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Retroviral Insertions Downstream of the Heterogeneous Nuclear Ribonucleoprotein A1 Gene in Erythroleukemia Cells: Evidence that A1 Is Not Essential for Cell Growth

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A large number of novel cellular proto-oncogenes have been identified and cloned by analysis of common integration sites in retrovirally induced malignancies. In the multistage erythroleukemias induced by the various strains of Friend leukemia virus, the analysis of proviral-integration events has led to the identification of two genes, *Fli*-1 and *Spi*-1, both novel members of the *ets* oncogene family of transcription factors. In this report, we describe the identification of another integration site, designated *Fli*-2 (Friend leukemia virus integration-2), in an erythroleukemia cell line induced by Friend murine leukemia virus (F-MuLV). Rearrangements at the *Fli*-2 locus were found in two erythroleukemia cell lines independently induced by F-MuLV and one leukemic cell line derived from the spleen of a mouse infected with the polycythemia strain of Friend leukemia virus. The deduced amino acid sequence of a cDNA corresponding to a transcript originating from genomic DNA adjacent to *Fli*-2 is identical to that of the human heterogeneous nuclear ribonucleoprotein A1 gene, a member of the gene family of RNA-binding proteins involved in RNA splicing. In one erythroleukemia cell line, A1 expression was undetectable as a result of F-MuLV integration in one allele and loss of the other allele. These results suggest that perturbations in RNA splicing mechanisms may contribute to malignant transformation and provide direct evidence that the A1 protein is not required for cell growth.

Friend leukemia is a multistage malignancy associated with an early stage characterized by the polyclonal proliferation of infected erythroid progenitors followed by a leukemic stage in which erythroleukemia cell clones emerge (2, 17). The early and late stages of Friend leukemia can be induced following a single injection of either the polycythemia- or anemia-inducing strain of Friend leukemia virus (FV-P or FV-A, respectively). Both FV-P and FV-A are complexes of a defective spleen focus-forming virus (SFFV-P and SFFV-A, respectively) and a replication-competent Friend murine leukemia virus (F-MuLV). F-MuLV itself can also induce erythroleukemia when injected into newborn mice of certain susceptible strains (34).

We have previously shown that the tumor suppressor gene p53 is inactivated in almost all of the erythroleukemia cell lines induced by various strains of Friend leukemia virus, as a result of deletion, truncation, retrovirus insertion, or point mutation (2, 27, 28). Moreau-Gachelin et al. have identified a common site for retroviral integration named *Spi*-1 (SFFV provirus integration-1) and shown that this locus is rearranged in the majority of the erythroleukemia cell lines induced by the FV-P and FV-A strains of Friend leukemia virus (24, 25). DNA sequences whose expression is highly activated as a result of these SFFV insertions have been isolated immediately adjacent to the *Spi*-1 insertion sites (26). The sequence of *Spi*-1 transcripts is identical to that of PU.1, a gene that encodes a DNA-binding protein related to the proto-oncogene *ets* family of transcription factors (18). Although *Spi*-1 is rearranged and its transcriptional domain is activated in FV-P- and FV-A-induced tumor cell clones, none of the erythroleukemia cell lines induced by helper virus F-MuLV show rearrangement of this locus. We have recently described a novel common proviral integration site named *Fli*-1 (Friend leukemia virus integration-1) in erythroleukemia cell lines induced by F-MuLV. The *Fli*-1 locus is rearranged in 75% of F-MuLV-induced erythroleukemia cell lines (3). In contrast, erythroleukemia cell lines induced by FV-P or FV-A all have a germ line configuration of the *Fli*-1 locus. A cDNA corresponding to the *Fli*-1 locus has also been isolated and, like *Spi*-1, encodes another novel member of the *ets* oncogene family of transcription factors (4). The high frequency of activation of the *Fli*-1 or *Spi*-1 *ets* genes and inactivation of the *p53* tumor suppressor gene in Friend erythroleukemia cell lines suggests that these genes play an important and obligate role during the evolution of the multistage leukemias induced by these viruses.

Although *Fli*-1 is rearranged and activated in a high proportion of F-MuLV-induced erythroleukemia cell lines, about 25% of these cell lines do not exhibit any structural rearrangement around either *Fli*-1 or *Spi*-1, nor do they express high levels of transcripts corresponding to these genes. Therefore, we sought to determine whether there are additional common integration sites utilized by F-MuLV in the late stages of erythroleukemia induction. In this paper, we describe the identification and isolation of another common site for retroviral integration named *Fli*-2 (Friend leukemia virus integration-2). This locus is rearranged in 3 of 30

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independently isolated erythroleukemia cell lines induced by either F-MuLV or FV-P strains of Friend leukemia virus. A cDNA corresponding to a transcript expressed in F-MuLV-induced erythroleukemic cells and derived from genomic DNA adjacent to *Fli*-2 was isolated. This cDNA is identical to mRNA coding for the human RNA-binding protein heterogeneous nuclear ribonucleoprotein (hnRNP) A1, which is involved in 5′ splice site selection.

MATERIALS AND METHODS

Cells. The erythroleukemia cell lines DP27-17, DP25-16, and DP28-9 were derived from methylcellulose colonies of spleen cells of DBA/2 adult mice that had been infected by FV-P, as described previously (5, 7). The CB3 and CB7 cell lines were derived from the spleens of BALB/c mice injected at birth with F-MuLV helper virus (33). The TP3 and TP1 cell lines were derived from the spleens of NIH/Swiss mice infected at birth with F-MuLV (30). The cells were maintained in α minimal essential medium supplemented with 10% fetal calf serum.

Construction of genomic libraries and isolation of proviral integration sites. High-molecular-weight DNA from the CB3 cell line was partially digested with *Eco*RI and ligated to the *Eco*RI arms of the bacteriophage vector EMBL-4. Phage plaques were screened with ^{32}P-labelled ecotropic envelope-specific probe from F-MuLV, as described previously (3). After three cycles of phage purification, the inserted fragments were subcloned into plasmid pUC18.

Construction of cDNA library and isolation of cDNA clones. A λgt11 cDNA expression library was constructed from 5 μg of poly(A)$^+$ RNA isolated from the erythroleukemia cell line CB7 with a Pharmacia cDNA synthesis kit as described previously (6). The library (10^6 phage) was amplified once and stored at 4°C. To isolate cDNA clones, 5×10^5 phage were screened with the genomic probe B; after three rounds of plaque purification, four colonies were recovered from the cDNA library.

Subcloning and nucleotide sequence determination. DNA was prepared from plaque-purified phage according to standard procedures with lambdasorb phage absorbent (Promega) and subcloned into pGEM-7Z(+) or pGEM-7Zf(−). The plasmid DNA was isolated with cesium chloride gradients, as described previously (27). For complete sequence determination, nested deletions spanning the cDNA insert of the desired clone were generated by the method described by Henikoff (15). These constructs were rescued as single-stranded DNA after superinfection of bacteria with the coliphage M13K07 and sequenced by the dideoxynucleotide chain termination method with Sequenase enzyme, reagents, and protocols supplied by U.S. Biochemical. Sequence was obtained from both strands.

Tumor DNA and molecular hybridization. High-molecular-weight DNA was isolated by a modification of the proteinase K phenol-chloroform method of Gross-Bellard et al. as described elsewhere (27). DNA was digested with restriction enzymes and electrophoresed on agarose gels. The DNA was acid depurinated before denaturation and transferred to nitrocellulose. The filters were hybridized with 2×10^6 cpm of random-primed probe per ml of hybridization mixture that contained 50% formamide, 10% dextran sulfate, 1.5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7]), 5× Denhardt's solution (1× Denhardt's solution is 0.02% bovine serum albumin–0.02% Ficoll 0.02% polyvinylpyrrolidone), and denatured salmon sperm DNA (110 mg/ml) at 42°C. The filters were washed with 0.1× SSC and 0.1% sodium dodecyl sulfate (SDS) at 65°C.

DNA probes. The F-MuLV envelope probe was an 830-bp *Bam*HI fragment derived from F-MuLV clone 57, as described previously (3). Probe A is a 3-kb *Hin*dIII-*Eco*RI fragment of λC6 phage clone (see Fig. 1). Probe B is a 3-kb *Sal*I-*Eco*RI fragment of λC2.2 genomic phage clone (see Fig. 1). The CB1 probe is a 1.4-kb cDNA corresponding to the *Fli*-2 locus derived from the λCB1 phage cDNA insert subcloned into pGEM-7ZF(+).

RNA extraction and Northern (RNA) blotting. Total cellular RNAs were isolated by the lithium chloride precipitation procedure. In brief, 10^8 cells were lysed in 0.1 M NaCl–0.1 M Tris (pH 7.9)–0.65% Nonidet P-40. RNA was isolated from the cell extracts by a 2-min centrifugation in a microcentrifuge (Eppendorf). An equal volume of buffer containing 7 M urea, 0.35 M NaCl, 0.01 M Tris (pH 7.4), 0.01 M EDTA, and 1% SDS was added to the lysate supernatant and extracted three times with water-saturated phenol-chloroform (1:1) and once with chloroform. The final aqueous phase was precipitated with ethanol and stored in 100% ethanol at −70°C. Poly(A)$^+$ RNAs were selected by passage of total RNAs through oligo(dT) cellulose (Pharmacia). A total of 5 μg of poly(A)$^+$ mRNA was fractionated by formaldehyde denaturing gel electrophoresis and then blotted onto nitrocellulose paper. The filter was hybridized with a randomly primed CB1 cDNA probe.

Western blot analysis. Approximately 10^7 cells were lysed in 400 μl of lysis buffer (150 mM NaCl, 5 mM EDTA, 50 mM Tris [pH 7.4], 1% Nonidet P-40, 0.1% SDS, 1 mM phenylmethylsulfonyl fluoride). Lysates were cleaned by centrifugation for 1 min and retention of supernatant. After protein concentrations were determined, 20 μg of extracted proteins from the erythroleukemia cell lines CB3, CB7, and DP27-17 and human HeLa cells was electrophoretically separated on an SDS–9% polyacrylamide gel and transferred to nitrocellulose. The filter was blocked in Tris-buffered saline containing 5% (wt/vol) nonfat dry milk and 0.5% Tween-20 (32). The membrane was probed with polyclonal anti-hnRNP A1 antibody (1/2,000), supplied by S. Wilson (National Institutes of Health), and developed with ^{125}I-protein A (1 μCi/ml; Amersham). Purified recombinant A1 protein was used as a positive control (11).

Nucleotide sequence accession number. The sequence shown in Fig. 4 has been deposited with GenBank under accession no. M99167.

RESULTS

Identification and isolation of a common proviral integration site (*Fli*-2) in F-MuLV-induced erythroleukemia cells. The CB3 erythroleukemia cell line was derived from the spleen of a BALB/c mouse infected at birth with F-MuLV (33). To search for putative oncogenes which might be activated in this cell line as a result of F-MuLV proviral integration, we constructed a library from the partially digested genomic DNA of the CB3 cell line and isolated all four integrated proviruses and their virus-cell junction fragments. We have previously shown that one of these loci, *Fli*-1, is activated by retroviral insertion in 75% of F-MuLV-induced erythroleukemias (3) and encodes a novel member of the *ets* family of DNA-binding proteins (4). Further analysis of a second integration site isolated from this genomic library revealed that it represents another common integration site, designated *Fli*-2 (Friend leukemia virus integration-2). The other two integration sites appear to be

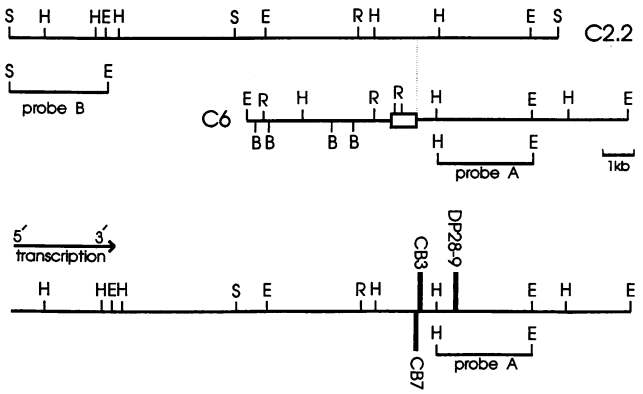

FIG. 1. Restriction map of the *Fli*-2 locus and its integrated provirus. The λC6 phage contains the 3′ region of the F-MuLV provirus and its junction fragment isolated from a genomic library constructed with DNA from the erythroleukemia cell line CB3 partially digested with *Eco*RI, as previously described (3). Probe A is a 3-kbp *Hin*dIII-*Eco*RI fragment from the λC6 insert. It was used to isolate the unrearranged allele of *Fli*-2 (λC2.2) from a normal BALB/c genomic library (Stratagene). The restriction maps of the isolated fragments were deduced from digestion of isolated phage DNA with the restriction enzymes S (*Sal*I), B (*Bam*HI), H (*Hin*dIII), E (*Eco*RI), and R (*Eco*RV). The sites of proviral integration into the *Fli*-2 locus of erythroleukemia cell lines CB3, CB7, and DP28-9 are indicated by solid bars on the deduced restriction map of *Fli*-2. Probe B is the 3-kbp *Sal*I-*Eco*RI fragment from λC2.2. It was used to screen a λgt11 cDNA library prepared from mRNA of the erythroleukemia cell line CB7 (6). The orientation of the *Fli*-2 transcriptional domain was determined from the sequence of the 401 nucleotides shown in the 3′ untranslated region of CB1 cDNA (Fig. 4, underlined).

unique and have not been characterized further. The restriction map of the phage containing a portion of the F-MuLV provirus and its 3′ junction fragment (λC6) is shown in Fig. 1. Probe A is a 3-kbp *Hin*dIII-*Eco*RI genomic fragment located upstream of the long terminal repeat in the λC6 DNA insert. This probe was used to determine whether *Fli*-2 was rearranged in other erythroleukemia cell lines induced by F-MuLV or FV-A and/or FV-P. Probe A was also used to isolate a phage clone containing the unrearranged *Fli*-2 locus from a BALB/c genomic library (λC2.2 [Fig. 1]). Genomic DNAs from various Friend cell lines were digested with the restriction enzymes *Bam*HI, *Eco*RI, *Hin*dIII, or *Eco*RV. As shown in Fig. 2, probe A detected DNA fragments of abnormal sizes in the F-MuLV cell lines CB3 and CB7 and the FV-P-induced cell line DP28-9 compared with normal genomic mouse DNA. Furthermore, this analysis showed that the CB3 cell line has lost the normal *Fli*-2 allele (Fig. 2). From the restriction enzyme analysis in Fig. 2, it appears that *Fli*-2 is rearranged in the CB3 and CB7 cell lines as a result of F-MuLV integration at closely located sites and in the same transcriptional orientation (Fig. 1).

Southern blot analysis of the *Fli*-2 locus in the FV-P-induced cell line DP28-9 also suggested that *Fli*-2 was rearranged as a result of a proviral integration event. Restriction enzyme digestion of DP28-9 genomic DNA with *Hin*dIII and *Eco*RI, followed by Southern blotting with the *Fli*-2 probe, gave novel 10.5-kbp *Hin*dIII and 15-kbp *Eco*RI fragments, as well as the 4.2-kbp *Hin*dIII and 9.5-kbp *Eco*RI DNA fragments present in normal DNA from the DBA/2J mouse strain (Fig. 2B and D). Because the SFFV-P provirus does not contain restriction sites for either *Hin*dIII or *Eco*RI and is 6.2 kbp in size, insertion of an SFFV provirus within a region bounded by the 4.2-kbp *Hin*dIII or 9.5-kbp *Eco*RI sites of the *Fli*-2 locus would be expected to increase the sizes of these fragments to approximately 10.5 and 15 kbp, respectively. Thus, these results are also consistent with the insertion of SFFV-P in the *Fli*-2 locus of the DP28-9 erythroleukemia cell line. From the sizes of the rearranged *Eco*RV and *Bam*HI DNA fragments in DP28-9, we have tentatively localized the site of SFFV-P proviral integration, as shown in Fig. 1.

Isolation of cDNA corresponding to the *Fli*-2 locus. To determine the transcriptional domain affected as a result of proviral integrations within *Fli*-2, we searched for expression of RNA transcripts from various regions that immediately flank the *Fli*-2 integration site. The ^{32}P-labelled 3-kbp

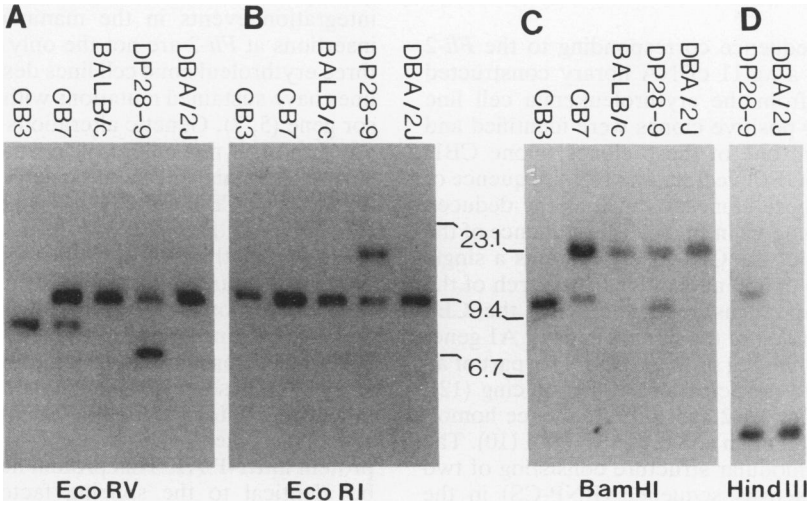

FIG. 2. Identification of the common integration site *Fli*-2 in Friend erythroleukemia cell lines. High-molecular-weight DNA (10 μg) was extracted from various erythroleukemia cell lines and digested with the restriction enzymes *Eco*RV (A), *Eco*RI (B), *Bam*HI (C), and *Hin*dIII (D). The DNA was separated electrophoretically on a 0.8% agarose gel and transferred to nitrocellulose. The filters were hybridized to probe A (Fig. 1).

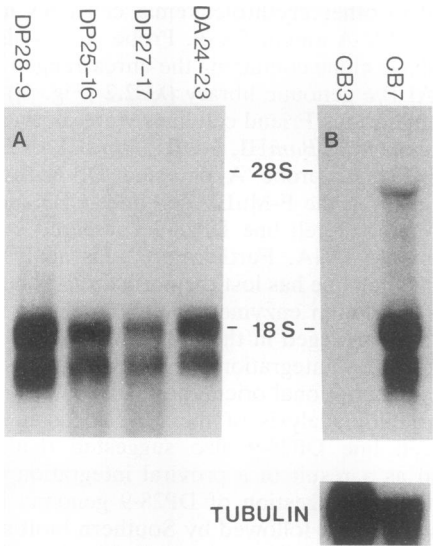

FIG. 3. *Fli*-2 expression in Friend erythroleukemia cell lines. (A and B) Poly(A)$^+$-selected RNA (5 μg) was prepared from various erythroleukemia cell lines, denatured with formamide, separated electrophoretically on a 1% agarose gel, transferred to nitrocellulose, and hybridized with the probe B, as described in the legend to Fig. 1. The filter was stripped by being washed twice in 10 mM Tris (pH 7.5)–1 mM EDTA–0.1% SDS at 70°C and rehybridized with a tubulin probe to check for uniform RNA loading.

*Sal*I-*Eco*RI fragment isolated from the phage DNA λC2.2 genomic insert (probe B in Fig. 1) detected 1.5- and 2.0-kb RNA transcripts in various erythroleukemia cell lines with (DP28-9 and CB7 cells) or without (DP25-16 and DP27-17 cells) *Fli*-2 rearrangement (Fig. 3A and B); however, transcripts were not detected with this probe in the CB3 cell line (Fig. 3B). Both the 2.0- and the 1.5-kb transcripts were highly expressed in Friend erythroleukemia cell lines, which was comparable to the levels of tubulin transcripts in these cells (Fig. 3A and B). The 1.5- and 2.0-kb transcripts appear to be expressed to equal levels in various erythroleukemia cells induced by different strains of Friend leukemia virus (Fig. 3A and B).

To characterize the sequence corresponding to the *Fli*-2 transcript, we screened a λgt11 cDNA library constructed from mRNA prepared from the erythroleukemia cell line CB7 with probe B. Four positive clones were identified and isolated. The insert from one of these clones (clone CB1) was subcloned into the pGEM vector. The DNA sequence of the insert from one of these clones, as well as the deduced amino acid sequence, is shown in Fig. 4. The sequence of the entire 1,443 nucleotides of the CB1 insert contains a single long open reading frame of 960 nucleotides. A search of the GenBank and EMBL data bases revealed that the CB1 cDNA is the mouse homolog of the human hnRNP A1 gene, which encodes an RNA-binding protein that forms part of an RNP complex thought to participate in RNA splicing (12). The open reading frame of *Fli*-2 has 100% sequence homology with the α isoform of human and rat hnRNP A1 (10). The A1 protein has a highly modular structure consisting of two copies of an RNP consensus sequence (RNP-CS) in the amino-terminal portion of the molecule involved in RNA recognition and binding as well as auxiliary domains possibly involved in interactions with other proteins (1). As shown in Fig. 4, the A1 protein contains two copies of an 80- to 90-amino-acid domain, each containing a very highly conserved RNP-CS octapeptide RGFG/AFVTY/F−.

The transcriptional orientation of hnRNP A1 mRNA within the *Fli*-2 locus. To determine the site of proviral integration within or adjacent to the transcriptional coding sequences of the *Fli*-2 gene, we sequenced part of the probe B that recognizes two expected hnRNP A1 transcripts on Northern blots (10). As shown in Fig. 1, the sequence of the 401-bp *Hin*dIII-*Eco*RI fragment located 3′ to probe B precisely matches the 401-bp *Hin*dIII-*Eco*RI sequences located in the 3′ untranslated region of CB1 cDNA (Fig. 4, underlined sequences). These results demonstrate that proviruses are integrated downstream of the *Fli*-2 transcriptional domain in the CB3, CB7, and DP28-9 cell lines. Furthermore, the CB3 cell line appears to be homozygous for the rearranged *Fli*-2 locus, suggesting that the integration of F-MuLV within the 3′ transcriptional domain of this locus results in complete loss of hnRNP A1 expression, as shown in Fig. 3B.

Expression of hnRNP A1 protein in erythroleukemia cell lines. To determine the effect of proviral integration within the *Fli*-2 locus on hnRNP A1 expression, we carried out Western blot analysis with polyclonal hnRNP A1 antibody (as described in Materials and Methods). In agreement with the RNA analysis, the Western blot analysis confirmed that CB3 cells fail to express A1 protein. In contrast, hnRNP A1 was expressed in both CB7, a cell line which contains both unrearranged and rearranged *Fli*-2 loci, and another FV-P-induced erythroleukemia cell clone, DP27-17, which has an apparently unaltered *Fli*-2 locus (Fig. 5). As positive controls, we used human HeLa cells and purified recombinant A1 protein in this analysis. The polyclonal A1 antiserum also recognizes two unknown high-molecular-weight bands.

DISCUSSION

By analyzing the sites of proviral integration in cell clones that emerge in the spleens of mice infected with F-MuLV, we have identified a novel integration site, *Fli*-2, that maps immediately downstream of the gene for murine hnRNP A1. Although not as frequent as integration events at the *Fli*-1 or *Spi*-1 locus, *Fli*-2 was an integration target in 3 of 30 independently derived erythroleukemic cell lines, a frequency that is much higher than expected for random integration events in the mammalian genome. Retroviral insertions at *Fli*-2 are not the only genetic alterations in the three erythroleukemic cell lines described here. All three cell lines have sustained mutations within the p53 tumor suppressor gene (5, 7). Genetic alterations in this gene appear to be a common, if not universal, event in the evolution of both Friend leukemia and a wide spectrum of human malignancies (2, 21). The CB3 and CB7 cell lines also have an activated *Fli*-1 locus as the result of F-MuLV integration immediately upstream of this *ets* oncogene family member (3). Thus, during the course of Friend leukemia, F-MuLV can act as a potent insertional mutagen, activating dominant oncogenes and inactivating tumor suppressor genes, as the result of independent proviral-integration events.

By cloning sequences immediately contiguous to the *Fli*-2 integration sites, we have identified a transcript whose sequence is identical to that of the gene for the RNA-binding protein hnRNP A1. This protein has recently been shown to be identical to the splicing factor SF5 (23). hnRNP A1 appears to act in concert with another splicing factor, SF2, to affect splice site selection. Given substrates that contain duplicated proximal and distal 5′ splice sites, SF2 favors proximal 5′ splice sites. Increasing the ratio of hnRNP A1 to

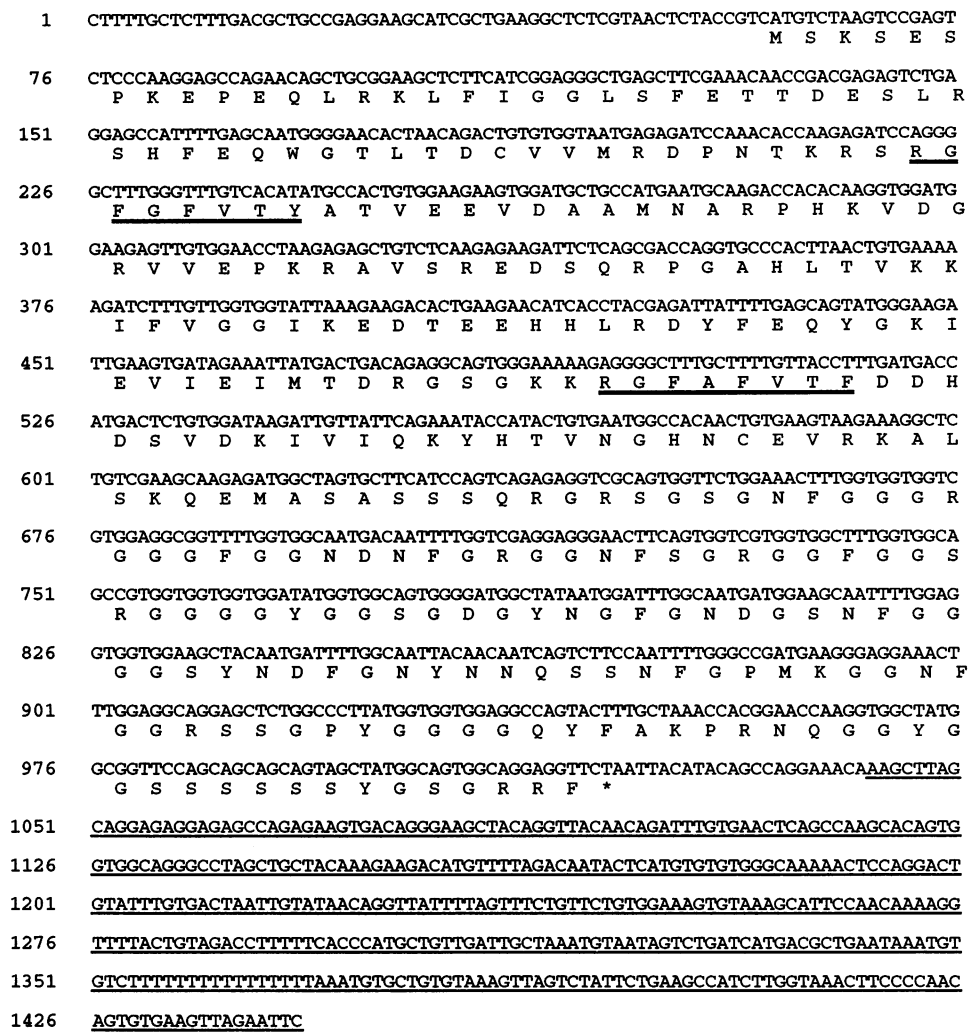

FIG. 4. Sequence analysis of a transcript corresponding to the *Fli*-2 locus. The sequence of the 1.4-kb CB1 insert cDNA corresponding to the *Fli*-2 junction fragment and its deduced amino acid sequence are shown. The termination codon is marked with an asterisk. The 401-bp carboxy terminus of CB1 cDNA corresponding to the 401-bp *Hin*dIII-*Eco*RI fragment of probe B is underlined (see Fig. 1). The two copies of the RNP-CS are doubly underlined.

SF2 favors the utilization of distal 5′ splice sites. Thus, hnRNP A1 appears to counterbalance the preference of SF2 for proximal 5′ splice sites; however, hnRNP A1 does not inhibit splicing reactions mediated by the essential splicing factor, SF2. Because neither SF2 nor hnRNP A1 exhibit specificity for particular splice sites, changes in the ratio of these two splicing factors might have a marked effect on gene expression by broadly regulating splice site selection. Thus, changes in the levels of hnRNP A1 expression might be expected to have a profound effect on cellular phenotype.

The CB3 cell line fails to express detectable hnRNP A1 RNA or protein and is homozygous for the retroviral insertion into the *Fli*-2/A1 locus. Thus, in this cell line, two events around *Fli*-2 have occurred: retroviral insertion and reduction to homozygosity. Two important conclusions can be drawn from analysis of the CB3 cell line. First, it is clear that A1 expression is not required for cell viability and cell growth. The loss of the unrearranged allele in this cell line makes it unlikely that CB3 cells are making low but undetectable amounts of A1 protein; rather, these data strongly argue that the insertion of F-MuLV in this cell has completely inactivated the A1 gene. The availability of a cell line that fails to express any A1 protein should be very useful for studying the normal role of this RNA-binding protein in RNA processing and splice site selection in an intact cell. Second, gene inactivation and reduction to homozygosity are a hallmark of tumor suppressor genes whose inactivation is required for evolution of the tumorigenic phenotype. Thus, the results with the CB3 cell line are consistent with the notion that the *Fli*-2/A1 locus is a tumor suppressor gene and raise the possibility that, at least in some cell lines, A1 expression may act in a negative way on the leukemic phenotype.

In contrast to these observations with CB3, the cell lines CB7 and DP28-9 expressed high levels of both A1 RNA and A1 protein and the sequence of the A1 RNA transcript from CB7 was identical to that of one of the isoforms of this gene. Although we have no simple interpretation of these results, they suggest that there is selection for alterations in A1 gene expression during the evolution of Friend leukemia. Another possible explanation of these results is that the expression of another transcriptional domain, located further downstream

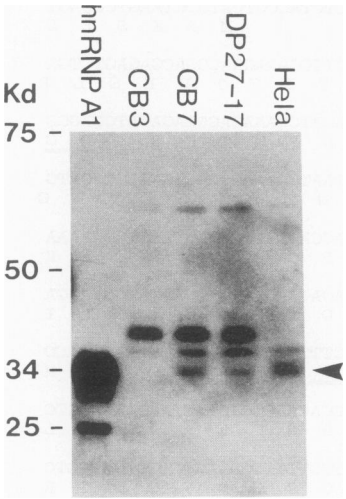

FIG. 5. Expression of A1 protein in Friend erythroleukemia cell lines. Total protein (20 μg) extracted from the erythroleukemia cell lines CB3, CB7, and DP27-17 and human HeLa cells were electrophoretically separated on an SDS–9% polyacrylamide gel and transferred to nitrocellulose. The membrane was probed with polyclonal anti-hnRNP A1 antibody (1/2,000) and developed with ^{125}I-protein A. Purified recombinant A1 protein was used as a positive control (11). The location of the 34-kDa hnRNP A1 protein is indicated with the arrow.

of the *Fli*-2 proviral integration site, may be altered because of retroviral insertions into *Fli*-2. To test this possibility, we have isolated a 10-kbp DNA fragment located downstream of the *Fli*-2 proviral integration site and shown that this DNA does not detect any transcripts in Northern blots prepared from RNA of various Friend cell lines (data not shown).

How might hnRNP A1 contribute to oncogenesis? Molecular characterization of other genes involved in malignant transformation has suggested that proteins that regulate the normal processes of cell proliferation and gene expression are key targets in the neoplastic process (8). These molecules include growth factors and their receptors, cytoplasmic signalling proteins involved in transducing extracellular signals to the nucleus, and nuclear transcription factors. In addition, overexpression of the RNA-binding protein and translation initiation factor eiF-4E results in malignant transformation of fibroblasts (19). Recent observations have suggested that nuclear RNA-binding proteins also participate in the control of gene expression by modulating splicing decisions during development. For example, sex determination in *Drosophila* species is controlled by sex-specific RNA splicing mediated by the products of the sex-lethal (*sxl*) and transformer-2 (*tra*-2) genes (9, 29). *sxl* and *tra*-2 proteins are RNA-binding proteins that regulate sex-specific alternative splicing by, respectively, blocking and activating distinct 3′ splice sites (14, 16). hnRNP A1 shares with *sxl* and *tra*-2 the conserved RNP-CS motif (1), and as discussed above, A1 appears to be identical to SF5, a protein recently shown to be involved in splice site selection (23). Perturbations in hnRNP A1 synthesis may therefore contribute to the malignant phenotype by differentially affecting the utilization of specific splice sites in transcripts that encode proteins involved in cell growth control (22, 35). Furthermore, increases in the levels of hnRNP A1 have been associated with the transition from a quiescent to a proliferating state (20, 31), and there are several examples of transformation-associated changes in the alternative splicing patterns of several genes (13, 36).

In summary, by analyzing the common sites of integration of F-MuLV in Friend erythroleukemia cell lines, we have identified a novel locus, *Fli*-2, that is rearranged in three independent Friend cell clones. The coding region immediately adjacent to *Fli*-2 encodes hnRNP A1, a protein recently shown to be involved in donor splice site selection. These observations raise the intriguing possibility that changes in alternative splicing directly contribute to the oncogenic phenotype and therefore that this class of genes might act as oncogenes or tumor suppressor genes in other malignancies. Finally, this analysis has also uncovered a Friend cell line that fails to express detectable levels of hnRNP A1 as the result of a retroviral insertional inactivation event of *Fli*-2. This latter observation provides direct evidence that hnRNP A1 is not essential for cell viability or cell growth.

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