Pregnancy-Specific Glycoprotein Gene Expression and the Induction by 5-Bromo-2′-deoxyuridine

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ABSTRACT: The pregnancy-specific glycoproteins (PSGs) of the placenta, members of the immunoglobulin superfamily, are encoded by multiple linked genes located on chromosome 19. To study the control of PSG expression, we have immortalized differentiated human placental cells (HP-A1) temperature-sensitive for transformation by a recombinant adenovirus-(ori⁻)-SV40 *ts*A mutant virus. We now show that expression of the PSG gene in HP-A1 cells is temperature-sensitive. At the permissive temperature (33 °C), these cells expressed low levels of PSG mRNA and synthesized a 64-kDa PSG. Shifting HP-A1 cells to a nonpermissive temperature (39.5 °C) increased PSG mRNA expression and biosynthesis with preferential increase in the synthesis of a 54-kDa and a low level of a 72-kDa PSG. Moreover, PSG expression was greatly induced by 5-bromo-2′-deoxyuridine (BudR), which selectively increased synthesis of PSGs of 72 and 54 kDa. In the presence of BudR, HP-A1 synthesized PSGs of 72, 64, and 54 kDa, similar to the pattern seen with placental PSGs. Ribonuclease protection assays demonstrated that HP-A1 cells express the majority of PSG mRNAs and BudR stimulated expression of PSG1 and PSG1-like transcripts. Reverse transcription and polymerase chain reaction analysis using PSG gene-specific primers demonstrated that untreated HP-A1 cells expressed primarily PSG1, PSG2, PSG4, and PSG5 mRNAs. BudR stimulated the expression of all PSG transcripts except PSG4. Moreover, in transient expression assays, BudR increased chloramphenicol acetyltransferase (CAT) expression directed by PSG1-I, PSG4, PSG5, PSG6, and PSG11 promoter-CAT fusion genes. Our data indicate that the increase in PSG expression by BudR is regulated at both transcriptional and post-transcriptional levels.

Pregnancy-specific glycoproteins (PSGs) are the major proteins synthesized by the human placenta and are found at levels as high as 200–400 μg/mL by the third trimester (Tatarinov & Masyukevich, 1970; Bohn, 1971; Lin et al., 1974; Lee et al., 1978). Serum levels of PSG proteins have been used as diagnostic tools for pregnancy and pregnancy-related complications (Wurz et al., 1981; MacDonald et al., 1983; Masson et al., 1983; Tamsen et al., 1983). In addition, PSG levels are elevated in patients with hydatidiform mole, invasive mole, and choriocarcinoma, and in the case of choriocarcinoma, PSG levels have been used to monitor treatment (Tatarinov, 1978; Sorensen, 1984).

The PSGs present in maternal serum are a heterogeneous group of closely related proteins (Chou & Plouzek, 1992). Molecular cloning studies have shown that these proteins are encoded by as many as 11 genes present in a cluster on chromosome 19 (Chan et al., 1988; Rooney et al., 1988; Streydio et al., 1988; Watanabe & Chou, 1988a, Khan et al., 1992). PSG proteins are closely related to another group of proteins, the carcinoembryonic antigens (CEAs) (Beauchemin et al., 1987; Oikawa et al., 1987). Interestingly, the genes for the CEA family of proteins are also located on chromosome 19 (Thompson et al., 1992). Both PSGs and CEAs are structurally similar to immunoglobulins (Igs) and thus are classified as members of the immunoglobulin superfamily [for reviews, see Thompson et al. (1991) and Chou and Plouzek (1992)]. The proteins of both subfamilies consist of an N-terminal variable domain (IgV-like) and a varying number of constant domains (IgC-like). The constant domains fall into two groups, A and B, based on their amino acid sequence similarity. Human PSGs are further classified as type I (L/N-A1-A2-B2-C) (two A domains) or II (L/N-A1/A2-B2-C) (one A domain) (Chou & Plouzek, 1992). The C-terminal domains are of varying length. It has been shown that the C-domains of PSG genes contain several splice sites which can generate multiple PSG mRNAs by alternative RNA splicing (Oikawa et al., 1988; Lei et al., 1992b). For instance, five PSG1 gene-encoded transcripts have been identified to date; their C-domains begin at one of the four splice sites (Lei et al., 1992b).

Despite the isolation and characterization of PSG cDNA and genomic clones, little is known about the control of PSG expression. To this end, we immortalized human placental cells with a recombinant adenovirus-(ori⁻)-SV40 virus carrying a temperature-sensitive (*ts*) mutation in the A gene of SV40 (Lei et al., 1992a). The A gene encodes the large tumor antigen which is required for maintenance of transformation (Martin & Chou, 1975). In earlier studies, we showed that the SV40 *ts*A chimera-immortalized placental cells are conditionally transformed cells that express the transformed phenotype only at a permissive temperature (Lei et al., 1992a). Moreover, the transformed placental cells express placenta-specific genes including PSG and the major placental glycoprotein hormone human chorionic gonadotropin. In the present report, we demonstrate that PSG biosynthesis and mRNA expression in the adenovirus-(or⁻)-SV40 *ts*A mutant-

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Table 1: PSG Gene Specific Oligonucleotide Primers

primer sequence 5′ to 3′	specific PSG gene[a]	complementary to cDNA sequence[b]	PCR product (bp) I	PCR product (bp) II
AACTTAAATCCCAGGGAGACCAT	5′-PSG1	537–558 hPSP11 (Chan et al., 1988)	544	265
GCCATGGAAACTGTGATCTTAAC	5′-PSG2	483–504 PSβGE (Streydio et al., 1988)	527	248
ATCATACGTAGTAGATGGTCAAATAA	5′-PSG3	477–502 pSP1-i (Rooney et al., 1988)	768	489
ACAAAGGGCAAATGACATACGTC	5′-PSG4	288–311 hPS133 (Chan et al., 1991)	805	526
TACAAAGGACAACTGATGGACC	5′-PSG5	287–308 hPS176 (Chan et al., 1991)	807	528
CAAATTATATATGGGCCTGCCTA	5′-PSG6	304–326 hPS12 (Zheng et al., 1990)	759	480
CATTATATCGTATATAGTTGATGGTAA	5′-PSG11	301–327 hPS91 (Chan et al., 1991)	776	497
TCATAGGATGCAGCTGTCTGAA	5′-PSG16	646–666 PSG9 (Plouzek & Chou, 1991)	441	162
CTGGGGAGGTCTGGACCAT	3′-universal	1062–1080 hPSP11 (Chan et al., 1988)		

[a] PSG gene nomenclature as described by Chou & Plouzek (1992). [b] Transcript clone names as described in the original publication indicated.

immortalized human placental cells can be stimulated to resemble expression in the human placenta by 5-bromo-2′-deoxyuridine (BudR). Furthermore, we show that BudR increases CAT expression directed by PSG promoter-CAT fusion genes.

MATERIALS AND METHODS

Biosynthesis of PSG. The HP-A1 human placental cell line (Lei et al., 1992a) was grown and maintained in α-modified minimal essential medium supplemented with streptomycin (100 μg/mL), penicillin (100 units/mL), and 4% fetal bovine serum. Four sets of cultures were grown initially at 33 °C (permissive temperature) for 2 days until reaching 40–60% confluence. Then two sets of cultures were treated with BudR (10 μg/mL). After an additional 2–3 days of incubation at 33 °C (reaching 90% confluence), one set each of control or BudR-treated cultures was shifted to 39.5 °C (nonpermissive temperature) for further experimentation. Medium was changed every 2–3 days.

Cultures grown in control or BudR-containing medium were labeled by incubation in the respective methionine-free medium containing [^{35}S]-L-methionine (100 μCi/mL, ICN Biochemicals, Inc., Lisle, IL) for 3 h at either 33 or 39.5 °C. PSGs in culture media were isolated by immunoprecipitation with rabbit anti-PSG serum (Watanabe & Chou, 1988a) and analyzed by polyacrylamide-SDS gel electrophoresis and fluorography. Apparent molecular weights were determined using [^{14}C]methionine-labeled protein standards (Amersham Corp., Arlington Heights, IL).

Northern Hybridization Analysis. RNA was isolated by the guanidinium thiocyanate/CsCl method (Chirgwin et al., 1979), separated by electrophoresis in 1.2% agarose gels containing 2.2 M formaldehyde (Lehrach et al., 1977), and transferred to Nytran membranes (Schleicher & Schuell, Keene, NH) by electroblotting. The filters were hybridized at 65 °C in the presence of an antisense PSG1a (Watanabe & Chou, 1988a) or human β-actin (Ponte et al., 1983) riboprobe (10^6 cpm/mL) as previously described (Lei et al. 1992a). Antisense riboprobes were synthesized following the procedures supplied by Promega Biotec (Madison, WI).

Ribonuclease Protection Assay. Ribonuclease protection assays were performed essentially as described (Ausubel et al., 1992). Briefly, total RNA from human placenta, poly-(A)$^+$ RNA from control or BudR-treated HP-A1 cells, or yeast tRNA was hybridized in a reaction mixture (30 μL) containing 80% formamide, 0.4 M NaCl, 0.04 M Pipes, pH 6.4, 1 mM EDTA, and an antisense PSG riboprobe (5×10^5 cpm) at 45 °C for 18 h. The hybrids were digested with RNase A (40 μg/mL) and RNase T1 (2 μg/mL) for 60 min at 30 °C and then electrophoresed on polyacrylamide–urea sequencing gels. Single base substitutions were normally not digested by these ribonucleases under the assay conditions used.

Probes. Molecular cloning studies indicate that the various PSG transcripts share over 90% nucleotide sequence identity in the 5′-domains, but differ in 3′-end coding and untranslated regions (Chou & Plouzek, 1992). The 3′-exon of a given PSG gene contains several RNA splice sites which generate multiple transcripts by alternative RNA splicing. For example, the 3′-exons of several PSG genes, including the PSG1 gene, contain four splice sites (C1, C2, C3, and C4) (Oikawa et al., 1988; Leslie et al., 1990; Lei et al., 1992b). To date, five PSG1 transcripts which contain identical sequence in the 5′-untranslated and -coding regions but diverge in the 3′-end coding and noncoding regions have been identified (Watanabe & Chou, 1988a; Lei et al., 1992b). The probes used in the ribonuclease protection analysis are derived from PSG1-encoded transcripts. They are PSG-5′ (nucleotides 1–142 of PSG1a) (Watanabe & Chou, 1988a), PSG3′-C1 (nucleotides 1411–1508 of PSG1e) (Leslie et al., 1990), PSG3′-C2 (nucleotides 1849-3′-end of PSG1e) (Leslie et al., 1990), PSG3′-C3 (nucleotides 15–85 of PSG1a) (Watanabe & Chou, 1988a), and PSG3′-C4 (nucleotides 1748-3′-end of PSG1b) (Watanabe & Chou, 1988a).

Reverse Transcription and Polymerase Chain Reaction. Reverse transcription and polymerase chain reaction (RT-PCR) were performed according to the procedure described by Kawasaki and Wang (1989) with minor modifications (Wu et al., 1993). One microgram of poly-(A)$^+$ RNA from control or BudR-treated HP-A1 cells or 1 μg of total RNA from human placenta was added to a reaction mixture (20 μL) containing 1 × PCR buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl, 2.5 mM $MgCl_2$, 0.1 mg/mL nuclease-free bovine serum albumin), 1 mM dNTPs, 20 units of RNasin, 100 pmol of random hexamers, and 20 units of AMV reverse transcriptase. The reaction mixture was incubated at 42 °C for 1 h, heated at 95 °C for 7.5 min, and then chilled on ice.

The sequences of the gene-specific 5′-primer and the universal PSG 3′-primer previously described (Wu et al., 1993) are shown in Table 1. The 3′-primer corresponded to a sequence of 21 bp at the 5′-end of the B2-domain, which is conserved in the identified cDNAs representing eight PSG genes. The oligonucleotide primers were designed such that their nucleotide composition will give a predicted melting temperature of about 65 °C (Thein & Wallace, 1986). Twenty four-cycle PCRs were performed. Additional cycles of PCR were performed for the PSG transcripts which were nondetectable after 24 cycles. After PCR, a 5-μL aliquot of the reaction mixture was analyzed by polyacrylamide gel electrophoresis. The sizes of PCR products corresponding to each cDNA are listed in Table 1. Primers for glyceraldehyde-3-

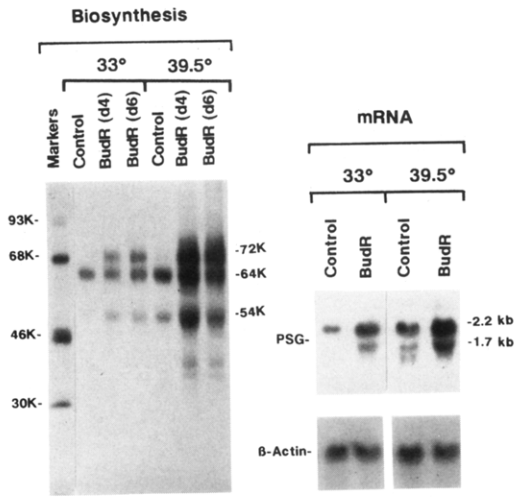

FIGURE 1: Effects of BudR on PSG biosynthesis and mRNA expression in HP-A1 placental cells grown at the permissive (33 °C) and nonpermissive (39.5 °C) temperatures. (A) PSG biosynthesis: HP-A1 cells were grown at 33 or 39.5 °C in control medium or medium containing 10 μg/mL of BudR as described in Materials and Methods. Cells were labeled with [^{35}S]-L-methionine for 3 h and anti-PSG-precipitable polypeptides in the medium were analyzed by polyacrylamide–SDS gel electrophoresis and fluorography. Polypeptides from 4×10^6 cells were applied to each lane. (B) PSG mRNA expression: cells were grown at 33 or 39.5 °C in control medium or medium containing 10 μg/mL of BudR for 4 days at the respective temperatures. Total RNA (20 μg/lane) was separated by electrophoresis on agarose gels, transferred to Nytran membranes, and hybridized with an antisense PSG1b (Watanabe & Chou, 1988a) or β-actin (Ponte et al., 1983) probe as described in Materials and Methods.

phosphate dehydrogenase (G3PDH) (Arcari et al., 1984), which yielded a 161 bp fragment, were included in each reaction as a control. The identity of the amplified DNA as PSG was confirmed by hybridization with a PSG1 cDNA probe.

Transfection and CAT Assays. HP-A1 cells in 150-cm^2 flasks were transfected with 50 μg of DNA by the calcium phosphate–DNA coprecipitate method as previously described (Lei et al., 1992b). The pCAT-Control (pSVCAT, Promega) and pCAT-Basic plasmids (Promega) were used as positive and negative controls, respectively. The PSG promoter-CAT fusion gene PSG1-I(–456/–34)CAT has been described (Lei et al., 1992b). PSG4(–288/–34)CAT, PSG5(–428/–34)-CAT, PSG6(–431/–34)CAT, and PSG11(–430/–34)CAT were constructed by PCR using the respective PSG gene as a template and inserted into the pCAT-Basic plasmid (unpublished results). Transient expression assays showed that these constructs directed the highest levels of CAT expression in HP-A1 cells.

RESULTS

BudR Induces PSG Gene Expression. We have previously shown that HP-A1 human placental cells are temperature-sensitive for maintenance of transformation and express the placenta-specific genes PSG and human chorionic gonadotropin (Lei et al., 1992a). To study temperature-dependent expression of PSG genes, we examined PSG biosynthesis in HP-A1 cells grown at the permissive (33 °C) and nonpermissive (39.5 °C) temperatures. Human PSGs are a family of closely related glycoproteins with apparent molecular masses of 72 (the major product), 64, and 54 kDa (Watanabe & Chou, 1988a). In the absence of an inducer, HP-A1 cells synthesized primarily a 64-kDa PSG at 33 °C (transformed phenotype) (Figure 1). Shifting these cells to 39.5 °C

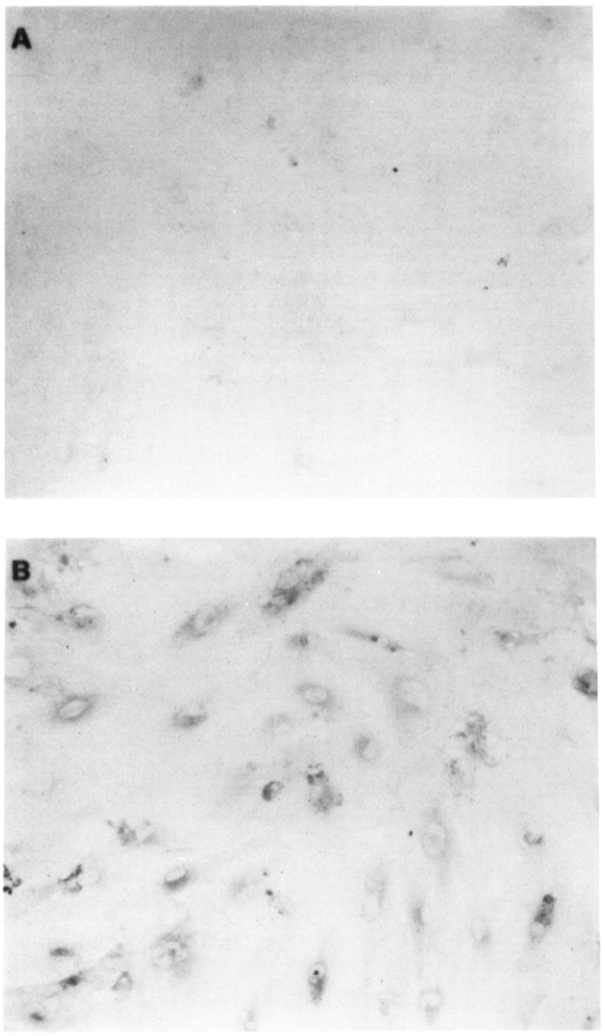

FIGURE 2: Immunocytochemical localization of PSG in HP-A1 cells. HP-A1 cells grown at 39.5 °C for 4 days in the presence of BudR were stained with (A) nonimmune or (B) rabbit anti-PSG serum as described in Materials and Methods.

(differentiated phenotype) increased PSG production. Moreover, at 39.5 °C, HP-A1 cells synthesized, in addition to the 64-kDa PSG, a 54-kDa PSG and a low level of a 72-kDa PSG (Figure 1).

BudR stimulated PSG production in HP-A1 cells at both 33 and 39.5 °C (Figure 1). This thymidine analog preferentially induced synthesis of PSGs of 72 and 54 kDa at both temperatures. HP-A1 cells grown at 39.5 °C in the presence of BudR synthesized greatly induced levels of PSGs of 72, 64, and 54 kDa, similar to those seen in the human placenta.

Human PSGs are encoded by mRNAs of 2.2 and 1.7 kb; these are in fact two groups of transcripts sharing high sequence identity (Watanabe & Chou, 1988a; Chou & Plouzek, 1992). To examine whether the increase in PSG biosynthesis resulted from a stimulation in PSG mRNA expression, we performed Northern-blot hybridization analysis (Figure 1). PSG mRNA expression was increased both by shifting HP-A1 cells to 39.5 °C and by BudR treatments, in agreement with our results on PSG biosynthesis.

Immunocytochemical Staining for PSG. Immunocytochemistry was performed on HP-A1 cells in order to determine the effect of BudR on PSG production in individual cells. HP-A1 cells grown at 39.5 °C in the presence of BudR were stained immunocytochemically with nonimmune (Figure 2A) or anti-PSG serum (Figure 2B). Panel B of Figure 2 shows that cells grown under these conditions actively

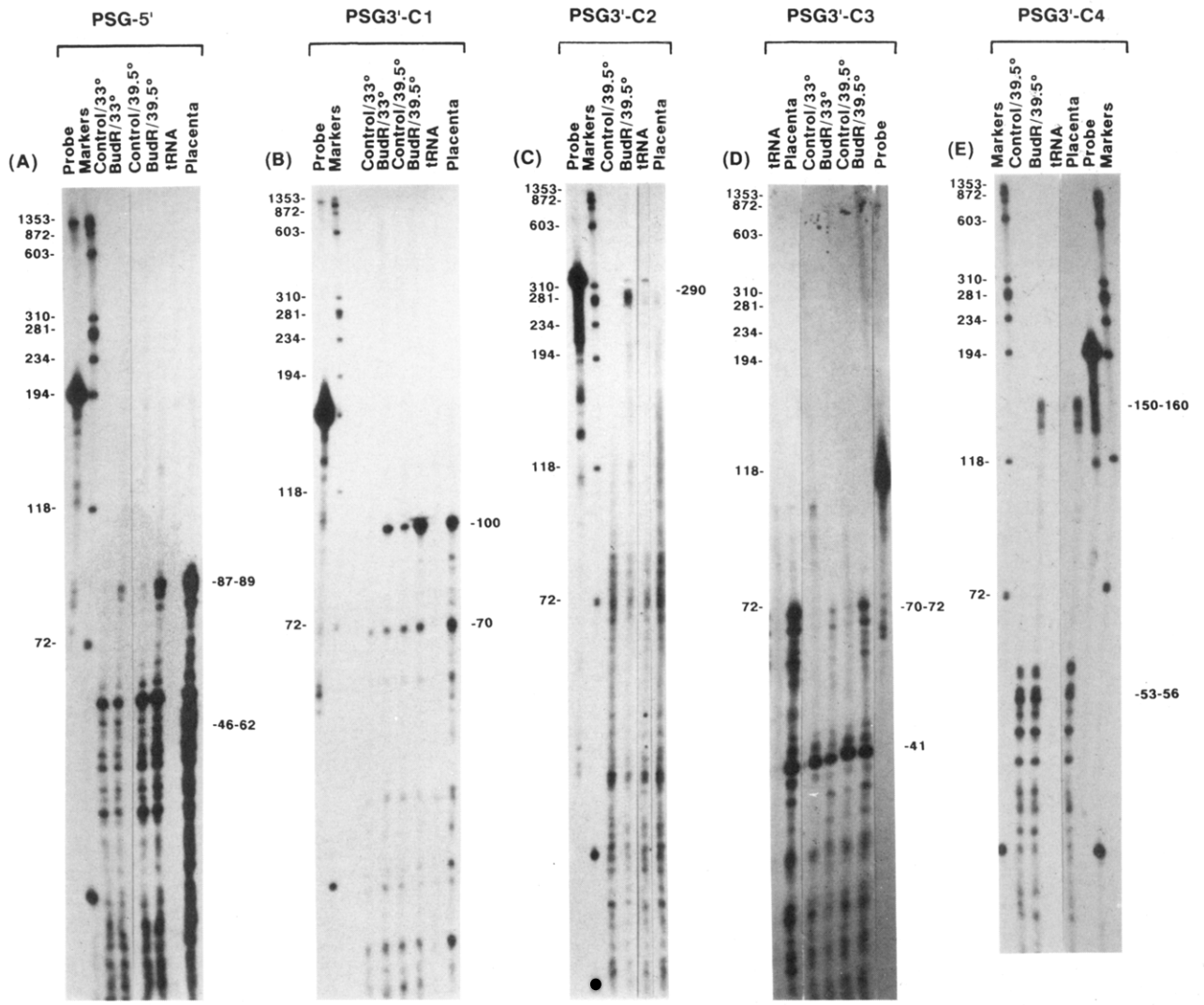

FIGURE 3: Ribonuclease protection analysis of PSG mRNAs in control and BudR-treated HP-A1 cells. Poly (A)$^+$ RNA (10 μg) from control or BudR-treated HP-A1 cells, total RNA (10 μg) from human term placenta, or yeast tRNA (10 μg) was annealed to five antisense riboprobes as described in Materials and Methods. The PSG-5′ probe detects the majority of PSG transcripts and the four PSG-3′ probes, which represent the five identified PSG1 transcripts, detect many but not all PSG mRNAs. The hybrids were digested with RNases A and T1 and the ribonuclease-protected fragments were separated by electrophoresis on polyacrylamide–urea sequencing gels. The *Hae*III digests of $\phi X174$ were used as markers.

synthesized immunoreactive PSG proteins. Furthermore, the majority of the cells in culture synthesized these proteins.

Ribonuclease Protection Analysis. For a more detailed analysis of the effects of BudR on PSG expression, a ribonuclease protection assay was employed. Antisense riboprobes derived from the PSG1 gene were hybridized with HP-A1 RNA and fragments protected from ribonuclease digestion were analyzed on an 8% polyacrylamide gel. PSGs are encoded by multiple transcripts that share 85%–95% nucleotide sequence homology in the 5′-untranslated and coding regions (Chou & Plouzek, 1992). Therefore, almost any PSG-5′ probe will hybridize with the majority of PSG transcripts. The 3′-end coding and untranslated regions of PSG members can be divided into several groups, depending upon the site of RNA splicing (Oikawa et al., 1988; Leslie et al., 1990; Lei et al., 1992b). In the present report, we employed four PSG-3′ probes (C1, C2, C3, and C4) derived from the PSG1 gene (Lei et al., 1992b). These PSG-3′ probes detect many, although not all, transcripts derived from the various PSG genes.

Two clusters of protected RNA species of 87–89 and 46–62 b were observed when ribonuclease protection assays were performed on placental RNA using the PSG-5′ probe (Figure 3A). The same clusters of protected RNAs were obtained in RNA isolated from HP-A1 placental cells grown at 33 °C, except the 46–62-*c* cluster was more prominent. An overall increase in PSG RNA synthesis was achieved both by shifting HP-A1 cells from 33 to 39.5 °C or by BudR treatments. However, these treatments selectively increased the larger (87–89 b) protected PSG RNA species. The relative amounts of both clusters of PSG mRNA species are similar to those observed in the human placenta only when the RNAs are isolated from HP-A1 cells grown in the presence of BudR at the nonpermissive temperature (39.5 °C).

Placental RNA hybridized with the PSG3′-C1 probe and digested with ribonucleases yielded a fully protected band of 100 b and a partially protected band of 70 b (Figure 3B); the 70-b species represented a PSG3′-C1-like transcript. At 33 °C, untreated HP-A1 RNA yielded primarily the partially protected band of 70 b (Figure 3B). RNAs from cells grown at 39.5 °C or in the presence of BudR show selective stimulation of a fully protected band of 100 b.

The PSG3′-C2 probe detected a very low level of a protected PSG RNA species of 290 b in human placental RNA (Figure 3C). This band was barely detectable in RNA from untreated HP-A1 cells. However, when the RNA was isolated from

HP-A1 cells treated with BudR at 39.5 °C, this species was greatly increased.

Placental RNA yielded fully protected bands of 70–72 b and a partially protected band of 41 b with the PSG3′-C3 probe (Figure 3D) and fully protected bands of 150–160 b and partially protected bands of 53–56 b with the PSG3′-C4 probe (Figure 3E). Few fully protected PSG species were observed in HP-A1 RNAs unless the RNAs were isolated from cells grown in the presence of BudR. However, clusters of smaller protected species (a 41-b fragment with the PSG3′-C3 probe and 53–56-b fragments with the PSG3′-C4 probe) similar to that observed in the human placenta were observed in RNAs from untreated cells as well as BudR-treated cells. This suggests that the same PSG3′-C3- and PSG3′-C4-like transcripts were expressed in HP-A1 cells as were in the human placenta.

In summary, the production of PSG1 and PSG1-like mRNAs was stimulated either by shifting HP-A1 cells to 39.5 °C or by BudR treatments. In general, HP-A1 cell RNA from cells grown in the presence of BudR at 39.5 °C shows a PSG transcription pattern most like that found in the human placenta. The exception was the RNA transcript detected with the PSG3′-C2 probe. BudR at 39.5 °C stimulated the production of this transcript manyfold over that seen in placental RNA.

PCR Analysis of PSG mRNA Expression in HP-A1 Cells. To further examine PSG genes expressed by HP-A1 cells and the effects of BudR, we performed RT-PCR analysis of RNA isolated from control and BudR-treated HP-A1 placental cells. A PSG gene-specific 5′-primer and a PSG universal 3′-primer were used to amplify a given PSG gene expressed by HP-A1 cells. A total of eight gene-specific 5′-primers corresponding to PSG1 (Chan et al., 1988; Watanabe & Chou, 1988a), PSG2 (Streydio et al., 1988), PSG3 (Rooney et al., 1988), PSG4 (Zimmermann et al., 1989; Chan et al., 1991), PSG5 (Khan & Hammarstrom, 1989; Chan et al., 1991), PSG6 (Zimmermann et al., 1989, Zheng et al., 1990), PSG11 (Chan et al., 1991), and PSG16 (Plouzek et al., 1991) were employed (Table 1). Studies have shown that two types of human PSGs, I (L/N-A1-A2-B2-C) and II (L/N-A1/A2-B2-C), exist (Chou & Plouzek, 1992). Type I contains an extra A-domain. Therefore, the presence of DNA fragments of predicted sizes (Table 1) in the PCR mixture indicates the expression of this PSG gene in HP-A1 cells. The production of the two bands predicted for types I and II transcripts showed that both types of PSG transcripts were expressed.

Using a semiquantitative RT-PCR analysis, we showed that the human placenta expressed high levels of PSG1, PSG2, PSG4, PSG5, and PSG6 messages and intermediate levels of PSG16 message (Figure 4A,C). Placenta expressed very low levels of PSG3 and PSG11 transcripts which can be seen either by increasing the PCR cycles or by using poly(A)$^+$ RNA (Wu et al., 1993).

HP-A1 cells expressed all eight PSG genes (Figure 4B,D) albeit at reduced levels as compared to the human placenta. The human placental PCR products were amplified from 1 μg of reversed transcribed total RNA whereas the HP-A1 PCR products were amplified from 1 μg of reversed transcribed poly(A)$^+$ RNA. In the absence of an inducer, PSG1, PSG2, PSG4, and PSG5 transcripts were detectable after 24 PCR amplification cycles. PSG16 transcripts could be detected after 28 cycles and PSG6 transcripts could be seen by re-amplifying a 24-cycle product for an additional 14 cycles. A 28-cycle reamplification of a 24-cycle PSG3 product was necessary to detect any PSG3 transcript and PSG11 was still undetectable in the absence of an inducer. With the possible exception of the PSG4 transcript, BudR stimulated the expression of all PSG mRNAs examined (Figure 4B,D). The anomalously high molecular weight products seen in the PCR products of PSG3 and PSG11 genes may result from artifacts of PCR amplification. However, the 340-bp fragment found in the PSG5 gene-specific amplification was detected in both placenta and HP-A1 cells, implying that this may be a PSG gene product yet to be discovered (Figure 4A–C). The 161-bp band represents the amplified DNA fragment of G3PDH (Arcari et al., 1984), which served as internal control of the PCR (Figure 4B).

BudR Increases PSG Promoter-CAT Fusion Gene Expression. In earlier studies, we have identified cis-acting DNA elements essential for the expression of PSG1-I gene and demonstrated that maximal promoter activity is contained within nucleotides –456 to –34 with respect to the translation initiation site (Lei et al., 1992b). To further examine regulation of PSG expression, we characterized additional PSG genes and showed that the peak promoter activity of PSG5 is contained within nucleotides –428/–34, PSG6 at –431/–34, and PSG11 at –430/–34 (unpublished results). The PSG4(–288/–34)CAT plasmid also directed high levels of CAT expression in HP-A1 cells (unpublished results). BudR increased CAT expression directed by PSG1-I, PSG5, PSG6, and PSG11 as well as PSG4 promoters (Figure 5), suggesting that the increase in PSG expression by BudR is regulated at the transcriptional level.

DISCUSSION

Human placental PSGs are the major secretory proteins found in maternal serum during pregnancy. These closely related glycoproteins have apparent molecular masses of 72 (major), 64, and 54 kDa (Watanabe & Chou, 1988a). Human choriocarcinoma cell lines established from malignant trophoblasts do not produce detectable levels of PSG protein or message (Lei et al., 1992a). In order to study the molecular mechanisms that regulate PSG gene expression, we used a recombinant adenovirus-(ori$^-$)-SV40 *ts*A mutant virus to immortalize human placental cells that do express PSG proteins and messages (Lei et al., 1992a). This chimeric virus carries a temperature-sensitive mutation in the SV40 large tumor (T) antigen which is required for maintenance of cell transformation (Martin & Chou, 1975). In earlier studies we showed that the SV40 *ts*A chimera-transformed human placental (HP-A1) cells are temperature-sensitive for the maintenance of transformation (Lei et al., 1992a). We now show that PSG biosynthesis and mRNA expression in HP-A1 cells are also temperature-dependent. Shifting HP-A1 cells from a permissive (33 °C, transformed phenotype) to a nonpermissive (39.5 °C, nontransformed phenotype) temperature increased both PSG synthesis and mRNA expression. At 33 °C, HP-A1 cells primarily synthesized a 64-kDa product. At 39.5 °C, these cells produced higher levels of PSGs of 72, 64 (major), and 54 kDa which more closely approximated the pattern seen in the human placenta.

Biochemical differentiation of HP-A1 cells could also be induced by the thymidine analog BudR, which is manifested by a marked increase in PSG biosynthesis and mRNA expression at both 33 and 39.5 °C. However, PSG gene induction was significantly greater at 39.5 °C than at 33 °C. BudR preferentially increased the synthesis of PSGs of 72 and 54 kDa, and in the presence of BudR, HP-A1 cells produced PSGs of 72, 64, and 54 kDa in proportions reminiscent of that found in the human placenta. Immuno-

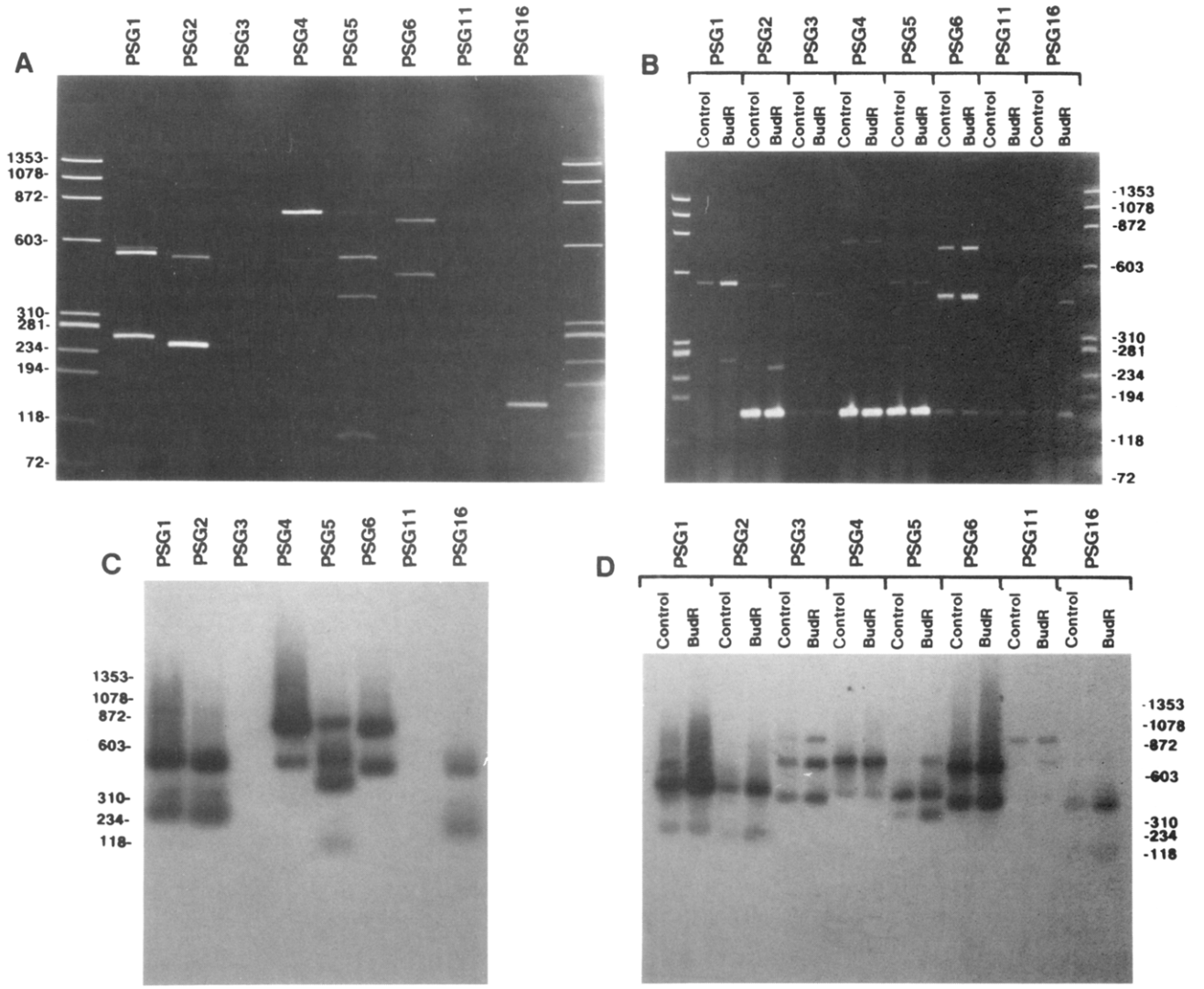

FIGURE 4: RT-PCR analysis of PSG transcripts expressed in the human placenta (A and C) and control and BudR-treated HP-A1 cells grown at 39.5 °C (B and D). One microgram of total RNA from human placenta or 1 μg of poly(A)$^+$ RNA from control or BudR-treated HP-A1 cells were reverse transcribed and amplified by PCR as described in Materials and Methods. Twenty-eight PCR cycles were performed with placental RNA. For PSG genes in HP-A1 cells, 24 PCR cycles were performed with PSG1, PSG2, PSG4, and PSG5 RNA and 28 PCR cycles with PSG16 RNA. PSG6 RNA was amplified for 24 PCR cycles, then 5 μL of the products was reamplified for an additional 14 cycles. PSG3 and PSG11 transcripts were amplified for 24 cycles, then 2 μL of the products was reamplified for an additional 28 cycles. (A and B) Amplified products on 6% polyacrylamide gels stained with ethidium bromide. (C and D) Southern hybridization analysis of the amplified products after electrophoresis in 2% agarose gels. The coding region of the PSG1 cDNA, which should hybridize with all predicted amplified fragments, was used as a probe.

cytochemistry performed on cells grown under the conditions that produce PSGs most like those seen in placenta (39.5 °C, BudR) demonstrated that the majority of the cells, and not a subset, produced immunoreactive PSG protein.

PSGs are encoded by transcripts of 2.2 and 1.7 kb; each is comprised of a group of transcripts sharing high sequence homology. We employed ribonuclease protection and RT-PCR assays to examine PSG expression in HP-A1 cells and the effect of BudR. These assays also enable us to determine if the majority of PSG genes are expressed by HP-A1 placental cells and their relative abundances in the presence and absence of BudR. Ribonuclease protection assays demonstrated that all PSG1 splice variants can be detected in RNAs from HP-A1 cells grown in the presence of BudR. In addition, our results showed that HP-A1 cells expressed all eight PSG genes analyzed, albeit at reduced levels. BudR induced expression of all PSG genes with the possible exception of PSG4. Moreover, BudR also increased CAT activity directed by five different PSG gene promoters including PSG4, suggesting transcriptional regulation of PSG expression by this analog. However, the selective stimulation of synthesis of 72- and 54-kDa PSG by BudR suggests additional regulation may occur at the post-transcriptional levels.

It is not surprising that some kind of stimulus is needed for HP-A1 cells to produce PSG mRNAs and proteins in patterns similar to those seen in the human placenta. The placenta most certainly is responding to large numbers of factors that are made primarily during pregnancy. BudR is readily incorporated into DNA in place of thymidine. The replacement of the 5-methyl group of thymidine with a bromine atom has been shown to alter the affinity of DNA to transcription activators or inhibitors as has been shown for the *lac* repressor (Lin & Riggs, 1972). Studies have shown that expression of a variety of mammalian genes can be positively or negatively modulated by BudR (Wright & Aronoff, 1983, Ito et al., 1984). Recently, Tapscott et al. (1989) and Lin et al. (1989) demonstrated that BudR inhibits myogenesis by blocking the expression of myogenic regulatory factors MyoD1 and CMD1. In the case of PSG gene induction, BudR may induce a regulator involved in PSG expression in HP-A1 cells.

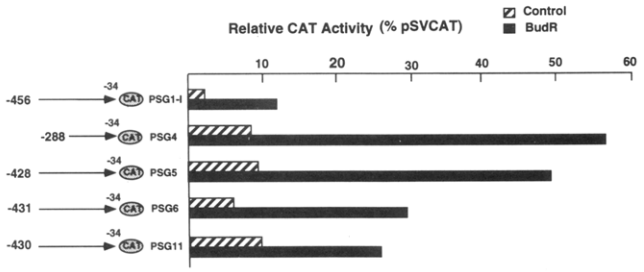

FIGURE 5: Effects of BudR on promoter activities of PSG genes. PSG1-I(–456/–34)CAT, PSG4(–288/–34)CAT, PSG5(–428/–34)-CAT, PSG6(–431/–34)CAT, and PSG11(–430/–34)CAT were transient transfected into HP-A1 cells as described in Materials and Methods. The pSVCAT (pCAT-Control) plasmid which contains both SV40 enhancer and promoter and the promoterless and enhancerless pCAT-Basic plasmid were used as positive and negative controls, respectively. The values obtained for the PSG promoter constructs were expressed as a percentage of the values obtained with the pSVCAT. The specific CAT activity expressed after transfecting an equivalent amount of pSVCAT into control or BudR-treated cells was 3.6 and 8.1 nmol/min per mg of protein, respectively.

Alternatively, incorporation of BudR into the DNA could render the regulatory regions of the PSG genes more susceptible to positive protein:DNA interactions. Studies to investigate the mechanisms of BudR induction are currently underway.

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REFERENCES

Arcari, P., Martinelli, R., & Salvatore, F. (1984) *Nucleic Acid Res. 12*, 9179–9189.

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., & Struhl, K. (Eds.) (1992) *Current Protocols in Molecular Biology*, pp 4.7.1.–4.7.8, Greene Publishing and Wiley-Interscience, New York.

Beauchemin, N., Benchimol, S., Cournoyer, D., Fuks, A., & Stanners, C. P. (1987) *Mol. Cell. Biol. 7*, 3221–3230.

Bohn, H. (1971) *Arch. Gynaek. 210*, 440–457.

Chan, W.-Y., Borjigin, J., Zheng, Q.-X., & Shupert, W. L. (1988) *DNA 7*, 545–555.

Chan, W.-Y., Zheng, Q.-X., McMahon, J., & Tease, L. A. (1991) *Mol. Cell. Biochem. 106*, 161–170.

Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., & Rutter, W. J. (1979) *Biochemistry 18*, 5294–5299.

Chou, J. Y., & Plouzek, C. A. (1992) *Sem. Reprod. Endocrinol. 10*, 116–126.

Ito, F., Sartwell, A. D., & Chou, J. Y. (1984) *Arch. Biochem. Biophys. 233*, 830–837.

Kawasaki, E. S., & Wang, A. M. (1989) In *PCR Technology* (Erlich, H. A., Ed.) pp 89–98, Stockton Press, New York, NY.

Khan, W. N., & Hammarstrom, S. (1989) *Biochem. Biophys. Res. Commun. 161*, 525–535.

Khan, W. N., Teglund, S., Bremer, K., & Hammarstrom, S. (1992) *Genomics 12*, 780–787.

Lee, J. N., Grudzinskas, J. G., & Chard, T. (1979) *Br. J. Obstet. Gynaecol. 86*, 888–890.

Lehrach, H., Diamond, D., Wozney, J. M., & Boedtker, H. (1977) *Biochemistry 16*, 4743–4751.

Lei, K.-J., Gluzman, Y., Pan, C.-J., & Chou, J. Y. (1992a) *Mol. Endocrinol. 6*, 703–712.

Lei, K.-J., Sartwell, A. D., Pan, C.-J., & Chou, J. Y. (1992b) *J. Biol. Chem. 267*, 16371–16378.

Leslie, K. K., Watanabe, S., Lei, K.-J., Chou, D. Y., Plouzek, C. A., Deng, H.-C., Torres, J., & Chou, J. Y. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 5822–5826.

Lin, S. Y., & Riggs, A. D. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 2574–2576.

Lin, T. M., Halbert, S. P., & Spellacy, W. N. (1974) *J. Clin. Invest. 54*, 576–582.

Lin, Z.-Y., Dechesne, C. A., Eldridge, J., & Paterson, B. M. (1989) *Genes & Dev. 3*, 986–996.

MacDonald, D. J., Scott, J. M., Gemmell, R. S., & Mack, D. S. (1983) *Am. J. Obstet. Gynecol. 147*, 430–436.

Martin, R. G., & Chou, J. Y. (1975) *J. Virol. 15*, 599–612.

Masson, G. M., Anthony, F., & Wilson, M. S. (1983) *Br. J. Obstet. Gynaecol. 90*, 146–149.

Oikawa, S., Nakazato, H., & Kosaki, G. (1987) *Biochem. Biophys. Res. Commun. 142*, 511–518.

Oikawa, S., Inuzuka, C., Kosaki, G., & Nakazato, H. (1988) *Biochem. Biophys. Res. Commun. 156*, 68–77.

Plouzek, C. A., Watanabe, S., & Chou, J. Y. (1991) *Biochem. Biophys. Res. Commun. 176*, 1532–1538.

Ponte, P., Gunning, P., Blau, H., & Kedes, L. (1983) *Mol. Cell Biol. 3*, 1783–1791.

Rooney, B. C., Horne, C. H. W., & Hardman, N. (1988) *Gene 71*, 439–449.

Sorensen, S. (1984) *Tumor Biol. 5*, 275–302.

Streydio, C., Lacka, K., Swillens, S., & Vassart, G. (1988) *Biochem. Biophys. Res. Commun. 154*, 130–137.

Tamsen, L., Johansson, S. G. O., & Axelsson, O. (1983) *J. Perinat. Med. 11*, 19–25.

Tapscott, S. J., Lassar, A. B., Davis, R. L., & Weintraub, H. (1989) *Science 245*, 532–536.

Tatarinov, Y. S. (1978) *Gynecol. Obstet. Invest. 9*, 65–97.

Tatarinov, Y. S., & Masyukevich, V. N. (1970) *Byull. Eksp. Biol. Med. 69*, 66–68.

Thein, S. L., & Wallace, R. B. (1986) In *Human Genetic Diseases* (Davies, K. E., Ed.) pp 33–50, IRL Press, Washington, DC.

Thompson, J. A., Zimmermann, W., Osthus-Bugat, P., Schleussner, C., Eades-Perner, A.-M., Barnert, S., Von Kleist, S., Willcocks, T., Craig, I., Tynan, K., Olsen, A., & Mohrenweiser, H. (1992) *Genomics 12*, 761–772.

Watanabe, S., & Chou, J. Y. (1988a) *J. Biol. Chem. 263*, 2049–2054.

Watanabe, S., & Chou, J. Y. (1988b) *Biochem. Biophys. Res. Commun. 152*, 762–768.

Wright, W. E., & Aronoff, J. (1983) *J. Cell Biol. 100*, 311–316.

Wu, S.-M., Bazar, L. S., Cohn, M. L., Cahill, R. A., & Chan, W.-Y. (1993) *Mol. Cell. Biochem.* In press.

Wurz, H., Geiger, W., Kunzig, H. J., Jabs-Lehmann, A., Bohn, H., & Luben, G. (1981) *J. Perinat. Med. 9*, 67–78.

Zimmermann, W., Weiss, M., & Thompson, J. A. (1989) *Biochem. Biophys. Res. Commun. 163*, 1197–1209.