Cyclic Nucleotide-Independent Protein Kinases Bound to Cytoplasmic and Nuclear Polyribosomes in Non-Infected and Adenovirus-Infected HeLa Cells

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Cyclic nucleotide-independent protein kinase activity bound to cytoplasmic and nuclear polyribosomes from non-infected and adenovirus-infected HeLa cells was compared. The enzymes catalysed the incorporation of phosphate from γ - ^{32}P -labelled ATP or GTP into acid-precipitable material in the absence of exogenous substrates. Their activity was not affected by cyclic AMP or cyclic GMP and was not inhibited by a cyclic nucleotide-dependent protein kinase-inhibitor protein. The kinases are tightly bound to polyribosomes of either origin from infected and non-infected cells, since treatment with 0.5 M-NaCl did not dissociate the activity. The enzymes and the enzyme-associated endogenous substrates of cytoplasmic polyribosomes are significantly different from those of the nucleus, and adenovirus infection of the cells did not alter the nature of the enzymes or the substrates at 18–20 h after infection. Nuclear kinases catalysed 3–4-fold more phosphate incorporation than did the cytoplasmic kinases. They did not phosphorylate endogenous substrates in the cytoplasmic preparations, and vice versa, which suggests that such substrates for cytoplasmic and nuclear kinases are specific. Polyacrylamide-gel electrophoresis of the phosphorylated proteins revealed the presence of a higher number of endogenous substrates in the nuclear preparation. The nuclear kinases phosphorylated all histones from HeLa cells, but the cytoplasmic ones phosphorylated predominantly the histone of mol.wt. 12000. Bovine heart kinase phosphorylated several low-molecular-weight cytoplasmic proteins and no nuclear proteins. With a DEAE-cellulose column either enzyme activity could be resolved into a number of peaks. The substrate specificities of these peaks indicate that there are at least two different forms of the enzyme in each preparation of polyribosomes.

Phosphorylation of proteins of nuclear and cytoplasmic origin is catalysed by a variety of kinases, not all of which are regulated by cyclic nucleotides. These enzymes seem to be important in a variety of regulatory and biosynthetic processes (Langan, 1973; Walsh & Ashby, 1973; Walsh & Krebs, 1973; Rubin & Rosen, 1975). Phosphorylation of nuclear proteins by various kinases may play a regulatory role in gene expression (Kleinsmith *et al.*, 1966; Langan, 1969; Teng *et al.*, 1971; Wang & Kostraba, 1975; Kleinsmith *et al.*, 1976), possibly at the level of RNA polymerase enzymes (Bell *et al.*, 1976; Hirsch & Martello, 1976) and mRNA biosynthesis (Blanchard *et al.*, 1977) and transport (Raskas, 1971).

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Cyclic nucleotide-independent protein kinases of cytoplasmic origin may regulate protein synthesis, presumably at the level of initiation (Levin *et al.*, 1976; Levin & London, 1978). Some of these kinases, induced by interferon treatment, phosphorylate cytoplasmic proteins of mol.wt. 64000–67000 and 37000 (Lebleu *et al.*, 1976; Jarvis *et al.*, 1978), which presumably leads to the drug-induced antiviral activity (Jarvis *et al.*, 1978). The association of cyclic nucleotide-independent protein kinases with many viruses suggests that protein phosphorylation may be important in the life cycle of these viruses (Rubin & Rosen, 1975).

Characterization of these enzymes and their endogenous substrates from a particular cell type would therefore be of major importance for understanding the regulation of various cellular processes.

Adenovirus 2 contains a cyclic nucleotide-inde-

pendent protein kinase, possibly of host-cell origin (Akusjarvi *et al.*, 1978; Blair & Russell, 1978). Although without a defined function, it can phosphorylate both exogenous protein substrates and adenoviral structural proteins. The enzyme is probably integrated into the structure of the virus and may be specifically incorporated during virus assembly in the host-cell nucleus. It has been reported that the transforming protein of adenovirus 5 (Lassam *et al.*, 1979), like that of Rous sarcoma virus (Collett & Erikson, 1978), either possesses or is associated with protein kinase activity.

During our investigation on ribosomes of adenovirus-infected HeLa cells, we observed that ribosomes of nuclear and cytoplasmic origin containing translatable mRNA have significant cyclic nucleotide-independent protein kinase activity and a variety of particle-associated endogenous acceptor proteins (Chatterjee *et al.*, 1977, 1979). Initial experiments suggested that the kinases and the acceptor proteins may be different in the two populations.

We have now compared the kinases bound to cytoplasmic and nuclear polyribosomes from non-infected and adenovirus-infected HeLa cells. Our study included enzyme-activity analysis, phosphorylation *in vitro* of endogenous and exogenous substrates (casein, phosvitin, histones and non-histones), and fractionation of the enzyme activity by DEAE-cellulose column chromatography to characterize substrate specificities of the enzyme fractions.

Experimental

Cell culture, growth and purification of adenovirus, infection of cells with adenovirus, preparation of nuclei and postnuclear supernatant from infected and non-infected HeLa cells

Growth of HeLa S-3 cells in suspension culture, growth of adenovirus type 2 in HeLa cells and purification of the virus by a published procedure (Maizel *et al.*, 1968) have been described (Chatterjee & Weissbach, 1974). Procedures for infecting cells with adenovirus (3000–4000 particles/cell) and for preparation of detergent (Triton N-101)-washed nuclei and postnuclear cytoplasmic supernatant from the infected cells have been described previously (Chatterjee *et al.*, 1977). The same procedures were used for non-infected HeLa cells.

Preparation of unwashed and high-salt-washed cytoplasmic and nuclear polyribosomes

To isolate nuclear particles, detergent-washed nuclei equivalent to 30–40 mg of protein were incubated in 16 ml of 10 mM-Tris/HCl, pH 7.5, containing 180 mM-sucrose, and 5 mg of poly(U) for 10 min at 0°C. After a low-speed centrifugation (3500g for 10 min at 3°C in a Sorvall RC-2

centrifuge) the nuclear-residue pellet was discarded, and the supernatant was adjusted to 5 mM-MgCl₂ and centrifuged at about 170 000g for 2.5 h at 3°C. The pellet was rinsed once with reticulocyte standard buffer (10 mM-NaCl/1.5 mM-MgCl₂/10 mM-Tris/HCl, pH 7.5) and suspended in sucrose buffer [5% (w/v) sucrose/20 mM-Tris/HCl (pH 7.5)/40 mM-KCl/5 mM-magnesium acetate/1 mM-dithiothreitol] to an A_{260} of 100–150 units/ml. The particles thus obtained were designated as unwashed nuclear polyribosomes.

To isolate cytoplasmic polyribosomes, the post-nuclear supernatant was adjusted to 5 mM-MgCl₂ and 0.5% Nonidet P-40 and homogenized gently in a Dounce homogenizer. The homogenate was centrifuged at 10 000g for 15 min at 3°C in a Sorvall RC-2 centrifuge. Cytoplasmic polyribosomes in the resulting supernatant were pelleted by centrifugation at about 170 000g for 2.5 h at 3°C in a Spinco 65 rotor. The pellet was rinsed and suspended in sucrose buffer to an A_{260} of 150–250 units/ml. This preparation represented unwashed cytoplasmic polyribosomes.

To prepare high-salt-washed preparations, unwashed polyribosomes from each source were treated with 0.5 M-NaCl for 2 h at 4°C and pelleted by another high-speed centrifugation. The protein content of the preparations was measured by the procedure of Lowry *et al.* (1951).

Protein kinase assay with endogenous and exogenous substrates

Enzyme activity was measured by a procedure of Blanchard *et al.* (1977), with the following modifications: the standard assay mixture (50 μ l) contained 10 mM-Tris/HCl (pH 8.3), 10 mM-MgCl₂, 10 mM-2-mercaptoethanol and 50–100 μ g of unwashed or high-salt-washed nuclear or cytoplasmic preparation. The reaction was initiated by adding [γ -³²P]ATP or [γ -³²P]GTP (Amersham Corp) to 0.1 mM (sp. radioactivity adjusted to 400–2500 c.p.m./pmol) with unlabelled triphosphates. After a 10 min incubation at 30°C the reaction was stopped by adding 1 ml of 7.5% (w/v) trichloroacetic acid containing 1 mM-NaH₂PO₄. Acid-precipitable material filtered through a nitrocellulose membrane was counted for radioactivity in Aquasol (New England Nuclear). The radioactive material was sensitive (>85%) to Pronase and insensitive to pancreatic ribonuclease.

The effect of protein kinase inhibitor on enzyme activity was measured by the procedure of Silberstein & August (1976).

To assay enzyme activity with exogenous substrates, the standard incubation mixture contained 50 μ g of substrate (histones or non-histones from HeLa cells, phosvitin or casein) and 30–50 μ g of nuclear or cytoplasmic polyribosomes. To deter-

mine whether the endogenous proteins in the nuclear preparation could function as substrates for the enzymes in the cytoplasmic preparation, and vice versa, mixing experiments were conducted with heat-inactivated (20 min at 65°C) polyribosomes as substrates. Heat treatment did not inactivate the substrates for phosphorylation by homologous enzymes.

Isolation of histones and non-histones from HeLa cells

Histones and non-histone proteins were isolated from HeLa cells by the procedure of McGillivray *et al.* (1972) as modified by Silver & Elgin (1976). The histones were eluted from the hydroxyapatite column with 0.001 M-sodium phosphate buffer, pH 7.0, in 2 M-NaCl/5 M-urea. Non-histones were eluted with 0.1 M-sodium phosphate, pH 7.0. The purity of the preparations was examined by polyacrylamide-gel electrophoresis.

Electrophoresis of phosphorylated proteins

Phosphorylated proteins were analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Maizel, 1971) in a discontinuous buffer system of Tris/glycine, pH 8.3. Samples were electrophoresed in 12.5% acrylamide slab gels at a constant 175 V until the tracking dye had migrated a fixed distance into the gel. The gels were then stained with 0.05% Coomassie Brilliant Blue and radioautographed (Laskey & Mills, 1975). Purified adenovirus was electrophoresed in a parallel track with each gel, and the capsid proteins (Maizel *et al.*, 1968) served as molecular-weight markers.

Fractionation of protein kinase on DEAE-cellulose column

Protein kinase activity was fractionated at 4°C by a modification of the procedure of Traugh *et al.* (1973). Unwashed nuclear or cytoplasmic polyribosomes were solubilized by sonication (three times for 30 s each at about 250 W in a model 140

sonifier, from Branson, Plainview, NY, U.S.A.) and centrifuged at 15000g for 5 min at 3°C in a Sorvall RC-2 centrifuge. The supernatant was dialysed overnight against 20 mM-Tris/HCl (pH 7.5)/10% glycerol/1 mM-dithiothreitol buffer. It was then applied to a DEAE-cellulose column (1.2 cm × 6 cm) equilibrated with the same buffer. The column was washed with 25 ml of this buffer and eluted with a linear gradient of 0–0.3 M-KCl (100 ml of each) in the same buffer. Fractions (2 ml) were measured for A_{280} and assayed for enzyme activity with [γ - 32 P]ATP. Phosphorylated proteins of the selected fractions were analysed by gel electrophoresis.

Results

Cyclic nucleotide-independent protein kinase activity in cytoplasmic and nuclear preparations

The kinases and the associated endogenous protein substrates were tightly bound (as discussed below) to cytoplasmic or nuclear polyribosomes isolated from non-infected or adenovirus-infected cells. The protein content of these ribosomes varied from 1.5 to 2.5 mg/10⁹ cells in unwashed preparations. In the standard assay mixture with no exogenous substrates, all four preparations incorporated radioactivity from [γ - 32 P]-ATP or -GTP into acid-precipitable protein (Table 1). The enzymes from the infected cells were comparatively less active, and the nuclear enzymes catalysed 3–4 times as much incorporation as the cytoplasmic enzymes. About twice as much phosphate was incorporated with ATP as with GTP. (In four experiments these results varied between 5 and 8%.)

Incorporation was maximum after a 10 min incubation and was linear up to 80 μ g of protein/50 μ l of assay mixture. In order to maximize the incorporation for the following experiments, a 10 min incubation period was therefore chosen. Incorporation was not stimulated by cyclic AMP or cyclic GMP (up to 15 μ M) and was not inhibited by purified protein kinase-inhibitor protein (25 μ g/50 μ l of assay mixture) from bovine heart.

Table 1. *Incorporation of 32 P from [γ - 32 P]ATP and [γ - 32 P]GTP into endogenous proteins*

The conditions for the protein kinase assay are described in the Experimental section. For this assay about 50 μ g of protein (containing the enzymes) from each cytoplasmic or nuclear preparation was used. The incubation was for 10 min at 30°C.

Preparation	Incorporation (pmol/ μ g of protein)		Ratio (ATP/GTP)
	ATP	GTP	
Cytoplasmic, non-infected	1.30	0.50	2.60
Cytoplasmic, adenovirus-infected	1.10	0.47	2.30
Nuclear, non-infected	5.20	2.90	1.80
Nuclear, adenovirus-infected	3.60	1.87	1.90

Differential phosphorylation of specific endogenous proteins

To identify the endogenous proteins phosphorylated in the preparations described above, cytoplasmic and nuclear preparations from infected and non-infected cells were incubated with [γ - 32 P]-ATP or -GTP, and the phosphorylated products were analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. The Coomassie-Blue-stained profiles of the endogenous proteins and the radioautograms of their phosphorylated components are given in Fig. 1. The stained gels show both high- and low-molecular-weight proteins (mol.wts. >120000 to 12000). The nuclear preparation contained fewer proteins than the cytoplasmic preparation. Cytoplasmic proteins were very similar in non-infected and virus-infected cells, but minor differences existed between nuclear proteins in the two cell groups.

In nuclear preparations, somewhat different endogenous proteins were phosphorylated with ATP and with GTP. For example, ATP phosphorylated intensely the two proteins of mol.wts. 27000 and 70000; with GTP the former was not phosphorylated at all, and the intensity of the latter was less. In cytoplasmic preparations the protein of mol.wt. 27000 does not appear to be phosphorylated by ATP or GTP, whereas a doublet protein of mol.wt. approx. 70000 seems to be phosphorylated by GTP only. The reason for this differential phosphorylation by ATP and GTP is not clear.

The phosphorylated proteins in non-infected and virus-infected cells were very similar, except for the intensely phosphorylated nuclear protein of mol.wt. 120000 that was present in the non-infected and practically absent from the infected cells. Since adenovirus infection, apart from decreasing cyclic AMP-independent protein kinase activity, does not appear to alter significantly the nature of the endogenous proteins (whether nuclear or cytoplasmic, non-phosphorylated or phosphorylated), the remaining experiments were carried out with non-infected cells unless otherwise stated.

Binding of protein kinase activity

Kinases bind differentially to polyribosomes in different cells. Cyclic AMP-independent protein kinase from rabbit reticulocyte ribosomes is tightly bound, and a significant amount remains bound even after treatment with 0.5M-NaCl (Traugh *et al.*, 1973). In HeLa cells, on the other hand, the enzyme isolated from nuclear ribonucleoprotein particles can be completely dissociated by the salt treatment (Blanchard *et al.*, 1977).

The Coomassie-Blue-stained endogenous proteins and radioautograms of the phosphorylated proteins of unwashed and high-salt-washed cytoplasmic and nuclear preparations are given in Fig. 2. Even after the treatment about 80% of the kinase activity and a significant number of endogenous proteins remained associated with the polyribosomes of either origin (compare phosphorylated

Fig. 1. Phosphorylated endogenous proteins of nuclear (N) and cytoplasmic (C) origin

Unwashed ribosomes from non-infected and adenovirus-infected HeLa cells were incubated with [γ - 32 P]ATP or [γ - 32 P]GTP as described in the Experimental section. The phosphorylated proteins were electrophoresed in 12.5% polyacrylamide gels containing sodium dodecyl sulphate, fixed, stained with 50% (v/v) methanol/10% (v/v) acetic acid containing 0.05% Coomassie Blue, destained, and radioautographed as described in the Experimental section. Adenovirus capsid proteins were co-electrophoresed in a parallel track as molecular-weight markers ('120K' etc. represents 120000 mol.wt. etc.).

proteins in 'unwashed' and 'salt-washed' tracks in Fig. 2). The intensely phosphorylated cytoplasmic protein of mol.wt. 120 000 virtually disappeared after high-salt treatment, and a new protein of mol.wt. about 48 000 appeared. In contrast, the phosphorylated nuclear proteins did not change appreciably. A similar tight binding has been reported in the virus-infected cells (Chatterjee *et al.*, 1979).

Phosphorylation of various substrates by nuclear and cytoplasmic kinases

Kinases from nuclear and cytoplasmic sources had similar substrate specificities with respect to phosphorylation of four exogenous acceptor proteins (results not shown). The acidic proteins casein and phosvitin, as well as histones from HeLa cells, were phosphorylated to some extent by the enzymes from infected and non-infected cells. As before, nuclear enzyme activity was considerably higher. Non-histones, on the other hand, were poor substrates.

The stained HeLa-cell histones and non-histones and their phosphorylated components are shown in Fig. 3. All five histones, mol.wts. 27 000–12 000, were phosphorylated by the nuclear kinases, whereas only the histone of mol.wt. 12 000 was pre-

dominantly phosphorylated by the cytoplasmic kinases. Moreover, the two highest-molecular-weight histones were phosphorylated considerably less by the cytoplasmic kinases than by the nuclear kinases. Although we had observed a little phosphate incorporation by non-histone proteins (mol.wts. 70 000–30 000), no specific phosphorylated component could be detected by radioautography.

In the mixing experiments, endogenous proteins of cytoplasmic polyribosomes did not act as substrates for the nuclear kinases, and vice versa (results not shown). To examine further this specificity of endogenous protein substrates for the nuclear and cytoplasmic enzymes, bovine heart kinase was used to phosphorylate the heated substrate proteins from nuclear and cytoplasmic sources. No ^{32}P incorporation was detected in the absence of exogenous substrate (results not shown). The bovine enzyme could not phosphorylate endogenous proteins of the nuclear preparation, but it phosphorylated such proteins of mol.wt. 30 000 and lower in the cytoplasmic preparation (Fig. 4).

Fractionation of protein kinases

To compare the multiplicity of cyclic AMP-independent protein kinases of cytoplasmic and nuclear origin, the enzyme activity in unwashed

Fig. 2. Phosphorylated endogenous proteins in unwashed (Unw) and high-salt-washed (Salt) preparations of nuclear and cytoplasmic origin from non-infected cells

The procedure for the preparation of unwashed and high-salt-washed ribosomes is described in the Experimental section. Procedures for phosphorylation using $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ and radioautography are described in Fig. 1. The assay mixture (50 μl) contained 32 μg of cytoplasmic ribosomes and 22 μg of nuclear unwashed or high-salt-washed ribosomes. Track M, adenovirus capsid proteins as molecular-weight markers ('120K' etc. represents 120 000 mol.wt. etc.).

Fig. 3. Phosphorylation of HeLa-cell histones (H) and non-histones (NonH) with kinases of cytoplasmic and nuclear origin from non-infected cells

The procedure for the isolation of histones and non-histones is described in the Experimental section. Procedures for phosphorylation with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ and radioautography are described in Fig. 1. The assay mixture (50 μl) contained 32 μg of unwashed cytoplasmic or 22 μg of unwashed nuclear preparation and 50 μg of histones or non-histones. None, No exogenous substrate. '120 K' etc. represents 120 000 mol.wt. etc., from molecular-weight markers.

preparations was fractionated on a DEAE-cellulose column. Each preparation had been sonicated and centrifuged. The supernatant was collected for column chromatography. Sonication released in the supernatant almost all of the enzyme activity in the cytoplasmic preparation and about half of that in the nuclear preparation (the rest was in the pellet).

Several additional interesting differences between cytoplasmic and nuclear kinases are evident from Fig. 5, which illustrates the separation of the enzyme activity assayed by $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ incorporation. In the cytoplasmic preparation about 95% of the total protein and 50% of the enzyme activity remained unadsorbed to the column and were collected as the flow-through fraction (peak I). The adsorbed activity was eluted as four major discrete peaks, at about 0.08 M-, 0.20 M-, 0.23 M- and 0.24 M-KCl. In the nuclear preparation, on the other hand, only about 15% of the total protein and 3% of the enzyme activity remained unadsorbed, and the adsorbed activity could not be resolved into four discrete peaks as in the cytoplasmic preparation.

A nuclear enzyme fraction (peak II) eluted at about 0.09 M-KCl was comparable with peak II of the cytoplasmic preparation. The nuclear fraction contained 10-fold more protein, but only about 30% as much enzyme activity. The three other enzyme fractions (peaks III-V) of the cytoplasmic pre-

paration and the comparable peak III of the nuclear preparation contained similar amounts of protein, but the enzyme activity of the nuclear peak was 2-3-fold higher than that of the cytoplasmic peaks. Freezing and thawing decreased the enzyme activity of the various peaks of both preparations by 30-60%.

To examine the substrate specificities of the enzymes in the various peaks, materials from peaks I-V of the cytoplasmic preparation and from peak III and the nuclear pellet fraction of the nuclear preparation were incubated with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ and radioautographed (Fig. 6). Because of the low concentrations of proteins in these peaks, heat-inactivated homologous proteins from the unfractionated preparations were added as endogenous substrates for phosphorylation. Enzyme activity was completely blocked by the heat treatment (Fig. 6).

In the cytoplasmic preparations the substrate specificities of the kinases of peaks III-V appear to be the same, as in each case the same proteins (mol.wts. 120 000-20 000) were phosphorylated. The proteins phosphorylated by peak-II enzyme, however, were quite different. The protein of mol.wt. 120 000 was barely phosphorylated, and at least four proteins appeared that were not present in peaks III-V (mol.wts. 27 000, 22 000, 20 000 and 15 000). Many of the phosphorylated proteins of peak I

Fig. 4. Phosphorylation of endogenous proteins from nuclear (N) and cytoplasmic (C) preparations by bovine heart protein kinase

Procedures for phosphorylation with [γ - 32 P]ATP and radioautography are described in Fig. 1. The assay mixture (50 μ l) contained 30–50 μ g of heat-inactivated nuclear or cytoplasmic proteins as substrate, 1 μ g of bovine heart protein kinase and 1.5 μ M-cyclic AMP. The bovine enzyme is cyclic AMP-dependent (Rosen *et al.*, 1975). '120K' etc. represents 120000 mol.wt. etc., from molecular-weight markers.

resembled those of peaks III–V; at least one (mol.wt. 120000) resembled that of peak II.

For the nuclear preparations the flow-through fraction (peak I) and peak II contained very little enzyme activity. Peak-I enzyme phosphorylated two proteins of mol.wts. 76000 and 24000; no such proteins could be found by using peak-II enzyme (results not shown). Peak III contained practically all of the adsorbed enzyme activity (see Fig. 5) and phosphorylated a number of proteins ranging from mol.wt. 76000 to 24000. In contrast with the cytoplasmic preparations, the pellet fraction contained about half of the total enzyme activity. A number of phosphorylated proteins, along with an intensely phosphorylated band at mol.wt. 70000, which was absent from peak III, were found in this fraction.

These results indicate that cyclic AMP-independent protein kinases exist in at least two different

Fig. 5. DEAE-cellulose column chromatography of protein kinase activity from (a) cytoplasmic and (b) nuclear preparations from non-infected HeLa cells

The procedures for the chromatography and phosphorylation are described in the Experimental section. About 35 and 12 mg of solubilized proteins of cytoplasmic and nuclear preparations respectively were used for the column. Enzyme activity was assayed by using 10 μ l of eluate (cytoplasmic fractions 3–5) or 35 μ l of eluate (cytoplasmic fractions 6–60 and all nuclear fractions). Heat-inactivated cytoplasmic (60 μ g) and nuclear (30 μ g) proteins were added as substrates for phosphorylation. ●, 32 P incorporation; ○, A_{280} (protein).

forms in cytoplasmic ribosomes (peak II, and peaks III–V) and in nuclear ribosomes (pellet and peak III). These results are further evidence that the polyribosome-bound cytoplasmic and nuclear kinases are different.

Discussion

Our experiments demonstrate that in HeLa cells the cyclic nucleotide-independent protein kinases bound to cytoplasmic and nuclear polyribosomes are different. The difference appears to exist in the enzymes as well as in the endogenous protein substrates associated with them.

Adenovirus infection of the cells, at 18–20 h after infection, does not appear to alter the nature of the enzymes or the substrates. This supports the

Fig. 6. Analysis of protein kinase activity by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of selected fractions of cytoplasmic and nuclear preparations separated by DEAE-cellulose column chromatography

Procedures for phosphorylation and radioautography are described in Figs. 5 and 1 respectively. The elution pattern of the peaks analysed for enzyme activity is shown in Fig. 5. For the cytoplasmic enzymes the assay mixture (100 μ l) contained 60 μ g of heat-inactivated cytoplasmic protein and 30 μ l of peak-I material or 75 μ l of that from the other peak fractions. For the nuclear enzymes the assay mixture (50 μ l) contained 36 μ g of heat-inactivated nuclear protein and 35 μ l of peak fraction. The nuclear pellet was obtained after solubilization. Heated, heat-inactivated unfractionated preparation. '120 K' etc. represents 120000 mol.wt. etc., from molecular-weight markers.

suggestion (Akusjarvi *et al.*, 1978; Blair & Russell, 1978) that adenovirus 2 is associated with a cyclic nucleotide-independent protein kinase activity of possibly host-cell origin.

Protein kinases have been classified into two types of activities: (i) cyclic nucleotide-dependent kinases, containing both the holoenzyme and a free catalytic subunit which has been dissociated from the regulatory component; and (ii) cyclic nucleotide-independent kinases (Langan, 1973; Walsh & Ashby, 1973; Walsh & Krebs, 1973; Rubin & Rosen, 1975). The former utilize ATP (Traugh & Traut, 1974; Rubin & Rosen, 1975) and the latter both ATP and GTP (Rodnight & Lavin, 1964; Goldstein & Hasty, 1973; Rubin & Rosen, 1975; Silberstein & August, 1976; Blanchard *et al.*, 1977; Akusjarvi *et al.*, 1978; Blair & Russell, 1978) as the phosphate donor. Cyclic nucleotide-independent kinases are not affected by cyclic nucleotide-dependent protein kinase inhibitor (Walsh & Ashby, 1973; Silberstein & August, 1976).

The cytoplasmic and nuclear kinases in the present study were not stimulated by various concentrations of cyclic AMP or cyclic GMP; they remained unaffected by the protein kinase-inhibitor protein; and they utilized GTP as well as ATP in the

phosphotransferase reaction. They are thus cyclic nucleotide-independent enzymes.

The nuclear kinases were 3–4-fold more active than the cytoplasmic ones; this difference was observed in unfractionated as well as fractionated preparations. The higher activity could mean that there are more enzyme molecules and/or substrate molecules per unit of protein in the preparation. Alternatively, the cytoplasmic preparation may contain more non-specific inhibitor of the enzymes. (Inhibitors of cyclic nucleotide-dependent and -independent kinases are reviewed in Szmigielski *et al.*, 1977.) Although the nuclear preparation contained fewer endogenous proteins than the cytoplasmic preparation, more proteins were phosphorylated. This suggests that there are more substrate molecules in the nuclear preparation.

DEAE-cellulose fractionation of the kinases from either preparation yielded a number of peaks, which indicates the presence of multiple kinases. The peak-II enzyme of the cytoplasmic preparation appears to be different from the rest, in that it phosphorylated predominantly the low-molecular-weight proteins (Fig. 6).

Substrate specificities of the polyribosome-bound nuclear and cytoplasmic kinases were different: the

nuclear kinases could not phosphorylate endogenous substrates in the cytoplasmic preparation, and vice versa. Furthermore, bovine heart kinase phosphorylated no endogenous proteins of the nuclear preparations and predominantly the proteins of 30000 and lower molecular weight in the cytoplasmic preparations. These results are a further indication that the endogenous substrates could be different.

Although both nuclear and cytoplasmic kinases phosphorylated to some extent the acidic proteins casein and phosvitin, as well as histones from HeLa cells, one difference was observed. All five histones from HeLa cells were phosphorylated by the nuclear kinases, but only the smallest one (mol.wt. 12000) was predominantly phosphorylated by the cytoplasmic ones. This observation suggests that the enzymes could be different, and it is evidence that nuclear kinases modify a component of similar origin, i.e. histones, which may control gene expression.

Cyclic nucleotide-dependent and -independent kinases and phosphate-acceptor proteins are believed to be integral parts of plasma membranes, sarcoplasmic reticulum, secretory vesicles, ribosomes and nuclei. The cyclic nucleotide-independent protein kinases phosphorylate various cytoplasmic and nuclear components, including histones (Langan, 1971), acidic proteins of chromatin (Kish & Kleinsmith, 1974), specialized membrane components (Bownds *et al.*, 1972) and ribosomal proteins (Traugh *et al.*, 1973; Ventimiglia & Wool, 1974). It seems reasonable to assume that such phosphorylation modulates the biological activity of these components. However, the nature of these enzymes in the cytoplasm and nucleus of a particular cell type has not been characterized.

Our study demonstrates a number of interesting differences between these enzymes bound to polyribosomes of nuclear and cytoplasmic origin in HeLa cells. Although a direct correlation between phosphorylation via these enzymes and a functional modification of a subcellular component cannot be made from this study, the difference in the nature of the enzymes and their associated endogenous protein substrates suggests that nuclear and cytoplasmic components may be modified by their respective kinases.

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