Vol. 203, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 30, 1994 Pages 190-199

CHARACTERIZATION OF THE GENOMIC STRUCTURE, CHROMOSOMAL LOCATION AND PROMOTER OF HUMAN PROSTAGLANDIN H SYNTHASE-2 GENE

Ryushi Tazawa, Xiao-Ming Xu, Kenneth K. Wu and Lee-Ho Wang*

Division of Hematology and Vascular Biology Research Center, Department of Internal Medicine, University of Texas-Houston, Houston, TX 77030

Received June 20, 1994

SUMMARY Prostaglandin H synthase (PGHS) is the rate-limiting enzyme in the conversion of arachidonic acid to prostanoids. The human PGHS has two isoforms. PGHS-1 is a house keeping gene whereas PGHS-2 is an inducible gene. We reported here the isolation of the entire PGHS-2 gene and its 5'-flanking region from a human bacteriophage P1 genomic library. The gene containing 10 exons is 7.5 kb in length and located at chromosome 1. The transcriptional start site was mapped at 134 bases upstream from the ATG start codon. Nucleotide sequence of 1.8 kb promoter region contains a TATA box and a number of potential regulatory elements including CRE, NF-κB, Sp1 and AP2 sites. Studies of the promoter activity showed that the first 460 nucleotides of 5'-flanking region efficiently drove transcription of the luciferase reporter gene in human umbilical vein endothelial cells upon stimulation with phorbor ester.

Prostaglandin H synthase (PGHS) catalyzes the first step of prostanoid biosynthesis (1-3). PGHS is a homodimer which exhibits two enzymatic activities; a cyclooxygenase activity that converts arachidonic acid to prostaglandin G_2 (PGG_2) and a peroxidase activity that transforms PGG_2 to PGH_2 (4). PGH_2 is the precursor of biologically important prostanoids such as thromboxane A_2, prostacyclin and PGE_2. Therefore, PGHS occupies a pivotal position in prostanoid biosynthesis and is also a target for non-steroidal anti-inflammatory drugs (5).

Recent studies showed the existence of two isoforms of PGHS. Type 1 (PGHS-1) is expressed constitutively in most tissues whereas type 2 (PGHS-2) is expressed only following cell activation

* To whom correspondence should be addressed at Division of Hematology, Department of Internal Medicine, P.O. Box 20708, University of Texas-Houston, Houston, TX 77225.

Abbreviations used: PGHS, prostaglandin H synthase; PGH_2, prostaglandin H_2; HUVEC, human umbilical vein endothelial cell.

0006-291X/94 $5.00

by growth factors, cytokines and mitogens. PGHS-1 was initially purified from ovine and bovine seminal vesicles (6-8). The isolation and sequence analyses of cDNAs encoding ovine, murine and human PGHS-1 showed a high degree of similarity (90%) in amino acid sequences among those species (9-13). The genomic DNAs for murine and human PGHS-1 have been shown to comprise 11 exons and 10 introns and are approximately of 22 kb in length (14, 15).

Although PGHS-2 has approximately 60% sequence identity with PGHS-1 and contains all the structural features important for PGHS enzymatic activity (16-21), this isoform is encoded by a distinct gene. The mRNA transcript of the PGHS-2 gene is about 4.0-4.5 kb in length compared to 2.8 kb for PGHS-1. In addition, the genomic DNAs of mouse, rat and chicken PGHS-2 have been isolated and the genes are distinct from those of PGHS-1 (22-24). It has been proposed that PGHS-1 is associated with production of prostaglandins involved in cellular "housekeeping" functions whereas PGHS-2 is in various biological events such as injury, inflammation and proliferation.

To understand the regulation of human PGHS-2 gene transcription, we have obtained from a human bacteriophage P1 genomic library a PGHS-2 gene with a 1.8-kb of 5'-flanking region. Here we reported characterization of structure of human PGHS-2 gene and its promoter activity.

MATERIALS AND METHODS

Tissue culture--Human umbilical vein endothelial cells (HUVECs) were prepared from freshly isolated umbilical veins as previously described (25, 26). The cells were grown in medium 199 supplemented with 20% heat-inactivated fetal calf serum, 50 μg/ml endothelial cell growth factor, 100 units/ml heparin, 100 μg/ml streptomycin, and 100 units/ml penicillin. Cells within three passages were used for the experiments.

Isolation of human PGHS-2 genomic DNA-- The high degree of similarity in genomic structures of murine PGHS-1 and PGHS-2 genes led us to assume that human PGHS-2 gene had genomic structure similar to PGHS-1 gene. Two primers, X30, 5'-AGCCTCGGCCAGATGGC-3' (1490- 1506 bp downstream from the ATG start codon; coding strand) and X31, 5'-AGGGACAGCCCTTCACG -3' (1671- 1687 bp downstream from the ATG start codon; complementary strand) were synthesized. The sequences of X30 and X31, as predicted, are in the last exon of PGHS-2 gene. PCR detection of a 198-bp fragment using primers X30 and X31 was carried out to screen a human genomic library constructed in bacteriophage P1 vector (Genome Systems, St. Louis, MO) (27). The conditions for PCR are: 3 cycles of 94 °C for 2 min, 52 °C for 1 min and 72 °C for 1 min followed by 27 cycles of 94 °C for 1 min, 52 °C for 1 min and 72 °C for 1 min. One positive clone, named #261 (or the company's official name DMPC-HFF#1-1415B), was isolated.

Exon-intron structure of PGHS-2 gene-- Twenty oligonucleotides corresponding to sequences throughout the human PGHS-2 cDNA were used to sequence the exon-intron regions of the PGHS-2 genomic gene. The P1 clone #261 was sequenced by dsDNA cycle sequencing using 1 μg of the P1 DNA and 1 pmol of ^{32}P end-labeled primer following the manufacture's protocol (BRL Life Technologies; Gaithersberg, MD). The sizes of introns were determined by PCR amplification

using primers from adjacent exons and followed by gel electrophoresis of the PCR products. PCR was performed at 95 °C for 1 min, 55 °C for 2 min and 70 °C for 2 min for 30 cycles.

Construction of cosmid library of the P1 clone #261-- The insert of the P1 clone #261 is approximately 60 kb in length. To facilitate the restriction mapping, a cosmid library of the P1 clone #261 was constructed. Five μg of P1 DNA were equally distributed to five tubes and treated with 10, 30, 50, 70 and 100 μU of Sau3A in 25 μl of digestion solution at 37 °C for 10 min. The solution was then extracted with phenol-chloroform. The DNAs in five tubes were combined, precipitated with ethanol and dissolved in 10 μl of TE buffer. Ligation and packaging were carried out according to manufature's protocol (Strategene; La Jolla, CA) using 2 μl of the partially-digested P1 DNA and 1 μg of BamHI-digested pWE15 vector. Cosmids containing the human PGHS-2 gene were isolated by hybridization with the human PGHS-2 cDNA (kindly provided by Dr. T. Hla). Restriction enzyme map of the inserts was determined using a cosmid mapping kit (Takara Biochemicals; Berkeley, CA) (28).

Primer extension analysis and DNA sequencing of the human PGHS-2 gene promoter-- Oligonuceotide X143 (5'-AGACGCCCTCAGACAGCAAAGCCTA-3') complementary to bases 55 to 81 upstream from the ATG start codon of human PGHS-2 gene was end-labelled with [γ-^{32}P]ATP by T4 polynucleotide kinase. Forty μg of total RNA from human foreskin fibroblasts were incubated with end-labelled X143 (1 x 10^6 cpm) in 20 μl of 40 mM PIPES (pH 4.6) containing 1 mM EDTA, 0.4 M NaCl and 80% formamide. The mixture was heated at 85 °C for 10 min and then 30 °C for 12 h. The resultant X143:RNA hybrid was precipitated with ethanol and resuspended in 15 μl of 50 mM Tris (pH 7.6), 60 mM KCl, 10 mM $MgCl_2$, 1 mM each of dNTP, 1 mM DTT, and 15 units of RNase inhibitor. Murine reverse transcriptase (25 units) was added and the mixture was incubated at 37 °C for 2 h. Following the digestion with RNase, the products were precipitated with ethanol and analyzed by electrophoresis on a 6% polyacrylamide sequencing gel to identify the transcriptional start site.

To sequence the promoter region, the 4-kb EcoRI fragment containing the 5'-flanking region of the PGHS-2 gene was subcloned into pGEM7Zf to generate pPGS4. The resulting plasmid was linearlized by KpnI and SacI, followed by digestion with exonuclease III to generate a series of 5'-deletion mutants for sequencing.

Construction of PGHS-2 reporter vector-- The deletion mutants of 5'-flanking region of PGHS-2 gene obtained as described above were used as templates in PCR amplification to construct the PGHS-2 reporter vector. Primers used were; XBT7, 5'-CGGGATCCTAATACGACTCACTATAG-GGCGA-3', T7 promoter sequence (BamHI site is underlined), and X70, 5'-CCAAGCTTGACAAT-TGGTCGCTAACCGAG-3', complimentary strand of the region -13 to +9 relative to the PGHS-2 transcriptional start site (HindIII site is underlined). The amplified DNA fragments containing the regions -891/+9 and -459/+9 were subcloned into the BamHI/HindIII site of firefly luciferase reporter vector pXP1 (29), resulting in the construct pPGS891LUC and pPGS459LUC, respectively.

Transfection and luciferase assay-- Two days prior to transfection, HUVECs were seeded at 30-40% confluence in 10-cm tissue culture dishes. Plasmid DNAs were isolated using a Quiagen DNA purification kit (Chatsworth, CA) and introduced into the cells by lipofectin method as previously described (30). Briefly, HUVECs were washed twice with serum free medium Opti-MEM (GIBCO), and then incubated with a lipofectin-DNA mixture containing 20 μg of plasmid DNA and 30 μg of lipofectin (BRL Life Technologies) for 5 h. Subsequently, the transfected cells were grown in the culture medium for 40 h and then treated with 50 nM phorbol 12-myristate 13-acetate (PMA) for 8 h.

For luciferase assay, the transfected cells were harvested, resuspended in 0.6 ml lysing buffer containing 25 mM Tris-phosphate, pH 7.8, 2 mM DTT, 2 mM 1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid, 10 % glycerol and 1 % Triton X-100 and subjected to brief sonication. Cell pellets were centrifuged and 100 μl of supernatant were used to determine the luciferase activities using a Monolight model 2010 luminometer (Analytical Luminescence laboratories).

Chromosomal localization of the human PGHS-2 gene by somatic cell hybrid panel--DNA isolated from a pre-defined panel of human/Chinese hamster somatic cell hybrids (BIOS Corporation, New Haven, CT) was screened for chromosomal determination of PGHS-2 gene by PCR detection of a 198-bp DNA fragment. Primers used were X30 and X31 as described above. PCR was performed using 50 ng of somatic cell hybrid DNA, 10 pmol each of primer and 2.5 units of Taq polymerase in 100 μl buffer. The PCR products were then analyzed on a 2% agarose gel.

RESULTS AND DISCUSSION

The bacteriophage P1 clone #261 was isolated by the PCR screening of a human genomic library using a pair of primers, X30 and X31, which were predicted to be located in the last exon of human PGHS-2 gene based on the genomic structures of the murine PGHS-1 and PGHS-2 genes. The clone #261 was subjected to double-stranded DNA cycle sequencing using the human PGHS-2-specific primers. The exon sequences thus determined matched the reported cDNA sequence (20). The sequences of exon-intron junctions were determined by comparing the cDNA sequence and genomic sequence. The human PGHS-2 gene is composed of 10 exons (Table I). All the exon-intron junctions follow GT-AG rule and agree with consensus sequences for the donor and acceptor sites (31). Furthermore, the polypyrimidine tracts which favor the RNA splicing were found within the 30 bases upstream from the 3' acceptor sites of the human PGHS-2 gene (data not shown) (32).

Table I. Exon-intron junctions of the human PGHS-2 gene. Capital letters in the DNA sequences represent nucleotides present in exons. Lowercase letters represent nucleotides present in introns. The numbers in the splice position correspond to the nucleotide sequences downstream from the ATG start codon in the human PGHS-2 cDNA as reported previously (20).

Intron No.	Size (kb)	Splice position	Exon	Intron donor	Intron acceptor	Exon
1	0.78	52/53	..TACAG	gtgagtacct.....	ttaattgcag	CAAAT..
2	0.12	169/170	..AACAC	gtaagtttgt.....	cgtcttgcag	CGGAA..
3	0.65	313/314	..GACAT	gtaagtacaa.....	aaaatttcag	CCAGA..
4	0.42	457/458	..CAAAG	gtgagtaaga.....	cattttcag	GTAAA..
5	0.73	639/640	..ATGGG	gtaagataga.....	cattttctag	GTGGA..
6	0.13	723/724	..ATCAG	gtatgcttcc.....	attttttag	ATAAT..
7	0.29	970/971	..GATAG	gtaaacaaga.....	gtgcaaatag	GAGAG..
8	0.52	1257/1258	..GCAGG	gtaagcatta.....	ctttccacag	GTTGC..
9	0.49	1405/1406	..TACAG	gtaagaaaca.....	gccttcacag	GAGAA..

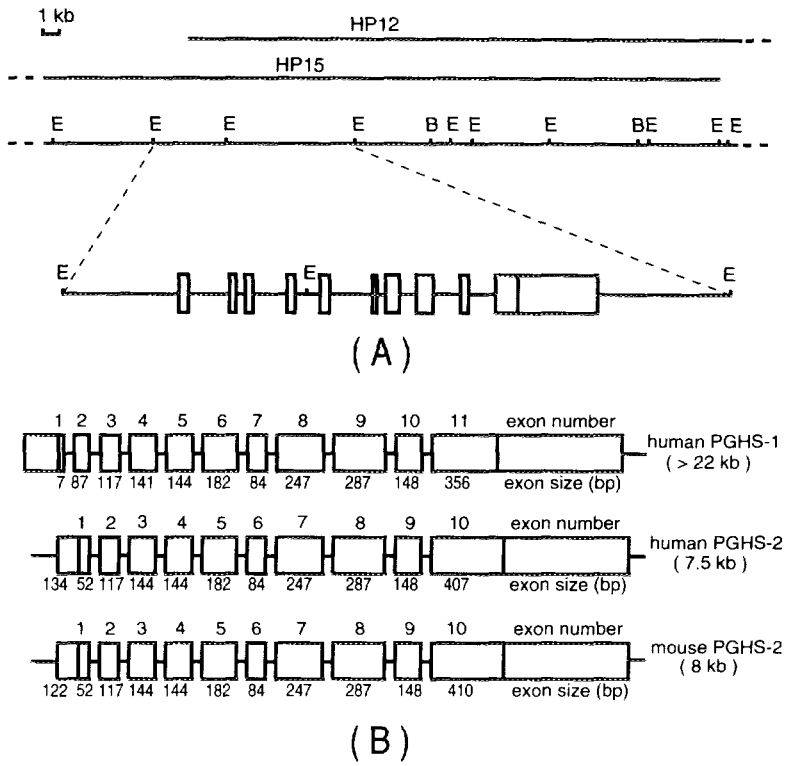

Fig. 1. Genomic structure of the human PGHS-2 gene. (A) Restriction enzyme map of PGHS-2 gene. Overlapping cosmid clones, HP12 and HP15, used to construct the restriction enzyme map are indicated at the top of the panel. Restriction enzymes shown are E, EcoRI; and B, BamHI. The exons are shown as open boxes at the bottom of the panel. (B) Comparison of the sizes of exons in human PGHS-1, human PGHS-2 and mouse PGHS-2 genes. The sizes of exons of human PGHS-1 (14) and those of mouse PGHS-2 (22) were compared with human PGHS-2 gene. The vertical lines dividing the first exons correspond to the ATG start codons whereas the lines within the last exons correpond to the translational stop codons.

The insert of clone #261 was estimated to be 60-70 kb in length which was too long for restriction enzyme mapping. A cosmid library of the P1 clone #261 was therefore constructed and screened using PGHS-2 cDNA as a probe. Two cosmid clones, HP12 and HP15, were isolated and confirmed by cycle sequencing for their authenticities. Restriction enzyme mapping revealed that both clones were overlapping and represented a contiguous genomic DNA of more than 40 kb. The sizes of introns were determined by PCR using the clone HP15 as a template. The maps constructed by these procedures are shown in Fig. 1 and summarized in Table I. Those results also show that the murine and human PGHS-2 genes have a similar structure in genomic organization.

Southern blot analysis of the clone #261 showed that the human PGHS-2 cDNA hybridized with two EcoRI fragments (4 and 8 kb) and one BamHI fragment (15 kb). High-stringency southern blot of human genomic DNA with the PGHS-2 cDNA probe identified bands identical to all of the restriction enzyme-digested clone #261 (data not shown), indicating a single copy of PGHS-2 gene is present in the human genome. The chromosomal localization of PGHS-2 gene was assigned by PCR screening for 198-bp DNA fragment in a panel of human/Chinese hamster somatic cell hybrid DNAs. The 198-bp amplified fragment was present in a human genomic DNA and in hybrid clones 867, 937 and 1099, and was absent in the remaining hybrid and hamster DNAs (data not shown). The authenticity of the 198-bp fragment was further confirmed by digestion with StyI which recognized an unique StyI site within the 198-bp fragment. The results allow us to assign the human PGHS-2 gene to chromosome 1, confirming the recent result that human PGHS-2 gene was localized at 1q25.2-q25.3 (33).

To determine the transcriptional start site, primer extension analysis was performed using total RNA isolated from PMA-stimulated and unstimulated human foreskin fibroblast cells. As expected, the PGHS-2-specific primer extension product was observed in the stimulated RNA sample, but not in the unstimulated sample. The transcriptional start site thus determined was located at 134 bases upstream from the ATG start codon (Fig. 2). The total length of human PGHS-2 gene from the

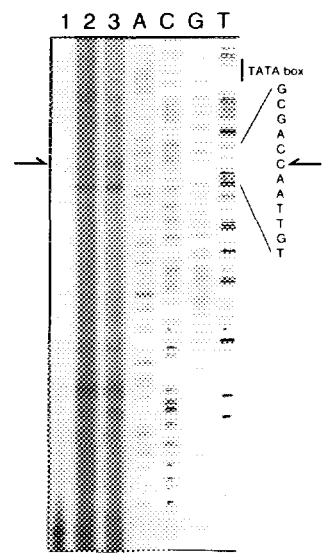

Fig. 2. Primer extension analysis of the human PGHS-2 mRNA. The ^{32}P-labeled primer X143 was hybridized with 40 μg of yeast tRNA (lane 1), total RNA from unstimulate (lane 2) and PMA-treated (lane 3) human foreskin fibroblasts, and then extended with murine reverse transcriptase. X143 was also used as a primer for DNA sequencing of pPGS4. The products of DNA sequencing and primer extension were resolved on a 6 % polyacrylamide sequencing gel. The sequence near the transcription start site is shown and the start site is indicated by arrow.

transcriptional start site to the polyadenylation tail identified in the cDNA clone is about 7.5 kb, consisting with the general view that the short size is a common feature among the primary response genes (34).

The 1.8-kb promoter sequence of human PGHS-2 is presented in Fig. 3. This region contained a canonical TATA box which is 31 bases upstream from the transcriptional start site and several

Fig. 3. Nucleotide sequence of the human PGHS-2 promoter region. Locations of potential response elements in the 5'-flanking region are marked. Nucleotides are numbered negatively to the right of the sequence with nucleotide +1 corresponding to the transcriptional start site (*).

putative response elements including Sp1, NF-κB, CRE, NF-IL6, GRE, PEA-3, AP2 and C/EBP sites (35-40), suggesting that the regulation of this gene involves a complex array of regulatory factors. In contrast, the promoter sequence of human PGHS-1, as we previously reported, displayed common features of housekeeping genes (41). Sequence comparison revealed that the first 200 bp of human PGHS-2 promoter sequence shared 67 % and 65 % identity with that of mouse and rat counterparts, respectively. In contrast, the 1.8-kb sequence of human PGHS-2 promoter region shares little sequence identity with the 1.5-kb sequence of chicken PGHS-2 promoter. It should be noted that the C/EBP element in the rat PGHS-2 promoter, which also conserved in the human PGHS-2 promoter, was recently shown to play an important role in induction of PGHS-2 gene in the granulosa cells (24). Analysis of the 5'-flanking regions of human PGHS-1 and PGHS-2 genes reveals little identity which likely contributes to their differential patterns of expression.

To demonstrate the promoter activity of the human PGHS-2 gene, two DNA fragments which are -891 to +9 and -459 to +9 nucleotides relative to the transcriptional start site of PGHS-2 were constructed into the luciferase reporter vector to generate pPGS891LUC and pPGS459LUC, respectively. These two constructs, the promoterless vector pXP1 and pSV2LUC which uses SV40 early promoter to drive the luciferase gene were transfected into HUVECs. The results of luciferase assays are shown in Table II. The basal levels of PGHS-2 expression are demonstrated as compared pPGS891LUC and pPGS459LUC with the negative control, pXP1. The transcriptional levels were further increased upon PMA treatment (4-fold increase in pPGS891LUC and 3-fold in

Table II. Promoter activities of the human PGHS-2 gene. HUVECs were transiently transfected with pXP1 (promoterless vector), pSV2LUC (SV40 early promoter-driven reporter vector), pPGS891LUC and pPGS459LUC. Details of transfection, PMA treatment and luciferase assays were described under "Materials and Methods."

		Luciferase activity (light units / μg protein)
-891 +9 LUC	pPGS891LUC (+PMA)	196±69
	pPGS891LUC (-PMA)	45±3
-459 +9 LUC	pPGS459LUC (+PMA)	185±30
	pPGS459LUC (-PMA)	63±10
SV40 LUC	pSV2LUC	257±103
LUC	pXP1	5±1

pPGS459LUC). The results clearly demonstrated that the 5'-flanking region of human PGHS-2 gene can confer mitogen-regulated accumulation of a reporter gene production.

ACKNOWLEDGMENT--This work was supported by grants NS-23327 and HL-35387 from National Institutes of Health.

REFERENCES

1. Needleman, P., Turk, J., Jakschik, B. A., Morrison, A. R., and Lefkowith J. B. (1986) Ann. Rev. Biochem. 55, 69-102
2. Kulmacz, R. J. (1987) CRC Handbook of Eicosanoids: Prostaglandins and Related Lipids, (Willis, A. L., ed) Vol. I, Part A, pp. 163-174, CRC Press, Boca Raton, FL
3. Smith, W. L. (1992) Am. J. Physiol. 263, F181-F191
4. Smith, W. L., and Marnett L. J. (1991) Biochim. Biophys. Acta 1083, 1-17
5. Vane, J., and Botting, R. (1987) FASEB J. 1, 89-96
6. Miyamoto, T., Ogino, N., Yamamoto, S., and Hayaishi, O. (1976) J. Biol. Chem. 251, 2629-2636
7. Hemler, M., and Lands, W. E. M. (1976) J. Biol. Chem. 251, 5575-5579
8. Van der Ouderaa, F. J., Buytenhek, M., Nugteren, D. H., and van Dorp, D. A. (1977) Biochim. Biophys. Acta 487, 315-331
9. DeWitt, D. L., and Smith, W. L. (1988) Proc. Natl. Acad. Sci. USA 85, 1412-1416
10. Merlie, J. P., Fagan, D., Mudd, J., and Needleman, P. (1988) J. Biol. Chem. 263, 3550-3553
11. Yokoyama, C., Takai, T., and Tanabe, T. (1988) FEBS Lett. 231, 347-351
12. DeWitt, D. L., El-Harith, E. A., Kraemer, S. A., Andrews, M. J., Yao, E. F., Armstrong, R. L., and Smith, W. L. (1990) J. Biol. Chem. 265, 5192-5198
13. Funk, C. D., Funk, L. B., Kennedy, M. E., Pong, A. S., and Fitzgerald, G. A. (1991) FASEB J. 5, 2304-2312
14. Yokoyama, C., and Tanabe, T. (1989) Biochem. Biophys. Res. Commun. 165, 888-894
15. Kraemer, S. A., Meade, E. A., and DeWitt, D. L. (1992) Arch. Biochem. Biophys. 293, 391-400
16. Xie, W., Chipman, J. G., Robertson, D. L., Erikson, R. L., and Simmons, D. L. (1991) Proc. Natl. Acad. Sci. USA 88, 2692-2696
17. Kujubu, D. A., Fletcher, B. S., Varnum, B. C., Lim, R. W., and Herschman, H. R. (1991) J. Biol. Chem. 266, 12866-12872
18. O'Banion, M. K., Sadowski, H. B., Winn, V., and Young, D. A. (1991) J. Biol. Chem. 266, 23261-23267
19. Sirois, J., and Richards, J. S. (1992) J. Biol. Chem. 267, 6382-6388
20. Hla, T., and Neilson, K. (1992) Proc. Natl. Acad. Sci. USA 89, 7384-7388
21. Jones, D. A., Carlton, D. P., McIntyre, T. M., Zimmerman, G. A., and Prescott, S. M. (1993) J. Biol. Chem. 268, 9049-9054
22. Fletcher, B. S., Kujubu, D. A., Perrin, D. M., and Herschman, H. R. (1992) J. Biol. Chem. 267, 4338-4344
23. Xie, W., Merrill, J. R., Bradshaw, W. S., and Simmons, D. L. (1993) Arch. Biochem. Biophys. 300, 247-252
24. Sirois, J., Levy, L. O., Simmons, D. L., and Richards, J. S. (1993) J. Biol. Chem. 268, 12199-12206
25. Maruyama, Y. (1963) Z. Zellforsch. Mikrosk. Anat. 60, 69-79
26. Jaffe, E. A., Nachman, R. L., Becker, C. G., and Minick, C. R. (1973) J. Clin. Invest. 52, 2745-2756

27. Sternberg, N. (1990) Proc. Natl. Acad. Sci. USA 87, 103-107
28. Rackwitz, H. R., Zehetner, G., Murialdo, H., Delius, H., Chai, J. H., Poustka, A., Frischauf, A., and Lehrach, H. (1985) Gene 40, 259-266
29. Nordeen, S. K. (1988) Biotechniques 6,454-456
30. Tazawa, R., Hirosawa, S., Suzuki K., Hirokawa, K., and Aoki, N. (1993) J. Biochem. 113, 600-606
31. Paggett, R.A., Grabowski, P.J. Konarska, M.M., Seller, S. and Sharp, P.A. (1986) Annu. Rev. Biochem. 55. 1119-1150
32. Chen, I.T. and Chasin, L.A. (1993) Mol. Cell. Biol. 13, 289-300
33. Kosaka, T. Miyata, A., Ihara, H., Sugimoto, T., Takeda, O., Takahashi, E. and Tanabe, T. (1994) Eur. J. Biochem. 221: 889-897
34. Herschman, H. R. (1991) Annu. Rev. Biochem. 60, 281-319
35. Gilmore, T. D. (1990) Cell 62, 841-843
36. Akira, S., Isshiki, H., Sugita, T., Tanabe, O., Kinoshita, S., Nishio, Y., Nakajima, T., Hirano, T., and Kishimoto, T. (1990) EMBO J. 9, 1897-1906
37. Wasylyk, C., Flores, P., Gutman, A., and Wasylyk, B. (1989) EMBO J. 8, 3371-3378
38. Mitchell, P. J., Wang, C., and Tjian, R. (1987) Cell 50, 847-861
39. Landschulz,W.H., Johnson,P.F., Adashi,E.Y., Graves,B.J. and McKnight,S.L. (1988) Genes Dev. 2:786-800
40. Jackson, S. P., MacDonald, J. J., Lees-Miller, S., and Tjian, R. (1990) Cell 63, 155-165
41. Wang, L.-H., Hajibeigi, A., Xu, X.-M., Loose-Mitchell, D., and Wu, K. K. (1993) Biochem. Biophys. Res. Commmun. 190, 406-411