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Isolation and Characterization of the Mouse PDGF β-receptor Promoter

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SUMMARY The PDGF β-receptor expression is tightly regulated during embryonic development and in several physiological and pathological situations. To determine the regulatory mechanism of the receptor, a 1.9 kb 5' flanking genomic fragment of the mouse PDGF β-receptor gene was cloned and analyzed by functional promoter assays. The fragment was shown to exert promoter activity in the luciferase expression vector system in mouse NIH 3T3 fibroblast and NB41 neuroblastoma cell lines as well as rat ST15A cerebellar cell lines. Functional studies on deletion mutants revealed several putative regulatory sequences. The deletion mutants acted similarly in NB41 cells and in ST15A cells, both of neuronal origin, but differently in the NIH 3T3 fibroblasts. No TATA box was found in the analyzed promoter region, however, site directed mutagenesis of a CCAAT motif, located 60 basepair upstream of the transcriptional start site, almost completely abolished the promoter activity in all cell types.

INTRODUCTION Platelet-derived growth factor (PDGF) is a multifunctional protein involved in several physiological as well as pathological processes (reviewed in refs. 1,2). PDGF and its receptors are expressed during mammalian development and the expression is developmentally regulated [3-7]. While PDGF α-receptor plays important roles early in development, PDGF β-receptor becomes the predominant receptor type later in development and in adults [8]. Stromal cells in normal tissues express only low levels of the β-receptor, but receptors are upregulated when cells are cultured *in vitro* [9]. Furthermore, induction of PDGF β-receptor occurs rapidly *in vivo* in conjunction with wound healing [10-12], atherosclerosis [13], proliferative glomerulonephritis [14], proliferative retinopathies [15], fibrosis [16] or rheumathoid arthritis [17]. In all these cases, marked induction of the receptor was found in the blood vessels and the surrounding stromal cells. Similarly strong upregulation was described in the stroma of certain tumors, particularly in the capillary endothelial cells [18,19]. Thus, PDGF β-receptor seems to play an important role in the reactive angiogenesis which occurs during inflammation and tumor development. Disruption of the β-receptor gene resulted in severe failure of the vascular system and in the lack of mesangial cells in the kidneys; the homozygotic mice died before birth [20].

PDGF β-receptor has also shown to be conditionally regulated in the nervous system. Neurons of newborn rat brain express PDGF β-receptor in a developmentally regulated

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fashion and respond to PDGF-BB with prolonged survival and increased neurite outgrowth [21]. SV40 T antigen immortalized cerebellar neuroepithelial stem cells, ST15A, express increasing amount of PDGF β-receptor during neuronal differentiation, and PDGF-BB further differentiates these cells [22]. Similar neurotrophic activity was demonstrated on rat fetal mesencephalic dopaminergic cells and striatal cells in culture [23,24]. Schwann cells in the peripheral nervous system also express PDGF β-receptor, which is also developmentally regulated [25], and can be induced *in vitro* [26].

Since regulation of the PDGF β-receptor expression seems to be of critical importance in several conditions *in vivo*, it is important to understand the exact mechanism of its transcriptional regulation. We have cloned 1.9 kb of the 5' flanking sequence of the mouse PDGF β-receptor gene, and characterized it using a luciferase reporter system.

MATERIALS AND METHODS

Genomic DNA cloning and sequencing. A 129 SV mouse genomic library created in l FIX II (Stratagene) was screened by a 463 bp long SacI fragment, containing part of exon 1, and the entire exon 2 and 3 of the PDGF β-receptor cDNA [27]. Positive clones were analyzed by restriction mapping. A 5.5 kb BamHI fragment, which was positively labeled by the cDNA probe, was subcloned into pUC 119 [28] for further analysis. A 1.9 kb long fragment, between the upstream SacI site and the exon 1/intron 1 boundary was sequenced by the dideoxy chain-termination method [29].

Primer extention analysis. We used an "*In vitro* eukaryotic transcription kit" from Stratagene according to the vendor's manual. We used the 5.5 kb long β13 fragment as a template for the *in vitro* transcription. A ^{32}P labeled 35 bp long oligonucleotide complementary to the nucleotides -381 and -346 was annealed to the transcribed mRNA and the extention products were run on polyacrylamide gel in parallel with sequence reactions according to Sanger using the same primer.

Constructs for the functional promoter assays. The constructs used for functional promoter assays were made by restriction enzyme digestion or polymerase chain reaction (PCR), and subcloned into a luciferase expression vector pGL2 basic (Promega). The 5.5 kb long BamHI (β13), as well as the 1.9 kb SacI and 1.5 kb XbaI/BbsI (β12) fragments were subcloned into pGL2 in front of the luciferase gene, and further deletion mutants were created from them by digestion with PstI and HindIII, and also with SacI in the case of β12, followed by religation. We also ligated the SacI and the β12 fragments in the opposite (3'-5') direction to test the orientation dependency of the promoter activity. Fragments 86 and 84 were generated by PCR, using the β12 construct as a template, with primers introducing an XhoI site to the 5' and BglII site to the 3'ends. The fragments were named after their respective 5'primer. The nucleotide sequence of 86 was 5'-CGACTCGAGGTAGCAGTGTCAGCCCTGGGTCT-3', and that of 84; 5'-CCACTCGAGGGGAGGGAGCAGGAGGGAAAGGAG-3'. The same downstream primer 5'-CGTAGATCTGAATCAGGGGAATGGAGAGGGTGC-3' was used for the amplification of both fragments. Following amplification, the products were purified from agarose gel, cut by XhoI and BglII, and ligated into the pGL2 vector. The PCR amplified fragments were sequenced to avoid the transfection of missmatched sequences.

Cell culture and transient transfection. The mouse fibroblast cell line NIH 3T3 (a gift from Sara A. Courtneidge, EMBL, Heidelberg) was cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco) containing 10% fetal calf serum (FCS) at 37°C, with 100 U/ml penicillin and 100 mg/ml streptomycin. The rat cerebellar ST15A cell line was a generous gift of Ronald D.G. McKay (NIH, Bethesda, MD). This cell line was established by introducing the temperature sensitive mutant of the SV40 T antigen into cerebellar cells isolated from 2 days old rats [30]. The cells were cultured in DMEM with 10% FCS and antibiotics at 33°C. The mouse NB41 neuroblastoma cells (American Type Culture Collection) were cultured in Ham's F10 medium supplemented with 15% horse serum, 2.5% FCS and antibiotics at 37°C.

The cells were seeded in 6-well plates at a density of 4×10^5/well about 12 hours before transfection, which was performed by the calcium-phosphate technique [31]. We used 15 μg of the luciferase expression constructs. The cells were incubated with the calcium-phosp-

hate-DNA precipitate for 4 hours, followed by a glycerol shock. The luciferase activity of the cell lysates was measured in a luminometer 1250 (LKB Wallac) after 48 hours, using a luciferase assay kit (Promega). All the transfections were repeated several times using at least two different batches of DNA. To control the transfection efficiency, we transfected 2 μg of the LacZ gene containing pCH110 vector (Pharmacia LKB Biotechnology) together with each luciferase construct. β-Galactosidase activity was determined by chemiluminescence, which was measured at 420 nm in a spectrophotometer (Titertek Multiskan Plus, Flow Laboratories).

Site-directed mutagenesis. Mutation of the CCAAT sequence was performed using the Altered Sites II *in vitro* Mutagenesis System from Promega according to their manual. The sequence of the mutagenic primer was 5'-CCAAGCTTGGGTACTGATCAGAATCGGCCCTGCAGC-CTTT-3'.

RESULTS

Organization of the 5'flanking genomic region of the PDGF β-receptor promoter. A mouse genomic library was screened by a 450 bp SacI fragment of the PDGF β-receptor cDNA, which contained a part of exon 1, and the entire exon 2 and 3. A 5.5 kb long BamH1 fragment (β13) of one of the several positive clones were analyzed by restriction enzyme mapping, and subcloned into the pUC 119 vector for further sequence determination (Fig. 1). Partial sequen-

Figure 1. DNA sequence of the 1.9 kb long 5' flanking region of the PDGF β-receptor gene. The first ATG codon, located in exon 2 is marked by a star. The intron 1/exon 2 boundary was found between nucleotides -5 and -6. Transcriptional start site located between nucleotide -421 and -411, and several putative transcription factor binding sites are overlined.

cing revealed that this fragment contained part of intron 1, exon 1, and about 3.5 kb of 5' flanking sequence. The β13 fragment did not contain the first ATG initiation codon, which is located at the beginning of exon 2. We determined the nucleotide sequence of a 1.9 kb long SacI/BbsI fragment of the β13, containing part of exon 1 and the 5' flanking region (Fig. 1).

To determine the exact transcription start site we performed primer extention assay with a 40 bp long primer, located in exon 1. We could detect one main product (Fig. 2), located around nucleotide -421 and -411, as indicated on Fig. 2. Although we detected more than one band during several repeated experiments, the above mentioned product was always visible, and its localization could be determined with a variation of less than 10 bases in independent experiments.

The nucleotide sequence of the PDGF β-receptor promoter within the SacI/BbsI fragment was compared with all the consensus sequences stored in the TRANSFAC database (GBF, Braunschweig). The sequence contained a very long CA track, and also some short GC rich regions, however, the promoter region does not seem to be particularly GC-rich. We could not find any TATA or TATA-like sequence close to the start site of transcription. However we could detect several consensus sequences for known transcription factors, such as SRF, AP-1, AP-2, CREB, NF-1, and GATA-1. Interestingly, there was a CCAAT box located about 60 bp from the initiation region, which could function as one of the binding sites for the transcriptional initiation complex and influence transcription efficiency [32,33].

Functional promoter analysis. All the promoter constructs were tested functionally by transient transfection into three different cell lines, NIH 3T3, NB41 and ST15A. The results of the

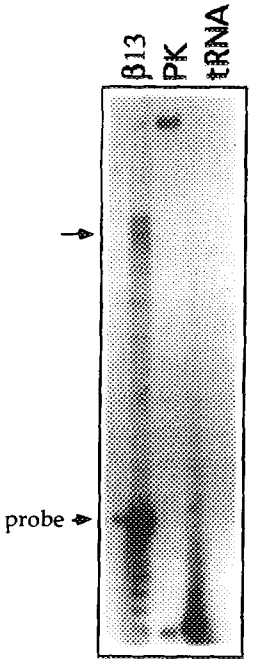

Figure 2. Determination of the transcriptional start site by primer extention analysis. The Krüppel gene (PK), provided in the Strategene kit,was used as a positive control, and tRNA as a negative one. A representative result out of five experiments is shown.

luciferase assays performed in the NIH 3T3 cells are summerized in Fig. 3. We could clearly show that the cloned 5' flanking promoter was active in a heterologous expression system. The strongest promoter activity of the β12 fragment was seen in the fibroblasts. Since deletion of the 3' end of the β12 fragment by digestion with SacI, did not change its activity, there is a negative regulatory element located between the XbaI and the upstream SacI sites at the 5' end of the promoter region. Furthermore, this negative element seemed to act in a cell type specific manner, since the SacI fragment yielded the highest promoter activity in the NB41 or ST15A cells. The PCR generated 86 fragment yielded luciferase activity of about 20% and fragment 84 showed only 5-10% activity of that of the β12 fragment. Deletion of the 5' end of the promoter to nucleotide -409, completely abolished the activity. The 5.5 kb long β13 fragment was tested in the NIH 3T3 cells, and gave an activity around 30% of that of the fragment with the strongest signal, suggesting the presence of negative distal elements. Since β13 contains about 750 bp of intron 1, it is possible that certain negative regulatory sequences might be in the intron.

The 3' deletion mutation by restriction enzyme digestion did not show any change in the luciferase activity using SacI, whose restriction site is located downstream of the initiation box. Restriction by PstI decreased the promoter activity to about 30% of the original ones. The PstI site is located at the position -54 from the most upstream point of

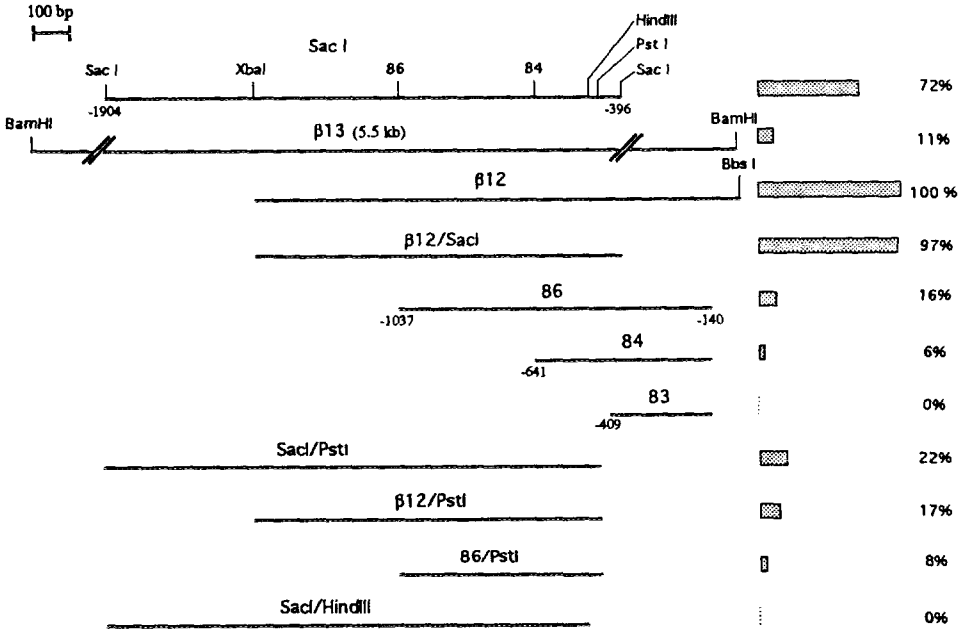

Figure 3. Functional promoter analysis of the different PDGF β-receptor promoter constructs in NIH 3T3 mouse fibroblasts using a luciferase reporter system. The restriction enzyme cleavage sites and the location of the PCR primers used for the construction of the deletion mutants are indicated. Bars represent the measured luciferase activities. The strongest activity of the β12 fragment was used as 100%, and all the data are normalized to the cotransfected β-galactosidase activity. The data show a representative result out of several repeated experiments. Duplicates of each sample were used in all experiments.

the transcriptional initiation. Finally, HindIII digestion completely abolished the luciferase activity of the constructs. Interestingly, the CCAAT box is located downstream of the HindIII site, between the HindIII and PstI restriction consensus sequences. We tested both β12 and SacI fragments ligated into the pGL2 expression vector in the 3'-5' orientation. The promoter activities were significantly decreased, retaining about 5% of those obtained by the 5'-3' orientation.

Site-directed mutagenesis of the CCAAT box. The results seen by the deletion mutants suggested the functional importance of the CCAAT box in the basic promoter activity. To further prove this, we introduced a mutation into this sequence using the SacI fragment as a template. The mutated construct showed a dramatic decrease of the promoter activity, retaining only about 5-10% activity of that of the wild type.

DISCUSSION

PDGF β-receptor belongs to the class III receptor tyrosine kinase family, together with PDGF α-receptor, colony stimulating factor-1 receptor (c-*fms*) and c-*kit* (reviewed in ref. 34). The genomic structures of the class III family members are all very similar. The PDGF β-receptor is localized in tandem upstream of the c-*fms* gene on human chromosome 5, and the two genes probably originate from the same anchestral gene [35]. The PDGF β-receptor gene has an untranslated exon 1, a very long intron 1 (around 25 kb), and the translation initiation codon is located in the second exon. The functional role of this particularly long intron 1 is not known. However, our results suggest the possibility that a negative regulatory element might exist within intron 1, since the longest genomic fragment β13, containing part of intron 1, exhibited much lower activity than the SacI or β12 fragments, which contain only upstream sequences. The similarly long intron 1 sequence of the c-*fms* gene is known to contain a promoter element, driving cell specific transcription of a reporter gene in macrophages [36]. In the case of PDGF β-receptor, there is only one reported case showing variations in the length of transcripts; a shorter mRNA variant was described in mouse teratocarcinoma and embryonic stem cells, however, the *in vivo* existence of this transcript coud not be proven [37].

Similarly to the case for the PDGF α-receptor promoter [38,39] the analyzed 5' flanking region of the PDGF β-receptor did not contain a TATA box or any TATA like sequences. Upstream regulatory elements located within 1.9 kb of the transcriptional start site on the PDGF β-receptor gene could direct expression of the luciferase gene in three different cell lines in an orientation-dependent manner. Sequence analysis and functional assays of deletion mutants suggested the presence of several putative regulatory elements. Two regions were found to be particularly interesting. One is the region close to the transcription initiation sites, containig a CCAAT sequence motif at position -60. Site-directed mutagenesis of the CCAAT box drastically reduced the basic promoter activity to about 5% of the wild type. The CCAAT is an important control element capable of binding protein factors that regulate the transcription efficiency [33,40]. Several transcription factors, such as C/EBP and CTF/NF-1 are known to bind to the CCAAT motif [41]. However, the most frequent protein, which binds to the CCAAT box at the position -60 to -80 is NF-Y [42]. Studies are

under way to determine which transcription factor that binds to this region. The other functinally interesting regulatory region is the one located between -1904 and -1495. We found that the activity of the β12 and SacI constructs transfected in the fibroblasts, differed from those transfected in the other two neuroepithelial cell lines. Thus, the Sac I construct, which contains a further upstream fragment of the β12, showed negative activity in fibroblasts, an activity which was not found in the cells of neuronal origin. These data raise the possibility that a cell type specific regulatory element reside in this region. Several putative transcription factor binding sites were found by computer analysis of this fragment. Further analysis is under way in our laboratory to define transacting proteins which bind to these consensus sequences.

Rapid upregulation of PDGF β-receptor expression has been demonstrated in various physiological and pathological processes *in vivo*. The expression of the β-receptor increases with time during embryonic development [8]. Induction of the receptor has been shown during retinoic acid (RA)-stimulated development of human embryonal carcinoma cells [43], and also during the differentiation of monocyte-derived macrophages [44]. The latter observation is of a particular importance in relation to the process of atherosclerosis. PDGF β-receptor possessing smooth muscle cells of the arteries are also key players during the development of atherosclerosis [44], as well as in wound healing [12]. Cell culture studies implicated several agents as inducers of the PDGF β-receptor expression. RA and cAMP induce the receptor specific mRNA level in Schwann cells [26], in ST15A cells [22] or in human neuroblastoma cell lines [45]. Phorbol-ester via activation of protein kinase C induces the β-receptor expression associated with the differentiation of neuroblastoma cell lines [45], as well as of ST15A neuroepithelial stem cells [22]. None of these compounds has yet been proved to induce the PDGF β-receptor mRNA expression at the transcriptional level, which remains to be determined by nuclear run-on assays as well as by the luciferase reporter system.

Defining the stimuli which directly induce the PDGF β-receptor promoter activity and understanding the mechanism of its regulation could give better insight into physiological regulation of the receptor, and also into the pathogenesis of several diseases where an aberrant expression of the receptor has been found. Studies are under way in our laboratory to find the positive and negative regulators of the PDGF β-receptor, as well as to characterize the transcription factors involved in these regulatory processes.

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REFERENCES

1. Raines, E. W., Bowen-Pope, D. F., Ross, R. (1990) Platelet-derived growth factor. In: Sporn and Roberts, eds. Handbook of Experimental Pharmacology. Peptide growth factors and their receptors. Vol. 95, Part I. Heidelberg: Springer-Verlag,173-262
2. Heldin, C.-H., Westermark, B. (1990) Cell Regulation 1, 555-566
3. Rappolee, D. A., Brenner, C. A., Schultz, R., Mark, D., Werb, Z. (1988) Science 241, 1823-1825

4. Orr-Urtreger, A., Bedford, M. T., Do, M.-S., Eisenbach, L., Lonai, P. (1992) Development 115, 289-303
5. Mercola, M., Wang, C., Kelly, J., Brownlee, C., Jackson-Grusby, L., Stiles, C., Bowen-Pope, D. (1990) Dev. Biol. 138, 114-122
6. Schatteman, G. C., Morrison-Graham, K., van Koppen, A., Weston, J. A., Bowen-Pope, D. F. (1992) Development 115, 123-131
7. Holmgren, L., Glaser, A., Pfeifer-Ohlsson, S., Ohlsson, R. (1991) Development 113, 749-54
8. Bowen-Pope, D. F., van Koppen, A., Schatteman, G. (1991) T.I.G. 7, 413-418
9. Terracio, L., Rönnstrand, L., Tingström, A., Rubin, K., Claesson-Welsh, L., Funa, K., Heldin, C.-H. (1988) J. Cell Biol. 107, 1947-1957
10. Robson, M. C., Phillips, L. G., Thomason, A., Robson, L. E., Pierce, G. F. (1992) Lancet 339, 23-25
11. Majesky, M. W., Reidy, M. A., Bowen-Pope, D. F., Hart, C. E. (1990) J. Cell. Biol. 111, 2149-2158
12. Reuterdahl, C., Sundberg, C., Rubin, C., Funa, K., B., G. (1993) J. Clin. Invest. 91, 2065-2075
13. Ross, R., Raines, E. W., Bowen-Pope, D. F. (1986) Cell 46, 155-169
14. Gesualdo, L., Di Paolo, S., Milani, S., Pinzani, M., Grappone, C., Ranieri, E., Pannarale, G., Schena, F. P. (1994) J Clin Invest. 1, p50-58
15. Robbins, S. G., Mixon, R. N., Wilson, D. J., C.E, H., Robertson, J., Westra, I., Planck, S. R., Rosenbaum, J. T. (1990) Invest Ophthalmol Vis Sci 35, 3649-63
16. Smits, A., Funa, K., Vassbotn, F. S., Beausang-Linder, M., af Ekenstam, F., Heldin, C.-H., Westermark, B., Nistér, M. (1992) Am. J. Pathol. 140, 639-648
17. Reuterdahl, C., Tingström, A., Terracio, L., Funa, K., Heldin, C.-H., Rubin, K. (1991) Lab. Invest. 64, 321-329
18. Funa, K., Papanicolaou, V., Juhlin, C., Rastad, J., Åkerström, G., Heldin, C.-H., Öberg, K. (1990) Cancer Res. 50, 748-753
19. Hermanson, M., Nistér, M., Betsholtz, C., Heldin, C.-H., Westermark, B., Funa, K. (1988) Proc. Natl. Acad. Sci. USA 85, 7748-7752
20. Soriano, P. (1994) 8, 1888-1896
21. Smits, A., Kato, M., Westermark, B., Nistér, M., Heldin, C.-H., Funa, K. (1991) Proc. Natl. Acad Sci. USA 88, 8159-8163
22. Ballagi, A. E., Funa, K. Submitted
23. Nikkhah, G., Odin, P., Smits, A., Tingström, A., Othberg, A., Brundin, P., Funa, K., Lindvall, O. (1993) Exp. Brain Res. 92, 516-523
24. Othberg, A., Odin, A., Ballagi, A. E., Åhgren, A., Funa, K., Lindvall, O. (1994) Exp. Brain Res. In Press
25. Eccleston, P. A., Funa, K., Heldin, C.-H. (1993) Dev. Biol. 155, 459-470
26. Weinmaster, G., Lemke, G. (1990) EMBO J. 9, 915-920
27. Yarden, Y., Escobedo, J. A., Kuang, W.-J., Yang-Feng, T. L., Daniel, T. O., Tremble, P. M., Chen, E. Y., Ando, M. E., Harkins, R. N., Francke, U., Friend, V. A., Ullrich, A., Williams, L. T. (1986) Nature 323, 226-232
28. Yannisch-Perron, C., Vieira, C. J., Messing, J. (1985) Gene 33, 103-119
29. Sanger, F., Nicklen, S., Coulson, A. R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467
30. Frederiksen, K., Jat, P. S., Valtz, N., Levy, D., McKay, R. (1988) Neuron 1, 439-448
31. Sambrook, J., Fritsch, E. F., Maniatis, T. E. (1989) Cold Spring Harbor Laboratory, Cold Spring Harbor
32. Jones, K. A., Kadonaga, J. T., Rosenfeld, P. J., Kelly, T. J., Tijan, R. (1987) Cell 48, 79-89
33. Chodos, L. A., Olesen, J., Hahn, S., Baldwin, A. S., Guarente, L., Sharp, P. A. (1988) Cell 53, 25-35
34. Claesson-Welsh, L. PDGF receptors: Structure and mechanism of action. In: Westermark, B. and Sorg, C. eds. Biology of platelet-derived growth factor. Cytokines. Vol. 5. Basel: Karger, S. AG; 1993, 31-43
35. Sherr, C. J. (1990) Blood 75, 1-12
36. Yue, X., Favoit, P., Dunn, T. L., Cassady, A. I., Hume, D. A. (1993) Mol. Cell Biol. 13, 3191-3201
37. Vu, T. H., Martin, G. R., Lee, P., Mark, D., Wang, A., Williams, L. T. (1989) Mol. Cell. Biol. 9, 4563-4567

38. Wang, C., Stiles, C. D. (1994) Proc. Natl. Acad. Sci. USA 91, 7061-7065
39. Afink, G. B., Nistér, M., Stassen, B. H. G. J., Rademakers, P. J. H., Bongcam-Rudloff, E., Van Zoelen, E. J. J., Mosselman, S. Genes & Chrom. & Cancer In Press
40. Jones, C. M., Simon-Chazottes, D., Guenet, J.-L., Hogan, B. L. M. (1992) Mol. Endocrinol. 6, 1961-1968
41. Dorn, A., Bollekens, J., Staub, A., Benoist, C., Mathis, D. (1987) Cell 50, 863-872
42. Bucher, P. (1990) J. Mol. Biol. 212, 563-78
43. Weima, S. M., van Rooijen, M. A., Mummery, C. L., Feyen, A., de Laat, S. W., van Zoelen, E. J. J. (1990) Exp. Cell Res. 186, 324-331
44. Inaba, T., Shimano, H., Gotoda, T., Harada, K., Shimada, M., Ohsuga, J., Watanabe, Y., Kawamura, M., Yazaki, Y., Yamada, N. (1993) J. Biol. Chem. 268, 24353-24360
45. Påhlman, S., Johansson, I., Westermark, B., Nistér, M. (1992) Cell Growth & Differentiation 3, 783-790