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The cDNAs coding for the α- and β-subunits of *Xenopus laevis* casein kinase II*

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Using a λgt10 cDNA library obtained from *Xenopus laevis* oocytes and probes derived from the known sequences of the human and *Drosophila* genes, a cDNA coding for the α-subunit of the *X. laevis* casein kinase II was isolated. The coding sequence of this clone determines a polypeptide of 350 amino acids. The *X. laevis* sequence is 98% identical to the human and rat proteins in the first 323 amino acids. Using the polymerase chain reaction to generate a 370-nucleotide-long probe, it was possible to clone and sequence a cDNA of 900 nucleotides that coded for the *X. laevis* β-subunit of casein kinase II. The derived protein sequence is 215 amino acids long and again shows an extraordinary degree of conservation with other species.

Protein kinase; Amphibian oocyte; Protein phosphorylation; Sequence motif; *Xenopus laevis*, casein kinase II

1. INTRODUCTION

Casein kinase II (CKII) is a protein kinase that is widely distributed in eukaryotes and that is not regulated by cyclic nucleotides, Ca^{2+} or phospholipids (for review see [1]). The interest in this enzyme has increased recently due to reports that demonstrate that CKII phosphorylates several oncogenes and antioncogenes [2–4], transcription factors [5,6], enzymes that participate in nucleic acid synthesis [7,8] as well as other important proteins [9–11]. In addition, the work of several laboratories has demonstrated that the activity of CKII fluctuates during the cell cycle and seems to be regulated by mitogenic signals [12–14].

The CKII enzymes are tetramers of the $\alpha_2\beta_2$ or of $\alpha\alpha'\beta_2$ configuration. The cDNAs for the α-, α'- and β-subunits of several mammalian species and of *Drosophila*, yeast and *C. elegans*, have been cloned and sequenced [15–19]. These sequences show a high degree of conservation.

Previous work from our laboratory has demonstrated that CKII is the most abundant protein phosphorylating activity present in the nuclei of *Xenopus laevis* oocytes and we have described its inhibition by nucleic acids and acidic peptides [20–22].

In this report, we present the sequence of the cDNAs coding for the α- and β-subunits of the CKII of *X. laevis*. These results demonstrate a remarkable degree of conservation of the sequence of these protein subunits in such widely separated species as humans and amphibians.

*Dedicated to the memory of Dr. Hermann Niemeyer, a pioneer of Chilean Biochemistry.

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2. MATERIALS AND METHODS

Oligonucleotides were synthesized with an Applied Biosystems 380A DNA Synthesizer in our laboratory. DNA sequences were determined using the method of Sanger et al. [23] and vectors M13mp18 and M13mp10 as described in the 'Sequenase' manual provided by U.S. Biochemical Corporation.

2.1. *PCR amplification*

PCR amplification was carried out as described [24] using Taq polymerase purchased from Promega. A *X. laevis* oocyte cDNA library constructed in the vector λgt10 was kindly donated by Dr. D.A. Melton of Harvard University [25].

2.2. *Screening for the α-subunit*

The λgt10 library was screened for the cDNA coding for the α-subunit of *X. laevis* CKII with several probes. Initially an oligonucleotide probe was synthesized based on the *Drosophila* alpha subunit gene [15]. This oligonucleotide had the sequence:

5'-GATATGTGGAGCCTGGGATGTATGCTGGCCAGCATGATC-3'

and was labelled with polynucleotide kinase. As described in the text, the clones isolated with this probe were truncated at the 5' end. The second screening that yielded clones with the full coding sequence of the *X. laevis* α-subunit employed two probes in replicate plating. The first probe was a 128 base-pair fragment corresponding to the 5' end of the coding region of the human α-subunit of CKII [26]. This fragment was generated by PCR amplification of human cDNA library with the primers:

upstream: 5'-ATGTCGGGACCCGTGCC-3'
downstream: 5'-CGAACCAGCTGGTAGTC-3'

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The second probe was a fragment derived from the truncated gene and contained 439 nucleotides which corresponded to the sequence between nucleotide + 104 and nucleotide + 543 of the sequence given in Fig. 1.

Both probes were labelled by random priming. The screening methods were essentially as described by Maniatis et al. [27].

2.3. *Screening for the β-subunit*

The λgt10 *X. laevis* oocyte cDNA library was screened with a probe generated by PCR amplification of the same library using two primers derived from the nucleotide sequence of the gene coding for the human β-subunit of CKII [24]. The two primers used were:

5′-CGTGGCAATGAGTTCTTCTG-3′
5′-ACATCCATGCACTTGGGGCA-3′

The probe generated in this fashion was 378 nucleotides long and corresponded to the sequence between nucleotide + 49 and nucleotide + 428 of the sequence presented in Fig. 3. This probe was also labelled by random priming. The positive clones obtained were purified through secondary and tertiary screening as described in Maniatis et al. [27].

2.4. *Reagents*

General chemicals and reagents were of analytical grade, obtained from commercial suppliers. [γ-^{32}P]ATP (7000 Ci/mmol) were purchased from ICN and [^{35}S]dATP-α-S (>1000 Ci/mmol) from Amersham. Restriction enzymes, M13 cloning kit and special buffers, were purchased from Promega Biotec (Madison, WI). Nitrocellulose was purchased from Schleicher and Schuell.

3. RESULTS

3.1. *Cloning of the cDNA coding for the α-subunit*

Using an oligonucleotide probe synthesized on the basis of the sequence determined for the α-subunit of the *D. melanogaster* gene [15], a λgt10 cDNA library of *X. laevis* oocytes was screened. Among the positive clones detected, two were purified through quaternary screening. These clones were inserted into a M13mp18 vector and sequenced by the method of Sanger et al. [23]. Both of these clones were found to code for the α-subunit of *X. laevis* CKII but they differed in length. One contained a 3′ non-coding segment of more than 1000 nucleotides while the other only extended 65 nucleotides after the stop codon. The longer fragment contained the polyadenylation signal and a poly-A segment. However, both cDNA clones were truncated at

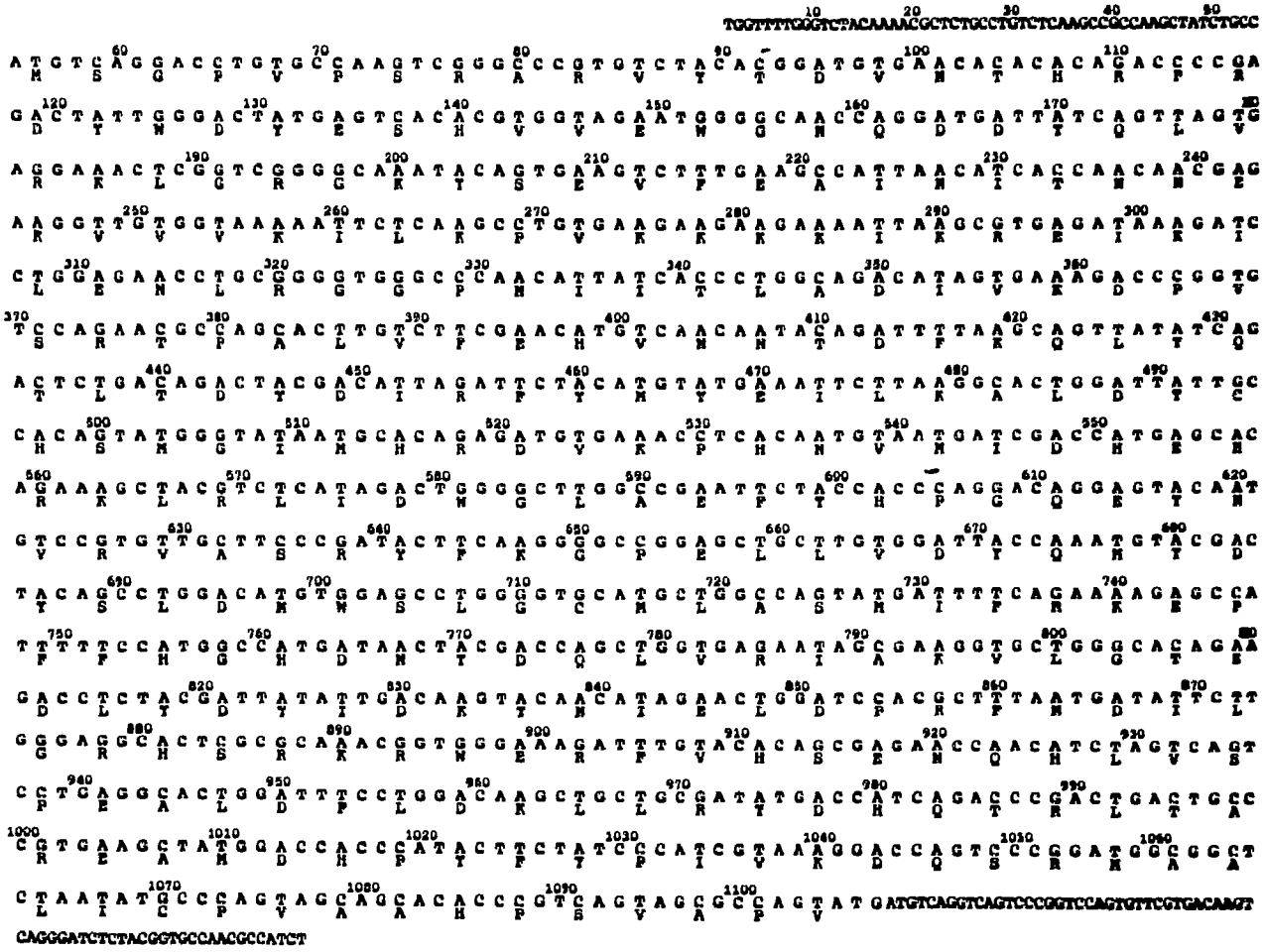

Fig. 1. The nucleotide and predicted amino acid sequence of the α-subunit of *X. laevis* casein kinase II. The Met initiation codon is at nucleotide 55 and the termination codon TGA is at nucleotide 1105.

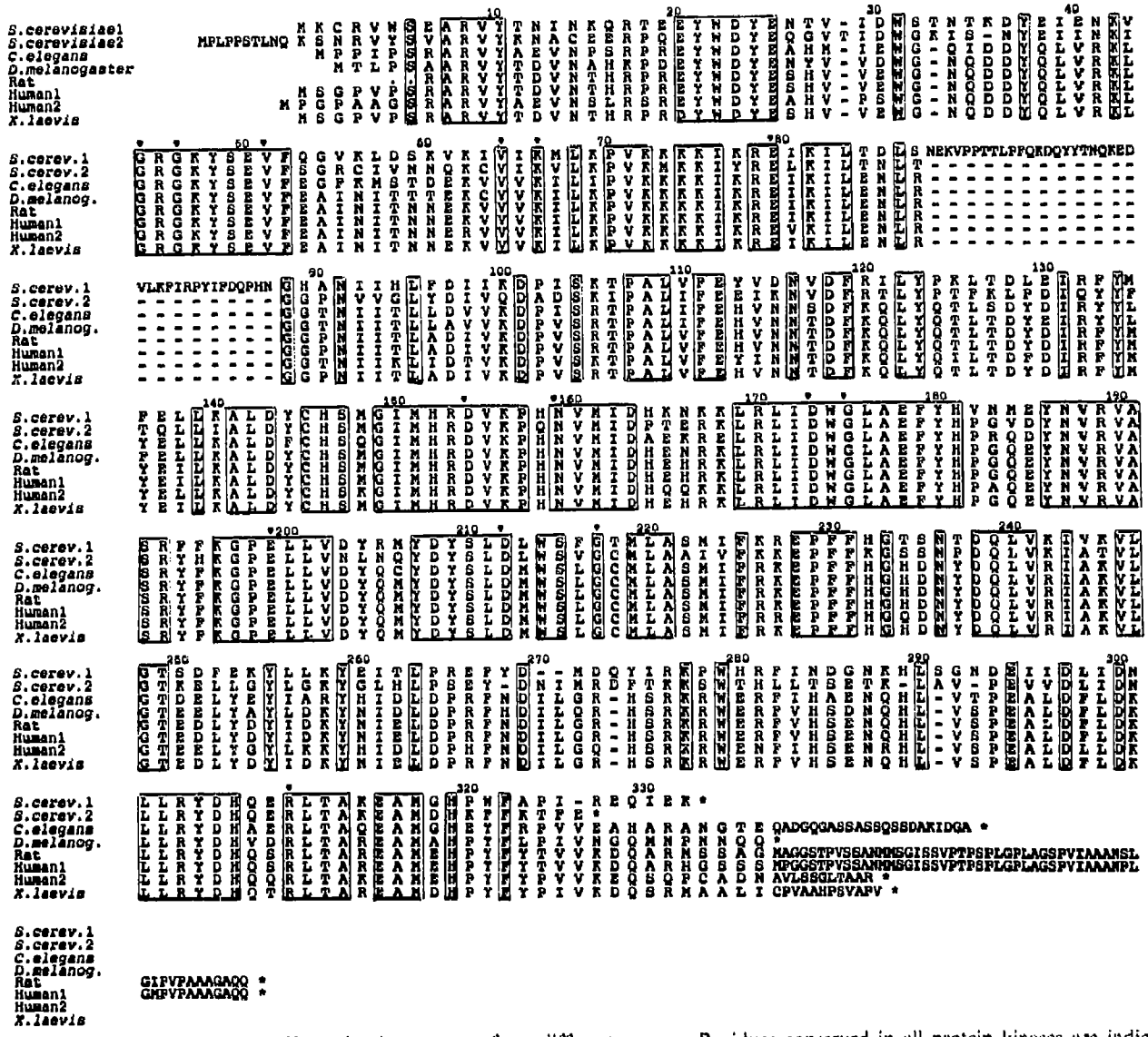

Fig. 2. Alignment of casein kinase II α-subunit sequences from different sources. Residues conserved in all protein kinases are indicated by arrowheads. Residues conserved in all casein kinase II are boxed.

an identical position which corresponded to nucleotide + 108 of the human αCKII subunit. This finding made it necessary to return to the cDNA library and re-screen, using two probes in replicate plating. The first probe corresponded to a fragment of the previously cloned truncated gene containing 439 base pairs, and the second probe was a 128 base pair PCR product from a human cDNA library and corresponded to the missing coding segment of the truncated genes. Only 2 out of the 5 clones analyzed were positive with both probes indicating that there is a relatively high incidence of truncated cDNAs for this gene in this cDNA library. However, it is interesting that other cDNA libraries seem to have a high number of truncated clones of this gene [28].

One of the doubly positive clones was purified, inserted into a M13mp10 vector and sequenced as described previously. Fig. 1 gives the nucleotide sequence of the coding region of this clone with short non-coding segments at the 5′ and 3′ ends. The sequence found in this clone was identical to the corresponding sequences previously determined in the truncated cDNAs. This figure also shows the derived amino acid sequence coded by this clone. The α-subunit of *X. laevis* CKII is a protein of 350 amino acids and a calculated M_r of 41,479 kDa.

A remarkable degree of sequence conservation is observed by comparing the amino acid sequence of the α-subunit of *X. laevis* CKII with the sequences of the α-subunits of other species (Fig. 2). In the first 323 amino acids, the *X. laevis* α-subunit sequence is identical to the human and rat sequences except for 4 conserva-

Fig. 3. The nucleotide and predicted amino acid sequence of the β-subunit of *X. laevis* casein kinase II. The Met initiation codon is at nucleotide 55 and the termination codon TGA is at nucleotide 700. The underlined bases correspond to the regions in the human CKII gene used to design the primers for PCR.

tive changes. In the same region the nucleotide sequences of human and *X. laevis* are 80% identical. On the other hand, it is clear that the sequences close to the carboxyl end are highly variable and have different lengths. The figure also shows that this α-subunit contains the typical motifs that have been described for protein kinase [29], however, some of the highly invariant amino acids in other kinases are altered in the α-subunit of CKII. This is the case of Val-64 which in most other kinases is Ala and of Trp-174 which in 64 other kinases is Phe. Another interesting feature of the α-subunit of CKII is the highly basic stretch going from Lys-72 to Arg-78 which contains 6 out of 7 basic amino acids. It has been proposed that this region might be involved in the interaction of the enzyme with the polyacidic peptide substrates that it recognizes [1]. It is also possible that this region may comprise part of the nuclear localization signal of this protein since long stretches of basic amino acids have been involved in the nuclear localization of other proteins [30].

So far, no results have been obtained to indicate the existence of a gene coding for an α-subunit in *X. laevis*, equivalent to those detected in human and in *S. cerevisiae* [18,28]. However, preliminary evidence obtained in this laboratory using genomic DNA indicates that *X. laevis* contains two or more variants of the α-subunit gene [V. Wilhelm et al., in preparation].

3.2. *The cloning and sequencing of the beta subunit*

A different strategy was used for the cloning of the cDNA coding for the *X. laevis* β-subunit. Synthetic oligonucleotides were prepared on the basis of the sequence determined for the human β-subunit [31]. These oligonucleotides were used as primers for the polymerase chain reaction as a means to amplify part of the β-subunit cDNA that is present in the λgt10 cDNA library. The amplified DNA, which corresponded to 370 base pairs, was inserted into an M13mp10 vector and sequenced to corroborate that it corresponded to the β-subunit gene. Subsequently, this PCR fragment was labelled by random priming and used as a probe to screen the λgt10 cDNA library. Twenty positive clones were detected and five were purified by ternary screening. One of these clones, containing an insert of 900 base pairs was sequenced and found to contain the entire coding region of the *X. laevis* β-subunit of CKII. The nucleotide and the derived amino acid sequences are

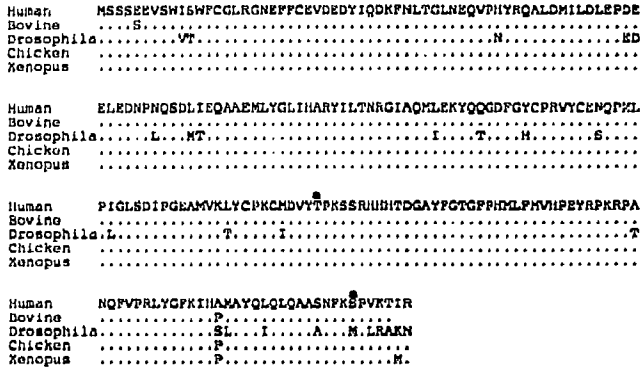

Fig. 4. Comparison of the amino acid sequence of the β-subunit of *X. laevis* casein kinase II with those of human, bovine, drosophila and chicken. The dots indicate probable $p34^{cdc-2}$ phosphorylation sites at amino acids 145 and 209.

shown in Fig. 3. The β-subunit of *X. laevis* CKII is a polypeptide of 215 amino acids with a calculated M_r of 24,960 Da.

Again, the gene for this subunit shows an amazing degree of sequence conservation. At the nucleotide level comparison of the coding regions of human and *X. laevis* shows 125 bases changed out of a total of 645. Almost all of these changes are in the third place positions or mutations that maintain codons for the same amino acid. Fig. 4 compares the amino acid sequences of *X. laevis* with those of 4 other species. It can be seen that the *X. laevis* β-subunit is identical to the human polypeptide in the first 193 amino acids and in the remaining of the molecule it only has 2 different amino acids. The *X. laevis* and chicken subunits only differ in 1 amino acid. This extraordinary conservation seems more remarkable because of the fact that this subunit is not known to carry out any catalytic function, its only known activity is the capacity to stimulate the phosphorylations catalyzed by the α-subunit [1].

Recently, there have been two reports that indicate that the β-subunit may be phosphorylated by the $p34^{cdc2}$ kinase that regulates key transitions in the cell cycle [32,33]. According to Litchfield et al. [33], the site of phosphorylation in the human β-subunit would be serine-209. However, Mulner-Lorillon et al. [32], who used purified *X. laevis* CKII in vitro found that $p34^{cdc2}$ phosphorylated the β-subunit in an unidentified threonine residue. Since serine-209 is present in the same position in the *X. laevis* subunit as in the human, these observations would suggest that the phosphorylation of the *X. laevis* by the $p34^{cdc2}$ kinase would be in a different position. This phosphorylation site may correspond to threonine-146 which is in a sequence that fulfills the consensus required by the $p34^{cdc2}$ kinase [34].

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REFERENCES

[1] Tuazon, P.T. and Traugh, J.A., in: Advances in Second Messenger and Phosphoprotein Research (P. Greengard and G.A. Robinson, Eds.) Vol. 23, Raven Press, New York, 1991, pp. 123–164.
[2] Luscher, B., Christenson, E., Litchfield, D., Krebs, E. and Eisenman, R. (1990) Nature 344, 517–522.
[3] Glineur, C., Bailly, M. and Ghysdael, J. (1989) Oncogene 4, pp. 1247–1254.
[4] Meisner, H. and Czech, M.P. (1991) Current Opinion in Cell Biology 3, 474–483.
[5] Manak, R., De Bisschop, N., Kris, R. and Prywes, R. (1990) Genes Dev. 4, 955–967.
[6] Lee, C., Yun, Y., Hoeffler, J. and Habener, J. (1990) EMBO J. 9, 4455–4465.
[7] Stetler, D.A. and Rose, K.M. (1982) Biochemistry 21, 3721–3728.
[8] Ackerman, P., Glover, C.V.C. and Osherhoff, N. (1988) J. Biol. Chem. 263, 12653–12660.
[9] Grande, J., Perez, M. and Itarte, E. (1988) FEBS Lett. 232, 130–134.
[10] Serrano, L., Diaz-Nido, J., Wandosell, F. and Avila, J. (1987) J. Cell. Biol. 105, 1731–1739.
[11] Hasler, P., Brot, N., Weissbach, H., Parnassa, A.P. and Elkon, K.B. (1991) J. Biol. Chem. 266, 13815–13820.
[12] Carroll, D., Santoro, N. and Marshak, D. (1988) Cold Spring Harbor Symp. Quant. Biol. 53, 91–95.
[13] Ackerman, P., Glover, C. and Osherhoff, N. (1990) Proc. Natl. Acad.Sci. USA 87, 821–825.
[14] Sommercorn, J. and Krebs, E.G. (1987) J. Biol. Chem. 262, 3839–3843.
[15] Saxena, A., Padmanabha, R. and Glover, C.V.C. (1987) Mol. Cell. Biochem. 7, 3409–3417.
[16] Meisner, H., Heller-Harrison, R., Buxton, J. and Czech, M.P. (1989) Biochemistry 28, 4072–4076.
[17] Tako, K., Kuenzel, E.A., Walsh, K.A. and Krebs, E.G. (1987) Proc. Natl. Acad. Sci. USA 84, 4851–4855.
[18] Hu, E. and Rubin, C.S. (1990) J. Biol. Chem. 265, 5072–5080.
[19] Chen-Wu, J.L.P., Padmanabha, R. and Glover, C.V.C. (1988) Mol. Cell. Biol. 8, 4981–4990.
[20] Leiva, L., Carrasco, D., Taylor, A., Veliz, M., Gonzalez, C., Allende, C.C. and Allende, J.E. (1987) Biochem. Int. 14, 707–717.
[21] Gatica, M., Allende, C.C. and Allende, J.E. (1989) FEBS Lett. 255, 414–418.
[22] Tellez, R., Gatica, M., Allende, C.C. and Allende, J.E. (1990) FEBS Lett. 265, 113–116.
[23] Sanger, F., Nicklen, S. and Coulson, A. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[24] Saiki, R., in: PCR Protocols. A Guide to Methods and Applications (M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White, Eds) Academic Press, San Diego, 1990, pp. 13–20.
[25] Rabagliati, M., Weeks, D., Harvey, R. and Melton, D. (1985) Cell 42, 769–777.
[26] Heller-Harrison, R.A., Meisner, H. and Czech, M.P. (1989) Biochemistry 28, 9053–9058.
[27] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
[28] Lozeman, F.J., Litchfield, D.W., Piening, C., Takio, K., Walsh, K.A. and Krebs, E.G. (1990) Biochemistry 29, 8436–8447.
[29] Hanks, S.K., Quinn, A.M. and Hunter, T. (1988) Science 241, 42–51.
[30] Silver, P.A. (1991) Cell 64, 489–497.
[31] Heller-Harrison, R.A., Meisner, H. and Czech, M.P. (1989) Biochemistry 28, 9053–9058.
[32] Mulner-Lorillon, O., Cormier, P., Labbe, J., Doree, M., Poulhe, R., Osborne, H. and Bellé, R. (1990) Eur. J. Biochem. 193, 529–534.
[33] Litchfield, D.W., Lozeman, F.J., Cicirelli, M.F., Harrylock, M., Eriesson, L.H., Piening, C.J. and Krebs, E.G. (1991) J. Biol. Chem. 266, 20380–20389.
[34] Langan, T.A., Gautier, J., Lohka, M., Hollingsworth, R., Moreno, S., Nurse, P., Maller, J. and Sclafani, R.A. (1989) Mol. Cell. Biol. 9, 3860–3868.