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Expression of the gene encoding the PR-like protein PRms in germinating maize embryos

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Summary. The PRms protein is a pathogenesis-related (PR)-like protein whose mRNA accumulates during germination of maize seeds. Expression of the *PRms* gene is induced after infection of maize seeds with the fungus *Fusarium moniliforme*. To further our investigations on the expression of the *PRms* gene we examined the accumulation of PRms mRNA in different tissues of maize seedlings infected with *F. moniliforme* and studied the effect of fungal elicitors, the mycotoxin moniliformin, the hormone gibberellic acid, and specific chemical agents. Our results indicate that fungal infection, and treatment either with fungal elicitors or with moniliformin, a mycotoxin produced by *F. moniliforme*, increase the steady-state level of PRms mRNA. PRms mRNA accumulation is also stimulated by the application of the hormone gibberellic acid or by treatment with silver nitrate, whereas acetylsalicylic acid has no effect. In situ RNA hybridization in isolated germinating embryo sections demonstrates that the *PRms* gene is expressed in the scutellum, particularly in a group of inner cells, and in the epithelium lying at the interface of the scutellum and the endosperm. The pattern of expression of the *PRms* gene closely resembles that found for hydrolytic enzymes, being confined to the scutellum and the aleurone layer of the germinating maize seed. Our results suggest that the PRms protein has a function during the normal process of seed germination that has become adapted to serve among the defence mechanisms induced in response to pathogens during maize seed germination.

Key words: Pathogenesis-related proteins – Maize – Seed germination – Fungi – In situ hybridization

Introduction

Elucidation of the molecular mechanisms underlying defence responses of plants against pathogens has become one of the major objectives in the field of phytopathology. Among the most frequently observed biochemical events following plant-pathogen interaction is the rapid accumulation of antimicrobial substances (phytoalexins) (Darvill and Albersheim 1984), reinforcement of cell walls by deposition of lignin and accumulation of hydroxyproline-rich glycoproteins (HRGPs) (Showalter et al. 1985; Mazau and Esquerre-Tugayé 1986), and production of a family of proteins termed PR (Pathogenesis-Related) proteins (van Loon 1985; Bol et al. 1990). Pathogenesis-related proteins were first detected in leaves from tobacco plants responding hypersensitively to a pathogen (tobacco mosaic virus, TMV) (van Loon 1985; Jamet and Fritig 1986; Matsuoka and Ohashi 1986). Since then, PR proteins have been found in a variety of infected plants such as tomato, potato, maize, bean, parsley, etc. (Camacho-Henriquez and Sanger 1984; de Tapia et al. 1986; Somssich et al. 1986; Granell et al. 1987; Kombrink et al. 1988; Nasser et al. 1988).

The expression of PR proteins can be induced by different infectious agents such as viruses, viroids, fungi or bacteria, and expression of many of them can also be induced by specific chemicals (i.e. polyacrylic acid, benzoic acid derivatives or silver nitrate) (van Loon 1983; Granell et al. 1987) or by hormones (Memelink et al. 1987).

The expression of PR proteins has also been reported in specific tissues of healthy plants, i.e. during the normal development of the tobacco flower or in parenchyma leaf tissue from healthy plants (Fraser 1981; Vera et al. 1988; Lotan et al. 1989; Castresana et al. 1990; Neale et al. 1990). These findings suggest that these proteins could also play a role in plant development.

We have recently reported the cloning of a gene coding for a PR-like protein, named PRms protein (Casacuberta et al. 1991), which is expressed in the aleurone layer of germinating maize seeds. The PRms protein is

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encoded by a single gene in the maize genome. Furthermore, its expression is induced in the aleurone layer by infection of maize seeds with a natural pathogen of maize, the fungus *Fusarium moniliforme*. Here we report a more detailed analysis of the site of expression of the *PRms* gene as measured by PRms mRNA accumulation. We present data on the effects of different factors such as fungal elicitors, the mycotoxin moniliformin, the hormone gibberellic acid and specific chemical agents on the steady-state level of the PRms mRNA.

Materials and methods

Plant material. Maize seeds (*Zea mays*, pure inbred line W64A) were used as the experimental material. Conidial suspensions were prepared from *F. moniliforme* and *Penicillium* ssp. as previously described (Casacuberta et al. 1991). Germination and fungal infection of maize seeds were performed as follows. Sterile seeds were germinated for 24 h and then inoculated with a conidial suspension of the fungus *F. moniliforme* by adding 50 μl (2000–3000 spores/ml) to each seed. Seeds were allowed to germinate for 3 more days. Vegetative tissues (radicle, coleoptile), embryo and the aleurone layer-enriched fraction were then dissected and immediately frozen in liquid nitrogen.

Fungal infection and treatment with different agents of isolated germinating embryos. Isolated embryos were also germinated in vitro and the effects of different agents on the expression of the *PRms* gene were analysed. For this purpose, embryos were dissected manually from dry sterilized seeds and germinated on wet filter paper in the dark at 25° C for the required period of time. The effect of fungal infection was tested by inoculating 18 h germinated embryos with fungal spores (*F. moniliforme, Penicillium* ssp.; 50 μl, 2000–3000 spores/ml). Infected (and sterile control) embryos were allowed to continue germination.

Germinating embryos were treated with the various agents as follows. Isolated embryos were germinated for 18 h, inoculated with 50 μl/embryo of aqueous solutions of the agent to be tested and then allowed to germinate for 6 h more. Moniliformin (1-hydroxycyclobut-1-ene-3,4-dione) was purchased from Sigma.

Two elicitors from different fungal pathogens were tested. First, a crude preparation of elicitor from *F. moniliforme* was prepared by standard procedures (Ayers et al. 1976). The fungus was grown on agar plates (Bacto-malt agar, Difco) at 28° C until the mycelium had covered the surface of the plate. Fungal mycelium was collected by shaking the plate with 10 ml of sterile water for 2 h at room temperature. The solution containing the whole mycelial suspension was sonicated at 100 W for 15 min, then autoclaved at 115° C (10 psi) for 40 min and finally concentrated by lyophilization. The second elicitor, from *Phytophtora megasperma*, was obtained as a purified preparation from Dr. M.T. Esquerré-Tugayé (Toppan and Esquerré-Tugayé 1984). Both elicitors were used as aqueous solutions to inoculate isolated germinating embryos (as indicated above) at the indicated concentrations (dry weight of elicitor per millilitre of water).

RNA isolation and blot analysis. RNA was extracted from aleurone layers, radicles and coleoptiles as described by Mundy et al. (1985) and from isolated germinating embryos (4–5 embryos) basically as described by Logemann et al. (1987). Total RNA was separated by electrophoresis on a 1.5% formaldehyde-containing agarose gel and blotted onto a nylon membrane (Hybond N, Amersham). The isolated *Eco*RI insert from the B8A2 cDNA clone (Casacuberta et al. 1991) was ^{32}P-labelled by random priming (using a kit from Boehringer) and used as the hybridization probe.

In situ hybridization. Sections of isolated germinating embryos (22 h) were prepared and hybridized as described by Langdale et al. (1988). The RNA transcripts were partially hydrolysed in 0.1 M $NaHCO_3$, pH 10.2, 0.01 M DTT, at 60° C to give an average length of 150 nucleotides. Sections were hybridized in situ with ^{35}S-labelled PRms transcripts using the pSPT18 vector system (Boehringer). Following autoradiography the sections were examined and photographed by light and dark-field microscopy (Zeiss Axioplan microscope and automated camera). Sections were also stained with acridine orange to check for the retention of RNA in the processed tissue.

Results

Expression of the PRms gene in F. moniliforme-*infected seedlings of maize*

Northern blot analyses were performed to analyse PRms mRNA accumulation in different tissues of 4 day germinated seedlings infected with the fungus *F. moniliforme*. Sterilized seeds were allowed to germinate for 24 h and then inoculated with spores of *F. moniliforme*. After 4 days of germination, young plants were dissected to obtain the aleurone layer-enriched fractions, embryos (axis plus scutellum), radicles and coleoptiles, which were subjected to Northern hybridization analysis. The results are shown in Fig. 1. A high level of PRms mRNA was observed in the embryo and the aleurone layer-enriched fraction, while no expression of the PRms gene was detectable in the radicle or the coleoptile of young infected plants. There was also no expression in radicle or coleoptile, or in adult leaves or roots from non-infected plants (Casacuberta et al. 1991). This Northern blot analysis indicates that, in *F. moniliforme* infected seedlings, the PRms gene is expressed in seed tissues and not in any of the other vegetative tissues tested here.

We analysed the time course of PRms mRNA accumulation during in vitro germination of isolated embryos. Northern blot analysis of RNAs extracted from germinating embryos revealed that the PRms gene is not expressed during the early stage of germination. In sterilized germinating embryos, the PRms mRNA becomes detectable after approximately 20 h of germina-

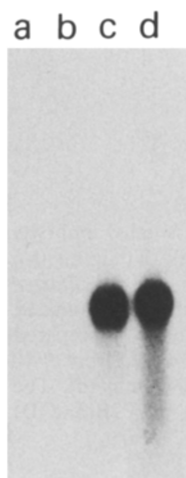

Fig. 1. RNA blot analysis of the maize seed PR-like protein (PRms). Total RNA was isolated from different tissues of 4 day germinated maize seedlings that had been infected with the fungus *Fusarium moniliforme*. Sterilized seeds were germinated for 24 h and then inoculated with fungal spores of the fungus *F. moniliforme*. Infected seeds were allowed to germinate for 3 more days. Lane a, radicle; b, coleoptile; c, aleurone layer; and d, embryo (axis plus scuttelum)

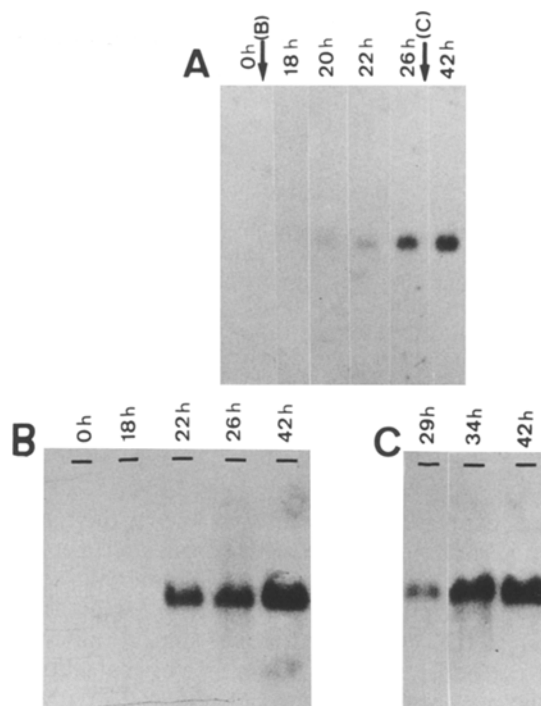

Fig. 2A–C. Time course of PRms mRNA accumulation during germination of isolated maize embryos. Effect of fungal infection on the PRms mRNA level. **A** Embryos were obtained from sterilized maize seeds and allowed to germinate. Total RNA was isolated at different times of germination (0 to 42 h) and subjected to Northern blot analysis. **B** and **C** Effect of infection by *Fusarium moniliforme* on PRms gene expression. Embryos were infected at different times of germination: 6 h (**B**) and 30 h (**C**) (as indicated by *arrows* in **A**) and total RNA samples were obtained at the indicated times of germination

tion and the level increases until the latest stage of germination analysed here (42 h) (Fig. 2A). For a more detailed analysis of the effect of infection by *F. moniliforme* on the expression of the PRms gene in isolated germinating embryos, the embryos were infected at three different times: after 6 h, 18 h and 30 h of germination. As is shown in Fig. 2B and C, the PRms mRNA acculates to high levels in germinating maize embryos upon infection with the fungus *F. moniliforme*. However, *PRms* gene expression but the enhancement of the level of this mRNA is only detected after the embryo has reached a certain stage in germination (after 20 h). When infection is carried out after 30 h of germination, induction is detected after 4 h of treatment, the shortest period of time analysed (Fig. 2C). The same results were obtained when isolated embryos were infected after 18 h of germination (results not shown). These results indicate that the *PRms* gene is expressed during in vitro germination of non-infected embryos, and its expression is further induced by fungal infection. Similar results were obtained on analysis of *PRms* gene expression and its induction by fungal infection in the aleurone layers of germinating maize seeds (Casacuberta et al. 1991).

The induction observed for the *PRms* gene in *Fusarium*-infected seeds was not restricted to this pathogen. Infection of isolated germinating embryos with another fungus, *Penicillium* ssp., a soil-inhabiting fungus that can invade maize seed embryos, also results in an increase in the level of the PRms mRNA (Fig. 3).

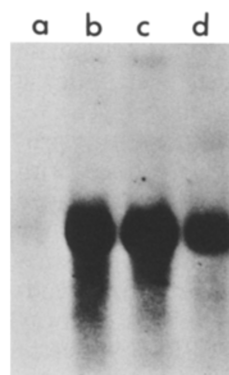

Fig. 3. Accumulation of PRms mRNA upon infection with fungal pathogens. Embryos isolated from dry sterilized seeds were germinated for 18 h and then inoculated with fungal spores. Infected (and control embryos) were allowed to germinate for 28 h more. RNA samples from isolated germinating embryos without (lane a) or with inoculation with a conidial suspension of *Penicillium* spp. (c), *Fusarium moniliforme* (d) or both fungi (b) were analysed

Effect of hormones and chemical agents on the level of PRms mRNA accumulation

We have investigated the possible role of the hormone gibberellic acid (GA_3) on the expression of the *PRms* gene. This hormone plays a key role during germination of cereal seeds, having an important regulatory function on gene expression in specific tissues, mainly in the aleurone layer (MacGregor et al. 1984; Mundy et al. 1985; Stuart et al. 1986; Fincher 1989). Knowing that the

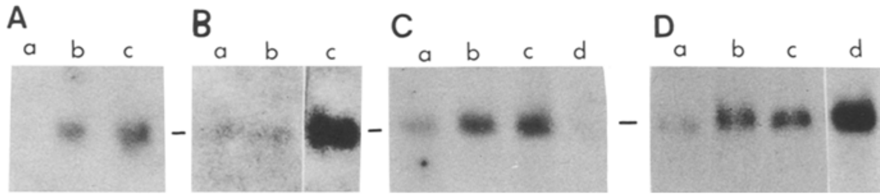

Fig. 4A–D. Accumulation of PRms mRNA in response to treatment with the hormone gibberellic acid (**A**), chemical agents (**B**), and fungal elicitors and the mycotoxin moniliformin (**C** and **D**). Isolated embryos were germinated for 18 h, inoculated with the agent to be tested and then allowed to germinate for 6 h more. **A** RNA samples from isolated embryos germinated in the presence of water (lane a), 1 μM gibberellic acid (b) and 10 μM gibberellic acid (c). **B** RNA samples from isolated embryos germinated in the presence of water (lane a), 5 mM acetylsalicylic acid (b), and 3 mM silver nitrate (c). **C** RNA samples from isolated embryos germinated in the presence of 30 μg/ml (lane a), 300 μg/ml (b), and 3 mg/ml (c) of elicitors prepared from *Fusarium moniliforme* and water (d). **D** RNA samples from isolated embryos germinated in the presence of water (lane a), 0.03 μg/ml of elicitors prepared from *F. moniliforme* (b), 0.03 μg/ml of elicitors prepared from *Phytophtora megasperma* (c) and 30 μg/ml of moniliformin (d). The autoradiographs were exposed for 2 (**A** and **C**) or 5 (**B** and **D**) days

PRms gene is expressed in the embryo and in the aleurone layer of germinating maize seeds (Casacuberta et al. 1991), we examined the possible effect of gibberellic acid on the accumulation of the PRMs mRNA. Northern blot analysis of RNAs obtained from isolated embryos germinated in the presence of different concentrations of gibberellic acid was performed (1 μM and 10 μM). Results shown in Fig. 4A indicate that gibberellic acid induces the accumulation of the PRms mRNA in isolated sterilized embryos germinated in vitro. Higher levels of PRms mRNA are found with increasing hormone concentration.

Expression of PRs may be artificially induced in leaves of different plant species by the application of a variety of chemicals, notably benzoic acid derivatives (i.e. acetylsalicylic acid; tobacco), silver nitrate (tomato and *Gynura aurantiaca*) and mercuric chloride (maize). For this reason, the possible effect of these specific chemicals on the level of *PRms* gene expression was studied. Thus, we observed that silver nitrate is able to stimulate accumulation of PRms mRNA whereas acetylsalicylic acid does not affect its level of expression (Fig. 4B).We were not able to induce the synthesis of the PRms protein in leaves of maize plants either after treatment with mercuric chloride or with any of the other agents assayed in this work (results not shown).

Another chemical compound, 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate natural end-product precursor in the ethylene biosynthesis pathway has been shown to induce expression of PRs in different plant species (van Loon 1985; Bellés et al. 1989). Accordingly, we tested the effect of ACC on PRms mRNA accumulation in isolated germinating maize embryos. Treatment with ACC does not result in a change in the PRms mRNA level (results not shown). This result seems to indicate that ethylene may not play a role in regulation of *PRms* gene expression during maize seed germination. However, additional experiments must be performed in order to test this hypothesis further.

PRms mRNA accumulation is induced by a mycotoxin and by fungal elicitors

Infection with the fungus *F. moniliforme* results in induction of accumulation of PRms mRNA in the aleurone layers of germinating maize seeds and in isolated germinating embryos. *F. moniliforme* (perfect state, *Gibberella fujikuroi*) is a gibberellin-producing fungus. In order to elucidate whether *PRms* gene expression is specifically induced by fungal infection, or as a response to the hormone produced by the fungus, we tested the effect of elicitors (and the mycotoxin, moniliformin) on the level of PRms mRNA accumulation. Two elicitors were tested. They were prepared from two different fungi that are either pathogenic (*F. moniliforme*) or non-pathogenic (*P. megasperma*) to maize. *P. megasperma* elicitors have been shown to induce phytoalexin production and the expression of PR proteins in tissues of other plant species that are not its natural hosts, including potato and parsley cells (Cline et al. 1978; Somssich et al. 1986). Since the active elicitor molecule(s) in the natural interaction of maize plants with *F. moniliforme* has not been identified, we used crude preparātions of cell-wall fragments from fungal mycelia. Crude preparations from fungal walls and culture fluids have been widely used as elicitors in other systems (Darvill and Albersheim 1984). Moreover, we also analysed the effect of moniliformin, a toxin produced by *F. moniliforme*.

Results on the effects of fungal elicitors and the mycotoxin on the expression of the *PRms* gene are presented in Fig. 4C and 4D. We tested various *F. moniliforme* elicitor concentrations (30 and 300 μg/ml and 3 mg/ml, Fig. 4C). Increase in the elicitor concentration by one order of magnitude has a considerable effect on the PRms mRNA level observed. At a 1000-fold lower elicitor concentration (0.03 μg/ml, Fig. 4D) induction is still observed that is comparable to that found after treatment with the same concentration of *P. megasperma* elicitors (Fig. 4D). It is interesting to note the strong induction observed by treatment with the mycotoxin moniliformin (Fig. 4D, lane d). It has been reported that *F. moniliforme* is able to produce about 600 μg of moniliformin per milligram of corn grit medium (Burmeister et al. 1979). Although the quantity of toxin elaborated

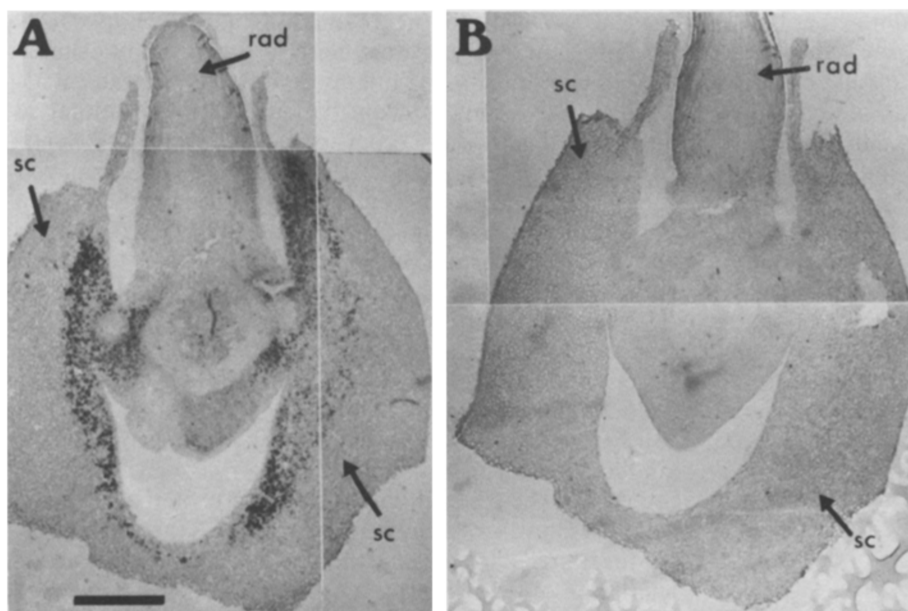

Fig. 5 A and B. Localization of PRms transcripts in sections of isolated germinating embryos of maize by in situ hybridization. Embryos were germinated for 24 h. Longitudinal sections were taken and hybridized with the in vitro synthesized anti-sense (**A**) or sense (**B**) PRms transcripts. Bar represents 600 µm. *sc* scutellum; *rad* radicle

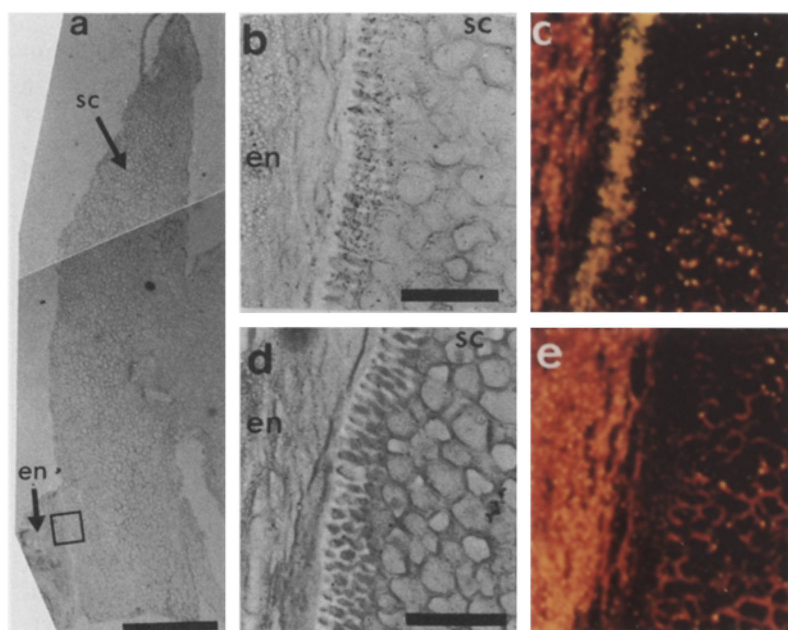

Fig. 6 a–e. Identification of the epitheliar scutelum cells containing mRNAs encoding the PRms protein by in situ hybridization. **a** Longitudinal section of isolated embryos containing residual endosperm (*en*) tissue attached to the scutellum (*sc*). **b**–**e** depict higher magnifications of the *boxed area* in **a**. Autoradiography (**b** and **d**) and dark-field illumination (**c** and **e**) are presented for hybridization with anti-sense (**b** and **c**) or sense (**d** and **e**) PRms transcripts. Bar represents 600 µm (**a**) and 100 µm (**b**–**e**)

by the fungus on infection of germinating maize seeds has not been determined, we assume that the concentration of the fungal toxin used in the present work (1.5 µg moniliformin/embryo, and an average embryo weight of approximately 50 mg) does not exceed the concentrations encountered in vivo. From our results we can conclude that, in isolated maize embryos germinated in vitro, PRms mRNA accumulation is specifically induced by fungal infection or by treatment with elicitor, isolated either from pathogenic or non-pathogenic fungi. A fungal toxin, moniliformin, also strongly increases the rate of PRms mRNA accumulation.

The PRms *gene is expressed in the scutellum of the germinating embryo*

In order to localize the site of expression of the *PRms* gene in the embryo during germination, we performed in situ hybridization experiments. Sections of isolated germinating embryos were probed with both sense and anti-sense ^{35}S-labelled RNA probes. The results are presented in Figs. 5 and 6.

The *PRms* gene is expressed in the scutellum of the germinating embryo (Fig. 5). The photograph shows that the probe hybridizes to a group of inner scutellar cells. Sections from isolated germinated embryos containing residual endosperm tissue attached to the scutel-

lum were also obtained (Fig. 6a) and examined at higher magnification (Fig. 6b–e). Only in this case was the structure of the epithelial scutellum cells well conserved after cutting and processing the samples. Figure 6 shows that, besides hybridization to the inner cells, the probe binds to the scutellar epithelial cells which lie at the interface of the scutellum and the endosperm (Fig. 6b). This can be better seen when sections covering the epitheliar scutellum area are viewed under dark-field illumination (Fig. 6c). The concentration of bound anti-sense RNA probe appears as green-yellow dots. The starch granules appear as bright yellow dots.

Hybridization of the PRms mRNA transcript to the anti-sense transcript was specific, as no increase in signal above background levels was observed when the sense transcript was used (Fig. 6d, e).

Discussion

We have previously reported the characterization of cDNA and genomic clones coding for a basic pathogenesis-related protein (PRms) that is expressed in the aleurone layers of germinating maize seeds (Casacuberta et al. 1991). Expression of the *PRms* gene is also induced after infection of seeds with a natural pathogen of maize, the fungus *F. moniliforme*. This is a seedborne fungus that causes stalk rot and kernel or ear rot. The present study was initiated to characterize further the effect of different agents (biotic or abiotic) on the level of expression of the *PRms* gene in germinating maize seeds. The fact that this gene is expressed in maize embryos has facilitated the analysis of possible factors affecting its expression since it is possible to germinate isolated embryos in vitro. Furthermore, as an initial step toward elucidation of the function of the PRms protein we have localized the cells in which the *PRms* gene is expressed in intact germinating maize embryos by in situ mRNA hybridization.

The protein encoded by the PRms mRNA is homologous to the known pathogenesis-related PR-1 group from tobacco and to the p14 protein from tomato leaves (Lucas et al. 1985; Cornelissen et al. 1986; Payne et al. 1989). In maize, a PR protein (PRm2) has been found to accumulate in leaves after mercuric chloride treatment or brome mosaic virus infection (Nasser et al. 1988). The PRm2 protein is related to the tobacco PR-1b protein based on its serological properties and amino acid composition. The PRm2 and PR-1b proteins are acidic proteins while the PRms protein is a basic protein. In contrast to what has been reported for the PRm2 protein, the synthesis of the PRms protein (as judged by measurement of the PRms mRNA level) is not induced by treatment with mercuric chloride either in seed tissues or in leaves.

It has been shown that, in leaves, the PR-1 group from tobacco is inducible by different chemicals (polyacrylic acid, benzoic acid derivatives, etc.). The induction of a PR-1a promoter by acetylsalicylic acid in transgenic tobacco plants has been reported (Ohshima et al. 1990; van de Rhee et al. 1990). More recently, it has been suggested that salicylic acid functions as the endogenous signal in the resistance response of tobacco and cucumber to viral infection (Malamy et al. 1990; Metraux et al. 1990). Our results indicate that the PRms promoter is not inducible by acetylsalicylic acid, as is the PR-1 group of tobacco. The possibility that, in maize seeds, salicylic acid may not play a role in signal transduction after fungal infection should also be considered. Further studies should be carried out to elucidate whether this compound can act as a natural transduction signal in maize.

The accumulation of PR proteins in tomato and *G. aurantiaca* leaves, and the enhancement of ethylene production, after silver nitrate treatment has been reported (Conejero and Granell 1986; Granell et al. 1987; Bellés and Conejero 1989). Silver nitrate treatment is able to induce the expression of both the *PRms* gene in maize seeds and the gene coding for the p14 protein in tomato leaves. However, treatment with ACC (the immediate natural precursor of ethylene) has no effect on the accumulation of PRms mRNA. Additional experiments must be performed in order to elucidate whether ACC or silver nitrate treatments increase the production of ethylene and whether ethylene treatment controls the expression of the *PRms* gene.

Our results demonstrate that PRms mRNA accumulation is specifically induced by fungal infection during germination of maize seeds. Induction is observed after direct inoculation with fungal spores (*F. moniliforme*, *Penicillium* ssp.) and also after treatment with fungal elicitors (from pathogenic or non-pathogenic fungi) or with a toxin produced by *F. moniliforme*. Moniliformin, and other toxins produced by several species of fungi, show important toxic effects on animals. Deleterious effects on plants have also been reported (Cole et al. 1973; Thiel et al. 1986). However, the possibility of induction of PRs by mycotoxins has not been explored so far. The role that mycotoxins may play in elicitation of the plant defence responses is a question of great interest in the field of molecular aspects of host-pathogen interactions.

The induction of PRms mRNA by the hormone gibberellic acid in sterilized germinating maize embryos, and also the level of expression found in sterilized germinating seeds (Casacuberta et al. 1991) reveals some interesting features. The important regulatory role of gibberellins in the expression of genes that are specifically induced during germination of cereal seeds in particular, genes coding for hydrolytic enzymes, such as α-amylases, proteases or cell-wall degrading enzymes, is well known. Various lines of evidence indicate that in response to the hormone gibberellic acid, the aleurone layer and the scutellum of the embryo secrete hydrolytic enzymes and, at least in barley seeds, it seems that the epithelial cells of the scutellum perform a secretory function early in germination (Gram 1982). Our results suggest the possibility of natural hormonal regulation of the *PRms* gene, which could be activated as a normal event in the biological cycle of the plant, during seed germination. The expression of PR proteins during natural processes in the development of healthy (non-infected) plants (flowering, leaf senescence) has been reported elsewhere

(Fraser 1981; Vera et al. 1988; Lotan et al. 1989; Castresana et al. 1990; Neale et al. 1990).

As previously mentioned, the PRms protein is homologous to PR proteins from tobacco (PR-1 group) and tomato (p14). Although these homologous proteins are well characterized from the biochemical point of view, no function has been assigned to any of them. It is possible that the functions of this family of proteins are similar. The PRms gene has been found to be expressed in the aleurone layer and embryo of germinating maize seeds. Within the embryo its expression is confined to the scutellum and to the scuttellar epithelial cells. We have not detected expression of the *PRms* gene in any other tissue under the various conditions tested here. Both the scutellum and the aleurone layer are specialized tissues in germinating cereal seeds. They are the sites of synthesis and secretion of hydrolytic enzymes such as α-amylases, proteases and cell-wall degrading enzymes (MacGregor et al. 1984; Hammerton and Ho 1986; Fincher 1989). In particular, the pattern of expression found for the *PRms* gene closely resembles that reported for a cell-wall degrading enzyme, (1–3, 1–4)-β-glucanase from germinated barley (McFadden et al. 1988). In summary, despite the fact that the function of the PRms protein remains unknown, our results indicate a correlation of the tissues in which PRms mRNA accumulates with tissues specialized in the production and secretion of hydrolytic enzymes. The hypothesis that enzymatic activities may be associated with PR proteins present in extracts of TMV-infected tobacco leaves had already been advanced by van Loon in the early seventies (van Loon 1972). Since then, several PR proteins have been found to have hydrolytic activities: various chitinases and β-1,3-glucanases from tobacco, potato, bean, maize, etc. (Legrand et al. 1987; Kauffmann et al. 1987). In tomato plants, one of the major PR proteins (P-69) is an endoproteinase (Vera and Conejero 1988). The question of a relationship between the possible hydrolytic activity of the PRms protein and its role in defence against pathogens will, however, require further studies.

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