Molecular cloning of a human polypeptide related to yeast sds22, a regulator of protein phosphatase-1

Sylvie Renouf[a], Monique Beullens[a], Stefaan Wera[a], Aleyde Van Eynde[a], James Sikela[b], Willy Stalmans[a], Mathieu Bollen[a,*]

[a]*Afdeling Biochemie, Faculteit Geneeskunde, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium*
[b]*Department of Pharmacology, University of Colorado Health Sciences Center, Denver, CO, USA*

Received 20 August 1995; revised version received 1 October 1995

Abstract **sds22 is a regulatory polypeptide of protein phosphatase-1 that is required for the completion of mitosis in both fission and budding yeast. We report here the cDNA cloning of a human polypeptide that is 46% identical to yeast sds22. The human homolog of sds22 consists of 360 residues, has a calculated molecular mass of 41.6 kDa and shows a tandem array of 11 leucine-rich repeat structures of 22 residues. Northern analysis revealed a major transcript of 1.39 kb in all 8 investigated human tissues. sds22 was detected by western analysis in both the cytoplasm and the nucleus of rat liver cells as a polypeptide of 44 kDa.**

Key words: Cell cycle; Dephosphorylation; Mitosis; Protein phosphatase

1. Introduction

The serine/threonine protein phosphatases of type-1 (PP-1) comprise an abundant and widely distributed group of enzymes that all consist of an isoform of the same catalytic subunit (PP-1_C) and one or more regulatory subunits [1–3]. The noncatalytic subunits of PP-1 not only determine the activity and substrate specificity of the holoenzymes, but also target the phosphatase to a particular cellular location. The primary structure of several mammalian regulatory polypeptides of PP-1 is already known; they include cytoplasmic (inhibitor-1/DARPP-32, inhibitor-2) and nuclear (NIPP-1) inhibitory polypeptides, a glycogen-binding subunit (G-subunit) and two regulatory subunits of the myosin-associated phosphatase of smooth muscle [1–6].

sds22 was originally identified in *Schizosaccharomyces pombe* as a suppressor of defects in mitosis that result from a particular mutation in a PP-1_C isoform [7]. A further functional analysis revealed that sds22 is essential for chromosome dysjunction during the metaphase/anaphase transition. sds22 does not itself display a protein phosphatase activity, but may facilitate PP-1 mediated dephosphorylation by interaction with PP-1_C [7,8]. The central part of sds22 consists of 11 leucine-rich repeats that are essential for the interaction with PP-1_C, while the N-terminal and C-terminal domains of sds22 are important for its stability and subcellular localization, respectively. Recent studies have led to the identification of an sds22 homolog in *Saccharomyces cerevisiae* that also appears to be essential for cell division and also forms a complex with PP-1_C [9,10]. A protein homologous to sds22 is also encoded by the *Caenorhabditis elegans* genome [11].

We report here the molecular cloning of a human protein that is highly homologous to sds22 from yeast. It is also shown that the human sds22 homolog is expressed in a variety of tissues and is present in both the cytoplasm and the nucleus.

2. Experimental

2.1. Nucleotide sequence and 5′-RACE analysis

Most of the nucleotide sequence encoding human brain sds22 was obtained by analysis of a cDNA clone that was deposited as an expressed sequence tag (see Results and Discussion). The first 47 nucleotides were obtained by 5′-RACE-PCR analysis using a human brain 5′-RACE-ready™ cDNA kit from Clontech. A primary PCR reaction (35 cycles) was conducted using an anchor-specific primer and an antisense primer (GATGTTTCTCAGCAGAT) corresponding to nucleotides 470–486 in Fig. 1. In a secondary PCR amplification (35 cycles) the anchor-specific primer was used together with a nested antisense primer (ACCTCAAATCCTTCAAT) corresponding to nucleotides 286–302 in Fig. 1. The products of the second PCR amplification were subcloned in the pGEM$_T$ vector (Promega), following the instructions of the manufacturer, and sequenced using T7 and Sp6 as primers.

Nucleotide sequencing was performed using the dideoxynucleotide chain termination method [12] in an A.L.F. sequenator (Pharmacia LKB) with the Pharmacia autoread sequencing kit. Both vector-specific and cDNA-specific oligonucleotide primers were used. Sequencing was performed on both strands.

2.2. Northern analysis

A Northern blot with poly(A)$^+$ RNA from eight different human tissues (2 μg each) was purchased from Clontech. The membranes were developed with a probe encompassing nucleotides 48–1299 of the full-length sds22 cDNA (Fig. 1) and ^{32}P-labeled to a specific radioactivity of 2×10^9 cpm/μg by random priming (Gibco BRL). The blots were prehybridized (3 h) and hybridized (overnight) at 42°C in the presence of 35% formamide, 5 × SSPE, 10 × Denhardt's, 2% SDS and 100 μg/ml denatured DNA from salmon sperm [13]. During hybridization the probe concentration amounted to 1 ng/ml. Subsequently, the membranes were washed twice for 10 min at room temperature in 2 × SSC plus 0.05% SDS, followed by a 40-min wash at 50°C in 0.1 × SSC plus 0.1% SDS [13]. Filters were autoradiographed by exposure at −80°C with intensifying screens for 72 h.

2.3. Immunological techniques

A synthetic peptide, with a sequence corresponding to the 14 C-terminal residues of human sds22 plus an additional N-terminal cysteine, was coupled to keyhole limpet hemocyanin and to bovine serum albumin, using the Pierce immunogen conjugation kit. Rabbit polyclonal antibodies to the hemocyanin-coupled peptides were affinity-purified on albumin-coupled peptide that was linked to CNBr-activated

*Corresponding author. Fax: (32) (16) 34-5995.
E-mail: mathieu.bollen@med.kuleuven.ac.be

The nucleotide sequence reported in this paper has been deposited in the EMBL/GenBank database under the accession number Z50749.

Abbreviations: LRR; leucine-rich repeat; PP-1, protein phosphatase-1; PP-1_C; catalytic subunit of PP-1; RACE; rapid amplification of cDNA-ends.

SSDI 0014-5793(95)01180-3

Sepharose-4B (Pharmacia LKB). The antibodies were eluted with 'Immuno Pure Gentle Ag/Ab Elution Buffer' (Pierce), extensively dialyzed against a buffer containing 20 mM Tris-HCl at pH 7.4 and 150 mM NaCl, and stored at −20°C.

Western analysis on cytosolic and nuclear fractions was essentially done as in [14], with the affinity-purified antibodies at a final concentration of 2 μg/ml. The nucleoplasm and the nuclear 0.3 M NaCl extract were prepared as described in [14]. The nucleoplasm is defined as the supernatant that is obtained by centrifugation (5 min at 13,000 × *g*) after dissolution of the nuclear pellet in a hypotonic medium (buffer C in [14]). For the preparation of the cytosolic fraction a liver was homogenized in 2 vols. of a buffer containing 15 mM PIPES at pH 7.2, 80 mM KCl, 15 mM NaCl, 0.5 mM dithiothreitol, 0.25 M sucrose and a cocktail of protease inhibitors including 0.5 mM phenyl methanesulphonyl fluoride (PMSF), 50 μM 1-chloro-3-tosylamido-7-amino-2-heptanone-hydrochloride (TLCK), 50 μM L-1,4′-tosylamino-2-phenylethyl-chloromethyl ketone (TPCK) and 5 μM leupeptin. The cytosolic fraction was obtained by succesive centrifugations at 8,000 × *g* (10 min) and 150,000 × *g* (35 min).

3. Results and discussion

3.1. Identification of a human homolog of sds22

The 3′-end of a randomly picked human infant brain cDNA clone, which was deposited as an expressed sequence tag (EMBL/GenBank accession number T16129), was found to be homologous to sds22 from *Schizosaccharomyces pombe*. Further sequence analysis of this clone, termed IB3548, demonstrated that the 1252 bp insert (nucleotides 48–1299 in Fig. 1) was homologous with the complete sds22 cDNA sequence from fission and budding yeast (see below). However, 5′-RACE-PCR analysis, using 3′-anchored human brain cDNA as template, showed that clone IB3548 lacked the 5′-end of the coding sequence. Indeed, the 5′-RACE-PCR cloning strategy yielded two cDNA fragments with a sequence identical to the 5′-end of clone IB3548 plus an upstream sequence of 47 nucleotides

```
   1 GAATTGGCAGCCAACATGGCGGCGGAACGCGGCGCGGGGCAGCAACAGTCGCAGGAGATG
                 M  A  A  E  R  G  A  G  Q  Q  Q  S  Q  E  M  15
  61 ATGGAGGTTGACAGGCGGGTCGAGTCTGAAGAATCCGGCGATGAAGAAGGGAAGAAACAC
     M  E  V  D  R  R  V  E  S  E  E  S  G  D  E  E  G  K  K  H  35
 121 AGCAGTGGCATCGTGGCCGACCTCAGTGAACAGAGCCTGAAGGATGGGGAGGAGCGGGGG
     S  S  G  I  V  A  D  L  S  E  Q  S  L  K  D  G  E  E  R  G  55
 181 GAGGAGGACCcAGAAGAAGAACATGAGCTGCCTGTGGACATGGAAACCATCAACCTGGAC
     E  E  D  P  E  E  E  H  E  L  P  V  D  M  E  T  I  N  L  D  75
 241 AGAGATGCAGAGGATGTTGATTTGAATCACTATCGCATAGGGAAGATTGAAGGATTTGAG
     R  D  A  E  D  V  D  L  N  H  Y  R  I  G  K  I  E  G  F  E  95
 301 GTACTGAAGAAAGTGAAGACTCTCTGCCTCCGCCAAAATTTAATTAAATGCATTGAGAAT
     V  L  K  K  V  K  T  L  C  L  R  Q  N  L  I  K  C  I  E  N  115
 361 CTGGAGGAGCTACAGAGTCTTCGAGAGCTGGATCTTTACGACAACCAGATCAAGAAGATT
     L  E  E  L  Q  S  L  R  E  L  D  L  Y  D  N  Q  I  K  K  I  135
 421 GAGAATCTGGAGGCGCTAACAGAGCTGGAGATTCTAGATATTTCTTTTAATCTGCTGAGA
     E  N  L  E  A  L  T  E  L  E  I  L  D  I  S  F  N  L  L  R  155
 481 AACATCGAAGGGGTTGACAAGTTGACACGACTGAAAAAACTCTTCTTGGTCAACAATAAA
     N  I  E  G  V  D  K  L  T  R  L  K  K  L  F  L  V  N  N  K  175
 541 ATCAGTAAAATTGAGAACTTAAGCAACTTACATCAACTACAGATGCTAGAGCTGGGATCT
     I  S  K  I  E  N  L  S  N  L  H  Q  L  Q  M  L  E  L  G  S  195
 601 AACCGCATCCGGGCAATCGAAAATATCGACACCTTAACCAACCTGGAGAGTTTGTTTTTG
     N  R  I  R  A  I  E  N  I  D  T  L  T  N  L  E  S  L  F  L  215
 661 GGGAAAACAAAATTACTAAACTTCAGAACCTGGATGCGCTCACCAACCTGACAGTCCTC
     G  K  N  K  I  T  K  L  Q  N  L  D  A  L  T  N  L  T  V  L  235
 721 AGTATGCAGAGCAACCGGCTGACCAAGATCGAGGGTCTGCAGAACCTGGTGAACCTGCGG
     S  M  Q  S  N  R  L  T  K  I  E  G  L  Q  N  L  V  N  L  R  255
 781 GAGCTGTACCTTAGCCACAATGGCATCGAGGTCATCGAGGGCCTGGAGAACAATAACAAA
     E  L  Y  L  S  H  N  G  I  E  V  I  E  G  L  E  N  N  N  K  275
 841 CTCACGATGTTGGACATTGCATCAAATAGAATCAAAAAGATTGAAAATATCAGCCATCTA
     L  T  M  L  D  I  A  S  N  R  I  K  K  I  E  N  I  S  H  L  295
 901 ACAGAGCTGCAAGAGTTCTGGATGAACGACAATCTCCTTGAGAGCTGGAGCGACCTCGAC
     T  E  L  Q  E  F  W  M  N  D  N  L  L  E  S  W  S  D  L  D  315
 961 GAGCTGAAGGGAGCCAGGAGCCTGGAGACAGTGTACCTGGAGCGGAACCCCTTGCAGAAG
     E  L  K  G  A  R  S  L  E  T  V  Y  L  E  R  N  P  L  Q  K  335
1021 GACCCCCAGTACCGGCGGAAGGTCATGCTCGCCCTCCCCTCCGTGCGGCAGATCGATGCC
     D  P  Q  Y  R  R  K  V  M  L  A  L  P  S  V  R  Q  I  D  A  355
1081 ACGTTCGTCAGGTTCTGAGTCCTTCTTGGCTCCTCATGTGGTCCCTCTCCTCGGAAGAAC
     T  F  V  R  F  *                                          360
1141 TGCCCAGCCACGGGTTTTTAACCCACCTGTTGCTCCTGAGGCTCGTCACTATATCAACAGT
1201 CACAAACCCAATGGCAATAAAGGCACTGACGATAGCTGGCGCGCGCGACGCCACACACCA
1261 TTTTCAGATGCCGTTGCAATTAAATCTTGCCACACTGTC
```

Fig. 1. cDNA and deduced amino acid sequences of sds22 from human brain. The nucleotide sequence is presented in the 5′-to-3′ direction and is numbered on the left. The deduced protein sequence (one-letter-code) is given below the nucleotide sequence and is numbered on the right. Also indicated are the putative consensus translation initiator codon (bold), the stop codon (asterisk) and two putative polyadenylation signals (boxed).

```
                                  M A A   3
E R G A G Q Q Q S Q E M M E V D R R V E S E  25
E S G D E E G K K H S S G I V A D L S E Q S  47
L K D G E E R G E E D P E E E H E L P V D M  69

E T I N L D R D A E D V D L N H Y R I G K I  91
E G F E V L K K V K T L C L R Q N L I K C I  113
E N L E E L Q S L R E L D L Y D N Q I K K I  135
E N L E A L T E L E I L D I S F N L L R N I  157
E G V D K L T R L K K L F L V N N K I S K I  179
E N L S N L H Q L Q M L E L G S N R I R A I  201
E N I D T L T N L E S L F L G K N K I T K L  223
Q N L D A L T N L T V L S M Q S N R L T K I  245
E G L Q N L V N L R E L Y L S H N G I E V I  267
E G L E N N N K L T M L D I A S N R I K K I  289
E N I S H L T E L Q E F W M N D N L L E S W  311

S D L D E L K G A R S L E T V Y L E R N P L  333
Q K D P Q Y R R K V M L A L P S V R Q I D A  355
T F V R F                                  360

Consensus repeat structure

E  L  L  L  L  L  N  I  I
```

Fig. 2. Human sds22 contains 11 leucine-rich repeat structures. The repeat motifs are boxed. Also indicated is the consensus sequence for the repeat structure.

that extends the open reading frame by 14 amino acids (nucleotides 1–47 in Fig. 1). The two PCR fragments differed however in the identity of nucleotides 4–6, which were either TTG (Fig. 1) or AGA (not shown). These differences may reflect an allelic variation and/or a PCR-amplification error.

The combined information, obtained by analysis of clone IB3548 and of the RACE-PCR products, suggested a complete human sds22 cDNA sequence of 1299 nucleotides (Fig. 1). The sequence surrounding the first ATG (bp 16–18) fulfills the Kozak [15] requirements for initiation of translation in eucaryotes. Using this ATG as start codon the sds22 cDNA sequence showed an open reading frame of 1080 nucleotides, encoding a polypeptide of 360 residues with a calculated molecular mass of 41,563 Da. Although initiation of translation at internal ATGs is rare [15], it cannot be excluded that the second (bp 58–60) and third (bp 61–63) ATG are also used as (alternative) initiator codons, since they also reside in a Kozak consensus sequence. The 3′-end of the human sds22 cDNA sequence contained two putative polyadenylation signals, but lacked a poly(A)$^+$-tail.

As has previously also been demonstrated for the yeast homologs [7–10], a large fraction of human sds22 (residues 70–311) consists of 11 leucine-rich tandem repeat structures (Fig. 2), also known as LRR motifs that may participate in protein–protein interactions [7–10,16]. In contrast to yeast sds22, however, all repeats in the human polypeptide are exactly 22 residues long. Analysis of the deduced primary structure also revealed that human sds22 is relatively rich in leucine (16.1%), glutamic acid (12.8%) and asparagine (8.3%), but con-

tains only 5 proline residues (1.4%). It is an acidic polypeptide (pI = 4.7), although the charge is not distributed evenly in the three regions that correspond to functional domains in the fission yeast homolog (see Introduction). Indeed, while the N-terminus (residues 1–69) is acidic (pI = 4.0), the LRR domain (residues 70–311) is somewhat less acidic (pI = 5.1), and the C-terrminus (residues 312–360) is very basic (pI = 10.2). It is also worth noting that human sds22 contains putative phosphorylation sites for protein kinases A and C, casein kinase-2 and tyrosine protein kinases (not shown), suggesting that the polypeptide, like the fission yeast homolog [8], is a phosphoprotein.

An alignment of human sds22 with the homologs from *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and *Caenorhabditis elegans* revealed an extensive homology between all polypeptides (not shown). Using the 'bestfit' program of the GCG software [17], the identity between human sds22 and the yeast or *C. elegans* homologs in the overlapping region amounted to 46%. The homology extends to the entire polypeptide chain but is particularly strong in the LRR domain. Unique for human sds22 is an extension of 32–38 residues at the N-terminus, which accounts for the larger mass of this homolog.

3.2. Tissue distribution and subcellular localization

Northern blot analysis showed one major mRNA species of 1.39 kb in all 8 investigated human tissues (Fig. 3), indicating a ubiquitous expression of sds22. At equal mRNA loading, the 1.39 kb transcript was particularly abundant in skeletal muscle, heart and brain. Compared to the size of this major mRNA species, the cDNA encoding sds22 was about 0.1 kb smaller (Fig. 1), which is likely to be explained by the missing poly(A)$^+$-tail (Fig. 1). In addition to the major 1.39 mRNA transcript, two minor transcripts of 2.0 kb and 1.1 kb were observed (Fig. 3).

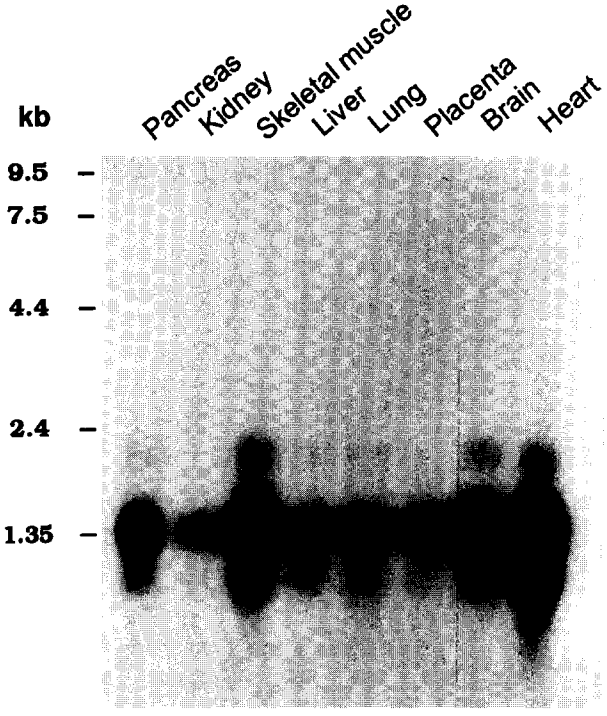

Fig. 3. Northern blot analysis in various human tissues. Each lane contained 2 μg poly(A)$^+$ RNA isolated from the indicated tissues. The blots were hybridized with a probe corresponding to bp 48–1299 of the full-length sequence of sds22 shown in Fig. 1.

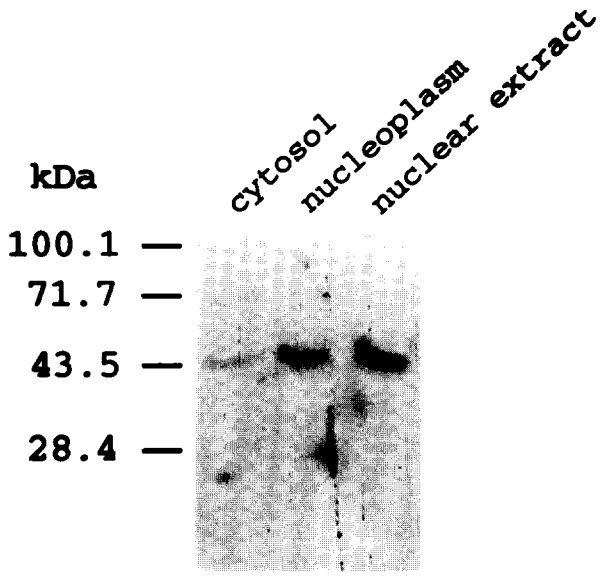

Fig. 4. Subcellular localization of sds22. A cytosolic fraction, the nucleoplasm and a nuclear 0.3 M NaCl extract were prepared from rat liver as indicated in section 2. About 15 μg protein of each fraction was subjected to 10% Tricine-SDS-PAGE, transferred to a membrane, and probed with antibodies against the C-terminus of sds22.

Antibodies raised against a synthetic peptide with a sequence encompassing the 14 C-terminal residues of human sds22 were used for western blotting of rat liver fractions (Fig. 4). The antibodies recognized a polypeptide of 44.4 ± 0.2 kDa (mean ± S.E.M; $n = 4$) in the cytosolic fraction, the nucleoplasm and the nuclear salt extract. However, at equal protein loading, the signal was much more intense in the nuclear fractions, indicating that sds22 is enriched in the nucleus, in agreement with its subcellular distribution in yeast [7,8].

3.3. Conclusions

Given the extensive overall homology (46%) with yeast sds22, we propose that the novel sequence reported here represents the human homolog of sds22. The high degree of phylogenetic conservation suggests that sds22 fulfills an essential role, which agrees with its proposed role in mitosis (see section 1). The present data constitute the basis for a functional analysis of mammalian sds22. It will be of particular interest to see whether the human protein, like the yeast homologs, is associated with PP-1_C. Preliminary evidence indicates that in a hepatic nuclear extract nearly all the type-1 catalytic subunit is associated with either NIPP-1 or an unidentified inhibitory polypeptide of 111 kDa [14]. However, it cannot be excluded that sds22 is only associated with a minor fraction of nuclear PP-1_C or that the association is lost during tissue fractionation. Alternatively, sds22 may not be a true regulatory subunit of PP-1 and may only be temporarily associated with the catalytic subunit, for example at the end of mitosis. Since hepatocytes are nearly all in G_0 phase of the cell cycle, such an association would have been missed.

Acknowledgments: The human infant brain cDNA library was kindly provided by Dr. Bento Soares at Colombia University. V. Feytons is

acknowledged for the preparation of the oligonucleotides and the peptide used in this study. Sequence analysis was done at the Belgian EMBnet Node Facility. This work was supported by the ASLK, by the Belgian Fund for Medical Scientific Research (Grant 3.0041.93) and by a Concerted Research Action of the Flemisch Government. S.R. was a postdoctoral fellow of the EU Human Capital and Mobility program.

References

[1] Bollen, M. and Stalmans, W. (1992) Crit. Rev. Biochem. Mol. Biol. 27, 227–281.
[2] Walter, G. and Mumby, M. (1993) Biochim. Biophys. Acta 1155, 207–226.
[3] DePaoli-Roach, A.A., Park, I.-K., Cerovsky, V., Csortos, C., Durbin, S.D., Kuntz, M.J., Sitikov, A., Tang, P.M., Verin, A. and Zolnierowicz, S. (1994) Adv. Enzyme Regul. 34, 199–224
[4] Shimizu, H., Ito, M., Miyahara, M., Ichikawa, K., Okubo, S., Konishi, T., Naka, M., Tanaka, T., Hirano, K., Hartshorne, D.J. and Nakano, T. (1994) J. Biol. Chem. 269, 30407–30411.
[5] Chen, Y.H., Chen, M.X., Alessi, D.R., Campbell, D.G., Shanahan, C., Cohen, P. and Cohen, P.T.W. (1994) FEBS Lett. 356, 51–55.
[6] Van Eynde, A., Wera, S., Beullens, M., Van Leuven, F., Stalmans, W. and Bollen, M. (1995) Abstract Book of the 20th European Symposium on Hormones and Cell Regulation, Mont St. Odile, France, September 22–25.
[7] Ohkura, H. and Yanagida, M. (1991) Cell 64, 149–157.
[8] Stone, E.M., Yamano, H., Kinoshita, N. and Yanagida, M. (1993) Curr. Biol. 3, 13–26.
[9] Hisamoto, N., Frederick, D.L., Sugimoto, K., Tatchell, K. and Matsumoto, K. (1995) Mol. Cell. Biol. 15, 3767–3776.
[10] MacKelvie, S.H., Andrews, P.D. and Stark, M.J.R. (1995) Mol. Cell. Biol. 15, 3777–3785.
[11] Wilson, R. et al. (1994) Nature 368, 32–38.
[12] Sanger, F., Nickel, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[13] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[14] Jagiello, I., Beullens, M., Stalmans, W. and Bollen, M. (1995) J. Biol. Chem. 270, 17257–17263.
[15] Kozak, M. (1987) Nucleic Acids Res. 15, 8125–8148.
[16] Kobe, B. and Deisenhofer, J. (1993) Nature 366, 751–756.
[17] Devereux, J., Haeberli, P. and Smithies, O. (1984) Nucleic Acids Res. 12, 387–395.