Structure of a Moloney Murine Leukemia Virus–Virus-Like 30 Recombinant: Implications for Transduction of the c-Ha-*ras* Proto-Oncogene

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Tumor progression locus 2 (*Tpl-2*) encodes a novel serine-threonine protein kinase which is activated by provirus integration in the late stages of oncogenesis in Moloney leukemia virus (MoMuLV) induced rat T-cell lymphomas. In this report, we present evidence that the provirus integrated in the *Tpl-2* locus in 1 of 10 T-cell lymphomas harboring a *Tpl-2* rearrangement (2779) is a recombinant between MoMuLV and virus-like 30 (VL30) sequences (Mo-VL30). Recombination between MoMuLV and VL30 may contribute to the transduction of *ras*, as suggested by the finding that VL30 flanks the *ras* oncogene in all of the *ras* transducing viruses isolated from rats to date. The Mo-VL30 recombinant described here represents evidence that recombination between MoMuLV and VL30 can be uncoupled from the transduction of *ras*, and it may precede the transduction. Sequence comparison between clones of Mo-VL30, Harvey sarcoma virus (Ha-MSV), and genomic c-Ha-*ras* revealed that all three share a 124-bp region of 87.3% homology. This region was detected at nucleotide positions –1845 to –1720 of c-Ha-*ras* and 20 bp 5' of the recombination breakpoint between VL30 and *ras* in Ha-MSV. On the basis of the sequence comparison between VL30, Ha-MSV, and c-Ha-*ras*, we are proposing a model which explains how VL30 may have facilitated the transduction of c-Ha-*ras* and perhaps the other *ras* proto-oncogenes. According to this model, the sequence homology between VL30 and c-Ha-*ras* targets this gene for transduction by promoting the integration of the provirus in this locus through homologous recombination.

Retroviral transduction of members of the *ras* proto-oncogene family in rats has been observed on three separate occasions to date. The outcome of these three independent events was the generation of three acute transforming viruses: Harvey murine sarcoma virus (Ha-MSV) (4), Kirsten murine sarcoma virus (Ki-MSV), and Rasheed murine sarcoma virus (Rasheed MSV) (10). Ha-MSV, Ki-MSV, and Rasheed MSV harbor the *ras* oncogene nested within virus-like 30 (VL30) sequences (2, 15, 17; this report). These observations collectively suggest that *ras* may be transduced by Moloney murine leukemia virus (MoMuLV)-VL30 recombinant viruses.

In this report, we present evidence that a provirus integrated in tumor progression locus 2 (*Tpl-2*) (9) in an MoMuLV-induced rat T-cell lymphoma is a recombinant between MoMuLV and VL30 (Mo-VL30). This finding suggests that the recombination between MoMuLV and VL30 can be uncoupled from the transduction of members of the *ras* proto-oncogene family and may precede the transduction. Sequence analysis of the VL30 portion of the Mo-VL30 recombinant revealed sequence homology with a 124-bp DNA region beginning at nucleotide position –1845 of c-Ha-*ras*. On the basis of this homology and the published sequence of Ha-MSV, we propose a model according to which the transduction of c-Ha-*ras*, and perhaps other members of the *ras* proto-oncogene family, is preceded by the integration of an MoMuLV-VL30 recombinant, by homologous recombination, 5' of the proto-oncogene.

MATERIALS AND METHODS

Tumors and cell lines. The MoMuLV-induced rat T-cell lymphomas and the cell lines derived from them have been described previously (7).

Southern blot analysis of genomic DNA. DNA isolation, restriction endonuclease digestion, agarose gel electrophoresis, Southern blotting, and hybridization were performed as previously described (12). The DNA was blotted and cross-linked by UV light onto nylon membranes (Hybond N; Amersham International). The probes were labeled with [α - 32 P]dCTP by using random priming (Boehringer Mannheim). Following hybridization, the filters were washed at a final stringency of 0.1 $\times$ SSC (1 $\times$ SSC is 0.15 M NaCl and 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate at 65°C for at least 1 h.

Construction of genomic DNA libraries and cloning of genomic DNA. Partial genomic DNA libraries were constructed in the bacteriophage lambda vector λ Dash by using 18- and 16-kb fractions of *Bam*HI-digested genomic DNA from tumor 2779 (12). The partial genomic libraries were screened by using probes 5'-RP and 3'-PP, from the DNA region flanking the 5' and 3' ends, respectively, of the provirus. A normal rat liver genomic DNA library constructed by cloning genomic DNA fragments, generated by partial *Sau*3AI digestion in λ Dash (Stratagene), was screened by using VL30 probe A. Genomic DNA clones were also generated by cloning the products of a polymerase chain reaction carried out by using rat genomic DNA, primers A (5'-CAGCTGCTTAGTATCTAGCA-3') and B (5'-GTCTTTCCTCAGTGCTAA-3'), and *Taq* polymerase (Stratagene).

DNA sequence analysis and sequence comparisons. The insert DNA of the lambda phage clones λ B18 and λ B16, obtained by screening the partial genomic DNA libraries constructed from tumor 2779, were digested with *Xho*I and

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*Bam*HI. The resulting DNA fragments were subcloned into plasmid vector pBluescript SK(–) (Stratagene) and subjected to sequence analysis. The sequences of the subclones were assembled on the basis of overlaps between them, using the Wisconsin sequence package, version 6.1. The sequencing was carried out by using T3 and T7 oligonucleotide primers on double-stranded DNA templates. The double-stranded DNA was denatured in alkali and then subjected to sequence analysis by the dideoxynucleotide chain termination method, using Sequenase version 2.0 (U.S. Biochemical) and [α - 35 S]dATP (NEN, Dupont). The products of the sequencing reactions were analyzed in 4 and 6% polyacrylamide–8.3 M urea gels. Sequence comparisons were performed by using the Wisconsin sequence package version 6.1.

Nucleotide sequence accession number. The nucleotide sequences in this report have been submitted to the GenBank data base under accession number L06433.

RESULTS

***Tpl-2* rearrangements in the MoMuLV-induced T-cell lymphoma 2779.** Provirus integration in a given genomic DNA region can be detected as a DNA rearrangement in Southern blots of restriction endonuclease-digested DNA hybridized to DNA probes derived from this region. Given that *Eco*RI does not cleave the genome of MoMuLV, *Eco*RI digestion of MoMuLV-induced tumor cell genomic DNA and hybridization to probes flanking the provirus should detect rearranged DNA bands larger than those derived from the corresponding unrearranged loci. However, this was not the case with the rearrangement of the *Tpl-2* locus detected in tumor 2779.

Southern blot analysis of *Eco*RI-digested 2779 tumor cell DNA and hybridization to *Tpl-2* probe 3'-PP revealed a rearranged DNA band which was shorter than the normal *Eco*RI *Tpl-2* band (Fig. 1A and C). Assuming that the *Tpl-2* rearrangement was due to the insertion of an altered MoMuLV genome, we proceeded to map the site of provirus insertion within the *Tpl-2* locus. To this end, tumor cell genomic DNA was digested with *Sac*I, which cuts within the MoMuLV long terminal repeat (LTR), and following agarose gel electrophoresis and transfer to nylon membranes, it was hybridized to *Tpl-2* probes 3'-PP and 5'-RP, derived from the cellular DNA regions 3' and 5' of the integrated provirus (Fig. 1). Subsequently, we generated a partial restriction endonuclease map of the *Tpl-2* provirus. To this end, 2779 tumor cell genomic DNA was digested with *Eco*RI, *Bam*HI, and *Hpa*I, and the resulting Southern blots were hybridized to probes 3'-PP and 5'-RP. The results are shown in Fig. 1A and B and are summarized in Fig. 1C.

The provirus integrated in the *Tpl-2* locus in tumor 2779 is a MoMuLV-VL30 recombinant. Partial genomic DNA libraries containing 18- and 16-kb *Bam*HI fragments were constructed in the bacteriophage lambda vector λ Dash. Clones of the rearranged 18- and 16-kb *Bam*HI *Tpl-2* bands (λ B18 and λ B16, respectively) were obtained by hybridization to probes 5'-RP and 3'-PP. Subsequently, insert DNA from the lambda clones λ B18 and λ B16 was digested with several restriction endonucleases to generate shorter DNA fragments, which were subcloned into plasmid vector pBluescript SK(–). The pBluescript subclones shown in Fig. 2 were sequenced in both directions. The sequence of the *Tpl-2* provirus assembled from the sequence of these subclones was compared with the sequence of Ha-MSV (17). The results in Fig. 3 show that the *Tpl-2* provirus is a recombinant between MoMuLV and VL30 (named Mo-VL30) and that the 5' and 3' sites of recombina-

FIG. 1. Site of provirus integration and partial restriction endonuclease map of the *Tpl-2* provirus in the MoMuLV-induced rat T-cell lymphoma 2779. (A) Southern blot analysis of normal rat (N) and tumor 2779 genomic DNA digested with *Eco*RI, *Bam*HI, and *Hpa*I and hybridized to the *Tpl-2* probe 3'-PP, derived from the region 3' of the site of provirus insertion. (B) Southern blot analysis of normal rat (N) and tumor 2779 genomic DNA digested with *Eco*RI, *Bam*HI, and *Hpa*I and hybridized to the *Tpl-2* probe 5'-RP, derived from the region 5' of the site of provirus insertion. The numbers on the left in both panels indicate the migration of *Hind*III-digested lambda size markers in kilobases. (C) Site of provirus integration within intron VII of *Tpl-2* in tumor 2779 and partial restriction map of the integrated provirus. Open boxes represent the proviral LTR elements; shaded boxes represent *Tpl-2* exons VII and VIII. Heavy lines identify *Tpl-2* intron and *Tpl-2* flanking sequences; light lines identify proviral DNA sequences. The arrow above the provirus indicates the transcriptional orientation of the provirus. The solid lines marked 5'-RP-Probe and 3'-PP-Probe at the bottom identify the DNA probes used in the experiments in panels A and B. The two *Bam*HI sites in the 5' half of the provirus were resolved as two separate sites by sequencing (see text and the legend to Fig. 3 for details). Restriction sites: R, *Eco*RI; P, *Pst*I; S, *Sac*I; B, *Bam*HI; Xh, *Xho*I; Hp, *Hpa*I.

tion between MoMuLV and VL30 in Mo-VL30 and Ha-MSV are identical. The genomes of Mo-VL30 and Ha-MSV differ in a region from nucleotide positions 1367 to 2115, which in Ha-MSV has been replaced by *ras*. The 3' end of clone λ B18 is separated from the 5' end of clone λ B16 by a gap which appears to be 25 bp long, as suggested from the sequence of the corresponding region of Ha-MSV.

The sequence from nucleotide positions 1367 to 2115 in Mo-VL30 is an integral part of VL30. The region between nucleotides 1367 and 2115 in Mo-VL30 is absent from Ha-MSV, in which it has been replaced by c-Ha-*ras*. This finding raised the question of whether this region is an integral part of VL30 or whether it was derived by recombination between VL30 and another cellular DNA sequence. To address this question, a normal rat genomic library

FIG. 2. Characterization of the *Tpl-2* provirus cloned from tumor 2779 and sequencing strategy. The recombination breakpoints between MoMuLV and VL30 are shown. The solid lines under the diagram of the provirus represent sequenced subclones. Probe A is a *PvuII* DNA fragment from nucleotide positions 1378 to 1987 representing the region of difference between Mo-VL30 and Ha-MSV. Probe B is a 2-kb *EcoRI* DNA fragment consisting of known VL30 sequences. Restriction sites: B, *Bam*HI; Hp, *Hpa*I; H, *Hind*III; X, *Xho*I; R, *Eco*RI; P, *Pst*I.

constructed from DNA partially digested with *Sau*3AI (Stratagene) was screened with probe A, extending from nucleotide positions 1378 to 1987 (Fig. 2 and 3). This screening yielded eight clones, of which seven hybridized to both probe A and probe B, derived from the VL30 region 3' of the *EcoRI* site at nucleotide position 2831. None of the clones hybridized to an MoMuLV LTR probe (Fig. 4), confirming that they were not the product of library contamination. Additionally, genomic DNA clones isolated by the polymerase chain reaction by using oligonucleotide primers A and B (Fig. 3) were shown to be identical in sequence with the region of the *Tpl-2* provirus between the primers (Fig. 3), except for scattered base differences between clones (data not shown).

Sequence comparison between Mo-VL30 and *ras* transducing viruses isolated from rats. The *ras* oncogene transduced by Ha-MSV and Ki-MSV was flanked by VL30 sequences (2, 15, 17). To determine whether the *ras* oncogene transduced by Rasheed MSV, the third *ras* transducing virus isolated from rats (10), was also flanked by VL30, the published sequence of Rasheed MSV (11) was compared with that of the recombinant described in this report. The comparison revealed that Rasheed MSV contains a 253-bp sequence ending 10 nucleotides 5' of the ATG codon of the Rasheed *v-ras* oncogene and exhibiting 80% homology with VL30. The 5' end of the VL30 sequence detected in Rasheed MSV corresponds to nucleotide position 734 in the Mo-VL30 recombinant and it is underlined in Fig. 3. Comparison of the sequence of the Mo-VL30 recombinant with that of Ha-MSV revealed that the 5' and 3' recombination breakpoints between MoMuLV and VL30 were identical in the two viruses. However, in Ha-MSV, a portion of VL30 beginning at position 1367 and ending at position 2115 was replaced by *ras*.

Sequence comparison between Mo-VL30 recombinant and a genomic clone of c-Ha-*ras*. Comparison of the sequence of the

Mo-VL30 recombinant with that of a rat genomic clone of c-Ha-*ras* (1) revealed a 124-bp region of 87.3% homology, which, in the recombinant, is located between nucleotides 1233 and 1356 (Fig. 3). The 5' end of this region in the genomic c-Ha-*ras* DNA was located at position -1845 (Fig. 5). The recombination breakpoint between VL30 and *ras* occurred 20 bp 3' of the homologous region. Immediately 5' of the recombination breakpoint, there is a 10-bp VL30 sequence, GTTCGCAGCC, which is closely related to the sequence GCCCGCAGCC detected in the first exon of c-Ha-*ras* between nucleotides -118 and -109 (Fig. 3 and 5).

DISCUSSION

Earlier studies had shown that *ras* oncogenes transduced by Ha-MSV and Ki-MSV were nested within VL30 sequences (2, 15, 17). In this report, we presented evidence that the third *ras* transducing virus isolated from rats, Rasheed MSV, also contains VL30 sequence 5' of the transduced *ras* oncogene. These data indicate that the recombination between MoMuLV and VL30 plays an important role in the transduction of *ras* but make no predictions regarding the temporal order of these events. The identification of a recombinant between MoMuLV and VL30, which is presented in this report, suggests that the recombination between MoMuLV and VL30 precedes the transduction of *ras*.

The coupling of the transduction of *ras* to the recombination of the replicating virus with VL30 can be given one of several explanations. One possibility is that the recombination between MoMuLV and VL30 occurs at high frequency so that *ras* oncogenes are transduced by these recombinants because they represent the predominant component of the replicating virus. However, this seems unlikely because the MoMuLV-VL30 recombinant that we described in this report is the only recombinant of this type described to date. Another possibility is that the VL30 sequences enhance the oncogenic potential of *ras*, and therefore viruses containing both are selected. Support for this hypothesis is provided by the finding that deletion of VL30 sequences located 3' of *ras* alters the transforming activity and oncogenic potential of Ha-MSV (14). A final possibility is that MoMuLV-VL30 recombinants are more likely to transduce *ras* oncogenes. The data presented in this report support the last hypothesis, without excluding the second one, and suggest a model describing the steps that may be involved in the transduction (Fig. 5).

Recombination between retroviruses or between retroviruses and cellular oncogenes is thought to occur during reverse transcription and therefore requires the copackaging of the two RNAs into the viral particles (16). Because mRNA transcripts of cellular proto-oncogenes do not contain packaging signals, it is thought that prior to the transduction of a given oncogene, provirus integration is required to occur at its 5' region (16). Transcriptional initiation from the introduced viral promoter gives rise to virus-

FIG. 3. DNA sequence comparison between Mo-VL30 (upper line) and Ha-MSV (lower line). The arrows define the boundaries of the R, U₅, and VL30 sequences as well as the open reading frame encoding p21^{ras}. The *Bam*HI sites representing the 3' and 5' ends of the two Mo-VL30 genomic clones (λ B18 and λ B16) are separated by a 25-bp region which has not been cloned. The region of homology with Rasheed MSV, mapping between nucleotides 734 and 986 of Mo-VL30 is underlined. The region of homology between VL30 and genomic of c-Ha-*ras*, mapping between nucleotides 1233 and 1356 of Mo-VL30, is boxed, as is the 10-bp region of homology between VL30 and the first exon of c-Ha-*ras*, which is located immediately 5' of the recombination breakpoint. The oligonucleotide primers A and B used for the cloning of genomic VL30 sequences by the polymerase chain reaction are indicated. To consolidate the sequence, a gap was introduced between nucleotides at positions 2428 and 4794. S.D., splice donor site.

450 **←R**
 GC~~CC~~CAAGTCTCCGATTGACTGAGTGGCCGGGTACCGGTGATACCAATAAACCCCTCTTGACGTTCAGACTTGTGCTCGCTGTCTCTGGGAGG 549
 1 GC~~CC~~CAAGTCTCCGATTGACTGAGTGGCCGGGTACCGGTGATACCAATAAACCCCTCTTGACGTTCAGACTTGTGCTCGCTGTCTCTGGGAGG 100

 550 **US**
 GTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGCTCTTCA~~TT~~CTGGGGGCTGCTCCGGATCGGAGACCCCTGCCACGGACACCGACCCACACC 649
 101 GTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGCTCTTCA~~TT~~CTGGGGGCTGCTCCGGATCGGAGACCCCTGCCACGGACACCGACCCACACC 200

 650 **S.D.** **VL 30**
 GGGAGGAGCGCCCGGGGCTTGTCTGTGCTGTGTCTGTGCTGTGAACGATCGATCAATAGGCTCAGATCTGGGACTATCTGGCGGGCCAGAG 749
 201 GGGAGGAGCGCCCGGGGCTTGTCTGTGCTGTGTCTGTGCTGTGAACGATCGATCAATAGGCTCAGATCTGGGACTATCTGGCGGGCCAGAG 300

 750 **BamHI**
 AAGGAGCTGACGAGCTCGGACTTCTCCCGCAGCCCTAGAGAGCGTTCAGGCGTGGTGGAGGAGCGGAGATCCGGATCC 849
 301 AAGGAGCTGACGAGCTCGGACTTCTCCCGCAGCCCTAGAGAGCGTTCAGGCGTGGTGGAGGAGCGGAGATCCGGATCCGTTGGCACCTCCGTCCTG 400

 850 **BamHI**
 GATTCGCGACCCCTTGATGACTCCGCTGAATCTTGGTTCAGTTTGGTACCGAAGCTCGCCGCGCTGCTGTTTACTGTTTGTACTGT 949
 401 TTTCCGAGGGATCCGACCCCTGGATGACTCCGCTGA. TTTTGGTTTCAGTTTGGTACCGAAGCTCGCCGCGCTGCTGTTTACTGTTTGTACTGT 500

 950 **TTGAAATGTTTGTCTTCTTTGTGACCTGACTGTGGT**
 501 TGGAAATGTTTGTCTTCTTTGTGACCTGACTGTGGTTTCTGAGCGTGTGGTGTCTGTAGTGTCTTTTGTACTTTTGTTCGTGTTTGAATTTGGACTG 1049
 TGGAAATGTTTGTCTTCTTTGTGACCTGACTGTGGTTTCTGAGCGTGTGGTGTCTGTAGTGTCTTTTGTACTTTTGTTCGTGTTTGAATTTGGACTG 600

 1050 **ACGACTGTGTTTAAATCTTGACCGACGACTGTGTTTAAATCATGAACACTGTTTGC**
 601 ACGACTGTGTTTAAATCTTGACCGACGACTGTGTTTGAATCATGAACACTGTTTGCCTTTGTTTCGTCGAGAGATTTTCTTGGTCCCTTTAAGCTTAC 1149
 ACGACTGTGTTTAAATCTTGACCGACGACTGTGTTTGAATCATGAACACTGTTTGCCTTTGTTTCGTCGAGAGATTTTACTTGGTCCCTTTAAGCTTAC 700

 1150 **TGAGTAAGAACTTAATTTGTAGACCCCGCTAGTGGCAGTGTGTTGCTTGATAGCCAAAGTAAATTTTAAACATAGT**
 701 TGAGTAAGAACTTAATTTGTAGACCCCGCTAGTGGCAGTGTGTTGTTGATAGCCAAAGTAAATTTTAAACATAGTGTTTGGGGGTGGGGAT 1249
 TGAGTAAGAACTTAATTTGTAGACCCCGCTAGTGGCAGTGTGTTGTTGATAGCCAAAGTAAATTTTAAACATAGTGTTTGGGGGTGGGGAT 800

 1250 **TTAGTCAGTGTAGACTCTTGCTAGCAAGCCCAAGGCCCTGGGTTCCGTCGCCAGCTCT. AAAAAAGGAAAGAAACAAAACAAACATATAGT** 1349
 801 TTAGTCAGTGTAGACTCTTGCTAGCAAGCCCAAGGCCCTGGGTTCCGTCGCCAGCTCTGAAAAAGGAAAGAAACAAAACAAACATATAGT 900

 1350 **GTTTATCTGCTTATCTTCGACGCGAGCTGTGATGAATGGGAAGATTTAAATTTCTTGGGACTAAGGAATCTAAAGCGGAGTTTACGCCGT** 1449
 901 GTTTATCTGCTTATCTTCGACGCGAGCTGTGATGAATGGGAAGATTTAAATTTCTTGGGACTAAGGAATCTAAAGCGGAGTTTACGCCGT 1000

 1450 **ATGGCACTAGTGCATAGCTGCCTAAAGATCAGAAATGCTGTTAAACAGGCTATAAAAAAGGACAGGCTGTGTTAGAATGCTACGAGGAATGATCTA** 1549
 1001 GGGCCCGGCCCCGGGGCAGTCCGCGCAGCAAGCGGTGGGCAAGAGCTCTGGTTTGGCAGCCCTGTAGAAGCATTGACGAAATCAAGCTTGTGGTG 1100

 1550 **AAAAGGCTGAGAGCTAAGTGGGCTATAAGAAACAGAACAGGAAAGAAATTTGCAATTTGCTACAGATAGGAGAGCGCTCATTTAGAGAACTTCTT** 1649
 1101 GTGGCGCTAGAGCGCTGGGAAGAGTGCCTGACCATCCAGCTGATCCAGAACCAATTTGTGGACGAGTATGATCCCACTATAGAGGACTCTACCGGA 1200

 1650 **TAAGGAAGCCTGCCCTATCTGCTGCTTATCCAAAGGAGGAGTCAGTCTCAACATTAATTAATTTCTTCTACTGATCATATTACATGGAGAGTGC** 174
 1201 AACAGGTAGTCATTGATGGGGAGAGCTGTTTACTGGACATCTAGACACAAAGGCTAAGAAGAGTATAGTGCCA..... 1274

 1750 **TTTGGTCTCTATCCCAACAGCAGCCAGTTCCTGCCCTATGTTTAAATGCCCAAGTTGGGGGACAAAAAGAGCCCTTAAATAGTTTCAAGAACTCTA** 1849
 1475GCTCGACGTATGGCATCCCTACATTTGAACATCAGCCAAAGACCCGCCAGGGTGTAGAGGATGCCCTTACACACTAGTAGCT 1558

 1850 **CAACTGTGAAGAACTTGTGTTTGGCAGCTCAGGAATTAATGTTTCTTTCCATGGGCTATAGCCCACTGAGACAGTTTGGTAAAGGGCTGTACTAAAGT** 1949
 1559 GAGATTGGGACGATCAAACTCGGAAATGAACCCGCTGATGAGATGGCCCTGGCTGCATGAGCTGCAAGTGTGTGCTGCTGCAGACCCAGGTGAGGC 1658

 1950 **CTTGAAAGCCCAAGGTGAACCTGGTTACAATTTCTTTTGTGACGTGCTTAGTATCTAGACCCAGGTTCTAACCATCTCTCTACCTTTCTGTTTAGTTGT** 2049
 1659 AGGGACCAAGCAAGCATCTGGGCGAGTGCCCTCAGCTAGCCAGATGAATCTATATCCACTTTGATGTCCTGCTCCCCCAATTTGCCAATCCCCCTGC 1758

 2050 **TACTAGAAGCCTAGTGTCTTTTGGAGGCTGGTCTCTTGAAGACAAAGCCACCAAGCTGACACTGAAGGAGGTAGGTACTCCTTAAGGAAAACTTAAG** 2149
 1759 CTGCACTGATCATGTCTTTTGTCTCGCTCCGCGACAGGCTCAGGACGAGGAGTCCGGATGACGAGGAGGTAGGTACTCCTTAAGGAAAACTTAAG 1858

 2150 **CCAGAGAGACAGCCGCTAACAGCTAGGCCTGCTCTGCTCGCTTTCTGTTTCAATAGACAAAGCTTGTGCTTATATTACAGTGGCTTTTGTGCCAA** 2249
 1859 CCAGAGAGACAGCCGCTAACAGCTAGGCCTGCTCTGCTCGCTTTCTGTTTCAATAGACAAAGCTTGTGCTTATATTACAGTGGCTTTTGTGCCAA 1958

 2250 **AAAAAGGCTCTGGTTAGCTGTGACTAAATGCTTATTTGCTCTCTCAGTGGACTCTCTCCTCTAGATTCCCTCTCTGTTTTCACACTCCACG** 2349
 1959 AAAAAGGCTCTGGTTAGCTGTGACTAAATGCTTATTTGCTCTCTCAGTGGACTCTCTCCTCTAGATTCCCTCTCTGTTTTCACACTCCACG 2058

 2350 **TGAGATGCTGTTAGTAACGTTTACAGATCTCTTTTACCCTAGGAAAGACAGAACTCTGCTAAAGGCCAGAAAGAA** 2427
 2059 TGAGATGCTGTTAGTAACGTTTACAGATCTCTTTTACCCTAGGAAAGACAGAACTCTGCTAAAGGCCAGAAAGAA..... 2136

 4795AGGACATCTGCCATTGCCCTGCAGGCTCGGTTATAGAGAAATTCAGAACTGCCATTGACTCAGATATAAAAGAAATAAAG 4875
 4505AGGACATCTGCCATTGCCCTGCAGGCTCGGTTATAGAGAAATTCAGAACTGCCATTGACTCAGATATAAAAGAAATAAAG 4585

 4876 **ACTTTTATAGCTGACCTCAAGAATACCTAACCTCCCTCTCAGAGGTAGTCTTTCAGAAATAGAAAGATTAGACCTGATATCTTCAAGAAAGAACCTG** 4975
 4586 ACTTTTATAGCTGACCTCAAGAATACCTAACCTCCCTCTCAGAGGTAGTCTTTCAGAAATAGAAAGATTAGACCTGATATCTTCAAGAAAGAACCTG 4685

 4786 **TGTTTGAGTCAACTCAAGATGGTTTGAGGAGCTGTTTAAAGATCCCTTGGTTTACCACCTTGATATCTACCAATTAGGACCCCTCATTTGATCTCT** 4885

 5176 **AATGATTTTGTCTTCGGAACCTGATCTTAAATCGATTAGTCCAATTTGTAAAGACAGGATATCAGTGTCCAGGCTCTAGTTTGTACTCAACAATAT** 5275
 4886 AATGATTTTGTCTTCGGAACCTGATCTTAAATCGATTAGTCCAATTTGTAAAGACAGGATATCAGTGTCCAGGCTCTAGTTTGTACTCAACAATAT 4986

 5276 **CACCAGCTGAAGCCTATAGAGTACGAGCCATAGATAAAATAAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAGACCCCACTGTAGGTTT** 5375
 4986 CACCAGCTGAAGCCTATAGAGTACGAGCCATAGATAAAATAAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAGACCCCACTGTAGGTTT 5085

 5376 GCACAAGCTAGCTTAAGTA..... 5393
 5086 GCACAAGCTAGCTTAAGTA..... 5103

FIG. 4. Evidence that the sequence from nucleotide positions 1367 to 2115 in the *Tpl-2* provirus is an integral part of VL30. Eight rat genomic DNA clones detected by using probe A, corresponding to the sequence from nucleotide positions 1378 to 1987 in Mo-VL30, were immobilized to nitrocellulose membranes and then hybridized to probes A and B (Fig. 2) and to an MoMuLV LTR probe.

oncogene hybrid mRNA transcripts containing packaging signals. VL30 sequences in MoMuLV-VL30 recombinants could contribute to the transduction of *ras* by promoting copackaging of the viral RNA with *ras* mRNA transcripts or recombination during reverse transcription. The promotion of copackaging, in turn, may be the direct result of provirus integration 5' of the proto-oncogene. Given that the *ras* mRNA contains neither packaging signals nor homology with VL30, it is unlikely that its packaging into viral particles or its recombination with viral sequences would be promoted by VL30 sequences in the transducing virus. Therefore, the VL30 sequences in MoMuLV-VL30 recombinants could contribute to the transduction of *ras* only by promoting provirus integration in the region 5' of the proto-oncogene. The model described in Fig. 5 predicts, indeed, that the frequency of provirus integration 5' of *c-Ha-ras* is enhanced because of homologous recombination promoted by the 124-bp homology between VL30 and *c-Ha-ras*. The presumed alignment of homologous regions could be resolved by recombination within, or alternatively 5' or 3' of,

the region of homology. Comparison between the sequences of Mo-VL30 and Ha-MSV (17) suggests that in this virus, the recombination breakpoint between VL30 and *ras* occurred 20 bp 3' of the region of homology. A similar comparison with the published sequence of Rasheed MSV (11) suggests that the recombination occurred 5' of the region of homology.

The hybrid RNA initiating in the LTR of the integrated provirus is expected to contain approximately 1.7 kb of cellular sequences between the provirus and the first exon of *c-Ha-ras* (1). Deletion of these sequences may occur by a variety of mechanisms. During the transduction of *c-Ha-ras* by Ha-MSV, the deletion appears to have occurred by homologous recombination between two partially homologous 10-bp sequences located immediately 5' of the VL30-*ras* recombination breakpoint and in the first exon of *ras* (Fig. 5). In Rasheed MSV, the 1.7-kb region appears to have been deleted by an illegitimate recombination event. Such deletions were suggested to occur when retrovirus constructs of *c-Ha-ras*, including the 1.7-kb region, were introduced by transfection into C3H/10T1/2 cells (1).

Our current data are confined to the transduction of *c-Ha-ras*. To address the question of whether the proposed model applies also to the transduction of *c-Ki-ras*, we need to define the recombination breakpoints between MoMuLV and VL30 and between VL30 and *c-Ki-ras* in Ki-MSV. Moreover, we need to know whether *c-Ki-ras* contains at its 5' end a DNA region similar to the 124-bp region of homology between VL30 and *c-Ha-ras*. The available sequencing information on Ki-MSV and *c-Ki-ras*, however, is limited (3, 5, 8, 13). On the basis of this information, we can only state that the MoMuLV-VL30 recombinant that transduced *c-Ki-ras* was almost identical to the one that transduced *c-Ha-ras* (8). The limited nature of the available information, however, does not allow us to address the question whether

FIG. 5. Model for the transduction of *c-Ha-ras*. This model is based on sequence comparisons between Mo-VL30, Ha-MSV, and *c-Ha-ras*. Therefore, its applicability to the transduction of *c-Ki-ras* is not known. Mo-VL30 is shown in its circular form at the top left. The open box represents the MoMuLV LTR, the solid lines represent MoMuLV sequences, and the interrupted line represents VL30 sequences. The shaded box, in the circular provirus and in the linear diagram of *c-Ha-ras* below, indicates the 124-bp region of homology between VL30 and genomic *c-Ha-ras*. The provirus integrates 5' of *c-Ha-ras* by homologous recombination mediated by the alignment of the 124-bp homologous DNA regions. The two solid perpendicular lines under the diagram showing the structure of the provirus indicate a 10-nucleotide partial homology between VL30 and the first exon of *c-Ha-ras*. These 10-bp homologous regions are underlined in the sequence under the diagram of the integrated provirus. Recombination between these repeats gives rise to RNA transcripts with a sequence at the recombination breakpoint identical to that of Ha-MSV.

c-Ki-*ras*, similarly to c-Ha-*ras*, contains at its 5' region a VL30-homologous sequence. Therefore, the applicability of the proposed model to the transduction of c-Ki-*ras* remains to be determined.

The data presented in this report uncouple the MoMuLV-VL30 recombination from the transduction of c-Ha-*ras* and perhaps other members of the *ras* oncogene family. The same data show that the recombination between MoMuLV and VL30 could precede and may promote the transduction of *ras* proto-oncogenes. The proposed model is consistent with the finding that the presumed enhancement of oncogene transduction by MoMuLV-VL30 recombinants is limited to members of the *ras* proto-oncogene family. Moreover, it is consistent with the structure of the recombinant and the available data on the structure of *ras* transducing viruses. Finally, the model is testable because it predicts that the introduction of the recombinant in rat cells will lead to provirus integration 5' of c-Ha-*ras* and predicts the structure of the integrated provirus. Future studies will use the Mo-VL30 recombinant described here to test the proposed model.

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REFERENCES

1. Chakraborty, A. K., K. Cichutek, and P. H. Duesberg. 1991. Transforming function of proto-*ras* genes depends on heterologous promoters and is enhanced by specific point mutations. *Proc. Natl. Acad. Sci. USA* **88**:2217-2221.
2. Ellis, R. W., W. DeFeo, T. Y. Shih, M. A. Gonda, H. A. Young, N. Tsuchida, D. R. Lowy, and E. M. Scolnick. 1981. The p21 *src* genes of Harvey and Kirsten sarcoma viruses originate from divergent members of a family of normal vertebrate genes. *Nature (London)* **292**:506-511.
3. George, D. L., A. F. Scott, S. Trusko, B. Glick, E. Ford, and D. J. Dorney. 1985. Structure and expression of amplified c-Ki-*ras* sequences in Y1 mouse adrenal tumor cells. *EMBO J.* **4**:1199-1203.
4. Harvey, J. J. 1964. An unidentified virus which causes the rapid production of tumours in mice. *Nature (London)* **204**:1104-1105.
5. Hoffman, E. K., S. P. Trusko, N. Freeman, and D. L. George. 1987. Structural and functional characterization of the promoter region of the mouse. c-Ki-*ras* gene. *Mol. Cell. Biol.* **7**:2592-2596.
6. Kirsten, W. H., and L. A. Myer. 1967. Morphologic responses to a murine erythroblastosis virus. *J. Natl. Cancer Inst.* **39**:311-335.
7. Lazo, P. A., A. J. P. Klein-Szanto, and P. N. Tschlis. 1990. T cell lymphoma lines derived from rat thymomas induced by Moloney murine leukemia virus: phenotypic diversity and its implications. *J. Virol.* **64**:3948-3959.
8. Norton, J. D., J. Connor, and R. J. Avery. 1984. Genesis of Kirsten murine sarcoma virus: sequence analysis reveals recombination points and potential leukaemogenic determinant on parental leukemia virus genome. *Nucleic Acids Res.* **12**:6839-6852.
9. Patriotis, C., A. Makris, S. E. Bear, and P. N. Tschlis. The tumor progression locus-2 (*Tpl-2*) encodes a protein kinase involved in the progression of rodent T cell lymphomas and in T cell activation. *Proc. Natl. Acad. Sci. USA*, in press.
10. Rasheed, S., M. G. Gardner, and R. I. Huebner. 1978. In vitro isolation of stable rat sarcoma viruses. *Proc. Natl. Acad. Sci. USA* **75**:2972-2976.
11. Rasheed, S., G. L. Norman, and G. Heidecker. 1983. Nucleotide sequence of the Rasheed rat sarcoma virus oncogene: new mutations. *Science* **221**:155-157.
12. Tschlis, P. N., P. G. Strauss, and M. A. Lohse. 1985. Concerted DNA rearrangements in Moloney murine leukemia virus-induced thymomas: a potential synergistic relationship in oncogenesis. *J. Virol.* **56**:258-267.
13. Tsuchida, N., T. Ryder, and E. Ohtsubo. 1982. Nucleotide sequence of the oncogene encoding the p21 transforming protein of Kirsten murine sarcoma virus. *Science* **217**:937-938.
14. Velu, T. J., W. C. Vass, D. C. Lowy, and P. E. Tambourin. 1989. Harvey murine sarcoma virus: influences of coding and noncoding sequences on cell transformation in vitro and oncogenicity in vivo. *J. Virol.* **63**:1384-1392.
15. Wei, C. M., D. R. Lowy, and E. M. Scolnick. 1980. Mapping of transforming region of the Harvey murine sarcoma virus genome by using insertion-deletion mutants constructed *in vitro*. *Proc. Natl. Acad. Sci. USA* **77**:4674-4678.
16. Weiss, R., N. Teich, H. Varmus, and J. M. Coffin. 1982. Molecular biology of tumor viruses. RNA tumor viruses, p. 719-734. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. Weiss, R., N. Teich, H. Varmus, and J. Coffin. 1985. Molecular biology of tumor viruses. RNA tumor viruses, 2nd ed., p. 928-931. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.