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Characterization of Protein Kinase Activity Associated with Rat Liver Polysomal Messenger Ribonucleoprotein Particles†

James Cardelli* and Henry C. Pitot

ABSTRACT: Poly(adenylic acid)-containing rat liver polysomal messenger ribonucleoprotein particles (pmRNP) were isolated and found to contain protein kinase activity. The association of the enzyme(s) with the particles was confirmed by experiments showing that the protein kinase activity comigrated with the pmRNP on metrizamide gradients and bound to oligo(dT)–cellulose columns only under conditions where the pmRNP bound. The following properties were determined for the pmRNP-associated kinase(s). Casein and phosvitin were preferred substrates over histone and protamine. The optimal $MgCl_2$ and KCl concentrations were found to be 12.5 and 50 mM, respectively. $MnCl_2$ and $CaCl_2$ could not replace $MgCl_2$ and were inhibitory at low concentrations. The optimum pH range was 7.7–9.0, and the enzyme activity was cAMP independent. A molecular weight of 55 000–60 000 was determined for the kinase(s) by sucrose gradient analysis. The enzyme(s) was capable of phosphorylating proteins endogenous to the pmRNP. Membrane-bound pmRNP contained much less kinase activity than free pmRNP while pmRNP from hepatoma 7777 contained an elevated level of the enzyme(s). The relationship between the protein kinase activity and one of the pmRNP proteins of molecular weight 66 000 is discussed.

Although polysomal and nonpolysomal messenger ribonucleoprotein particles (mRNP) have been detected and isolated from a wide variety of cell types, little is known about the function of the associated proteins (Lindberg & Sundquist, 1974; Kumar & Pederson, 1975; Jeffery, 1977; Cardelli & Pitot, 1977). Some groups have reported that these proteins may play a role in the translation of mRNA either as initiation factors or as translational control factors (Bag & Sarkar, 1975; Barrieux & Rosenfeld, 1977; Liautard, 1977; Ilan & Ilan, 1977). It has been reported that one of the mRNP proteins may be identical with the initiation factor e-IF2 (Barrieux & Rosenfeld, 1977; Hellerman & Shafritz, 1975) and that isolated mRNP form initiation complexes with ribosomes in the absence of added initiation factors (Liautard, 1977; Cashion & Stanley, 1974). Consistent with these findings are data showing that some initiation factors can bind to isolated mRNA (Shafritz et al., 1976; Kaempfer et al., 1978). Other groups have reported that mRNP contain no initiation factor activity and are translated with the same efficiency as mRNA

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when added to a cell-free protein synthesizing system (Freienstein & Blobel, 1974; Ernst & Arnstein, 1975; Chen et al., 1976).

Data have been presented that suggest that one of the mRNP proteins of molecular weight 78 000 which binds to the poly(adenylic acid) [poly(A)] region of the mRNA plays a role in the transport of mRNA from the nucleus to the cytoplasm (Schwartz & Darnell, 1976). This protein and others which bind near the poly(A) region of the mRNA appear to protect the molecule from endoribonuclease IV digestion (Müller et al., 1978). Furthermore, this protein has been shown to be antigenically similar to one of the poly(A) polymerases (Rose et al., 1979).

Investigators have recently reported that nuclear and cytoplasmic mRNP isolated from a number of different systems contain cAMP-independent protein kinase activity (Egly et al., 1976; Blanchard et al., 1977; Bag & Sells, 1979). We report here that purified rat liver polysomal mRNP also contain associated protein kinase activity with properties similar to those reported for the nuclear and cytoplasmic mRNP-associated kinase(s).

Materials and Methods

Animals, Chemicals, and Labeling. Male Holtzman rats weighing approximately 300 g were given food and water ad libitum. The compositions of the buffers employed in these studies are as follows: buffer 1, 10 mM Tris-HCl, 250 mM NaCl, and 10 mM Na_2EDTA, pH 7.5; buffer 2, 10 mM Tris-HCl, 1000 mM NaCl, and 10 mM Na_2EDTA, pH 7.5; and buffer 3, 10 mM Tris-HCl, 500 mM NaCl, 10 mM Na_2EDTA, and 25% formamide, pH 7.5.

Sucrose was purchased from the Schwarz/Mann Division of Becton; oligo(dT)–cellulose type T-2 was from Collaborative Research; [^{3}H]orotic acid was from Amersham Co. Metrizamide, casein, phosvitin, histone IIa, protamine, and phenylmethylsulfonyl fluoride were from Sigma; [γ-^{32}P]ATP (10 Ci/mmol) was obtained from New England Nuclear Corp.

Polysomal mRNP Preparations. Rat liver polysomes were prepared as previously described (Cardelli et al., 1976). Polysomal pellets (200–300 A_{260} units) were resuspended in buffer 1 and applied to oligo(dT)–cellulose columns (0.7 (i.d.) × 6 cm). After a 15-min incubation, columns were washed with 40 mL of buffer 1, and column-bound pmRNP were released by washing with buffer 3 at 25 °C (method 1). Alternately, the majority of the mRNA-associated proteins was released from the column by washing with buffer 2 at 4 °C (method 2). This method leaves the poly(A)-containing RNA and about 45% of the total protein still associated with the column-bound pmRNP. The released fractions were dialyzed for 3 h against 200 volumes of buffer consisting of 10 mM Tris-HCl (pH 7.5), 1 mM dithiothreitol (DTT), and 0.5 mM phenylmethylsulfonyl fluoride. The dialyzed fractions were concentrated to a final volume of 1–2 mL by using an Amicon apparatus and UM-10 filters. In some experiments, polysomes were isolated from livers of rats injected with 150 μCi of [^{3}H]orotic acid and 2–3 μmol of fluoroorotic acid. Only mRNA is labeled under these conditions (Cardelli et al., 1976).

Protein Kinase Assay. Standard assays were done in a final volume of 0.2 mL of "kinase buffer" (10 mM Tris-HCl, pH 8.2, 12.5 mM $MgCl_2$, 50 mM KCl, 5 mM NaF, and 1 mM DTT) containing 100 μg of phosvitin and the indicated amounts of pmRNP. Reactions were initiated by adding [γ-^{32}P]ATP (10 Ci/mmol) to a final concentration of 1.0 μM. After incubation at 30 °C for the indicated times, the reaction was terminated by the addition of 2 mL of ice-cold 10% trichloroacetic acid containing 1 mM ATP. Precipitates were collected on Whatman GF/C filters, washed with 30 mL of 5% trichloroacetic acid, dried, and counted in 5 mL of scintillation cocktail.

Kinase assays were scaled up for slab gel electrophoresis as follows. Final reaction volumes were 1.0–1.5 mL in "kinase buffer" with the indicated amounts of pmRNP. After a 15-min incubation at 30 °C, 2.0 mL of ice-cold 10% trichloroacetic acid with 1 mM ATP was added to stop the reaction. Samples were pelleted after the addition of 500 μg of yeast RNA, washed twice with 10% trichloroacetic acid, and dissolved in 200 μL of gel sample buffer [10% glycerol, 5% 2-mercaptoethanol, 3% sodium dodecyl sulfate ($NaDodSO_4$), and 62 mM Tris-HCl].

Gel Electrophoresis. Laemmli (1970) $NaDodSO_4$–polyacrylamide disc gel electrophoresis was performed as previously described (Cardelli & Pitot, 1977). Slab gels were run as follows. Solutions containing 10% acrylamide, 0.1% $NaDodSO_4$, 0.3% methylenebisacrylamide, 0.375 M Tris-HCl, pH 8.8, 0.025% *N,N,N′,N′*-tetramethylethylenediamine, and 0.3 mg/mL ammonium persulfate were cast in plates with dimensions of 16 × 9 × 0.15 cm. Stacking gels (3% acrylamide) were formed along one end of the gel. Gels were run at 25 mA per plate, stained in 0.1% Coomassie Brilliant Blue, 12.5% acetic acid, and 50% methanol, and destained in 4 parts of methanol, 1 part of acetic acid, and 9 parts of H_2O.

Gradient Analysis of pmRNP and Associated Kinase(s). Aliquots of dialyzed ^{3}H-labeled pmRNP isolated by using method 1 (see above) were layered on 20–60% metrizamide gradients containing 30 mM Tris-HCl, pH 7.6, and 25 mM KCl and centrifuged for 9 h at 4 °C in an SW56 rotor at 36 000 rpm. Fractions were collected and aliquots were removed for counting or assayed for protein kinase activity.

The molecular weight of the pmRNP-associated kinase(s) was determined by using sucrose gradients (Martin & Ames, 1961). Aliquots of concentrated pmRNP protein prepared by using method 2 were layered on 5–20% sucrose gradients made in 10 mM Tris-HCl, pH 7.6, and 1 mM DTT and centrifuged for 17 h at 4 °C in an SW56 rotor at 45 000 rpm. Fractions were collected and assayed for protein kinase activity.

Protein Assay. Protein concentration was determined by using the method of Esen (1978) with the following modifications. The chromatography paper containing the spotted protein was stained for 15 min with occasional shaking. After destaining briefly, the spotted proteins were cut out in equal-size squares, and the dye–protein complex was released by vortexing in 1.2 mL of 1% $NaDodSO_4$ for 30 s in a conical centrifuge tube.

Results

Evidence for a Direct Association of Protein Kinase Activity with pmRNP. In our initial experiments, we found that oligo(dT)–cellulose-isolated free polysomal messenger ribonucleoprotein particles (pmRNP) contained protein kinase activity. The following experiments were done to show that the protein kinase activity was actually associated with the pmRNP and not just copurifying with the particles during isolation. Free pmRNP labeled in vivo with [^{3}H]orotic acid in the presence of fluoroorotic acid were isolated by using method 1. This procedure allows the specific labeling of mRNA (Cardelli et al., 1976). Following dialysis, aliquots were fractionated on metrizamide gradients as described in Figure 1. Dialysis is necessary to remove the formamide which disrupts the mRNA–protein association (Cardelli & Pitot, 1977). Figure 1 shows that the dialyzed ^{3}H-labeled pmRNP bands in the gradient in a heterogeneous manner between 1.17 and 1.27 g/cm^3. The distribution of protein

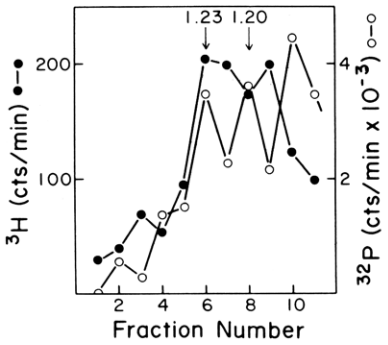

FIGURE 1: Metrizamide gradients of labeled polysomal mRNP. Ninety A_{260} units of mRNA-labeled free polysomes (see Materials and Methods) were fractionated on oligo(dT)–cellulose columns by following method 1. The isolated, dialyzed pmRNPs were layered on 20–60% metrizamide gradients containing 30 mM Tris-HCl and 25 mM KCl, pH 7.5, centrifuged for 9 h at 35 000 rpm in an SW56 rotor, collected, and processed as described under Materials and Methods. [^{3}H]mRNP (●) as well as kinase activity measured as ^{32}P incorporated (○) were determined in each aliquot.

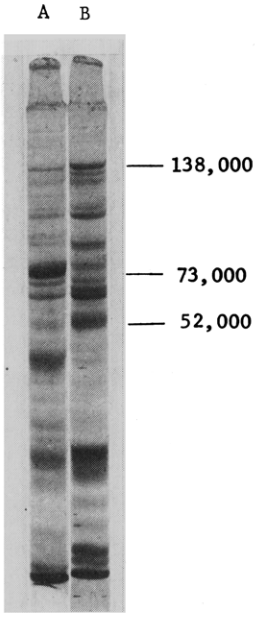

FIGURE 2: NaDodSO$_4$–polyacrylamide gel electrophoresis of various polysomal mRNP fractions. Free polysomes, 150 A_{260} units, were disrupted in buffer 1 and applied to an oligo(dT)–cellulose column. After being washed with buffer 1, columns were washed with buffer 2. The eluates from this wash and the following wash with buffer 3 were precipitated with ethanol and electrophoresed on 8.75% NaDodSO$_4$–polyacrylamide gels. Gels were stained and photographed. (A) Eluate of buffer 3 wash; (B) eluate of buffer 2 wash.

kinase activity also appears heterogeneous and appears to follow the distribution of labeled pmRNP fairly closely. The peak of kinase activity at the top of the gradient represents enzyme that did not reassociate with the pmRNP during dialysis. When pmRNP fractions are treated with low levels of ribonuclease prior to gradient analysis, most of the labeled RNA and kinase activity are shifted to the top of the gradient, suggesting that the banding of kinase activity in the region of 1.20–1.23 g/cm^3 is due to its association with RNA (data not shown). The following experiment also supports the idea of an association between the pmRNP and protein kinase activity. When polysomes are incubated with an excess of poly(uridylic acid) [poly(U)] [0.016 mg of poly(U)/mg of ribosomes] and then fractionated on an oligo(dT)–cellulose column, less than 10% of the labeled mRNP and protein kinase activity binds (unpublished results). This suggests that the

Table I: Substrate Specificity of pmRNP-Associated Kinase[a]

substrate	^{32}P incorporated (pmol)
–	0.01
phosvitin	1.78
casein	1.49
histone IIa	0.08
protamine	0.01

[a] Assays were done as described under Materials and Methods and contained 100 μg of the indicated substrates. Reactions were terminated after 7 min.

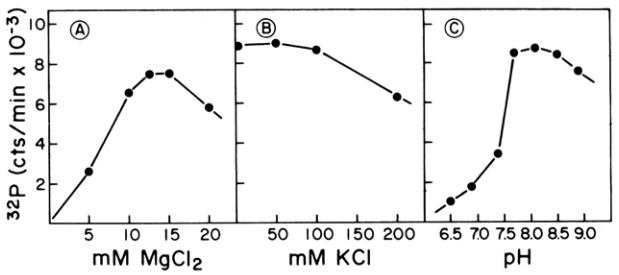

FIGURE 3: Effects of cations and pH on pmRNP-associated kinase. The effects of different $MgCl_2$, KCl, and H^+ concentrations on the pmRNP-associated kinase were determined by using the standard kinase assay. The reaction mixture containing 10 μg/mL isolated pmRNP protein (method 2) was allowed to proceed for 10 min at 30 °C before trichloroacetic acid precipitation, processing, and counting.

binding of the kinase activity to oligo(dT)–cellulose columns is because of its association with poly(A)-containing pmRNP. Since our polysome preparations are free of contaminating nuclear or cytoplasmic mRNP, these cannot be the source of kinase activity in our isolated free pmRNP (Cardelli & Pitot, 1977).

Enzymatic Properties of pmRNP-Associated Kinase(s). The experiments described above were performed by using pmRNP isolated in the presence of formamide (method 1). Since this chemical effects both protein–RNA interactions (Cardelli & Pitot, 1977) and protein kinase activity (unpublished data), we developed a technique to recover the majority of pmRNP protein and kinase without the use of this reagent. Figure 2B shows that most of the pmRNP proteins described in an earlier publication (Cardelli & Pitot, 1977) can be released from oligo(dT)–cellulose columns by washing with 1.0 M NaCl. Under these wash conditions, all of the pmRNP-associated kinase(s) is released (Table III). A major protein of M_r 73 000 as well as some minor ones remain associated with the pmRNP following this salt wash (Figure 2A). This protein probably represents the poly(A) specific binding protein (Schwartz & Darnell, 1976). The remainder of the experiments were done with pmRNP proteins prepared in this manner (method 2).

The following experiments were carried out to optimize the protein kinase assay conditions. Phosvitin and casein over a range of concentrations (0–100 μg/mL) were the preferred substrates for the kinase(s) as compared to protamine and histone IIa (Table I). We found that the phosphorylation reaction with phosvitin (100 μg/mL) as a substrate is linear for up to 30 min. The reaction is also linear up to 5 μg of added pmRNP (unpublished results). Above this concentration, the reaction plateaus and then gradually declines.

The reaction was absolutely dependent on Mg^{2+}, and the concentration curve for this cation showed a broad optimum between 11 and 15 mM (Figure 3A). Mn^{2+} and Ca^{2+} were ineffective as substitutes for Mg^{2+} in a range from 0 to 15 mM, and low levels of these cations actually inhibited the phos-

Table II: Effect of Cations and cAMP on pmRNP Kinase(s)[a]

reagent added	kinase buffer (mM)	^{32}P incorporated (pmol)
none		1.70
$MnCl_2$	2.5	1.06
	10.0	0.05
$CaCl_2$	2.5	1.48
	10.0	0.44
cAMP	0.005	1.73
	0.02	1.71
	0.10	1.64

[a] The indicated reagents were added to "kinase buffer" prior to the initiation of the reaction. See text for further details.

Table III: Salt-Dependent Release of pmRNP-Associated Kinase(s)[a]

salt concn (mM)	units of protein kinase activity released	protein released (μg)
350	10.50	150
450	2.25	100
600	1.15	230
1000	0.01	140
500 (25% formamide)	0.00	480

[a] One unit of kinase activity is defined as 1 pmol of ^{32}P incorporated per min into trichloroacetic acid precipitable material. Phosvitin was used as the substrate. See text for further details.

phorylation reaction in the presence of Mg^{2+} (Table II). KCl had little effect on the reaction at low concentrations and became inhibitory above 150 mM (Figure 3B). The kinase(s) showed little activity at a pH of less than 7.0. The greatest activity was found in the range of 7.7–8.9 (Figure 3C). Under normal assay conditions, little endogenous phosphorylation was detected. The kinase activity with phosvitin as a substrate was found to be cAMP independent over a range of concentrations (Table II).

Physical Properties and Distribution of the pmRNP-Associated Kinase(s). The following experiment was done to determine the salt concentration at which the protein kinase(s) was released from the pmRNP. EDTA-disrupted free polysomes were applied to oligo(dT)–cellulose columns, and the bound pmRNP was washed with increasing salt concentrations. The eluted fractions were collected and analyzed for protein and kinase activity. Table III shows that the majority of recoverable kinase activity is released from the pmRNP at 350 mM NaCl and little remains after washing with 600 mM. The various salt-released fractions were also analyzed on polyacrylamide gels. Proteins of M_r 66 000, 104 000, and 109 000 are released at lower salt concentrations, and then some of the other pmRNP proteins, i.e., M_r 52 000, are released (Figure 4). The gel profile of pmRNP-associated protein released by 500 mM NaCl and 25% formamide is shown in Figure 2A.

The molecular weight of the pmRNP-associated kinase was determined by using sucrose gradients (Martin & Ames, 1961). Figure 5 shows that the protein kinase activity sediments as one major and one minor peak. The calculated sedimentation rates were 3.8 and 10.6 S, respectively. The minor peak was seen only occasionally and may be due to aggregation of the 3.8 S species (unpublished data). The calculated molecular weight of the kinase(s) with bovine serum albumin, immunoglobulin G, and myoglobin as standards was determined to be 55 000–60 000.

The experiments described thus far were done with pmRNP isolated from free polysomes. We next examined membrane-bound pmRNP for the presence of protein kinase activity. Membrane-bound polysomes were released from isolated liver rough endoplasmic reticulum (Cardelli & Pitot, 1977) in the presence of 1% Triton X-100, pelleted through a 2.0 M buffered sucrose cushion, and fractionated on oligo(dT)–cellulose columns, following the procedure described under Materials and Methods for free polysomes. The column-bound pmRNP associated protein isolated by using

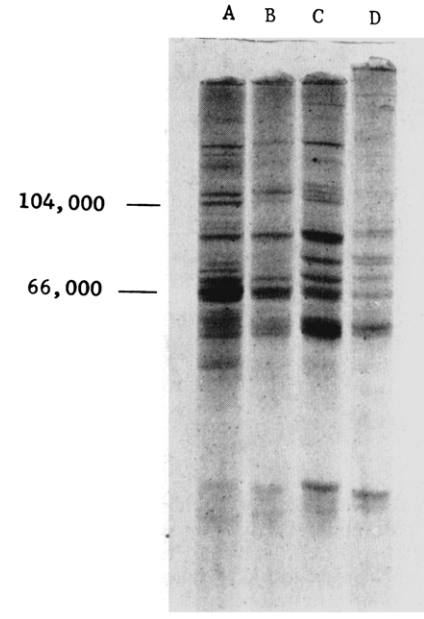

FIGURE 4: $NaDodSO_4$–polyacrylamide gel electrophoresis of salt-released pmRNP fractions. Polysomal mRNPs were bound to oligo(dT)–cellulose columns and washed with buffer 1. Columns were then washed with buffer 1 containing increasing amounts of NaCl. Fractions were collected and ethanol was precipitated, dissolved in gel sample buffer, and electrophoresed on 8.75% $NaDodSO_4$–polyacrylamide gels. Gels were stained, destained, and photographed. (A) 350 mM, (B) 450 mM, (C) 600 mM, and (D) 1000 mM NaCl eluates.

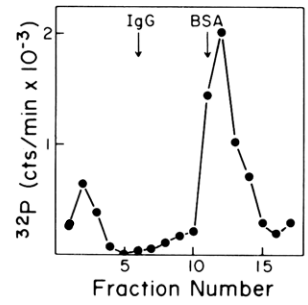

FIGURE 5: Molecular weight determination for the pmRNP-associated kinase(s). Polysomal mRNP protein (20 μg) isolated by method 2 was layered on 5–20% sucrose gradients made up in 10 mM Tris-HCl–1 mM DTT, pH 7.5, and centrifuged for 17 h at 45 000 rpm in an SW56 rotor. Parallel gradients contained 2 mg each of sperm whale myoglobin, IgG, and BSA. Gradients were fractionated and aliquots assayed for kinase activity or read a A_{280} as described under Materials and Methods.

Table IV: Specific Activity of pmRNP Kinase(s) from Different Sources[a]

source	sp act.
free pmRNP	95.9 (6)
membrane-bound pmRNP	23.5 (3)
hepatoma 7777 free pmRNP	131.2 (2)

[a] Numbers in parentheses are the number of preparations examined. See text for further details.

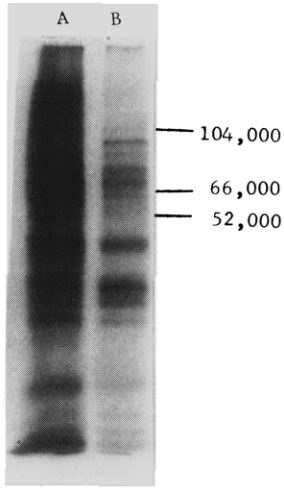

FIGURE 6: $NaDodSO_4$–polyacrylamide gel electrophoresis of ^{32}P-labeled pmRNP. pmRNP (10 μg) isolated by method 1 or 2 was incubated with [γ-^{32}P]ATP in "kinase buffer" for 15 min at 30 °C. Samples were trichloroacetic acid precipitated, washed, electrophoresed on slab gels, stained, destained, and then developed with Kodak X-Omat R. (A) pmRNP prepared by using method 1 or (B) method 2 (see Materials and Methods for details).

method 2 were assayed for the presence of kinase activity. The results tabulated in Table IV show that membrane-bound pmRNP when compared to free pmRNP on a specific activity basis contain only 25% of the amount of protein kinase activity. The amount of protein kinase activity actually associated with membrane-bound pmRNP preparations is probably less than that measured because 15–20% of the mass of our membrane-bound polysomes are contaminating free polysomes (Cardelli & Pitot, 1977). Furthermore, no loss (<5%) of free pmRNP associated kinase was observed if these particles were washed with 1% Triton X-100 prior to column fractionation, which suggests that the absence of kinase activity from membrane-bound pmRNP is not due to detergent treatment. We also examined free pmRNP isolated from hepatoma 7777 and found that the specific activity of the associated kinase was 50% greater than the free pmRNP associated kinase from normal liver (Table IV).

Phosphorylation of pmRNP-Associated Proteins. Experiments were then carried out to determine whether any of the pmRNP proteins were phosphorylated by the associated kinase(s) under our standard kinase assay conditions. Isolated pmRNP were incubated with [γ-^{32}P]ATP in the absence of an exogenous substrate, precipitated with trichloroacetic acid, washed, and electrophoresed on $NaDodSO_4$–polyacrylamide slab gels. An autoradiogram of these gels reveals the presence of a number of phosphorylated bands (Figure 6). The major phosphorylated bands (seen for free pmRNP, prepared by method 2) are at 30 000, 31 000, and 44 000 (Figure 6B). The minor bands range in molecular weight from 53 000 to 138 000. Of the pmRNP proteins described previously (Cardelli & Pitot, 1977), only those of M_r 52 000 and 104 000 and trace amounts of a 138 000 molecular weight protein are phosphorylated. When phosphorylated proteins associated with free pmRNP prepared by method 1 are examined on slab gels (this preparation includes all the column-bound protein and mRNA), one sees that the phosphorylated bands are qualitatively similar to those in Figure 6B but are quantitatively different (Figure 6A). Basically, the phosphorylated bands in the 30 000–45 000 molecular weight region are reduced in intensity relative to the proteins in the 52 000–138 000 molecular weight region. Furthermore, a phosphorylated band at M_r 59 000 is now seen.

We found no phosphorylated protein associated with pmRNP if the reaction was performed under conditions where the associated kinase showed little activity with phosvitin as a substrate (low pH, no Mg^{2+}). This suggests that the kinase(s) we have characterized is (are) responsible for phosphorylating the pmRNP-associated proteins.

Discussion

We have found that rat liver pmRNP isolated by using oligo(dT)–cellulose columns contain protein kinase activity. Previous work with a combination of sucrose and CsCl gradient approaches has essentially ruled out any contamination of such polysomes with nuclear or cytoplasmic mRNP (Cardelli & Pitot, 1977). This point is important because, as will be discussed below, both nuclear and cytoplasmic mRNP from other systems have been found to contain kinase activity (Egly et al., 1976; Blanchard et al., 1977; Bag & Sells, 1979). Our data also suggest that the kinase activity is associated with the pmRNP and not just copurifying with the particles. We showed that labeled pmRNP and kinase activity comigrated in metrizamide gradients. Furthermore, we found that little kinase activity bound to oligo(dT)–cellulose columns under conditions where pmRNP were inhibited from binding.

These results do not give us any information as to what percent of the kinase activity described here is found in the oligo(dT)–cellulose column flow through unassociated with pmRNP. This calculation is difficult because the oligo(dT)–cellulose flow through does contain protein kinase activity. This kinase(s) differs from the pmRNP-associated kinase in that it favors histone over phosvitin as a substrate, has a pH optimum of 7.4, and is partially cAMP dependent (data not shown). Our data suggest that the pmRNP-associated kinase is identical with a pmRNP protein of 66 000 molecular weight (vide supra) which is not found in the column flow through (Cardelli & Pitot, 1977). This suggests that the majority of the protein kinase described here is associated with pmRNP and not with ribosomes. Although these data suggest that the interaction of the kinase with the pmRNP may be specific, it does not prove it. Until we know the in vivo function of the protein kinase, the question of specificity will not be satisfactorily answered.

The pmRNP-associated kinase(s) has many properties similar to those described for the phosvitin-type protein kinase. Phosvitin and casein are the preferred substrates over protamine and histone. The enzyme(s) is (are) cAMP independent, and the activity is absolutely dependent on the presence of $MgCl_2$. $MnCl_2$ and $CaCl_2$ at low concentrations inhibit the phosphorylation reaction.

The pmRNP-associated kinase examined in this work also has properties similar to those described for the HeLa cell nuclear and plasma cell tumor cytoplasmic mRNP-associated kinases (Egly et al., 1976; Blanchard et al., 1977). All three kinase activities are Mg^{2+} dependent and cAMP independent. Like the pmRNP kinase(s) described here, the cytoplasmic mRNP-associated kinase utilizes casein as a substrate more effectively than histone. The HeLa cell nuclear RNP and rat liver pmRNP kinases have similar $MgCl_2$ and KCl activity curves. Kinases from both sources are inhibited by Ca^{2+} and show maximum activity above a pH of 7.8. The kinases are released from all three types of RNP by 500 mM NaCl. The molecular weights of the cytoplasmic and pmRNP kinases appear similar.

Although the evidence is not conclusive, we feel that the kinase and the pmRNP protein of M_r 66 000 (P66) may be one and the same protein. This idea is supported by the following evidence.

(1) P66 as revealed by $NaDodSO_4$–polyacrylamide gels is a minor protein associated with free pmRNP and is greatly reduced in membrane-bound pmRNP (Cardelli & Pitot, 1977). The data in Table IV show the membrane-bound pmRNP also has greatly reduced levels of protein kinase(s) relative to free pmRNP. After correcting for the contamination by free polysomes, this value is even less. Furthermore, pmRNP from hepatoma 7777 has an increased level of P66 relative to the level found in normal pmRNP (Cardelli & Pitot, 1978) and an increased level of protein kinase activity (Table IV).

(2) The relative amount of P66 seen in gels of different pmRNP salt-released fractions (Figure 4) correlates well with the amount of kinase activity in these fractions (Table III).

(3) Sucrose gradient analysis of pmRNP proteins suggests that the molecular weight of the kinase is 55 000–60 000, reasonably close to the molecular weight of P66. These data suggest that the kinase may be a single polypeptide chain of molecular weight 66 000.

(4) $NaDodSO_4$–polyacrylamide gel electrophoresis of the pmRNP-associated kinase(s) partially purified by using a DEAE-cellulose column shows the presence of a main band at 66 000 with a few other bands in the 50 000 molecular weight region (unpublished results).

When isolated pmRNP were incubated with [γ-^{32}P]ATP and then fractionated on slab gels, the resultant autoradiogram revealed a number of phosphorylated bands. Qualitatively, the phosphorylated proteins associated with pmRNP prepared by method 1 or 2 were similar, although a few quantitative differences were seen. An interesting finding was that a number of the phosphorylated pmRNP-associated proteins were barely detectable on slab gels when stained with Coomassie Blue. Furthermore, of the seven pmRNP-associated proteins described earlier, only three, P52, P104, and P138, were found to be phosphorylated (Cardelli & Pitot, 1977).

The functions of the pmRNP-associated kinase(s) and phosphate acceptor proteins are unknown but may be related to aspects of translation. Protein kinases have been implicated in the control of translation (Revel & Groner, 1978). The significance of the absence of protein kinase activity from membrane-bound pmRNP in regard to this point is not known. The absence of protein kinase activity from membrane-bound pmRNP may be an important factor in allowing the direct association of these particles with endoplasmic reticulum membranes (Cardelli et al., 1976). Alternatively, the presence of the protein kinase on free pmRNP may allow the translation of this class of mRNA to be regulated separately from the membrane-bound class. Another interesting point is that the pmRNP-associated kinase(s) described here has properties similar to the interferon-induced dsRNA-dependent protein kinase described by others (for review, see Baglioni, 1979). These similarities include cAMP independence, association with ribosomes or polysomes, KCl independence, and probably molecular weight (66 000?). Future work will have to be directed toward determining whether the nuclear, cytoplasmic, and polysomal pmRNP-associated protein kinases are related to the interferon-induced kinases and whether they play any role in protein synthesis or the control of mRNA metabolism.

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