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Platelet-derived Growth Factor Stimulates Membrane Lipid Synthesis Through Activation of Phosphatidylinositol 3-Kinase and Sterol Regulatory Element-binding Proteins*

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We analyzed the transcriptional program elicited by stimulation of normal human fibroblasts with platelet-derived growth factor (PDGF) using cDNA microarrays. 103 significantly regulated transcripts that had not been previously linked to PDGF signaling were identified. Among them, a cluster of genes involved in fatty acid and cholesterol biosynthesis, including stearoyl-CoA desaturase (SCD), fatty acid synthase, and hydroxymethylglutaryl-CoA synthase (HMGCS), was up-regulated by PDGF after 24 h of treatment, and their expression correlated with increased membrane lipid production. These genes are known to be controlled by sterol regulatory element-binding proteins (SREBP). PDGF increased the amount of mature SREBP-1 and regulated the promoters of SCD and HMGCS in an SREBP-dependent manner. In line with these results, blocking SREBP processing by addition of 25-hydroxycholesterol blunted the effects of PDGF on lipogenic enzymes. SREBP activation was dependent on the phosphatidylinositol 3-kinase (PI3K) pathway, as judged from the effects of the inhibitor LY294002 and mutation of the PDGF β receptor tyrosines that bind the PI3K adaptor subunit p85. Fibroblast growth factors (FGF-2 and FGF-4) and other growth factors mimicked the effects of PDGF on NIH3T3 and human fibroblasts. In conclusion, our results suggest that growth factors induce membrane lipid synthesis via the activation SREBP and PI3K.

Growth factors stimulate cell division by promoting the transition from G₀ to S in the cell cycle. One such factor, platelet-derived growth factor (PDGF),¹ was initially characterized as a

potent mitogen for fibroblasts and other cells of mesenchymal origin (1). PDGF consists of a family of dimeric ligands that are produced from four different genes, namely PDGF-A, -B, -C, and -D. They exert their cellular effects by binding to and dimerizing PDGF α - and/or β -receptors, which belong to the receptor-tyrosine kinase family (2, 3). The PDGF isoforms play important roles during embryonic development and in wound healing (1, 4). In addition, an improper activation of PDGF receptors contributes to the development of fibrosis, atherosclerosis, and certain forms of cancer (1).

Growth factor receptors, including the PDGF receptors, use a common set of signal transduction cascades (5). One of the best characterized pathways involves the sequential activation of Ras, RAF, MEK, and the ERK mitogen-activated protein kinase, which phosphorylates multiple substrates, many of which are transcription factors. A second important signaling event depends on the re-localization of phosphatidylinositol 3-kinase (PI3K) to the plasma membrane, which leads to the production of phosphatidylinositol 3,4,5-trisphosphate and the activation of downstream effectors such as protein kinase B (PKB, also called Akt), and p70 S6 kinase (6). Both ERK and PI3K play essential roles in the mitogenic response to growth factors (1, 7). Recently, Jones and Kazlauskas (8) proposed that the G₀ to S transition requires two waves of signaling; one that follows immediately after growth factor stimulation and is mediated by ERK and c-Myc, and a second wave that occurs about 8-h later and overlaps temporally with the cell cycle program (8). PI3K activation appeared to be essential during the second phase.

Most studies have focused on the mechanism by which growth factors stimulate DNA synthesis and mitosis. Besides these essential aspects of the cell cycle, cell proliferation also requires the replication of all other cell constituents. It was recently suggested that this process is in part mediated by PI3K, which was shown to stimulate protein synthesis and to contribute to cell size increase (9). The mechanisms of production of other cell constituents, such as membrane lipids, during proliferation are not well understood.

Lipogenic enzymes are controlled at multiple levels, such as gene expression, protein stability, and enzymatic activity. One of the most important groups of transcription factors that govern the expression of these enzymes is constituted by sterol regulatory element-binding proteins, *i.e.* SREBP-1a, -1c, and -2 (10, 11). SREBP-1c differs from SREBP-1a only in the N ter-

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The microarray gene expression data from this study has been submitted to the GEO database (Gene Expression Omnibus) and Array-Express under accession numbers: GSE1484 and E-MEXP-126, respectively.

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¹ The abbreviations used are: PDGF, platelet-derived growth factor; APRE, acute phase response element; EGF, epidermal growth factor; FAS, fatty acid synthase; FGF, fibroblast growth factors; HMGCR, hydroxymethylglutaryl-coenzyme A reductase; HMGCS, hydroxymethyl-

ylglutaryl-CoA synthase; PI3K, phosphatidylinositol 3-kinase; PKB, protein kinase B; RPLP0, ribosomal protein, large, P0; SCD, stearoyl-CoA desaturase; SLC20A1, solute carrier family 20, member 1; SREBP, sterol regulatory element-binding proteins; ERK, extracellular signal-regulated kinase.

minus, which is encoded by an alternative exon 1 of the *SREBP-1* gene. These factors are produced as precursor proteins embedded in the endoplasmic reticulum membrane as a complex with the cholesterol sensor SCAP (10, 12). SCAP is required to escort SREBP in the Golgi apparatus, where two specific proteases, S1P/SKI-1 and S2P, cleave SREBP, releasing a soluble active transcription factor that up-regulates the expression of genes involved in cholesterol and fatty acid biosynthesis, as well as NADPH production (13, 14). In a negative feedback mechanism, cholesterol promotes the binding of SCAP to INSIG-1 and -2, which prevents SREBP movement to the Golgi and its processing (12, 15). Using mutant mammalian cell lines, Goldstein *et al.* (16) have demonstrated that several components of the SREBP pathway are required for long term proliferation, unless the cells are provided with key lipid metabolism products, such as oleic acid, cholesterol, and mevalonate (16). Whether SREBP is regulated during cell growth is, however, not known.

In this report, we analyzed changes in gene expression in fibroblasts stimulated by PDGF, using cDNA microarrays. After 1–4 h of stimulation, we observed altered expression mainly of previously characterized immediate early genes (17, 18). Among the genes induced after longer periods of PDGF treatment were genes involved in lipid biosynthesis, which required the activation of SREBP transcription factors, and correlated with increased membrane lipid synthesis. This cascade of events was also triggered by other growth factors and was found to depend on the activation of the PI3K pathway.

MATERIALS AND METHODS

Cell Culture and Reagents—AG01518 foreskin fibroblasts (Coriell Institute for Medical Research, Camden, NJ) from passages 11–19 were cultured in modified Eagle's medium (Sigma) supplemented with 10% fetal calf serum and glutamine. For starvation and treatments, cells were washed and incubated in medium containing glutamine and 0.1% essentially fatty acid-free bovine serum albumin (Sigma). PDGF-BB was generously provided by Amgen. EGF, FGF-2, and FGF-4 were purchased from Peprotech (London, UK). HepG2 and NIH3T3 were obtained from ATCC and cultured in Dulbecco's modified Eagle's medium (Sigma) in the presence of 10% fetal calf serum or calf serum, respectively. NIH3T3 that stably express the EGF receptor were kindly provided by Katarzyna Kowanetz (Ludwig Institute for Cancer Research, Uppsala, Sweden). A rabbit antiserum raised against the CLARIKRTGDGNYKSG peptide, mapping at the C terminus of human SCD, was generated as described (19).

cDNA Microarray Analysis—Subconfluent AG01518 fibroblasts were starved for 16 h in the presence of 0.1% bovine serum albumin, and then stimulated for 1, 4, 10, or 24 h with PDGF-BB (10 ng/ml in starvation medium) or left untreated for the same periods of time. Total RNA was isolated using the RNeasy kit (Qiagen). The RNA quality was checked by formaldehyde-agarose gel electrophoresis (RNeasy protocol). Total RNA (40 μ g) from cells treated for a given period of time with PDGF or starvation medium alone was labeled in reverse transcription reactions (Superscript II kit, Invitrogen) with dCTP-Cy5 and dCTP-Cy3, respectively (Amersham Biosciences). In every second replicate experiment the fluorescent deoxynucleotides were swapped. Purified cDNA probes labeled with Cy3 and Cy5 were mixed per pair and hybridized to cDNA microarray chips (hver1.2.1) from the Sanger Institute/LICR/CRUK consortium (see www.sanger.ac.uk/Projects/Microarrays/ for details and hybridization protocols). For each time point, we performed at least four independent hybridizations and used at least two different batches of RNA. Chips were scanned in a PerkinElmer/GSI Lumonics ScanArray 4000 scanner, and spot intensities were measured using the QuantArray software (histogram method with background subtraction). Normalization and statistical analysis of the quadruplicate data sets were performed using GeneSpring 5.0 analysis software (Silicon Genetics). A Lowess non-linear normalization was applied, and the median of the ratio distribution for each array was set to 1. Regulated spots were selected based on the average ratio values ≥ 1.750 for up-regulated genes and ≤ 0.571 for down-regulated genes. In addition, we considered only genes that were significantly regulated (Student's *t*

test, $p < 0.05$) based on replicate hybridizations (global error model, GeneSpring). For all features selected using this protocol, the signal was significantly above the background, indicating that the expression of these genes was detectable. Finally, Hver1.2.1 microarrays contain replicate spots (1–6) corresponding to the same gene. Genes represented by spots that were not regulated in a similar manner were discarded. We show the average ratio of one representative spot for each regulated gene, with standard error calculated from multiple hybridizations and with the annotation provided by the microarray facility (Hver1.2.1_NCB133).

Quantitative Real Time PCR—These experiments were performed essentially as recommended by Applied Biosystems and described in a previous report (20). Briefly, cDNA was prepared from 5 μ g of total RNA using the Superscript II kit and diluted with water to 100 μ l. Quantitative real-time PCR experiments were performed with 2 μ l of cDNA, SYBR green master mix (Applied Biosystems), and oligonucleotides designed using the PrimerExpress software, according to the manufacturer's instructions. PCR reactions were run in triplicate on an ABI7000 sequence detection system. Quantitative data were calculated using a regression curve from serial dilutions of a standard cDNA, or the Ct method (for validated assays).

Luciferase Experiments—Cells were seeded 1 day before in 12-well plates (50,000 cells per well). NIH3T3 were transfected using LipofectAMINE PLUS (Invitrogen), and HepG2 with calcium phosphate precipitates, as described (20). The indicated promoters cloned in the pGL2 luciferase reporter (125 ng, Promega) were mixed with pDRIVE-chEF1-RU5' (β -galactosidase reporter, Invitrogen, 125 ng) and pTKCIII as carrier DNA. Cells were washed, incubated in serum-free medium for 36 h, in the presence or absence of PDGF-BB (50 ng/ml), lysed, and processed as described (20). Data are presented as the average ratio between the luciferase activity and the β -galactosidase content, with standard deviations from duplicate transfections. All experiments were repeated at least three times.

Lipid Synthesis Measurements—Subconfluent AG01518 fibroblasts were starved for 16 h, and treated with or without PDGF-BB (50 ng/ml) for 20 h. [14 C]acetate (5 μ Ci, 1 μ Ci/ml) was then added, and incubation was prolonged for 4 h. Cells were washed, trypsinized, centrifuged, resuspended in PBS, and solubilized by addition of 20 volumes of chloroform/methanol 2:1. Four volumes of 0.9% NaCl were added to separate the two phases. The chloroform phase was dried under a nitrogen flux, and either used for total lipid determinations (see below), or hydrolyzed for 1 h at 80 $^{\circ}$ C in 1 ml of methanol containing 1 M KOH. Cholesterol and other neutral lipids were extracted with hexane. The lower phase was then neutralized and mixed with chloroform to extract fatty acids. Fractions were dried and analyzed by thin layer chromatography using silica gel 60 plates (Merck), which were developed with hexane/diethyl ether/acetic acid (87:20:1 vol), in the presence of standards (oleic acid, stearic acid, cholesterol, lanosterol, and squalene, all from Sigma). Unsaturated fatty acids were separated from saturated ones using plates impregnated with AgNO₃, as described (21). Radioactivity was quantified using a FUJI phosphorimager, and standards were visualized in iodine vapors or by charring. The radioactivity of each fraction was also measured in a β counter. The analysis of non-hydrolyzed lipids was performed as described (22). The initial chloroform extract was loaded on an aminopropyl column (Supelco). Neutral lipids were eluted with chloroform/isopropyl alcohol (2:1) and phospholipids with methanol. Each fraction was dried and analyzed by thin layer chromatography. The plates were developed in chloroform/methanol (2:1) only to 2 cm from bottom edge, followed by development with chloroform/ethanol/triethylamine/water (30:34:30:8) for 4 cm, thoroughly dried, and then developed in hexane/diethyl ether (50:5). Spots were visualized as above, and identified by comparison with the following standards: phospholipid mix (Supelco), triolein, cholesterol oleate, cholesterol, lanosterol (all from Sigma). Total protein contents of the initial samples were measured using the BCA kit (Pierce) and used to normalize the results.

Immunoprecipitations and Western Blots—Subconfluent AG01518 cells were starved as described above and stimulated with PDGF-BB for the indicated period of time. *N*-Acetyl-leucyl-leucyl-norleucinal (25 μ g/ml, Calbiochem) was added to the medium 2 h before harvest (23). Cells were washed, scraped in 2 ml of PBS, centrifuged, and lysed (1% Triton, 0.2% sodium deoxycholate, 140 mM NaCl, 10% glycerol, 50 mM Tris, pH 8, 1 mM Pefabloc, 1% Trasylol, 1 mM NaF, 25 μ M *N*-acetyl-leucyl-leucyl-norleucinal). Lysates were homogenized by using a 20G syringe and cleared by centrifugation. SREBP-2 was immunoprecipitated from 1 mg of total proteins with 10 μ g of 1D2 antibody and protein G-Sepharose (Amersham Biosciences). Immunoprecipitates or equal amounts of total protein extract were analyzed by SDS-PAGE followed by Western blot-

TABLE I
Gene regulation by PDGF in AG01518 fibroblasts

AG01518 fibroblasts were stimulated with PDGF-BB for 1, 4, 10, or 24 h and analyzed using microarrays as described under "Materials and Methods." The value that corresponds to the largest change (peak) is shown with S.E. A more detailed table including all uncharacterized ESTs is available at www.icp.ucl.ac.be/mexp/pdgm.

Name	Synonyms/description	Ensembl reference	Peak		Published	
			Time	Value \pm S.E.	PDGF	SREBP
Transcription factors						
NR4A1	Nuclear receptor	ENSG00000123358	1	8.20 \pm 0.39 ^a	+ ^b	
EGR3	Pilot, early growth response 3	ENSG00000179388	1	4.44 \pm 0.54	+	
JUNB		ENSG00000171223	1	4.30 \pm 0.49	+	
IER3	Immediate early response 3 interleukin-3 promoter	ENSG00000182403	1	2.83 \pm 0.69	+	
NFIL3	Transcriptional activator	ENSG00000165030	1	2.81 \pm 0.28		
TIEG	TGF- β inducible early protein	ENSG00000155090	1	2.46 \pm 0.28	+	
IRF1	Interferon regulatory factor 1	ENSG00000125347	1	2.30 \pm 0.32 ^a	+	
JUN		ENSESTG00000601757	1	2.11 \pm 0.33	+	
BCL3	B-cell lymphoma 3-encoded protein	ENSG00000069399	1	1.87 \pm 0.67 ^a	+	
ZFP36L1	TIS11B	ENSG00000185650	1	1.84 \pm 0.13	+	
FOSL1	FRA1	ENSG00000175592	1	1.76 \pm 0.20	+	
MYC		ENSG00000136997	10	1.96 \pm 0.12	+	
SREBF2	Sterol regulatory element-binding factor 2	ENSG00000100152	24	2.11 \pm 0.47 ^a		+
PBX1		ENSESTG00000666656	24	0.56 \pm 0.04		
NR4A3		ENSG00000119508	24	0.52 \pm 0.03	+	
ID2	Inhibitor of DNA binding 2	ENSG00000115738	24	0.48 \pm 0.04		
Structural proteins						
Cytoskeleton						
TUBA8	Tubulin α 8	ENSG00000070490	24	5.19 \pm 0.44		
TUBA1	Tubulin α 1	ENSG00000167552	24	4.94 \pm 0.61		
TUBB	Tubulin β	ENSG00000137285	24	2.99 \pm 0.35		
TUBB5	Tubulin β 5	ENSG00000183805	24	2.85 \pm 0.09		
KRTHA3A	Keratin, hair acidic 3A	ENSG0000006059	24	2.03 \pm 0.31		
KRT15	Cytokeratin 15	ENSG00000171346	24	2.02 \pm 0.26		
MSN	Moesin	ENSESTG00000533257	24	2.01 \pm 0.16		
KRTHA1	Keratin, hair, acidic, 1	ENSG00000094796	24	2.00 \pm 0.26		
ARP3	Actin-related protein 3	ENSESTG00000842340	24	1.93 \pm 0.23		
PXN	Paxillin	ENSESTG000003020580	24	1.93 \pm 0.31		
LPXN	Leupaxin	ENSG00000110031	24	1.81 \pm 0.12		
GJA1	Connexin 43	ENSG00000152661	24	0.51 \pm 0.07		
Extracellular matrix						
SERPINB2	Plasminogen activator inhibitor 2	ENSG00000166401	10	1.91 \pm 0.16		
PLAU	Pro-urokinase precursor	ENSG00000122861	24	3.60 \pm 0.40	+	
SERPINE1	Plasminogen activator inhibitor, PAI	ENSG00000106366	24	2.16 \pm 0.22	+	
PLAT	Plasminogen activator	ENSG00000104368	24	2.11 \pm 0.17	+	
TNFAIP6	Hyaluronate-binding protein	ENSG00000123610	24	1.82 \pm 0.23	+	
PCOLCE	Type 1 procollagen C-proteinase enhancer	ENSG00000106333	24	0.55 \pm 0.03		
FBLN2	Fibulin 2	ENSG00000163520	24	0.54 \pm 0.10		
LUM	Lumican	ENSG00000139329	24	0.52 \pm 0.06		
FBLN5	Fibulin 5	ENSG00000140092	24	0.51 \pm 0.05		
MFAP4	Microfibril-associated glycoprotein 4	ENSG00000166482	24	0.43 \pm 0.05		
DCN	Decorin	ENSG00000011465	24	0.42 \pm 0.04		
Membrane structural proteins						
SLC20A1	Phosphate transporter 1, GLVR1	ENSG00000144136	24	5.68 \pm 0.78 ^a		
SLC20A2	Phosphate transporter 2, GLVR2	ENSG00000168575	24	2.57 \pm 0.52		
ITGA2	Integrin α 2	ENSG00000164171	24	2.46 \pm 0.12		
ITGA3	Integrin α 3	ENSG00000005884	24	2.12 \pm 0.32 ^a		
PLAUR	UPAR	ENSG00000011422	24	1.99 \pm 0.20	+	
Growth and survival						
MCL1	Myeloid cell leukemia sequence 1	ENSG00000143384	1	1.95 \pm 0.16	+	
CCNG2	Cyclin G2	ENSG00000138764	4	0.52 \pm 0.08		
DTR	Heparin-binding EGF-like growth factor	ENSG00000113070	10	2.22 \pm 0.22 ^a	+	
TSSC3	Tumor suppressing subtransferable candidate	ENSG00000181649	24	2.26 \pm 0.31 ^a		
IGF1R	IGF1 receptor	ENSESTG000002374024	24	0.54 \pm 0.02		
PDGFRA	PDGF receptor α	ENSG00000134853	24	0.42 \pm 0.04		
Immunity						
PTGS2	Prostaglandin endoperoxide synthase type II	ENSG00000073756	1	4.92 \pm 0.98	+	
GBP1	Guanylate-binding protein isoform I	ENSG00000117228	4	1.98 \pm 0.13		
MCP1	Small inducible cytokine A2	ENSG00000108691	4	1.95 \pm 0.28	+	
TAP1		ENSG00000185939	4	1.88 \pm 0.21		
LTBP1	Latent TGF β 1-binding protein	ENSG00000049323	24	1.85 \pm 0.14		
C1S	Complement component	ENSG00000182326	24	0.48 \pm 0.07		
Metabolism						
Cholesterol synthesis						
HMGCR	3-Hydroxy-3-methylglutaryl-CoA reductase	ENSG00000113161	24	2.81 \pm 0.33 ^a	+	+
DHCR24	24-Dehydrocholesterol reductase	ENSG00000116133	24	2.71 \pm 0.49		
HMGCS1	3-Hydroxy-3-methylglutaryl CoA synthase	ENSG00000112972	24	2.66 \pm 0.35 ^a		+
DBI		ENSG00000155368	24	2.18 \pm 0.28		+
DHCR7	7-Dehydrocholesterol reductase	ENSG00000172893	24	2.06 \pm 0.28	+	
EBP	Acyl-coenzyme A-binding protein	ENSG00000147155	24	1.95 \pm 0.16		+

TABLE I—continued

Name	Synonyms/description	Ensembl reference	Peak		Published	
			Time	Value \pm S.E.	PDGF	SREBP
SQLE	Squalene epoxidase	ENSESTG00001922663	24	1.90 \pm 0.25 ^a		+
SC4MOL	C4-Methyl-sterol oxidase, EGR25	ENSG00000052802	24	1.89 \pm 0.13		+
Fatty acid synthesis						
SCD	Stearoyl-CoA desaturase	ENSESTG00002135529	24	7.95 \pm 2.24 ^a		+
FASN	Fatty acid synthase	ENSG00000169710	24	3.45 \pm 0.65		+
ACAT2	Acetyl-Coenzyme A acetyltransferase 2	ENSG00000120437	24	2.77 \pm 0.22 ^a		+
ACLY	ATP citrate lyase	ENSESTG00002932335	24	2.75 \pm 0.32 ^a		+
MGLL	Lipophospholipase-like	ENSG00000074416	24	1.89 \pm 0.15 ^a		
BACH	Long chain acyl-CoA hydrolase	ENSG00000097021	24	1.86 \pm 0.13 ^a		
PKM2	Pyruvate kinase M1	ENSG00000067225	24	2.16 \pm 0.27		
PKLR	Pyruvate kinase type L	ENSG00000143627	24	1.92 \pm 0.31		+
PPAP2B	Phosphatidic acid phosphatase type 2B	ENSG00000162407	24	0.48 \pm 0.06 ^a		
Other metabolic pathways						
SFRS7	9G8 splicing factor	ENSG00000115875	4	2.11 \pm 0.07		
SFRS3	pre-mRNA splicing factor	ENSG00000112081	10	2.18 \pm 0.11		
ANPEP	Aminopeptidase N	ENSG00000166825	24	2.98 \pm 0.49		
ENO1	α -Enolase	ENSG00000074800	24	2.10 \pm 0.17		
NP	Purine nucleoside phosphorylase	ENSG00000100831	24	2.03 \pm 0.30		
TGM2	Transglutaminase	ENSG00000101418	24	1.93 \pm 0.44		
UBE2N	Ubiquitin-conjugating enzyme E2N	ENSG00000177889	24	1.91 \pm 0.25		
CYCS	Cytochrome c	ENSG00000172115	24	1.89 \pm 0.27		+
AMD1	S-Adenosylmethionine decarboxylase	ENSG00000123505	24	1.84 \pm 0.13		+
GOT1	Glutamic-oxaloacetic transaminase 1	ENSG00000120053	24	1.78 \pm 0.19		
ALDH1A1	Aldehyde dehydrogenase 1	ENSG00000165092	24	0.26 \pm 0.04		
Signaling						
DUSP1	MKP1 phosphatase	ENSG00000120129	1	6.45 \pm 0.48		+
ARHB	Rho B	ENSG00000143878	1	3.04 \pm 0.27		+
DUSP4	Dual specificity protein tyrosine phosphatase	ENSG00000120875	1	2.75 \pm 0.38		
DUSP5	Dual specificity protein tyrosine phosphatase	ENSG00000138166	1	2.51 \pm 0.10		+
STK15	Serine/threonine kinase 15	ENSG00000087586	24	2.65 \pm 0.52		
DUSP6	PYST1 phosphatase	ENSG00000139318	24	2.13 \pm 0.31 ^a		+
SH3BP5	SH3-domain binding protein 5	ENSG00000131370	24	0.56 \pm 0.05		
SGK	Serum/glucocorticoid regulated kinase	ENSG00000118515	24	0.54 \pm 0.07		
Stress & redox						
GADD45A	Growth arrest and DNA-damage-inducible α	ENSG00000116717	4	2.09 \pm 0.30		+
MT1	Metallothionein 1L	ENSG00000169688	24	2.82 \pm 0.39		
MT1B	Metallothionein 1B	ENSG00000173048	24	2.72 \pm 0.43		
MT3	Metallothionein-III	ENSG00000087250	24	2.53 \pm 0.38		
MT1K	Metallothionein-IK	ENSG00000125144	24	2.37 \pm 0.49		
GLRX	Glutaredoxin	ENSG00000118990	24	1.95 \pm 0.23		
HYOU1	Oxygen-regulated protein ORP150	ENSG00000149428	24	1.77 \pm 0.24		
DDB2	DNA damage-binding protein 2	ENSG00000134574	24	0.54 \pm 0.12		
GADD45B	Growth arrest and DNA damage inducible	ENSG00000099860	24	0.47 \pm 0.06		
TXNIP	Thioredoxin interacting protein	ENSG00000117289	24	0.39 \pm 0.03		
Miscellaneous						
NOL5A	Nucleolar protein 5A	ENSG00000101361	4	1.79 \pm 0.12		
DDX17	Probable RNA helicase	ENSG00000100201	10	0.56 \pm 0.04		
HIST3H2A	Histone H2A/L	ENSG00000180573	24	0.56 \pm 0.04		
CTSK	Cathepsin K	ENSG00000143387	24	0.56 \pm 0.04		
CTSF	Cathepsin F	ENSG00000174080	24	0.48 \pm 0.03		
ADM	Preproadrenomedullin	ENSG00000148926	24	0.52 \pm 0.04		
DAAM2	Dishevelled-ass. activator of morphogenesis	ENSG00000146122	24	0.49 \pm 0.09		
PSG11	Pregnancy-specific glycoprotein 13	ENSG00000170853	24	0.52 \pm 0.10		
PSG1	Pregnancy-specific glycoprotein-1d	ENSG00000131113	24	0.47 \pm 0.05		
PMP22	Peripheral myelin protein 22, gas3	ENSG00000109099	24	0.44 \pm 0.06		+
RDC1	Orphan G protein-coupled receptor	ENSG00000144476	24	0.42 \pm 0.05		

^a Data were confirmed by Northern blotting or quantitative PCR.

^b +, genes that were previously shown to be regulated by PDGF or SREBP.

ting performed with anti-SREBP-1 or anti-SREBP-2 (clone 2A4 and 1D2, respectively), and re-probed with anti- α -actin antibodies as a control (Sigma).

RESULTS

Regulation of Gene Expression by PDGF in Fibroblasts—In order to gain knowledge about gene regulation by PDGF, we performed microarray experiments with Hver1.2.1 chips, which were provided by the Sanger Institute Microarray facility, and contain 9932 single-stranded cDNAs. Human foreskin fibroblasts were starved in serum-free medium for 16 h, and then stimulated by PDGF-BB (10 ng/ml) for 1, 4, 10, or 24 h. Cells that were left untreated for the same period of time were used as control for each time point. RNA was extracted, and

used as a template to synthesize cDNA in the presence of dCTP-Cy3 or dCTP-Cy5. These probes were used for competitive hybridization of microarrays, as described under "Materials and Methods." We selected genes that were significantly regulated by PDGF-BB by at least 1.75-fold, at one or more time points. We found 127 genes that fulfilled these criteria, usually at only one time point. They included 24 that had previously been linked to PDGF signaling in various models. Notably, PDGF-AB elicited an almost identical transcriptional program (data not shown). To further validate our data, the expression of 18 genes was checked by Northern blotting and/or quantitative PCR. Overall, we found a close concordance between cDNA microarray data and PCR or Northern blot data,

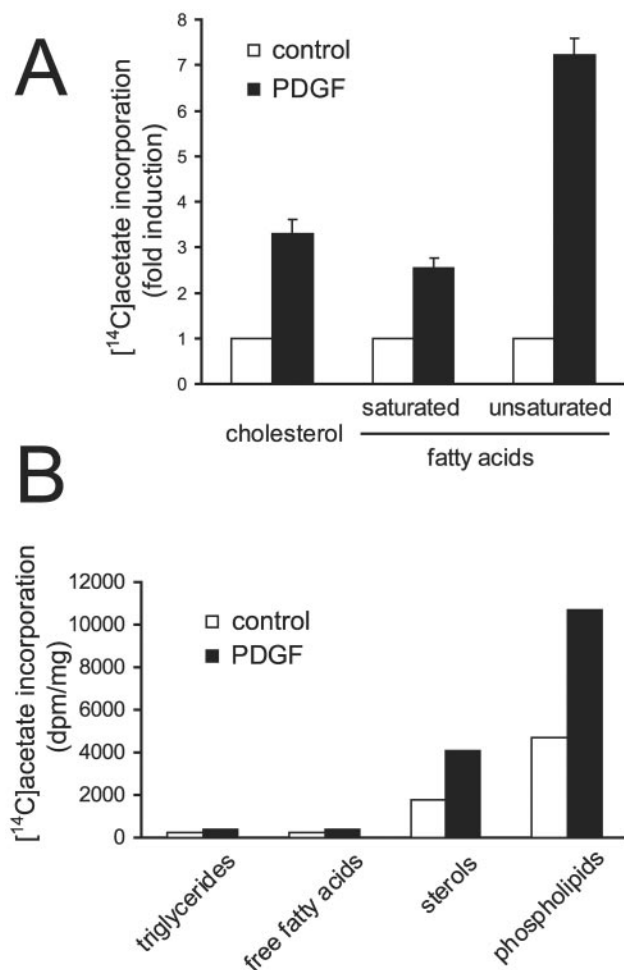


FIG. 1. Stimulation of lipid biosynthesis by PDGF in fibroblasts. AG01518 fibroblasts were brought to quiescence by culturing them in medium containing 0.1% bovine serum albumin for 20 h and then were stimulated with PDGF-BB for 24 h (black bars) or left untreated (open bars). [^{14}C]Acetate was added 4 h before harvest. *A*, lipids were extracted, hydrolyzed, and analyzed by TLC. Shown is the average of three independent experiments with S.E. *B*, initial lipid extract was fractionated on an aminopropyl column to separate neutral lipids from phospholipids, and each fraction was analyzed by TLC. Results are presented as the radioactivity incorporated in each spot divided by the total cell protein content. One representative experiment out of a set of three is shown.

with the microarray analysis underestimating the amplitude of each specific gene response.

Of the 127 genes regulated by PDGF-BB, 18 genes have no known cellular function, while the others could be classified into 12 clusters (Table I). Transcription factors constituted the most prominent class of genes regulated after 1 h of stimulation by PDGF and included well characterized proto-oncogenes such as *c-Jun*, *JunB*, and *c-Myc*. Several molecules involved in signaling were also up-regulated early, including the dual specificity phosphatases *DUSP-1*, *-4*, *-5*, and *-6*, which have been shown to inhibit ERK activation, in a negative feedback loop (24). Stimulation of fibroblasts for 24 h affected the expression of genes connected with the extracellular matrix and cytoskeleton, which might contribute to the effects of PDGF on connective tissue contraction. PDGF treatment also increased the expression of two phosphate transporters, *SLC20A1* and *SLC20A2*, which may account for the old observation that growth factors stimulate phosphate uptake by fibroblasts (25). The largest group comprised 28 genes connected to metabolism, including 17 genes implicated in the biosynthesis of fatty acids or cholesterol. Stearoyl-coenzyme A desaturase (*SCD*), which

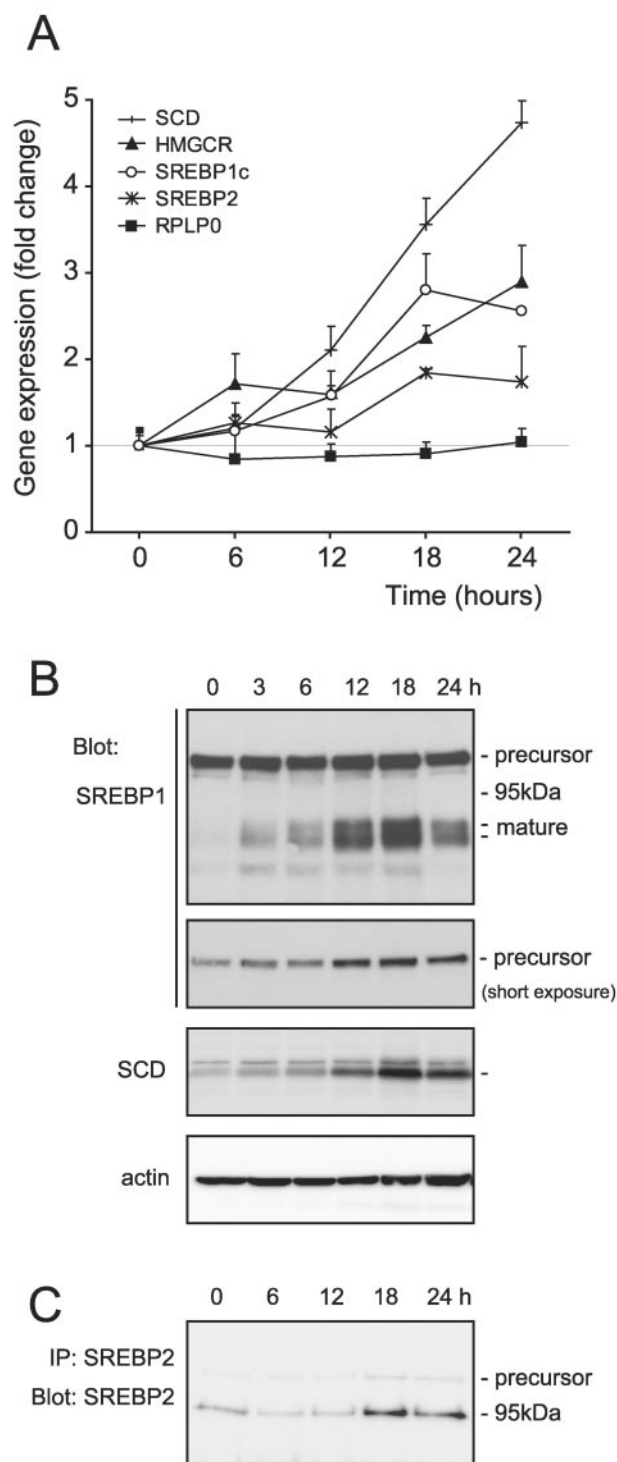


FIG. 2. Time course analysis of SREBP and stearoyl-coenzyme-A desaturase regulation by PDGF. AG01518 fibroblasts were kept in starvation medium for 40 h, or stimulated for 6, 12, 18, or 24 h with PDGF-BB before harvest. *A*, expression levels of *SCD*, *HMGCR*, *SREBP-1c*, and *SREBP-2* transcripts were analyzed by quantitative real-time-PCR. The housekeeping gene *RPLP0* (60 S acidic ribosomal protein P0) was used as a control. *B*, total cell lysates (corresponding to 40 μg of protein) were analyzed by Western blotting with anti-*SCD* or anti-*SREBP-1* antibodies. The filters were then blotted with anti- α -actin as a loading control. *C*, *SREBP2* was immunoprecipitated from cell lysates and analyzed by Western blot using a monoclonal antibody raised against the mature form of human *SREBP-2*.

catalyzes the transformation of saturated fatty acids into monounsaturated fatty acids, was the gene induced to the highest extent by PDGF after 24 h (8.0 ± 2.2 -fold). It was also regu-

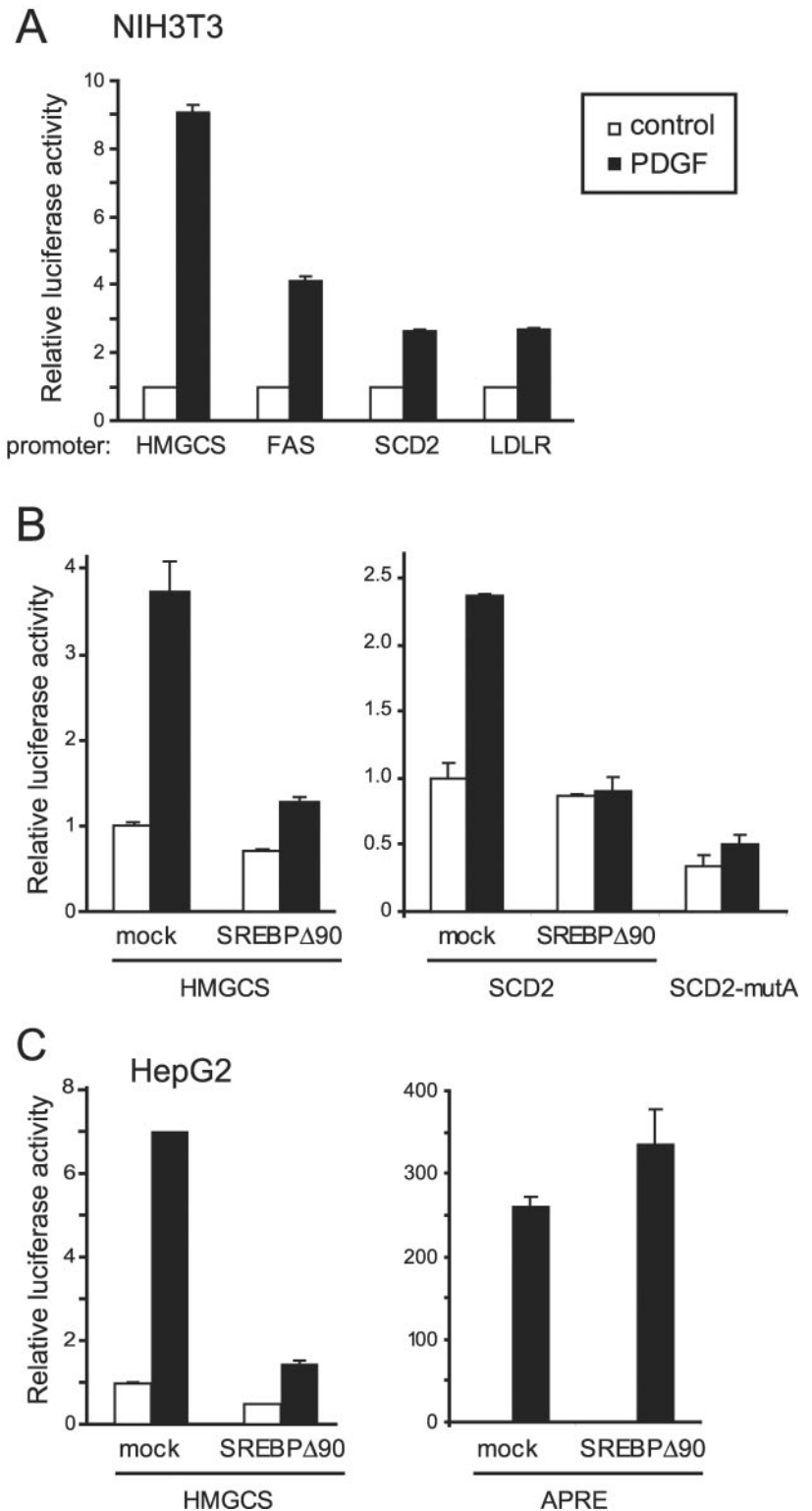


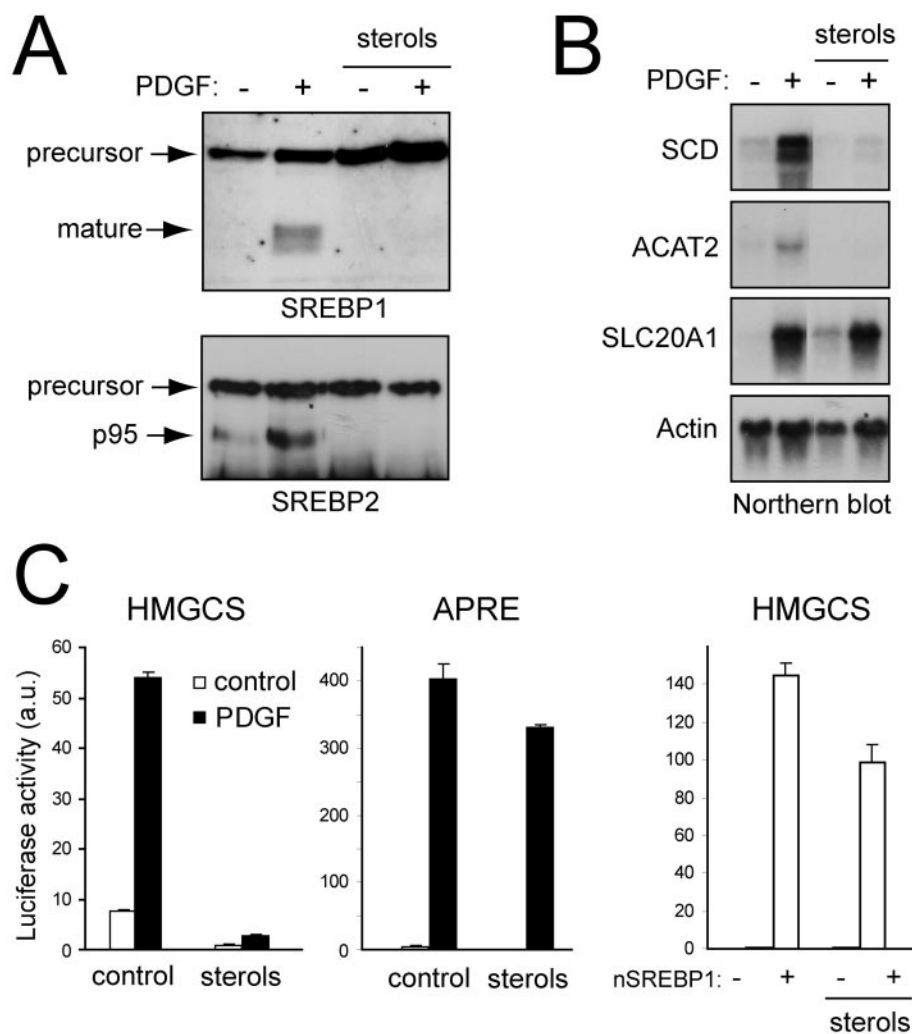
FIG. 3. Stimulation of SREBP-driven promoters by PDGF. *A*, NIH3T3 cells were transfected with constructs consisting of the indicated gene promoters cloned in front of a luciferase reporter. Cells were then cultured in serum-free medium in the presence or absence of PDGF-BB for 36 h. Luciferase content was measured from sample lysates. *B*, cells were co-transfected with a dominant negative SREBP-1 transgene (SREBP Δ 90, 100 ng) or a control plasmid (mock), and a reporter construct containing the *HMGCS* promoter, the *SCD2* promoter, or a mutated version of the *SCD2* promoter in which the sterol-responsive element has been disrupted (SCD2-mutA) (32). *C*, HepG2 cells were co-transfected with the PDGF β -receptor (20 ng) and either a luciferase reporter driven by the *HMGCS* promoter or by multiple STAT3-responsive elements (APRE) and treated as above.

lated by PDGF in NIH3T3 and AG01523 fibroblasts (data not shown). This cluster also included fatty acid synthase (*FAS*), hydroxymethylglutaryl-coenzyme A synthase (*HMGCS*), and reductase (*HMGCR*). Altogether, our microarray data point to a major effect of PDGF on lipid biosynthesis pathways.

Stimulation of Membrane Lipid Production by PDGF—We next investigated whether the effects of PDGF on the expression of lipogenic and cholesterogenic enzymes correlated with a change in lipid biosynthesis in AG01518 fibroblasts. To analyze newly synthesized lipids, cells were labeled with [14 C]acetate

for 4 h before harvest, after which lipids were extracted, hydrolyzed, and analyzed by thin layer chromatography. In line with our microarray results, PDGF had a striking effect on unsaturated fatty acid synthesis, and a moderate effect on the production of cholesterol and saturated fatty acids (Fig. 1A). To determine if fatty acids were incorporated into phospholipids or triglycerides, we also analyzed non-hydrolyzed lipid extracts. We observed that PDGF stimulated the synthesis of phospholipids and free sterols, *i.e.* cholesterol and lanosterol, a cholesterol precursor (Fig. 1B). Triglyceride levels were not affected

FIG. 4. Sterols block SREBP processing and the regulation of lipid metabolism genes by PDGF. AG01518 fibroblasts were starved in medium containing 0.1% bovine serum albumin, and stimulated with PDGF-BB in the presence of a mixture of sterols (cholesterol 10 $\mu\text{g}/\text{ml}$ and 25-hydroxycholesterol 1 $\mu\text{g}/\text{ml}$) or carrier alone (0.2% ethanol), as indicated, for 24 h. **A**, SREBP-1 and immunoprecipitated SREBP-2 were analyzed by Western blotting with specific antibodies. **B**, RNA was extracted and analyzed by Northern blotting (10 $\mu\text{g}/\text{lane}$) as described (43), with the radioactive probes corresponding to SCD, acetyl-coenzyme A acetyltransferase 2 (*ACAT2*), *SLC20A1* phosphate transporter, or β -actin. **C**, HepG2 cells were transfected with the PDGF β receptor and the indicated luciferase reporter constructs and processed as described in Fig. 3. As a control, cells were transfected with a construct encoding the mature nuclear form of SREBP-1 (nSREBP1, 5 ng).



by PDGF, and cholesterol esters were not detectable, indicating that PDGF did not promote fat storage, but rather membrane lipid synthesis. Importantly, the proportions of the different types of membrane lipids were not modified by PDGF. Altogether, these observations likely reflect the duplication of cellular membranes, which should accompany the mitogenic effect of PDGF on fibroblasts. Our data also suggest that PDGF affects the saturation index of fatty acids in these cells.

Activation of SREBP upon PDGF Treatment—To gain insights into the mechanism by which PDGF regulated the expression of lipid biosynthesis enzymes, we searched for common transcription factors that could control the corresponding genes. Based on published work (10, 11, 14), thirteen of them, including *SCD*, *HMGCR*, and *HMGCS*, are known targets of a group of closely related transcription factors, the sterol regulatory element-binding protein (SREBP)-1 and -2 (Table I). In addition, 24-dehydrocholesterol reductase (*DHCR24*) encodes an enzyme that lies in the cholesterol biosynthesis pathway and is therefore a likely target for SREBP, which has been suggested to regulate all genes involved in that process (26). Fibroblast stimulation with PDGF-BB also enhanced the expression of two other SREBP target genes, *i.e.* the cytochrome *c* (Table I), and glucose-6-phosphate dehydrogenase genes (1.71 ± 0.32). Based on quantitative PCR experiments, the low density lipoprotein receptor gene was also up-regulated about 5-fold (data not shown) as previously published (27). Altogether, PDGF had a significant effect on all genes that are known to be controlled by SREBP-1 and -2 and that are represented on the Hver1.2.1 arrays.

Our microarray data indicated that PDGF regulated the *SREBP-2* gene, itself. To analyze the effect of PDGF on the expression of SREBP genes, we set up a quantitative PCR experiment with probes specific for each SREBP family member. The results presented in Fig. 2A demonstrated that the abundance of the SREBP-1c and SREBP-2 transcripts were increased upon PDGF stimulation, at the same time or slightly after those encoding SCD and HMGCR. The alternative transcript encoding SREBP-1a was expressed at a low level in AG01518. We next checked whether PDGF could influence the expression of other components of the SREBP activation pathway. In Western blot experiments, we observed that PDGF did not modify the levels of the SCAP regulatory protein and the proteases S1P/SKI-1 and S2P (data not shown). INSIG-1, which acts as an inhibitor of the pathway in a negative feedback loop, was induced 4.5-fold by PDGF stimulation for 24 h, as one would expect if SREBP is activated (12, 15). INSIG-2 was not significantly affected (not shown). Altogether, our data suggested that PDGF regulated *INSIG-1*, *SREBP-1c*, and -2 genes. Since these genes are controlled by SREBP itself in a feedback manner, it is likely that their regulation in AG01518 cells is a consequence of the post-translational activation of SREBP by PDGF (15, 28, 29).

To test if PDGF treatment resulted in an increase in the amount of mature SREBP, we performed Western blots with specific monoclonal antibodies. The level of mature SREBP-1 was significantly increased as early as 3 h after PDGF treatment and reached a peak after 18 h (Fig. 2B). The SREBP-1 precursor protein was increased after 12 h at the same time as

SCD, in line with our observations at the RNA level. Two SREBP-2 species were immunoprecipitated from fibroblasts lysates: a 130-kDa protein that corresponds the precursor form, and a 95-kDa protein, which may represent a modified form of mature SREBP-2 (Fig. 2C). The latter was increased by PDGF stimulation for 12 h. The SREBP-2 precursor protein was also slightly increased. In conclusion, PDGF induces a rapid increase in mature SREBP-1 protein, which is followed by an increase in SREBP-2 and SCD. In addition, the amount of SREBP precursor proteins also increased upon prolonged PDGF stimulation.

Regulation of SCD and HMGCS Promoters by PDGF in a SREBP-dependent Manner—To investigate the effect of PDGF on SREBP transcriptional activity, we performed luciferase reporter experiments with the gene promoters of *HMGCS*, *FAS*, and *SCD*, which contain SREBP binding sites (12, 30). A construct containing the promoter of the low density lipoprotein receptor gene was also used because it had been shown to be regulated by PDGF (31). In NIH3T3 mouse fibroblasts, PDGF-BB enhanced the activity of all four promoters (Fig. 3A). In mice, two ubiquitous SCD isoforms have been described, SCD1 and SCD2, both of which were regulated by PDGF at the promoter level (not shown). We next used a dominant negative variant, SREBP Δ 90, which corresponds to the mature form of SREBP-1 devoid of its N-terminal transactivation domain, and which neutralizes both SREBP-1 and -2 (32).² When co-expressed, this construct inhibited the stimulation of *SCD2* and *HMGCS* promoters by PDGF (Fig. 3B). In addition, a *SCD2* promoter in which the SREBP binding site has been mutated did not respond to PDGF. Similar results were obtained in HepG2 cells transfected with the PDGF β -receptor (Fig. 3C and data not shown). As a control, SREBP Δ 90 did not affect the regulation by PDGF of a STAT3-responsive promoter containing multiple APRE sites.

To further demonstrate that PDGF activated lipogenic gene promoters in a SREBP-dependent manner, we used 25-hydroxycholesterol, which prevented the cleavage of SREBP-1 and -2 precursors in human fibroblasts (Fig. 4A), as shown previously in many cell lines (16). This compound abrogated the regulation of *SCD* and *ACAT2*, another SREBP target gene, by PDGF (Fig. 4B). By contrast, it did not interfere with the induction of the phosphate transporter *SLC20A1*. Addition of 25-hydroxycholesterol also blocked the PDGF-induced stimulation of the *HMGCS* promoter, but not that of the *APRE* promoter (Fig. 4C). As a further control, we showed that sterols had little effect on the activation of the *HMGCS* promoter by co-transfection of a construct encoding an active form of SREBP-1, which does not require endogenous processing (Fig. 4C). In conclusion, our data suggested that SREBP processing was required for the transcriptional effects of PDGF on lipid metabolism enzymes.

PI3K Mediates the Effects of PDGF on SREBP and Lipid Biosynthesis Genes—As mentioned above, two major signaling mediators have been linked to growth stimulation by PDGF, *i.e.* PI3K and ERK. To assess if these kinases were involved in the regulation of SREBP target genes by PDGF, we treated cells with specific small inhibitory molecules, LY294002 and U0126 that target PI3K and MEK, respectively. Although it completely blocked the phosphorylation of ERK (data not shown), U0126 had no effect on the stimulation of *SCD* and *HMGCS* expression by PDGF, and only partially inhibited *HMGCR* expression (Fig. 5). By contrast, LY294002 was as effective as sterols in blocking the effect of PDGF. In addition, inhibition of mTOR, which has also been shown to control cell

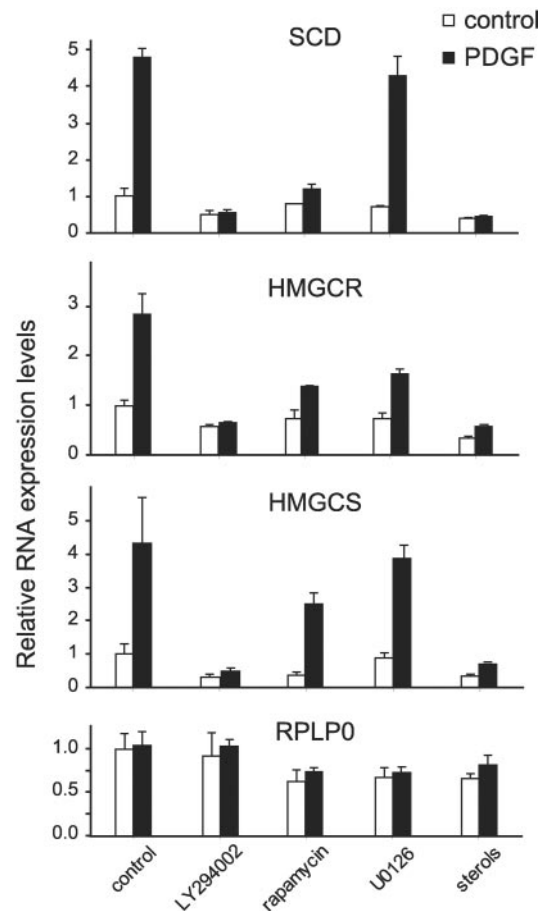


FIG. 5. LY294002 inhibits up-regulation of *SCD*, *HMGCR*, and *HMGCS* by PDGF. Starved AG01518 fibroblasts were stimulated for 24 h with PDGF-BB in the presence of 20 μ M LY294002, 10 μ M U0126, 20 ng/ml rapamycin, sterols, or carrier alone (0.1% Me₂SO), and lysed for RNA extraction. Equal amount of RNA were used for reverse transcription and real-time PCR analysis of the expression of *SCD*, *HMGCR*, *HMGCS*, and, as a control, *RPLP0*.

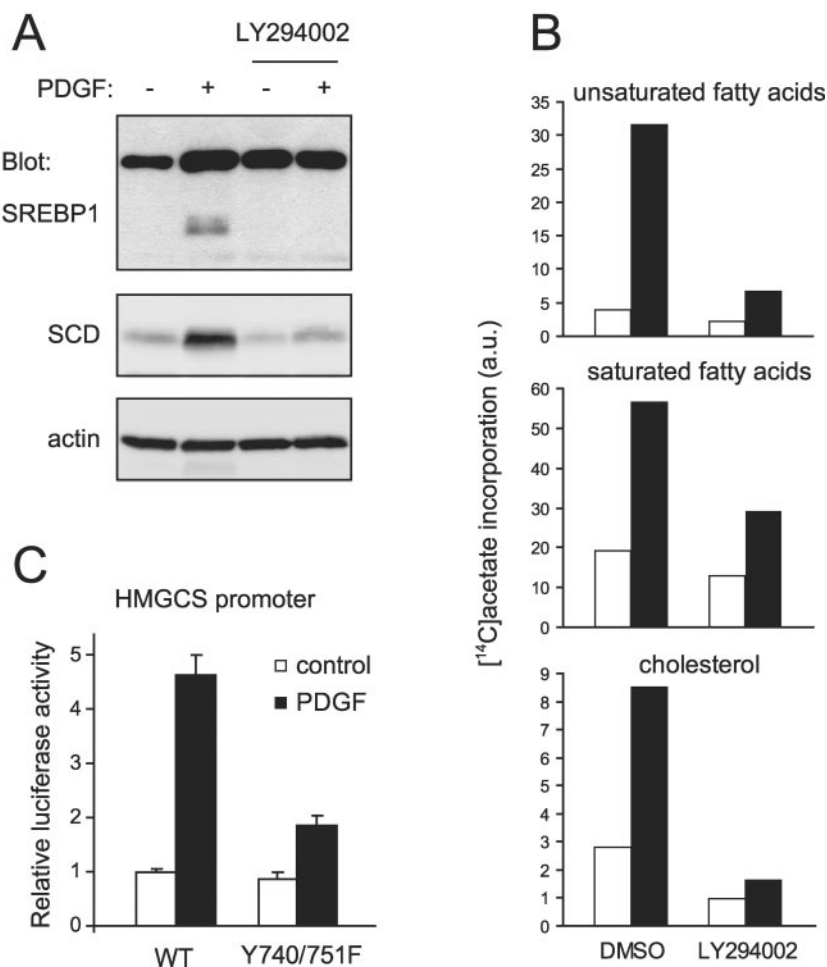
size, by rapamycin partially inhibited the up-regulation of *SCD*, *HMGCR*, and *HMGCS* by PDGF (Fig. 5). LY294002 also blocked the PDGF-induced increase in the amount of mature SREBP-1 protein, indicating that PI3K acted upstream of SREBP-1 (Fig. 6A). As expected, the same inhibitor decreased the biosynthesis of unsaturated fatty acids and cholesterol (Fig. 6B). The production of saturated fatty acids was also affected to a lesser extent.

The activation of PI3K by PDGF requires the binding of the regulatory subunit p85 to phosphorylated tyrosines 740 and 751 of the human PDGF β -receptor. In HepG2 cells transfected with the Y740/751F PDGF β -receptor, PDGF elicited a reduced activation of the *HMGCS* promoter, compared with cells transfected with the wild-type receptor (Fig. 6C). Taken together, these findings support the notion that PI3K is an important mediator of the activation of lipid biosynthesis by PDGF.

Stimulation of SREBP Target Gene Expression by other Growth Factors—If our observations reflect membrane lipid synthesis associated with cell growth, stimulation of fibroblasts with other growth factors would be expected to recapitulate the effects of PDGF. We first measured the activity of the *SCD2* and *HMGCS* promoters in a luciferase assay using a NIH3T3 clone stably transfected with the epidermal growth factor (EGF) receptor. Under these experimental conditions, EGF, fibroblast growth factor (FGF)-4 and high doses of insulin were at least as potent as PDGF-BB (Fig. 7A). In line with these results, treatment of human fibroblasts with FGF-4, insulin, as

² J. B. Demoulin, unpublished data.

FIG. 6. Role of the PI3K pathway in lipid biosynthesis stimulation by PDGF. **A**, starved AG01518 fibroblasts were stimulated for 24 h with PDGF-BB in the presence or absence of 20 μ M LY294002. SREBP-1, SCD, and α -actin expression were analyzed by Western blot. **B**, cells were labeled with [14 C]acetate for 4 h before harvest, and hydrolyzed lipids were analyzed as in Fig. 1. One representative experiment is shown out of two performed. **C**, HepG2 cells were co-transfected with an *HMGCR* promoter reporter construct and wild-type PDGF β -receptor (*WT*), or a mutant receptor in which the two tyrosines binding to p85 have been replaced by phenylalanine (*Y740/751F*). Cells were starved, stimulated with PDGF-BB for 36 h, and processed to measure luciferase activity, as described under "Materials and Methods."



well as FGF-2 and PDGF-AB, induced the expression of SCD, and increased the amount of mature SREBP-1, as demonstrated by Western blotting (Fig. 7B). PDGF-BB was, however, more potent than other growth factors in these cells. These results were mirrored at the RNA level, as illustrated by the regulation of SCD, HMGCR, and HMGCS by FGF-4 (Fig. 7C).

DISCUSSION

In the present report, we identified a sequence of events that lead to the synthesis of cellular membranes when fibroblasts enter the cell cycle. This pathway involves the PI3K-dependent activation of SREBP by growth factors, such as PDGF-BB. The role of SREBP was demonstrated by the following observations: (i) the transcriptional program elicited by PDGF presented an SREBP signature, with virtually all known SREBP target genes up-regulated by PDGF; (ii) PDGF stimulated the activity of SREBP-driven promoters, which could be inhibited by a dominant negative SREBP construct; (iii) PDGF treatment quickly increased the amount of mature SREBP-1; and (iv) blocking SREBP processing by sterols prevented PDGF action. A role for SREBP in membrane lipid production was recently demonstrated in *Drosophila*, where SREBP processing is directly controlled by phospholipids (33). In mammalian cells, the importance of the SREBP pathway in long term proliferation was suggested by analysis of mutant CHO cell lines which lack SCAP, S1P, or S2P and are auxotrophic for cholesterol, mevalonate, and unsaturated fatty acids (16, 23). Here, we show that SREBP-1 is activated early after growth stimulation, before mitosis. Importantly, our study does not exclude the existence of additional mechanisms of lipid metabolism regulation by growth factors, independently of SREBP.

Since SCD and FAS are regulated to a higher extent by PDGF, and the corresponding promoters are more sensitive to SREBP-1 compared with SREBP-2 (30), SREBP-1 might play a predominant role, which is compatible by its kinetics of activation after PDGF stimulation. However, the phenotype of SREBP-1-deficient mice, which are viable, argues against a unique role for SREBP-1 in cell growth *in vivo* (13). By contrast, inactivation of the gene encoding SREBP-2 results in early embryonic lethality (13). Whether PDGF also activates SREBP-2 should be further clarified. We observed the appearance of an intermediate molecular weight SREBP-2 protein, which was recognized by an antibody raised against mature SREBP-2, and disappeared upon addition of sterols, as one would expect from the *bona fide* mature form of SREBP-2. Recently, it was demonstrated that SREBP proteins are substrates for multiple post-translational modifications, including phosphorylation, acetylation, ubiquitination, and sumoylation, some of which significantly affect SREBP migration when analyzed by SDS-PAGE (20, 34, 35).

Our data point to the PI3K pathway as a link between PDGF receptors and activation of SREBP, based on the effect of LY294002 and the consequences of specific receptor mutations. In line with these results, SREBP regulation by insulin in hepatocytes depends on the PI3K pathway (36, 37). Several studies have also stressed the role of ERK in SREBP activation in cell lines and *in vitro* (31, 35, 38). However, the MEK inhibitor U0126 had no effect on SCD and HMGCS regulation by PDGF in fibroblasts. Finally, the effect of rapamycin suggested an involvement of mTOR, which cooperates with PI3K-dependent effectors to phosphorylate p70 S6 kinase and 4EBP1, and

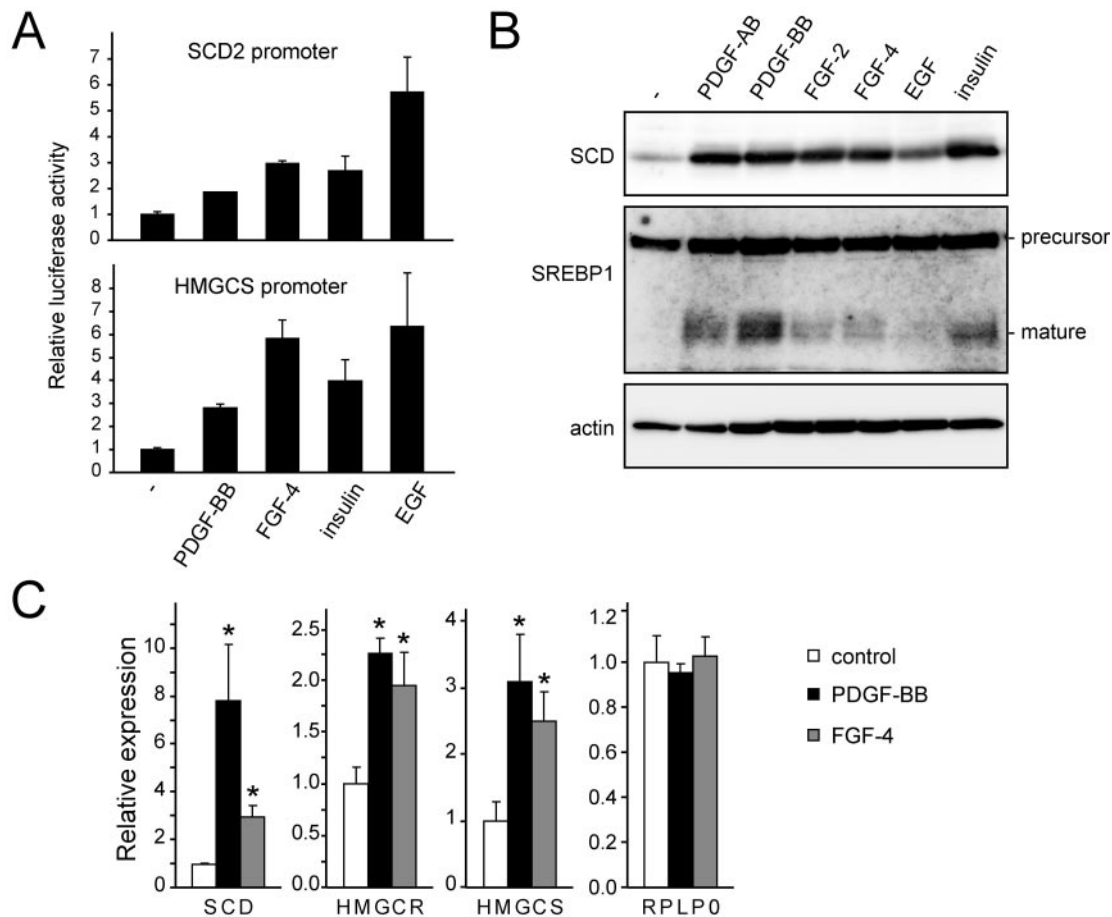


FIG. 7. Growth factor stimulation of SREBP target gene expression. A, NIH3T3 stably expressing the EGF receptor were transiently transfected with a reporter construct containing the *SCD* or *HMGCS* promoter. Cells were then washed and incubated with starvation medium containing 0.1% calf serum in the presence of PDGF-BB (25 ng/ml), EGF (25 ng/ml), FGF-4 (10 ng/ml, with 10 μ g/ml heparin), or insulin (5 μ g/ml). Luciferase activity was measured as above. B, AG01518 fibroblasts were starved in the presence of 0.1% bovine serum albumin and then incubated with PDGF-BB, EGF, FGF-2 (all at 25 ng/ml), or FGF-4 (10 ng/ml) for 24 h. As a control, cells were left untreated for the same period of time. Heparin was added in both FGF conditions (10 μ g/ml). Cell lysates were analyzed by Western blot using antibodies against SCD, SREBP-1, or α -actin. C, alternatively, RNA was extracted and used for measuring the levels of SCD, HMGCR, HMGCS, and RPLP0 expression by real-time quantitative PCR. Standard deviations are shown. *, statistically significant difference compared with non-treated controls (Student's *t* test, $p < 0.01$). The difference between PDGF and FGF-4 treatments was significant only for SCD ($p < 0.05$).

thereby controls protein synthesis and cell size (39). Although cell size increase obviously requires membrane synthesis, to our knowledge, that aspect had not been studied before. Our observations show that the same signaling effectors may control protein synthesis and membrane lipid production in a coordinate manner during cell growth. Further studies will have to determine how growth factor-activated PI3K affects SREBP.

The inhibitory effect of sterols indicated that SREBP activation by PDGF required SREBP precursor processing in a cholesterol-sensitive manner. It is possible that PDGF treatment increases SREBP cleavage, for instance by increasing the transport of cholesterol from the endoplasmic reticulum to the plasma membrane. Alternatively, since active SREBP proteins are quickly degraded, stabilization of mature SREBP by PDGF would produce the same effect. Given the importance of membrane synthesis for cell growth, it is possible that the regulation of the SREBP pathway occurs at multiple levels.

SCD was the gene that showed the largest change in expression after 24 h of PDGF treatment. As a result, PDGF induced a more pronounced increase in the production of unsaturated fatty acids compared with saturated fatty acids. The insertion of more rigid fatty acids in the membranes is expected to increase membrane fluidity, and might affect cell responsiveness to various stimuli (40). In addition, it was suggested that

SCD is expressed at a higher level in certain tumors compared with normal tissues (41), and that tumors have a higher oleic to stearic acid ratio. Our results imply that *SCD* induction by activation of growth factor-regulated pathways in tumors might be responsible for these observations. Interestingly, inhibition of *SCD* delayed tumor development in an *in vivo* murine model (42). Independently of *SCD* expression, SREBP activation has also been studied in various tumor cell lines. In that respect, Swinnen *et al.* (38) reported that overactivation of SREBP-1 by EGF in prostate cancer cells resulted in FAS overexpression and triglyceride accumulation. Based on the present data, we speculate that activation of SREBP by growth factors may play a broader role in tumor cell proliferation, by controlling membrane synthesis. Future work should assess if an increased lipid biosynthesis could contribute to the role of PDGF in the development of other diseases, such as atherosclerosis (1).

In conclusion, our data show that PDGF and other growth factors activate SREBP, which enhances the expression of lipogenic enzymes, leading to the synthesis of new membranes. This sequence of events appears to depend on PI3K activation, providing a link between lipid, protein, and DNA synthesis during cell growth.

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