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Oct-4 Regulates Alternative Platelet-derived Growth Factor α Receptor Gene Promoter in Human Embryonal Carcinoma Cells*

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Expression of the platelet-derived growth factor α -receptor (PDGF α R) gene is tightly controlled in mammalian embryogenesis. A well established model system to study human embryogenesis is the embryonal carci-

portance of the PDGF α R in mammalian development is also exemplified by the *Patch* (*Ph*) mouse mutant. The *Ph* mutant lacks part of the PDGF α R gene (5, 6) and displays severe developmental defects in mesodermal and neuroectodermal tissues, often resulting in prenatal lethality (7, 8).

noma cell line Tera2. We have shown previously that retinoic acid-differentiated Tera2 cells express two PDGF α R transcripts of 6.4 kilobase pairs (kb) (encoding the full-length receptor) and 3.0 kb, respectively, whereas in contrast, undifferentiated Tera2 cells express PDFG α R transcripts of 1.5 kb and 5.0 kb. Here we show that this switch in PDGF α R expression pattern during differentiation of Tera2 cells results from alternative promoter use. In undifferentiated cells, a second promoter is used, which is located in intron 12 of the PDGF α R gene. Functional analysis shows that this promoter contains a consensus octamer motif, which can be bound by the POU domain transcription factor Oct-4. **Oct-4** is expressed in undifferentiated Tera2 cells but not in retinoic acid-induced differentiated cells. Mutation of the octamer motif decreases promoter activity, while ectopic expression of Oct-4 in differentiated Tera2 cells specifically enhances the activity of this $PDGF\alpha R$ promoter. Therefore, we suggest that an important aspect in the maintenance of the undifferentiated state of human embryonal carcinoma cells results from Oct-4

An important model system for studying human early embryogenesis is that of testicular germ cell tumors. These tumors are derived from a derangement of a primordial germ cell in early life, which first develops into a noninvasive carcinoma-in situ and subsequently grow out as a seminoma or a nonseminomatous tumor (9). The stem cells of nonseminomatous tumors, also referred to as EC cells, strongly resemble cells of the early preimplantation human embryo. Various established human EC cell lines, among others the Tera2 cell line, can be induced to differentiate in vitro into a variety of mature, nontumorigenic cell types by the morphogen retinoic acid (10). We have recently shown that differentiation of Tera2 EC cells by retinoic acid (RA) is accompanied by a shift in expression of PDGF α R mRNA variants (11).² Four human PDGF α R transcripts have been identified as a result of a combination of alternative splicing and promoter use. Two $PDGF\alpha R$ mRNA species of 1.5 and 5.0 kb, respectively, are expressed in early human embryonic cells, including the undifferentiated Tera2 EC cells. Studies on surgically removed testicular germ cell tumors have shown that the 1.5-kb PDGF α R transcript can be used as a selective marker for carcinoma-in situ, seminoma, and undifferentiated nonseminomatous tumors in the human testis.² In differentiated cells, including RA-differentiated Tera2 (Tera2 RA) cells, two other PDGF α R transcripts of 6.4 kb, which encodes the functional full-length receptor, and of 3.0-kb, which potentially encodes a dominant negative isoform, have been identified. Aberrant expression of the full-length PDGF α R receptor, encoded by the 6.4-kb transcript, has also been implicated in tumorigenesis, *i.e.* it is overexpressed in various tumors, including gliomas (12). In a previous study we cloned and characterized the human PDGF α R gene promoter (P1), which gives rise to the 6.4- and 3.0-kb transcripts. Activity of this P1 promoter can be stimulated strongly by RA and cAMP (13). Similar studies with respect to the mouse and rat PDGF α R promoter have been published recently (14, 15). In the present study we have cloned and characterized the second PDGF αR gene promoter (P2), which gives rise to the 1.5- and 5.0-kb transcripts in early embryonic cells. We show here that the P2 promoter, located in intron 12 of the PDGF α R gene, is active in undifferentiated Tera2 cells and is controlled by the POU domain transcription factor Oct-4. Oct-4 expression is detected in Tera2 EC cells but not in Tera2 RA cells.

expression, which thereupon activates this PDGF α R promoter.

Platelet-derived growth factor $(PDGF)^1$ and its receptors play a prominent role during early mammalian development. Already in the preimplantation embryo of the mouse, from the two-cell stage onwards to the blastocyst stage, the PDGF-A chain is expressed (1), while both this gene and the cognate PDGF α -receptor (PDGF α R) gene are expressed in early postimplantation embryos (2). Murine embryonal carcinoma (EC) cells in culture secrete PDGF-AA (3) and express the PDGF α R following differentiation by retinoic acid (4). The im-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EMBL Data Bank with accession number(s) X95095. § Supported by Dutch Cancer Society Grant NUKC-9010.

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** To whom reprint requests should be addressed. Tel.: 31 24 3652707; Fax: 31 24 3652938; E-mail: vzoelen@sci.kun.nl. ¹ The abbreviations used are: PDGF, platelet-derived growth factor; PDGF α R and PDGF β R, platelet-derived growth factor- α and - β receptor, respectively; RA, retinoic acid; EC, embryonal carcinoma; kb, kilobase pair(s); kFGF, Kaposi's fibroblast growth factor; EMSA, electrophoretic mobility shift assay.

² Mosselman, S., Looijenga, L. H. J., Gillis, A. J. M., van Rooijen, M. A., Kraft, H. J., Van Zoelen, E. J. J., and Oosterhuis, J. W. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 2884–2888.



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MATERIALS AND METHODS

Ceil Culture – Tera2 clone 13 (Tera2) cells were grown in α -modification of minimal essential medium lacking nucleosides and deoxynucleosides, supplemented with 10% (v/v) fetal calf serum and 44 mM NaHCO₃ in a 7.5% CO₂ atmosphere at 37 °C. Differentiation of cells was induced by the addition of RA (5 μ M) 16 h after the cells were seeded at low density (5.0 \times 10³ cells/cm²) and maintained at this medium for 7 days, prior to further analysis or transfection.

 $PDGF\alpha R P2$ Promoter Constructs – Nucleotide sequence analysis was performed using the Pharmacia T7 sequencing kit. PDGF α R P2 promoter constructs were generated by standard cloning procedures (16), using either restriction fragments or DNA fragments obtained by the polymerase chain reaction. Mutation of the octamer binding site was performed with the Altered Sites system kit (Promega), according to the manufacturer's protocol. All fragments were inserted in the multiple cloning site of the pSLA4 luciferase reporter plasmid (13).

Transfection, Luciferase, and β -Galactosidase Assays-Promoterluciferase constructs were transiently transfected into either undifferentiated (Tera2 EC) or differentiated (Tera2 RA) cells using the calcium phosphate coprecipitation method (16). Luciferase activity was determined 48 h (Tera2 EC) or 72 h (Tera2 RA) post-transfection (Luciferase) assay kit, Promega). The luciferase activity was corrected for transfection efficiency by measuring the β -galactosidase activity of a lacZ gene driven by an SV40-promoter of a cotransfected pCH110 plasmid (17). Every experiment was done in duplicate and repeated at least twice with two batches of DNA. RNA Analysis – Total RNA was isolated from undifferentiated (EC) or differentiated (7 days of RA treatment) Tera2 cells, using the isothiocyanate method (18). After poly(A)⁺ isolation, the mRNA was quantitated spectrophotometrically and subjected to 1% agarose gel electrophoresis in formamide. The amount and integrity of loaded mRNA was controlled by ethidium bromide staining, after which it was transferred to Hybond-N (Amersham Corp.). Hybridization and washing procedures were carried out as described (11). A mouse Oct-4 cDNA probe (kindly provided by Dr. P. C. van der Vliet, University of Utrecht), was labeled by random priming (19) using a labeling kit (Amersham). Electrophoretic Mobility Shift Assay (EMSA) – DNA restriction fragments were filled in by Klenow polymerase treatment in the presence of $[\alpha^{-32}P]dCTP$. Oligonucleotides for EMSAs were end-labeled using $[\gamma^{-32}P]ATP$ and T4 polynucleotide kinase. Double-stranded oligonucleotides were separated from single-stranded oligonucleotides by polyacrylamide gel electrophoresis. The oct-1c consensus oligonucleotide was purchased from Promega. Nuclear extracts were prepared as described (20). Binding reactions and gel electrophoresis were performed essentially as described (21). The mouse anti-Oct-4-antibody has been described by Rosfjord and Rizzino (22).

842	t	cgagtaatag	tagtaatagt	agaataggtt	tccgtacagc
801	tggctctgtg	tgtaattaaa	ccetttttet	attgcaattc	ecctgtettg
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701	agagetgeee	cccaatttca	aatatttata	tctaagettt	ctttat <u>tttc</u>
651	<u>c</u> tgcctattt	cccaacaagg	gatgaggagc	ttagggagtt	aaaaagtagt
601	aaaatatgga	ggaaaagggc	ataattccca	ttataccaag	aggcattgct
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451	tggettgeta	tccgtggtga	gactgacacc	caaatgttct	ctcagagtct
401	ctttcagggt	ggaacaaaca	ggcttcaggg	tctttacgta	tgtateatea
351	caacatgaag PEA3	ctaattgctg	tgeteteggg	catgtttagc	tettggtaga
301	ataaatttee	taacaaataq	agaggagtag	aggecagect	gaagttttta

-251	t <u>ttaqtca</u> ct	ecttagaatc	gatgatattt	tgaatactga	agtatttcca
-201	gtggctagta	atttactaag	acaaaagatg	cccctytttg	catatggaaa
-151	acagaagggg AP2	agagagccag	gaggtgtggg	tgagagcccc	gaaggcaaga
-101	ggat <u>cccaqq</u>	<u>qq</u> ctggccca	gcacggaget	ggtaagacac	ggcgceteac
-51	acccagggag	ggctgcaccc	teetttetee 00	cgtctgtgtt T	ttettteect
l	TGCAAGTGTT	ATTCGACAAA	AGCAATTATG	CTAA <u>TTTCC</u> T PEA3	TCCTGTGGGC
51	TCAATTCCTT	TTTTTGACAC	GATGACTTAG	GAGGAGTCAT	TATGATTGCT
101	ĊCAAACAGGA	AAGACACTCG	CCCAGCTGTC	CGCCCGCAGA	GAGCTGGCTA
151	CGGTGCAGAA	AGCTGAGAGG	AGGCGTCTGG	AGTTTTTGGG	TGTTAATGAT
201	TCTGCCTGCC	CACAGGT			

FIG. 1. The nucleotide sequence of the PDGFaR gene P2 promoter and 5'-untranslated region of the 1.5-kb transcript. The transcription initiation site (11) is numbered nucleotide 1, and the transcribed region is given in *uppercase letters*. The sequence is given up to exon 13. Indicated in the figure are the consensus binding sites for transcription factors AP1, AP2, and PEA3, which are *underlined*, and for a POU domain transcription factor, which is depicted in *boldface*. Note the absence of a TATA box in the promoter. (GenBankTM/EMBL data base accession number X95095).

RESULTS

Sequence Determination and Characterization of the P2 Promoter of the PDGF αR Gene-Two alternative PDGF αR transcripts, of 1.5 and 5.0 kb, respectively, are specifically expressed in the undifferentiated Tera2 embryonal carcinoma cells. Although the transcripts terminate differently as a result of alternative splicing, both transcripts initiate in intron 12 of the PDGF α R gene (11).² In order to functionally characterize this putative promoter, which was designated P2 promoter, the region was cloned and sequenced (Fig. 1). The sequence upstream from the transcription initiation site lacks a TATA box, which is also the case for the PDGF α R P1 promoter in human (13), mouse (14), and rat (15), and is also not extremely GCrich. In the promoter region, several consensus binding sites for transcription factors could be detected, including AP1, AP2, and PEA3 motifs (23). A consensus octamer binding site is located in the transcribed region, at positions +28 to +35.

We first set out to determine the functional relevance of

cloned in front of a luciferase reporter gene, transiently transfected into undifferentiated Tera2 cells, and assayed for promoter activity (Fig. 2). The luciferase activity of the complete intron 12 promoter sequence of approximately 2.5 kb (clone -2500/+182) was comparable with that of the much smaller clone -668/+182, indicating that no important expression information is pertained upstream from nucleotide -668, up to exon 12 of the PDGF α R gene. A further deletion, down to position -102 (clone -102/+182), displayed only fractionally lower activity than the clones -2500/+182 and -668/+182, which further limits the region necessary for control of high level expression. The reverse orientation of the -668/+182fragment in the pSLA4 vector (clone -668/+182R) almost completely abolished activity, demonstrating the orientation dependence of the P2 promoter. These data show that intron 12 of the PDGF α R gene contains a bona fide promoter.

Deletion mutants generated at the 3' end (clones: -668/+14; -102/+14), which still included an intact transcription initiation site, reduced activity 4–5 times, compared with the parental clones -668/+182 and -102/+182. Thus, a cis-element determining high promoter activity is located in the transcribed part, within the region +14 to +182. The consensus octamer motif ATGCTAAT at position +28 to +35, which is present in all the constructs that show high promoter activity, was thereupon mutated to the sequence ACGCCAAT (clones -668/+182M and -102/+182M, respectively). This mutation

these putative binding sites and of other regions required for activity of the PDGF α R promoter. It is of interest to note that the P2 promoter region is demethylated in both undifferentiated and RA-induced differentiated Tera2 cells.³ A series of progressive deletion mutants of the PDGF α R P2 promoter was ³ H. J. Kraft, unpublished results.

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FIG. 2. Activity of PDGF α R gene P2 promoter mutants in Tera2 EC cells is dependent on an octamer motif. A series of 5' or 3' deletion mutants or of octamer motif point mutants was cloned in front of a luciferase reporter gene and transiently transfected into Tera2 EC cells. Exons (Ex) and restriction sites (B, BamHI; H, HinDIII; T, TaqI) are depicted in the figure. Luciferase activity was assayed 48 h post-transfection. Transfection efficiency was normalized for with β -galactosidase activity of a cotransfected pCH110 plasmid (Pharmacia Biotech Inc.). Values are presented as mean promoter activity relative to the clone -668/+182, which was arbitrarily set at 100% (S.D. is indicated).

is expected to abrogate all fortuitous binding of POU domainspecific proteins (24, 25). Upon changing these two nucleotides in the octamer motif, promoter activity drops by a factor of 3-4, comparable with deletion of the complete +14/+182 region (Fig. 2). This indicates that the octamer motif is indeed involved in directing P2 promoter activity in Tera2 EC cells.

In order to demonstrate that Tera2 EC nuclear proteins can actually bind to the octamer motif, an EMSA was performed. It is shown in Fig. 3A that the intact -102/+182 promoter fragment forms a complex with nuclear extracts of Tera2 EC cells. The formation of this complex can be specifically competed by excess (100 times) unlabeled probe itself, but not by the -102/+182M fragment containing the mutated octamer motif (*lane* 4). Moreover, the -102/+182M fragment does not form a complex in this EMSA (Fig. 3A, *lanes* 5-8). In addition, the -102/+182 fragment competed efficiently the four specific complexes of a consensus oct-1c oligonucleotide (Promega) with nuclear extracts of EC cells, while the -102/+182M fragment was refractory to competition (Fig. 3B). RA extracts was made possible, and is indicated in Fig. 4A. This shows that also during RA-induced differentiation of human Tera2 embryonal carcinoma cells the POU domain transcription factor Oct-4 is down-regulated.

To confirm the results obtained with the above described EMSAs, a Northern blot analysis was performed on mRNA of Tera2 EC and Tera2 RA cells. The blot was probed with a labeled Oct-4 cDNA, which showed that Oct-4 mRNA is present in Tera2 EC cells, contrasting with the absence of any detectable Oct-4 mRNA in Tera2 RA cells (Fig. 4B). Conclusive evidence that the indicated Oct-4 complex in the EMSAs is formed with this transcription factor comes from a supershift analysis. This analysis was performed with an anti-Oct4-antibody (22), which only supershifted the Oct-4-containing complex and not the Oct-1 complex (Fig. 4C).

Hence, undifferentiated human Tera2 embryonal carcinoma cells express Oct-4, which can complex with the P2-octamer motif. The cells cease to express Oct-4 upon RA-induced differentiation, which necessarily excludes complex formation between Oct-4 and the oct-P motif.

In conclusion, an octamer motif is involved in the regulation of the P2 promoter of the PDGF α R gene in undifferentiated human embryonal carcinoma cells.

Oct-4 Binds to the PDGFaR P2 Promoter Octamer Motif—As described previously, the major change in constituents of octamer binding proteins during retinoic acid-induced differentiation of murine embryonal carcinoma cells involves the down-regulation of Oct-4 expression (26). We hence hypothesized that also in the undifferentiated human embryonal carcinoma Tera2 cells the POU domain transcription factor Oct-4 is present and occupies the promoter P2 octamer motif, oct-P.

In order to test this hypothesis a series of EMSAs were performed with a double-stranded oligonucleotide (oct-P), which results in a higher resolution compared with the long promoter fragment. The oct-P contains the octamer sequence and flanking 7 nucleotides at the 3' side and 8 nucleotides at the 5' side of the P2 promoter (+20/+42). The resulting complexes were compared with the complexes formed by the consensus oct-1c oligonucleotide (see above). The oct-P as well as the oct-1c oligonucleotide gave rise to the formation of several complexes with nuclear proteins of Tera2 EC cells, Tera2 RA cells, or mouse F9 EC cells, which could be specifically competed by excess of the respective cold probe itself (Fig. 4; see also Fig. 5). In the EMSAs, oct-1c and oct-P displayed identical bandshift patterns (not shown). Based upon the complexes formed with the nuclear extracts of mouse F9 EC cells (27, 28) a positive identification of the Oct-4 complex with Tera2 EC or

Comparison of the oct-P Binding Site with the Consensus oct-1c Motif-The members of the POU transcription factor family are defined by their ability to bind to the octamer motif. This causes experimental pitfalls to distinguish between the binding of the different members to a particular motif in a specific cell type (29). In addition to the octamer consensus motif, however, the nucleotides juxtaposed to the motif are also important to the affinity and specificity of binding of a given Oct protein (24).⁴ Therefore, we set out to compare the binding properties of the consensus oct-1c and the PDGF α R genederived oct-P motifs with nuclear extracts from Tera2 EC cells in EMSAs. The oct-P-derived complexes could not be competed by a 500-fold excess of the consensus oct-1c oligonucleotide, while competition with oct-P itself was easily established. A 100-fold excess of oct-P competitor was sufficient for strong competition. No signal was detectable with a 500-fold excess of competitor, even upon prolonged exposure (Fig. 5). This indicates that the oct-P oligonucleotide is bound stronger by POU proteins from Tera2 cells than the consensus oct-1c oligonucleotide. The results with the oct-P oligonucleotide are confirmed by the reciprocal experiment, using the oct-1c oligonucleotide as probe. Even with 100-fold excess, the oct-P competitor abolished the specific binding to the oct-1c oligonucleotide, while the oct-1c oligonucleotide was needed in larger

⁴ H. R. Schöler, personal communication.

scriptional activation of the P2 promoter may still be dependactivity and Oct-4 expression. Developmentally regulated transtrong correlation has been observed between promoter P2 Oct-4 is sufficient in P2-initiated transcription, but so far a expressed in the testis (28). It is presently still unclear whether by Oct-6, since the POU domain transcription factor Oct-6 is vation makes it unlikely that PDGFaR expression is regulated does not contain the 1.5-kb PDGFaR transcript.2 This obsersidsed famion tant vhiroweton it is noteworthy that normal testis upon further differentiation in the testis but maintainereftib redruft nogu mordial germ cell marker, and its expression is down-regulated tiation dependent fashion. Moreover, Oct-4 is regarded a pririved from a primordial germ cell, expresses Oct-4 in a differenembryonal carcinoma cell line Tera2, which is ultimately dedown-regulated upon differentiation. We show here that the lines, e.g. F9 and P19, Oct-4 is expressed, and its expression is notype (24). Also, in several mouse embryonal carcinoma cell

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amounts than oct-P to compete efficiently with the labeled oct-IcPOU protein complex (not shown). Thus, the oct-P domain and flanking sequences suffice to bind Teras POU protein Oct-4 Enhances Expression by the PDGF of P2 Promoter in for high level transcription activity of the PDGF of gene P2 promoter in undifferentiated Teras cells. These Teras EC cells of Oct-4 decreases rapidly during RA-induced differentiation. This event coincides with ceasing amounts of promoter P2initiated PDGF of transcripts of 1.5 and 5.0 kb (11).² This suggests once again that Oct-4 acts on the oct-P domain. Hence, initiated PDGF of transcripts of 1.5 and 5.0 kb (11).² This initiated PDGF of transcripts of 1.5 and 5.0 kb (11).² This initiated PDGF of transcripts of 1.5 and 5.0 kb (11).² This initiated PDGF of transcripts of 1.5 and 5.0 kb (11).² This initiated PDGF of transcripts of 1.5 and 5.0 kb (11).² This initiated PDGF of transcripts of 1.5 and 5.0 kb (11).² this initiated PDGF of transcripts of 1.5 and 5.0 kb (11).² this initiated PDGF of transcripts of 1.5 and 5.0 kb (11).² this initiated PDGF of transcripts of 1.5 and 5.0 kb (11).² this initiated PDGF of transcripts of 1.5 and 5.0 kb (11).² this

maine inner cell mass and primordial germ cells, this tran-Considering the expression pattern of Oct-4 in e.g. cells of the level activation of promotel level the binding sites for PEAS, AP1, or AP2 are involved in high cells. Promoter deletion studies excluded the possibility that AP2, which are all known to play a role in the differentiation of scription factors are located, among others for PEA3, AP1, and In promoter P2 several consensation binding sites for other tranpromoter activity was restored by ectopic expression of Oct-4. over, in Tera2 RA cells, which contain no endogenous Oct-4, P2 mer motif resulted in a decrease of promoter activity. Moresus octamer motif in the promoter region. Mutating this octadomain transcription factor Oct-4, which binds to the consenof the PDGFaR P2 promoter depends critically on the POU promoter. The present study clearly demonstrates that activity gene may very well be related to the fact that it harbors a spectively (11, 30, 31). The size of intron 12 of the PDGFaR sponding introns have lengths of 280 and 127 base pairs, rerelated proto-oncogenes c-fms and c-kit, of which the correapproximately 2.5 kb, when compared with the structurally transcripts. Intron 12 of the PDGFoR gene is extremely large, promoter (P2), able to generate these alternative PDGFaR Isnown is known is of the PDGF about suits a functional strictly confined to undifferentiated Tera? EC cells. We have differentiated cells, expression controlled by the P2 promoter is paonbai. Which are exclusively expressed in RA-induced tiation. In contrast to the P1 promoter-controlled PDGF aR -nevelue bus there develoevel as human development and different promoter, which gives rise to two transcripts in Tera2 BC cells, We have examined the regulation of the human PDGF dR P2

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tustes a 4-fold induction of expression level in cells lacking endogenous Oct-4.

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transcription efficiency, the effect of ectopic Oct-4 expression was tested on P2 promoter luciferase constructs in Oct-4 deprived Tera2 cells. Tera2 cells were differentiated by retinoic acid for 7 days and cotransfected with an intact or a mutated promoter construct together with a CMV-Oct-4 expression vector (Fig. 6). The activity of either intact promoter construct (-668/+182 and -102/+182, only -102/+182 is shown) was enhanced 4-fold when compared with the mock-transfected clones. Moreover, the expression levels of the mutated promoter fragment (-102/ +182M) remained approximately the same, as expected. Notathe expression levels of the mutated promoter fragment (-102/ +182M) remained approximately the same, as expected. Notathe oct-P domain in the PDGFoR gene P2 promoter and effectine oct-P domain in the PDGFoR gene P2 promoter and effec-





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increasing concentrations to perform a supershift. The Oct-in the legend of Fig. 3. P. free probe. mouse F9 embryonal carcinoma cells (P9) with the PDGF legend of Fig. 3. The identified complexes are depicted undifferentiated EC or 7 days RA-differentiated Tera2 of PDGFAR promoter olgonucleotide oct-P was used in an

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Oct-4 Regulates PDGF a R Promoter in Human EC Cells

of the octamer flanking sequences of kFGF, REX-1, and PDGF α R shows, however, that the motifs cannot be aligned adequately to explain the preferential binding of Oct-4 to these sites. Accessory proteins might therefore explain Oct-4-dependent regulation (see above).

The role of proteins encoded by these alternative PDGF αR transcripts, if any, remains obscure, since presently none of these proteins have been detected in vivo. The sequence of the 5.0-kb messenger suggests a putative oncogene-like action, which may be important for autonomous growth of Tera2 EC cells. Undifferentiated Tera2 cells have been shown to proliferate in the absence of serum growth factors (34). A possible role in development or differentiation may also be inferred from the expression patterns of alternative PDGF αR transcripts. P2-initiated transcripts have been identified in human oocytes and preimplantation stages⁵ and in human testis tumors.² The down-regulation of the 1.5-kb transcript expression in spermatogenesis suggests that the P2-promoter is active in cells of the female primordial germ cell lineage, probably in the commitment of cells during embryonal development. Alternative transcripts, generated by differential promoter use and/or splicing, of other tyrosine kinase receptors have been described, including the genes for PDGF β R (4), FGF receptors (35), epidermal growth factor receptor (36), c-kit (37) and PDGF α R of the mouse (38). Interestingly, an alternative transcript of approximately 4.8 kb of the PDGF α R gene has been detected in the mouse embryonal carcinoma cell line F9, but only after RAinduced differentiation (38). This transcript has also been described by Lee et al. (39) and is regulated in a differentiationspecific manner. Any conclusive evidence concerning functions of the corresponding alternative proteins remains lacking, however. In conclusion, the POU transcription factor Oct-4 controls the developmentally regulated expression by the PDGF α R P2 promoter. Whether the down-regulation of Oct-4 during RAinduced differentiation is a prerequisite for the activation of the PDGF α R promoter P1 remains to be determined. We are currently working toward this goal.

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