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Retinoic Acid Promotes Transcription of the Platelet-Derived Growth Factor α-Receptor Gene

CHIAYENG WANG,[1] JAMES KELLY,[2] DANIEL F. BOWEN-POPE,[2] AND CHARLES D. STILES[1]*

Division of Cellular and Molecular Biology, Dana-Farber Cancer Institute, and Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115,[1] and Department of Pathology, University of Washington, Seattle, Washington 98195[2]*

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Retinoic acid together with dibutyryl cyclic AMP stimulated transcription of the platelet-derived growth factor α-receptor gene in embryonal carcinoma cells (line F9). Processed mRNA transcripts appeared within 4 h after exposure to these agents, and functional α:α homodimers appeared within 24 h.

Platelet-derived growth factors (PDGFs) (1, 5, 27) and their receptors (3, 8, 15, 28) have been linked to the biology of wound healing and connective tissue remodeling in vivo (13, 20, 23). However, a growing body of data suggests that the A:A isoform of PDGF has another function at early times in embryonic development. Transcripts for PDGF A are contained as maternal mRNA in the mouse and *Xenopus* eggs (16, 18). Mouse teratocarcinoma stem cell lines express the PDGF A gene preferentially, if not exclusively, and secrete a PDGF-like mitogen, which in all probability is PDGF A:A (9, 19).

The A:A homodimer of PDGF is the most selective form of PDGF. It can interact only with one of the three receptor isoforms, the α:α homodimer (2, 10, 22). In teratocarcinoma cells, expression of the PDGF α-receptor subunit mRNA is stimulated by the morphogen retinoic acid. Transcripts of PDGF α-receptor mRNA have been detected in early mouse embryos (17), and recent observations suggest that expression of the α-receptor gene is essential for correct development of the mouse embryo. Mice that inherit two copies of an embryonic lethal genetic locus termed patch express no PDGF α-receptor mRNA at a time when α-receptor transcripts are readily apparent in their wild-type or heterozygous siblings in utero. This lack of α-receptor mRNA reflects a deletion within the PDGF α-receptor gene (D. A. Stephenson, M. Mercola, E. Anderson, C. Wang, C. D. Stiles, D. Bowen-Pope, and V. M. Chapman, Proc. Natl. Acad. Sci. USA, in press).

In the face of these provocative links to the developmental process, expression of functional PDGF α-receptor protein has not been documented in embryos or in teratocarcinoma cells. The role of retinoids in promoting expression of the α-receptor gene has likewise gone unaddressed. In experiments described here, we focused on these gaps in the data base. We show that in teratocarcinoma cells at least, the α-receptor mRNA transcripts that are induced by retinoic acid are translated into functional PDGF α:α receptor homodimer. In addition, we show that retinoic acid functions at a transcriptional level to promote expression of the α-receptor gene.

Selective induction of PDGF α-receptor gene expression by retinoic acid. Expression of the PDGF α-receptor and β-receptor genes was monitored by Northern (RNA) blotting (21). To detect expression of the mouse α-receptor gene, we isolated and characterized a cDNA clone that contained the entire open reading frame of the protein. DNA sequence analysis (submitted to the GenBank data base) and COS cell expression studies (data not shown) confirmed that this cDNA encodes a murine homolog of the human and rat PDGF α receptors described by others (3, 14, 15). Murine PDGF β-receptor cDNA was a generous gift from L. Williams, University of California, San Francisco (28). Total RNA was isolated by modification of guanidinium chloride method (4).

The PDGF α-receptor subunit message was expressed in F9 cells after exposure to retinoic acid (Fig. 1A, lane 2). Dibutyryl cyclic AMP and theophylline cooperated with retinoic acid (a combination hereafter referred to as RACT) to induce higher levels of α-receptor mRNA (lane 4). In time course studies, we found that α-receptor message levels began to increase within 4 h after exposure to RACT (see Fig. 3) and remained elevated for at least 6 days in the presence of RACT (data not shown). RACT did not stimulate expression of the β-receptor within the time frame of these experiments (Fig. 1B). We have noted previously that some β-receptor mRNA can be seen within F9 cells after 6 days of exposure to RACT. Even at 6 days though, α-receptor transcripts are by far the predominant form of PDGF receptor mRNA (17).

Expression of functional PDGF α:α homodimer. Our next experiments addressed translation of the α-receptor mRNA. RACT-treated F9 cells, together with control cultures, were exposed to human recombinant PDGF A:A. The cultures were then harvested, lysed, and immunoprecipitated by using antiphosphotyrosine antibody. The immunoprecipitates were then tested for the ability to autophosphorylate by the in vitro kinase assay (6). Cells that had been pretreated with RACT and then exposed to PDGF expressed a kinase activity that was recognized by antiphosphotyrosine antibody (Fig. 2). The major substrate for this kinase was a protein of M_r 170,000, which corresponds in size to the processed PDGF α:α receptor homodimer (Fig. 2, lanes 4, 6, and 8). This kinase activity was not recognized by the antibody prior to PDGF treatment (lanes 3, 5, and 7); in control cultures not treated with RACT, the kinase was not detected under any conditions (lanes 1 and 2). Collectively, these results indicate that the α-receptor mRNA displayed in

* Corresponding author.

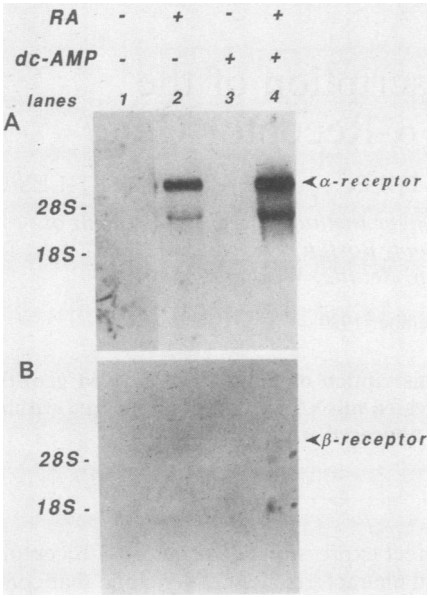

FIG. 1. Northern analysis of PDGF α-receptor expression in murine F9 teratocarcinoma cells. F9 cells cultured as described by others (24) were incubated for 72 h with 5×10^{-7} M retinoic acid (RA; lane 2) or 250 μM dibutyryl cyclic AMP (dc-AMP; lane 3) or both (lane 4). Theophylline (350 μM) was included when cells were treated with dibutyryl cyclic AMP. Northern blots were probed with ^{32}P-labeled DNA corresponding to the mouse PDGF α receptor (A) or the mouse PDGF β receptor (B). The expected mRNA lengths are 6.7 kb for the PDGF α-receptor subunit and 5.4 kb for the PDGF β-receptor subunit. A 20-μg sample of total RNA was electrophoresed through each lane. The two probes were a 1.8-kb *Eco*RI fragment of the mouse PDGF α-receptor cDNA and a 1.5-kb PstI fragment of the mouse PDGF β-receptor cDNA nick translated to 1×10^8 to 5×10^8 cpm/μg.

Fig. 1 is translated into functional PDGF α:α receptor homodimer within F9 cells.

Induction of α-receptor gene expression is not prevented by cycloheximide. Wang and colleagues have described two distinct kinds of retinoid-responsive genes in F9 cell cultures. Those encoding extracellular matrix proteins such as laminin B1, laminin B2, and collagen IV are induced at relatively late times (18 to 24 h) after exposure to retinoids (25, 26). The induction of these late genes may be secondary to the induction of primary response genes such as *ERA-1*, which encodes a homeobox protein (12). The *ERA-1* gene is induced rapidly (2 to 4 h) after exposure to retinoids, and the induction is protein synthesis independent (11). We attempted to determine whether the α-receptor gene responds in a primary or a secondary way to retinoic acid. The time course of α-receptor mRNA induction (Fig. 3) was more comparable to that noted for *ERA-1* than to that for laminin B1 (25). The induction of α receptor was somewhat delayed, but not prevented, by cycloheximide (Fig. 3). Further insights into the molecular mechanisms involved in transcriptional control of the α-receptor gene in F9 cells will require isolation and characterization of the promoter elements.

Induction of α-receptor gene expression reflects transcriptional changes. The induction of PDGF α-receptor mRNA by RACT could reflect differences in mRNA stability or gene transcription. To assess the role of transcription in the induction process, nuclear run-on assays were performed (Fig. 4). The laminin B2 gene, which is known to be induced

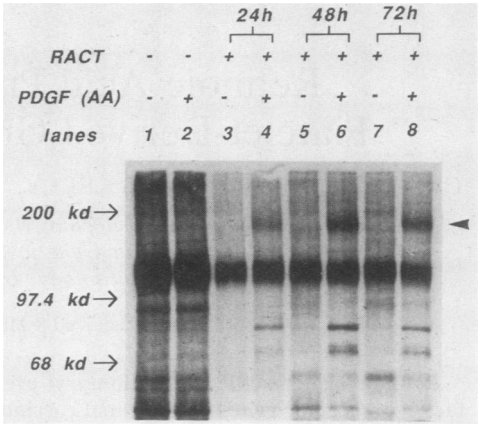

FIG. 2. Detection of functionally active PDGF α receptor in F9 cells. F9 cells were grown and exposed to RACT for 24 h (lanes 3 and 4), 48 h (lanes 5 and 6), or 72 h (lanes 7 and 8). Cells were then transferred to fresh medium supplemented with platelet-poor plasmid rather than serum and incubated in the presence of RACT for an additional 12 h. At the end of this time, some of the cultures (lanes 2, 4, 6 and 8) were exposed to human PDGF A:A (30 ng/ml) for 15 min. Cell lysates were prepared, and an in vitro assay for tyrosine kinase autophosphorylation was performed as described previously (6). Monoclonal antibodies specific for phosphotyrosine were a generous gift from Tom Roberts, Dana-Farber Cancer Institute. kd, Kilodaltons.

at the transcriptional level by retinoic acid and dibutyryl cyclic AMP in F9 cells (26), was included in our assays as a positive control. Run-on transcripts from RACT-treated F9 cells hybridized strongly with the antisense strand of the PDGF α-receptor gene but not to the sense strand (Fig. 4). The DNA strand selectivity indicates that labeled transcripts represent authentic α-receptor pre-mRNA rather than readthrough transcripts of other genes. Run-on transcripts from untreated F9 stem cells did not hybridize to either strand of the α-receptor DNA. α-Amanitin prevents produc-

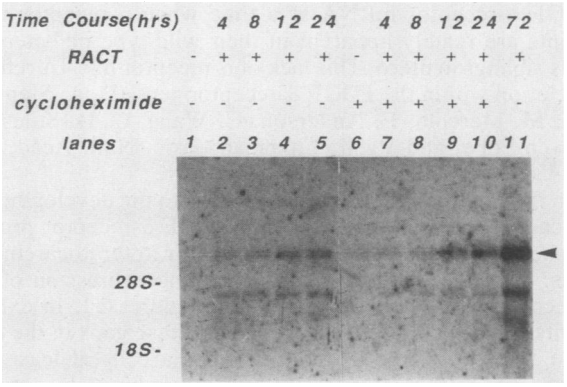

FIG. 3. Effect of cycloheximide on the expression of PDGF α receptor in F9 cells. F9 stem cells were exposed to RACT for 4, 8, 12, 24, or 72 h, as indicated (lanes 2 to 5 and 7 to 11). Control cultures (lanes 1 and 6) were maintained in the absence of RACT. Cycloheximide (10 μg/ml) was included in the culture medium where indicated (lanes 6 to 10). Total RNA was collected from the cells after treatment and examined by Northern blot analysis as described in the legend to Fig. 1. The F9 cells began to detach from the dish after 24 h in the presence of cycloheximide. For this reason, we could not assess the requirements for protein synthesis at later times.

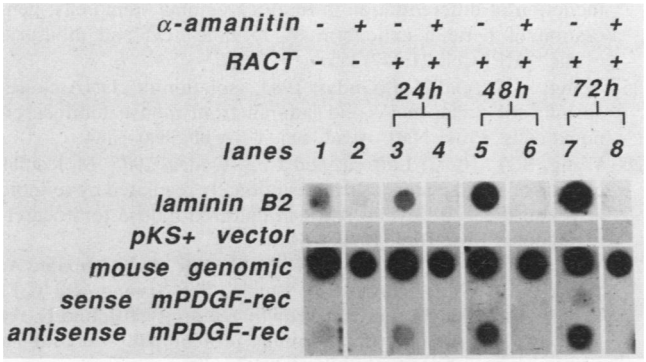

FIG. 4. Nuclear run-on analysis of PDGF α-receptor gene transcription. Nuclear run-on transcription reactions were done as described by Greenberg and Ziff (7). ^{32}P-labeled run-on RNA transcripts were made from either undifferentiated (lanes 1 and 2) or RACT-differentiated (lanes 3 to 8) F9 cells in the presence (lanes 2, 4, 6, and 8) or absence (lanes 1, 3, 5, and 7) of α-amanitin. The radiolabeled run-on transcripts were hybridized to 10 μg of the following linearized DNAs: laminin B2, pKS$^+$ vector, total mouse genomic DNA, PDGF α receptor (mPDGF-rec) (sense strand), and PDGF α receptor (antisense strand).

tion of the α-receptor pre-mRNA in RACT-treated F9 cells, suggesting that the transcripts are synthesized by RNA polymerase II.

Scanning laser densitometry of the run-on film (Fig. 4) indicated that at 72 h after addition of RACT, transcription was enhanced by a factor roughly proportionate to the induction of cytoplasmic mRNA at 72 h (Fig. 3). It is difficult to obtain quantitative data on gene induction by densitometric analysis of X-ray films. However, with due regard to the inherent inaccuracy of film scans, the run-on data indicate that enhanced transcription by RNA polymerase II plays a prominent role in the induction of PDGF α-receptor gene expression.

Collectively, these data explain earlier observations that undifferentiated F9 cells do not bind exogenous PDGF, whereas retinoic acid-differentiated F9 cells do (9, 19). After exposure to retinoids, F9 stem cell cultures express functional receptors for PDGF A:A. Transcriptional changes play a prominent role in the induction of PDGF α-receptor gene expression though not necessarily an exclusive role.

Two other issues await further study. The first of these is the nature of a faint 4.8-kb mRNA that is displayed by our probe for the mouse α-receptor subunit on Northern blots (Fig. 1 and 3). This 4.8-kb mRNA may be a truncated version of the authentic 6.7-kb α-receptor mRNA. Alternatively, the smaller mRNA may represent transcripts of a gene that is closely related to the PDGF α-receptor gene. The nature of this 4.8-kb transcript, which was observed also in the studies of Lee et al. (14), is currently under investigation.

The second issue is whether transcription of the PDGF α-receptor gene is regulated by retinoids within the developing mouse embryo. The developing embryo does express α-receptor mRNA (17). Expression of the α receptor may be necessary for correct embryonic development (Stephenson et al., in press). However, we cannot safely extrapolate our teratocarcinoma cell data to the developing mouse embryo. Direct analysis of retinoids and their role in regulating α-receptor gene expression in utero will be technically demanding but ultimately necessary.

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