Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 6408–6412, October 1984
Cell Biology

Phorbol ester, serum, and Rous sarcoma virus transforming gene product induce similar phosphorylations of ribosomal protein S6

(growth regulation/cell transformation/tumor promoters)

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ABSTRACT **The addition of phorbol 12-myristate 13-acetate (PMA), a potent tumor promoter, to serum-starved quiescent chicken embryo fibroblasts (CEF) or C127 murine cells resulted in increased phosphorylation of 40S ribosomal protein S6. The effect of PMA on S6 phosphorylation in quiescent CEF was half-maximal at ≈100 nM and was readily observed at 16 nM. In addition, S6 phosphorylation was increased in serum-starved CEF incubated with the diacylglycerol derivative, 1-oleoyl-2-acetylglycerol. S6 phosphorylation in PMA-stimulated, serum-stimulated, and serum-starved Rous sarcoma virus-transformed CEF was analyzed by phospho amino acid analysis, two-dimensional polyacrylamide gel electrophoresis, limited proteolysis with V8 protease, and two-dimensional thin-layer electrophoresis of chymotryptic digests. Comparison of S6 phosphorylation by these methods suggests that phosphorylation of S6 stimulated by PMA, serum, or oncogenic transformation with Rous sarcoma virus occurs through common pathways. This is further supported by the observation that the simultaneous addition of PMA and serum to CEF or of either PMA or serum to Rous sarcoma virus-transformed CEF did not significantly further increase the incorporation of phosphate into S6.**

The role of phosphorylation of 40S ribosomal protein S6 in protein synthesis and cell proliferation has recently received much attention. Interest in S6 phosphorylation was initially stimulated by observations of a temporal correlation between phosphorylation of this protein and the initiation of protein synthesis when quiescent cells are treated with serum or certain mitogens (1–8). Moreover, a similar result is obtained in *Xenopus* oocytes during progesterone- or insulin-induced maturation (9–11). The connection between S6 phosphorylation and initiation of protein synthesis is further supported by the observations that phosphorylated small ribosomal subunits are preferentially utilized during initiation complex formation (7, 12), that S6 can be crosslinked to polyuridylic acid by ultraviolet irradiation (13), and that S6-specific antibodies inhibit the formation of the initiation complex (14) required to initiate protein synthesis. Finally, it has recently been demonstrated that 40S subunits isolated from polysomes contain extensively phosphorylated S6 and exhibit conformational changes involving several ribosomal proteins. These changes are likely to be involved in the binding of initiation factors and mRNA to the small subunit (15).

In contrast to normal cells, serum starvation of many oncogenically transformed cells does not lead to a decrease in S6 phosphorylation (16, 17). In serum-starved Rous sarcoma virus (RSV)-transformed chicken embryo fibroblasts (CEF), expression of the *src* gene is responsible for S6 phosphorylation (16, 17). The RSV *src* gene product $pp60^{v\text{-}src}$ is a tyrosine-specific protein kinase (18, 19). However, S6 is phosphorylated at serine in biosynthetically labeled cells (16, 17), suggesting that S6 phosphorylation in RSV-transformed CEF is the result of a transformation-specific alteration in cellular metabolism. The ability of $pp60^{v\text{-}src}$ to alter S6 phosphorylation has also been demonstrated in *Xenopus laevis* oocytes after microinjection of purified enzyme (20). Based on these results it has been proposed that $pp60^{v\text{-}src}$ directly or indirectly interacts with a pathway operative in normal cells that regulates the state of S6 phosphorylation.

When added to normal cells, the tumor promoter phorbol 12-myristate 13-acetate (PMA) induces the expression of many properties common to oncogenically transformed cells and to cells treated with growth factors (21–23). In CEF for example, PMA, serum growth factors, and functional $pp60^{v\text{-}src}$ all are mitogenic, stimulate glucose uptake, induce uridine and thymidine utilization, and modulate the expression of plasminogen activator (21–23). Furthermore, these agents all induce phosphorylation of a 42-kDa polypeptide at tyrosine (24–26). Although $pp60^{v\text{-}src}$ and some polypeptide hormone receptors are associated with tyrosine-specific protein kinase activity (18, 19, 23), PMA is known to activate a phospholipid- and Ca^{2+}-dependent protein kinase (protein kinase C), that phosphorylates threonine and serine (27). PMA may be capable of activating a tyrosine-specific kinase, either directly or through the action of protein kinase C. However, PMA does not stimulate $pp60^{c\text{-}src}$ (normal cell homolog of $pp60^{v\text{-}src}$) kinase activity either *in vitro* or in cultured cells (28, 29) so another as yet unidentified tyrosine kinase is apparently involved. Interestingly, when PMA is added to RSV-transformed cells, the levels of plasminogen activator and ornithine decarboxylase are further increased (21, 22). The synergistic response observed for these parameters of transformation suggests that separate mechanisms are involved in obtaining the same response. However, PMA does not potentiate the increase in glucose transport or the decrease in collagen synthesis observed in RSV-transformed primary avian tendon cells (30). These results suggest that the common alterations in cellular structure and function resulting from oncogenic transformation or PMA treatment occur through both common and convergent biochemical pathways.

In view of the ability of PMA treatment of cultured cells to mimic many properties of transformation and growth factors, we investigated the ability of PMA to stimulate S6 phosphorylation in normal, quiescent CEF and C127 murine cells, and we compared S6 phosphorylation in PMA-stimulated CEF to that in CEF stimulated with serum growth factors and that in serum-starved RSV-transformed CEF. In

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Abbreviations; PMA, phorbol 12-myristate 13-acetate; OAG, 1-oleoyl-2-acetylglycerol; Me_2SO, dimethyl sulfoxide; RSV, Rous sarcoma virus; CEF, chicken embryo fibroblasts; ts-CEF, CEF infected with a temperature-sensitive transformation mutant of RSV.

this report we demonstrate that PMA treatment of serum-starved cells does lead to increased S6 phosphorylation and that, according to our analysis, this phosphorylation is similar to that observed in serum-stimulated CEF and CEF transformed with RSV. While this work was in progress, a report describing PMA-induced S6 phosphorylation in Reuber H35 hepatoma cells appeared (31). Our work supports and extends these findings by demonstrating similar S6 phosphorylation in serum-starved RSV-transformed CEF and in serum- and PMA-stimulated CEF.

MATERIALS AND METHODS

Cell Cultures. Uninfected CEF and CEF infected with temperature-sensitive (ts) mutants (NY68 or 72-4, obtained from H. Hanafusa, Rockefeller University, New York) of RSV (ts-CEF) were routinely cultured at 41°C in Richter's Imemzo medium (Associated Biomedic Systems, Buffalo, NY) containing 5% calf serum (GIBCO) and 10% tryptose phosphate broth (Difco). Transformation of ts-CEF was effected by transfer of cultures to the permissive temperature, 35°C; at the nonpermissive temperature (41°C) these cells exhibit a nontransformed phenotype because the $pp60^{v\text{-}src}$ is inactivated. CEF transformed with Schmidt–Ruppin A strain of RSV were cultured in Dulbecco's modified Eagle's medium (DME medium) supplemented with 5% calf serum and 10% tryptose phosphate broth. C127 murine cells were cultured in DME medium supplemented with 10% calf serum.

Confluent monolayers of cells were incubated in serum-free DME medium for 24–48 hr. After 1 hr in serum- and phosphate-free medium, the cultures were radioactively labeled for 1–3 hr with 1–2 mCi (1 mCi = 37 MBq) of $H_3{}^{32}PO_4$ (carrier-free, New England Nuclear) per 100-mm tissue culture plate. Appropriate cultures were incubated with dialyzed serum, PMA (Pharmacia P-L Biochemicals), 1-oleoyl-2-acetylglycerol (OAG; Molecular Probes, Plano, TX), or phorbol (Pharmacia P-L Biochemicals) during the biosynthetic labeling. Details of labeling and treatments are described in figure legends.

Gel Electrophoresis. Phosphorylated ribosomal proteins were prepared as described (16) and analyzed by $NaDodSO_4$/PAGE in 12% gels. To quantify the extent of S6 phosphorylation, the S6 band was cut out of the dried gel and the associated radioactivity was determined by liquid scintillation spectrometry. S6 phosphorylation was also analyzed by two-dimensional PAGE of ribosomal proteins as described by Kaltschmidt and Wittman (32) and modified by Thomas *et al.* (7). Carrier ribosomal proteins from unfertilized *X. laevis* eggs were used as reference markers for ribosomal protein S6. Analysis of S6 phosphopeptides by limited proteolysis was performed as described (33) by using *Staphylococcus aureus* V8 protease. Radiolabeled proteins and peptides were visualized using Kodak XAR-5 film or DuPont Cronex-4 film with DuPont Lightning Plus intensifying screens.

Two-Dimensional Thin-Layer Electrophoresis. ^{32}P-labeled S6 was excised from a wet one-dimensional $NaDodSO_4$/polyacrylamide gel, eluted, and prepared for proteolytic digestion as described (34) using 50 μg of carrier bovine serum albumin and α-chymotrypsin (49 units/mg, Worthington) at 80–100 μg/ml. Chymotryptic phosphopeptides were analyzed by cellulose thin-layer electrophoresis (Polygram Cel 300, Macherey & Nagel; Cellulose Chromogram, Eastman Kodak). Electrophoresis in the first dimension was run at pH 1.5 (acetic acid/formic acid/H_2O, 3:1:16) for 35 min at 37.5 V/cm. The second dimension was at pH 3.5 (pyridine/acetic acid/H_2O, 1:10:189) for 35 min at 50 V/cm. Migration was toward the cathode; Savant electrophoresis tanks cooled to 18°C were used. Phosphopeptides were visualized by autoradiography.

Phospho amino acid analysis of eluted S6 polypeptides was performed as described (18, 19) with the first dimension run at pH 1.5 for 1 h at 37.5 V/cm and the second dimension at pH 3.5 for 45 min at 50 V/cm.

RESULTS

PMA Stimulation of S6 Phosphorylation. The effect of PMA on S6 phosphorylation was evaluated in normal CEF and in a murine cell line, C127. Both cell types are quiescent when confluent and serum-starved. The S6 phosphoprotein in ^{32}P-labeled ribosomes isolated from these cells was analyzed by $NaDodSO_4$/PAGE and autoradiography (Fig. 1). Phosphorylation of S6 in serum-starved CEF (Fig. 1*A*, lane 1), serum-starved CEF incubated with phorbol (which is inactive as a tumor promoter) at 100 ng/ml (Fig. 1*A*, lane 4), serum-starved C127 murine cells (Fig. 1*B*, lane 1), and serum-starved C127 cells treated with phorbol (100 ng/ml; Fig. 1*B*, lane 4) was much less than that observed in CEF incubated in the presence of 5% (vol/vol) bovine calf serum or PMA at 100 ng/ml (Fig. 1*A*, lanes 2 and 3, respectively) and in C127 murine cells treated with 5% serum or PMA at 100 ng/ml (Fig. 1*B*, lanes 2 and 3, respectively). Fig. 1*C* shows the analysis of S6 phosphorylation in serum-starved ts-CEF (infected with RSV temperature-sensitive mutant 72-4) at the nonpermissive temperature (Fig. 1*C*, lane 1) and after incubation at the permissive temperature for 2 hr (Fig. 1*C*, lane 2). Stimulation of S6 phosphorylation occurred in PMA- and serum-stimulated cells, and in serum-starved oncogenically-transformed cells under the experimental conditions described in the legend to Fig. 1. Similar results were obtained when cells were prelabeled for 6–18 hr before stimulation of S6 phosphorylation.

In vitro, PMA interacts with and activates the Ca^{2+}- and phospholipid-dependent protein kinase, protein kinase C (27). This apparently occurs in a manner similar to that observed with diacylglycerol, the endogenous protein kinase C activator, to which PMA is structurally similar (27). We therefore analyzed the effect of an exogenously added di-

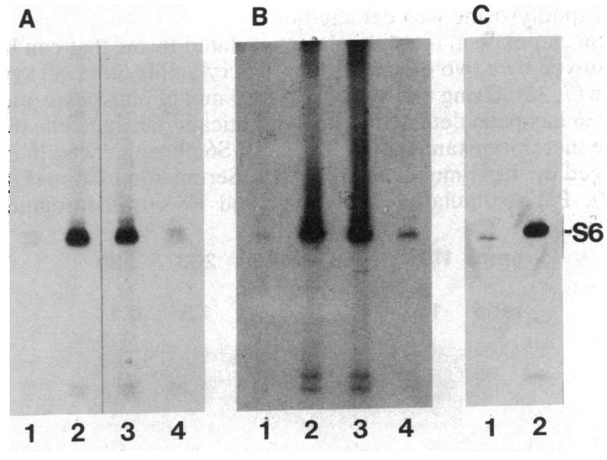

FIG. 1. Analysis of S6 phosphorylation stimulated by serum, PMA, and activated $pp60^{v\text{-}src}$. Confluent CEF, C127 murine cells, and ts-CEF were serum-starved at 41°C for 24 hr and labeled with 1 mCi of $H_3{}^{32}PO_4$ per 100-mm plate during treatment with serum, PMA, phorbol, or active $pp60^{v\text{-}src}$ for 2 hr. Ribosomes were isolated as described (16). Equal amounts of ribosomal protein were analyzed by $NaDodSO_4$/PAGE in 12% gels (35). Phosphorylated proteins were visualized by autoradiography. (*A*) S6 phosphorylation in serum-starved uninfected CEF that were untreated (lane 1), 5% serum-stimulated (lane 2), incubated with PMA at 100 ng/ml in medium containing 0.1% dimethyl sulfoxide (Me_2SO) (lane 3) and phorbol at 100 ng/ml in medium containing 0.1% Me_2SO (lane 4). (*B*) S6 phosphorylation in C127 murine cells treated as in (*A*). (*C*) S6 phosphorylation in serum-starved ts-CEF labeled for 2 hr at the nonpermissive temperature (lane 1) or during 2 hr at the permissive temperature (lane 2).

acylglycerol, OAG, on S6 phosphorylation. Fig. 2 shows the results obtained when serum-starved ts-CEF were prelabeled at 41°C (nonpermissive temperature) and were untreated (lane 1), transferred to 35°C (permissive temperature) (lane 2), or treated at 41°C with PMA at 100 ng/ml (lane 3), OAG at 250 μg/ml (lane 4) or OAG at 500 μg/ml (lane 5). OAG did stimulate phosphorylation of S6 in serum-starved cells. However, the stimulation (≈2-fold) of S6 phosphorylation obtained with these high concentrations of OAG was not as great as that obtained with PMA (≈4-fold in this experiment). This may be due to the poor solubility of OAG and/or its inability to enter the cell.

PMA Dose–Response Analysis. To biochemically compare PMA-stimulated S6 phosphorylation in CEF with serum- and transformation-induced S6 phosphorylation, it was necessary to obtain maximal S6 phosphorylation at PMA concentrations that were not toxic to the cells. Earlier work has demonstrated PMA effects on other cellular properties at nontoxic levels (10–100 ng/ml in CEF). PMA-stimulated S6 phosphorylation in quiescent CEF was readily observed at 10 ng/ml (16 nM; Fig. 3, lane 4) and was half maximal at ≈100 nM. Maximal S6 phosphorylation was obtained at PMA concentrations ranging from 100–250 ng/ml, levels that were not toxic to the cells. Maximal PMA-induced S6 phosphorylation was approximately equivalent to that obtained with 5% serum (Fig. 3, lane 8).

Comparison of S6 Phosphorylation Stimulated by PMA, Serum, and Oncogenic Transformation. Phosphorylation of S6 was characterized and compared by (*i*) analysis of phospho amino acids, (*ii*) two-dimensional polyacrylamide gel electrophoresis of phosphorylated S6, (*iii*) limited proteolysis with *S. aureus* V8 protease, and (*iv*) two-dimensional thin-layer electrophoresis of chymotryptic phosphopeptides.

Phospho amino acids obtained by acid hydrolysis of ^{32}P-labeled S6 from serum-stimulated, PMA-stimulated, and serum-starved RSV-transformed CEF were identical (data not shown). More than 95% of the total phospho amino acids was phosphoserine and <5% was phosphothreonine. No phosphotyrosine was detected.

S6 can exist in multiple phosphorylated forms that can be resolved by a two-dimensional polyacrylamide/urea gel system (7, 32). Using this system, up to 5 mol of phosphate/mol of S6 has been detected in biosynthetically labeled cells (8). We therefore examined the extent of S6 phosphorylation induced by the stimuli described here. Serum-stimulation (Fig. 4*B*), PMA-stimulation (Fig. 4*D*), and RSV-transformation

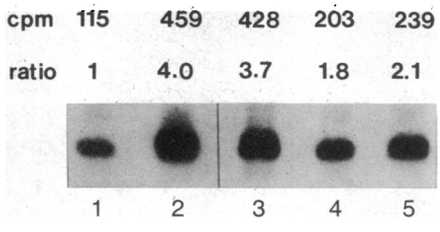

FIG. 2. Phosphorylation of S6 stimulated by incubation with diacylglycerol. Confluent ts-CEF were serum-starved at 41°C for 24 hr, prelabeled with 1.2 mCi of $H_3{}^{32}PO_4$ for 6.5 hr, and treated as described below for 2.5 hr. Purified ribosomes were subjected to $NaDodSO_4$/PAGE. Autoradiograms of the gels are shown; radioactivity associated with S6 was quantitated by liquid scintillation spectrometry. Equal amounts of ribosomal protein were analyzed. Lane 1, ts-CEF at 41°C; lane 2, ts-CEF incubated at 35°C; lane 3, ts-CEF at 41°C treated with PMA at 100 ng/ml; lane 4, ts-CEF at 41°C incubated with OAG at 250 ng/ml; lane 5, ts-CEF at 41°C incubated with OAG at 500 ng/ml. Incubations with PMA or OAG were done in medium containing 0.1% Me_2SO. OAG was suspended by sonication (3 × 3 min) in 1 ml of medium before addition to prelabeled cells. Ratios of radioactivity (cpm) in S6 bands to that in the S6 band in lane 1 are given.

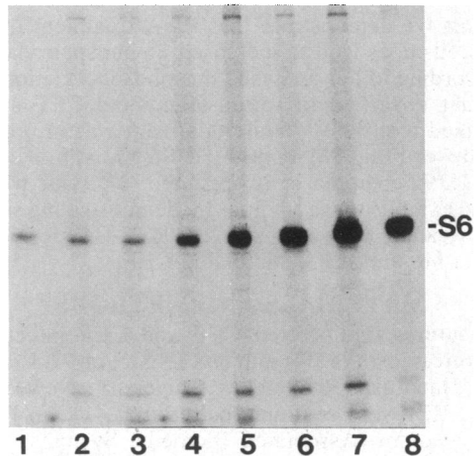

FIG. 3. Dose–response analysis of PMA effect on S6 phosphorylation. Confluent, uninfected CEF were serum-starved for 24 hr and radiolabeled. Equal amounts of ribosomal protein were analyzed as described in the legend to Fig. 1. Cultures were treated for 2 hr with phorbol at 100 ng/ml (lane 1), PMA at 0.1 ng/ml (lane 2), 1.0 ng/ml (lane 3), 10 ng/ml (lane 4), 50 ng/ml (lane 5), 100 ng/ml (lane 6), 500 ng/ml (lane 7), or with 5% serum (lane 8). Final concentration of Me_2SO in the medium was 0.1%. S6 was cut out of the dried gel and its radioactivity was determined in order to estimate the PMA concentration required for maximal stimulation.

(Fig. 4*E*) all produced the highly phosphorylated species of S6 (4–5 mol of phosphate/mol of S6, d + e), whereas in serum-starved (Fig. 4*A*) or phorbol-treated (Fig. 4*C*) cells, S6 containing 1–2 mol of phosphate/mol of polypeptide was the predominant form detected after a much longer exposure. Thus, by this analysis, the same degree of S6 phosphorylation was induced by all the stimuli under investigation.

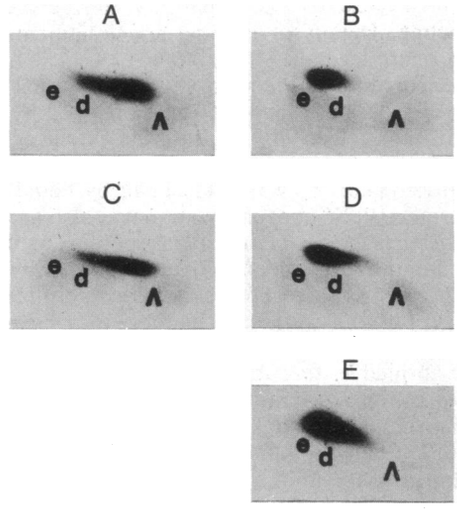

FIG. 4. Analysis of S6 phosphorylation by two-dimensional polyacrylamide gel electrophoresis (7, 32) of radiolabeled ribosomal proteins from confluent CEF and ts-CEF that were serum-starved for 24 hr, labeled with 2 mCi of $H_3{}^{32}PO_4$ for 2 hr in phosphate-free medium, and treated as described below. Ribosomes from unfertilized *X. laevis* eggs and ovarian tissue were used as carrier and marker for S6. d and e represent the positions of S6 phosphorylated with 4 and 5 mol of phosphate/mol of S6, respectively. Λ indicates the position of unphosphorylated S6. (*A*) Untreated CEF. (*B*) CEF treated with 5% serum. (*C*) CEF treated with phorbol at 100 ng/ml. (*D*) CEF treated with PMA at 100 ng/ml. (*E*) ts-CEF incubated at the permissive temperature for 2 hr. Incubations with phorbol and PMA were in medium containing 0.1% Me_2SO. Gels in *A* and *C* required 4–10 times longer exposures than those in *B*, *D*, and *E* to produce autoradiograms of approximately equal intensity.

To determine if identical sites were phosphorylated, S6 phosphopeptides were analyzed by (*i*) limited proteolysis with *S. aureus* V8 protease and separation by $NaDodSO_4$/PAGE (33) or by (*ii*) complete digestion with chymotrypsin and separation by two-dimensional thin-layer electrophoresis. Phosphopeptide patterns generated by limited proteolysis of ^{32}P-labeled S6 from PMA-, serum- and RSV-stimulated cells were identical to each other and to those previously observed for many serum-starved virally-transformed cells (data not shown; ref. 17). Analysis of chymotryptic S6-phosphopeptides (Fig. 5) showed that identical chymotryptic S6-phosphopeptides are phosphorylated in serum-stimulated CEF (Fig. 5*A*), PMA-stimulated CEF (Fig. 5*B*), and ts-CEF cultured at the permissive temperature for 2 hr (Fig. 5*C*). Fig. 5*D* shows the pattern of a mixture of S6 phosphopeptides from all three sources. In addition, high pressure liquid chromatography yielded identical elution profiles for the samples described above (data not shown). These experiments suggest that the same sites are phosphorylated in S6 from CEF stimulated by PMA, serum, or RSV transformation. However, quantitative differences in the degree of phosphorylation of some peptides were observed.

To further support the concept that the same sites are phosphorylated in S6 upon stimulation of CEF with PMA, serum, or transformation, the effects of a combination of the stimulatory factors were investigated. These studies again suggested that the same amino acids are phosphorylated in CEF under all three stimuli: any combination of two factors increased S6 phosphorylation only slightly over PMA-, serum-, or $pp60^{v\text{-}src}$-induced S6 phosphorylation alone. These small increases were not deemed significant when compared to the large increase observed with each stimulating factor alone.

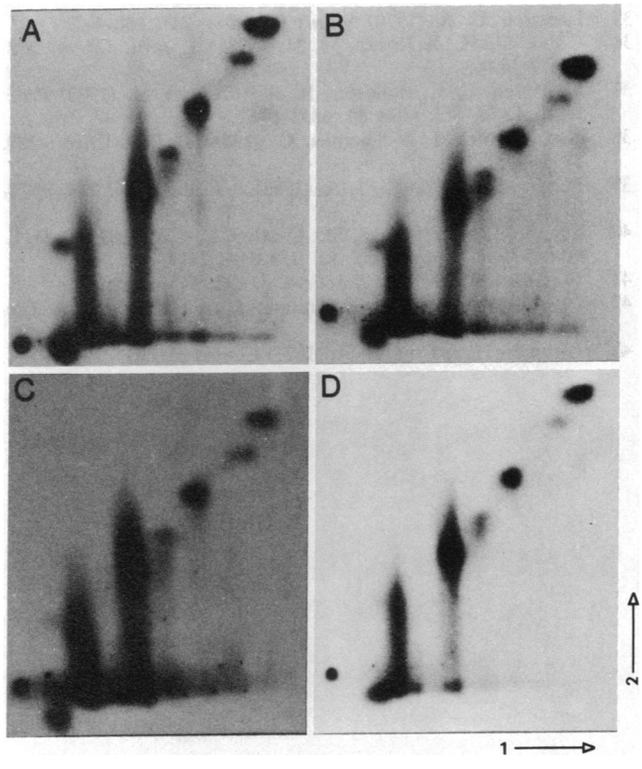

FIG. 5. Comparison of S6 chymotryptic phosphopeptides by two-dimensional thin-layer electrophoresis. Radiolabeled S6 was excised and eluted from $NaDodSO_4$/polyacrylamide gels, digested with chymotrypsin, and the chymotryptic phosphopeptides were analyzed. The directions of electrophoresis are indicated by arrows. Shown are autoradiograms of S6 chymotryptic phosphopeptides from 5% serum-stimulated CEF (*A*), CEF stimulated with PMA (100 ng/ml) (*B*), serum-starved ts-CEF (infected with the temperature-sensitive RSV mutant 72-4) incubated at the permissive temperature for 2 hr (*C*), and a mixture containing equal amounts (based on radioactivity) of the three samples (*D*).

DISCUSSION

In this study we report that tumor promoters, serum growth factors, or expression of the RSV protein $pp60^{v\text{-}src}$ stimulate the phosphorylation of 40S ribosomal protein S6 in a similar manner. Chymotryptic phosphopeptide and two-dimensional polyacrylamide gel analyses showed that the same sites are nearly maximally phosphorylated under the influence of each of the agents. Thus, the same serine-specific enzymes are regulated by these diverse stimuli or, alternatively, if more than one pathway is involved, they yield the same result.

Several kinases with the capacity of phosphorylate S6 *in vitro* have recently been described (36–39). The ability of PMA to stimulate S6 phosphorylation, coupled with the recent observation that $pp60^{v\text{-}src}$ phosphorylates phosphatidylinositol to phosphatidylinositol 4,5-bis(phosphate) (40), which can then be metabolized to diacylglycerol and inositol 1,4,5-tris(phosphate) (41), suggest a potential involvement of protein kinase C. In fact, it has recently been reported that protein kinase C can phosphorylate S6 *in vitro* (37). However, protein kinase C phosphorylates only 2 of 11 S6 tryptic phosphopeptides *in vitro* (37) whereas we observed similar chymotryptic S6 phosphopeptides from PMA- and serum-stimulated CEF and from RSV-transformed CEF. Furthermore, we are unable to consistently demonstrate significant diacylglycerol/phosphatidylserine stimulation of S6 phosphorylation with a highly purified preparation of protein kinase C (unpublished observations). Another protein kinase has been shown to phosphorylate S6 *in vitro* at sites in common with those obtained by biosynthetic labeling of insulin-stimulated 3T3-L1 cells (39). Interestingly, this kinase is activated by limited digestion with certain proteases (39) or by phospholipids and diacylglycerol in the absence of Ca^{2+} (42). Thus PMA or the $pp60^{v\text{-}src}$-stimulated increase in diacylglycerol could also exert their effects through such a protein kinase thereby regulating S6 phosphorylation. More recently, a comparison of extracts from serum-stimulated and quiescent cells has shown a highly specific S6 protein kinase activity. This activity, which is 25-fold greater in extracts from serum-stimulated cells, phosphorylates 9 of 11 possible sites in S6. To obtain maximum enzymatic activity, phosphatase inhibitors are required in all extraction buffers. This suggests that this particular protein kinase may be modified by phosphorylation, which may be involved in regulating its activity (38).

The stimuli used in this study have in common several biochemical properties that may be linked to their ability to stimulate cell proliferation. The RSV *src* gene product, $pp60^{v\text{-}src}$, has tyrosine kinase activity (18, 19) as well as the ability to phosphorylate phosphatidylinositol (40), which can then be metabolized to diacylglycerol and inositol tris(phosphate), apparent second messengers that may initiate a signal cascade (41). The possibility that $pp60^{v\text{-}src}$ functions in this manner *in vivo* is supported by the observation that phosphatidylinositol turnover is stimulated in RSV-transformed cells (43). Several growth-factor receptors also have associated tyrosine kinase activity (23) and interaction with the polypeptide growth factors not only activates the kinase activity but also alters phosphoinositide metabolism (41). Finally, the addition of PMA or diacylglycerol to normal cells alters the phosphorylation at tyrosine of a 42-kDa protein, as does the addition of certain growth factors or transformation with RSV (24–26). Furthermore, because of the structural similarity between diacylglycerol and PMA (27) the tumor

promoter may replace the putative second messenger in activating kinases involved in growth regulation. In view of these findings, it is clear that identification of the factors responsible for increased S6 phosphorylation may answer several key questions concerning growth control in both normal and cancerous cells.

We thank Eleanor Erikson and James Maller for help with the two-dimensional gels and David Alcorta, Robin Davies, and Marilyn Resh for critically reading this manuscript. This research was supported by U.S. Public Health Service Grant CA 34943 from the National Institutes of Health and by a grant from the American Business Cancer Research Foundation. J.B. was supported by a Damon Runyon–Walter Winchell Postdoctoral Fellowship, J.G.S. is a National Research Service Award Postdoctoral Fellow (CA07168-01), and R.L.E. is an American Cancer Society Professor of Cellular and Developmental Biology.

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