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PANG, a gene encoding a neuronal glycoprotein, is ectopically activated by intracisternal A-type particle long terminal repeats in murine plasmacytomas

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ABSTRACT **Plasmacytomagenesis provides a murine model to decipher progressive genetic events culminating in a B-cell neoplasia. Activation of the c-*myc* protooncogene by chromosomal translocation is considered an initiating event. Intracisternal A-type particles (IAPs) are defective retroviral-like structures present in the endoplasmic reticulum of plasmacytomas (PCTs). IAP proviral insertions have been documented to engender negative or positive effects on the expression of nearby cellular genes. We have isolated a gene, PANG (plasmacytoma-associated neuronal glycoprotein), that is ectopically transcribed in a number of PCTs due to IAP long terminal repeat (LTR) activation. A full-length PANG cDNA was isolated from an MPC-11 plasma cell tumor cDNA library and encodes a polypeptide of about 113 kDa with six immunoglobulin C2-like and four type III fibronectin-like domains. PANG bears a striking resemblance to axonal glycoproteins TAG-1 and F11 known to function in neuronal outgrowth. An extensive survey revealed a predominant 3.6-kb PANG transcript in 60% (30 of 50) of PCTs as well as unique smaller and larger species. All other normal and transformed lymphoid and nonlymphoid cell lines and normal tissues were negative for PANG expression except for the brain, wherein unique 4.0- and 6.1-kb transcripts were detected. Reverse transcriptase PCR analysis revealed IAP LTR fusion to PANG mRNAs in five PCTs and in a neuroblastoma line. The 5′ end of a mouse brain PANG cDNA was identical to the MPC-11 PANG transcript except for the precise replacement of its 5′ LTR sequence.**

Plasmacytoma (PCT) cells produce abundant quantities of intracisternal A-type particles (IAPs) budding from the endoplasmic reticulum (1). IAPs are defective retrovirus-like structures expressed in a number of murine tumor cells and in the developing murine embryo (1, 2). The mouse genome harbors several thousand IAP proviral DNAs and various deleted forms (3, 4). IAP proviruses exist as two major classes: type I (the most abundant variety) and type II (about 2 kb smaller with other unique sequences) (4). Recently, the mouse IAP-promoted placenta (MIPP) gene was shown to contain an inbred strain-specific, "solo" IAP long terminal repeat (LTR) insertion, suggesting that germ-line retrotransposition of IAPs can result in tissue-specific gene expression (5). PCTs express abundant quantities of type II IAP RNAs (6). IAP proviruses have been found to act as insertional mutagens in PCTs having both negative (7) and positive (8) effects on cellular gene expression. IAP proviral insertions within and nearby the c-*mos* gene and 3′ of a translocated c-*myc* gene have been documented in independent PCTs (8, 28). However, a more general role for IAPs in plasmacytomagenesis remains to be established.

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Here, we report the cloning and expression of PANG (plasmacytoma-associated neuronal glycoprotein) RNAs and the structural features of their encoded polypeptide.¶ Thirty of 50 PCTs expressed a 3.6-kb PANG transcript expressed with larger and smaller varieties in exceptional tumors, and up to 80% of PCTs expressing IAP LTRs were PANG positive. Remarkably, the normal site of PANG expression is the brain. An ≈3.6-kb, full-length PANG cDNA clone from an MPC-11 cDNA library was found to encode a new member of the family of neuronal adhesion molecules. These polypeptides generally contain immunoglobulin C2-like and fibronectin type III-like domains. They promote axonal growth and guidance (9–11) and include TAG-1 (11), L1 (12), F11/contactin (13), F3 (14), neural cell adhesion molecule (N-CAM) (15), *Drosophila* neuroglian (16), and myelin-associated glycoprotein (17). The 5′ end of PANG transcripts in MPC-11 and five other PCTs are fused to an IAP LTR in an identical fashion while this IAP LTR is not present in a murine brain PANG cDNA, implicating IAP LTRs in the ectopic activation of PANG in PCTs.

MATERIALS AND METHODS

Cell Lines. The Neuro 2A cell line was obtained from the American Type Culture Collection and grown in Eagle's minimum essential medium with 10% fetal bovine serum.

Polymerase Chain Reactions (PCRs). *PANG cloning.* PCR was performed with 5′ and 3′ amplimers (5′-GCGGCCGC-GAGGAAGAAATTGA-3′ and 5′-GCGGCCGCGAGGAT-CACTACCTTGGG-3′, respectively) homologous to two highly conserved coding segments in the c-*myc* third exon. Total cellular poly(A)$^+$ RNAs were converted into cDNAs in a 20-μl reverse transcriptase (RT) reaction containing 50 mM Tris·HCl (pH 8.3), 40 mM KCl, 6 mM $MgCl_2$, 1 mM of each of four dNTPs, 200 units of mouse mammary tumor RT (BRL), 12 units of RNasin (Promega), 2 μg of poly(A)$^+$ RNA, and 40 pmol of the 3′ amplimer and incubated at 37°C for 45 min. For the PCR, RT reaction volumes were increased to 100 μl in 10 mM Tris·HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 0.4 μM of each primer, and 5 units of *Taq* polymerase (Perkin–Elmer/Cetus). Samples were subjected to 5 cycles of amplification (1 min at 95°C, 1 min at 37°C, and 2 min at 72°C), followed by 50 cycles (1 min at 95°C, 1 min at 55°C, and 2 min at 72°C). The reaction mixtures were fractionated on a 6% polyacrylamide gel. PCR products were treated with Klenow DNA polymerase prior to cloning into the *Eco*RV site of pBluescript KS− (Stratagene).

Abbreviations: IAP, intracisternal A-type particle; LTR, long terminal repeat; PCT, plasmacytoma; PANG, plasmacytoma-associated neuronal glycoprotein; RT, reverse transcriptase; ORF, open reading frame; GPI, glycosylphosphatidylinositol.

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. L01991).

PANG expression. cDNA was made from 0.1 μg of poly(A)$^+$ mRNA by random hexamer priming with a Gene-amp RNA PCR kit (Perkin–Elmer/Cetus). PCR was initiated by a modified hot start protocol (Perkin–Elmer/Cetus), with 5′ and 3′ amplimers in 25 λ of 50 mM KCl/6 mM $MgCl_2$/10 mM Tris·HCl, pH 8.3, separated by AmpliWax PCR GEM 100 (Perkin–Elmer/Cetus) from 55 λ of an upper reagent with AmpliTaq polymerase, 73 mM KCl, 14.5 mM Tris·HCl (pH 8.3), and 20 μl of cDNA reaction mixture. Thermal cycling parameters for amplimers in Fig. 6 *A* and *C* were 2 min at 95°C, 30 cycles of 1 min at 95°C, 30 sec at 65°C, and 30 sec at 72°C followed by 5 min at 72°C, but in Fig. 6*B* the annealing temp was 60°C. The 3′ PANG amplimer in Fig. 6 was 5′-ACAGAGAAGCACCAC-3′ (bp 608–625 in Fig. 1). The 5′ amplimers were 5′-GACGGATCCCACCTCATTACAG-GTGGC-3′ (bp 363–380 in Fig. 1), 5′-TGTTGCGTCTT-TCCTGGC-3′, and 5′-GACGGATCCTGCCGCAGAAGAT-TCTGG-3′ in Fig. 6 *A*, *B*, and *C*, respectively. The latter pair of 5′ amplimers is from the IAP LTR in Fig. 2.

cDNA Libraries. An Okayama–Berg plasmid DNA library of MPC-11 poly(A)$^+$ RNAs (18) was kindly provided by Martin Julius. A λZAP cDNA library from postnatal day 30 whole mouse brain mRNAs was kindly supplied by Jim Boulter (Salk Institute).

DNA Sequencing. Single-stranded DNA was isolated via a modified rescue protocol described by Viera and Messing (19). Single-stranded templates were sequenced by the dideoxy chain-termination method with a sequenase kit (United States Biochemical).

Northern Blots. Poly(A)$^+$ RNAs were prepared with a Fast Track isolation kit (Invitrogen) and ≈1-μg samples were electrophoresed on 1.2% agarose gels in 1% formaldehyde. Gels were blotted (pp. 4.9.1–4.9.4 in ref. 20) onto Nytran filters (Schleicher & Schuell) and probed with a 2.29-kb *Eco*RI/*Stu* I fragment of PANG coding sequences or a 650-bp *Hin*dIII/*Sal* I fragment of IAP LTR plus PANG sequences. Filters were washed once in 2× SSC/0.1% SDS (1× SSC = 0.15 M NaCl/15 mM sodium citrate) for 5 min at room temperature, then washed twice in 0.2× SSC/0.1% SDS for 20 min at 65°C, and submitted to autoradiography with intensifier screens. A mouse multiple tissue Northern blot was purchased from Clontech.

RESULTS

Cloning and Characterization of a PCT-Specific Transcript. Two oligonucleotide primers, corresponding to small portions of an acidic region and the second amphipathic helix of the basic helix–loop–helix domain within the c-*myc* third exon, were employed in a RT-PCR based cloning strategy to attempt to clone novel *myc*-like genes (21) (see *Materials and Methods*). In addition to an expected c-*myc* DNA band, several other PCR products were visualized on a 6% polyacrylamide gel, including two major amplified bands of 220 and 240 bp detected with several PCR RNAs and the 240-bp species was PCT specific (21). The 220-bp PCR product was derived from an ≈3.7-kb mRNA encoding a helix–loop–helix polypeptide (M.A.C. and K.B.M., unpublished results). However, the 240-bp PCT-specific PCR product did not specify a helix–loop–helix-like domain and is the subject of this study.

The PCT-specific PCR product was used to screen an Okayama–Berg type MPC-11 cDNA library (18) that yielded a near full-length cDNA of ≈3.45 kb. The latter insert was subcloned into the *Bam*HI site of pBluescript (KS−), its complete sequence was determined, and the EMBO and GenBank nucleic acid and protein data bases were searched for related genes. The 5′ 112 bp precisely matched the 3′ portion of various IAP proviral LTRs (Figs. 1 and 2). Southern blot analysis with a DNA probe encompassing this 5′ 112 bp detected highly repetitive sequences in the BALB/c mouse genome and cross-hybridized with an IAP family probe (data not shown). The remainder of the cDNA sequence specified a 1029-amino acid polypeptide followed by a 166-bp 3′ noncoding terminus with an AAUAAA sequence 21 bp upstream of a poly(A) tail (Fig. 1). This unique open reading frame (ORF) programed the *in vitro* synthesis of an ≈113-kDa polypeptide with or without the 5′ LTR sequence (data not shown).

The Encoded Polypeptide Is an Additional Member of a Family of Neuronal Adhesion Molecules. Axonal glycoproteins TAG-1 (11) and F11 (13) displayed almost 50% identity over their entire length and up to 70% homology to the ORF of the PCT-specific cDNA (Fig. 3). We have named this neuronal-like adhesion molecule PANG. PANG contains six amino-proximal immunoglobulin C2-like (25) and four carboxyl-proximal fibronectin type III-like (26) domains analogous to those in TAG-1 (11), L1 (12), and F11 (13) (Fig. 3). The third immunoglobulin C2-like domain lacks a second cysteine that would form a hallmark disulfide bond. However, CD2, LFA3, CD4, CEA, and even an antibody molecule that are also missing cysteines in some of their V_H and C2 domains remain very immunoglobulin-related (25). In all of these cases, including PANG, cysteines are replaced by hydrophobic amino acids that would presumably serve as in-pointing residues to stabilize the immunoglobulin-like fold (25). PANG also contains 12 potential sites for N-linked glycosylation (Fig. 1). Many of the proteins in the adhesion molecule family are glycosylphosphatidylinositol (GPI) linked or have transmembrane as well as GPI-linked forms (9, 15). A hydrophobicity plot performed on the Macintosh PROTEAN program revealed no stretches of 20 or more hydrophobic amino acids that might form the α-helix of a transmembrane domain. Like TAG-1, PANG may be a peripheral membrane protein and/or linked to the plasma membrane by a GPI linkage and, short a hydrophobic sequence at its carboxyl terminus, does fit the general consensus for GPI-linked proteins (9).

A postnatal murine brain λZap cDNA library was probed with a restriction fragment of the PANG ORF. Twenty-four independent clones with PANG-like restriction patterns were obtained. Sequence obtained with a primer in the first immunoglobulin C2-like domain identified a subset of clones. Two such clones were identical to MPC-11 PANG and one extended beyond the ATG beginning the PANG ORF (Fig. 2). Its 5′ noncoding sequence including an in frame stop codon was identical to that of MPC-11 PANG. However, the IAP-derived LTR was replaced by an unrelated sequence. We conclude that brain and PCT PANG transcripts likely possess identical coding capacity but are transcriptionally activated by different mechanisms.

The Brain Is the Normal Site of PANG Expression. Northern blots of RNAs derived from various cell lines and normal tissues were hybridized with a PANG coding sequence restriction fragment. PANG transcripts were not detected in a large variety of normal or transformed cell lines of diverse cell types (data not shown) nor in most tissues (Fig. 4). However, two PANG transcripts of 4.0 and 6.1 kb were detected in normal adult murine brain tissue and a faint signal was also present in the testis (Fig. 4).

PANG Is Ectopically Activated in Murine Tumors Expressing Endogenous IAPs. Northern blots of poly(A)$^+$ RNAs prepared from a large panel of plasma cell tumors were screened with a 2.29-kb probe derived from the MPC-11 PANG cDNA clone. About 30 of 50 PCTs expressed variable levels of a 3.6-kb PANG mRNA and occasionally other larger (8.3 and 5.0 kb) and smaller (3.2, 2.2, and 1.8 kb) transcripts (see six representative samples in Fig. 5). Northern blots were stripped and reprobed with a TAG-1 cDNA fragment but only the brain was positive (data not shown), indicating

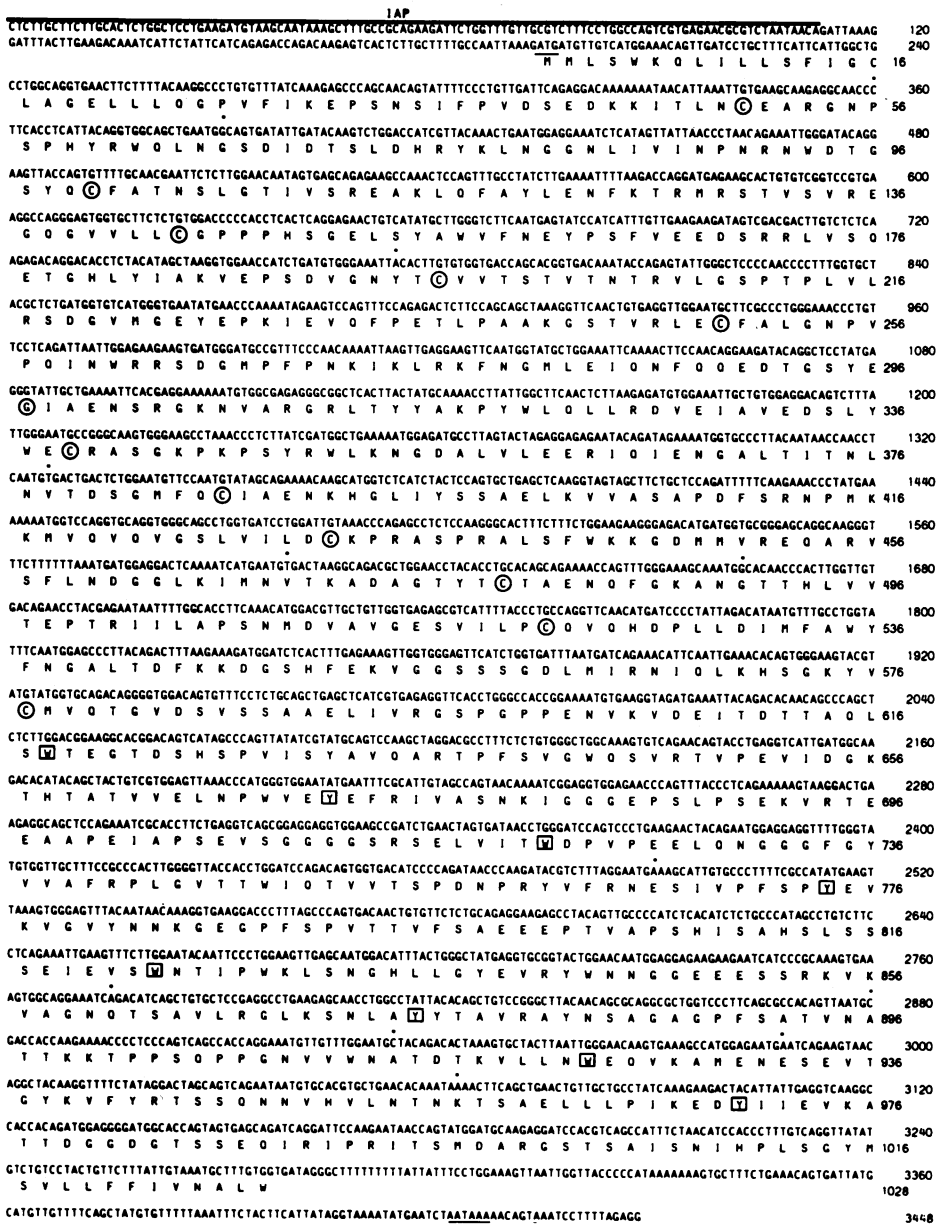

FIG. 1. Nucleic acid and predicted amino acid sequences of a near full-length MPC-11 PANG cDNA clone. IAP sequence, poly-(A) signal sequence (AATAAA), and possible initiator ATG are underlined. Circled cysteines and boxed tryptophans and tyrosines identify conserved residues of immunoglobulin C2-like and fibronectin-like type III domains. Potential sites of N-linked glycosylation are denoted by asterisks.

that expression of neuronal-specific adhesion molecules in PCTs may be unique to PANG. Northern blots were reprobed with an IAP LTR DNA fragment derived from the 5′ end of the MPC-11 PANG transcript. Various RNAs corresponding to different-sized IAP genomes were also detected in ≈73% of these PCTs and their expression was generally far greater than PANG (data not shown). Overall, about 80% (24 of 30) of the PANG-positive tumors also expressed IAP RNAs.

Ectopic PANG transcription in PCTs may be caused by an IAP LTR residing upstream or within the gene or, alternatively, via IAP insertion. Southern blots performed with various restriction enzymes and PANG DNA probes failed to reveal DNA rearrangements in MPC-11 or other PCTs, ruling out a nearby insertion event (data not shown). A RT-PCR protocol was used to determine if other cases of ectopic PANG activation also involved an IAP LTR. A pair of DNA primers derived from the first and second immunoglobulin

FIG. 2. Sequence comparisons of 5′ ends of PANG cDNAs from MPC-11 cells and murine brain and IAP LTRs including 3′ rc-*mos*, 3′ LTR inserted in the c-*mos* gene (8); the 5′ LTRs of IAP clones 3.2 and c5-1 (22); and an IAP LTR activating the murine interleukin 3 gene (23). Dashes indicate sequence identity, and common R and U5 regions in IAP LTRs (29) are shown. Boxed nucleotides in the PANG MPC-11 sequence are the 5′ PCR amplimers used in Fig. 6 *B* and *C*. In-frame TAA stop codon and downstream ATG initiator in the MPC-11 and brain PANG sequences are highlighted by a vertical box and an overhead arrow, respectively.

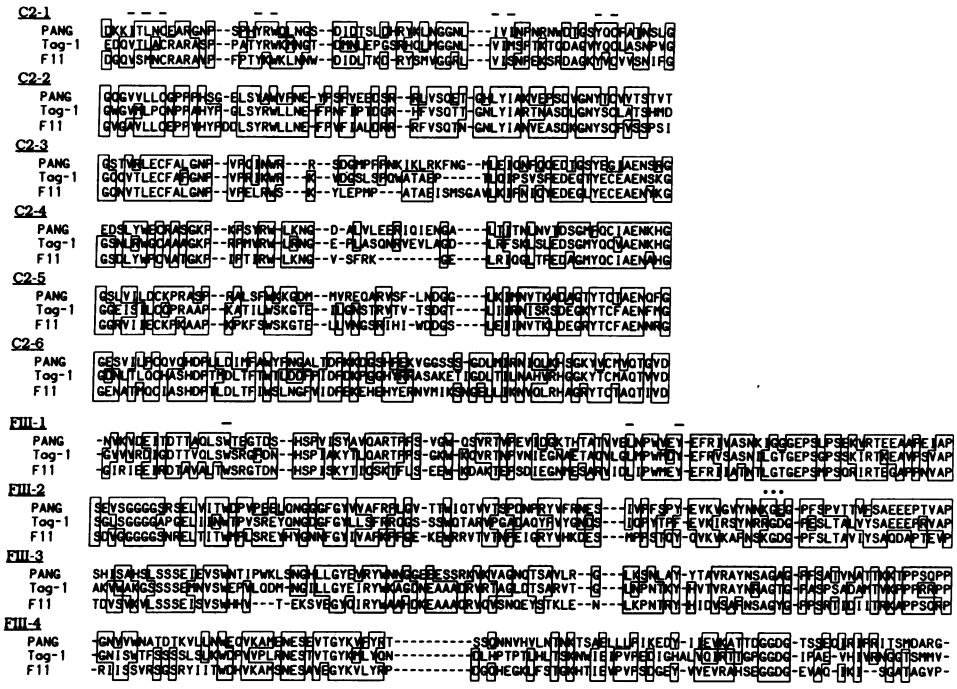

FIG. 3. Alignments of six immunoglobulin C2-like (C2-1–C2-6) and four fibronectin type III-like domains (FIII-1–FIII-4) in PANG, TAG-1, and F11. Shared amino acids are boxed. Prototypical residues are highlighted by dashes and a conserved RGD motif in the TAG-1 FIII-2 domain is identified by an asterisk.

C2-like domains detected a predicted band of 272 bp in seven PANG-positive PCTs and in the Neuro 2A line (C1300 neuroblastoma) (27) but, as expected, a liver sample was negative (Fig. 6*A*). PCTs negative for PANG on Northern blots were also negative by RT-PCR (data not shown). As a positive control, RT-PCR detected c-*myc* transcripts in each of these samples (data not shown). To reveal IAP LTR/PANG fusion transcripts, two LTR 5′ primers were used along with the PANG immunoglobulin C2-like domain 3′ primer (see Fig. 2 for primer sequences). A LTR U5 site 5′ primer yielded identical-sized PCR products in MPC-11, J558, PC7149, TEPC1198, TEPC1165, ABPC45, and Neuro 2A but no visible product in TEPC 1017 (Fig. 6*B*). A second, more 5′, R/U5 site primer yielded the identical-sized band in MPC-11, J558, PC7149, and ABPC45, but TEPC1198, 1017, and 1165 were all negative (Fig. 6*C*). Hybridization to a DNA probe, residing in between but not overlapping the 5′ and 3′ primers, yielded similar results (data not shown). Therefore, different IAP LTRs may be involved in PANG transcription in TEPC 1165 and 1198, while direct evidence is lacking for an IAP LTR in TEPC 1017 despite its robust PANG expression (Fig. 5). The presence of the same-sized LTR/PANG fusion product in each PCT and in the Neuro 2A neuroblastoma line (27) suggests that the 3′ end of the IAP LTR fuses to a PANG exon by RNA splicing. The brain and PCT PANG sequences diverged precisely at the IAP LTR/PANG boundary, supporting this idea (Fig. 2).

DISCUSSION

PANG is a member of the immunoglobulin/fibronectin superfamily of neuronal adhesion molecules whose closest relatives are TAG-1 and F11, which are known to promote axon migration (11, 13). The normal site of PANG expression is the brain, but, surprisingly, we report that atypically sized PANG transcripts exist in murine PCTs. PANG RNAs were

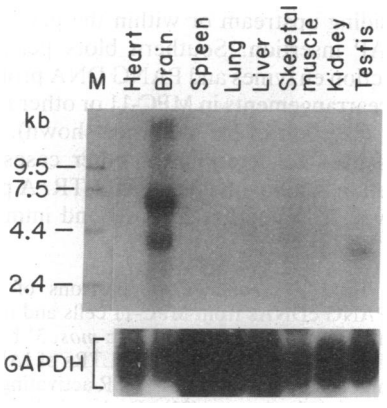

FIG. 4. Northern blot analysis of PANG in normal BALB/c murine tissues. A Clontech mouse multiple tissue Northern blot was hybridized with a ^{32}P-labeled, random-primed, 2.29-kb *Eco*RI/*Stu* I fragment containing PANG coding and 3′ noncoding sequences. It was subsequently stripped and reprobed with a rat glyceraldehyde 3′-phosphate dehydrogenase (GAPDH) (24) probe to provide an internal reference control for RNA quality and quantity.

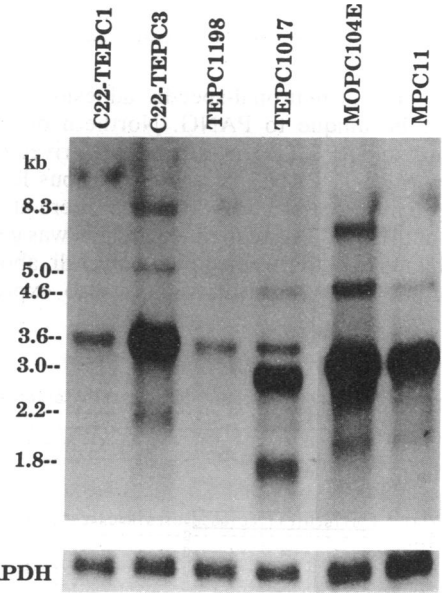

FIG. 5. Northern blot of six representative PCTs expressing PANG transcripts sequentially hybridized with PANG and glyceraldehyde 3′-phosphate dehydrogenase (GAPDH) probes as in Fig. 4.

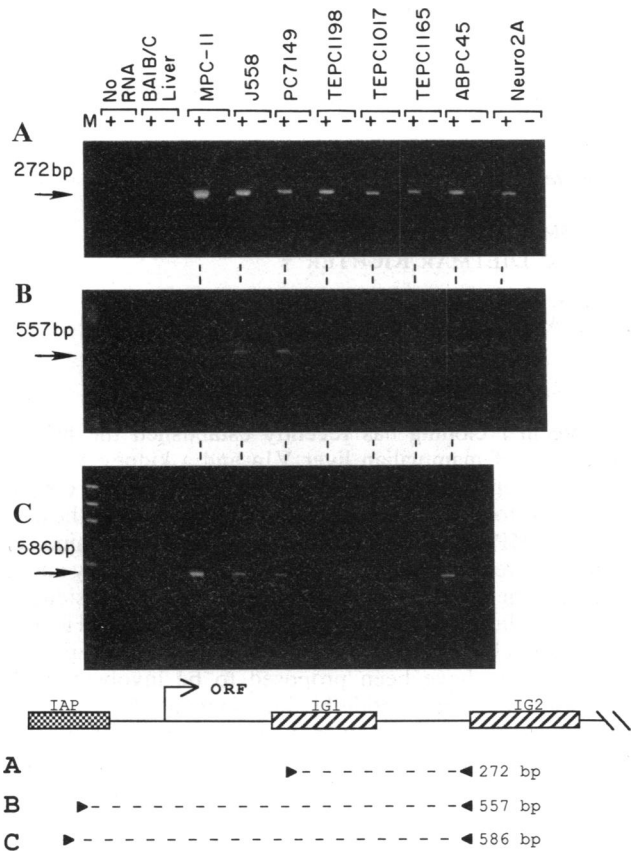

FIG. 6. RT-PCR analysis of PANG transcripts. (*A*) Transcripts revealed with 5′ and 3′ amplimers complimentary to PANG coding sequences. (*B* and *C*) IAP LTR/PANG fusion transcripts revealed with 3′ amplimers of *A* and abutting 5′ amplimers derived from the IAP LTR's U5 and R/U5 regions (see Fig. 2 for sequences of the LTR amplimers). + and −, With and without reverse transcriptase. Neuro 2A was not analyzed in *C*. Dashed vertical lines between the panels mark the same PCT RNA samples. M is a ϕX174 DNA-*Hae* III restriction digest.

not detected in other normal tissues or numerous normal and transformed cell lines. We find that PANG expression in PCTs is a consequence of ectopic gene activation by IAP LTRs. Cloning and sequence analysis of a normal brain PANG cDNA revealed complete identity with an MPC-11 PCT transcript except for the complete replacement of a 5′ IAP LTR by an unrelated noncoding sequence. RT-PCR analysis with IAP LTR and PANG-specific primers revealed ectopic PANG expression to be a common phenomenon in murine PCTs. A subline of an IAP-expressing neuroblastoma (Neuro 2A) also produced an IAP LTR/PANG fusion transcript, implying that the transcriptional activation of a subset of IAPs may lead to ectopic PANG expression independent of cell type. The absence of PANG transcripts in a subset of IAP-positive PCTs suggests that the differential activation of a subset of IAPs, presumably associated with the PANG locus, may be required for ectopic PANG expression.

TAG-1, a PANG-related glycoprotein, is expressed in fetal and adult brain and spinal cord and is transiently expressed on the surface of commissural neurons in the developing mammalian nervous system (11). TAG-1 is anchored to neurons through a GPI linkage but also exists as a peripheral membrane protein that is released from neurons (11). TAG-1 facilitated the extension of neurites *in vitro*, implying that it may play a role in axon growth and guidance *in vivo* (11). TAG-1 was suggested to promote the growth of commissural axons by homophilic interactions among its GPI-linked forms or by heterophilic binding to integrins or other axonal receptors (11). It will be interesting to determine if PANG and TAG-1 possess similar properties and expression patterns.

Since PANG was not detected in up to 40% of PCTs, it does not likely participate in tumor establishment. However, PANG could contribute to progressive, terminal phases of plasmacytomagenesis. PANG might enhance the metastatic phenotype and other adhesion molecules may substitute for it in PANG-negative PCTs. Experiments enforcing PANG expression during *in vivo* PCT development will hopefully illuminate this important issue.

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1. Kuff, E. L. & Lueders, K. K. (1988) *Adv. Cancer Res.* **51**, 183–276.
2. Biczysko, W., Pienkowski, M., Solter, D. & Koprowski, H. (1973) *J. Natl. Cancer Inst.* **51**, 1041–1050.
3. Lueders, K. K. & Kuff, E. L. (1977) *Cell* **12**, 963–972.
4. Shen-Ong, G. L. C. & Cole, M. D. (1982) *J. Virol.* **42**, 411–421.
5. Chang-Yeh, A., Mold, D. E., Brilliant, M. H. & Huang, R. C. C. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 292–296.
6. Cole, M. D., Ono, M. & Huang, R. C. C. (1982) *J. Virol.* **42**, 123–130.
7. Hawley, R. G., Shulman, M. J., Murialdo, H., Gibson, D. M. & Hozumi, N. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7425–7429.
8. Cohen, J. B., Unger, G., Rechavi, E., Canaani, E. & Givol, D. (1983) *Nature (London)* **306**, 797–799.
9. Ferguson, M. A. J. & Williams, A. F. (1988) *Annu. Rev. Biochem.* **57**, 285–320.
10. Jessel, T. M. (1988) *Neuron* **1**, 3–13.
11. Furley, A. J., Morton, S. B., Manalo, D., Karagogeos, D., Dodd, J. & Jessell, T. M. (1990) *Cell* **61**, 157–170.
12. Moos, M., Tacke, R., Scherer, H., Teplow, D., Fruh, K. & Schachner, M. (1988) *Nature (London)* **334**, 701–703.
13. Brümmendorf, T., Wolff, J. M., Frank, R. & Rathjen, F. G. (1989) *Neuron* **2**, 1351–1361.
14. Gennarini, G., Cibelli, G., Rougon, G., Mattei, M.-G. & Goridis, C. (1989) *J. Cell Biol.* **109**, 775–788.
15. Cunningham, B. A., Hemperly, J. J., Murray, B., Prediger, E. A., Brackenbury, R. & Edelman, G. M. (1987) *Science* **236**, 799–806.
16. Bieber, A. J., Snow, P. M., Hortsch, M., Patel, N. H., Jacobs, J. R., Traquina, Z. R., Schilling, J. & Goodman, C. S. (1989) *Cell* **59**, 447–460.
17. Arquint, M., Roder, J., Chia, L., Down, J., Wilkinson, D., Bayley, H., Braun, P. & Dunn, R. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 600–604.
18. Julius, M. A., Street, A. J., Fahrlander, P. D., Yang, J. Q., Eisenman, R. N. & Marcu, K. B. (1988) *Oncogene* **2**, 469–476.
19. Viera, J. & Messing, J. (1987) *Methods Enzymol.* **153**, 3–11.
20. Ausubel, F. A., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1992) *Current Protocols in Molecular Biology* (Greene/Wiley-Interscience, New York).
21. Connelly, M. A., Grady, R. C., Mushinski, J. F. & Marcu, K. B. (1992) *Curr. Top. Microbiol. Immunol.* **182**, 229–236.
22. Heidmann, O. & Heidmann, T. (1991) *Cell* **64**, 159–170.
23. Ymer, S., Tucker, W. Q. J., Campbell, H. D. & Young, I. G. (1986) *Nucleic Acids Res.* **14**, 5901–5918.
24. Fort, P., Marty, L., Piechaczyk, M., Sabrouty, S. E., Dani, C., Jeanteur, P. & Blanchard, J. M. (1985) *Nucleic Acids Res.* **13**, 32–38.
25. Williams, A. F. & Barclay, A. N. (1988) *Annu. Rev. Immunol.* **6**, 381–405.
26. Kornblihtt, A. R., Umezawa, K., Vibe-Pedersen, K. & Baralle, F. E. (1985) *EMBO J.* **4**, 1755–1759.
27. Klebe, R. J. & Ruddle, F. H. (1969) *J. Cell Biol.* **43**, 69A (abstr.).
28. Greenberg, R., Hawley, R. & Marcu, K. B. (1985) *Mol. Cell. Biol.* **5**, 3725–3728.
29. Weiss, R., Teich, N., Varmus, H. & Coffin, J. (1985) *RNA Tumor Viruses: Molecular Biology of RNA Tumor Viruses* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed., pp. 387–394.