Structure-Function Analysis of Casein Kinase 2 with Synthetic Peptides and Anti-peptide Antibodies*

(Lorin A. Charlton, Jaspinder S. Sanghera, Ian Clark-Lewis$, and Steven L. Pelech$)

From the Biomedical Research Centre and the Departments of Biochemistry and Medicine, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada

Casein kinase 2 (CK2) is a ubiquitous, multifunctional protein-seryl/threonyl kinase that has been implicated in cellular regulation. Synthetic peptides were patterned after three highly conserved regions in CK2: the N terminus (CK2-NT); the lysine-rich, kinase subdomain III segment (CK2-III) (nomenclature of Hanks et al. (Hanks, S. K., Quinn, A. M., and Hunter, T. (1988) Science 241, 42–52)); and a 10-residue segment located near kinase subdomain X that is shared between CK2 and p34cdc2 (CK2/cdc2). The CK2-III and CK2/cdc2 peptides markedly stimulated the autophosphorylation of the α- and α'-subunits of purified CK2 from sea star oocytes, and they elicited up to 2-fold increases in its casein or phosvitin phosphotransferase activity. These peptides completely reversed nearly total inhibition of CK2 phosphotransferase activity toward itself, casein, and phosvitin by either heparin or poly(Glu,Tyr; 4:1), whereas CK2-NT was ineffective. Elution of CK2 from heparin-agarose with the CK2-III peptide indicated that this region of CK2 might mediate heparin binding to CK2. Affinity-purified rabbit polyclonal antibodies developed against both CK2-III and CK2/cdc2, but not CK2-NT, also produced up to 1.8-fold enhancements of the casein and phosvitin phosphotransferase activities of purified CK2. All three of the antipeptide antibody preparations immunoreacted with the α- and α'-subunits of CK2 on Western blots. These studies indicate that kinase subdomains III and X are involved in the modulation of CK2 phosphotransferase activity.

Casein kinase 2 (CK2)$ appears to be universally distributed

* This research was supported by an operating grant from the Medical Research Council of Canada (to S. L. P.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

$$Recipient of a Medical Research Council of Canada scholarship award.

$ To whom correspondence and reprint requests should be addressed: Biomedical Research Centre, 2222 Health Sciences Mall, University of British Columbia, Vancouver, BC V6T 1Z3, Canada.

The abbreviations used are: CK2, casein kinase 2; CK2-NT, synthetic peptide patterned after the first, N-terminal 22 residues of Drosophila CK2; CK2-III, synthetic peptide patterned after a 20-residue region located in catalytic subdomain III region of human CK2; CK2/cdc2, a synthetic peptide that includes common 10-residue segments found in both Drosophila CK2 and S. pombe p34cdc2; p34cdc2, 34-kDa protein kinase encoded by the S. pombe cell division control 2 gene and its functional homologues in other species; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; TBS, Tris-buffered saline; Mops, MOPS, 4-morpholinepropanesulfonic acid.

in eukaryotes and has been detected in the cytosol, nucleus, mitochondria, and membranes of their cells (for reviews, see Refs. 1–3). CK2 occurs typically as a tetrameric complex (\(M_r \approx 130,000\)) with an αββα or ααββ configuration. For example, CK2 purified from sea star oocytes features a 44-kDa α-subunit, a 40-kDa α'-subunit, and two 28-kDa β-subunits (4). cDNA sequence analysis of the α- and α'-subunits from diverse species has confirmed that they are catalytic subunits encoded by distinct, but highly homologous genes (5–9). The extreme conservation of the primary structures of the α- and α'-subunits of CK2, as well as its β-subunit (9–11) during evolution, together with its ubiquitous distribution, points to a fundamental role for this protein kinase in eukaryotic cells.

CK2 is likely a pleiotropic enzyme in vivo, since it can phosphorylate over 50 proteins in vitro (2). Studies with synthetic peptide substrates have indicated that either an aspartyl, glutamyl, phosphoseryl, or phosphotyrosyl residue at the third position from the C-terminal side of the target phosphorylatable residue is necessary and sufficient for recognition by casein kinase 2 (12–16). Recent findings have implicated CK2 in the control of such nuclear events as gene expression and oncogenic transformation. Among others, Fos (17), Myb (18), Myc (19), p53 tumor suppressor protein (20), adenovirus E1A protein (17), papillomavirus E7 protein (21, 22), and SV40 large T antigen (1, 23) are some of the putative targets of CK2. In this regard, it is significant that modest stimulations of CK2 activity have been reported in response to growth factor treatments of cells (24–28) or during cell proliferation (29). The mechanisms by which these stimulations of CK2 activity are achieved are obscure, but might reflect posttranslational modification of the enzyme by phosphorylation.

Although CK2 is cyclic nucleotide-independent, it might be subject to regulation by other low molecular weight effectors. Polyamines have been shown to stimulate CK2 activity (30, 31), whereas 2,3-bisphosphoglycerate, heparin, and other sulfated glycosaminoglycans are inhibitory (32–34). Basic polypeptides, such as polylysine and polyarginine, also have a stimulatory effect, whereas acidic polypeptides, such as poly(Glu,Tyr; 4:1) potently inhibit CK2 activity (35–38).

Bacterially expressed, recombinant α-subunits of casein kinase 2 from Caenorhabditis elegans (8), Drosophila (39), and human (40) have been shown to feature most of the characteristics of the tetrameric form, including catalytic activity with either ATP or GTP and an extreme sensitivity to inhibition by heparin (\(IC_{50} \approx 0.1–0.3 \mu M\)). However, co-expression of the β-subunit appears to be necessary for stimulation of the phosphotransferase activity of the α-subunit toward casein by greater than 10-fold, and it is required to mediate the stimulatory effects of polyamines and polylysine on CK2 activity.

Relatively little is known about the locations of the precise
binding sites in CK2 for acidic inhibitors and basic activators of this protein kinase. In this study, synthetic peptides patterned after conserved regions in the primary structure of CK2 were evaluated for their effects on the phosphotransferase activity of CK2 in vitro. Two of these synthetic peptides, as well as antipeptide antibodies developed against these sequences, were particularly effective for activation of CK2 and reversal of nearly complete inhibition of CK2 activity by heparin and poly(Glu,Tyr; 4:1).

**EXPERIMENTAL PROCEDURES**

Materials—Sea star CK2 was purified to near homogeneity from maturing oocytes as described (4). Synthetic peptides based on the N terminus (SAARVYTDVNAHKDEYWDYEN-GGC, CK2-NT) of Drosophila CK2α-subunit, the subdomain III region (LKPVKKKKIKREIKILENL-RGGC, CK2-III) of human CK2α-subunit, and the subdomain X region (DQLVRIAKLGDQFIRFRALTGGC, CK2/cdc2) of Drosophila CK2α-subunit and Schizosaccharomyces pombe p34<sup>α</sup> were synthesized by P. Owen (Biomedical Research Centre), coupled to keyhole-limpet hemocyanin, and used to elicit antipeptide antibodies in rabbits. The peptides were purified to >97% by reverse-phase high performance liquid chromatography, and their amino acid compositions were consistent with their expected primary structures. Casein (type III), heparin-agarose, nitro blue tetrazolium, bromochloroindolyl phosphate, heparin, spermine, poly(Glu,Tyr; 4:1), and phosvitin were purchased from Sigma. Phosphoacceptors, pre-stained marker proteins, and alkaline phosphatase-conjugated goat anti-rabbit IgG were from Bio-Rad. [γ-<sup>32</sup>P]ATP was from ICN.

*Casein Kinase 2 Activity and Protein Assays*—CK2 activity in purified preparation of the enzyme or cytosolic extracts from sea star oocytes was assayed for 10 min at 30 °C in a final volume of 25 μl with 5 mg/ml partially dephosphorylated casein or 5 mg/ml phosvitin, 5 mM MgCl<sub>2</sub>, 22 mM Mops, pH 7.2, and 50 μM [γ-<sup>32</sup>P]ATP (2000 cpmpmol). The reaction was terminated by spotting 20 μl onto a 1.5-cm<sup>2</sup> piece of Whatman P-81 phosphocellulose paper. After the P-81 papers were washed extensively with 1% (w/v) phosphoric acid, they were transferred into 6-ml plastic vials containing 0.5 ml of Ecolome (ICN) scintillation fluid, and the radioactivity was quantitated in a Wallac (Pharmacia LKB Biotechnology Inc.) scintillation counter.

Protein was estimated by the method of Bradford (41) using bovine serum albumin as a standard (A<sub>280nm</sub> = 6.5).

**Electrophoresis—**SDS-PAGE was performed on 1.5-mm-thick gels, with acrylamide at 11% (w/v) in the separating gel and 4% (w/v) in the stacking gel, using the buffer system described by Laemmli (42). Samples in 5 μl in the presence of SDS-PAGE sample buffer (125 mM Tris-HCl, pH 6.8, 4% SDS, 0.01% bromphenol blue, 10% mercaptoethanol, and 20% glycerol) and electrophoresed for 4 h at 10 mA. For autoradiography, gels were exposed to Kodak XAR-5 film at room temperature.

**Immunological Studies**—After SDS-polyacrylamide gel electrophoresis of purified CK2, the separating gel was soaked in transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol) for 5 min and sandwiched with a nitrocellulose membrane, and then the proteins were transferred for 3 h at 250 mA. Subsequently, the nitrocellulose membrane was blocked with TBS (Tris-buffered saline) containing 5% skim milk for 2 h at room temperature. The membrane was washed twice with TBS containing 0.05% Tween 20 (TTBS) for 5 min before incubation with CK2-specific anti-peptide antibodies (in 1% skim milk/TTBS; 1:500 dilution) overnight at room temperature. Next day, the membrane was washed twice with TTBS before incubation with the second antibody (goat anti-rabbit IgG coupled to alkaline phosphatase in 1% skim milk/TTBS; 1:3000 dilution) for 2 h at room temperature. The membrane was rinsed twice with TTBS followed by one wash with TBS before incubation with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium color development solution (mixture of 3% nitro blue tetrazolium in 1 ml of 70% dimethylformamide and 1.5% 5-bromo-4-chloro-3-indolyl phosphate in 1 ml of 100% dimethylformamide before adding to 100 ml of 0.1 M NaHCO<sub>3</sub>, 10 mM MgCl<sub>2</sub>, pH 9.8). The color reaction was terminated after 5-15 min by rinsing the membrane in a large volume of water.

**RESULTS AND DISCUSSION**

Regulation of CK2 Activity by Kinase Catalytic Domain-based Peptides—Hanks et al. (43) have identified approximately 25 residues, located in 8 of 11 subdomains, which are highly conserved within the ~250-residue catalytic domain of nearly all sequenced protein-seryl/threonyl kinases (Fig. 1). We rationalized that other short sequences that are highly conserved in CK2 from diverse species, but not preserved in other members of the protein kinase family, might confer specific regulation or define the substrate selectivity of CK2. We focused our attention on three such uniquely conserved regions in CK2. These were the N-terminal 22 residues of the kinase (CK2-NT), a lysyl-rich 20-residue segment that encompassed a highly conserved glutamyl residue in kinase subdomain III (CK2-III), and a 10-residue segment located at kinase subdomain X (CK2/cdc2) (Fig. 1). To examine the effects of these peptides on CK2, we employed an essentially homogeneous preparation of the enzyme obtained from maturing sea star oocytes. We recently established that the echinoderm CK2 displays many of the distinguishing characteristics of the mammalian forms of CK2 (4).

Incorporation of purified CK2 with each of the synthetic peptides produced stimulation of its phosphotransferase activity toward either casein or phosvitin (Fig. 2). In the instance of casein, at 1 mg/ml concentrations of the peptides, nearly all of the effective peptide was CK2-III>CK2/cdc2>CK2-NT (Fig. 2A). At 2 mg/ml, CK2-III increased the casein kinase activity by greater than 2-fold, whereas the stimulation by CK2-NT was ~1.2-fold. With phosvitin as the phosphoacceptor, at 1 mg/ml concentrations, all of the peptides were equally effective in activation of CK2, but each of the stimulations were modest (~1.4-fold) (Fig. 2B). For both

---

**Fig. 1.** Location and structure of the CK2 peptides in the α-subunit of CK2. The positions of highly conserved amino acid residues in the catalytic domain of protein kinases are indicated in subdomains labeled according to the numbering system of Hanks et al. (43). Note that for CK2 in most species where primary sequence information is available, the residues denoted with asterisks are instead S in subdomain I, V in subdomain II, W in subdomain VII, and G in subdomain VIII. The boldface sequences in the synthetic peptides shown below are derived from CK2.

**Fig. 2.** Effect of CK2 peptides on the phosphotransferase activity of purified sea star CK2. Purified sea star CK2 was assayed for phosphotransferase activity toward casein (panel A) and phosvitin (panel B) with peptide conc. (mg/ml) of the CK2-NT (C), CK2-III (O), and CK2/cdc2 (△) peptides. Values are the means ± S.E. of at least three determinations. Similar results were obtained in at least three independent experiments.
casein and phosphoarginine, the increases in CK2 activity by CK2/cdc2 was reversed when the concentration of the peptide was raised to ~2 mg/ml. None of the peptides were found to be phosphorylated by sea star CK2 in control experiments (data not shown).

Stimulation of CK2 activity by each of the three peptides was surprising, although the CK2-III peptide was highly charged with 9 out of 23 residues derived from either arginine or lysine. At a hundredfold lower concentration, polylysine and polyarginine have been shown to potently enhance CK2 activity toward casein, and they stimulated a-subunit autophosphorylation at the expense of the β-subunit phosphorylation at the expense of the β-subunit phosphorylation (38, 44). As presented in Fig. 3B, CK2-III also enhanced α- and α’-subunit autophosphorylation, but without compromise of the β-subunit phosphorylation. Similar results were obtained with CK2-NT (Fig. 3B) and spermine (Fig. 3A). By contrast, CK2/cdc2, which was a very much less basic peptide, produced strong increases in α- and α’-subunit autophosphorylation at 0.4-1 mg/ml but a reduction of β-subunit phosphorylation (Fig. 3B). At higher concentrations of CK2/cdc2, the high levels of α- and α’-subunit autophosphorylation were reversed. Poly(Glu,Tyr; 4:1) and heparin also inhibited autophosphorylation of all three subunits of CK2 (Fig. 3A). Thus, there was a strong correlation between CK2 activity toward exogenous substrates and the α- and α’-subunit autophosphorylation but not with β-subunit autophosphorylation in the presence of the various peptides and other agents.

Regulation of CK2 Activity by Anti-CK2 Peptide Antibodies—A simple model for the stimulatory effects of the synthetic peptides on CK2 activity is that they disrupted internal interactions within CK2 that negatively modulate the kinase. The catalytic domain of CK2 might be rendered more accessible to substrates by a partial opening of the kinase in subdomains III and X, upon which CK2-III and CK2/cdc2 were based. Presumably, these peptides bound to those regions in the CK2 protein that normally interacted with subdomains III and X. Extremely high concentrations of the peptides could be necessary, rather than stoichiometric amounts, if the peptides only transitorily adopted the optimal conformations. It is more difficult to envision how CK2-NT exerted its marginal effect on CK2 activity. To test the above hypothesis, polyclonal rabbit antibodies were raised against the three CK2 peptides, and each of these preparations was affinity-purified on the appropriate peptide-agarose column. As shown in Fig. 4, each of these antibodies immunoreacted with the α- and α’-subunits of purified sea star CK2. They were also highly specific for these polypeptides in crude cytosolic extracts from sea star oocytes and various mammalian sources, with the exception of a ~55-kDa polypeptide, which may be a close relative of CK2 and has

Fig. 3. Autophosphorylation of sea star CK2 in the presence of CK2 peptides. Panel A, purified CK II was subjected to autophosphorylation in the absence (lane 13) and presence of 0.2-1.0 μg/ml poly(Glu,Tyr; 4:1) (lanes 1-4), 4-20 nM spermine (lanes 5-8), and 4-20 μg/ml heparin (lanes 9-12). Subsequently, SDS-PAGE and autoradiography were performed as described under “Experimental Procedures.” Panel B, prior to SDS-PAGE, purified CK2 was subjected to autophosphorylation in the absence (lane 14) and presence of 0.4-2 mg/ml CK2-NT (lanes 1-4), 0.4-2 mg/ml CK2-III (lanes 4-8), and 0.4-2 mg/ml CK2/cdc2 (lanes 9-13). Autoradiograms are shown. Migrations of marker proteins bovine serum albumin (67 kDa), ovalbumin (45 kDa), and carbonic anhydrase (31 kDa), as well as the α-, α’-, and β-subunits of CK2, are indicated. Similar results were obtained in two independent experiments.

Fig. 4. Immunoblotting of purified CK2 with antipeptide antibodies. Following SDS-PAGE, purified sea star CK2 was immunoblotted with affinity-purified rabbit polyclonal antipeptide antibodies raised against the CK2-NT peptide (lane 1), CK2/cdc2 peptide (lane 2), and CK2-III peptide (lanes 3 and 4). Lanes 1-3 correspond to samples where the antipeptide antibodies were incubated with the immunoblots in the absence of the corresponding immunogenic peptide, and the α and α’ subunits of CK2 were detectable. In lane 4, immunoreactivity of the CK2 catalytic subunits with the anti-CK2-III antibody was competed with 1 mg/ml of the CK2-III peptide. Similarly, the binding of the anti-CK2-NT and anti-CK2/cdc2 antibodies to CK2 were blocked by the appropriate synthetic peptides (data not shown). The migration positions of the prestained marker proteins bovine serum albumin (80 kDa), ovalbumin (50 kDa), carbonic anhydrase (33 kDa), soybean trypsin inhibitor (28 kDa), and lysozyme (19 kDa) are indicated.
not yet been described (Ref. 4 and data not shown). Although
the anti-CK2-NT antibodies had little or no effect on the
casein phosphotransferase activity of purified CK2, both the
anti-CK-III and anti-CK2/cdc2 antibodies facilitated almost
2-fold activations of CK2 (Fig. 5). This indicated that the
binding of the antibodies to subdomains III and X did not
interfere with the catalytic function of the kinase but appar-
ently relieved negative regulation of the enzyme in keeping
with the above model.

**Relief of Heparin and Poly(Glu,Tyr; 4:1) Inhibition of CK2
by Synthetic Peptides**—Heparin and poly(Glu,Tyr; 4:1) are
among the most potent inhibitors of CK2 that have been
described. We considered the possibility that the sites of
action of these inhibitors might involve the N terminus or
kinase subdomains III and X and tested the effects of the
CK2 peptides in concert with the heparin and poly(Glu,Tyr;
4:1) on the enzyme. CK2-III and CK2/cdc2 similarly reversed
almost complete inhibition of the casein and phosvitin phos-
photransferase activity of purified CK2 by 10 µg/ml heparin
or 1 µg/ml poly(Glu,Tyr; 4:1) (Fig. 6). CK2-NT had little
effect in this regard (Fig. 6). Likewise, CK2-III, but not CK2-
NT, reversed partial inhibition of the casein phosphotrans-
ferase activity of CK2 in cytosolic extracts from sea star
oocytes (Fig. 7).

The ability of the CK2 peptides to reverse inhibition of
CK2 autophosphorylation by 10 µg/ml heparin or 1 µg/ml
poly(Glu,Tyr; 4:1) was also examined (Fig. 8). CK2-III and
CK2/cdc2 both restored the autophosphorylation of all three
subunits in the presence of either inhibitor, whereas CK2-NT
only produced modest recoveries of the α′- and β-subunit
phosphorylations. This restoration of the β-subunit phos-
photransferase by CK2/cdc2 was particularly intriguing, as
this peptide reduced β-subunit phosphorylation in the absence
of heparin or poly(Glu,Tyr; 4:1) (Fig. 3B).

**Identification of Subdomain III as the Primary Heparin-
binding Site on CK2**—The high concentration of basic resi-
dues in kinase subdomain III of CK2 makes this an attractive
site for interaction with the polysulfonated glycosaminoglycan
heparin and poly(Glu,Tyr; 4:1), which are highly negatively
charged at neutral pH. The ability of CK2-III to reverse
inhibition by these polymers was consistent with this notion.
To further test whether this region of CK2 was important
for heparin binding, the ability of all three CK2 peptides to elute
purified CK2 bound to heparin-agarose beads was assessed.
Only the CK2-III peptide effectively caused the release of
bound CK2; ~33% of the adsorbed CK2 was eluted with 2.4
mg/ml CK2-III (Fig. 7).

The results of Hu and Rubin (8), in which two of the lysyl
residues in subdomain III of *C. elegans* CK2 were converted
to glutamyl residues by site-specific mutagenesis (i.e. KEE-
KIKR) and the IC50 for heparin inhibition of CK2 was in-
creased 70-fold, strongly support the assignment of sub-
domain III as the heparin-binding site. The preponderance
of basic residues in subdomain III could also facilitate nuclear
localization of CK2, as well as substrate binding, since pre-
ferred CK2 targets contain acidic residues in the vicinity of the
phosphoacceptor amino acid (2). However, CK2 from
*Saccharomyces cerevisiae* contains two nonconservative re-

---

**FIG. 5. Effect of anti-CK2 peptide antibodies on the phos-
photransferase activity of purified sea star CK2.** Purified sea
star CK2 was assayed for phosphotransferase activity toward casein
with 5-10 µg of affinity-purified anti-CK2-NT (C), anti-CK2-III (I),
and anti-CK2/cdc2 (A) peptide antibodies in a final volume of 25 µl.
Values are the means ± S.E. of at least three determinations. Similar
results were obtained in two independent experiments.

**FIG. 6. Reversal of heparin and poly(Glu,Tyr; 4:1) inhibi-
tion of purified CK2 by synthetic CK2 peptides.** Purified sea
star CK2 was assayed for phosphotransferase activity toward casein
(panel A) and phosvitin (panel B) peptides in the absence (O) and
presence of 10 µg/ml heparin (O) or 1 µg/ml poly(Glu,Tyr; 4:1) (panels
C and D) peptides in the presence of 0-2 mg/ml of the CK2-
NT (A), CK2-III (C), and CK2/cdc2 (D) peptides. Values are the means ± S.E. of
at least three determinations. In the absence of heparin, poly(Glu,Tyr;
4:1), and synthetic peptides, the casein and phosvitin phosphotrans-
ferase activities of purified CK2 were 1350 and 410 pmol/min/ml,
respectively. Similar results were obtained in three independent ex-
periments.
placements in 2 of these lysyl residues (i.e. KMKKIYR) (7). Likewise, the binding affinity for casein was not affected in the C. elegans KEKIKKR CK2 mutant developed by Hu and Rubin (8). Therefore, in view of these observations, it seems that at least 3 of the lysyl residues in subdomain III of CK2 are nonessential for substrate recognition.

For at least one other protein kinase, i.e. p34cdc2, it has been shown that synthetic peptides modeled after subdomain III can exert biological effects. Doree and collaborators (45, 46) have described the induction of sea star oocyte maturation by a peptide that contains the sequence EGVPSTAIREISLLKE. Antibodies developed against this sequence have been relatively selective probes for the anti-CKP/cdc2 antibod~dies. Nevertheless, subdomain X in p34cdc2 is likely to play a regulatory role in this kinase, as the present study has implicated for CK2.

Acknowledgments—Philip Owen, Greg Radigan, and Peter Borowski aided in the synthesis and purification of the synthetic peptides. Michael Williams and Faye Chow (Biomedical Research Centre) provided valuable technical assistance in the preparation of the anti-peptide antibodies.

REFERENCES

1. Krebs, E. G., Eisenman, R. N., Kuenzel, E. A., Litchfield, D. W., Lozeman, F. J., Luscher, B., and Sommercorn, J. (1988) Cold Spring Harbor Symp. Quant. Biol. 53, 77–84

2. L. A. Charlton, J. S. Sanghera, I. Clark-Lewis, and S. L. Pelech, unpublished data.

3. L. A. Charlton, J. S. Sanghera, I. Clark-Lewis, and S. L. Pelech, unpublished observations.
Regulation of Casein Kinase 2 by Peptides

25. Klarlund, J. K., and Czech, M. P. (1988) J. Biol. Chem. 263, 15872-15875
26. Carroll, D., and Marshak, D. R. (1989) J. Biol. Chem. 264, 7345-7348
27. Ackerman, P., and Osheroff, N. (1989) J. Biol. Chem. 264, 11958-11965
28. Villa-Moruzzi, E., and Crabb, J. W. (1991) Biochem. Biophys. Res. Commun. 177, 1019-1024
29. Schneider, H. R., and Isingger, O.-G. (1989) Biochim. Biophys. Acta 1014, 99-100
30. Cochet, C., Job, D., Piroillet, F., and Chambaz, E. M. (1980) Endocrinology 106, 750-757
31. Hathaway, G. M., and Traugh, J. A. (1984) J. Biol. Chem. 259, 7011-7015
32. Hathaway, G. M., Luben, T. H., and Traugh, J. A. (1980) J. Biol. Chem. 255, 8038-8041
33. Feige, J. J., Piroillet, F., Cochet, C., and Chambaz, E. M. (1980) FEBS Lett. 121, 139-142
34. Fischer, E. H., and Krebs, E. G. (1989) Biochim. Biophys. Acta 1000, 297-301
35. Meggio, F., Brunati, A. M., and Pinna, L. A. (1983) FEBS Lett. 160, 203-208
36. Meggio, F., Marchiori, F., Borin, G., Chessa, G., and Pinna, L. A. (1984) J. Biol. Chem. 259, 14576-14579
37. Meggio, F., and Pinna, L. A. (1988) Biochim. Biophys. Acta 1010, 128-130
38. Palen, E., and Traugh, J. A. (1991) Biochemistry 30, 5586-5590
39. Lin, W.-J., Tuazon, P. T., and Traugh, J. A. (1991) J. Biol. Chem. 266, 5664-5669
40. Granowsk, N., Boldyreff, B., and Issinger, O. G. (1991) Eur. J. Biochem. 198, 25-30
41. Bradford, M. M. (1976) Anal. Biochem. 72, 248-254
42. Leemln, U. K. (1970) Nature 227, 680-685
43. Hanks, S. K., Quinn, A. M., and Hunter, T. (1988) Science 241, 42-52
44. Traugh, J. A., Lin, W.-J., Takada-Axelrod, F., and Tuazon, P. T. (1990) Adv. Second Messenger Phosphoprotein Res. 24, 224-229
45. Picard, A., Cavadore, J.-C., Lory, P., Bernengo, J.-C., Ojeda, C., and Doree, M. (1990) Nature 247, 327-329
46. Gautier, J., Norbury, C. J., Lokha, M., Nurse, P., and Maller, J. (1988) Cell 54, 433-439
47. Pilech, S. L., Sanghera, J. S., and Daya-Makin, M. (1990) Biochem. Cell Biol. 68, 1297-1330
48. Knighton, D. R., Zheng, J., Eyck, L. F. T., Ashford, V. A., Xuong, N.-H., Taylor, S. S., and Sowadski, J. M. (1991) Science 253, 407-414

2. Pinna, L. A. (1990) Biochim. Biophys. Acta 1054, 267-284
3. Tuazon, P. T., and Traugh, J. A. (1991) Adv. Second Messenger Phosphoprotein Res. 23, 123-164
4. Sanghera, J. S., Charlton, L. A., Padden, H. B., and Pilech, S. L. (1992) Biochem. J., in press
5. Chen-Wu, J. L.-P., Padmanabha, R., and Glover, C. V. C. (1988) Mol. Cell. Biol. 8, 4981-4990
6. Heller-Harrison, R. A., Meisner, R., Buxton, J., and Czech, M. P. (1980) Biochemistry 28, 4072-4076
7. Padmanabha, R., Chen-Wu, J. L.-P., Hanna, D. E., and Glover, C. V. C. (1990) Mol. Cell. Biol. 8, 4089-4099
8. Hu, E., and Rubin, C. S. (1990) J. Biol. Chem. 265, 20609-20615
9. Maridor, G., Park, W., Krek, W., and Nigg, E. A. (1991) J. Biol. Chem. 266, 2362-2368
10. Takio, K., Kuenzel, E. A., Walsh, K. A., and Krebs, E. G. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 4851-4855
11. Jakobi, R., Voss, H., and Pyerin, W. (1989) Eur. J. Biochem. 183, 227-233
12. Kuenzel, E. A., Mulligan, J. A., Sommercorn, J., and Krebs, E. G. (1987) J. Biol. Chem. 262, 9136-9140
13. Marchiori, F., Meggio, F., Marin, O., Borin, G., Calderan, A., Ruzza, F., and Pinna, L. A. (1988) Biochim. Biophys. Acta 971, 352-358
14. Meggio, F., Perich, J. W., Johns, R. B., and Pinna, L. A. (1988) FEBS Lett. 237, 225-228
15. Meggio, F., Perich, J. W., Reynolds, E. C., and Pinna, L. A. (1991) FEBS Lett. 279, 307-309
16. Litchfield, D. W., Arends, A., Lozeman, F. J., Krebs, E. G., Hargrave, P. A., and Palczewski, K. (1990) FEBS Lett. 281, 117-120
17. Carroll, D., Santoro, N., and Marshak, D. R. (1988) Cold Spring Harbor Symp. Quant. Biol. 53, 91-95
18. Luscher, B., Christenson, E., Litchfield, D. W., Krebs, E. G., and Eisenman, R. N. (1990) Nature 344, 517-522
19. Luscher, B., Kuenzel, E. A., Krebs, E. G., and Eisenman, R. N. (1989) EMBO J. 8, 1111-1119
20. Meek, D. W., Simon, S., Kikkawa, U., and Eckhart, W. (1990) EMBO J. 9, 3253-3260
21. Finzi, J. M., Galloway, D. M., Eisenman, R. N., and Luscher, B. (1989) New Biol. 1, 44-53
22. Barbosa, M. S., Edmonds, C., Fischer, C., Schiller, J. T., Lowy, D. R., and Vouden, K. H. (1990) EMBO J. 9, 153-160
23. Grasser, F. A., Scheidtmann, K. H., Tuazon, P. T., Traugh, J. A., and Walter, G. (1988) Virology 165, 13-22
24. Sommercorn, J., Mulligan, J. A., Lozeman, F. J., and Krebs, E. G. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 4851-4855