Characterization of Two New Members of the Pregnancy-Specific β_1-Glycoprotein Family from the Myeloid Cell Line KG-1 and Suggestion of Two Distinct Classes of Transcription Unit‡

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ABSTRACT: Pregnancy-specific β_1-glycoproteins (PSGs) represent a large group (~12–15) of proteins, related to members of the carcinoembryonic antigen family, that are abundant in placental tissue and in the sera of pregnant women. We describe the isolation and characterization of two additional PSG cDNAs, PSG9 and PSG10, whose transcripts are largely expressed in placental tissue and to a lesser extent in some other cell types, including myeloid cells differentiated to granulocytes. PSG9 and PSG10 are representatives of two distinct classes of PSG protein that have N-termini with or without the Arg-Gly-Asp motif implicated in adhesion. In addition to this distinction at the amino acid level, our analysis of several PSG cDNAs suggests that the transcription units encoding these proteins may be further distinguished in their 3′ untranslated sequences, thus suggesting possibilities for transcriptional regulation of the two major protein classes.

The rapid growth of the syncytiotrophoblast and associated placental tissues requires extensive cellular interactions throughout all stages of pregnancy. Accompanying these interactions are the appearance of many novel protein species, including the large group of related proteins known as pregnancy-specific β_1-glycoproteins (PSGs).[1] These proteins have been reported to accumulate during pregnancy, eventually to high levels, in both placental tissue and maternal serum (Bohn, 1971).

Nearly 10 different cDNA members, potentially coding for as many different PSG proteins, have been described thus far [for a summary, see Barnett and Zimmermann (1990)]. These proteins have been shown to be related by sequence, by their overall domain and β-sheet structure, and by the sizes and positions of their disulfide loops to members of the carcinoembryonic antigen family (Watanabe & Chou, 1988b). In addition, the PSG genes, like those for CEA, map to human chromosome 19 (Barnett et al., 1989b; Niemann et al., 1989). A structural feature of the PSG proteins that does distinguish them from CEA family isoantigens is their overall lack of an obvious membrane attachment site. This implies that PSGs are largely secreted antigens, in keeping with their original detection in cell and tissue culture supernatants (Engvall et al., 1978; Chou, 1983). On the other hand, a recent report suggests that some PSβGs have hydrophobic C-terminal sequences, indicating the potential for cell membrane attachment (Khan & Hammarström, 1989).

We present the cDNA sequences of two new members of the pregnancy-specific β_1-glycoprotein family, PSG9 (formerly $PS_{K\alpha}$; Barnett et al., 1989b) and PSG10, that were isolated from a cDNA library of the human acute myelogenous leukemia line KG-1. We show here that despite their isolation from KG-1 cells, PSG9 and PSG10 are expressed predominantly in placenta, and to lesser extents in other cell types. The presence of an Arg-Gly-Asp (RGD) sequence in the N-termini of some PSGs implicates members of this protein group in adhesion-related events. However, other PSGs lack the RGD sequence, and analyses of 3′ untranslated region sequence from RNA transcripts that code for PSG proteins with and without the RGD motif permit us to distinguish two classes of transcriptional unit. This finding suggests possible transcriptional or posttranscriptional control of RNAs coding for pregnancy-specific β_1-glycoproteins.

Materials and Methods

Cells, Cell Lines, and Tissues. Cell lines were obtained from the American Type Culture Collection (Rockville, MD) and propagated according to ATCC recommendations. The cell lines were KG-1 (CCL 246), HEL 92.1.7 (TIB 180), U-037 (CRL 1593), K-562 (CCL 243), BT-20 (HTB 19), HL-60 (CCL 240), CCD-33Co (CRL 1539), SCaBER (HTB 3), BeWo (CCL 98), SW620 (CCL 227), HCMC (CCL239), NCI-H69 (HTB 119), and HT-1080 (CCL 121). Normal colonic epithelium was obtained from Dr. Peter Thomas (New England Deaconess Hospital). For induction of cell differentiation by phorbol ester, U-937 and HL-60 cells were stimulated by addition of 10 ng/mL PMA. After 2 days, nonadherent cells were discarded, while adherent cells were lysed directly for RNA isolation as described below. For KG-1 cells, sodium butyrate was added to a concentration of 200 nM for 2 days.

Isolation of cDNA Clones. To characterize the different members of the CEA gene family present in the KG-1 cell line, we screened 10^6 plaques of a λgt10/KG-1 cDNA library (HL1046b; Clontech Labs, Palo Alto, CA) with the DNA probe cLV7 (Kamarck et al., 1987) that identifies virtually all members of the CEA gene family. Positive plaques were rescreened with sequence-specific probes that would distinguish CEA and NCA55/95 cDNAs (3′ UTR probes; Barnett et al., 1988) and TM-CEA-related cDNAs (CYTO probe; Barnett et al., 1989b). Conditions for in vitro labeling of DNA and hybridization were as described previously (Barnett et al., 1987). Positive plaques were purified by three rounds of hybridization and then amplified by liquid growth on *Escherichia coli* NM514. DNA minipreps were prepared ac-

‡The nucleotide sequences reported in this paper have been submitted to the GenBank/EMBL Data Bank under Accession Numbers X17097 (PSG9) and X17098 (PSG10).

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[1] Abbreviations: PSG, pregnancy-specific β_1-glycoprotein; CEA, carcinoembryonic antigen; NCA, nonspecific cross-reacting antigen; BGP I, biliary glycoprotein I; UTR, untranslated region; PMA, phorbol 12-myristate 13-acetate; aa, amino acid.

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cording to Maniatis et al. (1982), and *Eco*RI inserts were subcloned into Bluescript plasmid vectors after purification from low melting temperature agarose gels.

DNA Sequencing. Both strands of plasmid DNA were sequenced by the dideoxynucleotide method (Sanger et al., 1977) using [^{32}P]dATP with a T7 DNA polymerase sequencing kit (U.S. Biochemicals, Cleveland, OH). DNA sequence analysis used the University of Wisconsin GCG sequence analysis software package.

RNA Analysis. For Northern blot analysis, poly(A+) RNAs were isolated and electrophoretically separated in denaturing 2.2 M formaldehyde–0.8% agarose gels. Nylon imprints of RNA gels were hybridized with ^{32}P-labeled DNA probes in 2× SSPE, 5× Denhardt's, and 1% SDS and washed in 0.2× SSPE and 0.25% SDS, all at 68 °C.

Polymerase Chain Reaction. For amplification by the PCR method, 2–5 μg of total RNA prepared from cell lines and from normal colonic epithelium (Chomczynski & Sacchi, 1987) was converted into single-stranded cDNA using random primers (pdN_6) and AMV reverse transcriptase in a 50-μL volume. Each 50-μL PCR reaction consisted of 5 μL of cDNA, 1 μM each of forward and reverse primer, 200 μM dNTP, 1× PCR buffer, and 1 unit of *Taq* DNA polymerase, essentially as directed by the manufacturer (Perkin-Elmer Corp., Norwalk, CT). For PSG9, primers were 5′CTCTACTTTTCCTGCTTCGGTGAG3′ (forward primer 1) and 5′GGGTAATATCCAGTCAGAGACTTT3′ (reverse primer 2); for PSG10, primers were 5′CTCGACTTGTCCTGCTTTGCGGAC3′ (forward primer 3) and 5′GTGTCTCTATTGTGGCAGCCATTA3′ (reverse primer 4). Thirty cycles were used for in vitro amplification of target cDNA with 30 cycles of 94 °C for 1 min, 64 °C for 2 min, and 72 °C for 3 min, except for the last cycle which was for 7 min. Five microliters of each sample was analyzed by electrophoresis on a 3% agarose gel.

Results

Isolation of Novel PSG Family Member cDNAs. We used differential hybridization to isolate cDNAs that were different from already identified members of the CEA gene family (see Materials and Methods). The cDNA clones were placed in two groups by restriction mapping (Figure 1A,B) and were shown by DNA sequencing to be members of the pregnancy-specific β_1-glycoprotein (PSG) family (Figure 1C,D). A representative of each cDNA group was designated PSG9 or PSG10, in keeping with the recently proposed conventions for CEA nomenclature (Barnett & Zimmermann, 1990).

The overall structural characteristics and likely domain organizations of PSG9 (Figure 1A) and PSG10 (Figure 1B) apoproteins are quite similar to other PSG proteins and to members of the CEA family. These include a probable 34 aa signal peptide sequence, an 110 aa N-terminal domain (compared to 108 amino acids for CEA, NCA, and BGP I), and A and B repeat regions containing potential 47 and 39 aa disulfide loops, respectively. The generally hydrophilic nature of PSG C-termini distinguishes the PSG family from other CEA-related isoantigens, since there are generally no obvious transmembrane nor membrane-anchoring sites that are likely to signal phosphatidylinositol glycan anchoring. This finding is consistent with the appearance of PSG proteins in culture supernatants and in maternal serum.

The nucleic acid and inferred amino acid sequences of the PSG9 apoprotein yield a domain structure that is highly similar to several previously described PSG proteins. By way of example, PSG9 and PSG93 (Watanabe & Chou, 1988a) both have *Eco*RI restriction sites at their translation termination codons and share similar 3′ UTR sequences. We suggest that the sequence coding for the five amino acid C-terminus and adjacent 3′ UTR of PSG9 is probably one of several alternatively spliced exons derived from the PSG9 transcription unit, just as PSG93 mRNA is one of four RNAs from the PSG1 primary transcript (Watanabe & Chou, 1988a). Overall, PSG9 is about 95% and 92% similar to the cDNA (including 5′ and 3′ UTRs) and polypeptide sequences, respectively, of PSG93. Recently, an incomplete cDNA coding for a PSβG with nearly 99% similarity to the PSG9 sequence has been described (Zimmerman et al., 1989). Whether or not PSG4 and PSG9 are allelic variants has yet to be elucidated.

The hypothetical PSG10 protein shares the 47–47–39 loop motif found in other PSG proteins and is highly similar in amino acid sequence to PSG6, for example (Zimmerman et al., 1989), but the two differ considerably at the presumed C-terminus. It is possible that PSG10 and PSG6 are alternatively spliced members of the same transcription unit, just as there are such members for the PSG1 transcription unit (Oikawa et al., 1989).

The amino acid sequence deduced from PSG10 cDNA has two specific characteristics of some other PSG isoantigens. First, within the N-terminal segment, there is an Arg-Gly-Asp (RGD) sequence at amino acid positions 93–95 that is found at a similar position in several other PSG proteins (Rooney et al., 1988; Khan & Hammarström, 1989; McLenachan & Manfield, 1989; Oikawa et al., 1989a; Zimmermann et al., 1989; Arakawa et al., 1990). Second, in comparing the PSG10 amino acid sequence with the sequences of other PSG proteins, a gap representing a single amino acid must be introduced at position +54 of the PSG10 N-terminus to maintain the linear sequence similarity of the extracellular domains. That this single amino acid difference is unlikely to be a simple cDNA cloning artifact is supported by the independent cloning and sequence analysis of a genomic N-terminal restriction segment of the CEA-related family member, hsCGM3 (Thompson et al., 1989), and of its cDNA, PSG6 (Zimmermann et al., 1989). In both cases, a comparable gap was seen. The presence of the deletion/insertion in PSG10 and PSG6 cDNAs may reflect a common DNA origin for these members. The effect on the tertiary structure of the resulting PSβG polypeptide(s) is not known.

Assortment of PSG mRNAs into Transcriptional Classes. The RNAs coding for RGD- and non-RGD-containing PSG proteins demonstrate differences in their 3′ untranslated regions, suggesting that PSG expression may be subject to regulation at the transcriptional level. Figure 2 aligns the 3′-terminal segments of PSG9 (A) or PSG 10 (B) with several published PSG cDNA sequences. This comparison suggests that most PSG mRNAs that code for RGD-containing proteins (like PSG10) have 3′ UTR sequences that differ substantially from those of PSG mRNAs not encoding an RGD sequence (like PSG9). This observation suggests that there are at least two different classes of PSG transcription units. The 3′ UTRs of PSG9-related molecules derived from different transcription units are highly conserved over the length of sequence shown (Figure 2A), and in several cases, beyond it. In performing this analysis, we consider only 3′ UTR sequences from those PSG9-related cDNAs that are known to be alternative splice products of a larger transcription unit. Alignment of sequences of PSG10-class transcription units (e.g., PSG10, PSG2, and PSG3 cDNAs) indicates that there is also considerable sequence homology between different segments of their 3′ UTRs. By way of example, much of the

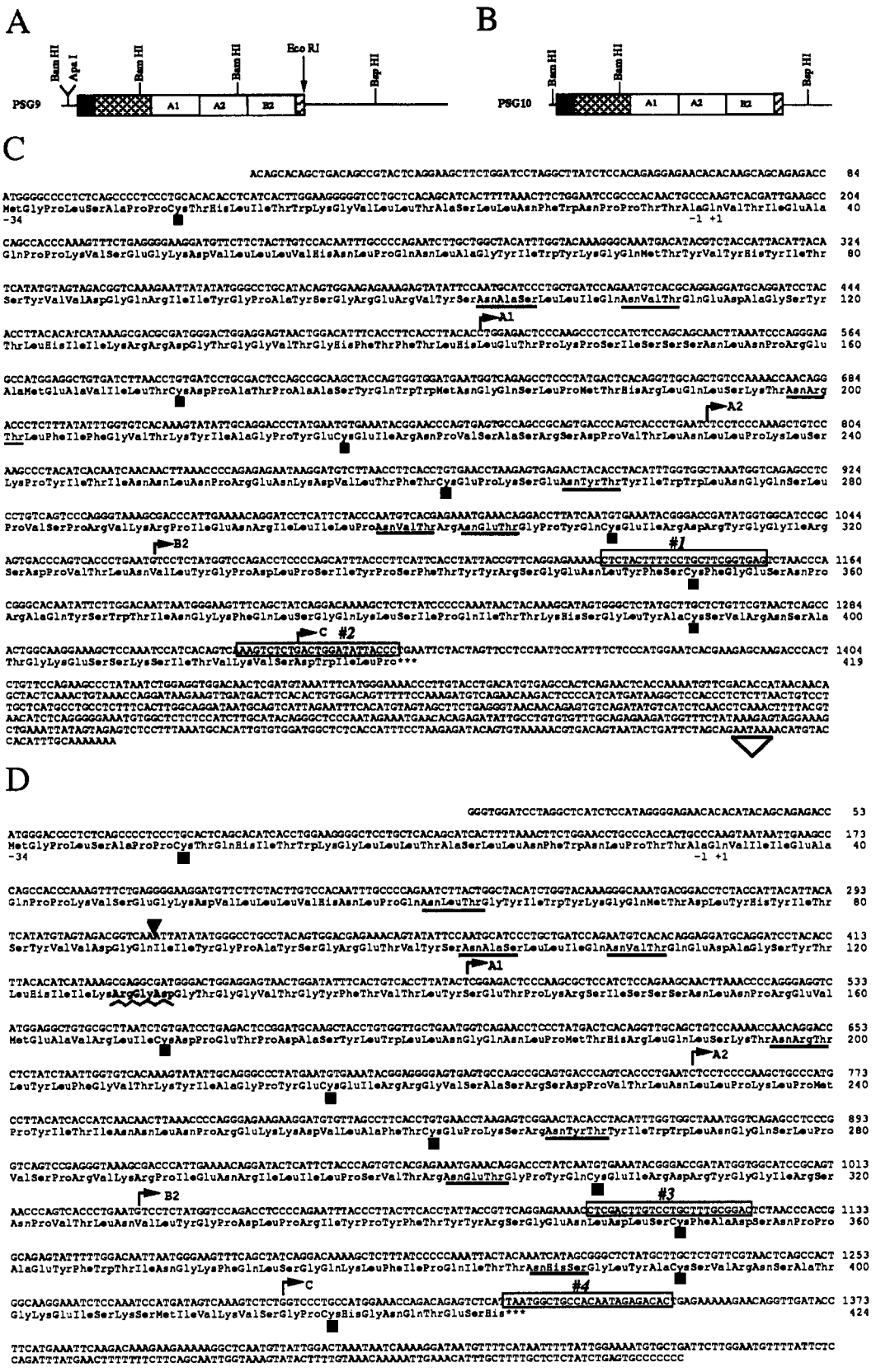

FIGURE 1: Nucleotide and inferred amino acid sequences derived from PSG9 (A) and PSG10 (B) cDNAs. The presumptive signal sequence extends from aa −34 to −1, and the N-terminus of the mature protein likely begins at +1. Consensus sequences for potential N-linked glycosylation sites are underlined, and cysteine residues are underboxed. A1, A2, and B2 demarcate the starts of likely domain regions with disulfide-linked loops, while C indicates the C-terminal domain; assignments of domain regions are based on previously published sequences of other PSG cDNAs (Oikawa et al., 1988). The probable polyadenylation site of PSG9 (C) is undermarked with an open triangle. For PSG10 sequence (D), the Arg-Gly-Asp (RGD) motif is bolded and indicated by a wavy line; the filled arrowhead (▼) marks the position of the amino acid insertion/deletion also found in PSG6 and hsCGM3 (see text). The oligonucleotide sequences used as primers in PCR reactions (see Figure 3) are boxed and numbered 1–4 as described under Materials and Methods.

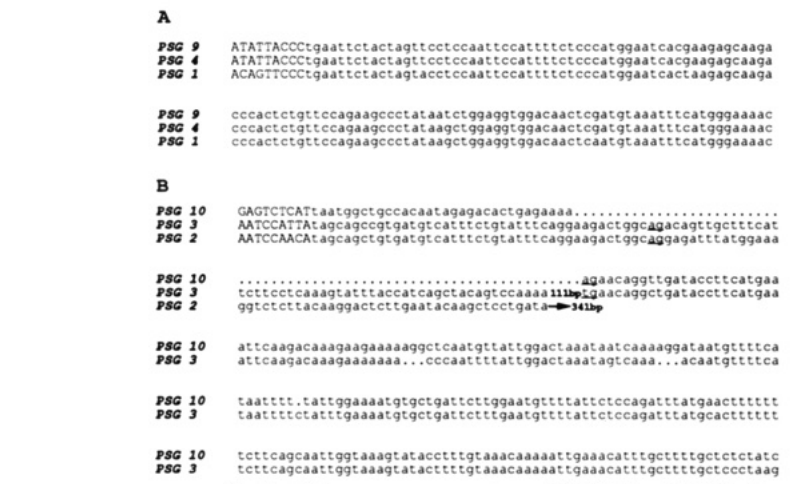

FIGURE 2: Partial sequence comparison of 3′ UTRs of PSG9-class (no RGD motif) and PSG10-class (with RGD motif) RNA transcripts. For both (A) and (B), the capitalized DNA sequence corresponds to the last three amino acids of the apoprotein, whiile lower case DNA sequence corresponds to 3′ UTR. For both (A) and (B), PSG9 and PSG10 sequences are from this paper. (A) PSG4 includes sequence information derived from hHSP2 (Chan et al., 1988) and PSG4 (Zimmermann et al., 1988) cDNAs; PSG1 includes sequence derived from PSG93 (Watanabe & Chou, 1988), PSβG-D (Streydio et al., 1988), and hPSP11 (Chan et al., 1988) cDNAs. (B) PSG3 includes sequence from pSP1-i (Rooney et al., 1988), hc17 (McLenachan & Mansfield, 1989), PS35 Arakawa et al., 1990), and FL-NCA-3 (Khan et al., 1989); PSG2 includes sequence from PSβG-E (Streydio et al., 1988) and PSβG HL (Oikawa et al., 1989a). For the purpose of aligning segments of different 3′ UTRs, dots are inserted to separate otherwise contiguous segments; for PSG2 and PSG3 sequences that are not shown, bold numbers indicate the extent of the missing sequence. Underlined nucleotide pairs represent typical sequences often found at the end of exons adjacent to a donor consensus sequence.

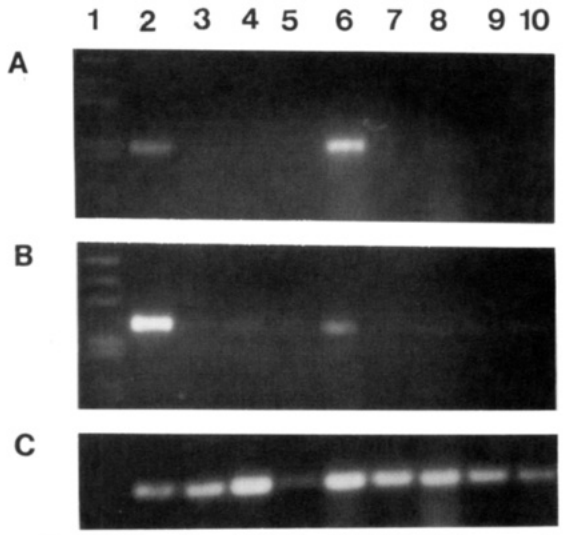

FIGURE 3: PCR detection of PSG9 and PSG10 RNA transcripts in cells of placental and myeloid origin. Approximately 10 μg of total RNA was used in a first-strand cDNA synthesis reaction; then 10% of this was subjected to 30 cycles of amplification by the polymerase chain reaction. Five-microliter samples were electrophoresed on 3% agarose gels and stained with ethidium bromide. Panels A, B, and C show the products of DNA amplification when using PSG9-specific (primer pairs 1 and 2), PSG10-specific (primer pairs 3 and 4), and ribosomal protein S14-specific oligonucleotides, respectively, as primers in the PCR. The size marker in lane 1 is part of a 1-kb DNA ladder (BRL, Gaithersburg, MD). PCR products in the remaining lanes were amplified from RNA derived from placenta (lane 2), HEL 92.1.7 cells (lane 3), K562 cells (lane 4), uninduced and phorbol ester induced HL-60 cells (lanes 5 and 6), uninduced and sodium butyrate induced KG-1 cells (lanes 7 and 8), and uninduced and phorbol ester induced U-937 cells (lanes 9 and 10). The sizes of the PCR products in (A), (B), and (C) are 210, 252, and 116 bp, respectively.

PSG10 3′ UTR is essentially identical only with the most distal sequence of the PSG3 3′ UTR (Rooney et al., 1988), while for PSG2 and PSG3, 3′ UTR sequence similarity ends at a likely consensus signal that suggests an intron/exon junction in genomic DNA just 37 nucleotides beyond translation termination. In each case, regions of similarity occur after potential splice junction signals (Figure 2B).

Specific Expression of PSG9 and PSG10 RNAs. We used the polymerase chain reaction (PCR) method to examine the specific expression in different cell types of mRNAs represented by PSG9 and PSG10 cDNAs. Two sets of specific primers were prepared from the sequence of each cDNA. Amplification with these primers is expected to yield fragment sizes of 210 and 252 bp, respectively. Because of the overall high sequence similarity among PSG members, we first amplified PSG10 and PSG9 cDNAs with PSG9 and PSG10 oligonucleotides, respectively, to determine whether heterologous priming might confuse our interpretation of the PCR products derived from total RNA. Because PSG10 oligonucleotides showed some nonspecific priming with PSG9 cDNA, we determined empirically that 64 °C, rather than the conventional 55 °C, was the ideal annealing temperature for suppressing oligonucleotide priming from heterologous templates.

Figure 3 shows that both sets of oligonucleotides yield PCR products of the expected size from placental RNA (panels A and B, lane 2), demonstrating that PSG9 and PSG10 are expressed as placental gene products. In addition, direct sequence analysis proves that the PCR products represent PSG9 and PSG10 sequences (data not shown).

Expression of PSG9- and PSG10-Related RNA in Myeloid and Nonplacental Tissue. Expression of CEA family isoantigens and their RNAs shows no clear tissue or cell specificity, but is largely a function of epithelial cells in general (Burtin et al., 1975). A notable exception is a CEA-related nonspecific cross-reacting antigen (NCA) in granulocytes (Huitric et al., 1976). Results of Northern analysis of RNAs from a number of cell lines derived from myelogenous leukemias indicated that one line in particular, KG-1, contained transcripts of the BGP I gene family (Barnett et al., 1989b) as well other CEA-related transcripts, including PSG9 (Barnett et al., 1989a).

To examine if PSG transcripts are a common feature of cells of myeloid origin, we isolated total RNA from control and phorbol ester induced leukemic cells and then amplified regions corresponding to PSG9 and PSG10 RNA. Figure 3A,B shows that of the cell lines examined, only HL-60 cells (lane 6) show a marked induction upon stimulation in the amount of PSG9 and PSG10 RNA, when compared to noninduced HL-60 cells (lane 5) or to other cell lines (lanes 3, 4, and 7–10). Closer examination of the amplified products reveals that a very small amount of PSG9 RNA is present in KG-1 cells treated with sodium butyrate (lane 8A) as well as in control (lane 9A) and phorbol-stimulated (lane 10A) U-937 cells. Similar observations can be made for the appearance of PSG10 RNA in other induced and noninduced cell types. Minor amplification of transcripts was detected in HEL 92.1.7 and K562 cell RNAs (lanes 3 and 4). To demonstrate that all first-strand cDNA reactions were productive, we show that RNA coding for ribosomal protein S14 (Rhoads et al., 1986) is amplified to approximately equivalent levels in all samples (Figure 3C).

The presence of PSG transcripts in nonplacental material prompted us to ask whether cells of normal or neoplastic origins contain PSG-related transcripts. We challenged Northern blots of poly(A+) RNA samples derived from a

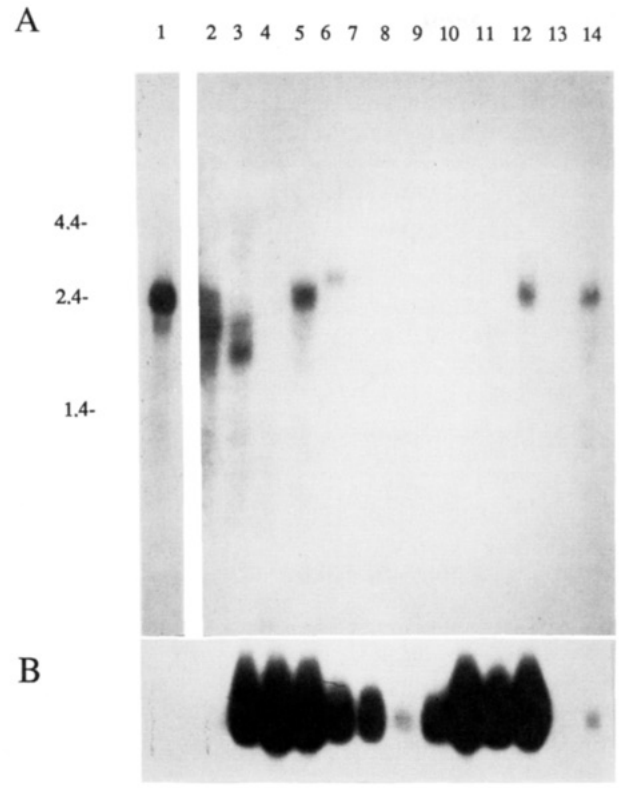

FIGURE 4: Northern blot detection of PSG RNA transcripts in nonplacental tissues. Three micrograms of poly(A+) RNA was electrophoresed on a 0.8% agarose–2.2 M formaldehyde gel and then blotted onto a nylon filter. Detection of RNA transcripts in (A) was by hybridization with a ^{32}P-labeled probe corresponding to the 3′ UTR of PSG9 cDNA (nucleotides 1343–2021). Sources of poly(A+) RNA in lanes 1–14 are placenta (1), CCD-33Co colonic fibroblasts (2), HT-1080 fibrosarcoma (3), NCI-H69 small cell lung carcinoma (4), SCaBER squamous bladder carcinoma (5), normal colonic mucosa (6), colon adenocarcinoma (7), BT-20 breast adenocarcinoma (8), KG-1 myelogenous leukemia (9), SW620 metastatic colon adenocarcinoma without (10) and induced with (11) transforming growth factor β, BeWo choriocarcinoma (12), normal liver (13), and HCMC colon epithelium (14). Autoradiographic exposure was 6 h for lane 1 and 14 days for lanes 2–14. (B) Rehybridization of (A) with ^{32}P-labeled ribosomal protein S14 cDNA. Sizes were determined by methylene blue staining of the 0.3–9.5-kb RNA ladder (BRL, Gaithersburg, MD).

variety of tissue or cell types with the 3′ untranslated region (UTR) of PSG9 cDNA. Since there is high similarity of the 3′ UTR of PSG9 cDNA with other PSG RNAs, positive hybridization signals would not necessarily reflect the expression of PSG9 mRNA in particular. Figure 4 shows that the placenta is indeed a major site of PSG mRNA expression (lane 1), but other sources also express PSG-related mRNA, albeit at considerably lower amounts than does placenta (lanes 2, 3, 5, 6, 12, and 14); still others are clearly negative even after normalizing for the amount of RNA present in each lane (lanes 4 and 7–11). It is interesting to note that fibroblastic and chorionic cells (lanes 2 and 12) do produce PSG-related RNA, in keeping with the results of Rosen et al. (1979) and Chou (1983).

Discussion

The present work describes the isolation of cDNAs from nonplacental RNA coding for novel pregnancy-specific β_1-glycoproteins, and the expression of these RNAs in myeloid and nonplacental cells. Although placental tissues appear to be the most abundant source of PSGs (Bischof, 1984), immunocytochemical analysis has shown that PSGs are also normal constituents of myeloid cells, particularly granulocytes (metamyelocytes and mature neutrophils), with little PSG-like protein detected in monocytic, erythroid, or megakaryocytic cells (Heikinheimo et al., 1987). On the other hand, a marked increase in the immunochemical fluorescence of PSG-related protein was detected after phorbol ester treatment of HL-60 cells, suggesting that synthesis of PSG(s) may be linked to cellular maturation and differentation. The particular program of cellular commitment may also be of some importance, since we show here that phorbol induction of HL-60, but not U-937, cells stimulates the synthesis of PSG RNA (Figure 3), yet both cell types with added phorbol ester adopt an adherent, macrophage-like morphology (Rovera et al., 1979). Consistent with these results is the finding of Heikinheimo et al. (1987), who detected PSG immunofluorescence in granulocytes (like stimulated HL-60 cells) but not in monocytes (like stimulated U-937 cells). Likewise, we find that extraplacental tissues do not appear to be prominent sources of PSG-related mRNAs (Figure 4), a finding that is supported by the results of Chan et al. (1988). The function of pregnancy-specific glycoproteins remains elusive. Members of this family may be implicated in the type of homotypic or heterotypic interaction that has been suggested for the related family members CEA and NCA (Benchimol et al., 1989; Oikawa et al., 1989b). Although members of the PSG family are high similar in sequence, there is some variation in the number and size of their disulfide loop as well as considerable heterogeneity in the sizes and sequences of their C-termini. This makes the assignment of a generalized function for the PSG proteins difficult. However, the finding of an RGD sequence at amino acid position 93–95 of the N-terminus of several PSG proteins suggests the potential involvement of this subclass of PSGs in extracellular matrix binding.

The RGD motif has been implicated in receptor-mediated cell adhesion, and cell migration and growth (Ruoslahti & Pierschbacher, 1987). If PSG proteins have a role in adhesion, as has been suggested for other CEA family members (Benchimol et al., 1989; Oikawa et al., 1989b), then RGD sequences in their N-termini may promote adhesion to or within the extracellular matrix. Recognition of other proteins could then occur via disulfide-linked loops. Because of their high concentrations in placental tissue, PSG proteins may have a specialized function in the promotion of cellular attachment and movement during development.

Alternative splicing within the PSG10-class transcription units, as it is within PSG9-class units (e.g., PSG1a-1d; Oikawa et al., 1988), is likely to account for the difference in linear arrangement of homologous sequences within the 3′ UTRs of different cDNAs. This is suggested by the potential splice donor sites at positions where sequence similarity ends and unrelated sequence begins (Figure 2B). Since there is no DNA sequence similarity between PSG9-class and PSG10-class 3′ UTRs, we speculate that the unique and class-specific untranslated regions represented by PSG9 and PSG10 cDNAs play an as yet undefined role in the regulation of expression of the two major PSG RNA classes. These UTR differences may, for example, serve to modulate the expression of the PSG mRNA class coding for RGD-containing proteins that could be integral components of the extracellular matrix. The distinct differences in 3′ UTR sequences could complex with particular proteins to provide a means for segregating or partitioning PSG mRNAs as ribonucleoprotein complexes.

Added in Proof

A recent report by Zheng et al. (1990) indicates that our PSG10 cDNA is virtually identical in sequence with their hPS12 cDNA, and their finding of the corresponding mRNA

in placenta is consistent with the results reported here.

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