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Linkage of two human pregnancy-specific β_1-glycoprotein genes: One is associated with hydatidiform mole

(protein family/cell adhesion molecule/placenta)

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ABSTRACT **A genomic clone containing two linked human pregnancy-specific β_1-glycoprotein (PSβG) genes has been isolated and characterized. The two genes are arranged in the same 5′ → 3′ orientation; the 3′ region (including the A2 and B–C exons) of the upstream gene, *PSGGA*, is linked to the 5′ region (including the 5′/L and L/N exons) of *PSGGB*, the downstream gene. Depending upon the domains compared, *PSGGA* and *PSGGB* share 92–98% nucleotide and 86–95% amino acid sequence identity with PSG93, the most abundant PSβG transcript. The 3′ exon (B–C) of *PSGGA* contains four alternative splice sites and three polyadenylylation sites, which account for the 3′ heterogeneity previously reported in the PSβG family. Each of the predicted *PSGGA*-encoded proteins would have a different carboxyl terminus. *PSGGB* corresponds to the previously identified cDNA PSG6, which encodes proteins containing a 34-amino acid leader peptide and a 108-amino acid N domain, which is one amino acid shorter than the majority of PSβG N domains. Additionally, the *PSGGB*-encoded proteins contain the cell-surface recognition tripeptide Arg-Gly-Asp, shared by several previously reported PSβGs as deduced from cDNA sequences. Northern blot hybridization performed with a *PSGGB*-specific oligonucleotide probe to the N domain revealed that *PSGGB* or a *PSGGB*-like gene encodes a major 1.7-kilobase mRNA in hydatidiform mole tissues and a major 2.0-kilobase mRNA in term placenta tissues. Moreover, the *PSGGB*-specific probe hybridized most strongly with mRNA from molar trophoblastic tissue, suggesting that the *PSGGB*-like species may be the gene preferentially expressed in gestational trophoblastic disease. Additionally, the sequence of a 2315-base-pair PSβG cDNA (PSG95) that contains an N-A1-A2-B2-C domain arrangement is reported. The coding region of PSG95 is identical to the previously reported cDNA clones PSG1d and FL-NCA, but PSG95 contains an additional 518 and 523 base pairs in the 3′ end as compared with PSG1d and FL-NCA, respectively.**

Pregnancy-specific β_1-glycoprotein (PSβG) was the first of a group of pregnancy-associated proteins identified (for reviews see refs. 1 and 2). It is produced by the placenta and detectable in maternal serum as early as 18 days after ovulation (3). Maternal serum concentrations reach 200–400 μg/ml by the third trimester, making PSβG a major secretory product of the human placenta (3, 4). PSβG has been used clinically to diagnose pregnancy and to predict some pregnancy-related complications. For example, low PSβG values are associated with poor pregnancy outcome in threatened abortions (5–7), intrauterine growth retardation (8), fetal hypoxia (9), and preeclampsia (10). The clinical uses of PSβG are not limited to pregnancy; it is found in the sera of most patients with hydatidiform mole, invasive mole, and choriocarcinoma (1, 4), and it has been employed as a marker for monitoring the treatment of choriocarcinoma (1).

Although PSβG has been used as a diagnostic marker, the function of PSβG is unknown. To understand more completely this potentially important protein and its function in pregnancy, our laboratory has undertaken a number of studies to characterize human PSβG. We found that placental PSβG represents a family of closely related glycoproteins of 72, 64, and 54 kDa and that placental poly(A)$^+$ RNA directed the synthesis of three polypeptides of 50, 48 (major), and 36 kDa as immunoprecipitated by anti-PSβG serum (11). Moreover, near-full-length cDNAs encoding members of the PSβG family were isolated and characterized by our laboratory (11, 12) as well as by others (13–20). Two gene fragments that share strong sequence similarity with the reported PSβG cDNAs have been reported (21, 22). We now report the structural organization of a genomic clone that contains two linked PSβG genes.§

A careful analysis of the structure of PSβG has shown that it is closely related to the carcinoembryonic antigen (CEA) family (23, 24). CEA has recently been shown to be an intercellular adhesion molecule mediating aggregation of cultured human colon adenocarcinoma cells (25). Both PSβG and CEA are members of the immunoglobulin superfamily (13–15, 23, 24, 26), which includes proteins that are arranged in domains and have constant and variable regions. A group of proteins, including neural cell adhesion molecule (N-CAM) (for a review see ref. 27), within the immunoglobulin superfamily contain the surface active tripeptide Arg-Gly-Asp, which acts in cell-surface recognition. Many members of the PSβG gene family (including *PSGGB* in this study) are closely related to N-CAM and encode proteins that contain the Arg-Gly-Asp tripeptide (13, 14, 17, 19, 22). In this paper, we explore the possibility that the *PSGGB*-encoded protein may be involved in the genesis of gestational trophoblastic disease by acting as an adhesion molecule. To support our hypothesis, we present data demonstrating that *PSGGB*-like mRNA is preferentially expressed in molar trophoblastic tissue. The *PSGGB*-like product may also serve as a more specific marker for molar pregnancy.

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Abbreviations: PSβG, pregnancy-specific β_1-glycoprotein; CEA, carcinoembryonic antigen; N-CAM, neural cell adhesion molecule; nt, nucleotide(s).

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§The sequences reported in this paper have been deposited in the GenBank data base [accession nos. M34715 (PSG95), M34716 (*PSGGA*), and M34717 (*PSGGB*)].

MATERIALS AND METHODS

Library Screening and Characterization of cDNA and Genomic Clones. A human placental cDNA library in λgt11 (Frank Gonzalez, National Institutes of Health) was screened with a probe containing the 5′ *Eco*RI–*Bam*HI fragment of the PSβG cDNA clone PSG16 (11), and a human leukocyte genomic library in λEMBL-3 (Clontech) was screened with a probe containing the entire PSG16 cDNA sequence. The cDNA and genomic inserts from positive clones were subcloned into pUC or pGEM vectors (Promega) for further characterization. Sequencing of cDNA and genomic clones was done by the dideoxy chain-termination method (28) using [α-[^{35}S]thio]dATP (400 Ci/mmol; Amersham; 1 Ci = 37 GBq). Both strands of the cDNA and genomic clones were sequenced and the sequences of the genomic clones were compared with cDNA sequences to identify intron–exon junctions.

Oligonucleotide Probes. Probes were synthesized (Cyclone Plus DNA synthesizer, Milligen Biosearch, Navato, CA) from areas of the *PSGGA* and *PSGGB* sequences that contain deletions or substitutions as deduced from comparisons with cDNAs PSG93 and PSG95. Probe 1 is a 30-mer containing nucleotides (nt) 619–648 of *PSGGB*. Probes 2 and 3 contain nt 45–68 and 323–355 of *PSGGA*, respectively. Oligonucleotides were labeled at the 5′-OH end with [γ-^{32}P]ATP by T4 polynucleotide kinase (BRL).

Trophoblastic Tissues. Human term placenta was obtained from normal pregnancy. Three hydatidiform mole tissue specimens were obtained from the University of Colorado Health Sciences Center, Denver. Samples 1 and 2 were noninvasive moles and sample 3 was an invasive mole.

Nucleic Acid Hybridization. Total RNA was extracted by the guanidinium thiocyanate method (29), and poly(A)$^+$ RNA was obtained by oligo(dT)-cellulose chromatography. RNA was electrophoresed in 1.2% agarose gels containing 2.2 M formaldehyde (30) and was transferred to Zetabind membranes (AMF Cuno) by electroblotting. The filters were hybridized at 50°C in the presence of an oligonucleotide probe (3×10^6 cpm/ml) in a previously described buffer (11) from which dextran sulfate was removed. The blots were washed twice in 2× SSC with 0.1% SDS for 30 min at room temperature, then three times in 0.2× SSC with 0.1% SDS for 30 min at 50°C. (SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7.)

RESULTS

Sequence of PSG95. Three PSβG cDNAs, PSG16, PSG93, and PSG95, have been isolated and characterized in this laboratory (11, 12). Sequence analysis indicates that PSG16 and PSG93 are highly similar and that PSG93 contains an additional 86 base pairs (bp) beginning at nt 1309 of PSG16 (11). Moreover, PSG93 is identical to cDNAs PSGD (13), hPSP11 (15), FL-NCA-2 (17), PSG1a (19), and PSβG81 (20), suggesting that PSG93 represents the major PSβG species. Like PSG93, the PSG95-encoded protein contains a 34-amino acid leader peptide, an N-A1-A2-B2-C domain arrangement, seven potential glycosylation sites, and six invariant cysteine residues, which are important in disulfide-bond formation (Fig. 1). Sequence analysis of cDNA PSG95 indicates that it is similar to PSGC (13), PSG1d (19), and FL-NCA (16) but is larger than all of them. The 5′ sequences of these four cDNAs are identical, except that PSG95 contains the longest 5′ untranslated region. The coding regions of PSG95, PSG1d, and FL-NCA are identical and encode a protein of 426 amino acids. However, PSG95 contains an additional 518 and 523 bp in the 3′ untranslated region as compared to PSG1d and FL-NCA, respectively. PSGC is identical to PSG95 from nt 45 to 1404 of PSG95; however, PSGC lacks nt 1405–1759 of PSG95. Homology between these two cDNAs again resumes from nt 1760 to the 3′ end of PSG95. Because of the 355-bp insertion in PSG95, the proteins encoded by PSG95 and PSGC differ in their sequences at the carboxyl termini. The 3′-end divergence may be generated by alternative splicing events, as discussed later. A complete analysis of the sequences of PSG95 compared to PSG93 and PSG16 reveals that PSG95 and PSG93 are identical until the alternative splicing junction in the carboxyl terminus, at nt 1404 of PSG95 (Fig. 1). However, PSG95 and PSG93 differ from PSG16 at nt 215, 287, 288, and 764, as well as 3′ to the alternative splice point (11). Thus, PSG16 is a polymorphic variant.

Isolation and Characterization of PSβG Genes. Nineteen PSβG genomic clones that strongly hybridized with a probe containing the entire PSG16 cDNA were isolated. These genomic clones were characterized by their restriction endonuclease digestion patterns and by their abilities to hybridize with PSβG-specific probes. Clone A, which hybridized strongly with a probe specific to the 5′ end of PSG93 (nt 1–142) and gave a restriction endonuclease pattern similar to that of human genomic DNA (data not shown), was extensively analyzed.

A careful analysis of clone A indicates that it contains two linked PSβG genes arranged in the same 5′ → 3′ orientation (Fig. 2). The upstream gene, *PSGGA*, contains the A2 and B–C exons in its 3′ region. The A2 exon is composed of 279 bp encoding 93 amino acids with 98% identity to the A2 protein domain of PSG16/PSG93/PSG95 at the nucleotide level and 95% identity at the amino acid level. The 3′ exon, B–C, contains 1950 bp and encodes the PSβG B2 domain and the 3′ untranslated regions of mRNAs similar to PSG16, PSG93, PSG95, PSG1d/FL-NCA, and PSGC. These appear to have been generated by alternative splicing. The majority of PSβG mRNAs, which differ in their 3′ region, diverge precisely at splice site 1 (C1 in Fig. 2). The *PSGGA* sequence immediately following this point shares 92% identity with PSG95 and contains the entire 3′ region of the PSG95-like sequence from nt 1405 to the 3′ end of PSG95. PSG1d (19) and FL-NCA (16) are identical to PSG95, except that the FL-NCA-like sequence ends just after splice site 2 (C2 in Fig. 2), and utilizes a polyadenylylation site beginning at *PSGGA* nt 902. PSGC (13) and PSG95 differ by the presence of an additional 355-bp sequence (nt 1405–1759) in PSG95 (Fig. 1). In *PSGGA*, this PSG95-like sequence appears to be an intron in the generation of a PSGC-like mRNA. This sequence contains the consensus splicing site GT/AG (31). Within *PSGGA*, the PSGC-like 3′ terminus begins at nt 889 (C2 in Fig. 2). The single polyadenylylation site shared by the putative PSG95- and PSGC-like transcripts begins at *PSGGA* nt 1409.

Fifty bases downstream from the ends of the PSG95- and PSGC-like sequences is another junction, splice site C3. This 50-bp sequence does not contain the intron consensus splicing site GT/AG; thus it may represent the true 3′ end of the PSG95- and PSGC-like transcripts. Beginning at splice site C3, the *PSGGA* sequence shares 94% sequence identity with the 3′ region of PSG93. PSG93 differs from PSG16 by the presence of an additional 86 bp beginning at nt 1309 of PSG16 (11). Splice site C3 marks the beginning of this 86-bp insert and, thus, nt 535–1482 (which contain the C1 and C2 sequences and the consensus splicing site GT/AG) act as an intron to generate a PSG93-like mRNA. The 86-bp region of the PSG93-like sequence is designated as C3 (Fig. 2) and begins at *PSGGA* nt 1483. Immediately following the 86-bp sequence is splice site C4, which represents where the PSG16-like message joins after splicing at site C1. Sequences in C4 are 94% identical with the shared 3′ sequences of PSG16 and PSG93. The shared polyadenylylation site for the putative PSG93- and PSG16-like messages begins at nt 2152 of *PSGGA*. Although similarity exists between *PSGGA* and previously reported PSβG cDNAs, *PSGGA* is not identical to any of them.

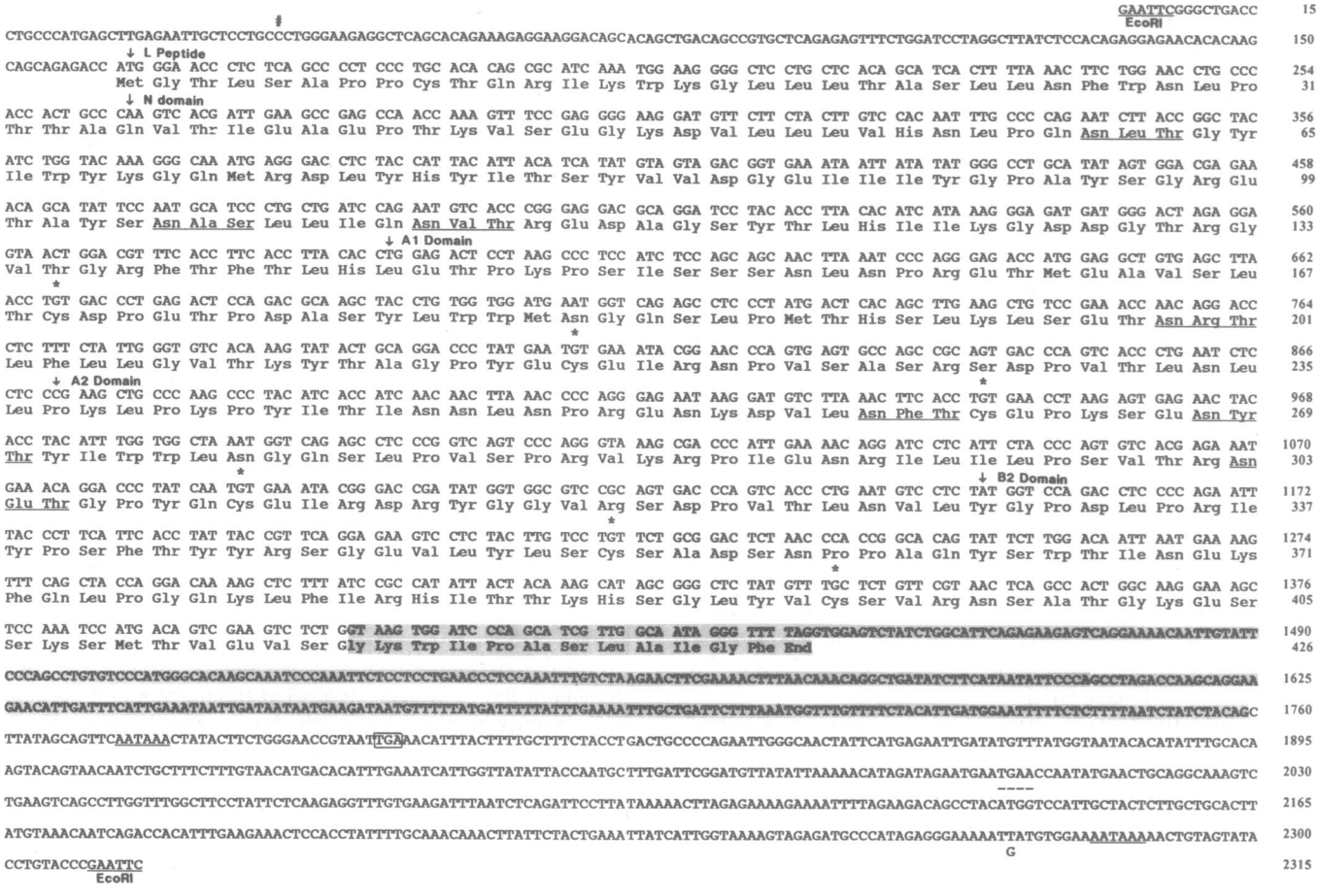


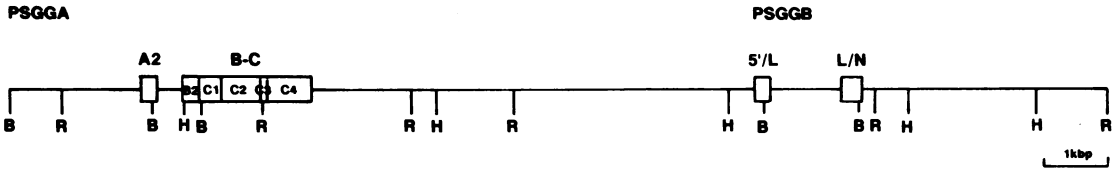
FIG. 1. Nucleotide and deduced amino acid sequences of cDNA PSG95, as compared to cDNA PSGC. The 5′ sequence of cDNA PSGC (13) begins at nucleotide 45 (indicated as #) of PSG95. Arrows indicate the leader (L) peptide and the N, A1, A2, and B2 protein domains of the PSG95-encoded protein. Potential asparagine-linked glycosylation sites and poly(A)-addition signals are underlined. Asterisks indicate cysteine residues involved in disulfide-bridge formation. The potential termination codon (TGA) for PSGC is boxed. The shaded area represents the sequence that is absent from PSGC (13). The nucleotide that differs from PSGC is shown below the substituted position, and dashes at nt 2003–2006 indicate deletions.

The nucleotide sequence of the downstream gene, *PSGGB*, is identical to cDNA PSG6 reported by Zimmermann *et al.* (19). We have obtained ≈180 bp of the *PSGGB* sequence upstream from the transcriptional start site of the PSG93-like sequence (the beginning of exon 5′/L). The 5′/L exon (≈250 bp) encodes the 5′ untranslated region and the first 21 amino acids of the leader peptide. The L/N exon (363 bp) encodes the remaining leader codon and the N protein domain (108 amino acid residues). Amino acids 126–128 of *PSGGB* are Arg-Gly-Asp. This tripeptide is the signal-surface active component of cellular adhesion molecules (27) and gives a possible clue to the function of this member of the PSβG family.

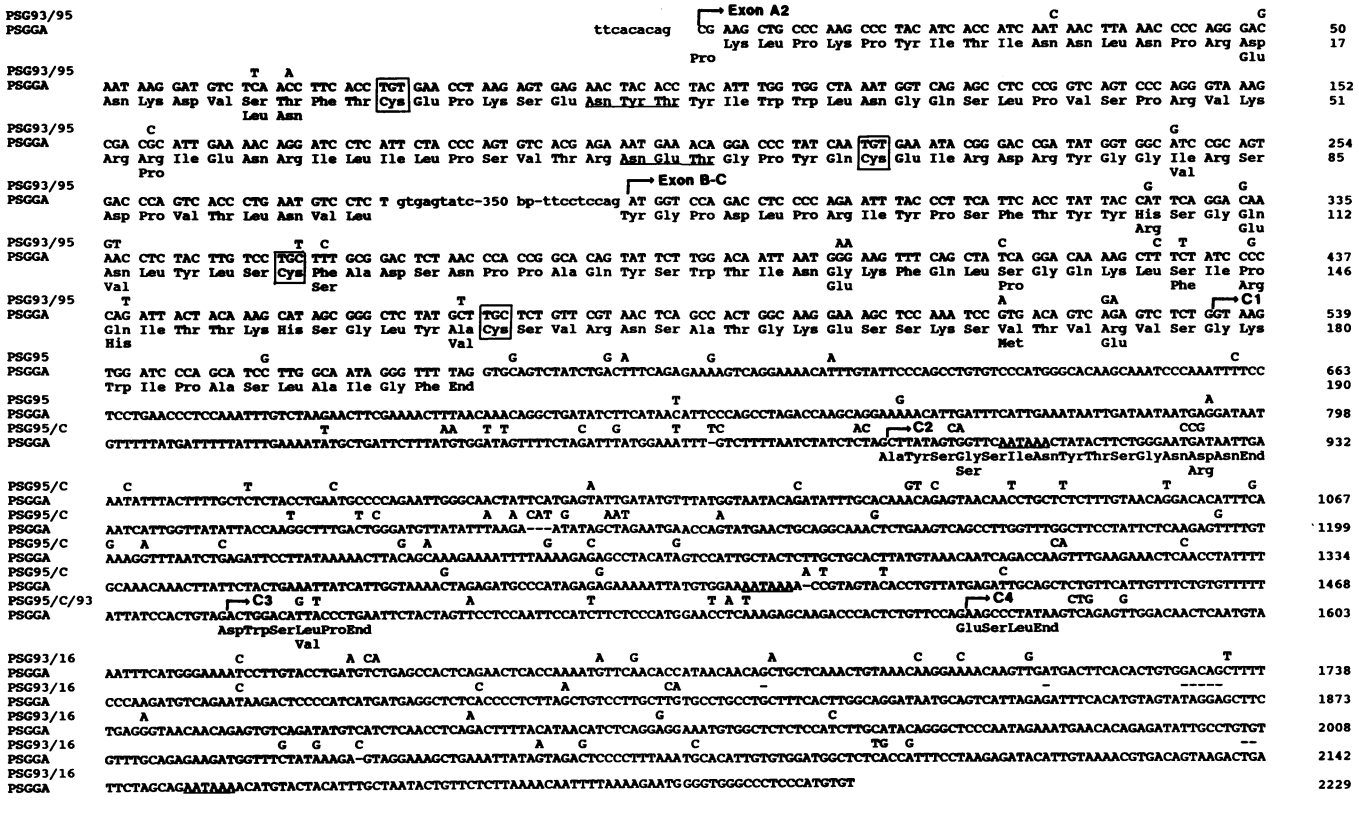
Northern Analysis. Fig. 2 illustrates the nucleotide sequences of *PSGGA* and *PSGGB* and the proposed exon and protein sequences compared to PSG16, PSG93, PSG95, and PSGC. To demonstrate that mRNAs encoded by *PSGGA* and *PSGGB* are actually expressed in placental tissue, we synthesized three oligonucleotides (probes 1–3) that would hybridize to the *PSGGA* and *PSGGB* sequences in areas that contain deletions or substitutions as compared with cDNA PSG93/PSG95. Probe 1 (30-mer) is located in the N domain of *PSGGB* and includes the 3-bp deletion present in this region as well as four base-pair substitutions when compared with PSG95/PSG93. Probe 2 (24-mer) is located in the A2 domain of *PSGGA*, where it contains three single base-pair substitutions when compared with PSG95/PSG93. Probe 3 (33-mer) is located in the B2 domain of *PSGGA*, where it contains four single base-pair substitutions and one double substitution when compared with PSG95/PSG93. Northern blots revealed that the *PSGGB*-specific probe, probe 1, hybridized with a major 1.7-kb mRNA and a minor 1.5-kb mRNA from the three molar tissues, but with a 2.0-kb mRNA (major) and a 1.5-kb mRNA from term placental tissue (Fig. 3). Moreover, the transcript recognized by probe 1 appears to be preferentially expressed in hydatidiform mole when compared with normal trophoblastic tissue (Fig. 3). Furthermore, the level of the transcript that hybridized with probe 1 was higher in an invasive mole than in two noninvasive moles. The *PSGGA* probe, probe 2, is the least specific probe and hybridized with two mRNAs of 2.2- and 1.5-kb in term placenta and with a 1.7-kb message in molar tissue. However, the relative amounts of probe 2-hybridizable message were higher in placenta than in hydatidiform moles. Probe 3, which is more specific to *PSGGA*, hybridized poorly with either placenta or molar RNA (data not shown).

DISCUSSION

We report the isolation and characterization of two linked genes (*PSGGA* and *PSGGB*) encoding members of the PSβG protein family and present the complete sequence of another PSβG cDNA, PSG95. PSβG is a family of proteins with many closely related individual members encoded by more than one gene on chromosome 19 (ref. 32; K.-J.L. and J.Y.C., unpublished results). In addition to this genomic clone, we



(A) PSGGA



(B) PSGGB

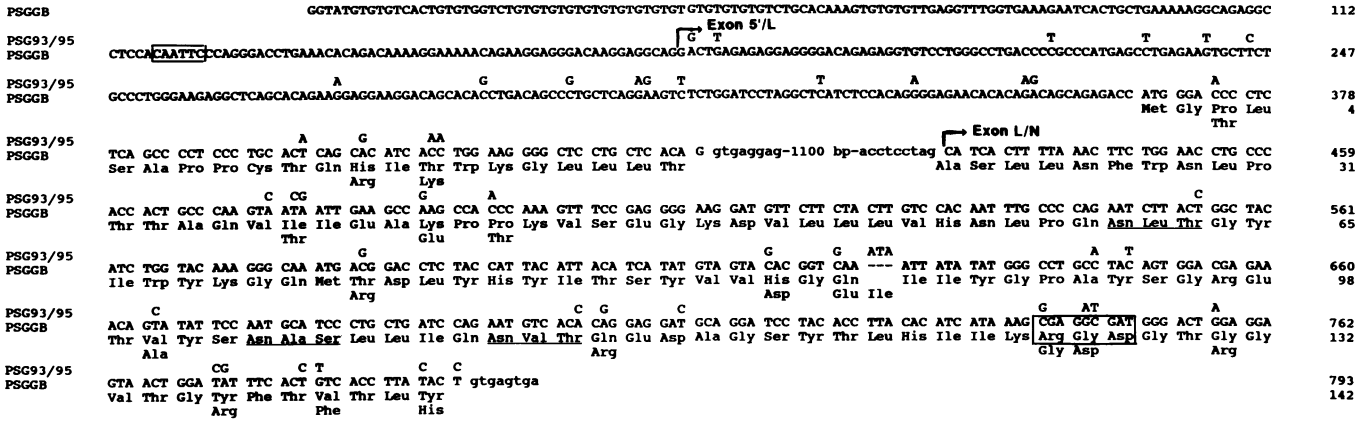


FIG. 2. Restriction map (B, *Bam*HI; R, *Eco*RI; H, *Hin*dIII) and the nucleotide and deduced amino acid sequences of two linked PSβG genes, *PSGGA* and *PSGGB*, as compared to cDNAs PSG16, PSG93, PSG95, and PSGC. (*A*) The upstream gene, *PSGGA*, contains the A2 and B–C exons. The B–C exon contains four alternative splicing sites (C1, C2, C3, and C4) that may be involved in the generation of four PSβG mRNAs, each encoding a protein with a different carboxyl terminus. The PSG1d-like 3′ sequence is composed of C1 (19), the PSG95-like 3′ sequence is composed of C1 and C2, the PSGC-like 3′ sequence is composed of C2 (13), the PSG93-like 3′ sequence is composed of C3 and C4 (11), and the PSG16-like 3′ sequence is composed of C4 (11). Cysteine residues are boxed, and potential asparagine-linked glycosylation sites and the poly(A) addition sequences are underlined. (*B*) The downstream gene, *PSGGB*, contains the 5′/L and L/N exons. The CAAT box and Arg-Gly-Asp tripeptide are boxed.

have identified another clone containing two different linked PSβG genes (clone B, unpublished results). Our results suggest that all of the PSβG genes may be linked. The B–C exon of *PSGGA* encodes the B2 protein domain and four alternative splicing points within the carboxyl terminus that could result in the generation of multiple transcripts. The

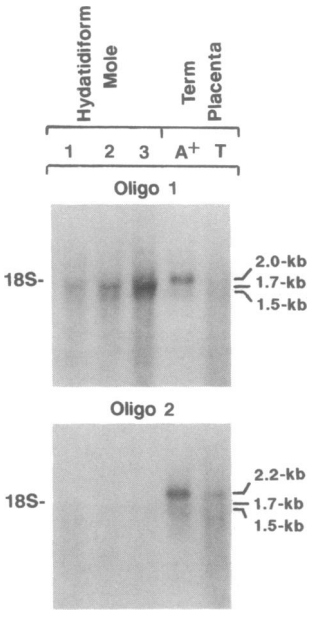


FIG. 3. Northern blot hybridization of placental and hydatidiform mole RNAs with *PSGGA*- and *PSGGB*-specific probes. Total (20 μg per lane, T) or poly(A)$^+$ (2 μg per lane, A$^+$) RNAs from human term placenta and hydatidiform moles were electrophoresed in formaldehyde/agarose gels. RNA was hybridized to ^{32}P-labeled oligonucleotide probes containing nt 619–648 (oligo 1) of *PSGGB* or nt 45–68 (oligo 2) of *PSGGA*. Samples 1 and 2 are total RNAs from two noninvasive moles, and sample 3 is total RNA from an invasive mole. Position of 18S rRNA is indicated.

similarity between the *PSGGA* 3′ sequences and the 3′ sequences of PSG95, PSGC (13), PSG1d (19)/FL-NCA (16), PSG93 (11), and PSG16 (11) suggests that these five PSβG mRNAs may be encoded by a gene with a similar B–C exon.

Oikawa *et al.* (21, 22) have isolated two other PSβG genomic fragments. The gene fragment CGM35 (21) contains the A1, B1, A2, and B–C exons. The B–C exon also contains several splice points, and alternative splicing is likely in this gene fragment as a means to generate different mRNA species; however, CGM35 does not encode any of the reported PSβG mRNAs. The second gene fragment, PSG-HL 12-2 (22), contains the 5′/L, L/N, A1, B1, and A2 exons with part of the B2 domain of the B–C exon. A PSβG cDNA (PSβG-HL clone 22) has been identified that has the N-A2-B2-C domain arrangement and shares complete sequence homology with genomic fragment PSG-HL 12-2 (22). This suggests that the A1 and B1 exons have been spliced out to generate this mRNA. A comparison of the intron sequences of *PSGGA* and *PSGGB* with the corresponding intron sequences of PSG-HL 12-2 reveals that they are very similar (data not shown). The similarity in intron sequences among the different PSβG genes indicates that divergence within this family is a relatively recent evolutionary event.

Although a putative CAAT box is located at nt 118–123 of *PSGGB*, there is no consensus TATA box-like sequence in the 5′ flanking region of this gene. The linkage of *PSGGA* and *PSGGB* indicates that an additional 5′ exon does not exist in *PSGGB*. This is supported by previous primer extension experiments, which showed that PSG93 may be a full-length cDNA clone (11), and the entire 5′-untranslated region of PSG93 is represented in *PSGGB*.

PSGGB encodes the PSG6 transcript reported by Zimmermann *et al.* (19). This PSβG species contains the Arg-Gly-Asp tripeptide (amino acids 126–128) in a position similar to that found in some other PSβG species (13, 14, 17, 19, 22). This tripeptide is also present in the well known cellular adhesion molecule N-CAM and in other proteins that are known to be important in the cellular recognition process (27, 33). The Arg-Gly-Asp-containing PSβG proteins could be important in maintaining the functional and structural integrity of the placenta, interacting between the trophoblastic and decidual cells, and mediating trophoblastic invasion. Although the association of PSSGB-like mRNA with hydatidiform moles was demonstrated with only three molar samples, our data support the theory that proteins encoded by *PSGGB* or a *PSGGB*-like gene may be acting as molar trophoblastic adhesion molecules in a manner analogous to CEA in adenocarcinoma of the colon (25). Additionally, the specificity of the *PSGGB*-like protein for gestational trophoblastic disease may be of use clinically in the diagnosis and monitoring of molar pregnancies.

In a broader sense, the concept that certain members of the PSβG family may be associated with specific disease entities could be of great importance. For instance, if a protein encoded by the *PSGGB*-like gene represents a specific marker for invasive mole or choriocarcinoma, does a specific PSβG marker exist for pregnancies that are destined to abort? Now that a variety of PSβG cDNAs have been identified, differential expression of these species should be investigated.

This work was done while C.A.P. held a National Research Council (Human Genetics Branch, National Institute of Child Health and Human Development) research associateship.

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