Identification of a Plant-Encoded Analog of PKR, the Mammalian Double-Stranded RNA-Dependent Protein Kinase

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Plant virus or viroid infection stimulates the phosphorylation of a plant-encoded protein of M, 68,000 to 70,000 (now termed pPKR) that is associated with double-stranded RNA-stimulated protein kinase activity. Using various biochemical and immunological comparisons, we have demonstrated that this plant protein is an analog of the mammalian PKR enzymes. pPKR is both cytosolic and ribosome associated, similar to mammalian PKR, and appears to be capable of phosphorylating exogenous histones. Monoclonal antiserum to the human PKR as well as antiserum to a conserved plant-encoded protein of M, 68,000 to 70,000 protein (Crum et al., 1988) demonstrates cross-reactivity with pPKR. Likewise, polyclonal antiserum to the pPKR detected the mouse and human PKR in western blot analysis. Northern blot analysis of a mammalian PKR cDNA detected a specific 2.5-kb transcript present in plant poly(A)⁺ RNA.

The significance of plant protein phosphorylation in signal transduction, metabolic regulation, and gene expression is now firmly established (Trewavas and Gilroy, 1991; Allen, 1992; Klimczak et al., 1992; Martin et al., 1993) despite inherent difficulties in studying phosphorylation in plant homogenates (Harter et al., 1994). However, structure-function relationships between animal and plant kinases remain to be clarified. Catalytic domains appear to be conserved between plant and animal kinases, although regulatory regions are significantly more diverse (Lawton et al., 1989) and few specific activators of plant kinases, with the exception of Ca²⁺, have been identified. Comparison with putative analogs in animal cells is one approach to study the regulatory significance of protein phosphorylation in plant cells.

Phosphorylation-dephosphorylation provides an efficient mechanism to regulate host-pathogen interactions. Recently, the resistance gene conditioning specific resistance in tomato to the bacterial pathogen Pseudomonas syringae pv tomato was cloned and determined to encode a protein kinase (Martin et al., 1993). Plant virus or viroid infection of susceptible host tissue induces the phosphorylation of an M, 68,000 to 70,000 protein (Crum et al., 1988; Hiddinga et al., 1988). In vitro phosphorylation of this protein is regulated by poly(rI)-poly(rC) or virus dsRNA isolated from infected tissues but not rRNA, DNA, or RNA-DNA hybrids (Roth and He, 1994). However, viroid ssRNA transcripts stimulate the phosphorylation of this plant-encoded protein (J.O. Langland and D.A. Roth, unpublished data). In vivo and in vitro phosphorylation in virus-infected protoplasts also is correlated with early events in viral replication, suggesting a role in pathogenesis (Hu and Roth, 1991). The in vivo phosphorylation of this protein in healthy protoplasts suggests a role in uninfected plants (Hu and Roth, 1991).

The biochemical characteristics of the plant M, 68,000 to 70,000 protein are similar to those of the vertebrate IFN-induced, dsRNA-dependent protein kinase, PKR (Hovanessian, 1989). Relative to the mPKR, the plant phosphoprotein is present at greater endogenous levels in healthy tissue, although expression of the plant enzyme is induced by virus infection (J.O. Langland and D.A. Roth, unpublished data). Furthermore, the increased level of the M, 68,000 to 70,000 protein phosphorylation is associated with the susceptible, disease-producing reaction rather than an antiviral response observed with IFN-induced PKR phosphorylation. Characterization of this plant phosphoprotein will contribute to understanding the functional significance of these apparent differences.

In the presence of dsRNA, mPKR autophosphorylates an M, 68,000 (mouse) or 72,000 (human) protein subunit of PKR, P₁. Autophosphorylation appears to be necessary for PKR activation. Once activated, PKR phosphorylates exogenous substrates, including histone proteins and eIF-2 (Berry et al., 1985; Pestka et al., 1987; Jacobs and Imani, 1988). Phosphorylation of eIF-2 on its α subunit can lead to inhibition of protein synthesis by preventing the exchange of GDP for GTP on the eIF-2 complex, thereby blocking formation of the ternary complex between eIF-2, Met-tRNA, and GTP (Pain, 1986). Through this mechanism, active PKR is likely responsible for the IFN-induced inhibition of replication of several viruses (Sam...

Abbreviations: ds, double-stranded; eIF-2, eukaryotic protein synthesis initiation factor 2; IFN, interferon; mPKR, mammalian PKR; pPKR, plant PKR; rA, adenosine; rC, cytosine; rI, inosine; S-100, supernatant of the 100,000g centrifugation; ss, single-stranded; TMV, tobacco mosaic virus.
Plant dissociation of I-KB from NF-KB and subsequent activation (Clemens, 1992; Kumar et al., 1994). Phosphorylation leads to a formed cells (Chong et al., 1992). PKR also phosphorylates I-κB, the inhibitor of the transcription factor NF-KB (Clemens, 1992; Kumar et al., 1994). Phosphorylation leads to a dissociation of I-κB from NF-κB and subsequent activation of a number of genes stimulated by NF-κB.

Our initial studies indicating a relationship between the plant M, 68,000 to 70,000 protein and mPKR were done using crude soluble cytoplasmic extracts and a relatively uncharacterized polyclonal anti-human PKR serum (Crum et al., 1988; Hiddinga et al., 1988). Further purification and characterization of the plant protein has been difficult because of its extreme lability and low abundance. Here we report the further characterization of this plant-encoded phosphoprotein and show that it is a dsRNA-dependent protein kinase (now termed pPKR). The biochemical and enzymatic properties of pPKR are very similar to those of the plant-encoded protein and antiserum to the plant enzyme recognized the mPKR. It is significant that northern blot analysis demonstrated that a cDNA probe to the mPKR recognizes a single poly(A)+ RNA from plant cells, which is approximately the same size as the PKR-RNA from mouse and human cells. Taken together, these data suggest the presence of a plant-encoded analog to the mPKR.

**MATERIALS AND METHODS**

**Materials**

Monoclonal antiserum to the human PKR has previously been characterized (Laurent et al., 1985) and was kindly provided by A. Hovanassian (Institut Pasteur, Paris, France). Rabbit polyclonal antiserum was generated against a synthetic peptide containing the C-terminal 69-amino acids of the rotavirus group C NSP3 protein that encodes a conserved dsRNA-binding domain (St. Johnston et al., 1992; Langland and Jacobs, 1992). For assays using barley fractions, extracts were first incubated for 5 min with 10 mg/mL poly(rA) before incubation with the dsRNA-agarose resin. For competition assays, soluble nucleic acids were incubated with the extract for 5 min at 4°C (at the indicated concentrations) prior to incubation with the dsRNA-agarose.

**Poly(rI)-Poly(rC)-Agarose-Binding Assays**

Binding assays were performed as previously described (Langland and Jacobs, 1992). For assays using barley fractions, extracts were first incubated for 5 min with 10 mg/mL poly(rA) before incubation with the dsRNA-agarose resin. For competition assays, soluble nucleic acids were incubated with the extract for 5 min at 4°C (at the indicated concentrations) prior to incubation with the dsRNA-agarose.

**Western Blot Analysis**

Proteins were separated by SDS-PAGE and transferred to nitrocellulose, and nonspecific binding sites were blocked with Blotto (Chu et al., 1989). The nitrocellulose was incubated with the primary antibody and secondary goat anti-rabbit IgG alkaline phosphatase conjugate and developed with 3-hydroxy-2-naphthoic acid anilide phospho-
buffer, pH 7.4) for 2 h at 42°C (Springer, 1990). The full-length mPKR gene (Meurs et al., 1990) and the mouse electrophoresed on a 1% (w/v) agarose-formaldehyde membrane was pretreated with RNA PT buffer (5X SET gel, transferred to nitrocellulose using a pressure blotter (Crum et al., 1988). Poly(A)$^+$ mRNA (500 ng) was using an oligo(dT) column matrix (Oligotex-dT kit; Qia-
RNA was purified from the resulting total RNA $^+$ Poly(A) followed by another precipitation with ethanol. 2 at 10,000g for 20 min and the pellet was resuspended in 2.5 mL of H
was precipitated by the addition of 2.5 mL of 4 M LiCl and precipitated by the addition of 2.5 mL of 4 M LiCl and 4-benzoylamino-2,5-diethoxybenzenediazonium chloride hemi[zinc chloride] salt.

Immunoprecipitation

For radioimmune precipitation, extracts were bound to dsRNA-agarose, subjected to in vitro phosphorylation with $[^{32}P]ATP$, and eluted in 1% (w/v) SDS with boiling. The supernatant was diluted to 0.1% (w/v) SDS, followed by the addition of 5 μL of antisera and incubation for 1 h on ice. *Staphylococcus aureus* cells (formalin-fixed, heat-killed, Cowan 1 strain) were added and incubation continued for 1 h. The immunocomplex was pelleted and washed three times in radiolabeled immunoprecipitation antigen buffer (50 mM Tris-Cl, pH 7.5, 150 mM NaCl, 1% [w/v] Nonidet P-40, 0.5% [v/v] sodium deoxycholate, 0.1% [w/v] SDS) and once in 0.1 mM Tris-Cl, pH 7.4, according to the method of Springer (1990). Bound proteins were eluted by addition of SDS-PAGE lysis buffer and incubation for 5 min in a boiling water bath prior to SDS-PAGE and autoradiography (Crum et al., 1988).

For immunoprecipitation-clearing experiments maximal clearing of PKR from extracts was achieved by incubating ribosomal salt wash fractions (0.5 mL) overnight at 4°C with anti-PKR sera (a mixture of a 1:10 dilution of anti-dsRNA-binding domain serum, 1:200 dilution of monoclonal human anti-PKR serum, and a 1:10 dilution of anti-pPKR serum). Separate fractions were similarly incubated with a 1:10 dilution of preimmune serum. *S. aureus* cells were then added and incubation continued for another 1 h at 4°C. The bacterial cells were pelleted and the supernatant fluid was used in standard poly(rI)-poly(rC)-agarose-binding assays.

Northern Blot Analysis

Leaf tissue (5 g) was harvested, frozen in liquid nitrogen, and ground to a fine powder. The tissue was imme-
diately added to 7.5 mL of NTES (100 mM NaCl, 10 mM Tris, pH 7.5, 1 mM EDTA, 1% [w/v] SDS) and 5 mL of phenol:chloroform:isoamyl alcohol (25:24:1, v/v) and vortexed for 30 s. The extract was centrifuged at 1000g for 5 min and the supernatant fraction was recentrifuged at 8000g for 10 min. Nucleic acid was precipitated with ethanol and resuspended in 2.5 mL of H$_2$O. Total RNA was precipitated by the addition of 2.5 mL of 4 M LiCl and overnight incubation at 4°C. The solution was centrifuged at 10,000g for 20 min and the pellet was resuspended in H$_2$O, followed by another precipitation with ethanol. Poly(A)$^+$ RNA was purified from the resulting total RNA using an oligo(dt) column matrix (Oligotex-dT kit; Qiagen, Inc., Chatsworth, CA). Poly(A)$^+$ mRNA (500 ng) was electrophoresed on a 1% (w/v) agarose-formaldehyde gel, transferred to nitrocellulose using a pressure blotter (Posiblot, Stratagene) and fixed by UV cross-linking. The membrane was pretreated with RNA PT buffer ($5 \times$ SET $[20 \times$ SET $= 3 \ M \ NaCl, 0.6 \ M \ Tris-Cl, pH \ 8.0, 40 \ mM \ EDTA], 10 \times$ Denhardt's solution, 50 mM phosphate buffer, pH 7.4) for 2 h at 42°C (Springer, 1990). The full-length mPKR gene (Meurs et al., 1990) was precipitated by the addition of 2.5 mL of 4 M LiCl and precipitation.

RESULTS
dsRNA-Dependent Autophosphorylation and Binding Properties of pPKR

Activation of the mPKR is dependent on the presence of dsRNA (Hovanessian, 1989). As shown in Figure 1B, autophosphorylation of an M, 68,000 to 70,000 protein (mouse PKR) in ribosomal salt wash extracts from mouse L cells occurred in the presence of 1 μg/mL dsRNA. This autophosphorylation event leads to the activation of mPKR (Samuel, 1993). The radiolabeled minor band at M, 80,000 in Figure 1B has been observed previously from mouse cell extracts, but the identity is not known (B.L. Jacobs, unpublished data). When similar assays were performed using barley leaf extracts, dsRNA-dependent phosphorylation of an M, 70,000 protein (pPKR) was observed (Fig. 1A). Much higher concentrations of dsRNA were required for pPKR phosphorylation (5–25 μg/mL) than for mPKR; however, this may be due to the presence of nuclelease activity in the crude barley extract.

As previously reported (Watson et al., 1991), both human and mouse PKR bind specifically to dsRNA-agarose and can subsequently be autophosphorylated in the presence of ATP (Fig. 2, lanes A and B). Since pPKR phosphorylation was dependent on the presence of dsRNA, pPKR binding

![Image](Figure 1. dsRNA-dependent phosphorylation of a barley M, 68,000 to 70,000 polypeptide (pPKR). Barley (A) or mouse L-cell (B) ribosomal salt wash extracts were prepared and incubated in the presence of $[^{32}P]ATP$ with increasing concentrations of soluble poly(rI)-poly(rC): lane A, 0 μg/mL; lane B, 1 μg/mL; lane C, 5 μg/mL; lane D, 25 μg/mL. The figure represents the autoradiograph of radiolabeled proteins separated by SDS-PAGE. Molecular mass mark-
to a dsRNA-linked affinity resin was determined. Initial attempts measuring pPKR binding to dsRNA-agarose were inconsistent. We determined that preincubation of the plant extracts with 10 μg/mL ssRNA prior to incubation with the dsRNA-agarose resin was necessary for binding and phosphorylation. This is likely due to the removal of nonspecific RNA-binding proteins that would otherwise inhibit pPKR binding and phosphorylation in this assay. Following ssRNA preincubation with barley leaf extracts, specific binding of pPKR protein to the dsRNA resin and subsequent autophosphorylation in the presence of ATP were observed (Fig. 2, lane C). Binding of the pPKR to the dsRNA-agarose could be prevented by the addition of excess soluble dsRNA (Fig. 2, lane D). As shown in Figure 2, the human and mouse PKRs have $M_r$ s of 72,000 and 68,000, respectively. The pPKR appears to have an $M_r$ of approximately 70,000 by SDS-PAGE.

Competition assays were performed to determine the specificity of dsRNA binding by pPKR. Barley leaf extracts were preincubated with 10 mg/mL ssRNA. Following this preincubation, increasing concentrations of soluble dsRNA or soluble ssRNA as indicated were added, and the extracts were incubated with dsRNA-agarose. pPKR binding and phosphorylation on the dsRNA-agarose was assayed after thorough washing of the resin. As shown in Figure 3, in the presence of soluble dsRNA subsequent pPKR phosphorylation levels decreased (top, cf. lane A with lanes B–E); however, no decrease in pPKR phosphorylation levels were observed in the presence of increasing amounts of soluble ssRNA (top, cf. lane A with lanes F–I). To confirm that the decrease in pPKR phosphorylation levels was due to a decrease in pPKR protein levels, antisierum specific to pPKR was used. As shown in the western blot in Figure 3 (bottom), pPKR protein levels decreased in a manner comparable to phosphorylation levels in the presence of soluble dsRNA (lanes B–E). Little or no decrease in pPKR protein levels was observed upon incubation when ssRNA was added prior to dsRNA-agarose-binding assays (lanes F–I). Taken together, these data indicate that soluble dsRNA but not ssRNA specifically competes for pPKR binding to dsRNA-agarose, causing subsequent inhibition of autophosphorylation.

**Subcellular Distribution of pPKR**

At the subcellular level, the mPKR is present both free in the cytosol and in association with ribosomes (Fig. 4, lanes
C and D; Langland and Jacobs, 1992). Presumably, this is important in the functional and physiological roles of the mPKR. Similar subcellular fractions were prepared from barley leaf tissue. When barley cytosolic (S-100) and ribosomal salt wash fractions were assayed for pPKR activity, a similar M<sub>r</sub> 70,000 phosphoprotein was detected in both fractions (Fig. 4, lanes A and B, respectively). Western blot analysis using antisera to pPKR and to the specific dsRNA-binding domain peptide also indicated the presence of the M<sub>r</sub> 70,000 radiolabeled pPKR protein in both subcellular fractions (data not shown).

**dsRNA-Dependent Histone Phosphorylation**

The mPKR is known to phosphorylate several substrates, including histone proteins (Jacobs and Imani, 1988). Histone phosphorylation requires activation of mPKR and, therefore, coincides with mPKR autophosphorylation. dsRNA-dependent phosphorylation of histone proteins by mPKR is shown in Figure 5B. Assays were performed to determine whether similar dsRNA-dependent phosphorylation of histone proteins could be observed in barley ribosomal salt wash extracts. Extracts of barley leaf tissue contained histone kinase activity even in the absence of dsRNA (Fig. 5A). However, addition of dsRNA led to an increase in histone phosphorylation, which was most evident with H1 histone (M<sub>r</sub> 38,000). Plant PKR autophosphorylation is not visible on the short film exposure shown in this figure but is visible upon longer exposure (data not shown). Maximal pPKR autophosphorylation was detected at 30 µg/mL dsRNA, a concentration similar to that which led to maximal histone phosphorylation. When cytosolic and ribosomal salt wash extracts from barley leaves were purified by dsRNA-agarose chromatography, the bound protein fraction was capable of catalyzing histone phosphorylation (data not shown).

To further demonstrate pPKR involvement in histone phosphorylation, immunoprecipitation clearing assays were performed. In this assay, anti-PKR or preimmune serum was incubated with extracts, followed by precipitation of the antiserum with S. aureus cells. The supernatant fraction was used in a poly(rI)-poly(rC)-agarose-binding assay to monitor PKR autophosphorylation and histone phosphorylation. As shown in Figure 6, levels of pPKR autophosphorylation were reduced upon clearing with immune but not with preimmune sera (cf. lanes A and B of Fig. 6A). When these fractions were assayed for the ability to phosphorylate exogenous histones, a reduction in the level of histone phosphorylation was also observed (Fig. 6A, lanes C and D). Similar results were obtained when...
human extracts were assayed. Immune sera reduced the level of mPKR autophosphorylation to some degree and reduced the phosphorylation of exogenous histone proteins, except histone H1 (Fig. 6B, lanes A-D). Such differential histone phosphorylation has been previously observed with the mPKR (Jacobs and Imani, 1988).

**Immunocharacterization of pPKR**

A highly specific, monoclonal antiserum to the human PKR (Laurent et al., 1985) and a rabbit polyclonal antiserum to the 69-amino acid dsRNA-binding motif of the rotavirus NSP3 protein (Langland et al., 1994) were used to characterize antigenic similarities between the mPKR and pPKR proteins. Immunoprecipitation analysis demonstrated that both the anti-dsRNA-binding motif serum and the monoclonal anti-human PKR serum recognized dsRNA-agarose-purified human PKR (Fig. 7, lanes B and D, respectively). Similar immunoprecipitation results were obtained with the dsRNA-agarose-purified barley pPKR (Fig. 7, lanes A and C). A co-precipitated protein of Mr approximately 48,000 was also detected in both the human and barley extracts. This protein has previously been reported in the human system as a degradation product of the full-length PKR protein (Galabru and Hovanessian, 1985).

Western blot analysis also demonstrated immunological similarity between mPKR and pPKR. An M, 70,000 band was recognized by antiserum to the conserved dsRNA-binding domain and co-migrated with the phosphorylated protein (Fig. 8, cf. lanes A and B with lanes E and F). This band was present on western blots using mouse and barley ribosomal salt wash extracts in which protein had first been purified on dsRNA-agarose (Fig. 8, lanes A and B, respectively). Furthermore, polyclonal antiserum to HPLC-purified pPKR (J.O. Langland, L.A. Langland, and D.A. Roth, unpublished data) was able to detect a dsRNA-agarose-purified protein present in both mouse and barley extracts (Fig. 8, lanes C and D, respectively). Doublet bands were detected on the western blot of barley and mouse ribosomal salt wash fractions using either the anti-dsRNA-binding motif serum or the anti-pPKR serum. The upper band co-migrated with the phosphorylated protein, and the lower band is thought to represent either the unphosphorylated form of PKR (Langland and Jacobs, 1992) or a degradation product of the protein. Preimmune sera did not cross-react with pPKR or mPKR (data not shown).

**Northern Blot Analysis of Tobacco and Wheat Germ RNA**

Radiolabeled probes were created by random-priming reactions using cDNA clones of the mouse (Baier et al., 1993) and human (Meurs et al., 1990) PKRs. Northern blot analysis using these probes revealed primarily a single transcript present in human poly(A)+ RNA (Fig. 9, lane D). A similarly sized 2.5-kb transcript was also detected from poly(A)+ RNA isolated from mock-inoculated and TMV-infected tobacco leaf tissue and from wheat germ using these probes (Fig. 9, lanes A and B, respectively).
Identification of a Plant-Encoded Double-Stranded RNA-Dependent Kinase

Phosphorylation/dephosphorylation is an important mechanism by which the functional activity of proteins can be regulated. The IFN-induced, dsRNA-dependent protein kinase PKR has been suggested to regulate multiple biological processes, including virus replication (Hovanessian, 1989; Samuel, 1991; Sen and Lengyel, 1992). Previous studies, using nucleotide photoaffinity labeling and immunoprecipitation assays, identified a plant-encoded protein whose phosphorylation is induced in the presence of virus or viroid infection or treatment with dsRNA (Crum et al., 1988; Hiddinga et al., 1988) but not dsDNA or DNA:RNA hybrids (Roth and He, 1994). Here we demonstrate that this protein, now termed pPKR, is analogous to mPKR. Physical characteristics of pPKR are similar to those observed with the mPKR, including dsRNA-binding activity and dsRNA-dependent phosphotransferase activity. Furthermore, three different specific antisera cross-react with the mPKR and pPKR. One of the antisera was specific to a conserved dsRNA-binding motif (St. Johnston et al., 1992; Langland et al., 1994), and its recognition of the pPKR suggests the presence of this conserved domain in the pPKR protein. Specific binding of pPKR to dsRNA-agarose further supports this contention. The presence of a specific dsRNA-binding site in the pPKR suggests conservation of a regulatory motif between plant and vertebrate kinases.

The hybridization of cDNAs from mouse and human PKR to a single 2.5-kb poly(A)⁺ RNA from plant cells provides further evidence establishing the homology between the mPKR enzymes and pPKR. PKR analog in plant tissue. Based on this study, we are currently screening plant cDNA libraries and have identified multiple positive clones using mouse and human cDNA probes. Sequencing of these clones will directly establish the homology between the mPKR enzymes and pPKR.

The demonstration of a PKR analog in plants has implications in the regulation of viral and viroid pathogenesis as well as in normal cellular function. Although the function of pPKR remains speculative, the enzyme may mediate the host response to viral or viroid infection through the presence of dsRNA in a system analogous to mPKR. It is unknown whether plant eIF-2α can be phosphorylated by pPKR, although our preliminary evidence suggests that human and plant eIF-2α may be phosphorylated in vitro by dsRNA-agarose-purified pPKR. The specificity and nature of this phosphorylation event remains to be studied. Clearly, a shutdown of protein synthesis in plant cells is not observed upon virus infection. However, Fraser and Gerwitz (1980) found that protein synthesis in TMV-infected tissues can be significantly inhibited during virus replication with subsequent recovery to levels comparable to those in uninfected plants following virus accumulation. No change in host mRNA levels was found, suggesting that inhibition was at the level of translation. In the mammalian system, many viruses have evolved regulatory mechanisms to inhibit mPKR activity and thus the antiviral effects associated with mPKR (Kitajewski et al., 1986; Jacobs and Imani, 1988; O'Malley et al., 1989; Watson et al., 1991; Katze, 1992). Thus, it is conceivable that although pPKR phosphorylation is significantly increased upon TMV infection of a susceptible host genotype (Crum et al., 1988), subsequent antiviral effects may be inhibited.

Alternately, our initial studies suggested that activation of pPKR may represent the triggering event in virus and viroid pathogenesis (Hiddinga et al., 1988; Hu and Roth, 1991). In vivo pPKR phosphorylation in tobacco protoplasts synchronously infected with TMV is correlated with virus replication and synthesis of viral proteins (Hu and Roth, 1991). Viroids replicate and induce disease in plants without encoding any proteins. This implies that the highly structured viroid RNA or replicative intermediates may interact with selected host factors to regulate replicative processes and symptom development. Recently, Diener et al. (1993) found that the mPKR is capable of being activated by viroid strains in a manner directly related to their ability to incite severe disease symptoms. These data, together with the observation that pPKR phosphorylation levels are induced during viroid infection (Hiddinga et al., 1988), support a possible role for pPKR in pathogenesis.

Although a major role of PKR in mammalian cells relates to the IFN-induced antiviral response, it is now apparent that mPKR is multifunctional. The mPKR is believed to be involved in control of gene expression through phosphorylation of I-κB. Phosphorylation induces the dissociation of I-κB from NF-κB and activation of the transcription of a number of genes stimulated by NF-κB (Clems, 1992; Jimenez-Garcia et al., 1993; Kumar et al., 1994). Recently, mPKR has been suggested to have apparent tumor-
suppressor activity (Clemens, 1992; Koromilas et al., 1992), possibly through translational and/or transcriptional regulatory mechanisms or phosphorylation of other regulatory substrates, perhaps including histone proteins. The distinctly different subcellular forms of mPKR have been suggested to be involved in these multiple physiological roles of mPKR (Koromilas et al., 1992; Langland and Jacobs, 1992). Since the pPKR has a subcellular distribution similar to that described for the mPKR, comparable physiological activities may be expected. In plant extracts, both ribosome-associated and cytosolic fractions containing pPKR were able to phosphorylate exogenous histones. Furthermore, pPKR has dsRNA-binding activity and could phosphorylate histones in a dsRNA-dependent manner. The dsRNA-dependent phosphorylation of histones parallel pPKR phosphorylation and immunoclearing of pPKR from extracts resulted in a reduction in the extent of histone phosphorylation. These results suggest that histone proteins may be a substrate for pPKR. The functional significance of histone phosphorylation by the mPKR remains unclear. However, recent evidence suggests the cell-cycle-dependent presence of mPKR within the nucleus (Jimenez-Garcia et al., 1993), thereby possibly giving mPKR access to histone substrates. The involvement of mPKR with apoptosis also suggests the regulatory significance of PKR and a possible role of histone phosphorylation (Lee and Esteban, 1994).

The multifunctional nature of mPKR suggests several possible roles for the analogous pPKR in infected as well as healthy plant cells. The cloning of pPKR will provide the opportunity to explore many of these potential effects.

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