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The *PVT* Gene Frequently Amplifies with *MYC* in Tumor Cells

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The line of human colon carcinoma cells known as COLO320-DM contains an amplified and abnormal allele of the proto-oncogene *MYC* (DM*MYC*). Exon 1 and most of intron 1 of *MYC* have been displaced from DM*MYC* by a rearrangement of DNA. The RNA transcribed from DM*MYC* is a chimera that begins with an ectopic sequence of 176 nucleotides and then continues with exons 2 and 3 of *MYC*. The template for the ectopic sequence represents exon 1 of a gene known as *PVT*, which lies 50 kilobase pairs downstream of *MYC*. We encountered three abnormal configurations of *MYC* and *PVT* in the cell lines analyzed here: (i) amplification of the genes, accompanied by insertion of exon 1 and an undetermined additional portion of *PVT* within intron 1 of *MYC* to create DM*MYC*; (ii) selective deletion of exon 1 of *PVT* from amplified DNA that contains downstream portions of *PVT* and an intact allele of *MYC*; and (iii) coamplification of *MYC* and exon 1 of *PVT*, but not of downstream portions of *PVT*. We conclude that part or all of *PVT* is frequently amplified with *MYC* and that intron 1 of *PVT* represents a preferred boundary for amplification affecting *MYC*.

Amplification of the proto-oncogene *MYC* occurs in a variety of human tumors (1). The structure of amplified *MYC* may not be otherwise disturbed. On occasion, however, amplification and structural rearrangement affect the same allele of *MYC*. A provocative example has been found in the line of human colon carcinoma cells known as COLO320 (2, 14). Two strains of these cells have been isolated and analyzed (2, 12, 14): COLO320-HSR, in which an apparently normal allele of *MYC* has been amplified within a homogeneously staining region (HSR); and COLO320-DM, in which a rearranged allele of *MYC* (DM*MYC*) has been amplified and is carried on double-minute chromosomes (DMs).

Previous work established that exon 1 and most of intron 1 of *MYC* are absent from DM*MYC* and that the locus gives rise to an anomalous mRNA (14). However, neither the composition of the DM*MYC* nor the nature of the rearrangement that engendered the allele was known. We now report that DM*MYC* is a chimeric gene formed by the insertion of ectopic DNA within intron 1 of *MYC*. The ectopic DNA includes exon 1 of a gene that we have provisionally designated as *PVT* because it includes a previously described domain of the human genome known by that name (E. Shtivelman, B. Henglein, P. Groitl, M. Lipp, and J. M. Bishop, Proc. Natl. Acad. Sci., in press). Our findings indicate that *PVT* and *MYC* may be linked in the human genome and that intron 1 of *PVT* may represent a frequent boundary for amplification affecting *MYC*.

MATERIALS AND METHODS

Construction of a cDNA library. First-strand cDNA was synthesized from 4 μg of oligo(dT)-selected RNA from COLO320-DM by using avian myeloblastosis virus reverse transcriptase (Life Sciences, Inc.) and oligo(dT) as a primer, as described earlier (9). After degradation of RNA with alkali, the second strand was synthesized by using *Escherichia coli* DNA polymerase I (New England BioLabs, Inc.). Ends of double-stranded cDNA were rendered blunt through treatment with T4 DNA polymerase (New England BioLabs). After methylation of *Eco*RI sites with *Eco*RI methylase and addition of *Eco*RI phosphorylated linkers (Bethesda Research Laboratories, Inc.), cDNA was ligated to *Eco*RI arms of bacteriophage λgt10 and transduced into *E. coli* C600 hfl (Stratagene Inc.).

Blot hybridization. Cellular DNA was digested with restriction endonucleases, separated on agarose gels, transferred to nitrocellulose or GeneScreen membranes (Du Pont), and hybridized by published procedures (17). Polyadenylated RNA was prepared from total cellular RNA by passage through oligo(dT)-cellulose (3), electrophoresed through formaldehyde-agarose gels, and blotted onto GeneScreen Plus membranes. Hybridization probes were prepared by using an oligonucleotide-primed labeling kit (Boehringer Mannheim Biochemicals).

Primer extension analysis. A single-stranded, end-labeled DNA probe was prepared by using T4 polynucleotide kinase (P-L–Pharmacia) and [γ-^{32}P]-ATP. Purified single-stranded probe was annealed in excess with 2 to 5 μg of polyadenylated RNA at 50°C overnight in 30 μl of hybridization buffer containing 80% formamide, 40 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES), 400 mM NaCl, and 1 mM EDTA. After ethanol precipitation, the annealed products were extended with avian myeloblastosis virus reverse transcriptase in the presence of all four deoxynucleotides. Extended products were analyzed on denaturing polyacrylamide gels.

Sequencing. Dideoxy sequencing was performed with single-stranded M13 DNA as described previously (13).

RESULTS

A chimeric RNA transcribed from DM*MYC*. Previous work has shown that the *MYC* component of the amplified DM*MYC* allele is truncated just upstream of exon 2 and joined to an ectopic nucleotide sequence of unknown origin (14). The principal polyadenylated RNA transcribed from DM*MYC* is shorter than the *MYC* RNA found in COLO320-HSR cells (2.2 versus 2.4 kilobases kb [Fig. 1A] [14]), but still too long to arise solely from exons 2 and 3 of *MYC*. It seems likely, therefore, that transcription from DM*MYC* creates a chimeric RNA, containing a 5′ domain initiated within and copied from the ectopic component of the allele.

To explore the location of the template for the ectopic

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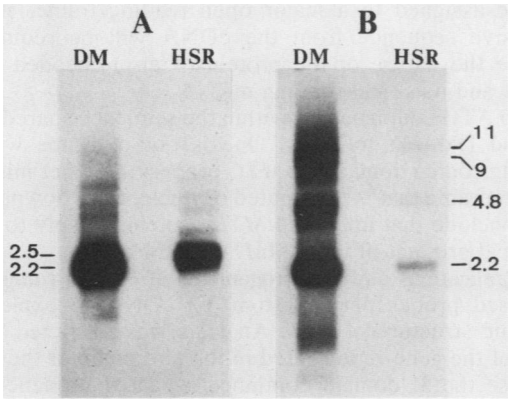

FIG. 1. Polyadenylated RNAs transcribed from *MYC* and *PVT* in COLO320-DM and COLO320-HSR cell lines. Polyadenylated RNAs were prepared from the cell lines COLO320-DM (lanes DM) and COLO320-HSR (lanes HSR), fractionated by electrophoresis (5 μg per lane), and hybridized with a probe for either exons 2 and 3 of *MYC* (A) or exon 1 of *PVT* (B). The positions of marker RNAs are given in kilobases.

component of DM*MYC* RNA, we exploited the presence of an *Apa*LI restriction site at the junction between the *MYC* and ectopic domains of DM*MYC* (14). We used *Apa*LI to cleave a genomic DNA clone that encompasses the junction and at least 7 kilobase pairs (kbp) on either side, fractionated the resulting fragments by electrophoresis, and hybridized these with a cDNA probe transcribed from the total polyadenylated RNA of COLO320-DM. *Apa*LI fragments containing *MYC* exons produced strong signals, whereas fragments representing a total of 7 kbp lying upstream of the *Apa*LI site did not hybridize (data not shown). We concluded that transcription from DM*MYC* must initiate more than 7 kbp upstream of the junction with *MYC* in the chimeric allele and that splicing joins the RNA transcribed from the upstream DNA to the transcript from exon 2 of *MYC* to create the chimeric RNA.

We then sought to obtain the 5′ domain of the DM*MYC* RNA by cloning the corresponding cDNA. A COLO320-DM cDNA library of 1.4×10^6 clones was constructed in λgt10, and 3×10^5 clones were screened in parallel with probes corresponding to exons 1 and 2 of *MYC*. Clones hybridizing only to the probe for exon 2 were further purified by plaque hybridization. Three apparently full-length clones with inserts of 2.1 kbp were found to have identical restriction maps. One of these clones was chosen for further study and designated DMmyc (Fig. 2A). The restriction map of this clone was also identical to that of a cDNA representing normal *MYC*, with the exception that exon 1 of *MYC* has been replaced by an ectopic nucleotide sequence of ca. 180 nucleotides (Fig. 2A). The ectopic component of the DM*MYC* cDNA was distinguished by the presence of *Sma*I and *Bgl*II restriction sites that were not found in the normal *MYC* cDNA.

We prepared a plasmid clone of the 160-nucleotide *Eco*RI-*Bgl*II fragment from the 5′ end of the DMmyc cDNA and used it as probe to analyze RNAs from COLO320 cell lines (Fig. 1B). The probe detected a 2.2-kb transcript that was abundant in COLO320-DM and also present (but much less abundant) in COLO320-HSR. The size of the RNA is identical to that previously assigned to the principal transcript from DM*MYC* (see above) (Fig. 1A). We concluded that the cDNA corresponds to the 2.2-kb chimeric DM*MYC* RNA.

An ectopic exon at the 5′ end of DM*MYC* RNA. The

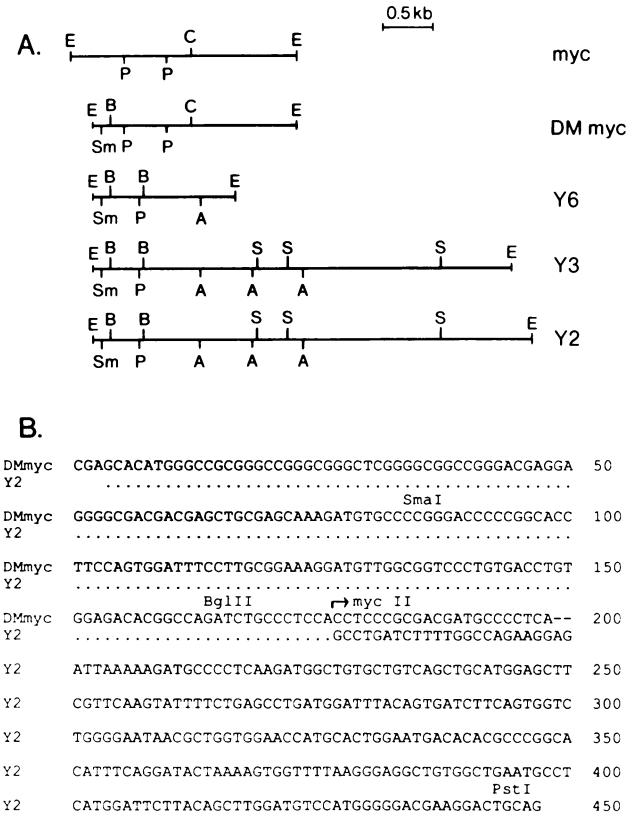

FIG. 2. Topography of cDNAs for *PVT* and DM*MYC*. Preparation and sequencing of the cDNA clones are described in the text. (A) Restriction maps of cDNA clones for normal *MYC* (myc), DM*MYC* (DM myc), and normal *PVT* (Y6, Y3, and Y2). Restriction sites shown are *Bgl*II (B), *Sma*I (Sm), *Pst*I (P), *Acc*I (A), and *Sac*I (S). (B) Partial nucleotide sequences of clones DMmyc and Y2, beginning at the 5′ termini. Dots indicate identical nucleotides. The beginning of exon 2 of *MYC* is marked by an arrow.

*Eco*RI-*Bgl*II probe also detected RNAs that had not reacted with a probe for *MYC*. The principal forms of these RNAs had lengths of ca. 4.8, 9, and 11 kb and were abundant in COLO320-DM, but were barely detectable at this exposure in COLO320-HSR (Fig. 1B). We deduced that these RNAs may be transcripts from the normal counterpart of the ectopic sequence in DM*MYC* and proceeded to isolate representative cDNAs.

We screened replicate filters of a COLO320-DM cDNA library for clones that contained the ectopic sequence at the 5′ end of DM*MYC* RNA, but not exon 2 of *MYC*. Three clones of this sort were isolated for further analysis and designated Y2, Y3, and Y6 (Fig. 2A). The cDNA insert in Y2 had a length of 4.3 kb and, thus, may be a full-length representation of one of the prominent RNA species. The clones have common 5′ domains and extend various distances in the 3′ direction, but appear identical in the regions where they overlap.

The commonality among the 5′ ends of the three cDNA clones suggested that they may include the 5′ termini of the RNAs from which they were transcribed. We tested this surmise by the use of primer extension. A single-stranded *Sma*I-*Bgl*II fragment from Y2 cDNA (Fig. 2A), labeled at the 5′ terminus of the *Bgl*II site, was annealed with polyadenylated RNAs from COLO320-DM cells and a small-cell lung carcinoma line, H82. The annealed products were extended

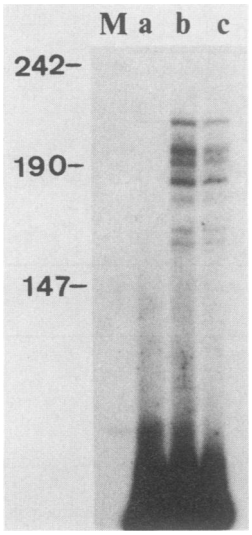

FIG. 3. Detection of 5′ termini for *PVT* RNAs. The single-stranded *Sma*I-*Bgl*I fragment of 78 nucleotides from the 5′ end of cDNA clone Y2 was labeled at the 5′ end of the *Bgl*II site, annealed with 20 μg of tRNA (lane a), 1 μg of polyadenylated RNA from COLO320-DM (lane b), or 5 μg of polyadenylated RNA from small-cell lung carcinoma line H82 (lane c), and then extended with avian myeloblastosis virus reverse transcriptase. Lengths of marker fragments are given in nucleotides.

with avian myeloblastosis virus reverse transcriptase and analyzed on a denaturing gel (Fig. 3, lanes b and c). At least seven major bands could be detected, ranging in size from 150 to 210 nucleotides. Since the 5′ ends of cDNAs Y2, Y3, and Y6 are ca. 180 nucleotides from the *Bgl*II site (Fig. 2A) and, consequently, within the domain covered by the extended products, we conclude that the cDNAs include authentic 5′ termini of RNAs. We reached the same conclusion by performing analyses with a riboprobe transcribed from a fragment of genomic DNA corresponding to the *Eco*RI-*Bgl*II fragment of cDNA (Shtivelman et al., in press) and by sequencing the same fragment of genomic DNA (Shtivelman et al., in press).

We conclude that the 5′ domains shared between the DM-myc and Y2 cDNAs represent exon 1 of a gene that contributes the ectopic sequence to DM*MYC*. In work to be reported elsewhere (Shtivelman et al., in press), we have designated this gene as *PVT* because it encompasses the human counterpart of a mouse locus known as *pvt-1* (7).

Nucleotide sequence across the fusion in the mRNA of DM*MYC*. The nucleotide sequences from the 5′ ends of the Y2 and DM*MYC* cDNAs are shown in Fig. 2B. The sequences are identical through the first 176 nucleotides, then diverge at a point that marks the beginning of exon 2 of *MYC* in the *MYC* cDNA. The findings sustain our view that the mRNA for DM*MYC* is formed by splicing from a site in the ectopic domain of DM*MYC* (i.e., from exon 1 of *PVT*) to the normal splice acceptor site for exon 2 of *MYC*.

The nucleotide sequence is rich in guanosine and cytosine immediately upstream of the gene proper (Shtivelman et al., in press) and through the first 60 nucleotides of the cDNAs (Fig. 2B). This finding, in combination with the heterogeneity at the 5′ end of the RNAs transcribed from DM*MYC* (see above), raises the possibility that the promoter for transcription belongs to the variety generally associated with housekeeping genes. There are a total of 13 ATG codons within the first 450 nucleotides of Y2 cDNA; at present, none of these can be assigned to a major open reading frame. A more extensive sequence from the cDNA will be required to deduce the nature of the protein (if any) encoded by the cDNA and its corresponding mRNA.

Two ATG codons occur within the sequence shared by the Y2 and DM*MYC* cDNAs. One is out of frame with the ensuing coding domain for *MYC* proper; the other initiates a reading frame that is terminated 63 nucleotides downstream. We conclude that unaltered *MYC* protein is likely to be the principal product of the DM*MYC* mRNA.

Amplifications and rearrangements of *PVT* in tumor cells. We used probes derived from Y2 cDNA to explore the genomic structure of *PVT*. Analysis was restricted to portions of the gene represented in the 5′ domain of the cDNA because the 3′ domain contained repeated sequences that obscured the specificity of hybridization. Figure 4 shows hybridization of different probes to Southern blots of DNA from COLO320-HSR, COLO320-DM, and a neuroblastoma cell line, LAN-1 (in which *MYC* is not amplified).

When the *Eco*RI-*Bgl*II fragment of 180 bp from the 5′ end of Y2 cDNA was used as a probe, it detected a single, 7.0-kbp *Eco*RI fragment in LAN-1 DNA, a highly amplified fragment of the same size in COLO320-DM and a much less amplified fragment in COLO320-HSR (Fig. 4A, lanes c, b, and a, respectively). We then extended the analysis to include at least exon 2 (and perhaps more) of *PVT*, by using the 0.45-kbp *Eco*RI-*Pst*I fragment from Y2 as probe. Hybridization now detected both the 7.0-kbp *Eco*RI fragment and an additional 7.7-kbp fragment (Fig. 4B, lanes d to f). The relative intensities of hybridization to the 7.0-kbp fragment were as before. In particular, the 7.0-kbp fragment was far more amplified in COLO320-DM cells than in COLO320-HSR cells. However, the 7.7-kbp fragment was amplified to a nearly equal extent in the two cell lines. Analysis of *Hin*dIII fragments revealed a similar configuration (Fig. 4B, lanes a to c), i.e., equivalent amplification of an 11.5-kbp fragment in the two cell lines, but disproportionately low amplification of a 6.4-kbp fragment in COLO320-HSR cells. (An apparently anomalous band in Fig. 4B, lane c, presumably represents a rearrangement affecting a portion of the amplified *PVT* in COLO320-DM cells.) We conclude that most of the amplified DNA in COLO320-HSR cells lacks exon 1 of *PVT*, but contains exon 2 and perhaps additional exons of the gene. This conclusion provides an explanation for the relative abundance of RNAs transcribed from exon 1 of *PVT* in COLO320-HSR and COLO320-DM (see above) (Fig. 1B).

When the *Pst*I-*Sac*I fragment of 1.0 kbp from the 5′ end of Y2 cDNA was used as a probe, it detected multiple fragments in restriction enzyme digests of genomic DNA (Fig. 4C). Digests of DNA from cell lines in which *PVT* and *MYC* are not amplified contained four fragments that hybridized with the probe (Fig. 4C, lanes g to i) (data not shown). We presume that these fragments represent a minimum of four different exons from *PVT*. The corresponding fragments were amplified in both strains of COLO320 cells (Fig. 4C, lanes a to f). Additional fragments were detected only in the DNAs of COLO320 cells; some of these were amplified, while others were not. We attributed the additional fragments to rearrangements within *PVT*, but we have no further information on the configuration of the rearrangements.

Transcription from *PVT* in human tumor cells. Using a 1-kb *Eco*RI-*Acc*I restriction fragment from Y2 cDNA as the probe (Fig. 2A), we detected an array of scarce polyadenylated RNAs that was similar in a variety of human cell lines (Fig. 5). The RNAs ranged in size from 1 to 9 kb (with a

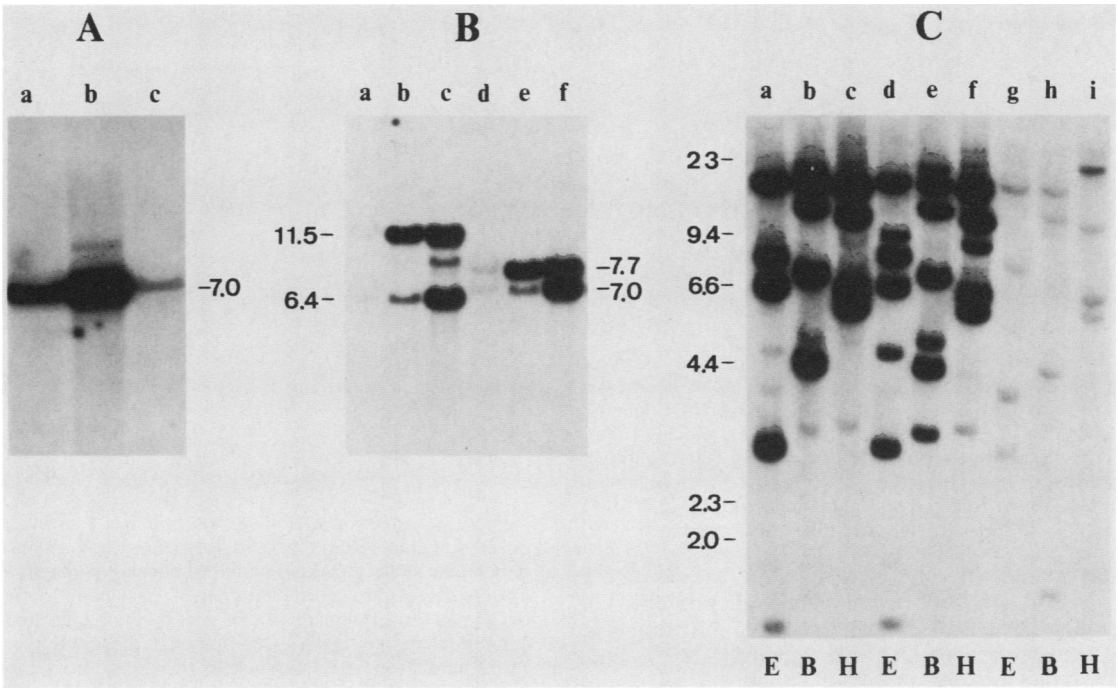

FIG. 4. Amplification and rearrangements of *PVT* in human cell lines. (A) Detection of exon 1 of *PVT*. DNAs (15 μg per lane) from the COLO320-HSR (lane a), COLO320-DM (lane b), and LAN-1 (lane c) cell lines were cleaved with *Eco*RI, fractionated by electrophoresis, and hybridized with a probe representing the *Eco*RI-*Bgl*II fragment from the 5′ end of Y2 cDNA. The sizes of hybridizing fragments are given in kilobase pairs, deduced from the mobility of markers. (B) Detection of exons 1 and 2. DNAs from cell lines LAN-5 (lanes a and d), COLO320-HSR (lanes b and e), and COLO320-DM (lanes c and f) were cleaved with either *Hin*dIII (lanes a to c) or *Eco*RI (lanes d to f). The probe was the *Eco*RI-*Pst*I fragment from the 5′ end of Y2 cDNA. Lanes a and d contained 15 μg of DNA; the remaining lanes contained 5 μg. The sizes of hybridizing fragments are given in kilobase pairs, deduced from the mobility of markers. (C) Detection of multiple exons. DNAs (15 μg per lane) from COLO320-HSR (lanes a to c), COLO320-DM (lanes d to f), and LAN-1 (lanes d to h) were cleaved with *Eco*RI (E), *Bam*HI (B), or *Hin*dIII (H). Hybridization was carried out with the 1.0-kbp *Pst*I-*Sac*I fragment of Y2 cDNA that detects multiple exons of *PVT*. The positions of marker fragments are given in kilobase pairs.

4.8-kb RNA being the most prominent) and were ca. 100-fold less abundant than their apparent counterparts in COLO320-DM cells (see above) (Fig. 1B). Although probes derived from the 3′ region of Y2 reacted only with the RNAs that were 2.5 kb and longer (data not shown), we have not deduced the details of how the various RNAs differ in their topographies.

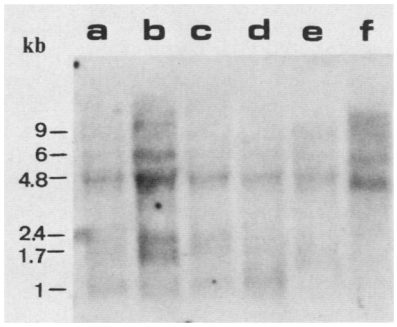

FIG. 5. RNA transcribed from *PVT* in human cell lines. Polyadenylated RNAs were fractionated by electrophoresis (5 μg per lane) and hybridized with a probe representing the 450-bp *Eco*RI-*Pst*I fragment from the 5′ end of Y2 cDNA. RNAs from the following cell lines were used: Daudi (Burkitt lymphoma) (lane a); Mg63 (osteosarcoma) (lane b); A204 (myosarcoma) (lane c); H160 (promyelocytic leukemia) (lane d); and LAN-5 and SK-N-SM (neuroblastomas) (lanes e and f, respectively). Lengths of RNAs are given in kilobases, as estimated from the migration of rRNAs.

Amplification of exon 1 of *PVT* in three additional cell lines carrying amplified *MYC*. We explored the frequency with which *PVT* and *MYC* might be coamplified by examining the DNA of four additional human cell lines in which *MYC* is amplified to various extents: HL60 (promyelocytic leukemia) (4, 6); H82 and N417 (small-cell carcinoma of the lungs) (11); and SK-N-MC (neuroepithelioma) (our unpublished data). For comparison, the analyses also included the neuroblastoma cell line LAN-1, in which *MYC* is not amplified. DNAs were cleaved with *Eco*RI, fractionated by electrophoresis, and hybridized to a probe prepared with the 0.45-kb *Eco*RI-*Pst*I fragment from the 5′ end of Y2 cDNA (Fig. 2A). This procedure permits exons 1 and 2 of *PVT* to be resolved and detected, as fragments of 7.0 and 7.7 kbp, respectively (Fig. 5A and B). Neither fragment was amplified in LAN-1 or HL60 cells (Fig. 6, lane a) (data not shown). As anticipated, both fragments were amplified in COLO320 lines (Fig. 6, lanes b and c), in the relative proportions described above. By contrast, only the fragment bearing exon 1 of *PVT* was amplified in H82, N417, and SK-N-MC cells (Fig. 6, lanes d to f). We conclude that *MYC* and exon 1 of *PVT* may be amplified in concert frequently. The domain of amplification can either terminate between exons 1 and 2 of *PVT*, as in H82, N417, and SK-N-MC, or extend farther into (or even beyond) *PVT*, as in the COLO320 lines. In concert, these findings raise the possibility that *MYC* and *PVT* are linked in the human genome—a possibility that we have substantiated elsewhere (Shtivelman et al., in press).

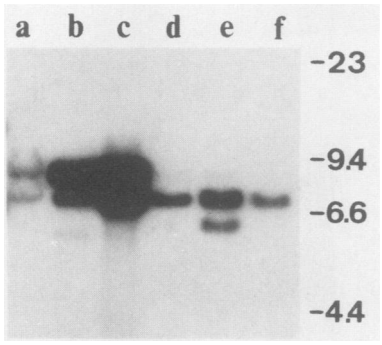

FIG. 6. Amplification of *PVT* sequences in cell lines carrying amplified *MYC*. *Eco*RI was used to cleave high-molecular-weight DNAs from the cell lines LAN-1 (lane a), COLO320-HSR (lane b), COLO320-DM (lane c), H82 (lane d), N417 (lane e), and SK-N-MC (lane f). The resulting fragments were separated by electrophoresis and hybridized with a probe for the 450-bp *Eco*RI-*Pst*I fragment at the 5′ end of Y2 cDNA. Analysis was performed with 15 μg of DNA from line LAN-1 and 5 μg of DNA from the other cell lines.

The data in Fig. 6 display two anomalies that do not bear on our major conclusions. First, the 7.7-kbp *Eco*RI fragment has been rearranged to a much larger size in SK-N-MC (Fig. 6, lane f). It remains evident, however, that this domain of *PVT* has not been amplified. Second, a novel and apparently amplified fragment of 5.5 kbp (containing sequences of exon 1 of *PVT* [data not shown]) appears in the digest of N417 DNA (Fig. 6, lane e). We presume that this fragment arose from a rearrangement within a portion of the amplified units in the cell line.

Abundant transcription from the 5′ domain of *PVT* in H82, N417, and SK-N-MC cell lines. A probe for exon 1 of *PVT* detected distinct sets of abundant polyadenylated RNAs in the H82, N417, and SK-N-MC cell lines (Fig. 7). None of these RNAs reacted with a probe for the 1.1-kb *Pst*I-*Sac*I fragment from Y2 cDNA, which represents sequences downstream of exon 1 in *PVT*; in contrast, the same probe detected abundant 4.8-, 9-, and 11-kb RNAs in COLO320-DM cells and smaller quantities of similar RNAs in COLO320-HSR cells (data not shown). We conclude that transcription initiates from the amplified exon 1 of *PVT* in the H82, N417, and SK-N-MC cell lines and then copies sequences not

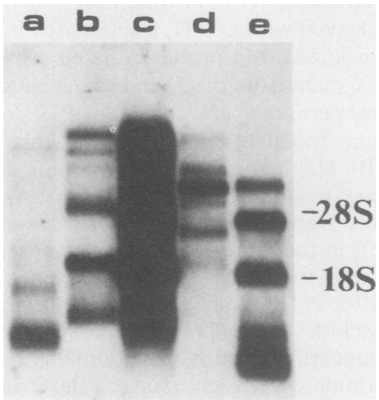

FIG. 7. Transcription from amplified alleles of *PVT*. Polyadenylated RNA (2 μg) from cell lines SK-N-MC (lane a), COLO320-HSR (lane b), COLO320-DM (lane c), H82 (lane d), and N417 (lane e) were fractionated by electrophoresis and hybridized with a probe for the 450-bp *Eco*RI-*Pst*I fragment at the 5′ end of Y2 cDNA.

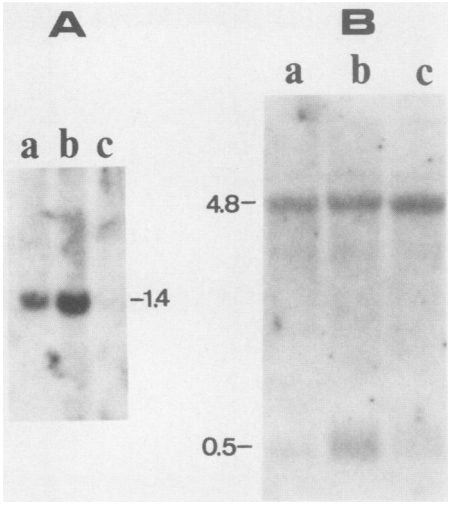

FIG. 8. Detection of *PVT* DNA and RNA in mouse cells. (A) *Eco*RI fragments from the DNAs of osteosarcoma cell lines 4031Tc3 (lane a) and Rec4Tc2 (lane b) and of normal mouse fibroblasts (lane c) were detected by hybridization in 35% formamide with a probe for the *Eco*RI-*Pst*I fragment at the 5′ end of Y2 cDNA. Each sample contained 20 μg of DNA. (B) Fractionated polyadenylated RNAs (5 μg per lane) from normal mouse liver (lane a) and the Rec4Tc2 (lane b) and 4031Tc3 (lane c) cell lines were hybridized in 35% formamide to the same probe as used in panel A.

normally found in *PVT* mRNA—perhaps portions of intron 1 of the gene. In contrast, a portion of the RNAs initiated from the amplified exon 1 of *PVT* in COLO320 cells continue into the subsequent exons of the gene (see Discussion).

Conservation, amplification and expression of *PVT* in the mouse genome. We used a probe prepared with the *Eco*RI-*Bgl*II fragment from the 5′ end of the Y2 cDNA in efforts to detect a mouse counterpart of *PVT* (Fig. 8A). When hybridized under conditions of intermediate stringency, the probe detected a single *Eco*RI fragment in the DNAs of normal mouse fibroblasts and two sublines of the murine osteosarcoma tumor SEWA (known to contain amplifications of *myc*). The fragment was amplified in SEWA lines 4031Tc3 and Rec4Tc2, derived from the same original tumor but differing in that they contain either double-minute chromosomes (4031Tc3) or C-bandless chromosomes (Rec4Tc2), the sites of amplified *myc* in the cells (15, 16). Both *myc* (15) and *PVT* (Fig. 8A) are amplified to a greater extent in Rec4Tc2 than in 4031Tc3. Probes for portions of *PVT* downstream of exon 1 failed to hybridize to normal mouse DNA or detect amplified fragments in the SEWA lines (data not shown). It is therefore possible that additional amplified fragments in mouse DNA escaped detection because of divergence between mouse and human *PVT*. We conclude that exon 1 of *PVT* is conserved in the mouse genome and is coamplified with *myc* in the SEWA cell lines. It appears likely that mice possess a genetic locus (*pvt*) akin to *PVT*.

By exploiting the conservation of sequences in exon 1 of *pvt*, we were able to detect RNAs that may be transcribed from the gene in mouse cells. A probe prepared with the 0.45-kb *Eco*RI-*Pst*I fragment from the 5′ end of Y2 cDNA detected 0.5- and 4.8-kb polyadenylated RNAs in normal mouse liver and the SEWA cell lines (Fig. 8B). In addition, the SEWA lines contained *pvt* RNA in the range of 0.6 to 0.7 kb. None of the *pvt* RNAs appeared abundant in the SEWA lines, despite appreciable amplification of the gene. We cannot account for this paradox at present.

DISCUSSION

Fusion of a previously unidentified human gene to *MYC* in COLO320-DM cells. The double minute chromosomes of COLO320-DM cells carry a chimeric gene (DM*MYC*) in which exon 1 and much of intron 1 of *MYC* have been displaced by ectopic DNA (14). In this paper we report that the RNA transcribed from DM*MYC* is also chimeric, containing exon 1 of a previously unidentified human gene fused to exon 2 of *MYC*. In work to be reported elsewhere (Shtivelman et al., in press), we have learned that the entirety of this gene normally lies downstream of *MYC* in the human genome, that it embodies a transcriptional unit covering more than 200 kbp, and that it includes the human counterpart of a mouse locus known as *pvt-1* (7). Thus, we have designated the human gene as *PVT*.

A domain within *PVT* is apparently a recurrent site for chromosomal translocations in mouse plasmacytomas (5, 20) and for insertions of retroviral DNA in lymphomas of mice (8) and rats (10, 19), in which settings the domain has been known variously as *pvt-1*, *Mlvi-1* or *mis-1*. The work described here and elsewhere (Shtivelman et al., in press) represents the first report of transcripts derived from this domain and, thus, the first demonstration that the domain is included within a gene.

We presume that the normal promoter for *PVT* initiates transcription from DM*MYC*. Splicing then joins exon 1 of *PVT* to exon 2 of *MYC*, utilizing normal donor and acceptor sites to eliminate a large but as yet unmeasured intron, and transcription continues to the normal site of termination for *MYC*. It seems most likely that the ectopic intervening sequences in DM*MYC* derive entirely from intron 1 of *PVT*, but it remains possible that the intervening sequences are a composite of several elements, assembled by a complex rearrangement of DNA.

Although the structure of DM*MYC* is anomalous, the gene may have retained the function of wild-type *MYC*. The coding domain for *MYC* protein apparently remains intact in the chimeric allele, and the *PVT* exon in the allele has no substantial coding potential of its own. The 5′ untranslated regions of DM*MYC* and *MYC* mRNAs are entirely different, however, and so the regulation of translation from the two forms of mRNA may differ. We have no evidence that bears on this possibility.

Amplification and rearrangement of *PVT*. The work reported here describes three distinctive configurations of *PVT* in amplified DNA (Fig. 9). (i) In COLO320-DM cells, *PVT* has been rearranged, so that exon 1 and an undetermined additional quality of DNA have been inserted within intron 1 of *MYC* to create the chimeric allele DM*MYC*. Exon 1 of *MYC* survived the rearrangement and has been amplified in concert with DM*MYC*. Thus, we presume that exon 1 and most of intron 1 of *MYC* lie upstream of DM*MYC*, where they may represent a futile transcriptional element; we have no evidence that initiation of transcription from the relocated portion of *MYC* gives rise to stable RNA.

(ii) In COLO320-HSR cells, *MYC* is intact and amplified. Exon 1 of *PVT* is not present in the principal amplicon of the cells, whereas downstream portions of *PVT* are. It appears that exon 1 was deleted from the HSR allele of *PVT* prior to the onset of amplification. (We attribute the modest amplification of exon 1 of *PVT* in COLO320-HSR cells to the presence of DM*MYC*, perhaps as a small population of DMs.)

FIG. 9. Configurations of amplified *MYC* and *PVT* in human tumor cells. The diagram portrays the arrangement and portions of *MYC* and *PVT* that are amplified in different human tumor cell lines. The drawing is not to scale and is otherwise stylized. The numbered open boxes represent the three exons of *MYC*; the solid boxes with Roman numerals represent exons of *PVT* whose number and exact arrangement remain unknown. The bold line that brackets exon 1 of *PVT* approximates an element that has been rearranged in COLO320-DM cells and deleted in COLO320-HSR cells.

(iii) In H82, N417, and SK-N-MC cells, *MYC* and exon 1 of *PVT* are amplified; in contrast, downstream elements of *PVT* are not amplified.

How did these three configurations of *MYC* and *PVT* arise? (i) We presume that the rearrangements found in COLO320 cells occurred in advance of amplification, since they seem too homogeneous to have arisen in any other manner. Since the two strains of these cells are clonal variants originating from the same tumor, we further presume that all of the amplicons have a common origin. It is then necessary to suppose that a single series of rearrangements occurring before or at the outset of amplification had two outcomes: relocation of exon 1 of *PVT* in COLO320-DM and deletion of exon 1 of *PVT* in COLO320-HSR. Whatever the mechanisms of the rearrangements might be, the distinctive and homogeneous configurations of *MYC* and *PVT* in the two cell lines suggest that the DMs and HSRs arose independently rather than by one serving as the precursor to the other.

(ii) The abnormal configurations of *PVT* in the H82, N417, and SK-N-MC cell lines presumably reflect the presence of a boundary for amplification between exons 1 and 2 of *PVT* in all three instances. We do not presently know how similar the boundaries are in the three cell lines.

Irrespective of how the configurations of *MYC* and *PVT* described here may have arisen, our findings should prove useful in future studies of gene amplification. The domains of DNA amplified in mammalian cells are typically very large, and their topographies and boundaries are poorly defined (18). The isolation of *PVT* and its chromosomal environs provides a set of molecular markers with which to explore the structure of the many amplicons that contain *MYC* (1).

Transcription from the normal and rearranged versions of *PVT*. Transcription from ostensibly normal alleles of human *PVT* gives rise to a heterogeneous array of polyadenylated RNAs with sizes ranging from 1 to 9 kb. Although these RNAs are scarce in most contexts, we believe that they are authentic transcripts of *PVT*: they react with probes from more than one domain of the gene, and we have in hand cDNAs whose lengths represent several of the distinct RNAs (e.g., 2.4 and 4.5 kb) and whose composition shows them to be derived from *PVT* (Shtivelman et al., in press). We presently have no information regarding which of these RNAs might be functional mRNAs, the proteins they might encode, and the means by which the diversity of RNAs is generated. Although we have examined the expression of

PVT only in tumor cells, it appears that the gene may be expressed in a variety of developmental lineages.

We have also made a provisional analysis of transcription from the mouse *pvt* locus. The results are puzzling: the products of transcription appear to be less heterogeneous than in human cells, and amplification of the gene in one line of tumor cells seems to have had no impact on the abundance of *pvt* RNA in the SEWA cell lines. Further consideration of these puzzles awaits the isolation and characterization of the mouse locus.

Exon 1 of *PVT* in DM*MYC* is represented in a series of abundant polyadenylated RNAs with sizes ranging from 2.2 to 11 kb. The abundance of the RNAs prompts the inference that they were transcribed from the amplified allele of *PVT*. Some of these RNAs contain sequences derived from *MYC*, whereas others do not (Fig. 1A and B). We presume that the latter initiate from the *PVT* promoter serving DM*MYC*, bypass the *MYC* component of the allele by splicing, and proceed through part or all of the amplified portions of *PVT* that lie downstream of DM*MYC* (Fig. 9). We cannot presently discern whether any of these RNAs have counterparts among the transcripts from wild-type *PVT*, nor can we account for the frequency with which the *MYC* component of DM*MYC* is apparently bypassed by splicing.

The H82, N417, and SK-N-MC cell lines contain an abundance of heterogeneous polyadenylated RNAs that include sequences representing exon 1 of *PVT* (Fig. 7). The abundance of the RNAs suggests that they were transcribed from the amplified version of the exon. Since it appears that other exons of *PVT* are not amplified in these cell lines, we suspect that the abundant RNAs arise from exon 1 of the gene and adjoining, amplified portions of intron 1. We cannot account for the fact that the array of sizes of the RNAs varies from one cell line to another.

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