

Cloning and chromosomal assignment of a widely expressed human receptor-like protein-tyrosine phosphatase

Frank R. Jirik, Nicole M. Janzen, Ian G. Melhado and Kenneth W. Harder

The Biomedical Research Center, University of British Columbia, Vancouver, BC V6T 1W5, Canada

Received 8 August 1990; revised version received 4 September 1990

Insight into the regulation of the actions of the protein-tyrosine kinases will be greatly facilitated by the full characterization of the family of protein-tyrosine phosphatases. A search for novel phosphatases resulted in the isolation of a cDNA, termed HLPR, encoding a member of the family of human receptor-like protein-tyrosine phosphatases: its cDNA sequence predicts a protein of 793 amino acids (unglycosylated *M*, 87500) and includes a 121 residue extracellular domain, a single transmembrane segment, and two tandem intra-cytoplasmic catalytic domains. The HLPR gene is located on human chromosome 20, and the protein it encodes likely plays a fundamental role in the physiology of all cells as its expression appears to be ubiquitous.

Protein-tyrosine phosphatase; Human chromosome 20; Protein phosphorylation; Hep G2 cell

1. INTRODUCTION

The phosphorylation of specific tyrosine residues within proteins catalysed by protein-tyrosine kinases (PTK) represents a pivotal component of the control of cellular functions such as signal transduction, cell proliferation, and oncogenesis [1]. The regulation of such cellular activities is likely determined in part by a balance between phosphorylation and dephosphorylation, the latter being mediated by protein-tyrosine phosphatases (PTPase). Heterogenous PTPase activities have been detected and characterized in both soluble and particulate fractions derived from a wide array of cell types and tissues [2,3] suggesting the existence of a diverse, ubiquitously expressed, PTPase gene family.

The first PTPase whose protein sequence was determined was a low molecular weight single catalytic domain species from human placenta, termed PTPase 1B [4,5]. This protein was discovered to possess homology with the intracellular imperfect tandem repeats of the leukocyte common antigen, (CD45), a receptor-like transmembrane molecule expressed on cells of hemopoietic origin [6]. In addition to CD45, several other receptor-like PTPases have been isolated, including: LAR [7], DLAR and DPTP [8], and LRP [9].

In T-lymphocytes, CD45 may catalyse the dephosphorylation of tyrosine-505 of *lck*, thus leading to a stimulation of this PTK [10-13]. Conversely, insulin-induced *Xenopus laevis* oocyte maturation is inhibited following the microinjection of PTPase 1B protein [14].

Thus, the activity of a given PTPase may regulate the actions of a PTK in a positive or negative manner. Furthermore, adding a further layer of regulation, the receptor-like PTPases are themselves likely regulated via interaction with specific ligands. Herein, we report the isolation of a widely expressed member of the family of human receptor-like PTPases which is homologous to the murine PTPase LRP [9].

2. MATERIALS AND METHODS

The human hepatoblastoma cell line Hep G2 (American Type Tissue Collection-ATCC, Rockville, MD) was used to obtain total RNA [15]. Poly(A)⁺ RNA isolated by oligo(dT)-cellulose chromatography [16] was used to construct a cDNA library in the bacteriophage vector ZAP (Stratagene, La Jolla, CA). Filter lifts of recombinant bacteriophage plaques, grown on *Escherichia coli* strain XL1-Blue (Stratagene), were screened by standard methods [16]. For probes DNA fragments were ³²P-labeled using a random labeling kit (Pharmacia, Uppsala, Sweden). The probe employed in library screening consisted of a 1.5 kb *BsmI* to *SphI* DNA fragment spanning the two PTPase domains of murine CD45 [17]. Filter lifts were washed at low stringency in 2 × SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0) with 0.1% sodium-dodecylsulfate at 45°C followed by autoradiography on XAR-5 film (Kodak, Rochester, NY).

Hybridizing clones were 'rescued' according to the manufacturers instructions (Stratagene). Double-stranded DNA sequencing was performed [18] using the T7, KS, T3, and SK oligonucleotide primers (Statagene), as well as synthetic oligonucleotide primers using a modified T7-DNA polymerase sequencing kit (Pharmacia). Overlapping sense and anti-sense strands were sequenced.

Correspondence address: F.R. Jirik, The Biomedical Research Center, 2222 Health Sciences Mall, Vancouver, BC, Canada V6T 1W5

Abbreviations: PTPase, protein-tyrosine phosphatase; PTK, protein-tyrosine kinase

Total cellular RNA from cell lines and tissues [15] was subjected to Northern blotting [16]. cDNA restriction fragments were labeled as above for use as probes. Washing and autoradiography were carried out as above except that membranes were washed at 50–55°C in 0.2 × SSC.

For chromosomal assignment, membranes containing transferred *Hind*III digested DNA from hamster-human hybrid panels (BIOS

Corp., New Haven, CT) were probed with labeled cDNA fragments and processed according to the manufacturer's instructions.

3. RESULTS AND DISCUSSION

Screening of the Hep G2 cell line library yielded several phage clones, which were 'rescued' as plasmids.

GTGGT**ATGGATGATG**CAGTTC**AAATAACTAAGGACACAT**GTTC**AAAGAGCATAATTA**ACTTTTT**AAAAGAAGCTAGACTTCTTC**
 AGAAGCTTGCCAGTTTTT**CAAGCTGATTTCTCTCACTGGCAACTCTCAGAGTGTCTCCTACTCCACCCTCCCCTGGTGATAA**
M D S W F I L V L L G S G L I C V S A N N A T T V A P S 28
GCATGGATTCTGGTTCATTCCTTGTCTGCTCGGCAGTGGTCTGATATGTGTGCTAGTCCCAACAATGCTACCACAGTTGCACCTTC
 V G I T R L I N S S T A E P V K E E A K T S N P T S S L 56
TGTAGGAATCCAAAGATTAATTAACTCATCAACGGCAGAACAGT**AAAGAAGAGGCCAAA**ACTTCA**AAATCCA**ACTTCTCACTA
 T S L S V V A P T F S P N I T L G P T Y L T T V N S S S 84
ACTTCTCTTTCTGTGGCACCAACTT**CAGCCCAA**TATAACTCTGGGACCCACCTATTTA**ACCAC**TGTCA**ATTCTTC**AGACTCTG
 D N G T T R T A S T N S I G I T I S P N G T W L P D N Q F 113
ACAATGGGACCACAAGCAAGCACCAATCTATAGGCATTA**ACTTCA**CCAAAT**GGAAC**TGGCTTCCAGATA**ACCAGT**
 T D A R T E P W P G N S S T A A T T P E T F P P S D E T 141
CACGGATGCCAGAACAGAACCCTGGCCGGGAAT**CCAGC**ACCGCAGCA**ACCCTCCAGAA**ACTTTCCCTCCTCAGATGAGACA
P I I A V M V A L S S L L V I V F I I I V L Y M L R F K 169
CCAATTATGCGGTGATGGTGGCCCTGTCCTCTCTGCTAGTGTGCTGTTATTATCATAGTTTTGTACATGTTAAGVTTAAGA
 K Y K Q A G S H S N S F R L S N A G R T E D G E P Q C V P L 198
AATACAAGCAAGCTGGGAGCCATTCCAATTCTTTCCGCTTATCCA**AGGCCG**CACTGAGGATGGTGAGCCCCAGTGTGTCACTC
 L A R S P S T N R K Y P P L P V D K L E E E I N R R M A 226
TCTGGCCAGATCCCCAAGCACCAACAGGAAAT**CCCACCCTG**CCCGTGGACAAGCTGGAAAGAGGAAAT**AACCGG**AGAAATGGCA
 D N N K L F R E F N A L P A C P I Q A T C E A A S K E 254
GACGACAATAAGCTCTTCAGGGAGGAATTCAACGCTCTCCCTGCATGTCTATCCAGGCCACCTGTGAGGCTGCTTCCAGGAGG
 E N K E K N R Y V N I L P Y D H S R V H L T P V E G V P D 283
AAAACAAGGAAAAAATCGATGTAAACATCTTGCCTTATGACCACTCTAGAGTCCACCTGACACCGGTTGAGGGGTTCCAGA
 S D Y I N A S F I N G Y E K N K F I A A Q G P K E E T 311
TTCTGATTACATCAATGCTTCATCAACGGTTACCAAGAAAAGAA**CAAAATC**ATTGCTGCACAAGGACCA**AAAAGA**AAACG
 V N D F W R M I W E Q N T A T I V M V T N L K E R K E C 339
GTGAATGATTTCTGGCGGATGATCTGGGAACAAACACAGCCACCATCGTCA**TGGTACC**AACTGAAGGAGAGAAAGGAGTGCA
 K C A Q Y W P Q G C W T Y G N I R V S V E D V T V L V D 368
AGTGCCCAGTACTGGCCAGCAAGGCTGCTGGACTYATGGAAATATCGGGTGTCTGTAGAGGATGTGACTGCTCCTGGTGGGA
 Y T V R K F C I Q Q V G D M T N R K P Q R L I T Q F H F 396
CTACACAGTACGGAAGTTCTGCATCCAGCAGGTGGGCGACATGACCAACAGAAAGCCACAGCGCCTC**ACTCAGTTC**ACTTT
 T S W P D F G V P F T P I G M L K F L K K V K A C N P Q 424
ACCAGCTGGCCAGACTTTGGGGTGCCTTTACCCCGATCGGCATGCTCAAGTTCC**CAAGAAGGTGA**AGCCTGTAA**CCCTCAGT**
 Y A G A I V V H C S A G V G R T G T F V V I D A M L D M M 453
ATCGAGGGCCATCGTGGTCCACTGCAGTGCAGGTGTAGGGCGTACAGGTACCTTGTGCTCATTGATGCCATGTGGACATGAT
 H T E R K V D V Y G F V S R I R A Q R C Q M V Q T D M Q 481
GCATACAGACCGGAAGTGTGATGGCTTTGTGAGCCGGATCCGGCCAGCGCTGCCAGATGGTGCA**AAACCG**ATGACAG
 Y V F I Y Q A L L E H Y L Y G D T E L E V T S L E T H L 509
TATGTCTTCATATACCAAGCCCTTCTGGAGCATTATCTCTATGGAGATACAGAACTGGAAGT**GACCTCTCTAGA**AA**CCACCTGC**
 Q K I Y N K I P G T S N N G L E E E F K K L T S I K I Q N 538
AGAAAATTACAAAAATCC**AGGGACC**CAACAATGGTTAGAGGAGGAGTTAAGAAAGTTA**ACATCAATCAA**ATCCAGAA
 D K M R T G N L P A N M K K N R V L Q I I P Y E F N R V 566
TGACAAGATGCGGACTGGAAACTTCCAGCCAACATGAAGAAGA**ACCGTGT**TTTTACAGATCATTCCATATGA**ATTCAAC**AGAGTG
 I I P V K R G E E N T D Y V N A S F I D G Y R Q K D S Y 594
ATCATTCCAGTTAAGCGGGCGAAGAGAATACAGACTATGTGAACGCATCCTTTATTGATGGCTACCGGCAGAAAGGACTCCTATA
 I A S Q Q P L L H T I E D F W R M I W E W K S C S I V M L 623
TCGCCAGCCAGGGCCCTCTTCTCCACACAATTGAGGACTTCTGGCGAATGATCTGGGAGTGGAAATCCTGCTCTATCGTATGCT
 T E L E E R G G Q E K C A Q Y W P S D G L V S Y G D I T V 651
AACAGAACTGGAGGAGAGGCGCAGGAGAAGTGTGCCAGTACTGGCCATCTGATGGACTGGTGTCTATGGAGATATTACAGTG
 E L K K E E E C E S Y T V R D L L V T N T R E N K S R Q 679
GAACTGAAGAAGGAGGAGGAATGTGAGAGCTACACCGTCCGAGACCTCCTGGTCA**CCAACACCAGGG**GAGAATAAGAGCCGGCAGA
 I R Q F H F H G W P E V G I P S D G K G M I S I I A A V Q 708
TCCGGCAGTTCCACTTCCATGGCTGGCC**TGAAGTGGG**CATCCCGAGTACCGGAAAGGGC**ATGATCAGCATCATCGCC**CGGTGCA
 K Q Q Q Q S G N P I T V H C S A G A G R T G T G C A L 736
GAAGCAGCAGCAGCAGTCAGGGAACCCCATCACCGTGCAGTGCAGCGCGGGGAGGAAGGACCGGGCACCTTCTGTGCCCTG
 S T V L E R V K A E G I L D V F Q T V K S L R L Q R P H 764
AGCACCGTCTGGAGCGTGTGAAAAGCAGAGGGGATTTGGATGTCTCCAGACTGTCAAGAGCCTGGCGCTACAGAGGCCACAK
 M V Q T L E Q Y E Q Y E V Q E A Y I D A F S D F C K 793
TGGTCCAGACTGGAACAGTATGAGTCTGCTACAAGGTGGTGCAGGAGTATATTGATGCATTCTCAGATTATGCCAACTTCAA
 *
GTAAGCGGC

Fig. 1. Nucleic acid sequence and translated amino acid sequence of the HLRP cDNA. Standard one letter code is used. Potential translational start codons, correct initiation AUG, and the termination codon, are indicated in bold print. Potential N-glycosylation sites are marked by a bold letter 'N'.

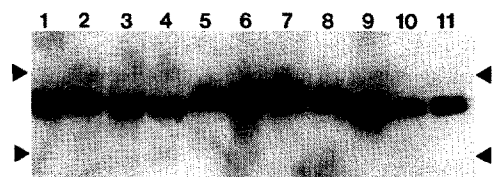


Fig. 2. Autoradiogram showing expression of HLRP in RNA of various sources. Lanes contain 15–25 µg of total cellular RNA. Arrows indicate the 28 S and 18 S ribosomal bands. Lanes 1–4, murine kidney, thymus, spleen, and liver; lanes 5–8, human Hep G2 (hepatoblastoma), T24 (bladder), AML193 (myeloid), FHs 173We (fibroblast); lanes 9–10, murine 3T3-Swiss, Pam 212 (keratinocyte); and lane 11, Rat 1.

One of these, having a 2.9 kb cDNA insert and a restriction enzyme map distinct from LAR was selected for analysis. The nucleotide sequence of this clone, designated HLRP, is shown in Fig. 1. Analysis of 36 human-hamster hybrids revealed HLRP-specific restriction fragments only in the DNA from 3 of 4 hybrids containing human chromosome 20, no discordancies were observed with all other human chromosomes. Interestingly, PTPase 1B has been mapped to a region of chromosome 20 containing the *src* and *hck* PTKs [19].

The 5' -untranslated sequence contains four upstream AUG initiation codons each of which is followed at a short distance by an in-frame termination codon. Upstream AUG codons, thought to regulate transla-

tion, are found in only a limited number of genes [20], although those encoding proto-oncogenes tend to be over-represented in this group. The fourth, and likely translationally efficient [20] AUG in the HLRP sequence is followed by a 2380 bp open reading frame. The position of this start codon is identical to that observed in the murine homologue LRP [9].

Northern blot analysis (Fig. 2) reveals HLRP transcripts in a variety of human cell lines. Transcripts were also detected in RNA from murine tissues and cell lines (Fig. 2). Although not shown, transcripts were detected in murine testes, brain, heart, and in primary smooth muscle and mast cells. Thus, transcripts hybridizing to HLRP probes appear to be ubiquitously expressed and in the rodent RNA likely result from LRP gene expression.

The human cell line RNA contains two classes of transcripts in the 3–3.5 kb range (Fig. 2). These are detected equally well by HLRP cDNA probes made from either the extracellular, or the catalytic domains. These transcripts may represent transcription from two different promoters, use of different polyadenylation signals, or alternate splicing of exons as has been observed in the first catalytic domain of the murine PTPase LRP [9].

The sequence of HLRP (Fig. 1) is predicted to encode a protein of 793 amino acids which on leader peptide removal [21] yields a predicted *M_r* 87 300 (unglycosylated). Four domains follow: an extracellular domain, a 26 residue transmembrane segment, and two tandem in-

		256		330
HePTP	D1	NKERNRVYNILPYDHSRVHLTFVEGV.....	PDSYDINASFINGYQEKNFIAAQGFKEETVNDVFRMIWEQNTATIVMV	
LAR	D1	--P---A-VIA-----I--SID-----	-G-----NY-D--RKQ-AY--T---LP--MG-----V---R---V--M	
DPTP	D1	-AC---PD-KA--QT--K-AVIN-L.....	QIT---N-VI--K-RK--C---M-S-ID-----HLEI--IL	
CD45	D1	-QN-----D-----YN--E-SEIN-D.....	AG-N-----Y-D-FK-PR-Y-----RD--D-----KATV----	
		549		623
HePTP	D2	NMKNRVLQIIPYFNRVIIIPVVRGE.....	ENTDYVNASFIDGYRQKDSYIASQGPLLHIIEDVFRMIWEWKSISIVML	
LAR	D2	-KF---LVN-M---LT--CLOPI--V.....	-GS--I---L-----QKA---T---AEST-----L--HN-TI----	
DPTP	D2	-NH---SQE---DR---LTP LPMR.....	---ST-I-----E--DNSETF-IA-D-FEN-G-----S-QSVTTL--I	
CD45	D2	-KS---NSNV---DY---PLKHELEMSKESEHDSDESSDDSDSE-PSK-I-----	HS-WKPEVM--A---KE--G--Q--FQR-VKV----	
		331		421
HePTP	D1	TNLKERKECKCAQYNP..DQGCNTYGNIRVSVEDVTVLVVDTYVRKFCIQQVGDH.TNRKFORLIQFHFTSWPFGVFPFTPIGMLKF LKKVKAC		
LAR	D1	-R-E-KSRV--DQ---.AR-TE-C-L-Q-TLL-TVE-AT---T-ALHKS-SS....EK-ELR-Q-MA---H---EY-TPI-A--RR----		
DPTP	D1	---E-YNKA---K---EKVFDTKQF-D-L-KFAQERKIG--IE-TLNVSKNKANVGEEEDR-Q---Y-YLT-K--MA-EH-H-II---IRQINSV		
CD45	D1	-RCE-GNRN---E---SMEE-TRAF-DV-V-KINQHKRCP-YIQKLN-VNKKK.....ATG-EVTHIQ-----H---ED-HLL--LRRR-N-F		
		624		708
HePTP	D2	TELEERGOEKAQYNP..SDGLVSYGDIIVELKKEEESYTVRDLVNTNTREN.....KSRQIRQFHFHGWV..EVGIPSDGKGMISIIAAVQ		
LAR	D2	-K-R-M-R---H---.AERSAR-QYFV-DPMA-YNMPQ-IL-EFK--DA-DG.....Q--T---Q-TD---Q-V-KT-E-F-DF-GQ-H		
DPTP	D2	S-IGD -FR--PR--A...-DE-Q-DH-L-KYVHS-S-PY--R-EFY---CKID.....DTLKVT--QYN---TVDGEV-EVCR-I-ELVDQAY		
CD45	D2	---KHGDQEI---G...E-RQT---E-D--DTKSS--L-VFELRHSKRK.....D--TVY-YQYTN-S..VEQL-AEP-EL--M-.Q-V		
		422		501
HePTP	D1	NPO.....YAGAIVVHCSAGVGRGTGFVVIDAMLDMMHTERKVDVYGFVSRIRARQCQMVDQYVFIYQALLESYLYGDTELE		
LAR	D1	--L.....D--PM-----C-I-----ER-KH-KT--I--H-TCM-S--NY---ED---HE---AATC-H--VP		
DPTP	D1	YSL.....QR-PIL-----TL-AL-SLIQQLLE-DS-SI-NT-CDL-H--NFL-SLK--I-L-R---DTGTF-N-DIC		
CD45	D1	SNF.....FS-P-----YIG-----EGLEA-N-----Y-VKL-R--L---VEA--IL-H---V-NQF-E--VN		
		709		791
HePTP	D2	KQQQQ.....SGNH.PITVHCSAGAGRTGTFVITVLERVKAEGILDVDFQTVKSLRLRQPHMVQTLQYEFYCVKVOEYIDAFSDYAN		
LAR	D2	-TKE.....F-QDG-----V---V-IT--I---MRY--VV-M-----T--T---A---ED--QL--RAAL--LGS-DH--T		
DPTP	D2	NHYKNNKN.....CRS-L---L-TD-SSI-V-MCIVQHLRL-KCV-ICA-TRK--S--TGLINSYA---LHRAIIN-S-LHHIAES		
CD45	D2	--KLPQKNSSEGNKHHKST-LLI--RD-SQ--I---LNL--SAET-EVV-I--V--A--KA-LG--S-F---Q-L-D-IASVTP-QNGQVK		

Fig. 3. Alignment sequences (one letter code) of HLRP, CD45, LAR, and DPTP. D1 and D2 indicate the first and second catalytic domains of each molecule. Residues identical to those of HLRP are represented by dashed lines. Gaps with dotted lines are for alignment optimization.

tracellular PTPase catalytic domains; an organization typical of receptor-like PTPases. Alignment of the intracellular sequences of HLRP, LAR, DPTP and CD45 reveals considerable amino acid sequence identity within the catalytic domains of all four molecules (Fig. 3). In domain one, for example, from residues 256–330, 331–421, and 422–501, HLRP shares 40%, 13%, and 34% identical residues, respectively, with the three other PTPases. The higher degree of conservation at either end of this catalytic domain suggests that the more degenerate central region subserves a structural function or determines substrate specificity. In domains one and two, HLRP and LAR share 56% and 52% identical amino acids, respectively. In the transmembrane and intracellular domains HLRP shares 99% identical residues with the murine LRP.

The 121 residue extracellular region of HLRP (and murine LRP), bears eight potential N-linked and multiple potential O-linked glycosylation sites, and is of modest size compared to the extracellular regions of T200/CD45 (541 residues), LAR and DLAR (1234 and 1345 residues), and DPTP (782 residues). This region of HLRP, while showing no similarity to these larger PTPases, demonstrates 76% identity with the corresponding region of the murine LRP. The modestly sized extracellular domain of HLRP likely does not serve as a receptor for a soluble ligand, it may instead interact with another membrane bound protein to form a complex.

HLRP represents a new member of the family of human receptor-like PTPases that, similar to the receptor PTKs, demonstrate conservation of their intracellular catalytic domains yet show considerable heterogeneity in their extracellular domains. Its widespread distribution suggests that HLRP is fundamental to the physiology of all cells. Furthermore, its expression may be coordinated with that of a ubiquitous PTK, such as the insulin receptor. The further study of these phosphatases, and particularly the characterization of the extracellular and intracellular molecules they interact with, may lead to the identification of novel regulatory mechanisms involving interactions between the PTKs and the PTPases.

Acknowledgements: We thank Dr J. Marth for the discussions that led to this study, and Drs S.L. Pelech and I. Clark-Lewis for their criticism of the manuscript. F.R.J. is supported by a Canadian Arthritis Society Associateship.

REFERENCES

- [1] Park, M. and Vande Woude, G.F. (1989) in: *Cancer: Principles and Practice of Oncology* (Rosenberg, S.A., De Vita V.T. and Hellman eds) J.B. Lippincott, Philadelphia, PA.
- [2] Lau, K.-H.W., Farley, J.R. and Baylink, D.J. (1989) *Biochem. J.* 257, 23–36.
- [3] Jones, S.W., Erikson, R.L., Ingebritsen, V.M and Ingebritsen, T.S. (1989) *J. Biol. Chem.* 264, 7747–7753.
- [4] Charbonneau, H., Tonks, N.K., Walsh, K.A. and Fischer, E.H. (1988) *Proc. Natl. Acad. Sci. USA* 85, 7182–7186.
- [5] Tonks, N.K., Diltz, C.D. and Fischer, E.H. (1988) *J. Biol. Chem.* 263, 6731–6737.
- [6] Thomas, M.L. and Lefrancois, L. (1988) *Immunol. Today* 9, 320–326.
- [7] Strueli, M., Krueger, N.X., Hall, L.R., Schlossman, S.F. and Saito, H. (1988) *J. Exp. Med.* 168, 1523–1530.
- [8] Strueli, M., Krueger, N.X., Tsai, A.Y.M. and Saito, H. (1989) *Proc. Natl. Acad. Sci. USA* 86, 8698–8702.
- [9] Matthews, R.J., Cahir, E.D. and Thomas, M.L. (1990) *Proc. Natl. Acad. Sci. USA* 87, 4444–4448.
- [10] Marth, J.D., Cooper, J.A., King, C.S., Zeigler, S.F., Tinker, D.A., Overell, R.W., Krebs, E.G. and Perlmutter, R.M. (1988) *Mol. Cell. Biol.* 8, 540–550.
- [11] Mustelin, T., Coggeshall, K.M. and Altman, A. (1989) *Proc. Natl. Acad. Sci. USA* 86, 6302–6306.
- [12] Pingel, J.T. and Thomas, M.L. (1989) *Cell* 58, 1055–1065.
- [13] Ostergaard, H.L., Shackelford, D.A., Hurley, T.R., Johnson, P., Hyman, R., Sefton, B.M. and Trowbridge, I.S. (1989) *Proc. Natl. Acad. Sci. USA* 86, 8959–8963.
- [14] Tonks, N.K., Cicirelli, M.F., Diltz, C.D., Krebs, E.G. and Fischer, E.H. (1990) *Mol. Cell. Biol.* 10, 458–463.
- [15] Chomczynski, P. and Sacchi, N. (1987) *Anal. Biochem.* 162, 156–159.
- [16] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) *Molecular Cloning: a Laboratory Manual*, Cold Spring Harbor University Press, Cold Spring Harbor, NY.
- [17] Thomas, M.L., Reynolds, P.J., Chain, A., Ben-Neriah, Y. and Trowbridge, I.S. (1987) *Proc. Natl. Acad. Sci. USA* 84, 5360–5363.
- [18] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) *Proc. Natl. Acad. Sci. USA* 74, 5463–5467.
- [19] Brown-Shimer, S., Johnson, K.A., Lawrence, J.B., Johnson, C., Bruskin, A., Green, N.R. and Hill, D.E. (1990) *Proc. Natl. Acad. Sci. USA* 87, 5148–5125.
- [20] Kozak, M. (1987) *Nucleic Acids Res.* 15, 8125–8148.
- [21] von Heijne, G. (1986) *Nucleic Acids Res.* 14, 4683–4691.