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Avian Oncovirus Mutant (SE21Q1b) Deficient in Genomic RNA: Characterization of a Deletion in the Provirus

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We have previously described a nonconditional mutant of avian sarcoma virus (SE21Q1b) which fails to package viral RNA (Gallis et al., Virology **94**:146-161, 1979; Linial et al., Cell **15**:1371-1381, 1978). Quail cells transformed by SE21Q1b contain normal amounts of intracellular viral mRNA's for *src*, *env*, and *gag-pol* and release particles with the density of normal virus containing a typical complement of virion proteins, including reverse transcriptase. These virions are noninfectious for both chicken and quail cells and contain primarily cellular rather than viral RNA. Analysis by gel electrophoresis of the cellular DNA of quail cells transformed by SE21Q1b after restriction endonuclease digestion indicated the presence of a single provirus. The provirus was located at one site in the genome of the host cell and was flanked by the characteristic terminally repeated sequences derived from the 3′ and 5′ ends of viral RNA. The only defect detected in the SE21Q1b provirus was a deletion of ca. 150 base pairs of DNA somewhere between 300 and 600 bases from the left (*gag-pol*) end of the provirus. Analyses of the proviral DNA of cells transformed by wild-type recombinants between SE21Q1b and leukosis viruses reveal that the recombinants no longer contain this deletion. The deletion, therefore, defines a region on the viral RNA which is required for correct packaging of the virion RNA.

The genome of avian sarcoma virus (ASV) can be divided into four genes: *gag* (group-specific antigens), *pol* (RNA-directed DNA polymerase), *env* (envelope glycoproteins), and *src* (fibroblast-transforming protein). In addition, the genome contains a region close to the 3′ end of the RNA, c, which is common to both transforming and nontransforming viruses. Nonconditional (deletion) mutants were crucial in determining the physical locations of both the *src* and *env* genes on the viral RNA (9, 15). The fact that these mutants were deletions was established by the electrophoretic mobility of their virion RNA in relation to wild-type 35 to 39S RNA. The location of the *src* and *env* genes was first determined by the loss of unique T1 oligonucleotides positioned with respect to the 3′-polyadenylic acid of virion RNA in these mutants (27).

We previously reported on the isolation of nonconditional mutants of ASV (12, 13). One mutant, SE21Q1b, arose spontaneously and had the unusual property of being incapable of packaging virion 35 to 39S RNA. Since the virus particle produced by SE21Q1b did not contain viral RNA, the conventional analyses for ASV deletion mutants were not available. We were, however, able to use restriction enzyme digestions coupled with the Southern transfer procedure (24) to show that the proviral DNA in SE21Q1b-transformed quail cells sustained a deletion of ca. 150 base pairs (bp). The deletion was located near the left (*gag-pol*) end of the provirus between the 3′5′ terminal repeat and the starting point for p19. The terminally repeated sequences characteristic of ASV DNA (7, 22) appeared intact in SE21Q1b. This deletion, therefore, defined a region of the genome which is required for recognition and packaging of the virion RNA.

MATERIALS AND METHODS

Virus and cells. SE21Q1b-transformed quail cells were derived from quail embryo fibroblasts transformed by PR-E21 virus as previously described (13). Wild-type clones of parental PR-E21 (PR-E21-18 and PR-E21-32) were obtained by cloning PR-E21-infected quail cells in soft agar as previously described.

For isolation of recombinants with leukosis virus, SE21Q1b-transformed quail cells were superinfected with either td108 or Rous-associated virus type 1 (subgroup A). After 2 weeks and two passages, the supernatant was plated on quail embryo fibroblasts. Foci were picked, and two cell clones (40a from the td108 superinfection and 32a from the Rous-associated virus type 1 superinfection) could be grown and demonstrated to be producing nondefective sarcoma virus.

DNA extraction and restriction endonuclease digestion. High-molecular-weight cellular DNA was prepared from transformed quail clones as described by Hughes et al. (6). DNA at concentrations of 50 to 100 μg/ml (measured by the diphenylamine reaction)

was digested with *Eco*RI and *Bam*HI in the buffers described by New England Biolabs. Usually 10 μg of DNA was used per analysis. To monitor the completeness of the digestion, 5% of the digest was removed at 15 min after the addition of the enzyme to a test sample containing 0.5 μg of covalently closed circular plasmid pBR313 DNA, which was then incubated in parallel with the main digestion. Since *Bam*HI and *Eco*RI each recognize a single site in pBR313 (1), we concluded that the digestion of cellular DNA was complete if electrophoresis in an agarose gel of the test reaction revealed complete conversion of pBR313 from a circular to a linear molecule.

Analysis of DNA in agarose gels. Electrophoresis of DNA in 0.8% agarose gels and transfer onto nitrocellulose filters were as previously described (22). A 10-μg amount of DNA was applied per lane. Viral DNA was detected by annealing the filter with ^{32}P-labeled complementary DNA (cDNA) under conditions previously described (22). [^{32}P]cDNA complementary to the entire viral RNA ($cDNA_{rep}$), the sequences between the primer binding site and the 5′ end of the viral RNA ($cDNA_{5'}$), and the 200 to 300 nucleotides adjacent to the polyadenylic acid in viral RNA ($cDNA_{3'}$) were prepared as previously described (22). After annealing, the filters were washed and exposed to X-ray film under conditions previously described (26).

Analysis of viral proteins. Virus-producing cells were labeled with [^{35}S]methionine, virus was purified by pelleting through sucrose, and viral proteins were analyzed by polyacrylamide gel electrophoresis as previously described (13).

RESULTS

Characterization of the defect in SE21Q1b. Since the cells transformed by SE21Q1b do not produce infectious virus, we characterized the proviral DNA sequences in the transformed cells, using the gel transfer procedure developed by Southern (24) and the strategy devised by Botchan et al. (2) to examine the structure of integrated simian virus 40 DNA. The integration of ASV DNA in quail cells exhibits little specificity with regard to the host cell site but has a high degree of specificity for the viral sequence (6, 19; S. H. Hughes, P. K. Vogt, E. Stubblefield, H. Robinson, J. M. Bishop, and H. E. Varmus, Cold Spring Harbor Symp. Quant. Biol., in press). Restriction fragments containing both virus and cell sequences ("junction" fragments) will be unique to each clone. We therefore used enzymes which produce several internal fragments.

*Eco*RI is a very useful enzyme for the characterization of both integrated and unintegrated ASV DNA (6, 7, 16, 19, 22) since it cleaves at two internal sites and within the 3′ sequences which are repeated at both ends of the linear viral and proviral DNA (Fig. 1). Digestion of transformed cell DNA with *Eco*RI produces three large well-resolved internal fragments

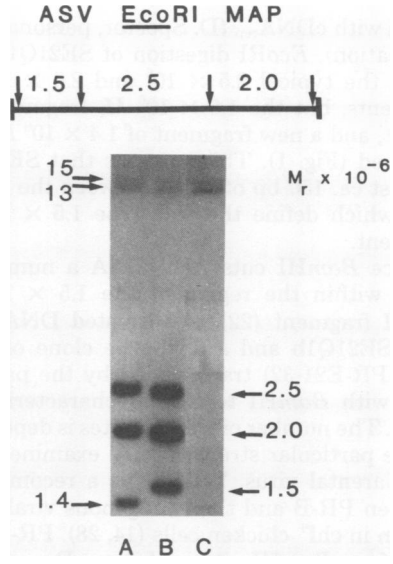

FIG. 1. *Evidence for a deletion in SE21Q1b. EcoRI cleaves ASV DNA at four sites as shown in the diagram of linear DNA. The provirus sits within the cell in essentially the same orientation as the linear DNA. High-molecular-weight whole cell DNA was prepared from uninfected quail cells, SE21Q1b cells, and PR-E21-18 cells. DNA (10 μg) was digested to completion with EcoRI and subjected to electrophoresis in a 0.8% agarose gel as described (22). The DNA was then denatured and transferred onto a nitrocellulose filter by the method of Southern (24). The filter was then annealed with 10^6 cpm of ^{32}P-labeled ASV (PRC) $cDNA_{rep}$ per ml (ca. 4×10^8 cpm/μg). After annealing, the nitrocellulose filter was washed extensively and exposed to X-ray film in the presence of an intensifying screen (26). The mobility of the HindIII fragments of bacteriophage lambda DNA in the same gel was used to determine the molecular weights: lane A, SE21Q1b; lane B, PR-E21-18; lane C, uninfected quail.*

which constitute over 97% of the provirus. The junction fragments which contain less than 3% of the provirus are not readily detected with $cDNA_{rep}$ but can easily be detected with cDNA's specific for the 3′ and 5′ ends of the viral RNA (6; Fig. 3). A further advantage of *Eco*RI is that the sites it recognizes have been at similar or identical locations in most strains of ASV so far characterized (22). In all cases we compared the cleavage pattern of SE21Q1b with that of wild-type clones (PR-E21-32 or PR-E21-18) of quail embryo fibroblasts transformed by the parental virus PR-E21.

*Eco*RI digestion of PR-E21-18 produced the three major fragments (2.5×10^6, 2.0×10^6, and 1.5×10^6 M_r) typical of the internal fragments of wild-type ASV. The large bands (ca. 13×10^6 and 15×10^6 M_r) represent the major endogenous *src* sequences in quail cells which are de-

tected with $cDNA_{rep}$ (D. Spector, personal communication). *Eco*RI digestion of SE21Q1b produced the typical 2.5×10^6 and 2.0×10^6 M_r fragments, but the 1.5×10^6 M_r fragment was absent, and a new fragment of 1.4×10^6 M_r was observed (Fig. 1). This suggests that SE21Q1b has lost ca. 150 bp of DNA between the *Eco*RI sites which define the wild-type 1.5×10^6 M_r fragment.

Since *Bam*HI cuts ASV DNA a number of times within the region of the 1.5×10^6 M_r *Eco*RI fragment (22), we digested DNA from both SE21Q1b and a wild-type clone of quail cells (PR-E21-32) transformed by the parental virus with *Bam*HI to further characterize the lesion. The number of *Bam*HI sites is dependent on the particular strain of ASV examined (22). The parental virus, PR-E21, is a recombinant between PR-B and the endogenous viral information in chf^+ chicken cells (14, 28). PR-B contains four *Bam*HI sites, whereas Rous-associated virus type 0 contains only the three sites nearest the left end (Fig. 2; P. R. Shank, S. H. Hughes, and H. E. Varmus, Virology, in press).

Cleavage of PR-E21-32 DNA with *Bam*HI produced internal fragments of 0.9×10^6 and 1.1×10^6 M_r (Fig. 2); fragments of identical size were produced by *Bam*HI cleavage of SE21Q1b. Since both clones produced identical internal fragments, the site of the deletion in SE21Q1b must be outside the terminal *Bam*HI sites. The fragment migrating at 3.0×10^6 M_r represents the major endogenous *src* sequences. The fragment migrating at ca. 5×10^6 M_r represents the right end of the SE21Q1b provirus joined to cell DNA. The smear of viral DNA extending upward from 3×10^6 M_r in the *Bam*HI digest of PR-E21-32 probably represents the right end of the provirus joined to different-sized cell fragments. PR-E21-32, therefore, does not represent a true clone. This problem has been encountered by others with permissive cells transformed by ASV (19; Hughes et al., in press). Presumably, the cells divide a few times in the agar before stable integration occurs (S. Hughes, personal communication). The problem is partially avoided by delaying the cloning for a few days after infection (Hughes et al., in press).

To confirm the location of the internal *Bam*HI fragments, we performed sequential double digestions with both *Eco*RI and *Bam*HI (Fig. 2). As predicted from our previous restriction maps, *Eco*RI digestion cut the 1.1×10^6 M_r *Bam*HI fragment, producing a second fragment of 0.9×10^6 M_r and a fragment of 0.2×10^6 M_r (not detected on this gel). The 2.0×10^6 M_r *Eco*RI fragment was produced in the double digest as was a fragment of 1.6×10^6 M_r from the 2.5×10^6 M_r *Eco*RI fragment. A third fragment of 0.9×10^6 M_r was also produced from the 2.5×10^6

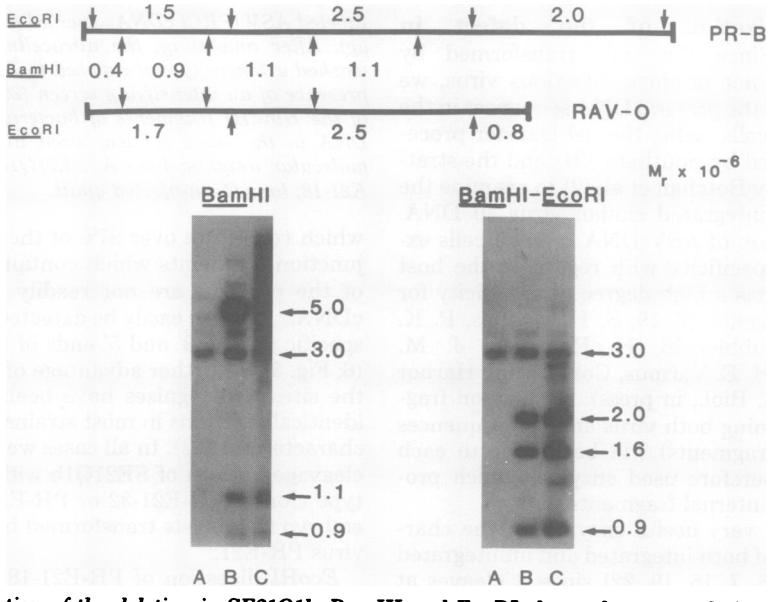

FIG. 2. *Location of the deletion in SE21Q1b. BamHI and EcoRI cleave the parental viruses which gave rise to SE21Q1b at different positions as shown in the diagram. High-molecular-weight DNA (10 μg) was digested to completion with BamHI or with BamHI and EcoRI and then subjected to electrophoresis in a 0.8% agarose gel. Virus-specific fragments were then detected with [^{32}P]$cDNA_{rep}$ as described in the legend to Fig. 1. Lane A, uninfected quail; lane B, SE21Q1b; lane C, PR-E21-32.*

M_r *Eco*RI fragment. These data indicate that SE21Q1b lost ca. 150 bp of DNA somewhere between the left-most *Eco*RI and *Bam*HI sites of the provirus (between 140 and 600 bp from the left end).

Characterization of terminal repeats and provirus-cell junctions. To determine whether SE21Q1b had a second lesion outside the terminal *Eco*RI sites and to characterize the number and structure of the provirus-cell junctions, we examined an *Eco*RI digest of SE21Q1b with $cDNA_{3'}$ and $cDNA_{5'}$ (Fig. 3). Since *Eco*RI cleaves ca. 60 bases into the 3′ sequence (22, 30) which is repeated at both ends of the provirus, the right-hand virus-cell junction should anneal strongly to $cDNA_{5'}$ and faintly to $cDNA_{3'}$, whereas the left-hand junction fragment should only anneal to $cDNA_{3'}$.

Analysis of the *Eco*RI digest of SE21Q1b with $cDNA_{3'}$ revealed a new fragment of 1.1×10^6 M_r which was not detected with $cDNA_{rep}$ (Fig. 3). $cDNA_{5'}$ detected a new fragment of 0.8×10^6 M_r which can also be seen faintly with $cDNA_{3'}$ (Fig. 3). This indicates that SE21Q1b contains a single, uniquely located provirus with *Eco*RI sites located ca. 1 kilobase into the cell DNA on the right and ca. 1.6 kilobases on the left of the provirus.

$cDNA_{3'}$ annealed strongly to the 2.0×10^6 M_r fragment and weakly to the 1.4×10^6 M_r fragment, whereas $cDNA_{5'}$ annealed as strongly to the 1.4×10^6 M_r fragment as it did to the 0.8×10^6 M_r fragment. This suggests that some of the 3′ and most of the 5′ sequences are located within the 1.4×10^6 M_r *Eco*RI fragment and indicates that the provirus of SE21Q1b has the configuration we have designated: CELL 3′5′ provirus 3′5′ CELL. Since the 1.4×10^6 M_r *Eco*RI fragment appeared to have the 5′ sequence, the deletion in SE21Q1b must occur somewhere between 300 and 600 bp from the left end of the provirus (see Fig. 6).

Isolation and analysis of wild-type recombinants. Although SE21Q1b did not recombine with high frequency with avian leukosis virus in contrast to wild-type ASV, two recombinants were isolated after superinfection of SE21Q1b cells by avian leukosis virus. The two recombinants, 32a (obtained from a cross with Rous-associated virus type 1) and 40a (from a cross with td108; reference 8) were obtained after superinfection and passage of the supernatants on quail cells until foci of transformation were obtained. The frequency of recombination was very low, estimated to be much less than 10^{-6}. Both of the recombinants behaved like wild-type nondefective ASV in biological experiments (data not shown). The integrated provi-

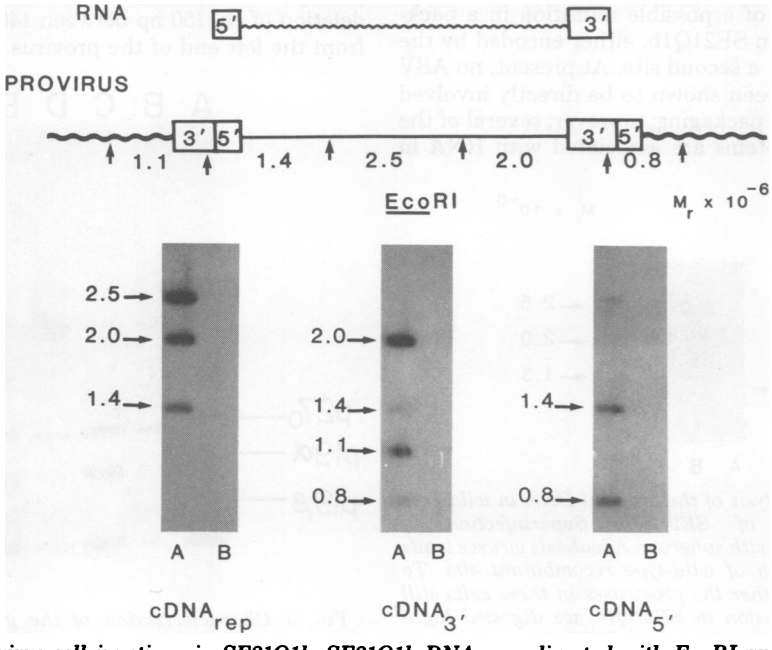

FIG. 3. *Provirus-cell junctions in SE21Q1b. SE21Q1b DNA was digested with EcoRI and processed as described in the legend to Fig. 1. The same filter was then reannealed with [^{32}P]$cDNA_{5'}$ and subsequently with [^{32}P]$cDNA_{3'}$. We show the EcoRI map of ASV DNA with the 3′ and 5′ sequences expanded to show the exact position of the EcoRI sites. Lane A, EcoRI-digested SE21Q1b DNA; lane B, EcoRI-digested uninfected quail DNA.*

rus of these recombinants was analyzed to determine whether they had regained the sequences missing in SE21Q1b, which would be expected if this deletion was responsible for the mutant phenotype.

Digestion of high-molecular-weight DNA from clone 40a with *Eco*RI produced the typical wild-type 1.5×10^6 M_r *Eco*RI fragment (Fig. 4). The wild-type phenotype is therefore correlated with reacquisition of the sequences which were lost in the 1.4×10^6 M_r *Eco*RI fragment of SE21Q1b. Clone 40a is a complex clone containing several copies of the viral DNA. In addition to the three wild-type *Eco*RI fragments, fragments of 2.4×10^6 M_r and 1.1×10^6 M_r were present. Likewise, wild-type clone PR-E21-32, which contained several copies of the viral DNA (see Fig. 2, lane C), produced an *Eco*RI fragment of 2.9×10^6 M_r (Fig. 4, lane B) in addition to the three wild-type fragments. The nature of these fragments has not been pursued.

We have previously shown that the mutation in SE21Q1b was both "cis"-acting and dominant, in that superinfection of SE21Q1b cells by avian leukosis virus produced avian leukosis virus but did not release infectious sarcoma virus. Furthermore, virus shed from superinfected cells contained about 90% cellular and 10% viral RNA, in direct contrast to wild-type virus which contained greater than 98% viral RNA (13). This is suggestive of a possible mutation in a packaging factor in SE21Q1b, either encoded by the deletion or at a second site. At present, no ASV protein has been shown to be directly involved in ASV RNA packaging; however, several of the *gag* gene proteins are associated with RNA in virions, and p19 is specifically bound to viral RNA (11, 20). Since we have previously shown (13) that SE21Q1b virions contain proteins with different electrophoretic mobilities than wild-type ASV or avian leukosis virus, which have been termed $p27_o$, $p19_\alpha$, and $p19_\beta$ (21), it was of interest to determine whether the wild-type recombinants 32a and 40a inherited the SE21Q1b-like proteins. Figure 5 shows that both recombinants inherited p27 and p19 from the leukosis virus parents rather than SE21Q1b. It can be noted that the electrophoretic mobilities of p19 from 40a and 32a were slightly different, corresponding to those of td108 and Rous-associated virus type 1, respectively. Therefore, we could not determine whether the *gag* proteins present in SE21Q1b were associated with the mutant phenotype. We do know that the SE21Q1b $p27_o$, $p19_\alpha$, and $p19_\beta$ are identical in electrophoretic mobility to those in the wild-type parental PR-E21 (13, 21). Furthermore, tryptic peptide analysis of the methionine-containing peptides of SE21Q1b p19's and those of PR-E21 wild type revealed no differences (R. Eisenman and M. Linial, unpublished data).

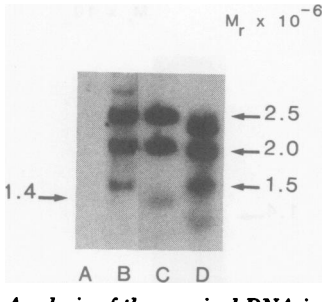

FIG. 4. *Analysis of the proviral DNA in wild-type recombinants of SE21Q1b. Superinfection of SE21Q1b cells with subgroup A leukosis viruses leads to the isolation of wild-type recombinant 40a. To determine whether the proviruses in these cells still retained the lesion in SE21Q1b, we digested high-molecular-weight whole cell DNA from clone 40a with EcoRI and processed the DNA as described in the legend to Fig. 1: lane A, uninfected quail DNA; lane B, PR-E21-32 DNA; lane C, SE21Q1b DNA; lane D, 40a DNA.*

DISCUSSION

We have presented evidence that the lesion in SE21Q1b is a deletion by characterization of the proviral DNA. Analysis with *Eco*RI showed a deletion of ca. 150 bp between 140 and 2,200 bp from the left end of the provirus. *Bam*HI anal-

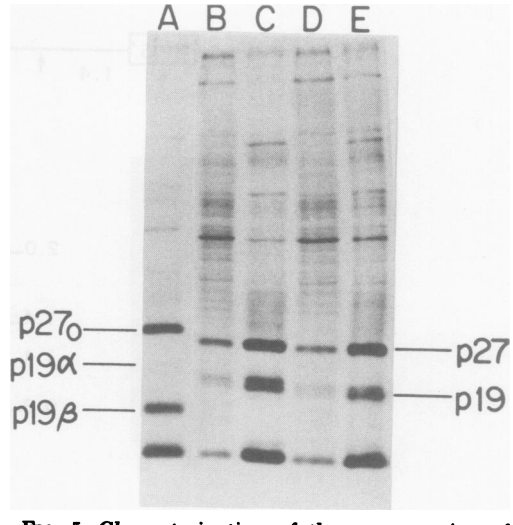

FIG. 5. *Characterization of the gag proteins of SE21Q1b and wild-type recombinants. Electrophoresis of [35S]methionine-labeled viral proteins on a 15% polyacrylamide-sodium dodecyl sulfate gel. Lane A, SE21Q1b; lane B, td108; lane C, 40a; lane D, Rous-associated virus type 1; lane E, 32a.*

ysis indicated that the deletion occurs within 600 bp of the left end of the provirus. The fact that SE21Q1b contains the normal 3'5' terminal redundancy at its left end further suggests that the deletion occurs internal to the redundancy or between 300 and 600 bp from the end of the provirus (Fig. 6).

Recently, Martin et al. examined a series of nonproducer quail clones transformed by UV-irradiated PRA ASV (16). These mutants represent extensive deletions of up to two-thirds of the proviral DNA; yet they also retain two copies of the 3'5' repeated structure. Both sets of data are consistent with the hypothesis that the left-hand repeated sequences are necessary for normal production of viral mRNA.

The fact that SE21Q1b produces virus particles with normal *gag* proteins suggests that the translated region of *gag* cannot begin for at least 450 bp from the left end of the provirus or 250 bp from the 5' terminus of the viral RNA. The deletion in SE21Q1b does not extend into the amino terminus of Pr76 since the amino terminal peptide of Pr76 p19 peptide 19a (18) is present in the $p19_\alpha$ and $p19_\beta$ of SE21Q1b (R. Eisenman and M. Linial, unpublished data). This is consistent with the observations of others (18) that the AUG codon located at the position 83 to 85 (5, 23) in the 5' region of the RNA is not the amino terminus of Pr76. If the AUG at position 83 to 85 in 5' is functional, our data suggest that a protein of at least 5,000 to 10,000 M_r could be encoded before the start of the amino terminal protein of Pr76. Our data do not rule out a second mutation (point or small deletion) within the *gag* gene or elsewhere in the genome which could account for the dominant effect of the SE21Q1b phenotype.

SE21Q1b produces apparently normal 28S and 21S mRNA's for the *env* and *src* genes, respectively (13). These subgenomic mRNA's are generated by a mechanism (splice) which transposes 5' sequences from the left end of the provirus to the 5' ends of the mRNA's (17, 29). The exact extent of the spliced sequence has not been defined, but it does extend beyond the 5' sequences (3). Analysis of simian virus 40 mutants has shown that sequences up to, but not including, the splice junction can be deleted yet retain proper splicing (10, 25). This would suggest that the region deleted in SE21Q1b cannot include the splice junction. However, part of the SE21Q1b deletion could encode sequences normally spliced onto messages but not necessary for translation.

It is tempting to speculate that the sequences missing in SE21Q1b which are required for packaging are normally not part of the 5' splice on the viral messages and that this accounts for the high efficiency of packaging 39S viral RNA and the low efficiency of packaging subgenomic mRNA's. Sequence analysis of SE21Q1b and wild-type genomes will be necessary to test this hypothesis.

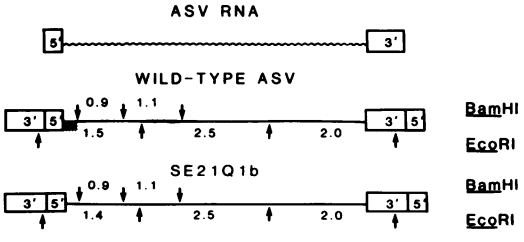

FIG. 6. *Location of the lesion in SE21Q1b. The structure of wild-type ASV DNA in relation to the viral RNA is shown with the terminal sequences expanded. The deletion of ca. 150 bp in SE21Q1b must be between the left-most EcoRI and BamHI sites as indicated by the hatched region.*

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LITERATURE CITED

1. **Bolivar, F., R. L. Rodriguez, M. S. Betloch, and H. W. Boyer.** 1977. Construction and characterization of new cloning vehicles. I. Ampicillin-resistant derivations of plasmid pMB-9. Gene **2**:75-93.
2. **Botchan, M., W. Topp, and J. Sambrook.** 1976. Arrangement of the simian virus 40 sequences in the DNA of transformed cells. Cell **9**:269-287.
3. **Cordell, B., S. R. Weiss, H. E. Varmus, and J. M. Bishop.** 1979. At least 104 nucleotides are transposed from the 5' terminus of the avian sarcoma virus genome to the 5' termini of smaller viral mRNAs. Cell **15**:79-91.
4. **Gallis, B., M. Linial, and R. Eisenman.** 1979. An avian oncovirus mutant deficient in genomic RNA: characterization of the packaged RNA as cellular messenger RNA. Virology **94**:146-161.
5. **Haseltine, W. A., A. Maxam, and W. Gilbert.** 1977. Rous sarcoma virus genome is terminally redundant: the 5' sequence. Proc. Natl. Acad. Sci. U.S.A. **74**:989-993.
6. **Hughes, S. H., P. R. Shank, D. H. Spector, H.-J. Kung, J. M. Bishop, H. E. Varmus, P. K. Vogt, and M. L. Breitman.** 1978. Proviruses of avian sarcoma virus are terminally redundant, coextensive with unintegrated linear DNA and integrated at many sites in rat cell DNA. Cell **15**:1397-1410.
7. **Hsu, T. W., J. L. Sabran, G. E. Mark, R. V. Guntaka, and J. M. Taylor.** 1978. Analysis of unintegrated avian RNA tumor virus double-stranded DNA intermediates. J. Virol. **28**:810-818.
8. **Kawai, S., P. H. Duesberg, and H. Hanafusa.** 1977. Transformation-defective mutants of Rous sarcoma virus with *src* gene deletions of varying length. J. Virol. **24**:910-914.

9. **Kawai, S., and H. Hanafusa.** 1973. Isolation of a defective mutant of avian sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **70**:3493–3497.
10. **Lai, C.-J., and G. Khoury.** 1979. Deletion mutants of simian virus 40 defective in biosynthesis of late viral mRNA. Proc. Natl. Acad. Sci. U.S.A. **76**:71–75.
11. **Leis, J. P., J. McGinnis, and R. W. Green.** 1978. Rous sarcoma virus p19 binds to specific double stranded regions of viral RNA: effect of p19 on cleavage of viral RNA by RNase III. Virology **84**:87–98.
12. **Linial, M., S. Brown, and P. Neiman.** 1978. A nonconditional mutant of Rous sarcoma virus containing defective polymerase. Virology **87**:130–141.
13. **Linial, M., E. Medeiros, and W. S. Hayward.** 1978. An avian oncovirus mutant (SE21Q1b) deficient in genomic RNA: biological and biochemical characterization. Cell **15**:1371–1381.
14. **Linial, M., and P. Neiman.** 1976. Infection of chick cells by subgroup E viruses. Virology **73**:508–520.
15. **Martin, G. S., and P. H. Duesberg.** 1971. The *a* subunit in the RNA of transforming avian tumor viruses. II. Spontaneous loss resulting in nontransforming variants. Virology **47**:494–497.
16. **Martin, G. S., K. Radke, S. Hughes, N. Quintrell, J. M. Bishop, and H. E. Varmus.** 1979. Mutants of Rous sarcoma virus with extensive deletions of the viral genome. Virology **96**:530–546.
17. **Mellon, P., and P. H. Duesberg.** 1977. Subgenomic, cellular Rous sarcoma virus RNAs contain oligonucleotides from the 3′ half and the 5′ terminus of virion RNA. Nature (London) **270**:631–634.
18. **Palmiter, R. D., J. Gagnon, V. M. Vogt, S. Ripley, and R. N. Eisenman.** 1978. The NH_2-terminal sequence of the avian oncovirus *gag* precursor polyprotein (Pr76*gag*). Virology **91**:423–433.
19. **Sabran, J. L., T. W. Hsu, C. Yeater, A. Kaji, W. S. Mason, and J. M. Taylor.** 1979. Analysis of integrated avian RNA tumor virus DNA in transformed chicken, duck and quail fibroblasts. J. Virol. **29**:170–178.
20. **Sen, A., and G. J. Todaro.** 1977. The genome-associated specific RNA binding proteins of avian and mammalian type C viruses. Cell **10**:91–99.
21. **Shaikh, R., M. Linial, S. Brown, A. Sen, and R. Eisenman.** 1979. Recombinant avian oncoviruses. II. Alterations in the *gag* proteins and evidence for intragenic recombination. Virology **93**:463–481.
22. **Shank, P. R., S. H. Hughes, H.-J. Kung, J. E. Majors, N. Quintrell, R. V. Guntaka, J. M. Bishop, and H. E. Varmus.** 1978. Mapping unintegrated avian sarcoma virus DNA: termini of linear DNA bear 300 nucleotides present once or twice in two species of circular DNA. Cell **15**:1383–1395.
23. **Shine, J., A. P. Czernilofsky, R. Friedrich, J. M. Bishop, and H. M. Goodman.** 1977. Nucleotide sequence at the 5′ terminus of the avian sarcoma virus genome. Proc. Natl. Acad. Sci. U.S.A. **74**:1473–1477.
24. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
25. **Subramanian, K. N.** 1979. Segments of simian virus 40 DNA spanning most of the leader sequence of the major late viral messenger RNA are dispensable. Proc. Natl. Acad. Sci. U.S.A. **76**:2556–2560.
26. **Swanstrom, R., and P. R. Shank.** 1978. X-ray intensifying screens greatly enhance the detection by autoradiography of the radioactive isotopes ^{32}P and ^{125}I. Anal. Biochem. **86**:184–192.
27. **Wang, L. H.** 1978. The gene order of avian RNA tumor viruses derived from biochemical analysis of deletion mutants and viral recombinants. Annu. Rev. Microbiol. **32**:561–592.
28. **Weiss, R. A., W. S. Mason, and P. K. Vogt.** 1973. Genetic recombinants and heterozygotes derived from endogenous and exogenous RNA tumor viruses. Virology **52**:535–552.
29. **Weiss, S. R., H. E. Varmus, and J. M. Bishop.** 1977. The size and genetic composition of virus-specific RNAs in the cytoplasm of cells producing avian sarcoma-leukosis viruses. Cell **12**:983–992.
30. **Yamamoto, T., G. Jay, and I. Pastan.** 1980. Unusual features in the nucleotide sequence of a cDNA clone derived from the common region of avian sarcoma virus messenger RNA. Proc. Natl. Acad. Sci. U.S.A. **77**:176–180.