JOURNAL OF VIROLOGY, June 1989, p. 2623–2628
0022-538X/89/062623-06$02.00/0

Vol. 63, No. 6

Effect of Proviral Insertion on Transcription of the Murine *B2m*^{b} Gene

WAYNE N. FRANKEL,[1]† TERRY A. POTTER,[2]‡ AND T. V. RAJAN[2]§*

Department of Genetics[1] *and Department of Pathology,*[2] *Albert Einstein College of Medicine, Bronx, New York 10461*

Received 27 October 1988/Accepted 14 February 1989

We describe three *B2m*^{b} mutants caused by integration of retroviral proviruses into the *B2m* gene. All three insertions occurred within 500 base pairs of each other in the 5′ end of the first intron of the *B2m*^{b} gene. Mutant cell line *i*1 contained a nonfunctional Abelson murine leukemia virus-initiated transcript that spliced into *B2m*^{b} exon II; mutant cell lines *i*7 and *i*18 contained transcripts complementary to *B2m*^{b} exon 1. Both *i*1 and *i*7 also contained small amounts of wild-type *B2m*^{b} transcript. The implications of these studies for the development of retroviral insertional mutagenesis as a strategy for cloning previously uncloned genes are discussed.

The function of a number of cell surface markers which typify various stages of B- and/or T-cell differentiation is unknown because of a paucity of biochemical information. A step toward the analysis of their function and precise role in lymphoid cell differentiation would be to clone the genes encoding these products. However, the lack of biochemical knowledge has hampered the cloning of many of these genes. We have been attempting to circumvent this lack and exploit the powerful selection scheme that can be used for isolating mutants (14). The premise of our technique is that retroviral infection of cells will result in essentially random integration of proviral DNA in the genome of the cells expressing the cell-surface marker. Once insertions have occurred, mutants caused by proviral insertion into the gene encoding the antigen can be isolated by using selection procedures. DNA sequences flanking the integration can be used as probes to isolate the intact gene from wild-type cells. This scheme has been attempted by several investigators using in vitro cultured cell lines (1, 6, 17). Retroviruses have also been used to insertionally inactivate genes in vivo (5; for a review, see reference 4).

We chose to use the *B2m* gene as a model system to determine whether retroviral mutagenesis is a viable scheme for isolating insertion mutants. Briefly, 23 independent, small cultures of an Abelson retrovirus-transformed *B2m*$^{a/b}$ heterozygous pre-B-lymphocyte cell line called 439.4.2 were subjected to infection with Moloney murine leukemia virus (Mo-MuLV). A week after the infection, the cultures were subjected to immunoselection with a monoclonal antibody directed against the *B2m*^{b} gene product to obtain mutants that do not express β2m^b protein on the cell surface. From each individual culture a single mutant was chosen to insure that a number of independent events would be screened. The structure of the *B2m*^{b} gene in these mutants was analyzed and compared with that in mutants which arose spontaneously (i.e., without prior infection with Mo-MuLV). The 23 mutants were analyzed by Southern blots by using a variety of *B2m* probes, and it was determined that three were due to proviral insertions: one an Abelson murine leukemia virus (Ab-MuLV) provirus and two (independent) Mo-MuLV proviruses (1). Here we describe some features of the integration of these three independent insertions, present some data relating to the effect of proviral integration on transcription of *B2m*^{b}, and discuss the potentials and limitations of this technique for the isolation of previously uncloned genes.

MATERIALS AND METHODS

Cell lines. The *B2m*$^{a/b}$ heterozygous cell line 439.4.2 and mutant cell lines *i*1 (referred to as mutant "x" in reference 1), *i*7, and *i*18, obtained from 439.4.2 by anti-*B2m*^{b} immunoselection after Mo-MuLV infection, have been previously described (1). All cell lines were maintained at 37°C in 10% CO_2 in RPMI 1640 medium supplemented with 10% fetal bovine serum and 5×10^5 M β-mercaptoethanol.

Hybridization probes. The probe B2m-1 contains the first exon of the *B2m*^{b} gene as an *Xho*I-*Hin*dIII fragment in pGemini-2 (Promega Biotec Co., Madison, Wis.); B2m-2 contains the second exon of the *B2m*^{b} gene as a *Hin*dIII-*Bam*HI fragment in pKC7. The probes were generated by subcloning appropriate fragments from a full-sized *B2m* gene generously provided by David H. Margulies, National Institutes of Health, Bethesda, Md. The probes were radiolabeled with ^{32}P by nick translation (7) or runoff transcription (8). The oligonucleotide probes, oligo-1 (5′-CGGTCGCT TCAGTCGTCAGC-3′) (representing the 5′ region of the sense strand of exon 1), oligo-4(5′-GGTGGCGTGAG TATACTTGAA-3′) (complementary to the 5′ end of the sense strand of exon 2), and oligo-5 (5′-GCCATACTG GCATGCTTAA-3′) (complementary to the sense strand at the 3′ end of exon 2 and, since it extends across the alleleic difference between *B2m*^{a} and *B2m*^{b}, specific for the latter), were made on a DNA synthesizer (Applied Biosystems, Foster City, Calif.), purified by polyacrylamide gel electrophoresis, and radiolabeled with ^{32}P by using T4 polynucleotide kinase (Boehringer Mannheim Biochemicals, Indianapolis, Ind.).

Isolation of RNA and DNA. Genomic DNA was prepared in microcentrifuge tubes by lysing 5×10^5 or more cells for 2 h at 37°C in lysis buffer (1% sodium dodecyl sulfate, 50 mg of protease K [Sigma Chemical Co., St. Louis, Mo.] per ml, 10 mM Tris [pH 8.0], 10 mM EDTA, 5 mM NaCl) and extracting twice with equal volumes of phenol-chloroform-isoamyl alcohol and then chloroform, followed by ethanol precipitation in 2.5 M ammonium acetate. Nucleic acids

* Corresponding author.
† Present address: Department of Microbiology and Molecular Biology, Tufts University School of Medicine, Boston, MA 02111.
‡ Present address: National Jewish Hospital Medical Center, Denver, CO 80206.
§ Present address: Room L 1006, Department of Pathology, University of Connecticut Health Centre, Farmington, CT 06032.

were immediately centrifuged, washed in 70% ethanol, and then suspended in an appropriate volume of 10 mM Tris (pH 8.0)–5 mM EDTA–20 mg of pancreatic RNase A (Sigma) per ml.

Cytoplasmic RNA was obtained by washing more than 10^8 cells in phosphate-buffered saline, suspending in 300 ml of phosphate-buffered saline containing 40 U of RNasin ribonuclease inhibitor (Promega Biotec), and transferring to microcentrifuge tubes. Nonidet P-40 and 10% deoxycholate were added to 0.5% each; the mixture was briefly vortexed, incubated on ice for 5 min, and then centrifuged for 1 min. The primary supernatant was transferred to a second microfuge tube. The pellet was washed with 1% Triton X-100–5 mM $MgCl_2$, and this supernatant was added to the primary supernatant. After the addition of sodium dodecyl sulfate and EDTA to 0.5% and 5.0 mM, respectively, the sample was extracted several times with 2 volumes of phenol-chloroform and then chloroform. RNA was precipitated by the addition of 2.5 volumes of ethanol. Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose (Collaborative Research, Inc., Lexington, Mass.) chromatography as described by Maniatis et al. (7), with minor modifications. Restriction digests, nucleic acid electrophoresis, capillary blot transfer, hybridizations, and washes were performed as previously described (13).

S1 mapping. S1 nuclease analysis was done by standard methods (7), with minor modifications. An *Ava*I-*Kpn*I *B2m* fragment was treated with calf intestinal alkaline phosphatase (Boehringer Mannheim) and electrophoresed in a 1.2% Seaplaque agarose (FMC Corp., Rockland, Maine) gel in Tris borate-EDTA. The appropriate fragment was excised from the gel, and DNA was purified by GENECLEAN (Bio101, La Jolla, Calif.) and 5′ end labeled with ^{32}P with T4 polynucleotide kinase; 60 mg of cytoplasmic RNA and approximately 10^5 cpm of probe were used per RNA sample. The hybridization was done at 50, 55, or 60°C for 5 h, and nuclease digestion was done for 45 min at 37°C with 1,500 U of S1 nuclease (Boehringer Mannheim) per ml. After isopropanol precipitation, the DNA was denatured and analyzed on a sequencing gel with ^{32}P-labeled *Sau*3A-digested pUC18 DNA as molecular weight markers.

Dideoxynucleotide sequencing of RNA. RNA sequencing was done as described by Geliebter et al. (2), with a few modifications. Cytoplasmic RNA was prepared as described above, and poly(A)$^+$ RNA was isolated and further purified by precipitation with 1 M LiCl and 7 volumes of ethanol. Between 15 and 20 mg of poly(A)$^+$ RNA was mixed with 5 ng of the ^{32}P 5′-end-labeled oligo-4 primer in annealing buffer, heated at 80°C, and then placed at 52°C (5°C less than the melting temperature for the oligonucleotide) for 45 min to allow annealing of the primer to the RNA. Reverse transcription was done for 1 h in the presence of dideoxynucleotides at 50°C; then the reaction was stopped, heated to 90°C for 3 min, and run on an 8% sequencing gel.

Dideoxynucleotide sequencing DNA. DNA sequencing was performed by the chain termination-length method (15), with modifications for [^{35}S]dCTP (Pharmacia Fine Chemicals, Piscataway, N.J.).

RESULTS

Site of integration of proviruses in the *B2m^b* gene. Using a number of restriction endonucleases and several DNA probes, we mapped the sites of integration and orientation of the proviruses. The composite of such data is shown in Fig. 1. The *B2m^b* gene in the mutant cell line *i*1 contains an

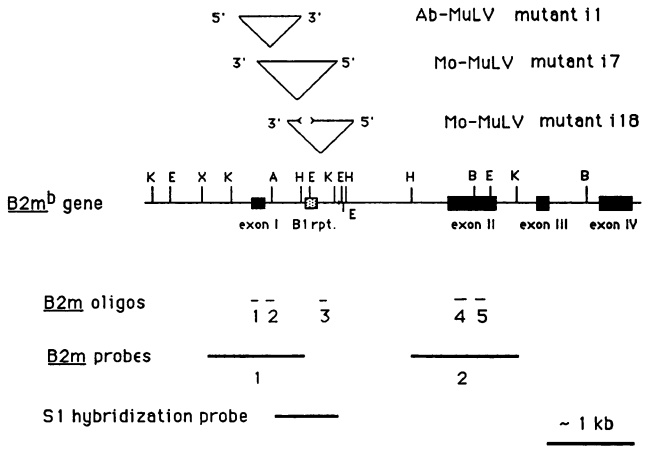

FIG. 1. Proviral insertions in the *B2m^b* gene. Molecular map of the *B2m^b* gene showing the exon structure, several restriction sites (determined mostly by Parnes and Seidman), the location and transcriptional orientation of each provirus, and B2m hybridization probes used in this paper. "B1 rpt" refers to a copy of the B1 family of repetitive DNA localized to the first intron by Parnes and Seidman. Abbreviations for restriction enzymes: A, *Ava*I; B, *Bgl*I, E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; S, *Sac*I; X, *Xho*I.

Abelson provirus, inserted in the same transcriptional orientation as the gene, 3′ of exon I; mutant *i*7 contains a Moloney provirus integrated in the reverse transcriptional orientation; and mutant *i*18 contains a Moloney provirus with a 1-kilobase deletion in the *env* gene, also oriented inversely relative to *B2m* transcription. It is noteworthy that all three insertions have occurred within 500 base pairs (bp) of each other in the 5′ half of the first intron of the gene.

Analysis of transcription of the *B2m^b* gene in mutant *i*1. Since loss of expression of the *B2m^b* gene in all of the mutants had occurred even though the insertions were not within the coding sequence, we sought to study the pattern(s) of transcription of this gene in the three mutants to gain insights into the mechanism by which this failure of expression had occurred. Transcription of the *B2m* gene in normal cells results in two polyadenylated species that differ in their 3′ termini (11). The two alleleic forms (*B2m^a* and *B2m^b*) are indistinguishable from each other except for a single-base change corresponding to codon 85.

When cytoplasmic RNA from mutants *i*1, *i*7, and *i*18 was hybridized to a *B2m^b*-specific oligonucleotide probe (oligo 5, which extends across the alleleic difference between *B2m^a* and *B2m^b* and is complementary to the coding sequence of the latter), we found that only mutant *i*1 contained a hybridizing species and that this RNA was slightly larger than the wild-type transcript (Fig. 2). To analyze the nature and size of this transcript further, it was necessary to delete the wild-type *B2m^a* allele. To do this, we exploited the fact that the H-2 antigens are not expressed in the absence of B2m. The mutant *i*1 has only one functional copy of *B2m*, namely, the *B2m^a* allele. We therefore subjected it to immunoselection with antibodies to H2K^b, H-2D^d, and H-2K^d antigens simultaneously. The mutant that resulted from such selection was tested to ensure that it was H-2 negative on the cell surface. We then analyzed the structure of the *B2m* gene by Southern blot analysis (Fig. 3). Using the *B2m* first exon probe on *Eco*RI-digested genomic DNA, we found that the wild-type cell has a single fragment (Fig. 3B). Mutant *i*1 had two fragments, one comigrating with that in the wild-type cell line and a novel fragment not present in the wild-type

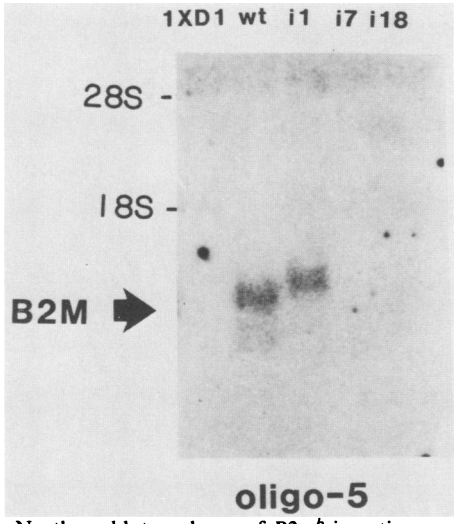

FIG. 2. Northern blot analyses of $B2m^b$ insertion mutants with the $B2m^b$-specific oligonucleotide oligo-5 (see Materials and Methods). Cytoplasmic RNA from negative control cell line 1XD1 (homozygous for $B2m^a$), wild type 439.4.2 (wt), and mutants *i*1, *i*7, and *i*18 was blotted to Gene Screen and hybridized to the $B2m^b$-specific oligonucleotide oligo-5. *B2m* mRNA was present in the wild type; none was detected in 1XD1, confirming the specificity of the probe for $B2m^b$. Only mutant *i*1 had an RNA species, slightly larger than that in the wild type, hybridizing to oligo-5.

cells. We have shown (1) that this novel fragment is generated by the proviral insertion and contains proviral and $B2m^b$ sequences. In the second-generation mutant referred to as $i1^{a-}$, the wild-type $B2m^a$ fragment is missing, leaving only

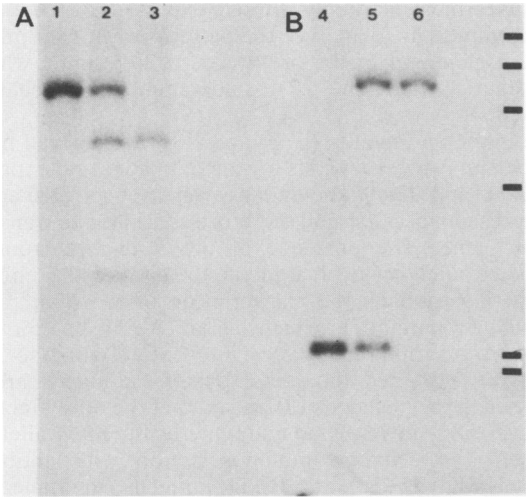

FIG. 3. *B2m* gene structure of the mutant $i1^{a-}$, as determined by Southern blot analysis. DNA was digested with the restriction endonuclease *Sac*I (A) or *Eco*RI (B). Molecular sizes are represented along the right margin and are (from top to bottom) 23, 9.4, 6.6, 4.4, 2.3, and 2.0 kilobases). In both panels, the wild-type cell line (lanes 1 and 4) contained a single fragment. The insertion mutant *i*1 (lanes 2 and 5) contained one fragment that comigrated with the wild-type fragment (derived from the unselected $B2m^a$ allele) and either one (*Eco*RI in B) or two (*Sac*I in A) novel fragments containing $B2m^b$ and MuLV proviral DNA. The second-generation mutant $i1^{a-}$ (lanes 3 and 6) no longer exhibited the wild-type fragment but retained the novel fragments derived from the inserted $B2m^b$ allele.

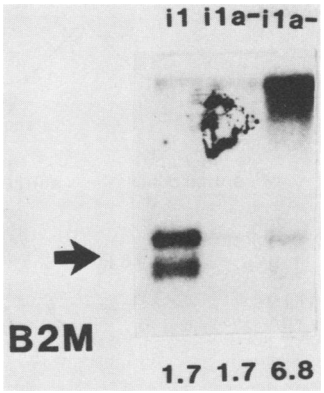

FIG. 4. Northern blot analysis of $i1^{a-}$ by using a high-specific-activity B2m-1 RNA probe. The probe was generated by runoff transcription and is complementary to *B2m* mRNA. The lanes contain poly(A)$^+$ RNA from mutants *i*1 (1.7 μg), $i1^{a-}$ (1.7 μg), and $i1^{a-}$ (6.8 μg). All lanes exhibited a large fragment that comigrated with 28S rRNA. The two lower-molecular-weight fragments correspond to the two alternately polyadenylated species of mRNA transcribed from the *B2m* gene. The lane with 1.7 μg of $i1^{a-}$ showed no *B2m* mRNA, and the lane with 6.8 μg of $i1^{a-}$ showed less than half that in *i*1. We therefore estimate that mutant $i1^{a-}$ contains less than 10% of the normal amount of *B2m* RNA.

the $B2m^b$ junction fragment. Similar results were obtained by using digestion with *Sac*I (Fig. 3A). We observed and described this loss of the *B2m* first exon in a class of deletion mutants that we (13) and Parnes et al. (12) independently described. Both groups have shown that these mutants have undergone large (>10-kilobase) deletions of the *B2m* gene, including the first exon. Based on the Southern blot analysis, we believe that the $i1^a$-mutant arises by the same mechanism.

To analyze the pattern of transcription of the $B2m^a$ gene in mutant $i1^a$-, we hybridized poly(A)$^+$ RNA to a high-specific-activity probe generated by runoff transcription of the first-exon probe (B2m-1), such that it would hybridize to transcripts that are of the sense orientation (Fig. 4). The high-molecular-weight fragment in Fig. 4 comigrated with 28S rRNA; the two lower-molecular-weight fragments corresponded to the two alternate transcripts derived from the B2m gene. Note that the larger-than-wild-type transcript visualized with the second exon probe is not visible. However, on prolonged exposure of the blot to X-ray film a very small amount corresponding to less than 10% of the wild-type transcript was visible. This transcript, presumably from the $B2m^b$ allele, was of normal size (Fig. 4).

We synthesized an oligonucleotide complementary to the sense strand of the 5'-most part of exon 2 (oligo 4 in Materials and Methods) and used it as a primer to sequence the novel RNA in mutant $i1^{a-}$. The sequence analysis revealed that the RNA was composed of proviral U5 and sequences upstream of the *gag* gene extending up to the *env* gene splice donor, followed by the $B2m^b$ second exon. This transcript was 62 bases longer than the wild-type *B2m* transcript and explains the larger-than-wild-type size of the novel RNA species observed in Northern (RNA) blots. The composition of this transcript is indicated in Fig. 5.

Transcription of the $B2m^b$ gene in mutants *i*7 and *i*18. When RNA from mutants *i*7 and *i*18 was hybridized to nick-translated B2m-1 probe (see Materials and Methods), a wild-type transcript corresponding to the $B2m^a$ gene was seen. In addition, a very large transcript greater than 9 kb

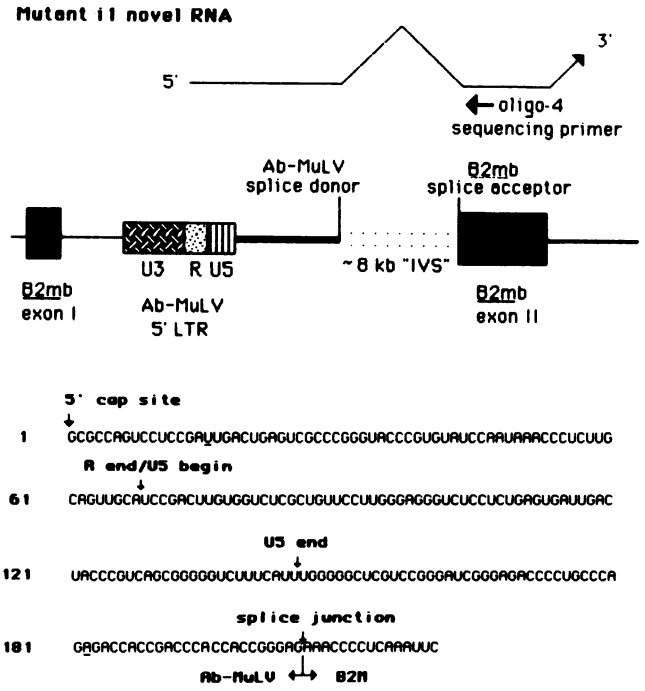

FIG. 5. Sequence and diagramatic representation of the 5′ end of the novel transcript in insertion mutant *i*1. Shown are the *B2m* exon II splice junction, the 5′ end of the novel transcript, and the 5′ LTR of the Ab-MuLV provirus. This RNA was 62 bp longer than normal *B2m* RNA. The two underlined nucleotides differed from the published Ab-MuLV DNA sequence (14a). Above the sequence is a schematic representation of the novel $B2m^b$ transcript in mutant *i*1, indicating that it initiates in the Ab-MuLV 5′ LTR and splices into the *B2m* sequence at the exon II splice acceptor.

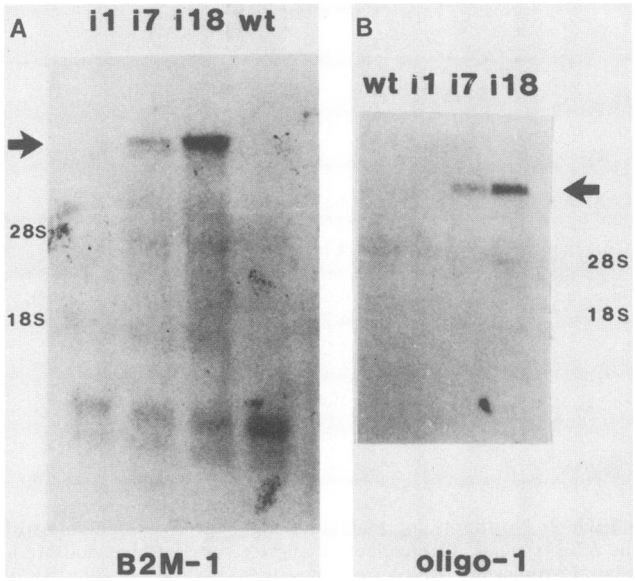

FIG. 6. Northern blot analysis of $B2m^b$ insertion mutants with *B2m* probes. Cytoplasmic RNA from mutants *i*1, *i*7, and *i*18 and the wild type was hybridized to either a nick-translated B2m-1 probe (A) or the *B2m* leader oligonucleotide oligo-1 (B). Mutants *i*7 and *i*18 had very-high-molecular-weight species not present in the wild type or in mutant *i*1. These species were NaOH sensitive and DNase I resistant and were larger than 9 kilobases. Since oligo-1 is from the *B2m*-coding strand, these novel transcripts must be complementary to the exon I coding sequence.

was noted (Fig. 6A, arrow), which was considerably more abundant in mutant *i*18 than in *i*7. Given the orientation of the provirus in *i*7 and *i*18, we felt that this RNA might arise by transcriptional readthrough into $B2m^b$ exon I. If this were the case, then the aberrant transcript should be opposite in orientation to the wild-type *B2m* transcript. We therefore synthesized a sense-strand oligonucleotide corresponding to the 5′ end of exon 1 (oligo 1 in Materials and Methods). Hybridization of RNA from *i*7 and *i*18 by using this oligonucleotide highlighted the aberrant transcripts (Fig. 6B) but, as expected, did not hybridize to the wild-type transcripts.

S1 mapping with the 5′-labeled 824-bp *Ava*I-*Kpn*I *B2m* fragment was done to determine the structure of these transcripts. Cytoplasmic RNA from mutant *i*7 specifically protected a major fragment of approximately 170 bp and two smaller, minor fragments of 165 and 140 bp. Cytoplasmic RNA from mutant *i*18 specifically protected a major fragment of approximately 540 bp and several minor fragments of approximately 485, 410, and 360 bp. An examination of the *B2m* intron sequence revealed that each of these *Ava*I end-labeled fragments mapped near a potential splice acceptor sequence in *B2m* exon I (Fig. 7).

DISCUSSION

In an earlier communication, we described the isolation and preliminary characterization of the *B2m* insertion mutants. There are two striking features about the insertions of the proviruses into the $B2m^b$ gene. First, the insertions have occurred within about 500 bp of each other in the 5′ end of the first exon. The reason for this clustering of insertions is unknown. The other remarkable feature is that none of the insertions has occurred within the coding region, and yet the proviruses have markedly altered expression of the gene. This prompted us to analyze the patterns of transcription of the *B2m* gene and of the proviruses to determine whether these data would allow us to understand the mechanisms involved.

The insertion mutants *i*1, *i*7, and *i*18 were derived from a cell line that is heterozygous for the *B2m* gene and expresses both $B2m^a$ and $B2m^b$. The mutants were not selected against the expression of $B2m^a$ and therefore continued to transcribe a $B2m^a$ gene. The presence of the transcript from this unselected allele made it difficult to analyze the effect of proviral insertion on the transcription of the $B2m^b$ allele, especially in mutant *i*1, which has an Ab-MuLV provirus-initiated transcript that splices into exon II on the $B2m^b$ allele. We exploited the facts that H-2 antigens are not expressed in the absence of B2m and that the only functional copy of *B2m* in the insertion mutants was the $B2m^a$ allele; we subjected the cells to immunoselection with antibodies against $H\text{-}2K^b$, $H\text{-}2K^d$, and $H\text{-}2D^d$ antigens simultaneously. Under these conditions, mutants of two genotypes may be expected to survive the selection. The first category would be those that had undergone mutations in $H\text{-}2K^b$, $H\text{-}2D^d$ and $K\text{-}2K^d$ genes and the second those that had undergone mutations in the $B2m^a$ allele and therefore failed epigenetically to express the H-2 products on the cell surface, even though the genes were intact. We reasoned that the second mechanism was statistically more likely. Indeed, this was found to be the case for all H-2-negative mutants that we isolated from *i*1 (and *i*7; data not shown), since Southern blot analyses of the second-generation mutants referred to as $i1^{a-}$ (and $i7^{a-}$; data not shown) revealed that they had

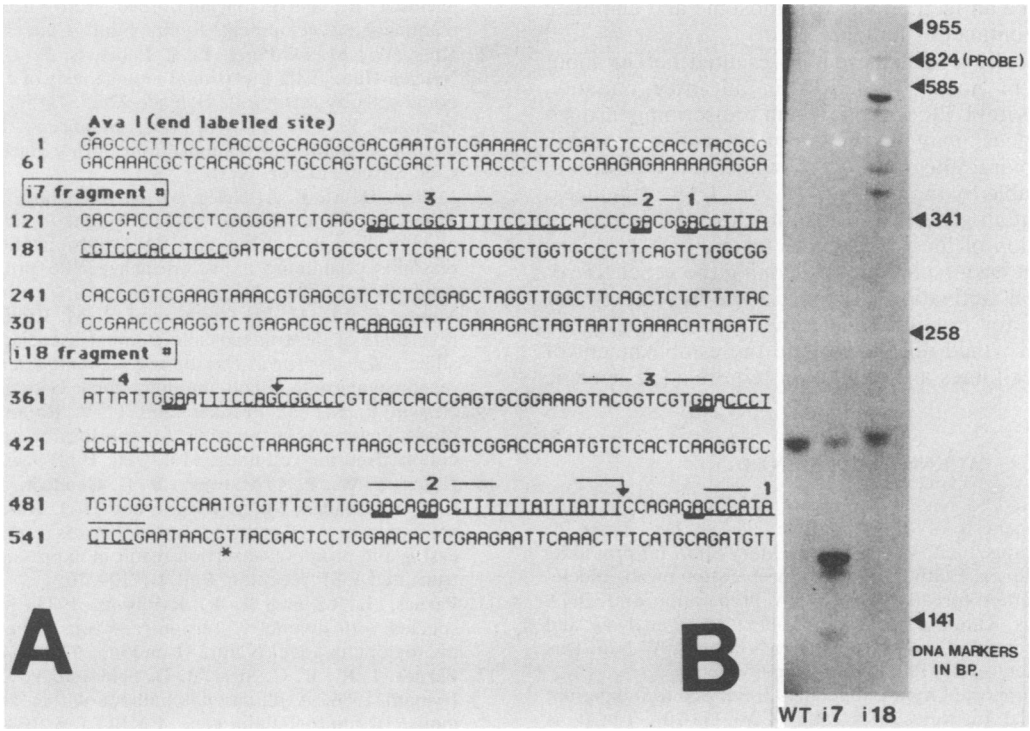

FIG. 7. (B) S1 nuclease mapping of the novel transcripts from mutants *i*7 and *i*18, by using the *B2m* intron *Ava*I-*Kpn*I hybridization probe, analyzed on a 5.5% polyacrylamide sequencing gel. Mutant *i*7 RNA protected a major fragment of approximately 170 bp and two smaller, minor fragments of approximately 165 and 140 bp. Mutant *i*18 protected a major fragment of approximately 540 bp and three smaller, minor fragments of approximately 510, 410, and 360 bp. (A) Nucleotide sequence of the *B2m* intron I region containing mutant *i*7 and *i*18 novel transcripts. Shown is the DNA sequence (3′ to 5′, noncoding strand relative to *B2m*) of *B2m* intron I from the *Ava*I site to the intronic *Kpn*I site. The *B2m* "origins" of S1-protected fragments from mutants *i*7 and *i*18 are shown, and potential splice acceptor sequences approximating the Py_{11} NAG consensus splice acceptor sequence are underlined. Notice that each of the protected fragments maps near a potential splice acceptor. The B1 repeat element is outlined with arrows.

undergone deletions of the first exon of the $B2m^a$ gene. We were unable to get this particular class of mutants from the *i*18 mutant.

Mutant *i*1 (and *i*7; data not shown) contains the $B2m^b$ transcript at less than 10% of the wild-type levels; since we were unable to obtain the $B2m^a$ deletion mutant from the mutant *i*18, it is unclear whether this cell line expresses any normal-sized *B2m* transcripts.

All three mutants have novel transcripts. The best characterized is the transcript in *i*1, which initiates in the proviral 5′ long terminal repeat (LTR) and, by using the Mo-MuLV-derived *env* splice donor (not normally used in Ab-MuLV-derived transcripts), splices into the physiological splice acceptor at the 5′ end of *B2m* exon II. It is likely that similar transcription occurs in mutants *i*7 and *i*18, reading through *B2m* exon I in the antisense orientation. However, unlike the situation with the transcript in *i*1, there would not be a physiological splice acceptor for these transcripts, since they are in the opposite orientation from that in the normal gene. Perhaps because of this, these transcripts use aberrant acceptor sites and splice into various positions in the cellular sequence within exon 1. Transcripts initiating in proviral LTRs and splicing into cellular sequences have also been seen in murine (16) and avian (10) oncogenes.

The mechanism of inactivation of the *cis B2m* gene by the proviruses is not clear. As noted above, aberrant transcripts initiated by the viral LTR are present in all three cases. Analyses of revertants of retroviruslike insertions in *Drosophila melanogaster* suggest that it is not merely the insertion of any DNA but the transcriptional activity of the inserted element that is responsible for the inactivation of the gene disrupted by the insertion (3, 9). In view of this, it is possible that the transcription of the MuLV proviruses that we observe in our mutants is responsible for the inactivation of the $B2m^b$ allele, affecting normal $B2m^b$ RNA synthesis by unknown mechanisms.

We demonstrated that retroviral insertion into a gene for a cell-surface antigen can turn off expression to the degree that mutants can be readily selected by immunoselection protocols. Given a cell line with the appropriate genotype for performing the selection and allele-specific monoclonal antibodies, this technique appears to be a viable procedure for cloning cell surface genes. The major problem is that only a fraction (3 of 23) of the mutants isolated by immunoselection contain proviruses in the gene of interest. It is obviously important to eliminate the spontaneous mutants. Over the past few years, we have conducted extensive studies on the mechanism of emergence of mutants in animal cells in culture, and it has been our observation that a majority of spontaneous mutants occur by mitotic recombination resulting in homozygosity for the unselected chromosome (9a, 13, 14). This phenomenon might be successfully exploited for this purpose. To take a specific example, wild-type 439.4.2 cells are heterozygous by RFLP for the *agouti* locus, which is linked to *B2m* (16a). Most $B2m^b$ loss mutants have lost the fragment characteristic of the C57BL/6 chromosome 2. We believe that a similar strategy can be used to screen mutants

for a cell-surface antigen on any chromosome and eliminate this class of spontaneous mutants.

Finally, we believe that the data presented herein about the existence of novel transcripts in all three mutants, initiated at the viral LTR sequences and transcribing through cellular sequences, may be an important and exploitable feature of retroviral integration. These aberrant transcripts, readily identifiable by the presence of viral LTR sequences, would also contain genetic information close to the gene of interest. Isolation of these sequences from a cDNA library may provide an easy technique for cloning the gene. Retroviral insertional activation may be another strategy for isolating genes for previously uncloned phenotypes, since such activation would not necessitate the establishment of heterozygous cell lines and would not require allele-specific selection.

ACKNOWLEDGMENTS

We are indebted to Naomi Rosenberg, whose expertise made insertional mutagenesis of *B2m*^{b} possible, and to Jack Lenz for advice and encouragement. We also thank Mary Short for providing Mo-MuLV subclones, Edith Palmieri for preparation of oligonucleotides, Ken Krauter for advice on RNA preparation and DNA analysis programs, Kate Montgomery for help with S1 analysis, and John Coffin for helpful discussions. The work done by W.N.F. was in partial fulfillment of his Ph.D. thesis requirements.

W.N.F. was supported by a Public Health Service training grant from the National Institutes of Health (CA09173-10); T.A.P. is supported by a grant from the American Cancer Society (CD-293). This work was also supported by Public Health Service grants from the National Cancer Institute (1 R01 CA29194 and CA13330). The oligonucleotide synthesis facility is supported by a grant from the National Science Foundation (PCM-8400114).

LITERATURE CITED

1. **Frankel, W., T. A. Potter, N. A. Rosenberg, J. Lenz, and T. V. Rajan.** 1985. Retroviral insertional mutagenesis of a target allele in a heterozygous cell line. Proc. Natl. Acad. Sci. USA **82:** 6600–6604.
2. **Geliebter, J., R. A. Zeff, R. W. Melvold, and S. G. Nattenson.** 1986. Mitotic recombination in germ cells generated two major histocompatiblity complex mutant genes shown to be identical by RNA sequence analysis: Kbm9 and Kbm6. Proc. Natl. Acad. Sci. USA **83:**3371–3375.
3. **Geyer, P. K., M. M. Green, and V. G. Corces.** 1988. Reversion of a gypsy induced mutation at the yellow locus of *Drosophila melanogaster* is associated with the insertion of a newly defined transposable element. Proc. Natl. Acad. Sci. USA **85:**3938–3942.
4. **Goff, S. P.** 1987. Gene isolation by retroviral tagging. Methods Enzymol. **152:**469–481.
5. **Jaenisch, R., and P. Soriano.** 1896. Retroviruses as tools for mammalian development. Symp. Fund. Cancer Res. **39:**59–65.
6. **King, W., M. D. Patel, L. I. Lobel, S. P. Goff, and M. C. Nyugen-Huu.** 1985. Insertional mutagenesis of embryonal carcinoma cells by retrovirus. Science **228:**554–558.
7. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green.** 1984. Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Res. **12:**7035–7056.
9. **Mount, S. M., M. M. Green, and G. M. Rubin.** 1988. Partial revertants of the transposable element associated suppressible allele *white apricot* in *Drosophila melanogaster*: structure and responsiveness to genetic modifications. Genetics **118:**221–234.

9a. **Nelson, F. K., W. Frankel, and T. V. Rajan.** 1989. Mitotic recombination is responsible for the loss of heterozygosity in cultured murine cell lines. Mol. Cell. Biol. **9:**1284–1288.

10. **Nilsen, T. W., P. A. Maroney, R. G. Goodwin, F. M. Rottman, L. B. Crittenden, M. A. Raines, and H.-J. Kung.** 1985. *c-erbB* activation in ALV-induced erythroblastosis: novel RNA processing and promoter insertion result in expression of an amino-truncated EGF receptor. Cell **41:**719–726.
11. **Parnes, J. R., and R. R. Robinson.** 1983. Multiple mRNA species with distinct 3′ termini are transcribed from the β2-microglobulin gene. Nature (London) **202:**443–452.
12. **Parnes, J. R., K. C. Sizer, J. G. Seidman, V. Stallings, and R. Hyman.** 1986. A mutational hotspot within an intron of the mouse β2-microglobulin gene. EMBO J. **5:**103–111.
13. **Potter, T. A., W. Frankel, R. A. Zeff, and T. V. Rajan.** 1987. Spontaneous deletion at the *B2m* locus: evidence for site-specific genetic rearrangement. J. Immunol. **138:**1270–1274.
14. **Rajan, T. V.** 1977. H-2 antigen variants in a cultured heterozygous mouse leukemia cell line. Immunogenetics **6:**105–115.

14a. **Reddy, E. P., M. J. Smith, and A. Srinivasan.** 1983. Nucleotide sequence of Abelson murine leukemia genome: structural similarity of its transforming gene product to other *onc* gene products with tyrosine-specific kinase activity. Proc. Natl. Acad. Sci. USA **80:**3623–3627.

15. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain termination inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
16. **Shen-Ong, G. L., H. C. Morse III, M. Potter, and J. F. Mushinski.** 1986. Two modes of *c-myb* activation in virus-induced mouse myeloid tumors. Mol. Cell. Biol. **5:**380–392.

16a. **Siracusa, L. D., L. B. Russell, E. M. Eicher, D. J. Corrow, N. G. Copeland, and N. A. Jenkins.** 1987. Genetic organization of the *agouti* region of the mouse. Genetics **117:**93–100.

17. **Varmus, H. E., N. Quintrell, and S. Ortiz.** 1981. Retroviruses as mutagens: insertion and excision of a non-transforming provirus alter expression of a resident transforming provirus. Cell **25:** 23–26.