Cyclic AMP-binding Proteins and Protein Kinase during Regression of Walker 256 Mammary Carcinoma

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Cytoplasmic and nuclear cAMP-binding and protein kinase activities in N6O'-dibutyryl cyclic AMP (Bt,cAMP)-responsive and -unresponsive Walker 256 mammary carcinomas (W256) have been studied during Bt,cAMP treatment in vitro. Total nuclear CAMP binding in the responsive tumor is increased 3-fold within 1 day after Bt,cAMP treatment. This increase in nuclear binding is accompanied by a 50% decrease in total cytoplasmic CAMP binding. The same treatment produces no change in the binding by unresponsive tumor cytosol or nuclei. The predominant species of CAMP-binding proteins which is increased in the nuclei of the responsive tumor has a sedimentation constant of 3.6 S. This peak also shows protein kinase activity which is not stimulated by cAMP but is inhibited by the inhibitor protein of cAMP-dependent protein kinase. The protein substrate specificity of this increased protein kinase is similar to that of cytosol cAMP-dependent protein kinase and distinct from that of kinase present in control nuclei. Scatchard analysis of CAMP-binding data shows that the increased binding proteins in Bt,cAMP-stimulated nuclei exhibit two types of binding: one is identical with those present in the unstimulated nuclei, and the other is similar to one of the two types of binding found in the cytosol.

Cytosol from the responsive tumor exhibits three major peaks of CAMP-binding activity, sedimenting at 4.3 S, 5.8 S, and 6.9 S, respectively. The cytosol also contains CAMP-independent and -dependent forms of protein kinase, sedimenting at 3.3 S, 5.6 S, and 6.9 S, respectively. In the unresponsive tumor cytosol, the binding component of 4.3 S and the protein kinase fraction of 3.3 S decreased while the higher molecular weight species of these proteins (>7 S) increased. Dibutyryl cAMP treatment results in a shifting of the heavier binding and kinase components to their respective lighter components in the responsive tumor but not in the unresponsive tumor. The major CAMP-binding components of the responsive tumor cytosol show electrophoretic mobilities distinctive from those in the unresponsive tumor cytosol.

These data suggest that the nuclear accumulation of CAMP-binding proteins and protein kinase may play an important role in the cAMP-mediated control of growth and that this event may be related to the characteristics of the binding proteins and the kinase present in the cytosol.

The extensive analysis of CAMP binding has shown that one type of Walker 256 mammary carcinoma (W256) which regresses after Bt,cAMP treatment (Bt,cAMP-responsive) contains CAMP-binding proteins with different physicochemical properties from those of other W256 which grows despite Bt,cAMP treatment (Bt,cAMP-unresponsive). Thus, a correlation exists between the qualitative differences in CAMP-binding proteins, the regulatory subunit of cAMP-dependent protein kinase (1), and the difference in Bt,cAMP responsiveness in vitro. If the alteration of cAMP-binding proteins would account for "unresponsiveness" to Bt,cAMP treatment, then a comparison of response of the binding proteins between responsive and unresponsive W256 should help to explain the role of CAMP-binding proteins or protein kinase in growth control. These studies demonstrate the differences in the response of CAMP-binding proteins and protein kinase in responsive and unresponsive W256 to Bt,cAMP treatment in vitro.

EXPERIMENTAL PROCEDURES

Materials - Dibutyryl cAMP was obtained from Schwarz/Mann, Orangeburg, N.Y., α-casein, mixed histone (type II-A), histone-F1, bovine serum albumin, ovalbumin, and γ-globulin were from Sigma Chemicals Co., St. Louis, Mo.; other materials were the same as described in the preceding paper (1).

Cyclic AMP-binding Assay - The binding of cAMP to proteins was measured by a modification of the membrane filtration method of Gilman (2) at CAMP exchange conditions (3-5) as described in detail in the preceding paper (1): binding was measured at 23°C for 3 h, pH 6.5, with 10−6 M [3H]cAMP + 10−4 M nonradioactive cAMP.

Protein Kinase Assay - Protein kinase activity was determined by measuring 32P incorporation from γ-labeled ATP into histone as described in the preceding paper (1).

Preparation of Tumor Cytosol and Nuclear Extracts - All procedures were performed at 0-4°C. Tumors removed from animals were homogenized immediately in a Teflon-glass homogenizer with 3 volumes of 10 mM Tris/HCl buffer, pH 7.5. Supernatants (105,000 × g for 1 h) were used as cytosols. The resulting pellets were homoge-

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nized in 3 times the original volume of Buffer A (0.25 M sucrose, 2 mM MgCl₂, 1 mM CaCl₂, 10 mM KCl, 20 mM Tris/HCl, pH 7.5), containing 0.2% (v/v) Triton X-100, then passed through three layers of gauze, and centrifuged at 770 × g for 10 min. These pellets were resuspended and homogenized in the same volume of Buffer A, and centrifuged at 770 × g for 10 min. The nuclear pellets (consisting of morphologically intact nuclei with minimal cytoplasmic contamination as seen by phase-contrast microscopic examination) thus obtained were homogenized lightly in 10 mM Tris/HCl, pH 7.5 (0.5 mg of original tissue), added to equal volumes of 2.0 M KCl in 10 mM Tris/HCl, pH 7.5, and extracted at 0°C for 90 min. The suspensions were then centrifuged at 105,000 × g for 45 min and the clear supernatants used as nuclear extracts.

Sucrose Density Gradient Centrifugation—Sucrose density gradient studies were conducted by the method of Martin and Ames (6) using a Beckman SW 50.1 rotor at 45,000 rpm at 2°C for 17 h. Linear sucrose gradients from 5 to 20% sucrose in 10 mM Tris/HCl, pH 7.0, in the presence or absence of 1.0 mM MgCl₂ and 1 mM CaCl₂, were employed. Samples of cytosols and nuclear extracts containing 1.0 mg of protein were applied to the gradients in a volume of 0.1 to 0.2 ml. The gradient tubes were emptied by withdrawing the contents of the tube from the bottom with a long hypodermic needle connected to a peristaltic pump (Syringe Micro buret, model SB2, Micro-Metric Instrument Co., Cleveland, O.). γ-Globulin, s₂₀,w = 7.1 S, bovine serum albumin, s₂₀,w = 4.5 S, and ovalbumin, s₂₀,w = 3.6 S, were used as internal standards.

Polyacrylamide Gel Electrophoresis—Disc gel electrophoresis on 7% acrylamide gel was carried out essentially as described by Ornstein (7) and Davis (8) except that 50 mM Tris/HCl, 10% glycerol (pH 8.5) was used in place of Tris/glycine/HCl.

Preparation of Protein Kinase Inhibitor—The inhibitor was prepared essentially by Gilman's modification (2) of the method of Appleman et al. (9). Rat skeletal muscle was homogenized in 5 volumes of 10 mM Tris/HCl, pH 7.5, and boiled for 10 min. After removal of particulate material by centrifugation at 16,000 × g for 10 min, activity was precipitated with 1/10 volume of 50% trichloroacetic acid. The precipitate was collected by centrifugation at 16,000 × g for 10 min, dissolved in water, and the pH adjusted to 7.0 with 1 N NaOH. This fraction was dialyzed against distilled water at room temperature, and the precipitate which formed was discarded. The preparation was used at this stage of purity.

Other Methods—Protein concentration was determined by the method of Lowry et al. (10). Glucose-6-phosphate dehydrogenase was assayed according to the method of Glock and McLean (11). Maintenance of Bt,cAMP-responsive and -unresponsive W256 in Sprague-Dawley female rats (3 to 4 months old, 200 g average body weight) was the same as described in the preceding paper (1). Dibutyryl cAMP (10 mg/0.2 ml of 0.85% NaCl solution per rat subcutaneously) was injected daily as previously described (12).
the sedimentation patterns (Fig. 2, lower portion). In the presence of CAMP, the heaviest binding fraction (6.9 S) in the binding and kinase components (>7 S) which were not apparent in the responsive cytosol increased. Sucrose density gradient centrifugation of the cytosols was also performed in the presence of 10^{-6} M CAMP and in the absence of KCl was performed as described under "Experimental Procedures." Aliquots of each fraction assayed for CAMP-binding (•) and protein kinase activity (○) or absence (△) of 10^{-6} M CAMP as described under "Experimental Procedures." The values represent one of three similar experiments. γ-GLOB, γ-globulin; BSA, bovine serum albumin.

S decreased, while the higher molecular weight species of the binding and the kinase components (>7 S) which were not apparent in the responsive cytosol increased. Sucrose density gradient centrifugation of the cytosols was also performed in the presence of 10^{-6} M CAMP to examine the effect of CAMP on the sedimentation patterns (Fig. 2, lower portion). In the presence of CAMP, the heaviest binding fraction (6.9 S) in the responsive cytosol decreased and the lightest binding peak (4.3 S) increased. Simultaneously, the heavier fraction of CAMP-dependent protein kinase (6.9 S) decreased and the fraction of CAMP-independent kinase (3.3 S) increased. The shifting of the heavier binding and kinase fractions to their respective lighter fractions was not perceptible in the unresponsive cytosol that sedimented in the presence of CAMP. These data suggest that CAMP-dependent dissociation of the holoenzyme or the inactive form of protein kinase, CR, into an active, catalytic subunit, C, and a regulatory component, R (the inactive form of protein kinase, CR, into an active, catalytic subunit, C, and a regulatory component, R (the lightest fractions of cytoplasmic and nuclear extracts of Bt,cAMP-responsive and -unresponsive W256 is described under "Experimental Procedures." Aliquots of each fraction assayed for CAMP binding (•) and protein kinase activity either in the presence (○) or absence (△) of 10^{-6} M CAMP by the method described in the text. The values represent one of three similar experiments. γ-GLOB, γ-globulin; BSA, bovine serum albumin; OVAL, ovalbumin.

The effect of Bt,cAMP treatment on the sedimentation profiles of cytoplasmic and nuclear CAMP-binding proteins and protein kinase in the responsive and unresponsive W256 is shown in Fig. 3. Dibutyryl CAMP treatment resulted in a marked decrease in the major fractions of CAMP-binding and protein kinase activities in the cytoplasm of the responsive tumor (C). Moreover, the sedimentation profiles of the binding and kinase activities in the treated responsive cytosols (C) were different from those of the control cytosol (A), i.e. an increase in the lighter components of the binding protein and protein kinase and a decrease in the heavier fractions. These changes in the sedimentation profiles of the binding and kinase activities indicate a possible conversion in vivo of heavier binding and kinase components to their respective lighter components (15-21) due to the increased endogenous CAMP following Bt,cAMP treatment (see Fig. 2 for CAMP effect).

However, Bt,cAMP treatment did not affect the sedimentation profiles of CAMP-binding and protein kinase activities in the unresponsive tumor cytosol (compare E and G), despite the increased endogenous CAMP concentration in the tumor (12). The decrease in cytoplasmic binding and kinase activities in the treated responsive tumor was accompanied by an increase of these protein species into the nuclei. The nuclear extract from the treated responsive tumor (D) gave a sharp sedimentation profile composed mainly of a 3.6 S species containing both CAMP-binding and CAMP-independent protein kinase activities. This nuclear accumulation of CAMP-binding and kinase activities may be due to an experimental artifact, the following experiments were performed: the isolated nuclei (see "Experimental Procedures") from Bt,cAMP-treated responsive tumors were added to 10 volumes of cytosol from Bt,cAMP-treated responsive and unresponsive tumors, respectively; the mixtures were rehomogenized in 10^{-6} M CAMP, and the nuclei resolated, then the specific activities of CAMP-binding and protein kinase in the original nuclear extracts were compared to those in the resolated nuclear extracts. Similar activities were obtained for the
protein kinase components was not found in the treated unresponsive tumor (H), i.e. the sedimentation profiles of these protein species in the nuclear extract were the same as those in the untreated control tumor. The sedimentation profiles of cAMP binding and protein kinase of control nuclei for both responsive and unresponsive tumors showed small broad peaks (B and F). This result presumably reflects the several species of nuclear protein kinase that have been described (22-25).

The differences observed between Bt,cAMP-treated responsive and unresponsive tumor nuclei with respect to cAMP-binding and protein kinase activities may be related to the presence of an activator in the responsive tumor nuclei or, alternatively, an inhibitor in the unresponsive tumor nuclei. To investigate this possibility, cAMP binding and protein kinase were assayed in the mixtures of responsive and unresponsive nuclei. When mixed in a ratio of 1:1, the resulting nuclear fraction exhibited these cAMP-binding and protein kinase activities predicted from the activities of the individual fractions (Fig. 4). When mixed in various ratios, the nuclear fractions showed the same binding and kinase activities as expected from an additive effect. These data, therefore, provided no evidence of the presence of an inhibitor in the unresponsive nuclei nor of an activator in the responsive nuclei.

Comparison between Cytoplasmic and Nuclear cAMP-binding Proteins and Protein Kinase

Polyacrylamide Gel Electrophoresis of Cytoplasmic and Nuclear cAMP-binding Proteins – The electrophoretic resolution of cAMP-binding components in the cytosol of both responsive and unresponsive W256 is shown in Fig. 5. It shows three major cAMP binding components in the responsive cytosol and four major binding components in the unresponsive cytosol. Moreover, the two binding components separated in the unresponsive cytosol showed distinctly slower mobilities toward anode than those of the responsive cytosol. Thus cytoplasmic cAMP-binding components from responsive and unresponsive tumors are distinctive with respect to their electrophoretic mobilities.

The comparison between cytoplasmic and nuclear cAMP-binding components of Bt,cAMP-treated responsive W256 is shown in Fig. 6. The cAMP-binding components in Bt,cAMP-stimulated nuclei consist of a single major species which migrates toward the anode faster than those of the cytoplasmic species. These data together with the data of sucrose density gradient centrifugation suggest the physicochemical difference between cAMP-binding proteins of cytoplasm and those of Bt,cAMP-stimulated nuclei.

Scatchard Analysis of Cytoplasmic and Nuclear cAMP Binding in Control and Bt,cAMP-treated Responsive W256 – To compare the cAMP-binding components in the cytosol and nucleus in terms of binding affinity toward cAMP, the binding activity was measured as a function of cAMP concentration and analyzed by Scatchard plots (26). The total binding activity was measured at cAMP exchange conditions (1, 3-5) and the results are shown in Fig. 7. The cytosols from Bt,cAMP-treated and untreated tumor exhibited two major types of binding sites: a higher affinity binding (Kd ~ 2 x 10^-7 M) and a lower affinity binding (Kd ~ 1 x 10^-7 M). The amount of total binding in both cytosols was similar. The nuclear extract from untreated tumor exhibited a single class of binding with a high affinity (Kd ~ 3.5 x 10^-9 M), but the amount of total binding was about 2.5% of that in untreated tumor cytosol. Dibutyryl cAMP treatment resulted in the appearance of a new type of the binding (Kd ~ 2.5 x 10^-9 M) in the nuclei. The affinity of the newly appeared binding sites in the nuclei of treated tumor is similar to that of the higher affinity binding sites present in the cytosol. The binding data suggest indirectly the transfer of cytoplasmic binding components into the nucleus following Bt,cAMP treatment.
Substrate Specificity of Cytoplasmic and Nuclear Protein Kinase Activities in Control and Bt2cAMP-treated Responsive and Unresponsive W256—Histones and casein were tested as phosphate acceptors for cytoplasmic and nuclear protein kinase from control and treated responsive and unresponsive tumors (Table I). Cytoplasmic protein kinase from the control responsive tumor showed that histones were the preferred protein substrate when assayed in the presence of cAMP, but in the absence of cAMP, the enzyme preferentially utilized casein. Cyclic AMP stimulated the histone phosphorylation about 5-fold but did not stimulate the casein phosphorylation. The nuclear protein kinase of the control responsive tumor preferentially utilized casein both in the presence and absence of cAMP. Dibutyryl cAMP treatment resulted in a decrease of total cytoplasmic protein kinase in the responsive tumor and the kinase preferentially utilized histones either in the presence or absence of cAMP. The decrease of cAMP stimulation of histone phosphorylation in the treated responsive tumor cytosol may indicate the possible in vivo conversion of the inactive form of protein kinase to its active form (15–21) due to the increased endogenous cAMP following Bt2cAMP treatment. Dibutyryl cAMP treatment also resulted in a change in the protein substrate specificity of nuclear protein kinase in the responsive tumor, i.e. the histone kinase activity assayed in the presence or absence of cAMP increased 4-fold over the

![Fig. 6. Electrophoretic resolution of cytoplasmic and nuclear cAMP-binding components of Bt2cAMP-treated responsive W256.](image)

Pools of five tumors were immediately homogenized after removal from rats (1 day after Bt2cAMP treatment (12)) and cytosol and nuclear extract were prepared by the method described in the text. The preincubation of cytosol and nuclear extract with [3H]cAMP, the following electrophoresis, and determination of radioactivity in the gel slices were the same as those described in the legend to Fig. 5. The values are specific binding (see the legend to Fig. 1) and represent one of three similar experiments.

![Fig. 7. Scatchard plots for cAMP binding in cytosol and nuclei of Bt2cAMP-treated and untreated responsive W256.](image)

The enzyme assay was performed as described under “Experimental Procedures” with the exception that a wide range of cAMP concentration was used in the reaction to construct a Scatchard plot (26). The values are specific binding (see the legend to Fig. 1) and represent triplicate determinations.

### Table I

Protein substrate specificity of cytoplasmic and nuclear protein kinase activities in control and Bt2cAMP-treated responsive and unresponsive W256

| Tumor and substrate | Cytoplasmic | Nuclear |
|---------------------|-------------|---------|
|                     | Control +cAMP | Bt2cAMP-treated +cAMP | Control +cAMP | Bt2cAMP-treated +cAMP |
| α-Casein            | 0.39         | 0.36     | 0.09 | 0.19 | 0.65 | 0.60 | 0.18 | 0.25 |
| Histone mix-        | 0.12         | 0.60     | 0.24 | 0.38 | 0.11 | 0.19 | 0.40 | 0.58 |
| ture                | 0.18         | 1.00     | 0.32 | 0.74 | 0.12 | 0.22 | 0.50 | 0.60 |
| Histone F1          | 0.40         | 0.35     | 0.37 | 0.40 | 0.65 | 0.62 | 0.67 | 0.65 |
| Unresponsive        | 0.17         | 0.40     | 0.12 | 0.26 | 0.14 | 0.25 | 0.10 | 0.20 |
| α-Casein            | 0.15         | 0.45     | 0.12 | 0.40 | 0.20 | 0.30 | 0.15 | 0.30 |
| Histone mix-        |              |         |      |      |      |      |      |      |
| ture                |              |         |      |      |      |      |      |      |
| Histone F1          |              |         |      |      |      |      |      |      |

*nmol/min/mg protein*
control nuclear kinase activity, whereas the casein phosphorylation decreased to 50% of the control activity. Thus histone is the preferred protein substrate for both the nuclear kinase and the cytoplasmic kinase in the responsive tumor following BtCAMP treatment. Cytoplasmic and nuclear protein kinase in the control unresponsive tumor showed a similar substrate specificity as that of the control responsive tumor but cAMP stimulation of histone phosphorylation was appreciably lower than that in the responsive tumor. Furthermore, BtCAMP treatment produced no change in the protein substrate specificity of either cytoplasmic or nuclear protein kinase in the unresponsive tumor.

**Effect of Protein Kinase Inhibitor on Protein Kinase of Control and BtCAMP-treated Responsive W256 Nuclei—Dibutyryl cAMP treatment resulted in an increase of histone phosphorylation in the nuclei of the responsive tumor, but the phosphorylation was not stimulated by cAMP. Whether this increased protein kinase is the catalytic subunit derived from cAMP-dependent protein kinase was examined using a protein kinase inhibitor prepared (2,9) from rat skeletal muscle. Table II shows that protein kinase activity from the nuclei of BtCAMP-treated responsive W256 nuclei showed a similar substrate specificity as that of the control responsive tumor but cAMP stimulation of histone phosphorylation was appreciably lower than that in the responsive tumor. Furthermore, BtCAMP treatment produced no change in the protein substrate specificity of either cytoplasmic or nuclear protein kinase in the unresponsive tumor.

**DISCUSSION**

The present studies have shown that the growth response of W256 to cyclic nucleotide treatment is closely related to the response of cAMP binding and protein kinase of tumors. Little or no salt-extractable nuclear cAMP-binding and protein kinase activities can be detected in W256 nuclei before their exposure to exogenous BtCAMP. The binding protein and protein kinase appears to be present exclusively in the cytoplasm of untreated tumors. After the administration in vivo of BtCAMP a progressive increase occurs in the nuclear cAMP-binding and protein kinase activities with a concomitant decrease of these protein species in the cytoplasm. It appears, therefore, that cAMP promotes the nuclear accumulation of cAMP-binding protein and protein kinase, originally present in the cytoplasm, in much the same way as steroid hormones promote the nuclear accumulation of receptor proteins. This event of nuclear accumulation of cAMP binding and protein kinase with BtCAMP treatment occurs only in responsive W256 which regresses following treatment. Dibutyryl cAMP-unresponsive W256 which possesses a significant amount of cytoplasmic cAMP-binding and protein kinase activities fails to accumulate these protein species into the nuclei after in vivo administration of cyclic nucleotide. Although the biological relevance of the nuclear accumulation of cAMP binding and protein kinase can only be speculated at the present time, the correlation between the nuclear accumulation of these macromolecules and BtCAMP-responsive growth arrest is striking. The same correlation was also found with other BtCAMP-responsive rat tumors, i.e. dibutyryl cAMP-responsive R230 AC mammary tumor and 3123 hepatoma (27) and DMBA mammary tumor.

Nuclei-bound protein kinase in responsive W256 after BtCAMP treatment was found to be different from the enzymes in control (untreated) nuclei but similar to those in the cytosol of treated responsive tumor. The nuclear kinase found in the BtCAMP-treated responsive W256 showed a preference for histones as the protein substrate just as the cytoplasmic kinase, but the nuclear kinase of the control tumor (untreated) preferentially utilized casein as the substrate. The increased histone kinase of responsive tumor nuclei following BtCAMP treatment was not stimulated further by cAMP but was strongly inhibited by the protein inhibitor of protein kinase, suggesting that the kinase is probably the catalytic subunit derived from the cAMP-dependent protein kinase (9, 28-30). Scatchard analysis (26) of cAMP-binding data shows that the increased binding proteins in the nuclei of BtCAMP-treated responsive W256 exhibit two types of binding sites; one is identical with that present in the unstimulated nuclei and the other is similar to one of the two binding sites found in the cytosol. This further suggests that cAMP stimulation causes translocation of cytoplasmic cAMP-binding proteins into the nuclei. Sucrose density gradient centrifugation and electrophoresis on polyacrylamide gel, however, showed nonidentity between cytoplasmic cAMP-binding proteins, and protein kinase and these protein species in the stimulated nuclei. One possible explanation of this apparent physicochemical difference is that the binding proteins and the kinase in the cytoplasm were modified at some step during the nuclear translocation.

Translocation of cytoplasmic cAMP-binding protein and protein kinase into the nucleus has also been suggested in other tissues. Jungmann et al. (31, 32) showed that cAMP formed in a complex with a calf ovary cytosol protein (cAMP-binding protein) bind to ovary nuclei and chromatin and that concomitantly with the nuclear association of cAMP-binding protein, cAMP-independent protein kinase activity increases in the nuclei. Bhalla et al. (33) found that as a consequence of hormonal stimulation of uterine adenylate cyclase, a significant decrease of cytosol cAMP-binding and protein kinase activities occurs. This hormone-induced decrease of cytosol kinase was associated with a large increase in protein kinase activity in the nuclei 500 g pellet. Palmer et al. (34) and Castagna et al. (35) showed that glucagon or BtCAMP elicit a redistribution of the protein kinase catalytic subunit from the cytoplasm to the nuclear fraction in rat liver.

The failure of cAMP-binding proteins to accumulate into the nuclei in BtCAMP-unresponsive tumors correlated with their altered physicochemical properties of the binding proteins

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from those of responsive tumors. Cyclic AMP-binding proteins from unresponsive tumors showed different sedimentation properties and electrophoretic mobilities from those of responsive tumors. These altered properties of cAMP-binding proteins are reflected by both their lower apparent affinity toward CAMP and instability toward temperature and pH (1). These data imply that Bt+AMP-unresponsive tumors may possess CAMP-binding proteins which are structurally different from those in responsive tumors. A correlation between the structural difference in CAMP-binding proteins and the difference in the biological effect of cyclic nucleotides has also been implicated in other systems (36). Whether the failure of nuclear accumulation of CAMP-binding proteins and protein kinase of the unresponsive tumor could be, indeed, the result of a defect in the cytoplasmic binding proteins is presented in the following paper (37).

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Cyclic AMP-binding proteins and protein kinase during regression of Walker 256 mammary carcinoma.

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J. Biol. Chem. 1977, 252:6342-6348.
Additions and Corrections

Vol. 252 (1977) 1739-1744

Function of initiation factor 1 in the binding and release of initiation factor 2 from ribosomal initiation complexes in Escherichia coli.

Evan A. Stringer, Probir Sarkar, and Umadas Maitra

Page 1741. Legend to Fig. 2, line 3

No IF-1 was added in these experiments. The line should read:

Two initiation reaction mixtures, A and B (0.2 ml each) were prepared as described in legend to Fig. 1 except that 60 units of IF-2, 30 units of IF-3, and 2.4 A260 units of 30 S subunit were added to each reaction mixture.

Vol. 252 (1977) 3525-3532

Characterization of polypeptides associated with messenger RNA and its polyadenylate segment in Ehrlich ascites messenger ribonucleoprotein.

William R. Jeffery

Pages 3528 and 3529, Figs. 3 and 5

Photograph of gels shown in Fig. 3 is actually Fig. 5, while gels shown in Fig. 5 are actually those of Fig. 3.

Page 3530

In the last paragraph before "Discussion," references to Fig. 6, F and E are interchanged. Thus, line 11 from the end of the paragraph should read:

...Figure 6F, the salt wash proteins extracted from oligo(dT)-cellulose mixed with salt wash alone (Fig. 6E)...

Vol. 252 (1977) 6169-6176

Induction of different rat liver supernatant aldehyde dehydrogenases by phenobarbital and tetrachlorodibenzo-p-dioxin.

Richard A. Deitrich, Pequita Bludeau, Thomas Stock, and Michael Roper

Page 6169. Paragraph 3, line 14

Y enzyme should be T enzyme. The lines should read:

...the enzyme occurs. (d) The $\phi$ enzyme has a $K_m$ for acetaldehyde at pH 7.4 of about 0.22 mM, while the T enzyme has a $K_m$ of about 2.5 mM. (e) The molecular weight as determined by...

Vol. 252 (1977) 6342-6348

Cyclic AMP-binding proteins and protein kinase during regression of Walker 256 mammary carcinoma.

Yoon Sang Cho-Chung, Timothy Clair, and Rochelle Porper

Page 6345, Fig. 4

Due to a printer's error in the final photographing stage, part of Fig. 4 was covered by type that had slipped. The correct figure appears below.

![Corrected Figure 4](image-url)

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9081
Additions and Corrections

Vol. 252 (1977) 1739-1744

Function of initiation factor 1 in the binding and release of initiation factor 2 from ribosomal initiation complexes in Escherichia coli.

Evan A. Stringer, Probir Sarkar, and Umadas Maitra

Page 1741, Legend to Fig. 2, line 3

No IF-1 was added in these experiments. The line should read:

Two initiation reaction mixtures, A and B (0.2 ml each) were prepared as described in legend to Fig. 1 except that 60 units of IF-2, 30 units of IF-3, and 2.4 A[^50] units of 30 S subunit were added to each reaction mixture.

Vol. 252 (1977) 3525-3532

Characterization of polypeptides associated with messenger RNA and its polyadenylate segment in Ehrlich ascites messenger ribonucleoprotein.

William R. Jeffery

Pages 3528 and 3529, Figs. 3 and 5

Photograph of gels shown in Fig. 3 is actually Fig. 5, while gels shown in Fig. 5 are actually those of Fig. 3.

Page 3530

In the last paragraph before "Discussion," references to Fig. 5, E and F are interchanged. Thus, line 11 from the end of the paragraph should read

phoresis. As shown in Fig. 6 F, the salt wash proteins ex-

Line 6 from the end of the paragraph should read

from oligo(dT)-cellulose mixed with salt wash alone (Fig. 6E)

Vol. 252 (1977) 6169-6176

Induction of different rat liver supernatant aldehyde dehydrogenases by phenobarbital and tetrachlorodibenzo-p-dioxin.

Richard A. Deitrich, Pequita Bludeau, Thomas Stock, and Michael Roper

Page 6169, Paragraph 3, line 14

Y enzyme should be T enzyme. The lines should read:

the enzyme occurs. (d) The T enzyme has a K[^m] for acetalde-

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Page 6345, Fig. 4

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