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Functional Expression and Characterization of the Interferon-Induced Double-Stranded RNA Activated P68 Protein Kinase from *Escherichia coli*†

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ABSTRACT: The P68 protein (referred to as P68 on the basis of its molecular weight of 68000 in human cells) is a serine/threonine kinase induced by interferon treatment and activated by double-stranded (ds) RNAs. Although extensively studied, little is currently known about the regulation of kinase function at the molecular level. What is known is that activation of this enzyme triggers a series of events which lead to an inhibition of protein synthesis initiation and may, in turn, play an integral role in the antiviral response to interferon. To begin to understand P68 and its biological functions in the eukaryotic cell, we have expressed the protein kinase in *Escherichia coli* under control of the bacteriophage T7 promoter. In rifampicin-treated cells, metabolically labeled with [^{35}S]methionine and induced by IPTG, the P68 kinase was the predominant labeled product. Further, P68 was recovered from extracts as a fully functional enzyme, shown by its ability to become activated and phosphorylate its natural substrate, the α subunit of eukaryotic protein synthesis initiation factor 2 (eIF-2). Moreover, P68 was phosphorylated in vivo in *E. coli*, providing conclusive evidence that the kinase has the capacity to phosphorylate and activate itself in the absence of other eukaryotic proteins. In contrast, a mutant P68 protein, containing a single amino acid substitution in the invariant lysine in catalytic domain II, was completely inactive. Interestingly, both the mutant and wild-type protein kinases efficiently bound activator dsRNAs despite the fact that only the latter was activated by these RNAs. Finally, the expressed kinase could be isolated from contaminating *E. coli* proteins in an active form by immunoaffinity chromatography with a monoclonal antibody specific for P68.

The P68 protein kinase (referred to as P68 from its molecular weight of 68000 but also referred to by others as DAI, dsl, or PI/eIF2) is a member of the serine/threonine kinase family and one of several genes that can be induced by interferon (Lebleu et al., 1976; Lengyel, 1982; Petska et al., 1982; Samuel, 1979). Two activities are characteristic of P68. The first involves activation by dsRNA or heparin (resulting in the autophosphorylation of the enzyme), and the second is that once phosphorylated, P68 catalyzes the phosphorylation of its natural substrate, the α subunit of eukaryotic protein synthesis initiation factor (eIF-2) (Galabru & Hovanessian, 1987; Hovanessian, 1989). This event prevents the recycling of eIF-2–GDP to eIF-2–GTP by the guanine nucleotide exchange factor eIF-2B, due to the latter being sequestered in an inactive complex with eIF-2–GDP (Konieczny & Safer, 1983; Panniers & Henshaw, 1983; Safer, 1983). Resultant limitations in functional eIF-2 impede the initiation step of protein synthesis by preventing the transfer of initiator Met-tRNA (via the ternary complex eIF-2·GTP·Met-tRNA) to the 40S ribosomal subunit before mRNA is bound (Jagus et al., 1981). Both activation of P68 and phosphorylation of eIF-2 α have been reported to be independent of cAMP and cGMP; both activites, however, are markedly stimulated by the divalent cation manganese and ATP (Hovanessian, 1989).

Activation of P68 and enhanced phosphorylation of eIF-2 α have been documented in virus-infected, interferon-treated cells, suggesting a possible role for P68 in the antiviral response mediated by interferon [for a review, see Hovanessian (1989)]. Indeed, viral-specific RNAs, including those of HIV-1, have the capacity to activate the kinase (Maran & Mathews, 1988; Black et al., 1989; SenGupta et al., 1989; Bischoff & Samuel, 1989; Roy et al., 1991). To counteract the potentially harmful effects caused by this activation, certain viruses have devised strategies to down-regulate the P68 [e.g., see Katze et al. (1987), Lee et al. (1990), Black et al. (1989), Roy et al. (1990), and Mellits et al. (1990)]. Despite these studies, however, little is currently known about the molecular mechanisms involved in activation and repression of P68 or of the potential physiological role of the kinase in the cell.

The gene coding for the human P68 kinase has recently been cloned and sequenced (Meurs et al., 1990). The availability of the cloned gene has enabled us to utilize recombinant expression systems to express P68 for use in structure/function

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analysis and to gain further insights into P68 regulation. We report here the expression of the P68 protein kinase in *Escherichia coli* using the bacteriophage T7 promotor. Our results show wild-type P68, as expressed in a soluble form, was fully functional, capable of activation and autophosphorylation, whereas a mutant P68, differing in only a single amino acid, was not. Further, the P68 efficiently phosphorylated its natural substrate, the α subunit of eIF-2. The *E. coli* expressed P68 molecules, which also efficiently bind activator dsRNAs, will allow us to define regulatory regions of the kinase necessary for interaction with virally encoded activators and repressors. Finally, bacterial-expressed P68 also will be of value in elucidating the role of P68 in the regulation of cellular gene expression.

Experimental Procedures

Bacterial Strains and Recombinant Plasmids. Bacterial hosts and vectors used for expression were BL21(DE3)pLysS and pET T7 vectors, respectively (Novogen, Madison, WI; Studier et al., 1990). The cloning of plasmid pBS-8.4R, containing the complete coding region for the P68 protein, has been previously described (Meurs et al., 1990). To facilitate cloning into pET vectors, an *Nde*I restriction site was introduced at the P68 initiating ATG using uracil template M13 site-directed mutagenesis (Kunkel, 1985). DNA sequence analysis was performed to verify the mutation. A 1819 bp *Hin*dIII–*Pst*I fragment containing the coding sequence of the P68 in M13, including the *Nde*I site, was then inserted into the *Hin*dIII–*Pst*I site of Bluescript KS+ to generate the plasmid pBS-68N. To eliminate the possibility of sequence alteration occurring as a result of mutagenesis, pBS-68Nwt was created by substituting a 1500 bp *Nco*I–*Pst*I fragment containing the carboxyl-terminal region of the P68 in pBS-68N with a wild-type P68 *Nco*I–*Pst*I fragment from pBS-8.4R. The expression plasmid pET295K (the wild-type P68 with a lysine at position 295) was constructed by inserting a 1805 bp *Nde*I–*Bam*HI fragment from pBS-68Nwt into pET11a such that the P68 was under control of the T7 promotor. Expression plasmids containing a mutant P68 protein (an invariant lysine to arginine substitution at position 295; Katze et al., 1991) were generated by substituting a 518 bp *Bgl*II wild-type fragment from pBS-68Nwt with the same fragment containing the mutation. Correct orientation was confirmed by *Nco*I–*Acc*I digestion. The 1805 bp *Nde*I–*Bam*HI fragment containing the mutant kinase was then inserted into similarily digested pET11a to generate the expression plasmid pET295R.

Production of P68 Protein Using T7 Polymerase. Plasmids containing wild-type P68 or mutant variants of P68 were transformed into *E. coli* strain BL21(DE3)pLysS and grown at 37 °C in TY medium containing ampicillin (75 μg/mL). After cultures had grown to an A_{550} of 0.5, they were induced with 0.5 mM IPTG with growth continued for up to 2 h. If rifampicin was to be included in the medium, 200 μg/mL was added 30 min after IPTG induction and left for a further 2 h. Cultures were then placed on ice and cells harvested by centrifugation. The cells were lysed and extracts prepared as described below.

Electrophoresis and Immunoblotting Analysis. Extracts of recombinant *E. coli* cells were pelleted, washed once in TE [10 mM Tris-HCl (pH 7.5) and 1 mM EDTA], and treated with lysis buffer I [10 mM Tris-HCl (pH 7.5), 50 mM KCl, 1 mM dithiothreitol, 2 mM EDTA, 50 mM NaF, 0.2 mM phenylmethanesulfonyl fluoride (PMSF), 1000 units of aprotinin/mL, and 1% Triton X-100]. Cell extracts were then added to an equal volume of 2× disruption buffer [5% SDS, 20% β-mercaptoethanol, 20% glycerol, and 150 mM Tris-HCl (pH 6.8)] and boiled for 5 min prior to being loaded onto 10% SDS–polyacrylamide gels. For protein analysis, gels were stained with 0.1% Coomassie Brilliant Blue G-250 in 10% (v/v) acetic acid containing 20% ethanol. For immunoblot analysis, proteins were transferred to nitrocellulose membranes [Hybond-C (Amersham); Towbin et al., 1979] and incubated with a monoclonal antibody to to P68 (Laurent et al., 1985). After the membranes were washed, blots were incubated with ^{125}I-labeled anti-mouse IgG (ICN) and subjected to autoradiography.

Immunoprecipitation Analyais. Lysed cell extracts were centrifuged, and the soluble portion was diluted in buffer II [20 mM Tris-HCl (pH 7.5), 50 mM KCl, 400 mM NaCl, 1 mM EDTA, 100 units of aprotinin/mL, 1 mM dithiothreitol, 0.2 mM phenylmethanesulfonyl fluoride, 20% glycerol, and 1% Triton X-100] prior to the addition of anti-P68 monoclonal antibody. After 1-h incubation at 4 °C, protein G–Sepharose was added to the lysate and left to incubate for a further hour at the same temperature. Precipitates were then washed 4× with buffer II and 3× with buffer III [10 mM Tris-HCl (pH 7.5), 100 mM KCl, 0.1 mM EDTA, 100 units of aprotinin/mL, and 20% glycerol]. Washed immunoprecipitates were boiled for 5 min in 2× disruption buffer, separated by SDS–PAGE, and subjected to autoradiography.

Radiolabeling of Bacterial-Expressed P68. Bacterial proteins were labeled by growing the cells to an A_{550} of 0.5 in TY medium at 37 °C. The cells were then harvested by centrifugation and washed once in TE before being resuspended in an equal volume of labeling media. Methionine labeling media consisted of 5× M9 media containing 20 μg/mL thiamin and 0.5 mM amino acids (minus methionine). In vivo phosphate labeling media consisted of 20 mM Tris-HCl (pH 7.5), 100 mM NH_4Cl, 40 mM NaCl, 20 μg/mL thiamin, and 0.5 mM total amino acids. Cultures were starved for 20 min in the respective media prior to induction with IPTG (0.5 mM) and the addition of rifampicin (200 μg/mL). Twenty microcuries of [^{35}S]methionine or 50 μCi of carrier-free [^{32}P]orthophosphate was added per milliliter of culture and incubated at 37 °C for 1 h. Following harvesting by centrifugation, cells were washed once in cold TE buffer. Cell extracts were prepared and analyzed by SDS–PAGE.

Assay of Protein Kinase Activity. Protein kinase activity was measured as described earlier (Katze et al., 1987, 1988). Briefly, soluble fractions of *E. coli* were immunoprecipitated as described above and incubated in a final volume of 50 μL of buffer III containing 2 μM [γ-^{32}P]ATP (50 Ci/mmol), 2 mM $MgCl_2$, 2 mM $MnCl_2$, and reovirus dsRNA. Purified eIF-2 (0.5 μg; a gift of Brian Safer) was added to the mixture and left to incubate for 30 min at 37 °C. After the addition of 2× disruption buffer, samples were electrophoresed and autoradiographed.

Binding of Reovirus dsRNA to P68. 5′ end labeling with T4 kinase of the dsRNAs of reovirus type 3 (generously provided by Dr. Aaron Shatkin) was carried out in the presence of [γ-^{32}P]ATP. P68 was immunoprecipitated from the bacterial extracts using MAb–Sepharose and then reacted with the radiolabeled RNAs in binding buffer [10 mM Tris-HCl (pH 7.5), 100 mM KCl, 0.1 mM EDTA, 2 mM $MgCl_2$, 2 mM $MnCl_2$, 7 mM β-mercaptoethanol, 100 units/mL aprotinin, 10 μM ATP, 10 μg/mL tRNA, 0.5% Triton X-100, and 20% glycerol] for a 20-min incubation at 30 °C. After appropriate washing, the P68–RNA complex was resuspended in NET [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.05% Nonidet P-40], carrier tRNA, and 1% SDS. After phenol–chloroform extraction and ethanol precipitation, RNAs specifically bound

to P68 were analyzed on a 10% SDS–polyacrylamide gel (Shatkin et al., 1968).

Results and Discussion

Construction and Expression of Recombinant P68 Plasmids. The procedures for cloning wild-type and mutant P68 kinase cDNAs into the pET series of vectors (Studier et al., 1990) involved removal of all untranslated sequences that may adversely affect the efficiency of mRNA translation. This was performed by introducing a *Nde*I restriction site at the location of the P68 ATG by site-directed mutagenesis (Kunkel, 1985). Creation of the *Nde*I site further facilitated cloning into the expression vector such that the wild-type P68 gene was under control of the bacteriophage T7 promotor (pET295K). A mutant variant of P68, differing from the wild-type kinase by an arginine substitution for the lysine at position 295 (Katze et al., 1991), was also expressed in this system (pET295R). Substitutions for the domain II lysine in other kinase species, including v-src, v-mos, and epidermal growth factor receptor (EGFR) kinases, have been shown to result in loss of protein kinase activity (Hanks et al., 1988).

Expression plasmids containing kinase clones were then introduced into *E. coli* BL21(DE3)pLysS, a lysogen containing the λ derivative prophage DE3, in which the T7 RNA polymerase gene is under the control of the lacUV5 promotor. Strain BL21(DE3)pLysS also carries the plasmid pACYC184 containing the T7 lysozyme gene under control of the phage O3.8 promotor. This plasmid expresses low levels of lysozyme which binds basal levels of T7 polymerase and helps prevent premature transcription until induction by IPTG (Studier et al., 1990). Extracts of *E. coli*, expressing wild-type and mutant kinases, were prepared and analyzed after IPTG induction by Coomassie Brilliant Blue stained SDS gels (Figure 1A). As a control throughout this study, extracts from bacteria transformed by the plasmid lacking the P68 gene were analyzed. To increase the yield of P68 kinase relative to the background levels of host protein synthesis, rifampicin (which inhibits transcription by the host RNA polymerase but not the T7 polymerase) was added post-IPTG induction to the culture medium. A novel protein of M_r 68 000 was apparent in extracts of *E. coli* expressing the mutant (pET295R) and wild-type (pET295K) kinase molecules while, as expected, this protein was absent in extracts of IPTG-induced *E. coli* BL21(DE3)pLysS which contain the empty pET 11a vector (see arrow, Figure 1A).

To verify that these proteins were identical to the authentic P68, immunoblot analysis with the P68 monoclonal antibody (Laurent et al., 1985) was performed on these extracts at various times post-IPTG induction (Figure 1B). As a positive control, extracts from interferon-treated 293 cells were electrophoresed on the same gel. These cells are routinely used as a source of the human native P68 kinase (Katze et al., 1987). As expected, levels of the bacterially expressed P68 proteins were found to increase over the induction period. Interestingly, although the construction of P68 wild-type and mutant expression plasmids was identical, save for a single amino acid change at position 295, immunoblot analysis revealed that during induction, the mobility of the wild-type P68 slowed. In contrast, the mutant P68 did not exhibit this shift and electrophoresed only as the faster migrating species. Previous work has shown that such an alteration in mobility is likely due to extensive phosphorylation of the P68 kinase (Katze et al., 1987; Galabru & Hovanessian, 1987; see below). The native human P68 kinase migrated slightly slower than the mutant P68, suggesting that the kinase may be partially phosphorylated in the interferon-treated 293 cells.

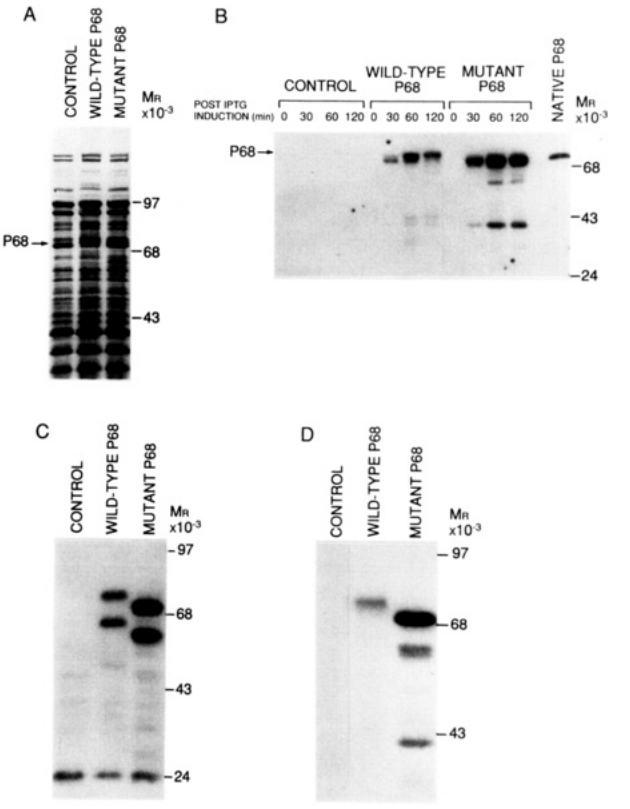

FIGURE 1: Expression of the wild-type and mutant P68 kinase proteins in *E. coli*. (A) Coomassie Brilliant Blue stained 10% SDS–PAGE of extracts (200 μg) prepared from control, wild-type, and mutant-expressing *E. coli* extracts. Extracts were prepared 2-h post-IPTG induction with rifampicin added 30-min post-induction. The arrow denotes the position of the P68 kinase protein; the positions of molecular weight standards are shown on the right. (B) Western blot analysis of extracts (50 μg) prepared from control, wild-type, and mutant-expressing cells at various times post-IPTG induction. Rifampicin was added at 30-min post-induction. The blots were hybridized with the P68 MAb as described under Experimental Procedures. The native human P68 was detected in extracts (500 μg) prepared from the interferon-treated human 293 cell line. (C) Control, wild-type, and mutant cells were labeled with [^{35}S]methionine as described under Experimental Procedures and the total extracts subsequently analyzed on a 10% SDS–PAGE. (D) Immunoprecipitation analysis of the radiolabeled extracts described in panel C utilizing the P68 monoclonal antibody.

To further analyze the synthesis of the mutant and wild-type proteins, cultures of bacteria expressing wild-type or mutant kinases were propagated in methionine-free media and induced with IPTG. After addition of rifampicin, [^{35}S]methionine was added to the media for 1 h with total cell extracts subjected to gel electrophoresis and autoradiography. Figure 1C revealed the synthesis of two major P68-specific proteins with approximate M_r 68 000 and 60 000. The larger protein represented the intact P68 protein (with the mutant protein again migrating faster than the wild type) whereas the smaller likely represented a degradation product of the full-length P68. Degradation fragments of the unstable kinase have been routinely observed in studies of P68 in mammalian cells (Galabru & Hovanessian, 1985). The smaller radiolabeled protein (approximately 24 000 daltons) represented an *E coli* protein since it was also observed in control extracts. Immunoprecipitation analysis of the labeled extracts confirmed the identity of the larger intact proteins as those of P68 origin (Figure 1D). The slower migrating degradation products were precipitated with reduced efficiency, possibly due to their reduced stability or their lacking the epitope recognized by the P68 monoclonal antibody (Katze et al., 1991). It is rel-

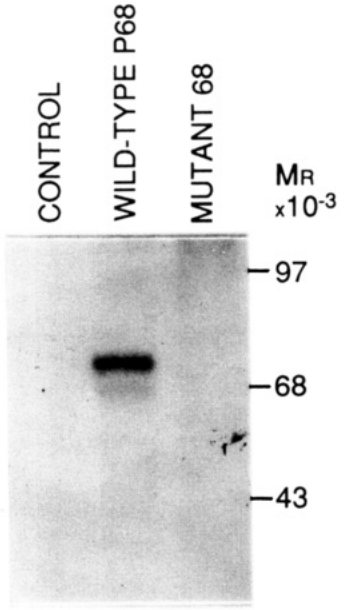

FIGURE 2: Wild-type P68 protein kinase is phosphorylated in *E. coli* during induction. Control, wild-type, and mutant-expressing cells were labeled with [^{32}P]orthophosphate and immunoprecipitated with the P68 MAb as described under Experimental Procedures. The samples were analyzed on a 10% SDS–polyacrylamide gel.

evant to note that in repeated experiments, the mutant kinase appeared to be even less stable than the wild type, also giving rise to a specific degradation product of approximately 40 000 daltons (see Figure 1B,D).

Both the Western blot and immunoprecipitation analysis showed that higher levels of the mutant kinase were present relative to the wild type. We are currently determining whether this is due to decreased protein stability or a decrease in wild-type P68 gene expression. Curiously, the expression of the wild-type and mutant kinases was more comparable in non-rifampicin-treated cultures (G. N. Barber and M. G. Katze, unpublished observations). This latter observation raises the possibility that an *E. coli* host protein, present in reduced amounts in rifampicin-treated cells, can specifically influence wild-type P68 expression. Finally, there is the alternative intriguing possibility, presently untested, that the wild-type P68 is down-regulating its own synthesis. Indeed, under certain experimental conditions, the presence of the wild-type kinase appeared to inhibit the growth of *E. coli* harboring the plasmid (G. N. Barber and M. G. Katze, unpublished observations).

Functional Activity of the E. coli Expressed P68. To examine the in vivo activation state of the mutant and wild-type P68 molecules and directly test whether the mobility shift observed in immunoblots was due to phosphorylation during synthesis, cultures of recombinant bacteria were labeled with [^{32}P]orthophosphate. The radiolabeled bacteria were lysed with disruption buffer containing EDTA to prevent artifactual phosphorylation of the kinase during extract preparation. NaF (50 mM), a phosphatase inhibitor, also was included routinely in all buffers to prevent dephosphorylation of the P68 kinase during extraction. Immunoprecipitation of the ^{32}P-labeled extracts revealed the presence of a phosphorylated P68 protein kinase in extracts from wild-type-expressing cells (Figure 2). No labeled P68 protein was observed in control of mutant lanes although equal amounts of cellular extracts (containing approximately equal cpm) were added to the immunoprecipitations. Since other eukaryotic cellular kinases are not present in *E. coli*, we can conclude that P68 is responsible for its own phosphorylation. Although earlier data had suggested this to

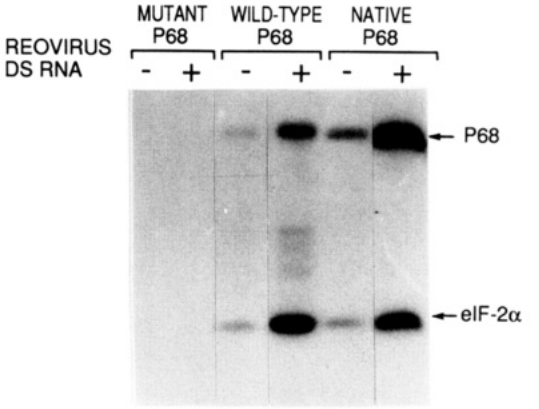

FIGURE 3: Wild-type *E. coli* P68 kinase is functional and phosphorylates the eIF-2α subunit in vitro. The wild-type and mutant extracts (15 μg) as well as extracts (500 μg) from interferon-treated 293 cells were subjected to immunoprecipitation with the P68 MAb as described under Experimental Procedures. The kinase assays were performed in the absence or presence of reovirus dsRNAs (0.10 μg/mL) and in the presence of 0.5 μg of purified eIF-2. The samples were then analyzed by 10% SDS–PAGE. The positions of P68 and the eIF-2 α subunit are indicated.

be the case (Galabru et al., 1989; Hovanessian, 1989), these studies now provide direct evidence. We cannot, however, absolutely rule out that an *E. coli* kinase is phosphorylating P68 although we consider this unlikely particularly since the mutant protein is not phosphorylated.

We have not yet identified the activator(s) of the P68 protein kinase activation in *E. coli*. It is well established that dsRNAs are efficient activators as are polyanions such as heparin (Hovanessian, 1989). In addition, eukaryotic viral RNAs are effective activators of P68 (Black et al., 1989; Maran & Mathews, 1988). Further, we and others have shown that single-stranded RNAs can activate the protein kinase through double-stranded regions in their secondary structure (Edery et al., 1989; SenGupta & Silverman, 1989; Bischoff & Samuel, 1989; Roy et al., 1991). It is probable therefore that bacterial dsRNAs or single-stranded RNAs with extensive secondary structure are activating P68. This type of in vivo activation of P68 would be minimal in eukaryotic cells due to the presence of cellular phosphatases or other natural regulators of the P68 kinase (Szyka et et., 1989; Lee et al., 1990).

Although the above experiments assured us that the P68 produced in *E. coli* was functional, it was necessary to test whether the kinase could phosphorylate its natural substrate, the α subunit of protein synthesis factor, eIF-2. The soluble portions of extracts from recombinant bacteria (containing the wild-type and mutant kinases) were therefore prepared, and P68 was immunoprecipitated as described under Experimental Procedures. The immunopurified kinase was then activated with reovirus dsRNAs in the presence of [γ-^{32}P]ATP and exogenously added purified eIF-2 (Figure 3). Even though the wild-type recombinant P68 was already partially activated when extracted from *E. coli*, the activator induced the further phosphorylation of the kinase itself as well as the phosphorylation of the eIF-2 α subunit. In contrast, no phosphorylation or catalytic activity was found for the mutant kinase. As a control, the activity of the human native P68 kinase, immunopurified from interferon-treated 293 cells, was examined in parallel and found to phosphorylate eIF-2 α.

Since a long-range goal of our analysis is to define the regions of P68 which interact with activators and repressors of the kinase, we next tested whether reovirus dsRNAs bound to the wild-type and mutant constructs. We chose to study the binding of these RNAs since they are efficient activators

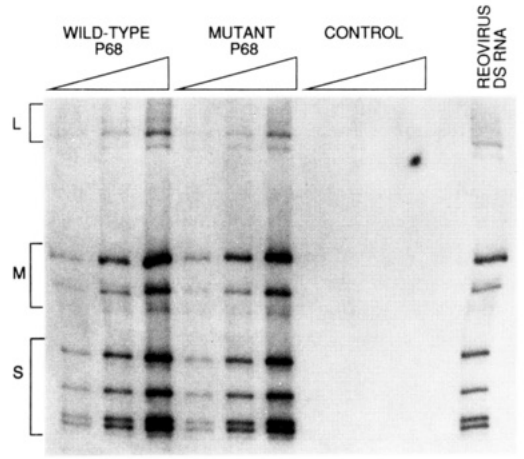

FIGURE 4: Binding of reovirus dsRNAs to wild-type and mutant P68 kinases. Increasing amounts of ^{32}P-labeled reovirus dsRNAs (12.5, 25, and 50 ng) were added to wild-type and mutant P68 kinases immunopurified from *E. coli* extracts as described under Experimental Procedures. As a control, RNA was added to MAb–Sepharose which was prereacted to control extracts, lacking P68. The lane marked REOVIRUS DS RNA depicts 25 ng of the starting material. RNAs were analyzed on a 10% SDS–PAGE. Migration of large (L; ca. 3500–3900 nt), medium (M; 2200–2300 nt), and small (S; 1200–1400 nt) reovirus dsRNA species is shown on the left.

of P68, and can be end-labeled with [^{32}P]ATP and readily analyzed on a polyacrylamide gel. The P68 mutant and wild-type extracts (and the control extracts lacking the kinase) were subjected to immunoprecipitation with the P68 MAb–Sepharose. The immunopurified kinase was then reacted with increasing concentrations of reovirus ^{32}P-labeled dsRNAs (in the presence of carrier tRNA) as described under Experimental Procedures. After binding and appropriate washings, complexed reovirus dsRNAs were electrophoresed on a 10% SDS–polyacrylamide gel (Figure 4). Approximately equal amounts of dsRNA bound to the mutant and wild-type kinases despite the fact that only the latter can be activated by these RNAs. No detectable RNA binding to the MAb–Sepharose, previously reacted with control extracts, was observed, confirming that the RNAs were not binding nonspecifically either to the monoclonal antibody itself or to contaminating *E. coli* proteins. Recent evidence has shown that the amino terminus contains the domains critical for dsRNA binding (Katze et al., 1991). That the *E. coli* expressed molecules can effectively bind these RNAs provides further proof not only of the bona fide nature of the overexpressed protein but also of the utility of this system for structure–function analysis.

Conclusions

In the present report, we described the expression of a functional P68 protein kinase in *E. coli*. The wild-type P68 was able to activate itself and phosphorylate the α subunit of eIF-2 whereas a mutant with a single amino acid substitution was completely inactive. We also have succeeded in purifying the wild-type P68 protein kinase in an active form from contaminating *E. coli* proteins with the P68 MAb–Sepharose as earlier described by Galabru et al., 1989 (data not shown). It is interesting to note that *E. coli* expression of other kinases has met with mixed success. In some cases, the kinase either was insoluble or was not fully active [e.g., see Seth and Vande Woude (1985), Lacal et al. (1984), and Gilmer and Erikson (1981)]. We estimate by Western blot analysis of fractionated extracts that approximately 50–60% of P68 was present in the soluble fraction of *E. coli* (data not shown). Furthermore, by comparing the physical amounts and the activity of P68 present in *E. coli* and interferon-treated cells, we approximate that similar proportions of the recombinant P68 were active relative to the native kinase isolated from human cells. Studies are now in progress to utilize this *E. coli* system both to prepare P68-specific antisera and to identify regulatory kinase domains which interact with RNA activators and repressors.

Acknowledgments

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