Phosphorylation of Plant Eukaryotic Initiation Factor-2 by the Plant-encoded Double-stranded RNA-dependent Protein Kinase, pPKR, and Inhibition of Protein Synthesis in Vitro*

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The initiation phase in eukaryotic protein synthesis is characterized by complex interactions between numerous initiation factors, ribosomal subunits, nucleotides, and Met-tRNA. Framework events in initiation-reinitiation involve ternary complex (elF-2-GTP-tRNA) binding to free 40 S ribosomal subunits to form a 48 S preinitiation complex. The subsequent binding of mRNA and 60 S ribosomal subunits is dependent upon GTP hydrolysis, yielding a viable initiation complex and releasing elF-2GDP (1, 2). In order for another round of initiation to begin, GDP must be exchanged with GTP. This guanine nucleotide exchange is catalyzed by elF-2B. It is generally believed that phosphorylation of the α subunit of elF-2 on Ser51 stabilizes the elF-2GDP-elF-2B complex, effectively sequestering elF-2B. This elegant regulatory mechanism is induced by various stresses including virus infection (3), heat shock (4), and deprivation of amino acids (5) or heme (6).

The elF-2α kinases comprise a specific protein kinase subfamily (7). They include the heme-deficient kinase, HRI (6, 8); the interferon-induced, double-stranded RNA (dsRNA)-dependent kinase, mPKR (9, 10); and yeast GCN2 (11, 12). Although their noncatalytic regions differ significantly, all contain inviolate amino acid domains associated with Ser/Thr kinase activity and elF-2α phosphorylation (7). Collectively, the mammalian interferon-induced dsRNA-dependent protein kinases are termed PKR or mPKR. Low levels (1–10 μg/ml) of dsRNA or appropriately structured single-stranded RNA (ssRNA) bind to and activate mPKR via autophosphorylation, whereas higher levels have an inhibitory effect (7, 13). Recently, a plant-encoded analog of mPKR has been identified and characterized from monocot and dicot tissues (14, 15). Plant PKR, pPKR, is an M₆, 68,000–70,000 Ser/Thr kinase present in both cytosolic and ribosome-associated fractions. Although it is constitutively expressed in healthy cells, pPKR protein and phosphorylation levels are significantly stimulated by dsRNA, select polyanions, or virus/viroid infection (16, 17).

The regulation of plant protein translation by elF-2α phosphorylation has not been demonstrated. Indeed, there is considerable confusion in the literature regarding the phosphorylation of elF-2, in large part due to discrepancies in subunit identification (18–21). elF-2α is composed of three subunits, the M₆, 36,000 α subunit, the M₆, 38,000 β subunit, and the M₆, 52,000 γ subunit (22). Plant elF-2 (peIF-2) has been purified and is also composed of three subunits of M₆, 38,000, M₆, 42,000 (doublet), and M₆, 50,000 (18–20, 23–26). Several studies have indicated that kinases that phosphorylate the α-subunit of mPKR also phosphorylate the M₆, 42,000 subunit of peIF-2, although often this subunit has been designated as elF-2β (18, 19, 21, 27). It has not been conclusively shown that phosphorylation of the M₆, 42,000 subunit affects translational activity in wheat germ systems. Previous failure to detect an effect of phosphorylation in vitro (28) or in vivo (29), the lower dissociation constant of elF-2 for GDP in plants (20) compared with mammals, and the apparent absence of plant recycling factor activity (elF-2B) have led to the general assumption that plants do not use phosphorylation of elF-2α as a means to regulate protein translation (20). However, the wheat germ M₆, 42,000 subunit has now been conclusively identified as the α-subunit and the M₆, 38,000 as the β-subunit by molecular cloning and cDNA sequencing. Further, Arabidopsis thaliana...
expressed sequence tags (EST, GenBank<sup>TM</sup> accession numbers T45955 and T22066) that encode functional equivalents of mEF-2α contain the inviolate phosphorylation sites corresponding to Ser<sup>51</sup>. In addition, an A. thaliana EST (GenBank<sup>TM</sup> accession number T44879) and a rice EST (GenBank<sup>TM</sup> accession number D25052) appear to encode the functional equivalents of yeast GCN3 and GCD2, respectively, that are subunits of recycling factor eEF-2B. Thus, it appears highly likely that plants contain the necessary factors for an eEF-2α phosphorylation pathway.

Here we show that the plant-encoded pPKR is capable of phosphorylating the α subunit of both plant and mammalian eEF-2. In addition, in vitro protein synthesis in wheat germ lysates is specifically inhibited by phosphorylated eEF-2α, dsRNA, an inducer of pPKR activity, or activated mPKR.

**EXPERIMENTAL PROCEDURES**

**Materials—**Plant eEF-2 was purified according to Ref. 30. Purified human eEF-2 was supplied by W. Merrick (Case Western Reserve University, Cleveland, OH) and N. Gupta (University of Nebraska, Lincoln, NE). Poly(r)poly(C)-agarose was made according to Langland et al. (31). In vitro translation systems were obtained from Promega (Madison, WI) and Ambion (Austin, TX). Anti-pPKR serum was prepared according to Langland et al. (15), antiserum to a conserved dsRNA binding domain (that recognizes pPKR) (15) was supplied by B. Jacobs (Arizona State University, Tempe, AZ), and the monoclonal antibody to mPKR was supplied by A. Hovanessian (Institut Pasteur, Paris, France) (32). The peptide corresponding to residues 45–56 of mEF-2α was synthesized according to the method of Mellor and Proud (33). All other reagents and chemicals were purchased from Sigma unless otherwise indicated.

**Barley (Hordeum vulgare L. “Stephoe”)** was grown in the dark for 7 days at 25 °C. Leaves were homogenized in 150 mM PIPES, pH 7.5, 50 mM EDTA, 5 mM dithiothreitol, 100 mM KCl, 1 μg/ml aprotinin, 1 μg/ml leupeptin, and 2 mM phenylmethylsulfonyl fluoride. The samples were dried and resuspended in 6% (v/v) formic acid, 1.25% (v/v) pyridine followed by electrophoretic thin layer chromatography separation (using Silica gel G) in the same buffer at 400 V for 2 h. The peptides were visualized by autoradiography.

**Immunodepletion Analysis—**For immunoprecipitation clearing experiments, maximal clearing of pPKR from extracts was achieved by incubating ribosome salt wash fractions (0.5 ml) overnight at 4 °C with anti-PKR sera (containing a mixture of dsRNA-binding domain anti-serum (1:1 dilution), monomolecular human PKR antisemur (1:100 dilution), and anti-pPKR serum (1:10 dilution). Separate fractions were similarly incubated with preimmune serum. Staphylococcus aureus cells were then added, and incubation continued for 1 h at 4 °C. The bacterial cells were pelleted, and the supernatant was used in standard poly(r)poly(C)-agarose binding assays.

**In Vitro Translation—**Protein synthesis assays were done at 25 °C for 60 min in standard wheat germ lysate reaction mixtures according to the manufacturer’s directions using BMV RNA. Aliquots were denatured in SDS-sample buffer and separated by SDS-PAGE. Where indicated, poly(r)poly(C), poly(rI), or poly(rI) was added to the translation reaction prior to the addition of BMV RNA.

In vitro translation assays to evaluate the effect of phosphorylated eEF-2-α on protein synthesis were modified as follows: mPKR or buffer (20 μm HEPES, pH 7.5, 10% glycerol, 100 μm KCl, 5 μm MnCl<sub>2</sub>, 5 μm MgOAc, 14 μm β-mercaptoethanol) was incubated with poly(r)poly(C)-agarose and thoroughly washed in 1 μl KCl buffer followed by a 10 μm KCl wash (31) prior to the addition of phosphorylation mix alone or plant or mammalian eEF-2α (3 μg) in phosphorylation mix. Phosphorylation mix contained 100 μM unlabelled ATP, 5 μM MnCl<sub>2</sub>, 5 μM MgOAc, 20 μM HEPES, pH 7.5, and 10 μM KCl. After incubation for 20 min at 30 °C, the resin containing the bound mPKR or the resin incubated with buffer was pelleted, and the supernatant was removed and aliquots were fractionated by SDS-PAGE. An autoradiogram of in vivo translation mix manufactured by Ambion, and BMV RNA translation assays were performed according to the manufacturer’s directions.

**RESULTS AND DISCUSSION**

Specific kinases present in animals and yeast phosphorylate eEF-2α, resulting in translational regulation (3). A corresponding eEF-2α phosphorylation pathway has not been identified in plants, although a plant analog of the mammalian eEF-2α kinase, PKR, has been characterized (14–16). Fig. 1A demonstrates that a kinase present in the ribosomal salt wash fractions from barley leaves is capable of specific in vitro phosphorylation of the M. 36,000 subunit of mEF-2 and the M. 42,000 doublet of pEF-2, now termed pEF-2α (lanes B and C, respectively). Phosphorylation of pEF-2α by the plant kinase appears better than phosphorylation of mEF-2α, given that equal protein amounts were used. Purified human PKR likewise phosphorylates mEF-2α and pEF-2α (Fig. 1B, lanes B and C) (36). These results suggest that pEF-2α may contain a domain similar to the phosphorylation domain of mEF-2α. Supporting this conclusion is the existence of an Arabidopsis EST (T45955) having 83% identity at the amino acid level with the phosphorylation domain of human eEF-2α corresponding to residues 45–56. Further this plant EST has 100% identity with a domain (residues 72–90) that is highly conserved between eEF-2α and K3L. K3L is a vaccinia virus-encoded protein that is a competitive inhibitor of PKR-mediated phosphorylation of eEF-2α (37). The labeled bands at M. 48,000 and 55,000 in lane A are degradation products of pPKR based upon peptide mapping (data not shown), and it is likely that proteolytic activity not controllable by the inhibitors used in extract preparation is also responsible for the apparent degradation in the eEF-2 preparations observed in Fig. 1A, lanes B and C.

The fact that plant kinase fractions were incubated with dsRNA-agarose and thoroughly washed prior to in vitro phosphorylation assays suggests that the responsible kinase is a...
The positions of molecular mass markers (in kDa), pPKR, mPKR, and meIF-2 are shown. The only source of kinase was partially purified mPKR from HeLa cells. Therefore, the role of pPKR in eIF-2α phosphorylation levels is directly correlated with decreased pPKR levels from immunoclearing (lanes B and C).

To further define the role of pPKR in eIF-2α phosphorylation, dsRNA-agarose-purified ribosomal salt wash fractions from barley containing pPKR were incubated with 2-aminopurine, a potent inhibitor of PKR (38). The inhibition of meIF-2α and pPKR phosphorylation by barley and HeLa cell ribosomal salt wash fractions in the presence of 2-aminopurine was directly correlated with inhibition of pPKR and mPKR phosphorylation (data not shown).

The eIF-2α kinases specifically phosphorylate Ser51 of meIF-2α (1, 2, 7). To confirm pPKR as a member of the eIF-2α kinase family, the ability of pPKR to specifically phosphorylate Ser51 of eIF-2α was evaluated in vitro phosphorylation reactions using a synthetic eIF-2α peptide substrate. A peptide substrate with the sequence ILLSELRRRIR corresponding to residues 45–56 of meIF-2α (33) was synthesized and subjected to in vitro phosphorylation in the presence of either purified mPKR or partially purified pPKR. Mellor and Proud (33) reported that only eIF-2α kinases are capable of phosphorylating the residue corresponding to Ser51 in this peptide in the absence of Ca2+ and phosphatidyserine. Both mPKR and a dsRNA-binding kinase present in the partially purified plant extract from barley tissues phosphorylated the peptide substrate (Fig. 4A, lanes A and C, respectively). The M, 68,000–70,000 pPKR band was visible in lane C only after prolonged exposure. The level of peptide phosphorylation decreased when purified mPKR or plant extracts were incubated with soluble dsRNA (lanes B and D) but not ssRNA (lanes A and C) prior to dsRNA-agarose binding and in vitro phosphorylation. The identity of pPKR as the kinase responsible for peptide phosphorylation was also indicated by immunoclearing experiments. Immunoabsorption of plant extracts with specific anti-pPKR sera prior to dsRNA-agarose binding resulted in decreased pPKR phosphorylation levels and a corresponding decrease in peptide phosphorylation (Fig. 4B, lanes A and B).

The synthetic eIF-2α peptide contains two Ser residues cor-

![Figure 1](http://www.jbc.org/)

**Fig. 1.** Phosphorylation of plant and mammalian eIF-2. Panel A, ribosome salt wash fractions were prepared from barley tissue followed by incubation with poly(rI)-poly(rC)-agarose and in vitro phosphorylation in the presence of γ-32P-ATP. Bound proteins were eluted, separated by SDS-PAGE, and visualized by autoradiography. In lane A, there were no further additions. In lanes B and C, 3 μg of mammalian eIF-2 and plant eIF-2, respectively, were added prior to the addition of [γ-32P]ATP. Panel B, similar assays to those in panel A were performed, only the source of kinase was partially purified mPKR from HeLa cells. The positions of molecular mass markers (in kDa), pPKR, mPKR, peIF-2α, and meIF-2α are shown.

![Figure 2](http://www.jbc.org/)

**Fig. 2.** Phosphorylation of eIF-2α by a dsRNA-binding protein kinase. Barley ribosome salt wash fractions (lanes A, B, E, and F) or partially purified HeLa mPKR (lanes C, D, G, and H) were incubated with poly(rI)-poly(rC)-agarose followed by the addition of [γ-32P]ATP and plant eIF-2α (lanes A–D) or mammalian eIF-2α (lanes E–H). In lanes B, D, F, and H, extracts were incubated with 500 μg of soluble poly(rI)-poly(rC) prior to incubation with poly(rI)-poly(rC)-agarose. Radiolabeled proteins were eluted, separated by SDS-PAGE, and visualized by autoradiography. The positions of molecular mass markers (in kDa), PKR, pPKR, and meIF-2α are shown.
resin, poly(rC)-agarose binding assays. After thorough washing of the bacterial cells were pelleted, and the supernatant was used in standard S. aureus (C). Following incubation, mPKR bound to poly(rI)-poly(rC)-agarose followed by the addition of [γ-32P]ATP and the eIF-2 peptide (3 μg). Extracts were incubated with 500 μg of soluble poly(rI)-poly(rC) (lanes B and D) or 500 μg of soluble poly(rI) (lanes A and C) prior to incubation with poly(rI)-poly(rC)-agarose. Radiolabeled proteins were eluted, separated on a Tricine gel, and visualized by autoradiography. Panel B, barley ribosome salt wash fractions were incubated with preimmune serum (lane A) or anti-PKR sera (lane B). S. aureus cells were then added, and incubation continued. The bacterial cells were pelleted, and the supernatant containing the phosphorylation mix alone (lanes A and D), mix and unphosphorylated eIF-2 (lanes B and C), or mix and phosphorylated eIF-2 (lanes E and F) was removed and added to standard wheat germ lysate in vitro translation reactions in the presence of BMV RNA and [35S]methionine. Translated proteins were separated by SDS-PAGE and visualized by autoradiography. The positions of the molecular mass markers (in kDa), PKR, and the eIF-2 peptide are shown.

Since phosphorylation of eIF-2α by mPKR inhibits protein synthesis and pPKR is capable of phosphorylating plant eIF-2α, we were interested in determining if plant protein synthesis is inhibited in the presence of phosphorylated eIF-2. Phosphorylation of meIF-2 and peIF-2 was accomplished by incubation with mPKR bound to poly(rI)-poly(rC)-agarose in the presence of unlabeled ATP and phosphorylation buffer. Following incubation, mPKR bound to poly(rI)-poly(rC)-agarose was pelleted, and the supernatant containing the phosphorylated meIF-2 or peIF-2 was added to translation reactions. Controls consisted of 1) phosphorylation mix (containing only buffer and ATP), 2) phosphorylation mix containing unphosphorylated eIF-2, 3) phosphorylation mix containing unphosphorylated meIF-2, and 4) phosphorylation mix alone incubated previously with mPKR bound to poly(rI)-poly(rC)-agarose. Controls 1–3 were incubated with poly(rI)-poly(rC)-agarose in the absence of mPKR. Fig. 5 shows that in vitro translation of BMV RNA is unaffected in the presence of phosphorylation mix alone (lane A), unphosphorylated meIF-2α (lane B), unphosphorylated peIF-2α (lane C) or phosphorylation mix supernatant that was incubated with mPKR bound to poly(rI)-poly(rC)-agarose (lane D). However, the addition of meIF-2 or peIF-2 that was phosphorylated by mPKR dramati-

**Fig. 3.** Decreased eIF-2α phosphorylation in pPKR immune-cleared extracts. Barley ribosome salt wash fractions (lanes B and C) were incubated with preimmune serum (lane B) or anti-PKR sera (lane C). Controls 1–3 were incubated with poly(rI)-poly(rC)-agarose followed by the addition of [γ-32P]ATP and plant eIF-2 (panel A) or mammalian eIF-2 (panel B) were added. In lane A, partially purified HeLa mPKR was incubated with poly(rI)-poly(rC)-agarose followed by the addition of [γ-32P]ATP and plant eIF-2 (panel A) or mammalian eIF-2 (panel B). Radiolabeled proteins were eluted, separated by SDS-PAGE, and visualized by autoradiography. The position of molecular mass markers (in kDa), PKR, and eIF-2α are shown.

Responding to positions 48 and 51 of meIF-2α. These are separated by a single cleavage site for V8 protease between residues corresponding to Glu49 and Leu50. The positively charged fragment (LSRRRIR) containing Ser51 can be separated from the negatively charged fragment containing Ser52 (ILLSE) by electrophoretic thin layer chromatography (33). When the peptide was in vitro phosphorylated in the presence of [γ-32P]ATP and mPKR or the plant dsRNA-binding kinase followed by V8 digestion and peptide separation, results show that the residue corresponding to Ser51 of the eIF-2α was specifically phosphorylated by mPKR and the plant dsRNA binding kinase (data not shown).

Taken together, these results demonstrate that the M14,000 doublet that is the α subunit of plant eIF-2 and the M36,000 α subunit of meIF-2 can be specifically phosphorylated in vitro by the M68,000–70,000 plant-encoded dsRNA-dependent protein kinase, pPKR, as well as the eIF-2α kinase, mPKR.

**Fig. 4.** pPKR phosphorylation of a synthetic eIF-2α substrate. Panel A, partially purified HeLa mPKR (lanes A and B) or barley ribosome salt wash fractions (lanes C and D) were incubated with poly(rI)-poly(rC)-agarose followed by the addition of [γ-32P]ATP and the eIF-2 peptide (3 μg). Extracts were incubated with 500 μg of soluble poly(rI)-poly(rC) (lanes B and D) or 500 μg of soluble poly(rI) (lanes A and C) prior to incubation with poly(rI)-poly(rC)-agarose. Radiolabeled proteins were eluted, separated on a Tricine gel, and visualized by autoradiography. Panel B, barley ribosome salt wash fractions were incubated with preimmune serum (lane A) or anti-PKR sera (lane B). S. aureus cells were then added, and incubation continued. The bacterial cells were pelleted, and the supernatant was used in standard poly(rI)-poly(rC)-agarose binding assays. After thorough washing of the resin, [γ-32P]ATP and the eIF-2 peptide (3 μg) were added. Radiolabeled proteins were eluted, separated on a Tricine gel, and visualized by autoradiography. The positions of molecular mass markers (in kDa), PKR, and the eIF-2 peptide are shown.

**Fig. 5.** Inhibition of BMV RNA in vitro translation in wheat germ lysates by phosphorylated eIF-2α. Buffer (lanes A–C) or mPKR (lanes D–F) were incubated with poly(rI)-poly(rC)-agarose, washed extensively with 1 M KCl buffer and then 10 mM KCl buffer, and incubated with phosphorylation mix (lanes A and D) or phosphorylation mix containing meIF-2 (lanes B and E) or peIF-2 (lanes C and F). The supernatant containing phosphorylation mix alone (lanes A and D), mix and unphosphorylated eIF-2 (lanes B and C), or mix and phosphorylated eIF-2 (lanes E and F) was removed and added to standard wheat germ lysate in vitro translation reactions in the presence of BMV RNA and [35S]methionine. Translated proteins were separated by SDS-PAGE and visualized by autoradiography. The positions of the molecular mass markers (in kDa) are shown. Asterisks indicate the positions of BMV translation products of M94,000, 35,000, 20,000, and 15,000.
cally inhibited BMV RNA translation in wheat germ lysates (lanes E and F, respectively).

The observations that dsRNA inhibits in vitro translation in rabbit reticulocyte lysates via a mPKR-mediated mechanism (39) and that pPKR is constitutively present in wheat germ (15) encouraged us to determine if dsRNA addition to wheat germ would similarly inhibit protein synthesis. In the absence of exogenous dsRNA, BMV RNA is efficiently translated in vitro in wheat germ lysates (Fig. 6, lane A). The addition of dsRNA to wheat germ in vitro translation lysates inhibited translation of BMV RNA in a concentration-dependent manner (lanes B–E). In rabbit reticulocyte lysates, 0.1 μg/ml dsRNA is sufficient to totally inhibit protein synthesis (39), whereas in Fig. 6A, 10 μg/ml dsRNA was required to significantly inhibit protein synthesis in wheat germ lysates. However, this is consistent with the dsRNA levels necessary to activate pPKR in vitro and in vivo in plant protoplasts (40). As shown in Fig. 6B, the specificity of inhibition is indicated by the fact that translation was not inhibited by ssRNA. It should be noted that we found significant variation between wheat germ lysate preparations in terms of dsRNA levels necessary for inhibition, with several showing no response to dsRNA (data not shown). This effect, we believe, is due to varying levels of a PKR inhibitor present in the lysates.

We next determined if exogenous mPKR is capable of inhibiting in vitro translation in wheat germ lysates. In the absence of mPKR, translation of BMV RNA was comparable with Fig. 6, lane A. The addition of purified mPKR alone decreased in vitro translation of BMV RNA in wheat germ lysates (compare Fig. 7, lane A with Fig. 6, lane A). Only the major BMV translation products of M, 94,000 and 20,000 were detectable. This is likely due to the activation of mPKR that is typically observed during the purification process. However, BMV translation in wheat germ lysates was completely inhibited in the presence of mPKR and low dsRNA concentrations (0.1 μg/ml) (Fig. 7, lane B). This concentration of dsRNA was much lower than that required in Fig. 6, suggesting interaction with mPKR rather than pPKR. Furthermore, this is the optimal dsRNA concentration required for mPKR shutdown of rabbit reticulocyte translation. The addition of ssRNA or buffer alone had no effect on translation (data not shown).

The findings that plant protein translation is inhibited by phosphorylated eIF-2, activated mPKR, or dsRNA indicates that components necessary for the in vivo regulation of protein synthesis via an eIF-2α phosphorylation mechanism are present in plants similarly to other eukaryotes. The dsRNA-dependent inhibition of protein synthesis data is in apparent conflict with previous findings (20, 41). However, we believe that the existence of a plant-encoded mPKR is responsible to the lack of a consistent dsRNA-mediated inhibition of protein translation in wheat germ lysates. The mammalian analog of p67 is tightly associated with meIF-2α and inhibits phosphorylation (42). In separate studies, we found that mammalian p67 inhibited mIF-2α and pIF-2α phosphorylation in the presence of either purified mPKR or partially purified plant fractions containing pPKR. Phosphorylation of mPKR and pPKR in the presence of p67 was correspondingly decreased. Based upon Western blotting using monoclonal antisera, we determined that the plant-encoded p67 inhibitor is temporally regulated during plant growth and development. Differential activation of the plant p67 analog may account for variations in dsRNA sensitivity between wheat germ lysate preparations and the apparent difficulty in characterizing the plant eIF-2α phosphorylation pathway. These results suggest that plants have a previously unappreciated mechanism to regulate cellular response to various environmental signals.

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REFERENCES

1. Hershey, J. W. B. (1991) Annu. Rev. Biochem. 60, 717–755
2. Merrick, W. C. (1992) Microbiol. Rev. 56, 291–315
3. Samuel, C. E. (1991) Virology 183, 1–11
4. Scorsone, K. A., Panners, R., Rowlands, A. G., and Henshaw, E. C. (1987) J. Biol. Chem. 262, 14538–14546
5. Dever, T. E., Feng, L., Wei, R., Cigan, A. M., Donahue, T. F., and Hinnebusch, A. G. (1992) Cell 68, 585–596
6. Pal, J. K., Chen, J. J., and London, M. I. (1991) Biochemistry 30, 2555–2562
7. Samuel, C. E. (1993) J. Biol. Chem. 268, 7603–7606
8. Chen, J. J., Throop, M. S., Gehrike, L. L., Kuo, I., Pal, J. K., Brodsky, M., and London, M. I. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 7729–7733
9. Mikes, E., Chong, K., Galabru, J., Thomas, N. S. B., Kerr, I. M., Williams, B. R. G., and Hovanessian, A. G. (1993) Cell 62, 379–390
10. Thomas, D. C., Doohan, J. P., and Samuel, C. E. (1992) Virology 188, 33–46
11. Wei, R. C., Jackson, B. M., and Hinnebusch, A. G. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 4579–4583
12. Hinnebusch, A. G. (1990) Trends Biochem. Sci. 15, 148–152
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13. Hovanessian, A. G. (1989) J. Interferon Res. 9, 641–647
14. Hiddinga, H. J., Crum, C. J., and Roth, D. A. (1988) Science 241, 451–453
15. Langland, J. O., J in, S., Jacobs, B. L., and Roth, D. A. (1995) Plant Physiol. 108, 1259–1267
16. He, X., and Roth, D. A. (1994) Prog. Mol. Subcell. Biol. 14, 28–47
17. Crum, C. J., Hiddinga, H. J., and Roth, D. A. (1988) J. Biol. Chem. 263, 13440–13443
18. Benne, R., Kasperaitis, M., Voorma, H. O., and Ceglarz, E. (1980) Eur. J. Biochem. 104, 109–117
19. Seel, S. N., Schmidt, A., and Marcus, A. (1983) J. Biol. Chem. 258, 10573–10576
20. Shaikhin, S. M., Smailov, S. K., Lee, A. V., Kozhanov, E. V., and Iskakov, B. K. (1992) Biochimie 74, 447–454
21. Ranu, R. S. (1980) Biochem. Biophys. Res. Commun. 97, 1124–1132
22. Ernst, H., Duncan, R. F., and Hershey, J. W. B. (1987) J. Biol. Chem. 262, 1206–1212
23. Sprenuill, L. L., Waltheall, B. J., Lax, S. R., and Ravel, J. M. (1979) J. Biol. Chem. 254, 143–148
24. Clark, R. D., and Ranu, R. S. (1987) Mol. Cell. Biochem. 74, 129–135
25. Treadwell, B. V., Mauser, L., and Robinson, W. G. (1979) Methods Enzymol. 60, 181–193
26. Lax, S. R., Browning, K. S., Maia, D. M., and Ravel, J. M. (1986) J. Biol. Chem. 261, 15632–15636
27. Mehta, H. B., Dholakia, J. N., Roth, W. W., Parekh, B. S., Montelaro, R. C., Woodley, C. L., and Wahba, A. J. (1986) J. Biol. Chem. 261, 6705–6711
28. Browning, K. S., Yan, T. F. J., Lauer, S. J., Aquino, L. A., Tao, M., and Ravel, J. M. (1985) Plant Physiol. 77, 370–373
29. Dezoeten, G. A., Penswick, J. A., Horisberger, M. A., Ahl, P., Schultze, M., and Hohn, T. (1989) Virology 172, 213–222
30. Lax, S. R., Lauer, S. J., Browning, K. S., and Ravel, J. M. (1986) Methods Enzymol. 118, 109–128
31. Langland, J. O., Pettiford, S., and Jacobs, B. L. (1995) Protein Expression Purif. 6, 25–32
32. Laurent, A. G., Krust, B., Galabru, J., Svab, J., and Hovanessian, A. G. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 4341–4345
33. Mellor, H., and Proud, C. G. (1991) Biochem. Biophys. Res. Commun. 178, 430–437
34. Langland, J. O., and Jacobs, B. L. (1992) J. Biol. Chem. 267, 10729–10736
35. Schagger, H., and von Jages, G. (1987) Anal. Biochem. 166, 368–379
36. Samuel, C. E., Knutson, G. S., Berry, M. J., Atwater, J. A., and Lasky, S. R. (1986) Methods Enzymol. 119, 499–516
37. Davies, M. V., Elroy-Stein, O., Jاجری, R., Moss, B., and Kaufman, R. J. (1992) J. Virol. 66, 1943–1950
38. Samuel, C. E. (1992) Pharmacol. Ther. 54, 307–317
39. Sarre, T. F. (1989) Biosystems 22, 311–325
40. Hu, J., and Roth, D. A. (1991) Biochem. Biophys. Res. Commun. 179, 229–235
41. Rejinders, L., Aalbers, A. M. J., Van Kammen, A., and Berns, A. J. M. (1975) Biochim. Biophys. Acta 390, 69–77
42. Ray, M. K., Datta, B., Chakraborty, A., Chattopadhyay, A., Meza-Keuthen, S., and Gupta, N. K. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 539–543
