Volume 283, number 2, 210–214 FEBS 09757 June 1991

ADONIS 001457939100466K

rig encodes ribosomal protein S15

The primary structure of mammalian ribosomal protein S15

Motoo Kitagawa[1,*], Shin Takasawa[1], Norihisa Kikuchi[2], Takako Itoh[2], Hiroshi Teraoka[2], Hiroshi Yamamoto[1,**] and Hiroshi Okamoto[1]

[1] *Department of Biochemistry, Tohoku University School of Medicine, Sendai 980, Miyagi, Japan* and [2] *Shionogi Research Laboratories, Shionogi & Co. Ltd., Fukushima-ku, Osaka 553, Osaka, Japan*

Received 4 March 1991

rig, a gene originally isolated from a rat insulinoma cDNA library, codes for a basic 145 amino acid protein ((1986) Diabetes 35, 1178–1180). Here we show that the immunoreactivity to a monoclonal antibody against the deduced *rig* protein and the translation product of *rig* mRNA comigrated with ribosomal protein S15. The amino acid sequence of ribosomal protein S15 purified from rat liver coincided with that deduced from the nucleotide sequence of *rig* mRNA, but there were indications that the initiator methionine was removed and the succeeding alanyl residue was monoacetylated. From these results, we conclude that the product of *rig* is ribosomal protein S15.

rig; *rig* protein; Ribosomal protein S15; Amino acid sequence; N-terminal modification; Rat liver

1. INTRODUCTION

rig (rat insulinoma gene) was first isolated from a cDNA library of chemically induced rat insulinomas [1–3]. A higher expression of *rig* has been observed in a variety of tumor cells such as insulinomas [4], esophageal and colon cancers [5] and during the proliferative phase of liver regeneration [6]. The 145 amino acid sequence deduced from rat, hamster, human, mouse and chicken cDNAs [1–4,7] remained invariant, suggesting that *rig* has evolved under extraordinarily strong selective constraints. Human genomic *rig* has features characteristic of housekeeping genes [5], but no function for *rig* has yet been found.

In this study, ribosomal protein S15 is demonstrated by immunoblotting, translation of *rig* mRNA and amino acid sequencing to be the product of *rig*.

2. EXPERIMENTAL

2.1. *Materials*

Male Wistar rats were used throughout the experiments. A mouse monoclonal antibody against the synthetic peptide corresponding to the 20 amino acid sequence of the *rig* protein (amino acid residues 46–65) [1] was kindly supplied by Drs. S. Yoshida, K. Obata, and K. Iwata (Fuji Chemical Industries Ltd., Takaoka, Japan). Horseradish peroxidase-conjugated goat anti-mouse IgG (H + L) antibody was purchased from Zymed (San Francisco, CA); polyvinylidene difluoride (PVDF) membrane (Immobilon-P) from Millipore (Bedford, MA); [^{35}S]methionine (37 TBq/mmol) from the Amersham Corp.; lysyl endopeptidase from Wako Pure Chemical Industries (Osaka, Japan).

2.2. *Subcellular fractionation and ribosome purification*

Mitochondrial, microsomal and cytosolic fractions of rat liver were prepared essentially according to the method of De Duve et al. [8]. Ribosomes and their subunits were isolated according to Ogata and Terao [9]. Protein was determined by the method of Bradford [10].

2.3. *Polyacrylamide gel electrophoresis and immunoblotting*

SDS- and two-dimensional-PAGE was carried out as described in [11,12]. After PAGE, proteins were transferred to PVDF membranes and immunodetected [13,14] with the anti-*rig* peptide antibody (10 μg/ml) and with the horseradish peroxidase/anti-mouse IgG conjugate (1:500).

2.4. *In Vitro expression of* rig *and fluorography*

The transcript of rat *rig* cDNA was translated in a rabbit reticulocyte lysate containing [^{35}S]methionine as described previously [4]. The ^{35}S-labeled product was extracted with 67% acetic acid and precipitated with rat 40 S ribosomal proteins in acetone. Then the mixture was analyzed by two-dimensional-PAGE, followed by Coomassie brilliant blue staining and fluorography.

2.5. *Isolation and amino acid sequencing of ribosomal protein S15*

Spots of ribosomal protein S15 were cut out from three-dimensional gels [9], and the protein was extracted into 70% formic acid [15]. Lysyl-endopeptidase fragments of ribosomal protein S15 were separated by reverse-phase HPLC, and underwent automated Edman degradation using an Applied Biosystems Model 477A protein sequencer. Amino acid compositions were determined with a Hitachi Model 835 amino acid analyzer. Mass spectra were measured by liquid secondary ion mass spectrometry with a Hitachi M-90 mass spectrometer.

Correspondence address: H. Okamoto, Department of Biochemistry, Tohoku University School of Medicine, 2-1 Seiryomachi, Aoba-ku, Sendai 980, Miyagi, Japan. Fax: (81) (22) 273 7273

* *Present address*: Institute for Molecular and Cellular Biology, Osaka University, Suita-shi, Osaka 565, Japan

** *Present address*: Department of Biochemistry, Kanazawa University School of Medicine, 13-1 Takaramachi, Kanazawa 920, Japan

Abbreviations: PVDF, polyvinylidene difloride; PTH, phenylthiohydantoin

Published by Elsevier Science Publishers B.V.

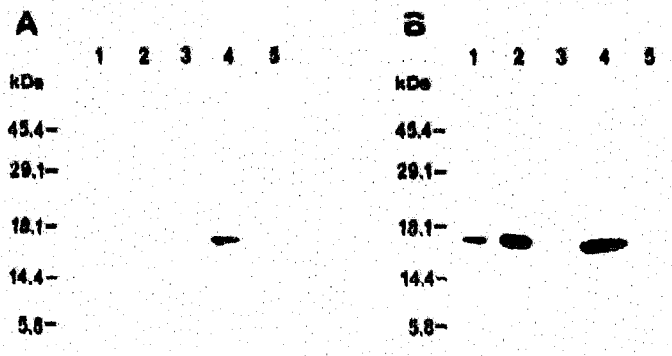

Fig. 1. Distribution of the 17-kDa protein in rat liver subcellular fractions. (A) Subcellular fractions. Lanes: (1) homogenate; (2) nuclear fraction; (3) mitochondrial fraction; (4) microsomal fraction; (5) cytosolic fraction. 100 μg protein/lane were loaded. A very faint immunoreactive band was observed in lane 1 of the original membrane. (B) Sub-microsomal fractions. Lanes: (1) microsomal fraction; (2) ribosomal fraction; (3) post-ribosomal microsome fraction; (4) 40 S ribosomal subunit; (5) 60 S ribosomal subunit. 50 μg protein/lane were loaded. Positions of the size markers are presented on the left of each panel.

3. RESULTS

As shown in Fig. 1, most of the immunoreactivity to a monoclonal antibody against the synthetic 20 amino acid sequence of the *rig* protein was recovered in the ribosomal fraction. The immunoreactive protein had an apparent molecular weight of 17000, which is consistent with that expected from the open reading frame of the *rig* mRNA [1]. The 17-kDa protein was recovered in the 40 S subunit of the ribosome, but not in the 60 S subunit (Fig. 1B, lanes 4 and 5). When the 40 S subunit proteins were run in the two-dimensional gel electrophoresis, and immunostained with the monoclonal antibody, only one spot was stained, which corresponded to ribosomal protein S15 (Fig. 2). The in vitro transcription/translation product of rat *rig* cDNA comigrated with S15 on the two-dimensional gel electrophoresis (Fig. 3). These results suggested that the *rig* protein corresponds to ribosomal protein S15.

Ribosomal protein S15 has been defined as the constituent of a spot on the two-dimensional gel [16], but its primary structure has not yet been determined. We isolated the S15 protein from rat liver and analyzed its amino acid sequence. No PTH-amino acid was liberated at the first cycle of Edman degradation, indicating that there is a blockage on the amino-terminal. As shown in Fig. 4, with lysyl endopeptidase fragments (*b–h* and *j–n*), the degradation reaction proceeded and the sequence determined coincided with that deduced from the nucleotide sequence of *rig* cDNA [1]. Fragment *a* was resistant to Edman degradation, and its amino acid composition was A:E:V:Q:K = 1:2:1:1:1, which corresponded only to the amino acid residues 2–7 deduced from rat *rig* cDNA [1]. Thus, fragment *a* was regarded as representing the amino-terminal fragment without the initiator methionine. The residue next to the initiator methionine was alanyl [1], which has been reported [17] to favor NH_2-terminal processing. The amino acid composition of the S15 protein agreed with that deduced from the *rig* cDNA, except that one methionyl residue was missing (data not shown). The mass spectrum of the protonated molecular ion of fragment *a* was *m/z* 745, a value consistent with that for monoacetyl-A-E-V-E-Q-K. These results suggested that

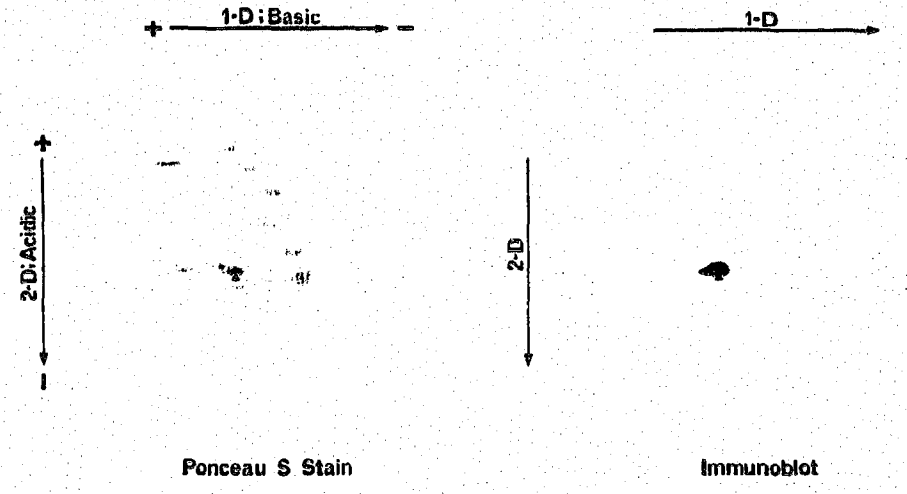

Fig. 2. Identification of the reactivity of the anti-*rig* peptide antibody in 40 S subunit proteins. A 100 μg of 40 S subunit proteins were applied to two-dimensional-PAGE, and transferred to membranes. Electrophoresis was from left to right in the first dimension and top to bottom in the second. (Left panel) Ponceau S-stained membrane transfer. (Right panel) Immunoblot of the same membrane as left panel. The triangle which indicates the position of ribosomal protein S15 was marked directly on the membrane. Spot positions of the S15 and other ribosomal proteins were identified according to [16].

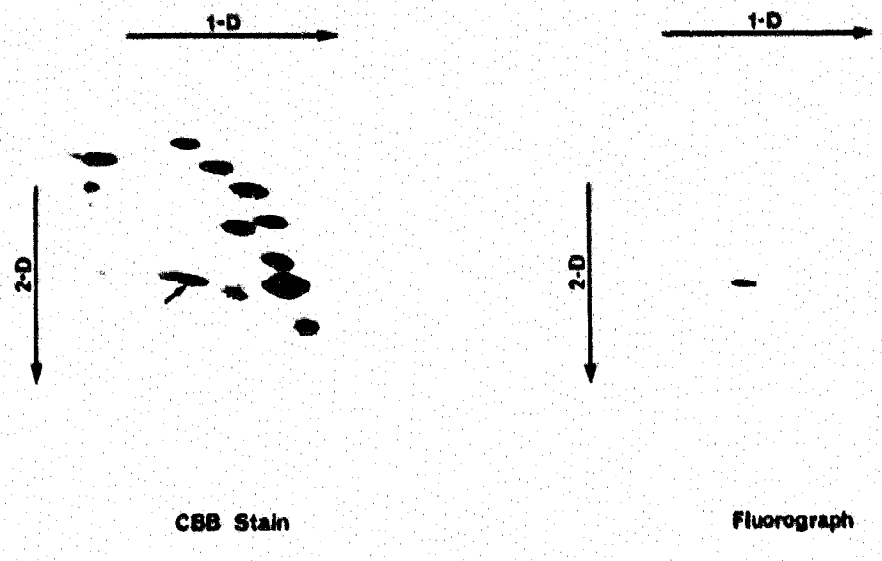

Fig. 3. Two-dimensional gel electrophoresis of the in vitro synthesized product of *rig* cDNA. In vitro transcription/translation product of *rig* cDNA was applied to the two-dimensional-PAGE with 50 μg 40 S subunit proteins. Electrophoresis was from left to right in the first dimension and top to bottom in the second. (Left panel) Coomassie brilliant blue (CBB) stain. The arrow indicates the spot of S15 protein. (Right panel) Fluorography.

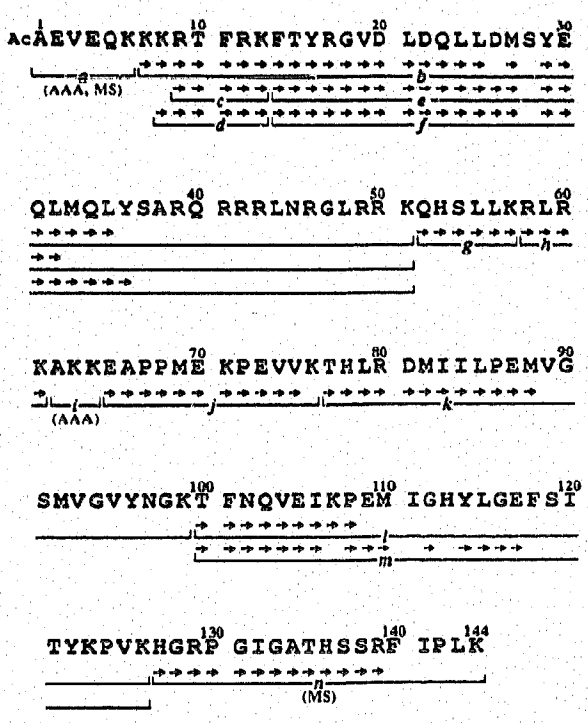

Fig. 4. The primary structure of rat liver ribosomal protein S15. The amino acid sequence deduced from rat *rig* cDNA [1] except for the initiator methionine is presented with the single letter code. The residues in rat ribosomal protein S15 identified by Edman degradation are marked with arrows. Brackets (*a–n*) indicate the endopeptidase fragments. Fragments *a*, *i* and *n* are assigned to the sequences based on the results of the analyses designated in parentheses. AAA, amino acid analysis; MS, mass spectrometry; AcA, acetyl alanine. Amino acid residues are numbered beginning with acetyl alanine.

in rat ribosomal protein S15 the initiator methionine is removed and the succeeding alanine is monoacetylated.

4. DISCUSSION

The present results led to the conclusion that *rig* encodes ribosomal protein S15 and to the determination of the previously unknown primary structure of S15 protein. Screening for relationships to proteins stored in a collection of ribosomal protein sequences revealed that *rig*/S15 protein has a significant degree of homology with prokaryotic and chloroplast ribosomal S19 proteins (Fig. 5), a most highly conserved ribosomal protein among species [18]. *Escherichia coli* S19 protein is reported to be located at the top of the 'head' of the small ribosomal subunit and to be situated close to the peptidyl transferase center and the binding sites for the initiation factors, suggesting the involvement of the S19 protein in the initiation and elongation steps of translation [18]. Evidence from recent cross-linking experiments suggests that eukaryotic S15 protein has a similar role in the ribosome functions [19]. The correspondence of *rig*-encoded protein to the ribosomal protein agrees well with our previous speculation that *rig* belongs to the class of housekeeping genes [5]. The increased expression of *rig* in tumor cells such as insulinomas [1,4] and esophageal cancers [5] also agrees with the observation that ribosomal protein synthesis increases in growing cells as compared with resting cells [20].

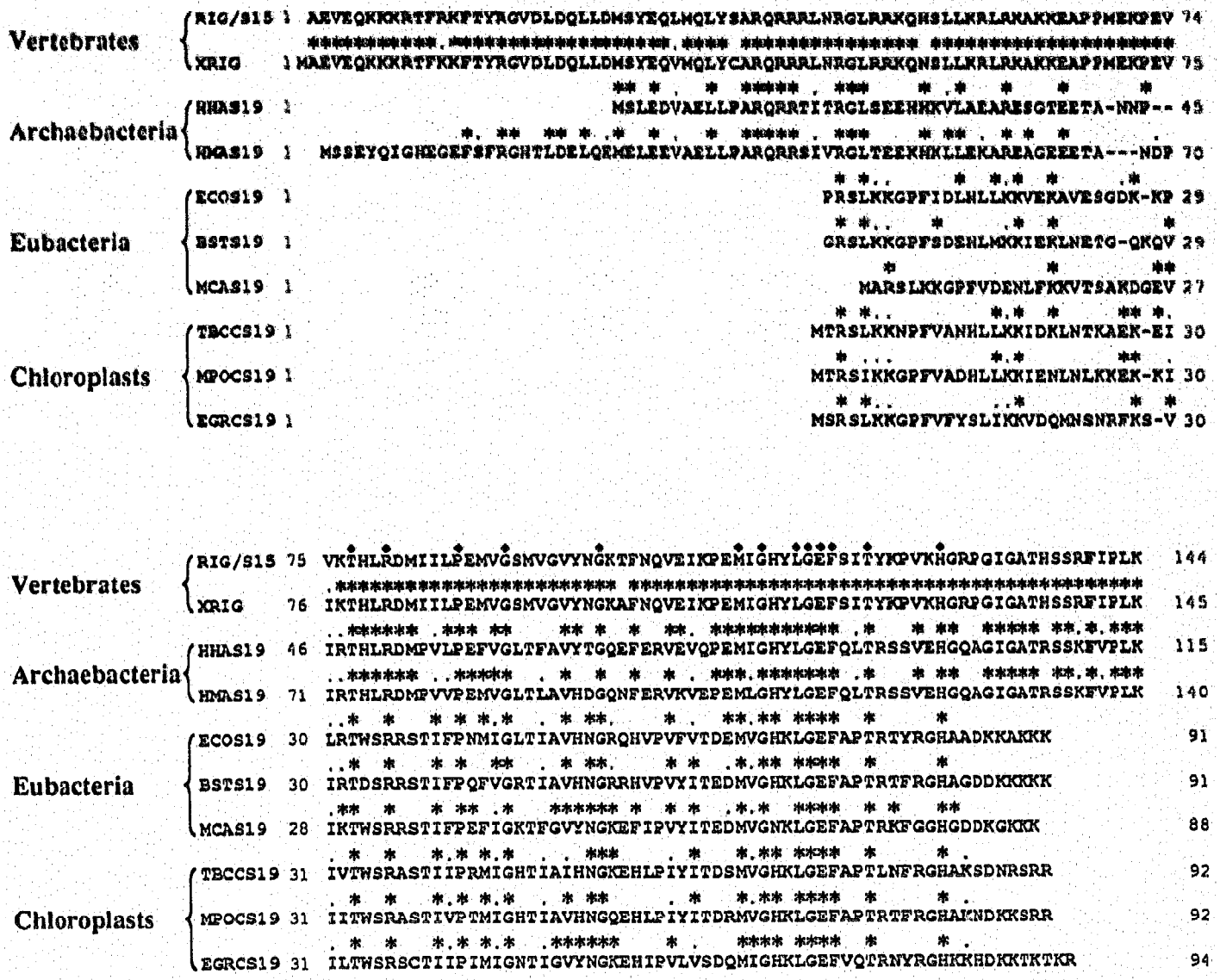

Fig. 5. Alignment of the amino acid sequences of *rig*/S15 protein from homoiotherms and *Xenopus rig* protein with the sequences of ribosomal S19 proteins from prokaryotes and chloroplasts. Amino acid residues are numbered on both sides. Residues that are identical to *rig*/S15 protein are designated by asterisks and those that are conservative substitutions are designated by dots over the one letter symbols. Residues that are conserved among all the species are designated by diamonds. RIG/S15, mammalian and chicken *rig*/S15 proteins [1,4,7]; XRIG, *Xenopus laevis rig* protein [7]; HHAS19, *Halobacterium halobium* S19 protein [21]; HMAS19, *Halobacterium marismortui* S19 protein [22]; ECOS19, *Escherichia coli* S19 protein [23]; BSTS19, *Bacillus stearothermophilus* S19 protein [18]; MCAS19, *Mycoplasma capricolum* S19 protein [24]; TBCCS19, *Nicotiana tabacum* (tobacco) chloroplast S19 protein [25]; MPOCS19, *Marchantia polymorpha* (liverwort) chloroplast S19 protein [26]; EGRCS19, *Euglena gracilis* chloroplast S19 protein [27]. Sequences of ECOS19 and BSTS19 were obtained from the proteins and sequences of XRIG, HHAS19, HMAS19, MCAS19, TBCCS19, MPOCS19 and EGRCS19 were deduced from the cDNA or genes.

Acknowledgements: We are indebted to Dr. Tatsuo Tanaka, Department of Biochemistry, School of Medicine, University of the Ryukyus for providing the data base and valuable advice, and to Drs. Yuji Ikenishi and Nobuo Yoshida for their assistance and encouragement. This work has been supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

REFERENCES

[1] Takasawa, S., Yamamoto, H., Terazono, K. and Okamoto, H. (1986) Diabetes 35, 1178–1180.

[2] Okamoto, H. (1989) in: Diabetes 1988 (Larkins, R., Zimmet, P. and Chisholm, D. eds) pp. 55–62, Elsevier, Amsterdam.

[3] Takasawa, S., Inoue, C., Shiga, K. and Kitagawa, M. (1990) in: Molecular Biology of the Islets of Langerhans (Okamoto, H. ed.) pp. 287–299, Cambridge University Press, Cambridge.

[4] Inoue, C., Shiga, K., Takasawa, S., Kitagawa, M., Yamamoto, H. and Okamoto, H. (1987) Proc. Natl. Acad. Sci. USA 84, 6659–6662.

[5] Shiga, K., Yamamoto, H. and Okamoto, H. (1990) Proc. Natl. Acad. Sci. USA 87, 3594–3598.

[6] Inoue, C., Igarashi, K., Kitagawa, M., Terazono, K., Takasawa, S., Obata, K., Iwata, K., Yamamoto, H. and Okamoto, H. (1988) Biochem. Biophys. Res. Commun. 150, 1302–1308.

[7] Sugawara, A., Nata, K., Inoue, C., Takasawa, S., Yamamoto, H. and Okamoto, H. (1990) Biochem. Biophys. Res. Commun. 166, 1501–1507.
[8] De Duve, C., Pressman, B.C., Gianetto, R., Wattiaux, R. and Appelmans, F. (1955) Biochem. J. 60, 604–617.
[9] Ogata, K. and Terao, K. (1979) Methods Enzymol. 59, 502–515.
[10] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[11] Laemmli, U.K. (1970) Nature 227, 680–685.
[12] Lastick, S.M. and McConkey, E.H. (1976) J. Biol. Chem. 251, 2867–2875.
[13] Harlow, E. and Lane, D. (1988) in: Antibodies (Harlow, E. and Lane, D. eds) pp. 471–510, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[14] Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
[15] Odani, S., Kenmochi, N. and Ogata, K. (1988) J. Biochem. 103, 872–877.
[16] McConkey, E.H., Bielka, H., Gordon, J., Lastic, S.M., Lin, A., Ogata, K., Reboud, J.-P., Traugh, J.A., Trnut, R.R., Warner, J.R., Welfle, H. and Wool, I.G. (1979) Mol. Gen. Genet. 169, 1–6.
[17] Flinta, C., Persson, B., Jörnvall, H. and von Heijne, G. (1986) Eur. J. Biochem. 154, 193–196.
[18] Hirano, H., Eckart, K., Kimura, M. and Wittmann-Liebold, B. (1987) Eur. J. Biochem. 170, 149–157.
[19] Nygård, O. and Nilsson, L. (1990) Eur. J. Biochem. 191, 1–17.
[20] Meyuhas, O. (1984) in: Recombinant DNA and Cell Proliferation (Stein, G.S. and Stein, J.L. eds) pp. 243–271, Academic Press, Orlando, FL.
[21] Mankin, A.S. (1989) FEBS Lett. 246, 13–16.
[22] Arndt, E., Krömer, W. and Hatakeyama, T. (1990) J. Biol. Chem. 265, 3034–3039.
[23] Yaguchi, M. and Wittmann, H.G. (1978) FEBS Lett. 88, 227–230.
[24] Ohkubo, S., Muto, A., Kawauchi, Y., Yamao, F. and Osawa, S. (1987) Mol. Gen. Genet. 210, 314–322.
[25] Sugita, M. and Sugiura, M. (1983) Nucleic Acids Res. 11, 1913–1918.
[26] Ohyama, K., Fukuzawa, H., Kohchi, T., Shirai, H., Sano, T., Sano, S., Umesono, K., Shiki, Y., Takeuchi, M., Chang, Z., Aota, S., Inokuchi, H. and Ozeki, H. (1986) Nature 322, 572–574.
[27] Christopher, D.A., Cushman, J.C., Price, C.A. and Hallick, R.B. (1988) Curr. Genet. 14, 275–286.