MOLECULAR AND CELLULAR BIOLOGY, Nov. 1994, p. 7604–7610
0270-7306/94/$04.00+0

Vol. 14, No. 11

Breakpoint Junctions of Chromosome 9p Deletions in Two Human Glioma Cell Lines

HELEN M. POMYKALA, STEFAN K. BOHLANDER, PAMELA L. BROEKER, OLUFUNMILAYO I. OLOPADE, AND MANUEL O. DÍAZ*

Section of Hematology/Oncology, Department of Medicine, and The University of Chicago Cancer Research Center, The University of Chicago Pritzker School of Medicine, Chicago, Illinois 60637

Interstitial deletions of the short arm of chromosome 9 are associated with glioma, acute lymphoblastic leukemia, melanoma, mesothelioma, lung cancer, and bladder cancer. The distal breakpoints of the deletions (in relation to the centromere) in 14 glioma and leukemia cell lines have been mapped within the 400 kb *IFN* gene cluster located at band 9p21. To obtain information about the mechanism of these deletions, we have isolated and analyzed the nucleotide sequences at the breakpoint junctions in two glioma-derived cell lines. The A1235 cell line has a complex rearrangement of chromosome 9, including a deletion and an inversion that results in two breakpoint junctions. Both breakpoints of the distal inversion junction occurred within AT-rich regions. In the A172 cell line, a tandem heptamer repeat was found on either side of the deletion breakpoint junction. The distal breakpoint occurred 5′ of *IFNA2*; the 256 bp sequenced from the proximal side of the breakpoint revealed 95% homology to long interspersed nuclear elements. One- and two-base-pair overlaps were observed at these junctions. The possible role of sequence overlaps, and repetitive sequences, in the rearrangements is discussed.

Several chromosomal mechanisms leading to the loss of function of putative tumor suppressor genes and the subsequent abnormal growth and proliferation of cancer cells have been described previously (16). Such mechanisms include point mutations, somatic crossing over, deletions and unbalanced translocations, and chromosome nondisjunction. The molecular cloning and characterization of several tumor suppressor genes, such as *TP53* on chromosome 17p13 (19, 43), *RB1* on chromosome 13q14 (13), *WT1* on chromosome 11p13 (5), and *APC* on chromosome 5 (25), have greatly expanded our understanding of the role of tumor suppressor genes in the development of cancer. However, the molecular mechanisms underlying the interstitial deletions and unbalanced translocations associated with tumor suppressor genes have not been well studied.

Recent data indicate that unbalanced translocations or interstitial deletions of the short arm of chromosome 9 [del(9p)] are recurring chromosomal abnormalities in a variety of tumor types, including acute lymphoblastic leukemia, glioma, melanoma, lung cancer, head and neck cancer, mesothelioma, ovarian cancer, and bladder cancer (1, 2, 7, 8, 23, 28, 29, 35, 40, 42). Through molecular analysis, homozygous deletions of DNA sequences or losses of heterozygosity on 9p in a significant proportion of these tumors have been described (4, 10–12, 20, 24, 29, 31–33). Although the lengths and locations of these deletions vary, there is a common region of deletion at band 9p21. This suggests the presence of a tumor suppressor gene in this region, whose inactivation contributes to the malignant process in all these different tumor types. The molecular studies with the different tumor types have demonstrated that the deletions involving 9p are sometimes interstitial and often include homozygous deletions of all or part of the interferon (*IFN*) gene cluster (10, 11, 20, 32, 33). A number of the cell lines and patient samples with *IFN* gene deletions also lack methylthioadenosine phosphorylase enzyme activity (11). The gene coding for methylthioadenosine phosphorylase (*MTAP*) was previously mapped to 9p (6). Because homozygous submicroscopic deletions often include both the *IFN* gene cluster and the *MTAP* gene, we suggested that these genes, or a gene very closely linked to them, are the critical tumor suppressor genes involved in the deletions (10, 11, 32).

In an effort to clone and to characterize the putative tumor suppressor gene, we have constructed a detailed long-range restriction map of the *IFN* gene cluster on 9p21 by using yeast artificial chromosome (YAC) clones and cosmid clones (9) (Fig. 1). We were able to identify 26 *IFN* genes and pseudogenes which were clustered within a 400-kb region. We identified several cell lines with deletions which extended from the *IFN* gene cluster to include more proximal sequences. Many of the distal deletion breakpoints mapped within, or distal to, the interferon gene cluster (distal and proximal are used in relation to the centromere). However, a few cell lines had deletions that excluded the *IFN* gene cluster, suggesting that some other gene or genes proximal to it are the relevant genes. *CDKN2* (previously called *INK4*, *MTS1*, or *CDK4I*), a gene encoding an inhibitor of CDK4 (38), a cyclin-dependent protein kinase involved in cell cycle control, was recently mapped to the smallest region of overlap of 9p deletions in a large variety of tumor cell lines (24, 31). The approximate locations of the deletion breakpoint junctions in several neoplastic cell lines were mapped within the *IFN* gene cluster, allowing us to clone and analyze the sequences at the breakpoints.

This report describes the molecular analysis of the deletion breakpoint junctions in two glioma-derived cell lines. Analysis of the nucleotide sequences directly involved in the breakpoints has provided information which might be generally applicable to interstitial deletions associated with tumor suppressor genes.

MATERIALS AND METHODS

Cell lines. S. A. Aaronson provided the A1235 glioma cell line. The A172 cell line was isolated from a patient with a

* Corresponding author. Present address: Loyola University Cancer Center, 2160 South First Ave., Maywood, IL 60153. Phone: (708) 327-3172. Fax: (708) 327-3173.

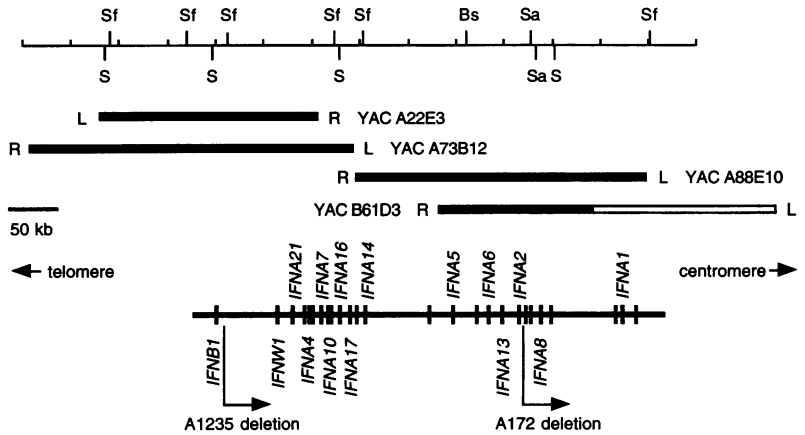

FIG. 1. Diagram of the YAC clones used in this work. The filled bars represent inserts from chromosome 9 containing *IFN* gene sequences. Open bars represent inserts from another chromosome artifactually linked during cloning. The YAC inserts are aligned with a restriction map of the region and with a map of the interferon genes. The vertical black bars represent true *IFN* genes (their names are indicated); vertical gray bars represent *IFN* pseudogenes. R and L indicate the right and left ends of the YACs, respectively. Bs, *Bss*HI; S, *Sal*I; Sa, *Sac*II; Sf, *Sfi*I. Additional YACs from this region have been studied; a comparison of their restriction maps (9) with maps from phage and cosmid clone contignations (17) suggests that the inserts in these YACs are not rearranged.

malignant glioma and was obtained from the American Type Culture Collection. Abnormalities involving the *IFN* gene cluster in A1235 and A172 cells have been described previously (29, 33). The BV173 cell line isolated from a patient with chronic myelogenous leukemia in blast crisis (34) has a normal configuration of the *IFN* gene cluster (11).

DNA isolation and analysis. Total cellular DNAs from the cell lines were prepared as previously described (39). YAC clones were obtained from the St. Louis YAC library after screening of the library with a consensus primer for the *IFNA* genes (9). A complete physical map of these YACs has been described previously (9). YAC cultures were grown and harvested as described previously (21). YAC DNAs in solution and in agarose were prepared according to previously described protocols (21). High-molecular-weight DNAs were digested with restriction endonucleases, electrophoresed in a 1% agarose gel for separation, transferred to a nylon membrane (GeneScreen Plus; New England Nuclear), and hybridized to radiolabeled probes as previously described (39).

Genomic clones. A human genomic library, prepared from partially *Sau*3AI-digested Caucasian male placenta DNA ligated to the *Xho*I sites of lambda Fix II vector, was obtained from Stratagene Cloning Systems, La Jolla, Calif. All lambda bacteriophage and pBluescript SK+ plasmid vectors were obtained from Stratagene Cloning Systems. Genomic libraries from the A1235 and A172 cell lines were prepared according to the manufacturer's protocol. A1235 cell line DNA was digested to completion with *Xba*I and ligated to the *Xba*I-digested arms of the lambda DASH bacteriophage vector. A172 cell line DNA was digested to completion with *Hin*dIII and ligated to the *Hin*dIII-digested arms of the lambda DASH bacteriophage vector. YAC B61D3 DNA was ligated to *Hin*dIII-digested arms of the lambda DASH bacteriophage vector after being completely digested with *Hin*dIII; YAC A73B12 and YAC A88E10 were digested to completion with *Bam*HI or *Hin*dIII and ligated to the arms of the EMBL3 bacteriophage vector. The ligated DNAs were packaged into bacteriophage by using Gigapack II Gold packaging extract (Stratagene Cloning Systems) and were then plated and screened by using Colony/Plaque Screen hybridization filters (New England Nuclear). To block repetitive sequences, Cot 1 human DNA (Bethesda Research Laboratories) was added to the labeled probe and to the hybridization mixture according to the manufacturer's instructions. Bacteriophage clones were digested with the appropriate enzyme (New England Nuclear), and the fragments of interest were ligated to the plasmid vector pBluescript SK+.

DNA probes. The human *IFNA2* and *IFNB* cDNA inserts in the pL-fA and pHFb plasmids, respectively (30, 36), were obtained from P. Pitha-Rowe. Phage and plasmid clones were digested with appropriate enzymes and electrophoresed on an agarose gel, and the fragments of interest were primed with random hexanucleotides and labeled with Klenow fragment and [α-^{32}P]dCTP by using an Oligolabeling kit (Pharmacia).

Method of sequence determination. Double-stranded sequencing was performed by the Sanger dideoxy chain termination method by using a Sequenase version 2.0 kit (United States Biochemical). Sequencing reactions were run on 6% polyacrylamide gels (Sequagel; National Diagnostics, Atlanta, Ga.).

The following primers used for sequencing were synthesized on an automated Applied Biosystems synthesizer: Nt2, 5′-CG CGCAATTAACCCTCACTA-3′; A172.Bam.2, 5′-AGACTCT CAGAGATTAA-3′; and A1235G, 5′-GATACAGTGTTACA GCTAT-3′.

RESULTS

We showed that all *IFN* genes proximal to *IFNB* are homozygously deleted in A1235 cells and that all *IFN* genes proximal to *IFNA2* are homozygously deleted in A172 cells by using a *Hin*dIII Southern blot of these cell lines (32). Both cell lines retain one allele of the *IFNB* gene, whereas A172 retains one allele of all *IFNA* and *IFNW* genes distal to *IFNP11*, with the breakpoint occurring between *IFNA2* and *IFNP11* (29, 32). We cloned the breakpoint junctions in both cell lines.

Cloning of the A1235 breakpoint region. (i) Unrearranged *IFNB* clone. The region surrounding the *IFNB* gene was previously mapped by Gross et al. (14). As reported by Miyakoshi et al. (29), the 1.8-kb *Eco*RI fragment containing *IFNB* was not rearranged in A1235 cells; however, the 10-kb *Hin*dIII fragment was rearranged to become a 3.3-kb fragment

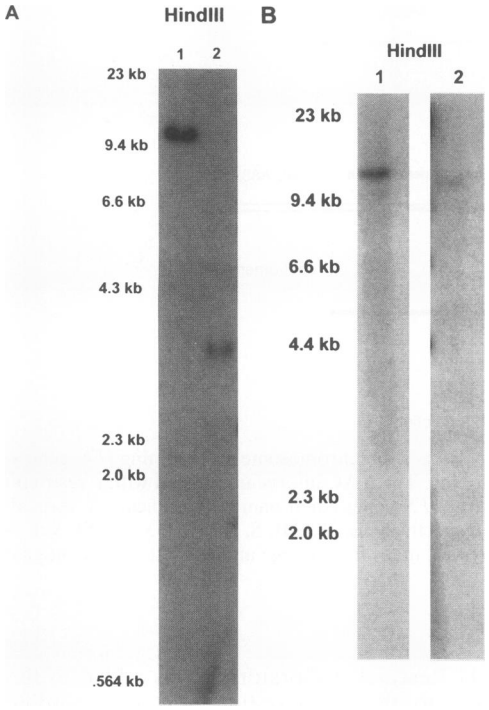

FIG. 2. (A) Southern blot of *Hin*dIII digests hybridized to an *IFNB* gene probe. Lane 1, genomic placenta DNA showing a normal 10-kb fragment; lane 2, A1235 cell line DNA showing a rearranged 3.3-kb fragment. (B) Southern blot of *Hin*dIII digests hybridized to an *IFNA2* gene probe. Lane 1, human placenta DNA showing a normal 11.8-kb fragment; lane 2, A172 cell line DNA showing a rearranged 11.1-kb fragment.

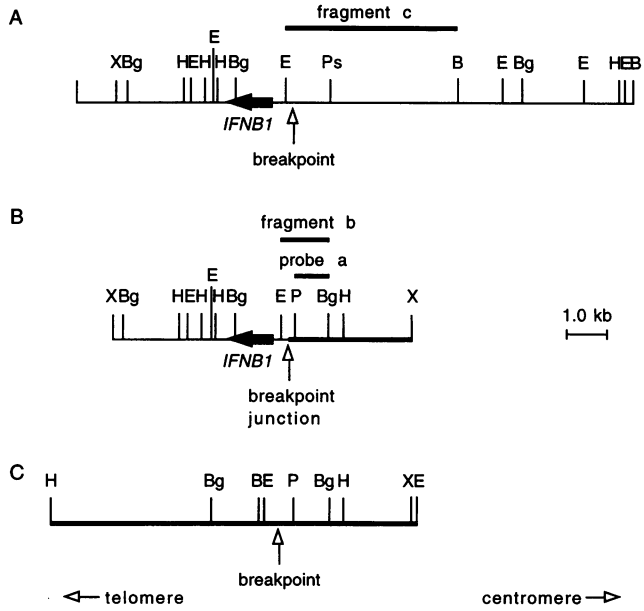

FIG. 3. (A) Restriction map of a normal *IFNB* clone containing the A1235 inversion-deletion breakpoint located within the 5′ *Eco*RI fragment 653 kbp 5′ of the ATG start codon of the *IFNB* gene (fragment c). (B) Restriction map of rearranged A1235 clone containing the inversion-deletion breakpoint junction. (C) Distal normal clone with the A1235 inversion-deletion breakpoint region located 3′ of the *Pvu*II site. H, *Hin*dIII; Bg, *Bgl*II; B, *Bam*HI; P, *Pvu*II; Ps, *Pst*I; E, *Eco*RI; X, *Xba*I. Not all restriction sites have been mapped.

(Fig. 2A). This places the breakpoint junction in A1235 cells within the 4.2-kb *Eco*RI fragment (fragment c) 5′ of *IFNB* and not more than 1.3 kb 5′ of the promoter region of the gene (Fig. 3A). Using the *IFNB* cDNA probe, we screened a phage library prepared from the YAC clone A73B12 (9) (Fig. 1), obtaining a 13.7-kb *Bam*HI insert that contained the unrearranged *IFNB* region (with one of the A1235 breakpoints). We subcloned fragment c into a plasmid, and starting from the *Eco*RI site immediately upstream of the *IFNB* gene, we sequenced 441 bp. This sequence was compared with the sequence of the rearranged chromosome to localize the breakpoint junction.

(ii) A1235 breakpoint junction. By using the *IFNB* cDNA probe, a 7.5-kb rearranged *Xba*I *IFNB* clone was isolated from a genomic phage library prepared from the A1235 cell line. This 7.5-kb *Xba*I insert was subcloned into a plasmid and mapped with restriction endonucleases (Fig. 3B). The 1.25-kb *Eco*RI-*Bgl*II fragment 5′ of *IFNB* (fragment b) was subcloned into pBluescript SK+ and partially sequenced. The sequence diverged from that of the *IFNB* unrearranged clone 300 bp after the *Eco*RI site, indicating the location of the breakpoint junction.

(iii) Normal DNA sequences containing the A1235 proximal breakpoint. To clone the proximal normal genomic sequences around the A1235 breakpoint, the proximal portion of the 1.25-kb rearranged clone was isolated by digestion with *Pvu*II and *Not*I. *Pvu*II cuts within the proximal region of the insert, 23 bp away from the breakpoint junction, and *Not*I cuts within the polylinker of the plasmid vector. The resulting 900-bp fragment (probe a in Fig. 3B) was used to screen a lambda FIX genomic library from human placental DNA. A phage clone containing a 9-kb human DNA insert was isolated. A 6.5-kb *Bgl*II fragment from within the isolated phage (Fig. 3C) was subcloned into a plasmid. By using primer A1235G from the upstream sequence of the rearranged A1235 clone, 347 bp spanning the breakpoint region was sequenced. The breakpoint occurred after 151 bp. To localize the position of probe a on our YAC contig, the probe was hybridized to Southern blots containing the various YACs (9). The results showed hybridization to a region of YAC A73B12 (9) (Fig. 1) which contains sequences distal to *IFNB*. This suggests a more complicated mechanism of rearrangement consisting of a deletion of material proximal to *IFNB* and inversion of a distal fragment containing *IFNB* (Fig. 4A). The breakpoint junction and normal sequences are aligned in Fig. 6A.

Cloning of the A172 breakpoint regions. (i) Normal *IFNA2* clone. Because all *IFN* genes proximal to *IFNA2* are deleted in A172 cells, we used the *IFNA2* gene to screen a phage library prepared from *Hin*dIII fragments of YAC A88E10. We isolated a phage clone with a 10-kb *Hin*dIII insert. The insert was restriction mapped (Fig. 5A), and the transcriptional direction of the gene within the clone was determined after comparison with the partial map produced by Henco et al. (17). The transcriptional orientation of the *IFNA2* gene is towards the telomere, i.e., it is the same as that of the *IFN* genes distal to it (9); therefore, the deletion breakpoint region was predicted to fall 5′ of *IFNA2*. Because Southern blot analysis of *Hin*dIII-digested A172 DNA showed the presence of the normal unrearranged 10-kb fragment, we walked further 5′ by screening a *Bam*HI library prepared from YACA88E10 (9) with probe d (Fig. 5A). A 12-kb normal clone was isolated and mapped (Fig. 5B). Probe e, isolated from the *Bam*HI clone, detected an 11.8-kb normal *Hin*dIII band in human placental

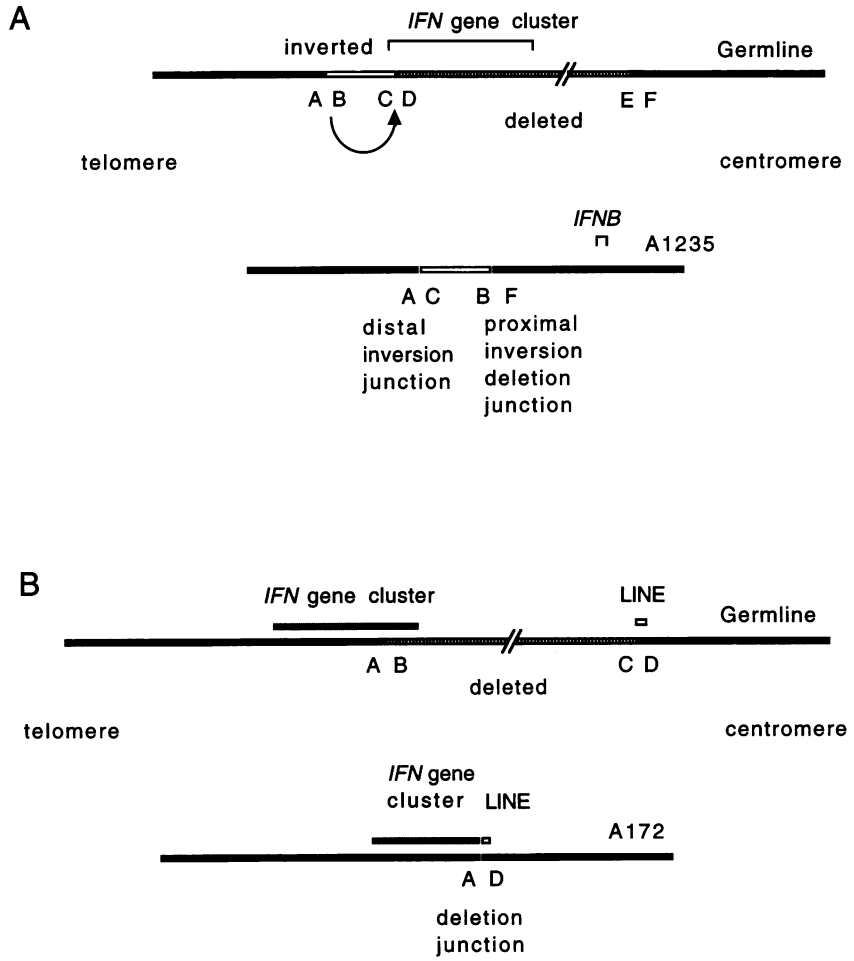

FIG. 4. Diagram of the rearrangements observed in the cell lines A1235 and A172, according to our interpretation. The ends of the inverted and deleted segments have been labeled with letters to better show their fate. The deleted segment in both cell lines is relatively large, while the inverted segment in A1235 is smaller than 200 kb. The *IFN* gene cluster is represented by a gray solid bar, and the LINE sequence is represented by an empty box (not drawn to scale).

DNA and an 11.1-kb rearranged *Hin*dIII band in A172 DNA (Fig. 2B). Probe f (Fig. 5B) was used to isolate the normal clone from the YAC B61D3 library and the rearranged clone from the A172 genomic library. A restriction map of both clones (Fig. 5C and D) places the distal breakpoint of A172 cells within the 3.0-kb *Eco*RI-*Bgl*II fragment downstream of interferon pseudogene 11 (*IFNP11*).

(ii) Subcloning and analysis of the A172 breakpoint junction. The rearranged 3.6-kb *Bam*HI fragment (p3.6BA72) and the distal normal 3.0-kb *Bgl*II-*Eco*RI fragment (p3BgEA72) were subcloned into a plasmid. We sequenced from the proximal *Bam*HI site of p3.6BA72 for a distance of 366 bp. A primer (A172.Bam.2) for the inverted complement of the most distal sequence read was used to sequence p3BgEA72 in an effort to cross the breakpoint region of the normal clone. Comparison of sequences from both clones showed that the breakpoint junction of A172 cells falls 256 bp distal from the proximal *Bam*HI site of p3.6BA72. At the breakpoint junction of the rearranged clone we found two tandem heptamer repeats (TGTAGGT) on either side of the breakpoint. Comparison with GenBank sequences showed that the 256 bp of proximal sequence had significant homology to long interspersed nucleotide elements (LINEs). The nucleotide sequences distal to the breakpoint junction are AT rich. The normal and breakpoint junction sequences, aligned with a LINE sequence, are shown in Fig. 6B.

DISCUSSION

Tumor suppressor genes can either be inactivated by point mutation, their normal alleles being lost by somatic recombination or chromosome nondisjunction, or be partially or entirely lost by interstitial chromosomal deletions. Among the chromosomal regions that contain known or putative tumor suppressor genes, some, such as band 9p21 in leukemias and gliomas and band 3p14 in small-cell lung cancer, are associated with deletions at a relatively high frequency compared with other regions containing tumor suppressor genes, such as band 17p13 (*TP53*). The higher frequencies of chromosomal deletions associated with neoplasia at 3p14 and 9p21 may be due to intrinsic fragility or recombinogenicity of closely linked DNA sequences or to the presence of more than one tumor suppressor gene in the region. If the inactivation of two tumor suppressor genes in a small genomic region is necessary to provide the neoplastic cell with a significant advantage, dele-

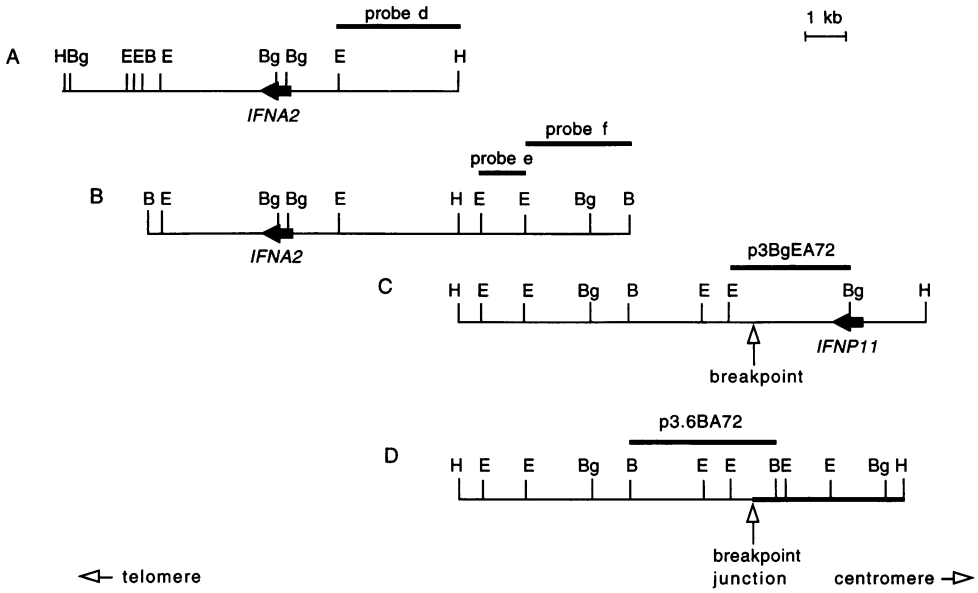

FIG. 5. (A to C) Restriction maps of overlapping phage clones of the *IFN* gene cluster containing *IFNA2* and *IFNP11*. The distal deletion breakpoint region in A172 is located 3′ of *IFNP11* within a 3.0-kb *Eco*RI-*Bgl*II fragment (p3BgEA72). (D) Restriction map of A172 rearranged phage clone containing the breakpoint junction located within the proximal 1.14-kb *Eco*RI-*Bam*HI fragment of p3.6BA72. Probes d, e, and f were used for walking from the distal clone to the proximal clone. H, *Hin*dIII; Bg, *Bgl*II; B, *Bam*HI; E, *Eco*RI. Not all restriction sites have been mapped.

tion will be favored over point mutation as the oncogenic event. As an example, if we assume that inactivating mutations for each of two tumor suppressor genes at 9p21 occur with a frequency of 10^{-8}, the frequency of double mutation will be 10^{-16}; if deletions including both genes occur at a frequency of 10^{-10}, the frequency of deletion will be lower than the frequency of single mutations but much higher than the frequency of double mutation.

In the 9p21 region, deletion breakpoints associated with leukemias, gliomas, melanomas, and other tumors show clustering within a region of 400 kb that contains the type I *IFN* genes (9, 32). Clustering of deletion breakpoints may indicate the presence of particularly fragile or recombinogenic sequences, or it may indicate a boundary region between a tumor suppressor gene and a gene with positive selective value.

The deletion in the glioma cell line A1235 is part of a complex rearrangement that also involves an inversion (Fig. 4A). The sequences proximal to the breakpoint upstream of the *IFNB* gene are deleted. The deletion extends centromerically to include the remainder of the *IFN* gene cluster and other sequences. The telomeric side of this breakpoint has been joined to sequences at distal locations, less than 300 kb downstream of the *IFNB* gene, resulting in an inversion. The breakpoint junction analyzed by us represents the distal junc-

A

```
                                  breakpoint
< cen                            /                          tel >
TTTTATAAGAGGGAATTGAGCATCCTCTGATTTT G GTATCTGTGGAGGCAGCGGGATGGTGGTCCTAGAA IFNB 5' flanking
TTTTATAAGAGGGAATTGAGCATCCTCTGATTTT G AATTTTGTATATTTTTGAAGTTCAGCTGGCATAAG A1235 del. junction
ACTTAAGGAGTGATAGACAGAGATATAAAGAGCA G AATTTTGTATATTTTTGAAGTTCAGCTGGCATAAG distal germline seq.
< tel                           / \                           cen >
                     breakpoint  one nucleotide
                                 overlap
```

B

```
                      breakpoint    heptamer
< tel                           \    repeat                        cen >
GTCAGATGAGTAGGTTGCGAAAATTTTCTCCCAT GT TGTAGGTTGCCTGTTCACTCTGATGGTAGTTTCT  HSLINE-10
TTGTCATTTTGTCAACACATCTACATATTTGTAG GT TGTAGGTTGCCTGTTCACTCTGATGGTAGTTGCT  A172 del. junction
TTGTCATTTTGTCAACACATCTACATATTTGTAG GT GAGGCAAATGGTCAAGATATTTCCTTGTTTTGTC  IFNA2-IFNP11 spacer
< tel                        heptamer| \                           cen >
                             repeat  |  breakpoint
                                     |
                                    dinucleotide
                                    overlap
```

FIG. 6. (A) Alignment of the two normal sequences and the inversion-deletion junction sequence from A1235. There is a 1-nucleotide overlap between the two recombining sequences at the junction. (B) Alignment of the normal sequence from the *IFNA2-IFNP11* spacer region, the deletion junction sequence from A172, and a LINE sequence. There is a 2-nucleotide overlap at the junction. Heptamer repeats present at both recombining ends are underlined.

tion of this inversion-deletion. There is a 1-bp overlap at the junction (Fig. 6A). This overlap may have participated in the recombination process (see below).

The deletion in the glioma cell line A172 seems to be a simple interstitial deletion (Fig. 4B). The breakpoint junction of this deletion joins a LINE sequence at the proximal side, with AT-rich sequences located on the distal side of the *IFNA2* gene. After the normal sequence at the *IFN* gene cluster and the LINE consensus sequence were compared with the junction sequence, a 2-bp overlap involving a GT dinucleotide was found at the junction (Fig. 6B). On the distal side of the junction this GT dinucleotide (underlined) is part of a heptamer, TGTAGGT. The same heptamer is found on the consensus LINE sequence at the proximal side of the junction, preceded by the GT dinucleotide. The deletion results in a tandem repeat of the heptamer at the junction. Overlaps of 1 or more nucleotides are common during nonhomologous recombination in mammalian cells, and they probably play a role in the repair of double-stranded breaks in chromosomes (37). Short sequence overlaps have been observed at the breakpoint junctions of translocations associated with neoplasia, as well as at the junctions of normal gene rearrangements that occur in lymphoid cells (15). In the case of A172 cells, the heptamers may have played a role in transient pairing of the two recombining strands, followed by slippage (to pairing of the dinucleotide) and subsequent repair.

The involvement of a LINE sequence in this deletion poses some questions about the possible role of LINEs in general in chromosome rearrangements. Several deletions in the β-globin gene locus have one breakpoint within a LINE sequence (18). Nevertheless, the frequency of participation of LINE sequences in β-globin deletions is not higher than expected on the basis of their relative length, assuming random scattering of the deletion breakpoints. LINEs use AT-rich DNA sequences as preferred sites of integration (26). The distal side of the breakpoint junction in A172 cells is an AT-rich region, and it is possible that a LINE sequence was transposed into this site and later recombined with another LINE sequence located proximal to the insertion. Deletions of the low-density lipoprotein receptor gene, in the metabolic disorder familial hypercholesterolemia, can be mediated by recombination between *Alu* repetitive sequences (27). Alternatively, it is possible that a proximal LINE sequence had recombined with the distal AT-rich sequences during an abnormal transposition event that did not use a reverse-transcribed LINE sequence as a substrate, but rather a genomic one. Deletions due to abnormal transposition events in bacteria can be mediated by the bacterial transposon Tn*5* (22). These deletions are flanked by one copy of the transposon.

The features common to the deletion breakpoint junctions of A1235 and A172 cells are the presence of small sequence overlaps at the junctions and the association of the junctions with scaffold attachment sites (SARs), AT-rich regions that are enriched in the nuclear-matrix-attached DNA fraction (3). We have shown that most of the individual *IFN* genes are flanked by SARs (3a), and we are at present investigating the relationship of these SARs to the deletion breakpoints in the region. SARs are associated with both ends of the A1235 breakpoint junction and with the distal side of the breakpoint junction in A172 cells (3a). The significance of these associations is unclear at present. However, an association between SARs and breakpoints involved in chromosome rearrangements has been proposed before (41).

ACKNOWLEDGMENTS

We are grateful to S. A. Aaronson and P. Pitha-Rowe for providing cell lines and probes for this work and to Janet D. Rowley for providing support and useful comments.

This work was supported by grants CA49133 (to M.O.D.), CA42557 (to J. D. Rowley), and CA14599 (Cancer Center Core Grant, University of Chicago) from the National Cancer Institute, National Institutes of Health, and by grants from the Louis Block Fund (to O.I.O.) and the J. S. McDonnell Foundation (to O.I.O.).

REFERENCES

1. **Bello, J. M., S. Moreno, and J. A. Rey.** 1990. Involvement of 9p in metastatic ovarian adenocarcinoma. Cancer Genet. Cytogenet. **45**:223–229.
2. **Bigner, S. H., J. Mark, D. E. Ballard, M. S. Malhaley, and D. D. Bigner.** 1986. Chromosomal evolution in malignant gliomas starts with specific and usually numerical deviations. Cancer Genet. Cytogenet. **22**:121–135.
3. **Bode, J., and K. Maass.** 1988. Chromatin domain surrounding the human interferon-B gene as defined by scaffold-attached regions. Biochemistry **27**:4706–4711.

3a. **Broeker, P. L., et al.** Unpublished results.

4. **Cairns, P., M. E. Shaw, and M. A. Knowles.** 1993. Preliminary mapping of the deleted region of chromosome 9 in bladder cancer. Cancer Res. **53**:1230–1232.
5. **Call, K. M., T. M. Glaser, C. Y. Ito, A. J. Bockler, J. Pelletier, D. A. Haber, E. A. Rose, A. Kral, H. Yager, W. H. Lewis, C. A. Jones, and D. E. Housman.** 1990. Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus. Cell **60**:509–520.
6. **Carrera, C. J., R. L. Eddy, T. B. Shows, and D. A. Carson.** 1984. Assignment of the gene for methylthioadenosine phosphorylase to human chromosome 9 by mouse-human somatic cell hybridization. Proc. Natl. Acad. Sci. USA **81**:2665–2668.
7. **Carrol, A., P. P. Castleberry, and W. Crist.** 1987. Lack of association between abnormalities of the chromosome 9 short arm and either lymphomatous features or T cell phenotype in childhood acute lymphoblastic leukemia. Blood **69**:735–784.
8. **Cowan, J. M., R. Halaban, and U. Francke.** 1988. Cytogenetic analysis of melanocytes from premalignant nevi and melanomas. J. Natl. Cancer Inst. **80**:1159–1164.
9. **Diaz, M. O., H. M. Pomykala, S. K. Bohlander, E. Maltepe, K. Malik, B. Brownstein, and O. I. Olopade.** 1994. Structure of the human type-1 interferon gene cluster determined from a YAC clone contig. Genomics **22**:540–552.
10. **Diaz, M. O., C. M. Rubin, A. Harden, S. Ziemin, R. A. Larson, M. M. LeBeau, and J. D. Rowley.** 1990. Deletions of interferon genes in acute lymphoblastic leukemia. N. Engl. J. Med. **322**:77–82.
11. **Diaz, M. O., S. Ziemin, M. M. Le Beau, P. Pitha, S. D. Smith, R. R. Chilcote, and J. D. Rowley.** 1988. Homozygous deletion of the α- and β1-interferon genes in human leukemia and derived cell lines. Proc. Natl. Acad. Sci. USA **85**:5259–5263.
12. **Fountain, J. W., M. Karayiorgou, M. S. Ernstoff, J. M. Kirkwood, D. R. Vlock, E. L. Titus-Ernstoff, B. Bouchard, S. Vijayasaradhi, A. N. Houghton, J. Lahti, V. J. Kidd, D. E. Housman, and N. C. Dracopoli.** 1992. Homozygous deletions within human chromosome band 9p21 in melanoma. Proc. Natl. Acad. Sci. USA **89**:10557–10561.
13. **Friend, S. H., R. Bernards, S. Rogelj, R. A. Weinberg, J. M. Rapaport, D. M. Albert, and T. P. Dryja.** 1986. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature (London) **323**:643–646.
14. **Gross, G., U. Mayr, W. Bruns, F. Grosveld, H. M. Dahl, and J. Collins.** 1981. The structure of a thirty-six kilobase region of the human chromosome including the fibroblast interferon gene IFN-beta. Nucleic Acids Res. **9**:2495–2507.
15. **Gu, H., I. Förster, and K. Rajewsky.** 1990. Sequence homologies, *N* sequence insertion and J_H gene utilization in V_HDJ_H joining: implications for the joining mechanism and the ontogenetic timing of LY1B cell and B-CLL progenitor generation. EMBO J. **9**:2133–2140.
16. **Hansen, M. F., and W. K. Cavenee.** 1987. Genetics of cancer

predisposition. Cancer Res. **47:**5518–5527.
17. **Henco, K., J. Brosius, A. Fujisawa, J. I. Fujisawa, J. R. Haynes, J. Hochstadt, T. Kovacic, M. Pasek, A. Schambock, S. Schmid, K. Todokoro, M. Walchli, S. Nagata, and C. Weissman.** 1988. Structural relationship of human interferon alpha genes and pseudogenes. J. Mol. Biol. **185:**227–260.
18. **Henthorn, P. S., O. Smithies, and D. L. Mager.** 1990. Molecular analysis of deletions in the human β-globin gene cluster: deletion junctions and locations of breakpoints. Genomics **6:**226–237.
19. **Hollstein, M., D. Sidransky, B. Vogelstein, and C. C. Harris.** 1991. p53 mutations in human cancers. Science **253:**49–53.
20. **James, C. D., V. P. Collins, M. J. Allalunis-Turner, and R. S. Day.** 1993. Localization of chromosome 9p homozygous deletions in glioma cell lines with markers constituting a continuous linkage map. Cancer Res. **53:**3674–3676.
21. **Janssen, K. (ed.).** 1993. Current protocols in molecular biology. Greene Publishing Associates and John Wiley and Sons, New York.
22. **Jilk, R. A., J. C. Makris, L. Borchardt, and W. S. Reznikoff.** 1993. Implications of Tn*5*-associated adjacent deletions. J. Bacteriol. **175:**1264–1271.
23. **Jin, Y., F. Meltens, N. Mondahl, S. Heim, C. Olegard, J. Wennerberg, A. Biorklund, and F. Mitelman.** 1993. Chromosome abnormalities in eighty-three head and neck squamous cell carcinomas: influence of culture conditions on karyotypic pattern. Cancer Res. **53:**2140–2146.
24. **Kamb, A., N. A. Gruis, J. Weaver-Feldhaus, Q. Liu, K. Harshman, S. V. Tavtigian, E. Stockert, R. S. Day III, B. E. Johnson, and M. H. Skolnick.** 1994. A cell cycle regulator potentially involved in genesis of many tumor types. Science **264:**436–439.
25. **Kinzler, K. W., M. C. Nilbert, L. K. Su, B. Vogelstein, T. M. Bryan, D. N. Levy, K. J. Smith, A. C. Preisinger, P. Hedge, D. McKechnie, R. Finniear, A. Markham, J. Groffen, M. S. Boguski, S. S. Altschul, A. Horii, H. Ando, Y. Miyoshi, I. Nishisho, and Y. Nakamura.** 1991. Identification of FAP locus genes from chromosome 5q21. Science **253:**661–665.
26. **Korenberg, J. R., and M. C. Rykowski.** 1988. Human genome organization: *Alu*, LINEs, and the molecular structure of metaphase chromosome bands. Cell **53:**391–400.
27. **Lehrman, M. A., D. W. Russell, J. L. Goldstein, and M. S. Brown.** 1986. Exon-Alu recombination deletes 5 kilobases from the low density lipoprotein receptor gene, producing null phenotype in familial hypercholesterolemia. Proc. Natl. Acad. Sci. USA **83:** 3679.
28. **Lukeis, R., L. Irving, and M. Garson.** 1990. Cytogenetics of non-small cell lung cancer. Analysis of consistent non-random abnormalities. Genes Chromosomes Cancer **2:**116–124.
29. **Miyakoshi, J., K. D. Dobler, J. Allalunis-Turner, J. D. S. McKean, K. Petruk, P. B. R. Allen, K. N. Aronyk, B. Weir, D. Huyser-Wierenga, D. Fulton, R. Urtasun, and R. S. Day III.** 1990. Absence of IFNA and IFNB genes from human malignant glioma cell lines and lack of correlation with cellular sensitivity to interferons. Cancer Res. **50:**278–283.
30. **Mizoguchi, J., P. M. Pitha, and N. K. Raj.** 1985. Efficient expression in *Escherichia coli* of two species of human interferon-a and their hybrid molecules. DNA **4:**221–232.
31. **Nobori, T., K. Miura, D. J. Wu, A. Lois, K. Takabayashi, and D. A. Carson.** 1994. Deletions of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. Nature (London) **368:**753–756.
32. **Olopade, O. I., S. K. Bohlander, H. Pomykala, E. Maltepe, E. Van Melle, M. M. LeBeau, and M. O. Diaz.** 1992. Mapping of the shortest region of overlap of deletions of the short arm of chromosome 9 associated with human neoplasia. Genomics **14:** 437–443.
33. **Olopade, O. I., R. B. Jenkins, D. T. Ransom, K. Malik, H. Pomykala, T. Nobori, J. M. Cowan, J. D. Rowley, and M. O. Diaz.** 1992. Molecular analysis of deletions of the short arm of chromosome 9 in human gliomas. Cancer Res. **52:**2523–2529.
34. **Pegoraro, L., L. Matera, J. Rits, A. Levis, A. Palumbo, and G. Biagini.** 1983. Establishment of a Ph-positive human cell line (BV173). JNCI **70:**447–451.
35. **Pollak, C., and A. Hagemeijer.** 1987. Abnormalities of the short arm of chromosome 9 with partial loss of material in hematological disorders. Leukemia (Baltimore) **1:**541–548.
36. **Raj, N. B., and P. M. Pitha.** 1981. Analysis of interferon mRNA in human fibroblast cells induced to produce interferon. Proc. Natl. Acad. Sci. USA **78:**7426–7430.
37. **Roth, D. B., and J. H. Wilson.** 1986. Nonhomologous recombination in mammalian cells: role for short sequence homologies in the joining reaction. Mol. Cell. Biol. **6:**4295–4304.
38. **Serrano, M., G. J. Hannon, and D. Beach.** 1993. A new regulatory motif in cell cycle control causing specific inhibition of cyclin D/CDK4. Nature (London) **366:**704–707.
39. **Shima, E., M. M. LeBeau, T. W. McKeithan, J. Minowada, L. C. Showe, T. W. Mack, M. D. Minden, J. D. Rowley, and M. O. Diaz.** 1986. T cell receptor alpha chain gene moves immediately downstream of c-myc in a chromosomal 8;14 translocation in a cell line from a human T-cell leukemia. Proc. Natl. Acad. Sci. USA **83:**3439–3443.
40. **Smeets, W., R. Pauwels, L. Laarakkers, F. Debruyne, and J. Geraedts.** 1987. Chromosomal analysis of bladder cancer. III. Nonrandom alterations. Cancer Genet. Cytogenet. **29:**29–41.
41. **Sperry, A. O., V. C. Blasquez, and W. T. Garrard.** 1989. Dysfunction of chromosomal loop attachment sites: illegitimate recombination linked to matrix association regions and topoisomerase II. Proc. Natl. Acad. Sci. USA **86:**5497–5501.
42. **Taguchi, T., S. C. Hhanwar, J. M. Siegfried, S. M. Keller, and J. R. Testa.** 1993. Recurrent deletions of specific chromosomal sites in 1p, 3p, 6q, and 9p in human malignant mesothelioma. Cancer Res. **53:**4349–4355.
43. **Takahashi, T., M. Nau, I. Chiba, M. Birrer, R. Rosenberg, M. Vinocour, M. Levitt, H. Pass, A. Gazdar, and J. Minna.** 1989. p53: a frequent target for genetic abnormalities in lung cancer. Science **246:**491–494.