPOAS    | 0.297 ± 0.033 0.127 ± 0.013 0.137 ± 0.009 0.130 ± 0.014 | 2.21 ± 0.18 32.30 ± 1.75 25.09 ± 1.10 19.80 ± 2.60 |          |          |          |          |          |          |

*Leaf.

### Table III

|          | Ratio necrotic/non-necrotic leaf areas |          |          |
|----------|---------------------------------------|----------|----------|
|          | SA (free) | SA (conjugated) |          |          |
| URODAS   | 1.09 ± 0.07 | 3.12 ± 0.21 |          |          |
| CPOAS    | 1.00 ± 0.09 | 4.05 ± 0.59 |          |          |

Necrotic and non-necrotic leaf areas of UROD (URODAS) and CPO (CPOAS) antisense plants were separated as described for Fig. 4. Content of free and conjugated SA was analyzed as given in Table II. Values represent the means ± S.D. from three independent experiments.
between wild-type plants and UROD or CPO antisense plants without a necrotic phenotype. This finding is correlated with the lack of PR-1 protein expression in these plants (Fig. 7A). Quantification of hybridization signals corresponding to the two viral RNAs (Fig. 7B) revealed a 70–90% reduction of TMV in UROD and CPO antisense plants showing necrotic lesions in comparison to wild type (Fig. 7C).

**DISCUSSION**

**Accumulation and Localization of Scopolin—**Transgenic plants with reduced activity of either UROD or CPO accumulated considerable amounts of a fluorescent compound in close proximity to necrotic leaf areas (Fig. 4). This compound was identified as scopolin based on its spectroscopic and fluorescence properties, HPLC analysis, and by comparison of the aglycone obtained by b-glucosidase digestion. The structure of the product was further confirmed by chemical synthesis of scopolin which gave identical results. The data excluded isoskopelin which has been detected in leafy galls of tobacco (30). Results from fluorescence microscopy suggested that scopolin was localized in the vacuoles (Fig. 5). Scopoletin and scopolin accumulation has previously been demonstrated in cell suspension cultures of tobacco (31). Although scopoletin has mainly been recovered from the culture filtrate, scopolin accumulated within the cells (31). Vacuolar localization is consistent with the rapid uptake of the coumarin glucoside esculin into isolated barley leaf vacuoles (32).

There is substantial evidence that scopolin and scopoletin play an important role in disease resistance. It has been shown that scopoletin possesses antimicrobial activity (34–37). In incompatible plant-pathogen interactions a rapid and pronounced synthesis of scopoletin was observed, and a slower and reduced formation was found in compatible interactions (36–38). Elevated constitutive levels of scopoletin and scopolin were found in a disease-resistant *Nicotiana* hybrid (35). Spraying of tobacco plants with scopoletin but not with scopolin prior to TMV inoculation reduced lesion formation (35). In potato tubers infected with *Phytophthora infestans* a ring of blue fluorescence derived from accumulate scopolin was observed around the site of infection (39). Scopolin also accumulated during the hypersensitive reaction of TMV-infected local lesion host tobacco varieties (10, 11). Its low toxicity compared with the aglycon scopoletin and its putative vacuolar localization classifies scopolin as a preformed antimicrobial compound (40). Upon tissue disruption following pathogen attack, scopoletin is released by the action of b-glucosidases originating from the plant itself or from the attacking microorganism and exerts its inhibitory effects. In analogy, the action of a b-glucosidase is required to exert the antimicrobial effects of the cyanogenic glucoside dhurrin or the isoflavonoid derivative maackiain, which is stored as a glucoside in roots of red clover (41, 42).

**Lesion Formation in Leaves of UROD and CPO Antisense Plants Resembles the Hypersensitive Reaction Observed after TMV Infection—**The strong accumulation of scopolin in leaves of UROD and CPO antisense plants suggests similarities between the formation of necrotic lesions upon accumulation of photosensitizing porphyrins and the hypersensitive reaction following TMV infection. Tobacco leaves infected with TMV similarly show substantial increase in the levels of both free and conjugated SA (25) and have stimulated PR protein expression (17, 43) with a gradual distribution and a maximum near the center of the lesions (25, 16). It is widely accepted that in infected plants concentration of SA and induction of PR proteins correlate with enhanced resistance against pathogens (29, 44–47). It has been shown that treatment of tobacco with

![Image](http://www.jbc.org/)

**FIG. 6.** Accumulation of PR (pathogenesis-related) proteins in leaf extracts of wild-type UROD (URODAS), or CPO (CPOAS) antisense plants. Protein extracts were separated by SDS-polyacrylamide gel electrophoresis and blotted onto nitrocellulose. Immunodetection was performed with antibodies raised against tobacco PR-1, -2, -Q, and -S (Heitz et al. (16)). A, PR protein expression in leaves of wild-type and UROD antisense plants at different developmental stages. B, PR protein expression in necrotic and non-necrotic leaf areas of UROD and CPO antisense plants. Areas with and without necrotic lesions were obtained from leaves of UROD or CPO antisense plants (see Fig. 4).

![Image](http://www.jbc.org/)

**FIG. 5.** Fluorescence microscopy of a necrotic and a non-necrotic leaf area of a UROD antisense plant. Hand-cut sections of tobacco leaves from UROD antisense plants were mounted in water. Excitation with UV light and a long-pass LP 397 filter allowed monitoring of both blue and chlorophyll-derived red fluorescence. The bar represents 100 mm. A, necrotic area; B, non-necrotic area.
acumulation of tetrapyrroles in a susceptible tobacco variety lacking the N gene contributes a systemic pathogen resistance in uninoculated tissue.

Deregulation of Chlorophyll Biosynthesis Can Initiate Defense Responses Against Pathogen Attack—Cellular responses in plants similar to those observed after pathogen attack can be provoked by a wide range of other stress situations in plants. UV light, heavy metal, or ozone treatment, or genetic modification of metabolic pathways can lead to the induction of PR protein expression (50–53). In addition, increased resistance to some pathogens has been observed in transgenic plants expressing cholera toxin (54), yeast invertase (55), bacterio-opsin (56) as well as in response to reduced catalase activity (57, 58). Our results are consistent with these and other examples of altered cell death control by transgene expression (53, 59).

Free phytorexins are potent photosensitizers and have been used in photodynamic therapy for the treatment of cancer (60). They are also involved in the mode of action of diphenyl ether type herbicides (e.g. acifluorfen), which act on protoporphyrinogen oxidase (61). The phototoxicity of tetrapyrroles is exerted via reactive oxygen species. Singlet oxygen is generated from molecular oxygen after UV irradiation of coproporphyrin (62). By using the electron spin resonance technique a range of free radicals is observed when phorphyrin solutions are illuminated (63). Reduced activities of UROD or CPO led to the accumulation of tetrapyrroles (4, 5). The limited capacity of the antioxidative defense mechanisms in UROD and CPO was indicated by decreased levels of low molecular weight antioxidants (6). Reactive oxygen species derived from excessive tetrapyrroles could mimic signaling molecules formed after pathogen attack in triggering defense responses (64–66). However, other cellular processes resulting from tetrapyrrole-induced oxidative stress could contribute to the induction of antimicrobial defense responses.

Evidence was recently provided that lesion formation after TMV inoculation of tobacco can be attributed to programmed cell death (67). Programmed cell death was inhibited in plants grown under low oxygen pressure suggesting that ambient oxygen levels are required for this response (68). Based on the similarity of antimicrobial defense responses triggered in UROD or CPO antisense plants and TMV-infected tobacco, one could speculate that lesion formation in the porphyric transgenomes is exerted via reactive oxygen species. Singlet oxygen is generated from molecular oxygen after UV irradiation of coproporphyrin (62). By using the electron spin resonance technique a range of free radicals is observed when phorphyrin solutions are illuminated (63). Reduced activities of UROD or CPO led to the accumulation of tetrapyrroles (4, 5). The limited capacity of the antioxidative defense mechanisms in UROD and CPO was indicated by decreased levels of low molecular weight antioxidants (6). Reactive oxygen species derived from excessive tetrapyrroles could mimic signaling molecules formed after pathogen attack in triggering defense responses (64–66). However, other cellular processes resulting from tetrapyrrole-induced oxidative stress could contribute to the induction of antimicrobial defense responses.

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Expression of Uroporphyrinogen Decarboxylase or Coproporphyrinogen Oxidase Antisense RNA in Tobacco Induces Pathogen Defense Responses Conferring Increased Resistance to Tobacco Mosaic Virus

Hans-Peter Mock, Werner Heller, Antonio Molina, Birgit Neubohn, Heinrich Sandermann, Jr. and Bernhard Grimm

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