Hormone-induced Protein Phosphorylation. III. Regulation of the Phosphorylation of the Secretagogue-responsive 29,000-dalton Protein by Both Ca\(^{2+}\) and cAMP in Vitro

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ABSTRACT In the preceding papers, we demonstrated that the endogenous phosphorylation of a 29,000-dalton protein is stimulated in response to secretagogue application to intact cells from the rat exocrine pancreas and parotid and dephosphorylated upon termination of secretagogue action. One- and two-dimensional gel analysis of \(^{32}\)P-labeled pancreatic and parotid lobules as well as their respective subcellular fractions revealed that the same protein was covalently modified in both tissues and was localized to the ribosomal fraction.

To identify the intracellular second messengers which may mediate or modulate the phosphorylation of the 29,000-dalton protein in intact cells, the effects of Ca\(^{2+}\), cAMP, and cGMP on the endogenous phosphorylation of this protein were assessed in subcellular fractions from the rat pancreas and parotid. Our results demonstrate that the phosphorylation of the 29,000-dalton polypeptide may be regulated by both Ca\(^{2+}\) and cAMP in the pancreas and in the parotid. No cGMP-dependent protein phosphorylation was found in either tissue. As in the \textit{in situ} phosphorylation studies, the Ca\(^{2+}\)- and cAMP-dependent phosphorylation of this same protein was localized to the ribosomal fraction. The cAMP-dependent protein kinase activity was found primarily in the postmicrosomal supernatant in contrast to the Ca\(^{2+}\)-dependent protein kinase that appeared to be tightly associated with the substrate in addition to being present in the postmicrosomal supernatant. The data suggest that, in cells from the exocrine pancreas and parotid, secretagogues may regulate the phosphorylation of the 29,000-dalton protein through Ca\(^{2+}\) and/or cAMP.

We have demonstrated that the endogenous phosphorylation of a ribosomal protein (Mr 29,000) is reversibly altered in a hormone-dependent fashion in intact pancreatic and parotid acinar cells (1, 2). Since the binding of secretagogues with their receptors in these cells results in the release of intracellular messengers such as Ca\(^{2+}\) and/or cyclic nucleotides, the effects of these putative second messengers on protein phosphorylation were assessed in homogenates from the parotid and pancreas in pancreatic subcellular fractions to gain a better understanding of how hormones regulate the phosphorylation of the Mr 29,000 protein. The present experiments demonstrate that the 29,000-dalton protein, which has been identified as ribosomal protein S6, can act as a major substrate for both Ca\(^{2+}\) - and cAMP-dependent protein kinases. A preliminary note on this research has been published (3).

MATERIALS AND METHODS

Materials

All chemicals used were of reagent grade and were obtained from the following sources: adenosine 3':5'-cyclic monophosphate, guanosine 3':5'-cyclic monophosphate, ATP, Tris, and maleic acid were obtained from Sigma Chemical Co. (St. Louis, MO); \(^{32}\)P-ATP (5-10 Ci/mmol, 1 Ci = 3.7 x 10\(^{10}\) Bq) was obtained from New England Nuclear (Boston, MA); all other reagents were obtained as described previously (1).

Methods

SAMPLE PREPARATION: Female Sprague-Dawley rats weighing 100-180 g and fed \textit{ad libitum} were killed by a blow to the head, and the pancreas or parotid was excised within 2 min and immersed in 0.3 M sucrose containing 0.1 mg/ml soybean trypsin inhibitor (STI) at 4\(^\circ\)C. All subsequent steps were carried out at 4\(^\circ\)C. The tissue was minced finely with scissors and homogenized in a final tissue homogenate with 0.1 M Hepes, pH 7.5 (1:5, w/v).

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kinases in the pancreatic homogenate is found in the total phosphorylated by both Ca\(^{2+}\)- and cAMP-dependent protein kinases. In Fig. 2, the 29,000-dalton polypeptide which is endogenously focused on in this study.

Initial experiments to determine the effects of Ca\(^{2+}\), cAMP, and cGMP on the endogenous phosphorylation of the 29,000-dalton polypeptide were performed using tissue homogenates. Fig. 1 shows that, in both the parotid and pancreas, the endogenous phosphorylation of the 29,000-dalton protein is dramatically stimulated by Ca\(^{2+}\) and to a lesser extent by cAMP. No cGMP-dependent protein phosphorylation was observed under these conditions at concentrations ranging from 1 to 100 \(\mu\)M and incubation times of 0.5, 1 and 10 min at either 37°C or 4°C.

As seen in Fig. 1, other substrates were also phosphorylated by Ca\(^{2+}\)-dependent protein kinases; however, the phosphorylation of these proteins was not consistently affected by secretagogues in situ (1). Consequently, proteins phosphorylated in broken cell preparations may not reflect the physiological situation in intact cells where spatial integrity is maintained and, for this reason, the 29,000-dalton phosphoprotein was focused on in this study.

Since subcellular fractions from the pancreas have been well characterized, this tissue was used for all subsequent studies. In Fig. 2, the 29,000-dalton polypeptide which is endogenously phosphorylated by both Ca\(^{2+}\) and cAMP-dependent protein kinases in the pancreatic homogenate is found in the total microsomal fraction. The phosphorylation of this protein was not observed to any significant extent in either the nuclear, zymogen granule, mitochondrial, or soluble fractions despite their contamination to various degrees with rough microsomes (data not shown). Upon separation of total microsomes into smooth and rough microsomal fractions by discontinuous sucrose gradient centrifugation, the 29,000-dalton phosphoprotein copurified with rough microsomes (RER). Although not shown, smooth microsomes exhibited no endogenous phosphorylation of any substrates by Ca\(^{2+}\) or cAMP-dependent protein kinases either in the presence or in the absence of the soluble fraction.

When comparing the total microsomal fraction to the rough microsomal fraction (Fig. 2), it can be seen that in the latter the phosphorylation of the 29,000-dalton polypeptide in the control condition was enhanced, which would be expected to occur as the substrate is enriched. However, the cAMP effects were minimal. Since it is possible that the cAMP-dependent protein kinase was present in less than optimal quantities in the starting total microsomes and partitioned into soluble supernatant(s) during preparation of rough microsomes, the soluble fraction was added to the rough microsomal fraction. This caused a dramatic increase in the cAMP-dependent phosphorylation of the 29,000-dalton protein as well as of several other phosphoproteins while having no appreciable effects on the Ca\(^{2+}\)-dependent phosphorylation of the 29,000-dalton polypeptide. The other proteins in the soluble fraction whose phosphorylation is dependent on Ca\(^{2+}\) or cAMP will not be discussed in this paper as their phosphorylation was not routinely observed in secretagogue-stimulated intact cells. Accordingly, their patterns of phosphorylation should be “subtracted” when examining the \(^{32}\)P\(_{\text{r}}\)-labeled protein pattern in the rough
FIGURE 2 Autoradiographs of SDS polyacrylamide gels showing the effects of water (−), Ca²⁺ (Ca), and cAMP (cA) on the endogenous phosphorylation of proteins from pancreatic homogenates, a total microsomal fraction, a rough microsomal fraction (RER), RER plus soluble fraction, and the soluble fraction alone.

FIGURE 3 Dose-response curve demonstrating the effects of the free Ca²⁺ concentration on the stimulation of phosphorylation of the 29,000-dalton polypeptide in pancreatic microsomes. For quantification, the radioactive band was cut out of the gel, put into Aquasol, and counted for 5 min in a Beckman LS-150 scintillation counter. Results are expressed as percent of [γ-³²P]ATP incorporation into the 29,000-dalton protein where 100% equals the number of cpm incorporated into this protein in the absence of Ca²⁺ (~85 cpm) and are representative of three experiments. 5 mM Ca²⁺, which is used throughout the study, represents a Ca²⁺ activity of ~200 μM.

maximal phosphorylation of this protein was determined to be ~12 μM which is comparable to calculated intracellular levels of Ca²⁺ found during induction of discharge by secretagogues (5). The stimulation of phosphorylation of the 29,000-dalton protein was sensitive to a relatively narrow range of free Ca²⁺ concentrations; above 300 μM, the effects of Ca²⁺ became inhibitory.

Analysis of the extent of phosphorylation of the 29,000-dalton protein and of the levels of [γ-³²P]ATP as a function of time and of Ca²⁺ addition is shown in Table I. The Ca²⁺-dependent phosphorylation of this polypeptide is rapid, reaching a maximum within 1 min and showing little change over time, with only a 17% decrease in labeling over control values being evident at 30 min. In contrast, [γ-³²P]ATP levels dropped markedly by 5 min at 37°C, with 5% of the original activity remaining at 30 min. Thus, in agreement with the data shown in the previous paper (2) using [³²P]-labeled intact cells, the phosphate incorporated into this protein by the Ca²⁺-dependent protein kinase is metabolically stable over the time period examined. Similar results were obtained for cAMP-stimulated phosphorylation in a postmitochondrial supernatant (data not shown).

The localization of the 29,000-dalton phosphoprotein using the in vitro phosphorylation system was further defined by treating a total microsomal suspension with 0.5% deoxycholate to dissolve away the membranes and centrifuging the ribosomes through a 2 M sucrose cushion to obtain a highly enriched ribosomal fraction. As seen in Fig. 4, it is clear that the ribosomal fraction (which exhibits no endogenous protein phosphorylation by itself) shows both Ca²⁺- and cAMP-dependent phosphorylation of a 29,000-dalton polypeptide in the presence of the soluble fraction. Thus, the soluble fraction can act as a donor for both the Ca²⁺- and cAMP-dependent protein kinases while this highly enriched ribosomal fraction contains principally one substrate which is endogenously phosphorylated. We do not know at present whether the lack of Ca²⁺-dependent phosphorylation in the ribosomal fraction in the microsomal fraction to which was added the soluble fraction. Further studies revealed that the rough microsomal fraction could be washed three times with the sucrose medium without noticeable change in the Ca²⁺-dependent protein phosphorylation, demonstrating that the substrate and Ca²⁺-dependent protein kinase are tightly bound.

This specific covalent modification of the M₉ 29,000 protein was further characterized using the microsomal fraction. The relationship between the free Ca²⁺ concentration and the incorporation of [³²P] into the 29,000-dalton polypeptide is shown in Fig. 3. The free Ca²⁺ concentration which induced half-
absence of soluble fraction is due to the loss of the Ca\textsuperscript{2+}-dependent protein kinase or absence of regulatory proteins such as calmodulin or other factors. The ribosomal nature of the phosphoprotein, however, is reinforced by the fact that hepatic polysomes purified according to Bloemendal et al. (6) show endogenous Ca\textsuperscript{2+}- and cAMP-dependent phosphorylation of a 29,000-dalton protein in the presence of pancreatic soluble fraction (data not shown).

It is important to note that, in contrast to the rough microsomal fraction which has Ca\textsuperscript{2+}-dependent protein kinase activity tightly associated with it, the ribosomal fraction uses the soluble fraction as the source of the kinase. Thus, the soluble fraction contains cAMP-as well as Ca\textsuperscript{2+}-dependent protein kinases, whereas only the latter is bound to the rough microsomal fraction. The Ca\textsuperscript{2+}-dependent protein kinase activity in the rough microsomal preparation is most likely at saturating levels since addition of the soluble fraction caused no further enhancement of the Ca\textsuperscript{2+}-dependent phosphorylation of the 29,000-dalton protein.

Since the 29,000-dalton polypeptide phosphorylated in the presence of Ca\textsuperscript{2+} exhibits a slightly slower mobility in one-dimensional SDS polyacrylamide gels than the band whose phosphorylation is stimulated by cAMP, the question arose as to whether in fact the same protein was being covalently modified. To resolve this issue and also to relate the identity of this phosphoprotein to the one described in our in situ studies with rat pancreatic lobules (1, 2), two-dimensional PAGE of pancreatic ribosomes phosphorylated in vitro was performed according to Lastick and McConkey (4). The 29,000-dalton protein phosphorylated in the presence of Ca\textsuperscript{2+} in the ribosomal fraction (Fig. 5A) possesses the same mobility as the protein whose phosphorylation was stimulated by cAMP (Fig. 5B). These data strongly suggest that Ca\textsuperscript{2+}- and cAMP-dependent protein kinases are both modifying the same protein. Examination of the Coomassie Blue patterns (data not shown) indicates that these phosphoproteins comigrate with ribosomal protein S6, in agreement with the results obtained in the preceding paper (2) using \textsuperscript{32}P-labeled intact cells. The other phosphorylated species seen in this figure are of lower molecular weight and are unrelated to ribosomal protein S6. Thus, the 29,000-dalton protein which exhibits enhanced phosphate incorporation in a hormone-dependent fashion in intact cells can be phosphorylated by both Ca\textsuperscript{2+}- and cAMP-dependent protein kinases in vitro, its identity being ribosomal protein S6, based on two-dimensional gel analyses.

In the preceding paper (2), the phosphopeptide pattern of the 29,000-dalton protein obtained from Staphylococcus aureus V8 protease (SAP) digestion was described for pancreatic lobules and for subcellular fractions derived therefrom. To gain further insight into the question of whether Ca\textsuperscript{2+}- and cAMP-dependent protein kinases are covalently modifying the same sites on the 29,000-dalton protein in vitro and to relate this species to the 29,000-dalton protein whose phosphorylation is affected by secretagogues in \textsuperscript{32}P-labeled pancreatic lobules, the SAP mapping technique was applied to the substrates phosphorylated in response to Ca\textsuperscript{2+} and cAMP as shown in Fig. 6. For comparative purposes on the same gel, the SAP map of the 29,000-dalton protein from the ribosomal fraction derived from \textsuperscript{32}P-labeled pancreatic lobules (rib) stimulated with cholecystokinin-octapeptide (CCK-8) is shown. From this substrate three peptides are generated, the 15,000-dalton fragment showing a 16-fold greater \textsuperscript{32}P incorporation than the other two peptides. Using \textsuperscript{[\gamma-\textsuperscript{32}P]}ATP as the phosphate donor, cAMP stimulates the endogenous phosphorylation of these same three peptides (Fig. 6, cA). The \textsuperscript{32}P, incorporated into the 5,000- and 10,000-dalton fragments is still in an equimolar ratio as is observed in the ribosomes from \textsuperscript{32}P-labeled cells; however, the radioactivity in the 15,000-dalton peptide is only three times that of the other two bands.

The phosphopeptide pattern produced from the Ca\textsuperscript{2+}-dependent phosphorylation of the 29,000-dalton protein presents both similarities and differences when compared to the peptide patterns from the cAMP-stimulated substrate (cA) and the in situ labeled ribosomal fraction (rib). Thus, while the 10,000- and 5,000-dalton fragments are phosphorylated and comigrate with the respective fragments from both the cAMP lane as well as with proteolytic fragments from the in situ phosphorylated ribosomal fraction (rib), the 15,000-dalton phosphopeptide migrates slightly slower than the comparable fragment in the other lanes. Whether this 15,000-dalton band represents the same peptide whose phosphorylation is stimulated by cAMP or is a different phosphopeptide cannot be ascertained at present. The slight shift in migration may be due to a difference

| Table 1 |
|---------------------------------|
| Quantification of \textsuperscript{[\gamma-\textsuperscript{32}P]}ATP Levels and \textsuperscript{32}P, Incorporation into the 29,000-dalton Protein in the Absence and Presence of Ca\textsuperscript{2+} as a Function of Time |
|---------------------------------|
| Time | \textsuperscript{[\gamma-\textsuperscript{32}P]}ATP | \textsuperscript{32}P, Incorporation into 29,000-dalton Protein |
|---------------------------------|
| 10 sec | 1 min | min | min |
| H\textsubscript{2}O | 4,161 | 3,924 | 2,347 | 633 | 217 |
| Ca\textsuperscript{2+} | 4,136 | 3,965 | 2,396 | 624 | 228 |
| 29,000-dalton protein | | | | |
| H\textsubscript{2}O | 141 | 162 | 171 | 152 | 126 |
| Ca\textsuperscript{2+} | 384 | 592 | 615 | 608 | 409 |

A total pancreatic microsomal fraction was phosphorylated with 17 \textmu M \textsuperscript{[\gamma-\textsuperscript{32}P]}ATP as described in Materials and Methods. For quantification, the 29,000-dalton phosphoprotein was cut out from the gel and subjected to scintillation spectrometry. \textsuperscript{[\gamma-\textsuperscript{32}P]}ATP levels were determined on samples of the same reaction (terminated with SDS) by thin-layer chromatography on PII-cellulose sheets using 1 M LiCl as the resolving solvent as described previously (1). After drying, the \textsuperscript{[\gamma-\textsuperscript{32}P]}ATP spot was localized by autoradiography using authentic \textsuperscript{[\gamma-\textsuperscript{32}P]}ATP as a standard and quantified by scintillation spectrometry. Values are expressed as cpm from one experiment and are quantitatively comparable to results obtained in two additional separate experiments.

FIGURE 4 Autoradiographs showing the localization of the 29,000-dalton protein to the pancreatic ribosomal fraction. Ribosomes plus soluble fraction, and the soluble fraction alone, were phosphorylated in the presence of water (—), Ca\textsuperscript{2+} (Ca), or cAMP (cA) as described previously.
FIGURE 5 Autoradiographs from two-dimensional polyacrylamide gels run according to (4) showing the phosphorylation pattern from ribosomes phosphorylated in the presence of (A) Ca$^{2+}$ or (B) cAMP. The arrows indicate the position of the 29,000-dalton protein. The water control is not shown since the 29,000-dalton phosphoprotein was not visible even after a 7-d exposure to x-ray film in contrast to these 1-d exposures shown in this figure.

FIGURE 6 Phosphopeptide pattern of the 29,000-dalton protein from the ribosomal plus soluble fraction phosphorylated in the presence of water (+), cAMP (cA), or Ca$^{2+}$ (Ca) for 1 min at 37°C. Rib, ribosomal fraction prepared from $^{32}$P-labeled pancreatic lobules stimulated in situ with CCK-8 (2). In all cases, the 29,000-dalton phosphoprotein was excised from one-dimensional SDS polyacrylamide gels and digested with 10 μg of SAP as described (2). Quantification of the $^{32}$P incorporated into each band after SAP digestion and electrophoresis was performed by cutting the spot out of the dried gel and subjecting it to liquid scintillation spectrometry.

in the number or sites of phosphorylation on the peptide since the intrinsic charge and shape of peptides probably are important factors determining their mobility in SDS gels. In addition, three other phosphopeptides are observed in the Ca$^{2+}$- and cAMP-dependent phosphorylated 29,000-dalton proteins (indicated by arrows in Fig. 5) excised from one-dimensional SDS polyacrylamide gels (data not shown). More extensive studies with different types of proteases followed by two-dimensional thin-layer chromatography will be required to reveal all phosphorylated sites on the molecule and their relative dependencies on Ca$^{2+}$- and cAMP-stimulated protein kinases. Comparison of such results from the 29,000-dalton protein phosphorylated in situ and in vitro should enable us to determine whether the differences in the $^{32}$P incorporated into the SAP-generated peptides shown in Fig. 6 are in fact a result of differential site phosphorylation.

DISCUSSION

In this study, we have examined the effects of the putative intracellular messengers Ca$^{2+}$, cAMP, and cGMP on the endogenous phosphorylation of the 29,000-dalton protein in subcellular fractions of the pancreas to gain a better understanding of how secretagogues may regulate the phosphorylation of this protein in intact cells (1, 2). The results indicate that the phosphorylation of the 29,000-dalton polypeptide in vitro can be induced by physiological concentrations of Ca$^{2+}$ as well as by cAMP. The endogenous Ca$^{2+}$-dependent protein kinase activity was associated both with the substrate in the rough microsomal fraction and with the soluble fraction while the cAMP-dependent protein kinase activity partitioned predominantly into the latter under the conditions used in our fractionation procedure. To determine whether there are multiple Ca$^{2+}$- and cAMP-dependent protein kinases in the pancreas will require further investigation. Unpublished observations indicate that calmodulin enhances the Ca$^{2+}$-dependent protein kinase activity on the phosphorylation of ribosomal protein S6, although these results are open to interpretation as phosphatidylserine appears, in our hands, to mimic the effects of calmodulin.

In agreement with the results shown in the preceding paper (2), where the in situ phosphorylation of the 29,000-dalton polypeptide was localized to a highly enriched ribosomal fraction from the pancreas and parotid, the data reported here strongly suggest that this same protein, which has been identified as ribosomal protein S6 by two-dimensional polyacryl-
amide gel analyses, is endogenously phosphorylated in vitro in a Ca\textsuperscript{2+} as well as cAMP-dependent manner. Taken together, these results support the hypothesis that in intact exocrine cells secretagogues may regulate the phosphorylation of this protein by modulating the levels of Ca\textsuperscript{2+} and/or cAMP.

In the pancreatic acinar cell, secretagogues appear to induce protein discharge through at least two different second messenger systems (7, 8). Carbachol, CCK-8 and bombesin elevate intracellular levels of Ca\textsuperscript{2+} and cGMP, although the latter response does not correlate with protein discharge from acini (9). On the other hand, secretin, vasoactive intestinal peptide, and cholecystokinin activate adenylate cyclase and cause a rise in cAMP levels in acinar cells, with no measurable effect on \textsuperscript{45}Ca\textsuperscript{2+} fluxes. This also appears to be the mechanism by which isoproteorol evokes discharge from the parotid acinar cell (10). We previously demonstrated (1) that secretin, CCK-8, and carbachol in the excocrine pancreas and isoproteorol and dibutyryl cAMP in the parotid all stimulate the endogenous phosphorylation of the same 29,000-dalton polypeptide in \textsuperscript{32}P-labeled lobules under conditions where protein discharge is elicited. The data presented in the present study suggest that carbachol and CCK-8 may induce the phosphorylation of this molecule in the pancreas by elevating intracellular levels of Ca\textsuperscript{2+} thereby activating a Ca\textsuperscript{2+}-dependent protein kinase. In contrast, secretin and isoproteorol presumably increase only the cAMP concentration within pancreatic and parotid acinar cells, respectively, leading to activation of a cAMP-dependent protein kinase which could then phosphorylate this same substrate, which is also consistent with our data. However, the possibility exists that both Ca\textsuperscript{2+} and cAMP may be mobilized in response to any secretagogue applied, but since this may occur in discrete cellular compartments, these effects may be difficult to observe by examining \textsuperscript{45}Ca\textsuperscript{2+} fluxes and total cAMP levels in intact cells. To assess this possibility, it will be of interest to compare proteolytic fragments from the 29,000-dalton protein phosphorylated in situ in response to these various secretagogues and in vitro by Ca\textsuperscript{2+} and cAMP-dependent protein kinases. In this way, it should be possible to determine the extent to which Ca\textsuperscript{2+} and cAMP stimulate the phosphorylation of the same or different sites on the same molecule (as has been demonstrated for Protein I from brain [11]). From this type of data, it should then be possible to deduce the nature of the intracellular messenger(s) which mediate the secretagogue’s effect. The fact that similar phosphopeptide patterns are generated by protein discharge of the 29,000-dalton protein phosphorylated in response to CCK-8 in vitro or derived from ribosomes phosphorylated in situ in response to CCK-8 (Fig. 6) already suggests that this cyclic nucleotide may indeed be mobilized by CCK-8 in contrast to current dogma (7).

The data from these three papers have shown that the phosphorylation of a protein with an apparent \( M_r \) of 29,000 is stimulated in a hormone-dependent fashion in intact cells, is mediated by both Ca\textsuperscript{2+} and cAMP in vitro, and is localized to the ribosome, its identity being ribosomal protein S6. In many cell types, the 4OS ribosomal protein designated S6 has been demonstrated to be phosphorylated by a variety of stimuli both in vivo and in vitro as reviewed in (12). This protein has been cross-linked to the synthetic mRNA poly(U) in ultraviolet-irradiated 40S ribosomal subunits (13) and becomes less accessible to reductive methylation when monomers are assembled into polysomes (14). These results have led to the proposal that S6 may comprise part of the mRNA binding domain of the ribosome, the extent of phosphorylation regulating the translational activity of ribosomes possibly by affecting the binding and/or selection of certain classes of mRNA (12, 15, 16). Although the phosphorylation of S6 has not been previously shown to be Ca\textsuperscript{2+}-dependent, it has been demonstrated to be affected by cAMP-dependent as well as cAMP-independent protein kinases (17, 18). Our results demonstrate for the first time that a ribosomal protein, in particular S6, can be covalently modified in a Ca\textsuperscript{2+}-specific manner. The fact that the 29,000-dalton protein possesses multiple sites of phosphorylation may provide a means of “fine tuning” the system since phosphorylation of the sites in different combinations could regulate the binding and/or selection of certain classes of mRNAs.

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REFERENCES

1. Freedman, S. D., and J. D. Jamieson. 1982. Hormone-induced protein phosphorylation. I. The relationship between secretagogue action and endogenous protein phosphorylation in intact cells from the exocrine pancreas and parotid. J. Cell Biol. 93:903-908.

2. Freedman, S. D., and J. D. Jamieson. 1982. Hormone-induced protein phosphorylation. II. Localization to the ribosomal fraction from rat exocrine pancreas and parotid of a 29,000-dalton protein phosphorylated in situ in response to secretagogues. J. Cell Biol. 95:909-917.

3. Freedman, S. D., and J. D. Jamieson. 1981. Ribosomal localization of a 29,000-dalton protein whose phosphorylation is stimulated in situ by secretagogues in the rat exocrine pancreas and parotid. J. Cell Biol. 91 (2, Pt. 2): 213a (Abstr.).

4. Lessick, S. M., and E. H. McConkey. 1976. Exchange and stability of HeLa ribosomal proteins in vivo. J. Biol. Chem. 251:2867-2875.

5. Kreiswenger, R. H. 1979. The informational role of calcium in the cytosol. Adv. Cyclic Nucleotide Res. 11:1-26.

6. Biesemendui, H. E. L. Bennett, and W. S. Bonn. 1974. Preparation and characterization of free and membrane-bound polysomes. Methods Enzymol. 30:333-337.

7. Gardner, J. D., and R. T. Jensen. 1980. Receptor for secretagogues on pancreatic acinar cells. Am. J. Physiol. 238:C63-C64.

8. Schultz, I., and H. H. Stolze. 1980. The exocrine pancreas: the role of secretagogues, cyclic nucleotides and calcium in enzyme secretion. Annu. Rev. Physiol. 42:127-156.

9. Kretsinger, R. H. 1979. The informational role of calcium in the cytosol. Adv. Cyclic Nucleotide Res. 11:1-26.

10. Freedman. S. D., and J. D. Jamieson. 1982. Hormone-induced protein phosphorylation. I. The relationship between secretagogue action and endogenous protein phosphorylation in intact cells from the exocrine pancreas and parotid. J. Cell Biol. 93:903-908.

11. Hutner, S. W., L. J. DeGennaro, and P. Greenberg. 1980. Differential phosphorylation of multiple sites in purified protein I by cyclic AMP-dependent and calcium-dependent protein kinases. J. Biol. Chem. 256:1482-1488.

12. Freedman, S. D., and J. D. Jamieson. 1982. Hormone-induced protein phosphorylation. I. The relationship between secretagogue action and endogenous protein phosphorylation in intact cells from the exocrine pancreas and parotid. J. Cell Biol. 93:903-908.

13. Terao, K., and K. Ogata. 1979. Proteins of small subunits of rat liver ribosomes that are phosphorylated in situ in response to secretagogues. J. Cell Biol. 91 (2, Pt. 2): 213a (Abstr.).

14. Gunther, G. S., and L D. Jamieson. 1979. Increased intracellular cyclic GMP does not correlate with protein discharge from pancreatic acinar cells. Nature (London). 280:318-320.

15. Kretsinger, R. H. 1979. The informational role of calcium in the cytosol. Adv. Cyclic Nucleotide Res. 11:1-26.