Anal. Biochem. 76, 442.
Karlin, A., McNamee, M. G., Weill, C. L., & Valderrama, R. (1976b) in *Methods in Receptor Research* (Blecher, M., Ed.) pp 1-32, Marcel Dekker, New York, N.Y.
Karlin, A., Damle, V., Valderrama, R., Hamilton, S., Wise, D., & McLaughlin, M. (1978) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 37,* 529.
Karlsson, E., Arnberg, H., & Eaker, D. (1971) *Eur. J. Biochem. 21,* 1.
Klett, R. P., Fulpius, B. W., Cooper, D., Smith, M., Reich, E., & Possani, L. D. (1973) *J. Biol. Chem. 248,* 6841.
Maelicke, A., Fulpius, B. W., Klett, R. P., & Reich, E. (1977) *J. Biol. Chem. 252,* 4811.
McNamee, M. G., Weill, C. L., & Karlin, A. (1975) *Ann. N.Y. Acad. Sci. 264,* 175.
Ong, D. E., & Brady, R. N. (1974) *Biochemistry 13,* 2822.
Prives, J. M., Reiter, M. J., Cowburn, D. A., & Karlin, A. (1972) *Mol. Pharmacol. 8,* 786.
Raftery, M. A., Vandlen, R. L., Reed, K. L., & Lee, T. (1975) *Cold Spring Harbor Symp. Quant. Biol. 40,* 193.
Reiter, M. J., Cowburn, D. A., Prives, J. M., & Karlin, A. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69,* 1168.
Reynolds, J., & Karlin, A. (1978) *Biochemistry 17* (preceding paper in this issue).
Rice, R. H., & Means, G. e. (1971) *J. Biol. Chem. 246,* 831.
Weber, M., & Changeux, J.-P. (1974) *Mol. Pharmacol. 10,* 1.
Weill, C. L., McNamee, M. G., & Karlin, A. (1974) *Biochem. Biophys. Res. Commun. 61,* 997.

Two-Step Purification of the Major Phosphorylated Protein in Reticulocyte 40S Ribosomal Subunits†

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ABSTRACT: In reticulocytes, a single ribosomal protein, S13, has been shown to be phosphorylated by the cAMP-regulated protein kinases. The 40S ribosomal subunits were phosphorylated in vitro with [γ-^{32}P]ATP to facilitate the identification of S13 during the two-step purification procedure. Total ribosomal protein from the 40S subunit was fractionated by phosphocellulose chromatography in urea, and S13 was purified to homogeneity by gel filtration on Sephadex G-100. The protein was identified by the radioactive phosphate, by molecular weight, and by the migration characteristics in a two-dimensional polyacrylamide gel electrophoresis system. Thin-layer electrophoresis of partial acid hydrolysates of S13 showed that more than one phosphorylated residue was present in the same oligopeptide, indicating at least some of the phosphoryl groups were clustered in the protein molecule.

Studies on the incorporation of radioactive inorganic phosphate into ribosomal proteins in mammalian cells have indicated the major site of phosphorylation was a single 40S ribosomal protein. This protein has been designated S6 in rat liver (Gressner & Wool, 1974a) and S13 in rabbit reticulocytes (Traugh & Porter, 1976), and has been shown to contain multiple phosphoryl groups (Gressner & Wool, 1974a; Traugh & Porter, 1976; Lastick et al., 1977; Treloar et al., 1977; Roberts & Ashby, 1978). In rat liver, phosphate incorporation into S6 was stimulated by glucagon (Blat & Loeb, 1971; Gressner & Wool, 1976a) dibutyryl cAMP (Gressner & Wool, 1976a), cyclohexamide, puromycin (Gressner & Wool, 1976b), ethionine (Treloar et al., 1977), and under conditions of regeneration (Gressner & Wool, 1974a). Stimulation has also been shown in rabbit reticulocytes (Cawthon et al., 1974), pituitary slices (Barden & Labrie, 1973), hamster islet cell tumor (Schubart et al., 1977), and rat cerebral cortex (Roberts & Ashby, 1978) with cAMP, and in baby hamster kidney fibroblasts (Leader et al., 1976), and HeLa cells (Lastick et al., 1977) by addition of serum.

The protein, S13, which was phosphorylated intracellularly by incubating intact reticulocytes in a nutritional medium with radioactive inorganic phosphate, was modified in vitro by cAMP-regulated protein kinases isolated from rabbit reticulocytes (Traugh & Porter, 1976). The incorporation of radioactive phosphate into S13 has been used as a means of rapidly identifying the protein during this two-step purification to homogeneity. The purification of S6 from rat liver has been described previously by Wool & co-workers (Collatz et al., 1976).

Experimental Procedures

Materials. Urea (Mallinckrodt) was purified and used fresh, or stored in aliquots and frozen. Sodium dodecyl sulfate and pyronine G were obtained from BDH Chemicals Ltd. The [γ-^{32}P]ATP was prepared as described previously (Hathaway et al., 1978).

Preparation of Ribosomal Subunits. Reticulocytes were prepared from New Zealand doe rabbits, and ribosomal subunits were purified by zonal centrifugation as described previously (Traugh & Traut, 1974) and stored in small aliquots at −70 °C.

Phosphorylation of 40S Ribosomal Subunits. The phosphorylation mixture contained in a reaction volume of 25 mL: 50 mM Tris-HCl, pH 7.5; 3 mM $MgCl_2$; 0.0014 mM cAMP; 0.14 mM [γ-^{32}P]ATP; 1.22 mg/mL of 40S subunits; protein kinase. Phosphorylation was started by addition of 1000 enzyme units of type I cAMP-regulated protein kinase per mg of 40S subunits and incubated at 30 °C for 100 min. The

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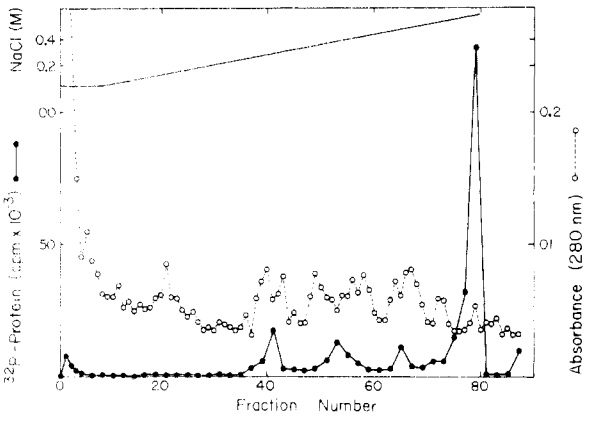

FIGURE 1: Total protein from 40S ribosomal subunits chromatographed on phosphocellulose. Chromatography was as described in Experimental Procedures. ^{32}P-labeled protein (●—●); absorbance at 280 nm (O- -O); NaCl concentration (—).

cAMP-regulated protein kinase was purified by chromatography on DEAE-cellulose and phosphocellulose as described elsewhere (Hathaway et al., 1978). In order to quantify the amount of phosphate incorporated into 40S subunits, aliquots of 0.02 mL were removed from the incubation mixture, and the protein was precipitated in 10% trichloroacetic acid, washed and counted as previously described (Traugh & Traut, 1974).

Preparation of Ribosomal Proteins. Phosphorylation of 40S ribosomal subunits was terminated by the addition of an equal volume of 6 M LiCl:8 M urea and kept at 4 °C overnight. The RNA was pelleted by centrifugation at 10 000 rpm for 20 min in a Sorvall SS-34 rotor and discarded. The supernatant was made to 20% trichloroacetic acid and placed in an ice bath for 45 min to precipitate the proteins. The protein precipitate was collected by centrifugation at 10 000 rpm for 20 min and washed twice with cold ethanol:diethyl ether (1:1), and once with diethyl ether. The pellet was dried and redissolved in 1 to 1.5 mL of 8 M urea (pH 5.0) and brought to 50 mM NaH_2PO_4, 12 mM methylamine hydrochloride.

Chromatography on Phosphocellulose. A stock of cellulose phosphate (Whatman P-11) was equilibrated with buffer I (6 M urea; 50 mM NaH_2PO_4, pH 6.5; 12 mM methylamine hydrochloride; 0.005% 2-mercaptoethanol), and a column (1 × 15 cm) was prepared. Chromatography was carried out according to a modification of the procedure of Hardy et al. (1969), with buffer I. The flow rate was 12 mL per h, and all operations were carried out in the cold. After application of the ribosomal protein sample, the column was washed with one column volume of buffer I, and a linear gradient ranging from 0 to 0.6 M NaCl in buffer I (total volume of 400 mL) was applied. Five-milliliter fractions were collected. Covalently bound radioactive phosphate was detected by monitoring the trichloroacetic acid precipitable counts in 0.05-mL samples of each fraction as described earlier. Absorbance at 280 nm was measured on undiluted fractions using a Varian Techtron UV-VIS spectrophotometer Model 635.

Sephadex G-100 Gel Filtration. Sephadex G-100 was prepared as described by Pharmacia and equilibrated with buffer I and a column (1.75 × 102 cm) was packed at a flow rate of 20 mL per h. Solutions of blue dextran 2000 (4 mg/mL), ovalbumin (10 mg per mL), and bromphenol blue (0.01%) were used for calibration of the column.

Phosphocellulose fractions containing S13 were pooled, and the proteins precipitated with 20% trichloroacetic acid. The precipitate was collected by centrifugation and the pellet was redissolved in a minimum volume (0.5 to 1.5 mL) of 8 M urea (pH 5.0). The sample was applied to the top of the column, and the proteins were eluted with buffer I and collected in 5-mL fractions.

Electrophoresis in Polyacrylamide Gels Containing Sodium Dodecyl Sulfate. Electrophoresis in 10% polyacrylamide gels containing sodium dodecyl sulfate was carried out according to the procedures of Weber & Osborn (1969). Samples (0.1 mL) of the column fractions were analyzed; electrophoresis was at 3 mA per gel for 12 to 13 h. Approximately 0.1 mg of 40S protein was run concurrently as a standard. After electrophoresis, the proteins were stained with Coomassie Brilliant Blue (Schwarz/Mann), destained, and stored in water. Cellophane-wrapped gels were autoradiographed on No-Screen Medical x-ray film.

Molecular weights of the fractionated 40S ribosomal proteins were determined with external and internal protein standards using the following proteins: catalase (57 500), ovalbumin (45 000), yeast alcohol dehydrogenase (37 000), lactoglobulin (17 150), lysozyme (14 400), trypsin (25 000), ribonuclease A (13 600), and ribonuclease S (11 400).

Two-Dimensional Polyacrylamide Gel Electrophoresis. The procedure for two-dimensional polyacrylamide gel electrophoresis has been previously described (Traugh & Porter, 1976). The fractions containing S13 or total protein were concentrated by precipitation with 20% trichloroacetic acid as indicated earlier and redissolved in 0.025 mL of 8 M urea with 1 mM 2-mercaptoethanol. Approximately 0.02 to 0.05 mg of purified S13 or 0.2 mg of total 40S protein was applied in a volume of 0.06 to 0.07 mL using pyronine G as a dye marker. Immediately prior to electrophoresis, the sample was adjusted to pH 8.4 with 1 M Tris-HCl. Electrophoresis in the first dimension was conducted at 2 mA per gel for 10 h, and in the second dimension for approximately 16 h at 80 V. Electrophoresis in both dimensions was carried out at room temperature, and the gels were stained and destained as described earlier.

Partial Acid Hydrolysis. Partial acid hydrolysis of purified phosphorylated S13 and phosvitin was carried out according to the procedures of Traugh & Traut (1972) and Williams & Sanger (1959). Phosvitin was phosphorylated with cAMP-independent protein kinase, using conditions described previously for casein (Traugh & Traut, 1974).

Results

The 40S ribosomal subunits were phosphorylated in vitro using a cAMP-regulated protein kinase partially purified from rabbit reticulocytes and [γ-^{32}P]ATP. It has been shown previously that S13 is the major ribosomal protein modified in vitro and upon incubation of intact reticulocytes in a nutritional medium with radioactive phosphate (Traugh & Porter, 1976). Under the conditions of the reaction, 1.6 mol of radioactive phosphate was incorporated per mol of 40S ribosomal subunits. It had previously been estimated that 1 to 2 phosphoryl groups were already bound per subunit in the intact cell (Traugh & Porter, 1976). Thus, the subunits contained an average of 3 mol of phosphate after phosphorylation in vitro.

Total ribosomal protein prepared from the phosphorylated 40S subunits was fractionated by phosphocellulose chromatography in urea. Figure 1 shows the elution profile from a typical phosphocellulose chromatography run. One major peak of radioactivity eluted near the end of the linear gradient, between 0.55 and 0.60 M NaCl. The individual fractions were analyzed by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate to determine the degree of purification and to identify the proteins by molecular weight. As shown in

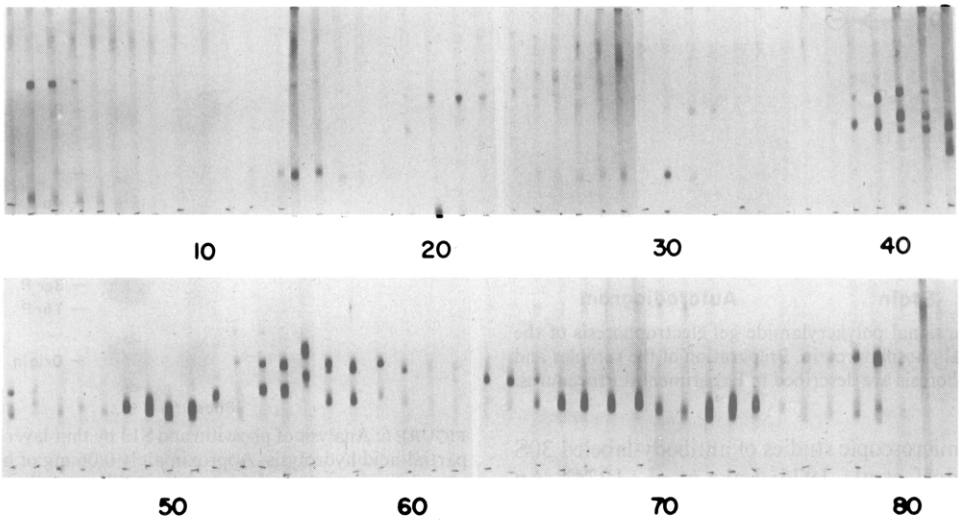

FIGURE 2: Electrophoretic analysis of the phosphocellulose column fractions in polyacrylamide gels containing sodium dodecyl sulfate. Preparation of the samples and electrophoretic conditions are described in Experimental Procedures. The numbers below each gel indicate the phosphocellulose column fraction (from Figure 1).

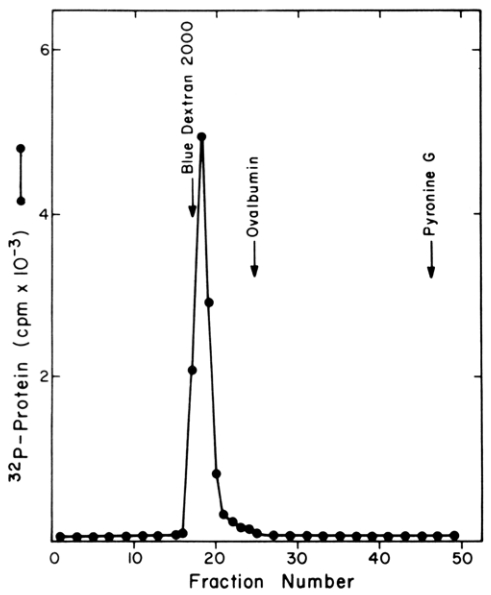

FIGURE 3: Elution profile of S13 on Sephadex G-100. Chromatography was as described in Experimental Procedures. Arrows indicate the position of markers used to calibrate the column. ^{32}P-labeled protein (●—●).

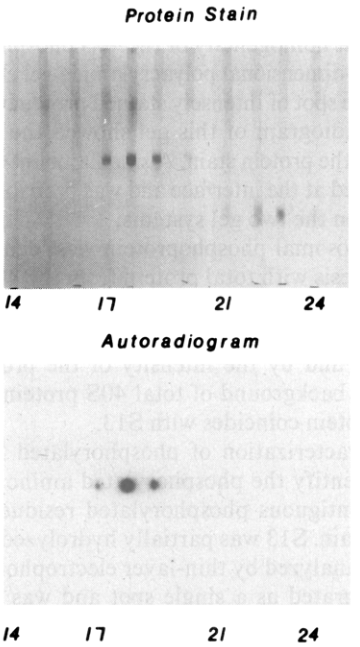

FIGURE 4: Analysis of proteins after Sephadex G-100 chromatography by polyacrylamide gel electrophoresis. Preparation of the samples and conditions of electrophoresis are described in Experimental Procedures. The numbers represent the fraction numbers (from Figure 3).

Figure 2, 28 protein bands were observed. The molecular weight range of these proteins agreed with the previously determined values ranging from 8000 to 39 000 (Howard et al., 1975).

When the protein profile corresponding to the major peak of radioactivity (fractions 76–80) was examined, three protein species were detected. The slowest migrating protein band, with an apparent molecular weight of 34 000 to 35 000, was shown to contain the covalently bound phosphate when analyzed by autoradiography. This value agrees well with the value of 35 000 obtained by Traugh & Porter for S13 (1976). The lower two bands of protein, neither of which were phosphorylated, migrated with apparent molecular weights of 19 000 and 16 000 and were tentatively identified as S29 and S30, respectively.

Since the molecular weight difference between the phosphorylated and nonphosphorylated proteins appeared to be of the order of 15 000 to 20 000, final purification of the radioactive phosphoprotein was accomplished by the use of Sephadex G-100 gel filtration in urea. A single symmetrical peak of radioactivity was observed to elute from Sephadex G-100 in the void volume, as shown in Figure 3. The fractions were analyzed by polyacrylamide gel electrophoresis to identify the position of elution for the three proteins. The radioactivity was shown to coincide with a protein with a molecular weight of 34 000 (Figure 4).

The three ribosomal proteins exhibited abnormal elution characteristics from those expected for globular proteins. One possible explanation is that these ribosomal proteins were ellipsoid in shape; thus, these molecules would appear to occupy a larger volume and to possess a larger molecular weight on gel filtration. Support for this argument comes from studies with purified ribosomal proteins from *E. coli*. S4 was shown to have an elongated conformation in solution, as demonstrated by hydrodynamic methods and low angle x-ray scattering studies (Paradies & Franz, 1976), and in the ribosome, as

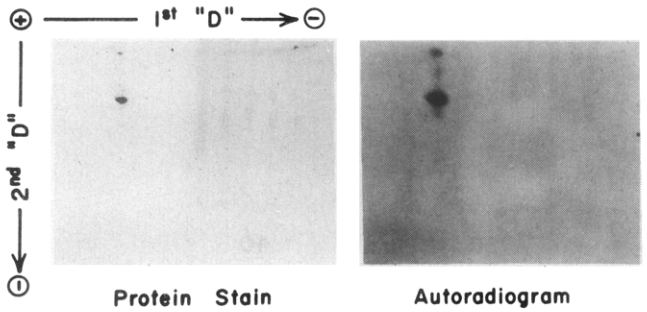

FIGURE 5: Two-dimensional polyacrylamide gel electrophoresis of the purified 40S ribosomal phosphoprotein. Preparation of the samples and conditions of electrophoresis are described in Experimental Procedures.

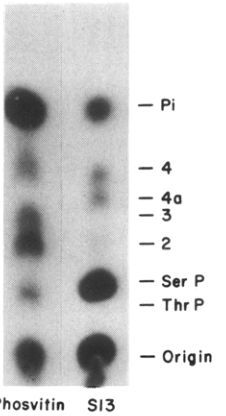

FIGURE 6: Analysis of phosvitin and S13 by thin-layer electrophoresis after partial-acid hydrolysis. Approximately 0.06 mg of homogeneous S13 or phosvitin was hydrolyzed using the Williams & Sanger technique as described in Experimental Procedures. The dried samples were applied to the chromatography plates with phosphoserine and phosphothreonine as standard markers. P_i, ^{32}P-labeled inorganic phosphate; SerP, phosphoserine; ThrP, phosphothreonine; 2, the postulated position of an oligopeptide with two phosphorylated residues; 3, three phosphorylated residues; 4 and 4a, oligopeptides with four phosphorylated residues. The autoradiogram is shown.

shown by electron microscopic studies of antibody-labeled 30S subunits (Tischendorf et al., 1975; Lake et al., 1974). An elongated conformation has also been suggested for a number of other 30S ribosomal proteins (Engelmann et al., 1975; Tischendorf et al., 1975; Lake et al., 1974; Lake & Kahan, 1975). An alternative explanation would be that aggregates of the individual proteins had formed under the conditions of purification.

The apparent homogeneity of the 40S phosphoprotein was verified by two-dimensional polyacrylamide gel electrophoresis (Figure 5). One spot of intensely stained protein was observed, and the autoradiogram of this gel showed the radioactivity coincided with the protein stain. A small amount of radioactive protein remained at the interface and was probably due to poor contact between the two gel systems.

The 40S ribosomal phosphoprotein was identified as S13 by electrophoresis with total protein from the 40S ribosomal subunit by two-dimensional polyacrylamide gel electrophoresis (data not shown). The purified protein was identified by autoradiography and by the intensity of the protein stain in relation to the background of total 40S proteins. The single radioactive protein coincides with S13.

Partial characterization of phosphorylated S13 was undertaken to identify the phosphorylated amino acids and to analyze for contiguous phosphorylated residues within the polypeptide chain. S13 was partially hydrolyzed, and the hydrolysate was analyzed by thin-layer electrophoresis. The radioactivity migrated as a single spot and was identified as phosphoserine (data not shown). To examine for the occurrence of runs of phosphoserine in S13, the milder hydrolysis conditions of Williams & Sanger (1959) were used. Phosvitin, a protein with multiple phosphorylation sites, was used as a standard. The covalently bound radioactivity in the partially hydrolyzed sample of phosvitin migrated as phosphoserine and as three distinct spots which migrated between phosphoserine and radioactive inorganic phosphate (Figure 6). These results correspond well with those of Williams & Sanger (1959), who reported the separation of phosphopeptides containing up to six phosphoserine residues. The fastest phosphopeptide (4), shown in Figure 6, apparently corresponds to a tetrapeptide (Williams & Sanger, 1959). Accordingly, a dipeptide (2) and a tripeptide (3) containing two and three residues of phosphoserine, respectively, were also detected. Thin-layer electrophoresis of phosphorylated S13 yielded phosphoserine and three phosphopeptides migrating between phosphoserine and radioactive inorganic phosphate. Although the amount of hydrolyzed protein was too low to permit a determination of the amino acid composition of these peptides, the increased mobility of these phosphopeptides, over that of phosphoserine, suggests the presence of greater than one phosphorylated residue per fragment. As a control, the free amino acid cysteine was exposed to 12 N HCl at 37 °C for 30 h and analyzed by thin-layer electrophoresis as indicated above to monitor the level of cysteic acid formation. Under these conditions cysteine is not oxidized to cysteic acid since no ninhydrin staining material was observed to migrate towards the anode. Thus, those peptides with mobilities greater than phosphoserine apparently correspond to increased levels of phosphorylation with apparently no contribution from the sulfonic acid group of cysteic acid.

Discussion

Recently a number of proteins from both the 40S and 60S ribosomal subunits from rat liver have been purified to homogeneity using different chromatographic techniques (Westermann et al., 1971; Collatz et al., 1976, 1977; Tsurugi et al., 1976, 1977). Purification of S6 from rat liver has been described previously (Collatz et al., 1976). Using a procedure adapted from Hardy et al. (1969) for ribosomal proteins from bacteria, a two-step purification of S13 from reticulocyte 40S ribosomal subunits we have described. This included chromatography on phosphocellulose at pH 6.5 followed by chromatography on Sephadex G-100. S13, phosphorylated by a cAMP-regulated protein kinase and [γ-^{32}P]ATP, was rapidly identified during purification by monitoring radioactivity. Identification of the protein was established by two-dimensional electrophoresis.

The role of the cAMP-regulated phosphorylation of S13 in the control of protein synthesis has not been identified, although increased phosphorylation has been observed in cell cultures following serum stimulation (Leader et al., 1976; Lastick et al., 1977), in rat liver under conditions of induced diabetes (Gressner & Wool, 1976b), and in rat liver (Blat & Loeb, 1971; Gressner & Wool, 1976a) and hamster islet cell tumors (Schubart et al., 1977) by glucagon. Recently a second component of the protein synthesizing complex, initiation factor 3 (eIF-3), has also been shown to be modified by the cAMP-regulated protein kinases (Traugh & Lundak, unpublished results). The ability to obtain homogeneous S13 opens up a number of experimental approaches to examining the role of cAMP in the control of protein synthesis.

Localization of the [^{32}P]phosphoryl groups within the

polypeptide chain of S13 was determined by partial acid hydrolysis. Three phosphopeptides with increased mobilities over that of phosphoserine were identified. This suggests the possible occurrence of contiguous phosphorylated residues within the polypeptide chain of S13. It has been assumed that the serine residues modified in vitro by the cAMP-regulated protein kinase were the same as those modified in whole cells; further experiments will be required to establish this fact.

A clustering of phosphate groups on S13 could enhance alterations in the binding of RNA, such as differential stabilization of Met-tRNA$_f$ and aminoacyl-tRNA, or selection of specific classes of mRNA. Additionally, phosphorylation could alter the conformation of the ribosome, or modify the association between species of RNA.

References

Barden, N., & Labrie, F. (1973) *Biochemistry 12,* 3096-3102.

Blat, C., & Loeb, J. E. (1971) *FEBS Lett. 18,* 124-126.

Cawthon, M. L., Bitte, L. F., Krystosek, A., & Kabat, D. (1974) *J. Biol. Chem. 249,* 275-278.

Collatz, E., Wool, I. G., Lin, A., & Stoffler, G. (1976) *J. Biol. Chem. 251,* 4666-4672.

Collatz, E., Ulbrich, H., Tsurugi, K., Lightfoot, H. N., MacKinlay, W., Lin, A., & Wool, I. G. (1977) *J. Biol. Chem. 252,* 9071-9080.

Engelmann, D. M., Moore, P. B., & Schoenborn, B. P. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72,* 3888-3892.

Glynn, I M., & Chappell, J. B. (1964) *Biochem. J. 90,* 147-149.

Gressner, A. M., & Wool, I. G. (1974a) *J. Biol. Chem. 249,* 6917-6925.

Gressner, A. M., & Wool, I. G. (1974b) *Biochem. Biophys. Res. Commun. 60,* 1482-1490.

Gressner, A. M., & Wool, I. G. (1976a) *J. Biol. Chem. 251,* 1500-1504.

Gressner, A. M., & Wool, I. G. (1976b) *Nature (London) 259,* 148-150.

Hardy, S. J. S., Kurland, C. G., Voynow, P., & Mora, G. (1969) *Biochemistry 8,* 2897-2905.

Hathaway, G. M., Lundak, T. S., Tahara, S. M., & Traugh, J. A. (1978) *Methods Enzymol.* (in press).

Howard, G. A., Traugh, J. A., Croser, E. A., & Traut, R. R. (1975) *J. Mol. Biol. 93,* 391-404.

Lake, J. A., & Kahan, L. (1975) *J. Mol. Biol. 99,* 631-644.

Lake, J. A., Pendergast, M., Kahan, L., & Nomura, M. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71,* 4688-4692.

Lastick, S. M., Nielsen, P. J., & McConkey, E. H. (1977) *Mol. Gen. Genet. 152,* 223-230.

Leader, D. P., Rankine, A. D., & Coia, A. A. (1976) *Biochem. Biophys. Res. Commun. 71,* 966-974.

Lin, A., & Wool, I. G. (1974) *Mol. Gen. Genet. 134,* 1-6.

Paradies, H. H., & Franz, A. (1976) *Eur. J. Biochem. 67,* 23-29.

Roberts, S., & Ashby, C. D. (1978) *J. Biol. Chem. 253,* 286-296.

Schubart, U. K., Shapiro, S., Fleischer, N., & Rosen, O. M. (1977) *J. Biol. Chem. 252,* 92-101.

Sherton, C. C., & Wool, I. G. (1972) *J. Biol. Chem. 247,* 4460-4467.

Terao, K., & Ogata, K. (1972) *Biochim. Biophys. Acta 285,* 473-482.

Tischendorf, G. W., Zeichardt, H., & Stöffler, G. (1975) *Mol. Gen. Genet. 134,* 187-208.

Traugh, J. A., & Porter, G. G. (1976) *Biochemistry 15,* 610-616.

Traugh, J. A., & Traut, R. R. (1972) *Biochemistry 11,* 2503-2509.

Traugh, J. A., & Traut, R. R. (1974) *J. Biol. Chem. 249,* 1207-1212.

Treloar, M. A., Treloar, M. E., & Kisilevsky, R. (1977) *J. Biol. Chem. 252,* 6217-6221.

Tsurugi, K., Collatz, E., Wool, I. G., & Lin, A. (1976) *J. Biol. Chem. 251,* 7940-7946.

Tsurugi, K., Collatz, K., Todokoro, K., & Wool, I. G. (1977) *J. Biol. Chem. 252,* 3961-3969.

Walsh, D. A., Perkins, J. P., Brostrom, C. O., Ho, E. S., & Krebs, E. G. (1971) *J. Biol. Chem. 246,* 1968-1976.

Weber, K., & Osborn, M. (1969) *J. Biol. Chem. 244,* 4406-4412.

Westermann, P., & Bielka, H. (1974) *Acta Biol. Med. Ger. 33,* 531-537.

Westermann, P., Bielka, H., & Bottger, M. (1971) *Mol. Gen. Genet. 11,* 224-234.

Williams, J., & Sanger, F. (1959) *Biochim. Biophys. Acta 33,* 294-296.