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# $NH_2$-terminal sequences of two src proteins that cause aberrant transformation

(Rous sarcoma virus/$p60^{src}$)

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**ABSTRACT** **Two isolates of recovered avian sarcoma viruses (rASVs), rASV157 and rASV1702, transform cells in culture, but have greatly reduced *in vivo* tumorigenicity. The src proteins of rASV157 and rASV1702 have alterations within their $NH_2$ termini, are not myristoylated, and have an altered subcellular localization. We have molecularly cloned and determined the nucleotide sequences of the *src* genes of rASV157 and rASV1702. We found that their src proteins have unusual $NH_2$ termini: the rASV157 src protein $NH_2$ terminus consists of 30 amino acids of the env signal peptide attached to Ser-6 of the src sequence, while the rASV1702 src protein $NH_2$ terminus consists of 45 amino acids of the env signal peptide attached to Ala-76 of the src sequence. Expression of recombinant Rous sarcoma virus constructs containing the molecularly cloned rASV *src* genes produced src proteins with the same properties as those of the parental viruses. Our results suggest that the $NH_2$-terminal structures are responsible for many unusual properties of the mutant src proteins.**

Cellular transformation by Rous sarcoma virus (RSV) is mediated by the *src* gene product, $p60^{src}$, whose transforming activity is critically dependent upon both tyrosine kinase activity (1, 2) and $NH_2$-terminal myristoylation, which is required for plasma membrane association (3–7). Two isolates of recovered avian sarcoma viruses (rASVs), rASV157 and rASV1702, encode size-variant (62.5 kDa and 56 kDa, respectively) kinase-active src proteins (8, 9). These src proteins are exceptional in that they are not myristoylated (10) but are active in transformation with respect to morphological changes, colony formation in soft agar, deoxyglucose transport, and cytoskeletal disorganization (8, 11, 12). Cells infected with rASV157 or rASV1702 have a partially transformed phenotype with respect to cell-surface membrane changes and extracellular fibronectin (11). rASV157 and rASV1702 src proteins have an altered membrane association, localized by indirect immunofluorescence to adhesion plaque clusters and scrolls (11) and displaying a salt-sensitive membrane association by subcellular fractionation (12). The *in vivo* tumorigenicity of rASV157 and rASV1702 is greatly reduced; they induce benign, noninvasive tumors that regress rapidly (9, 12).

Previous studies have shown that the src proteins of rASV157 and rASV1702 contain unique $NH_2$-terminal sequences (9) as a result of unusual recombination events between viral (v-*src*) and cellular (c-*src*) DNA during the generation of these rASVs (13–15). rASV157 and rASV1702 are derived from transformation-defective (td) mutant td109, which retains 3′ v-*src* information but lacks 5′ v-*src* and upstream c-*src*-derived sequences (16). td109-derived rASV157 and rASV1702 are replication-defective because of deletion of the *env* gene upon their generation (13). To identify the unique $NH_2$-terminal sequences of the *src* genes of rASV157 and rASV1702 and the basis for the unusual properties of their src proteins, we have molecularly cloned and analyzed the nucleotide sequences of their *src* genes. Finding that their src proteins indeed lacked the ordinary $NH_2$-terminal structure, we constructed recombinant viruses containing the molecularly cloned rASV *src* genes. The biochemical properties of the src proteins produced upon transfection of the *in vitro* constructed recombinants were identical to those of the parental rASVs.

## MATERIALS AND METHODS

**Cell Culture and Viruses.** Chicken embryo fibroblasts (CEF) were prepared, maintained, and infected as described (17). Cell cultures were passaged once after infection to allow virus spread before DNA isolation and biochemical analyses. The isolation and characterization of td109-derived rASV157 and rASV1702 have been described (8–14).

**Molecular Cloning of Proviral DNA Fragments.** High molecular weight DNA was extracted (18) from CEF transformed by rASV157 or rASV1702. The DNA was digested to completion with *Eco*RI and fractionated by agarose gel electrophoresis. Fractions of DNA eluted from gel slices enriched for the 4.6- to 4.8-kilobase (kb) proviral *pol-src* fragments were identified by Southern blotting, ligated to purified λgtWES.λB *Eco*RI arms, and packaged *in vitro* into infectious λ phage by using standard procedures (18). Plaque screening identified phages containing DNA that hybridized strongly with a 0.9-kb *Pvu* II 3′ v-*src* probe, several of which were purified and amplified. The cloned *Eco*RI fragments (see Fig. 1*A*) were isolated from purified phage DNA and subcloned into the *Eco*RI site of pBR322. Restriction enzyme analysis of the recombinant plasmids confirmed that the *Eco*RI fragments contained the mutant *src* genes and that the alterations were confined to the 5′ end of the *src* gene.

**DNA Sequence Analysis.** The *Eco*RI fragments containing the entire *src* sequences of rASV157 and rASV1702 were isolated from recombinant plasmids and were digested with *Kpn* I and *Pst* I. Five of the seven fragments generated contained *src* information. Three *Pst* I–*Pst* I fragments of 345, 255, and 612 base pairs (bp) were subcloned into the *Pst* I site of phage M13mp18 (19). The 456-bp *Pst* I–*Eco*RI fragment containing the 3′ end of *src* was subcloned between the *Pst* I and *Eco*RI sites of M13mp18 and M13mp19. The *Kpn* I–*Pst* I fragments containing the 5′ end of *src*, measuring 461 bp for rASV157 and 296 bp for rASV1702, were subcloned between the *Kpn* I and *Pst* I sites of M13mp18 and

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Abbreviations: rASV, recovered avian sarcoma virus; RSV, Rous sarcoma virus; SR-RSV, Schmidt–Ruppin A strain of RSV; td, transformation-defective; CEF, chicken embryo fibroblasts; kb, kilobase(s); TBR, tumor-bearing rabbit.

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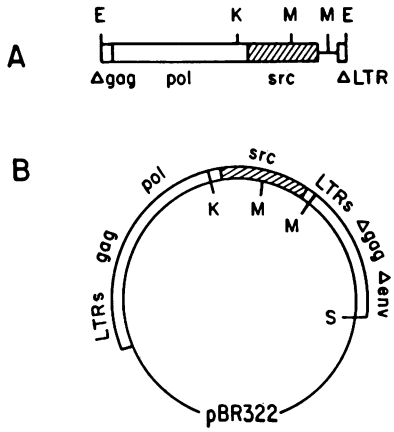


FIG. 1. Structures of the cloned rASV157 and rASV1702 *src*-containing proviral DNA fragments and the construction of recombinant plasmids. (*A*) The 4.6- to 4.8-kb *Eco*RI *src*-containing proviral fragments were molecularly cloned from *Eco*RI-digested total cellular DNA from rASV-transformed CEF into λ phage vectors and subcloned into pBR322. The plasmid clones are referred to as pE157 and pE1702. (*B*) Construction of variant *src*-containing plasmids pS157 and pS1702. Recombinant plasmids contain the molecularly cloned mutant *src* genes, indicated by the hatched box, in a replication-defective *env*-deleted proviral structure. The open boxes represent viral DNA sequences containing long terminal repeats, *gag*, *pol*, and portions of *gag* and *env* sequences, and the thin line represents pBR322 sequences. Restriction enzyme sites used in the construction are designated E (*Eco*RI), K (*Kpn* I), M (*Mlu* I), S (*Sal* I).

M13mp19. The dideoxy chain-termination method of Sanger *et al.* (20) was used to sequence pairs of clones with opposite polarity.

**Plasmid Construction.** Standard recombinant DNA techniques (18) were used to construct plasmids containing the molecularly cloned *src* genes (see Fig. 1*B*). These plasmids, called pS157 and pS1702, were made by a three-part ligation of the following fragments: a 9-kb *Sal* I–*Kpn* I fragment from pSR-REP (21, 22) containing pBR322 sequences, two long terminal repeats, *gag*, and Bryan strain RSV-derived *pol* sequences; a 1.8-kb (for pS157) or 1.7-kb (for pS1702) *Kpn* I–partial *Mlu* I fragment from pE157 or pE1702 containing the entire variant *src* gene; and a 2-kb *Mlu* I–*Sal* I fragment from pSR-XD2 (21) containing two long terminal repeats and portions of *gag* and *env* sequences. The structures of the recombinant plasmids were verified by restriction analysis.

Plasmids were cotransfected with *Sst* I-cut and religated UR2AV helper DNA (23) into CEF as described (21, 22). Fusiform morphological transformation like that induced by the parental rASVs was detected 8–10 days after transfection. Virus stocks were harvested 10–13 days after transfection.

**Protein Analysis.** Labeling of infected cells, cell fractionation, immunoprecipitation of proteins by tumor-bearing rabbit (TBR) serum, *in vitro* protein kinase assay of immune complexes, and NaDodSO$_4$/PAGE analysis were as described (1, 3, 6). One-dimensional partial proteolytic mapping (6) and two-dimensional mapping with *Staphylococcus aureus* V8 protease (24) were as described.

## RESULTS

**Molecular Cloning of rASV157 and rASV1702 *src* Genes.** The proviral *src*-containing *Eco*RI DNA fragments (4.8 kb for rASV157 and 4.6 kb for rASV1702) were isolated from CEF infected with rASV157 or rASV1702 (Fig. 1*A*) and cloned into λgtWES.λB. The 4.6- to 4.8-kb *pol-src* fragments were predicted from the genomic structure of the replication-defective *env*-deleted td109-derived rASVs (13, 14). The *src*-containing *Eco*RI fragments were purified from recombinant λ phage clones and subcloned into pBR322 to generate plasmids pE157 and pE1702.

**DNA Sequence of the *src* Genes of rASV157 and rASV1702.** Comparative restriction analysis of pE157, pE1702, and pBH-β, a plasmid containing the *pol-src* *Eco*RI fragment from the Bryan strain of RSV (25), confirmed that the alterations in the rASV157 and rASV1702 *src* genes were restricted to the extreme 5′ end and that the *src* gene of rASV157 was slightly larger than that of rASV1702, and the analysis indicated that the usual *Nco* I site at the *src* translation initiation site and the upstream flanking regions (26, 27) were absent from the *src* genes of the rASVs. The DNA sequences of the coding regions of the entire *src* genes of rASV157 and rASV1702 were determined to identify the nature of the mutations leading to the size-variant src proteins.

Fig. 2 shows the nucleotide sequences of the *Kpn* I–*Pst* I fragments containing the 5′ end of the *src* genes of rASV157 and rASV1702, the region to which the alterations were localized. Comparison of these sequences with those of the *pol-env* and *src* regions of td109 (15), Prague C RSV (28), or the Schmidt–Ruppin A strain of RSV (SR-RSV) (26, 27) and with the c-*src* sequence (29) revealed that the rASV *src* sequences were fused to various lengths of the *env* signal sequence, which overlaps with the 3′ end of the *pol* gene (28). Previous analysis has indicated that the src mRNAs of rASV157 and rASV1702 are spliced subgenomic mRNAs (13, 14). Translation of subgenomic mRNAs using the env splice acceptor site results in initiation at the same AUG used to initiate translation of gag (28, 30), generating a coding sequence in which the first six amino acids of the env signal peptide are derived from the $NH_2$ terminus of Pr76$^{gag}$ (28, 30). Translation of the sequences shown in Fig. 2 thus yields src proteins with unusual $NH_2$ termini: rASV157 src protein has an $NH_2$ terminus containing 30 amino acids of env signal peptide (including 6 amino acids of gag) attached to Ser-6 of the src sequence, while rASV1702 src protein has an $NH_2$ terminus consisting of 45 amino acids of env signal attached to Ala-76 of the src sequence, using the p60$^{src}$ amino acid sequence numbering of Takeya and Hanafusa (26, 27, 29).

From the codons for Ser-6 in rASV157 and Ala-76 in rASV1702 to the codon for Met-466, the *src* sequences are identical to the c-*src* sequence (29). From the codon for Gly-467 to the termination codon following Glu-526, the rASV157 and rASV1702 *src* sequences are identical to the v-*src* sequence (27, 31). A few nucleotide differences within the 3′ noncoding sequences flanking *src* were observed between the rASVs and SR-RSV. These results are consistent with previous analyses of the rASV157 and rASV1702 genomic RNAs (13, 14) and src proteins (9).

**$NH_2$ Termini of rASV157 and rASV1702 Proteins.** The predicted structures of the src proteins of rASV157 and rASV1702 are shown schematically in Fig. 3*A*. Detailed amino acid sequence comparisons of the $NH_2$ termini of the env protein and the src proteins of RSV, rASV157, and rASV1702 are shown in Fig. 3*B*. The absence of an acceptor Gly-2 is consistent with the lack of N-myristoylation of the src proteins of rASV157 and rASV1702 (3, 5, 7, 10). One-dimensional partial proteolytic mapping with *S. aureus* V8 protease of [$^{35}$S]methionine-labeled src proteins showed that the initiator methionine was not removed from the $NH_2$ terminus of the src proteins of rASV157 and rASV1702 (data not shown), consistent with the presence of the initiator methionine on mutant nonmyristoylated src proteins with an acidic second residue (6, 7).

To verify that the extreme $NH_2$ termini of the rASV157 and rASV1702 src proteins are derived from the $NH_2$ terminus of gag by mRNA splicing to the env splice acceptor site, we performed two-dimensional analysis of *S. aureus* V8 prote-

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rASV157 KpnI - PstI fragment:
                                                                    env | splice
  KpnI                                                                ↓AlaPheLeuThrGlyHisProGlyLysAlaSerLys
 GGTACCCTCTCGAAAAGTTAAACCGGACATCACCCAAAAGGATGAGGTGACTAAGAAAGATGAGGCGAACCCTCTTTTTGCAGGCATTTCTGACTGGGCACCCTGGGAAGGCGAGCAAG
  ValProSerArgLysValLysProAspIleThrGlnLysAspGluValThrLysLysAspGluAlaAsnProLeuPheAlaGlyIleSerAspTrpAlaProTrpGluGlyGluGlnG

                                  src→        10                                  20                                  30
LysAspSerLysLysLysProLeuAlaThrSerLysSerLysProLysAspProSerGlnArgArgArgSerLeuGluProProAspSerThrHisHisGlyGlyPheProAlaSerGln
AAGGATTCCAAGAAGAAACCGCTAGCAACAAGCAAGAGCAAGCCCAAGGACCCCAGCCAGCGCCGGCGCAGCCTGGAGCCACCCGACAGCACCCACCACGGGGGATTCCCAGCCTCGCAG
luGlyPheGlnGluGluThrAlaSerAsnLysGlnGluGlnAlaGlnGlyProGlnProAlaProAlaGlnProGlyAlaThrArgGlnHisProProArgGlyIleProSerLeuAlaA

                  40                                  50                                  60                                  70
ThrProAsnLysThrAlaAlaProAspThrHisArgThrProSerArgSerPheGlyThrValAlaThrGluProLysLeuPheGlyGlyPheAsnThrSerAspThrValThrSerPro
ACCCCCAACAAGACAGCAGCCCCCGACACGCACCGCACCCCCAGCCGCTCCTTTGGGACCGTGGCCACCGAGCCCAAGCTCTTCGGGGGCTTCAACACTTCTGACACCGTCACGTCGCCG
spProGlnGlnAspSerSerProArgHisAlaProHisProGlnProLeuLeuTrpAspArgGlyHisArgAlaGlnAlaLeuArgGlyLeuGlnHisPhe***

                  80                                  90                                  100
GlnArgAlaGlyAlaLeuAlaGlyGlyValThrThrPheValAlaLeuTyrAspTyrGluSerArgThrGluThrAspLeuSerPheLysLysGlyGluArgLeuGln
CAGCGTGCCGGGGCACTGGCTGGCGGCGTCACCACTTTCGTGGCTCTCTACGACTACGAGTCCCGGACTGAAACGGACTTGTCCTTCAAGAAAGGAGAACGCCTGCAG
                                                                                                        PstI


rASV1702 KpnI - PstI fragment:
                                                                    env | splice
  KpnI                                                                ↓AlaPheLeuThrGlyHisProGlyLysAlaSerLys
 GGTACCCTCTCGAAAAGTTAAACCGGACATCACCCAAAAGGATGAGGTGACTAAGAAAGATGAGGCGAACCCTCTTTTTGCAGGCATTTCTGACTGGGCACCCTGGGAAGGCGAGCAAG
  ValProSerArgLysValLysProAspIleThrGlnLysAspGluValThrLysLysAspGluAlaAsnProLeuPheAlaGlyIleSerAspTrpAlaProTrpGluGlyGluGlnG

                                                                    src→        80
LysAspSerLysLysLysProLeuAlaThrSerLysLysAspProGluLysThrProLeuLeuProThrArgValAsnTyrAlaGlyAlaLeuAlaGlyGlyValThrThrPheValAla
AAGGATTCCAAGAAGAAACCGCTAGCAACAAGCAAGAAAGACCCGGAGAAGACACCCTTGCTGCCAACGAGAGTTAATTATGCCGGGGCACTGGCTGGCGGCGTCACCACTTTCGTGGCT
luGlyPheGlnGluGluThrAlaSerAsnLysGlnGluArgProGlyGluAspThrLeuAlaAlaAsnGluSer***

   90                                  100
LeuTyrAspTyrGluSerArgThrGluThrAspLeuSerPheLysLysGlyGluArgLeuGln
CTCTACGACTACGAGTCCCGGACTGAAACGGACTTGTCCTTCAAGAAAGGAGAACGCCTGCAG
                                                        PstI
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FIG. 2. DNA sequences of the *Kpn* I–*Pst* I fragments containing the 5′ ends of the variant *src* genes. The predicted amino acid sequences for src are shown above the DNA sequence, while the predicted sequences for pol are shown below the DNA sequence. Asterisks denote termination codons. Arrows denote the env splice acceptor site and the beginning of src homology. Numbers indicate amino acid positions in src relative to the wild-type sequence (26, 27).

ase-derived fragments of [$^{35}$S]methionine-labeled src and Pr76$^{gag}$ proteins (Fig. 4). Since V8 protease cleaves at the COOH-terminal side of glutamic acid residues, digestion of both Pr76$^{gag}$ and the src proteins of rASV157 and rASV1702 should yield a negatively charged $NH_2$-terminally derived AcMet-Glu peptide (24). Both Pr76$^{gag}$ and the src proteins from rASV157-infected cells contained a comigrating negatively charged [$^{35}$S]methionine-labeled V8 peptide (Fig. 4 *B–D*) that was absent in wild-type RSV p60$^{src}$ (Fig. 4*A*). The negatively charged peptide was also observed upon analysis of the rASV1702 src protein (data not shown).

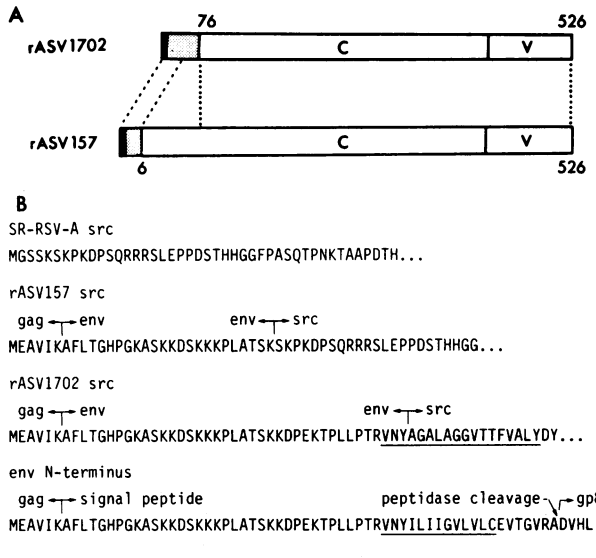


FIG. 3. Structure of rASV src proteins. (*A*) Schematic drawing of the structure of the src proteins encoded by rASV157 and rASV1702. Black boxes denote 6 amino acids derived from the $NH_2$ terminus of Pr76$^{gag}$, stippled boxes denote the env signal sequence (39 residues for rASV1702 and 24 residues for rASV157), and white boxes denote c-src (C) and v-src (V) sequences. Numbers indicate amino acid positions in src. Regions of exact homology are indicated by the sets of parallel dotted lines. (*B*) Comparison of the $NH_2$-terminal sequences of the src proteins of wild-type SR-RSV, rASV157 and rASV1702, and the env protein. Amino acids are designated by the single-letter code. Long hydrophobic regions are underlined.

**Biological Activity of the Cloned *src* Genes.** We constructed recombinant RSV DNAs having a structure similar to the *env*-deleted proviral structure of the parental rASVs by using the molecularly cloned *src* genes (Fig. 1*B*). The proviral structures of these plasmids, pS157 and pS1702, are replication-defective and should not produce virus upon transfection into CEF unless helper virus DNA is cotransfected. CEF were cotransfected with rASV recombinant DNA and *Sst* I-cut and religated UR2AV helper DNA (23) and were maintained as described (21, 22). Eight to 10 days after transfection, fusiform-transformed morphology like that induced by the parental rASVs was observed in cultures transfected with both rASV and helper UR2AV DNAs, whereas no morphological changes were observed in cultures transfected by helper or rASV DNA alone. Viral stocks, designated rASV157-m and rASV1702-m, were harvested from the transformed cultures.

Cells infected with rASV157-m or rASV1702-m produced size-variant src proteins identical in size to the parental rASVs (Fig. 5*A*). These mutant src proteins were not detectably labeled with [$^3$H]myristic acid (Fig. 5*B*). The src proteins displayed a salt-sensitive subcellular fractionation of kinase activity identical to the pattern shown by the parental viruses (Fig. 5*C*).

## DISCUSSION

We have molecularly cloned the *src* genes of two td109-derived isolates of rASV. DNA sequence analysis revealed that the *src* sequences in rASV157 and rASV1702 were fused to various lengths of the *env* signal sequence. The

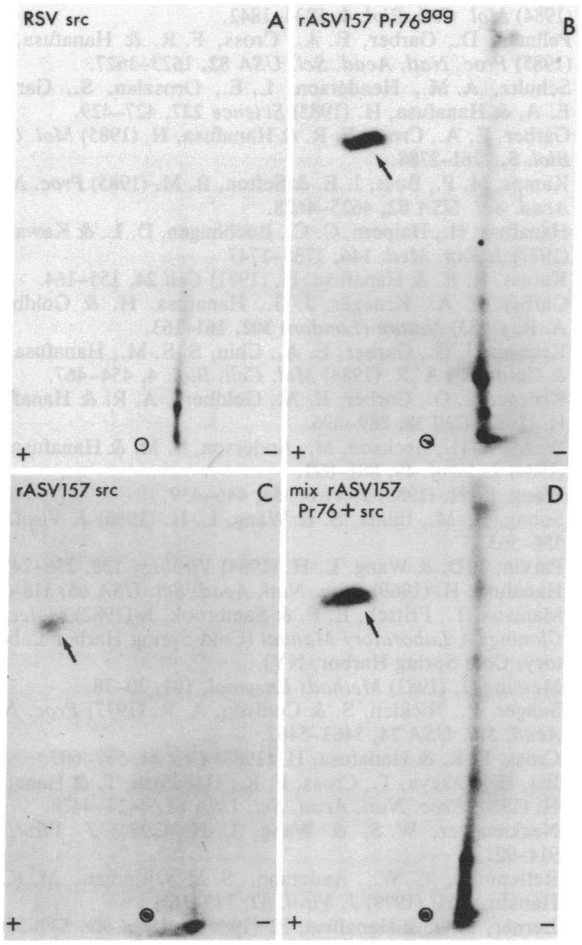


FIG. 4. Two-dimensional analysis of V8 protease digestion products. Infected cells were labeled for 4 hr with [$^{35}$S]methionine, cell extracts were immunoprecipitated with TBR serum, and immunoprecipitates were analyzed by NaDodSO$_4$/PAGE. Gel bands were excised, and proteins were subjected to V8 proteolysis and two-dimensional analysis as described (24). (*A*) SR-RSV p60$^{src}$. (*B*) rASV157 Pr76$^{gag}$. (C) rASV157 src protein. (D) A mixture of rASV157 Pr76$^{gag}$ and src proteins. Samples were spotted at the origin (O), and electrophoresis at pH 6.5 was performed in the horizontal direction with the anode at the left. Ascending chromatography was from bottom to top. Arrows mark the position of the negatively charged $NH_2$-terminal peptide.

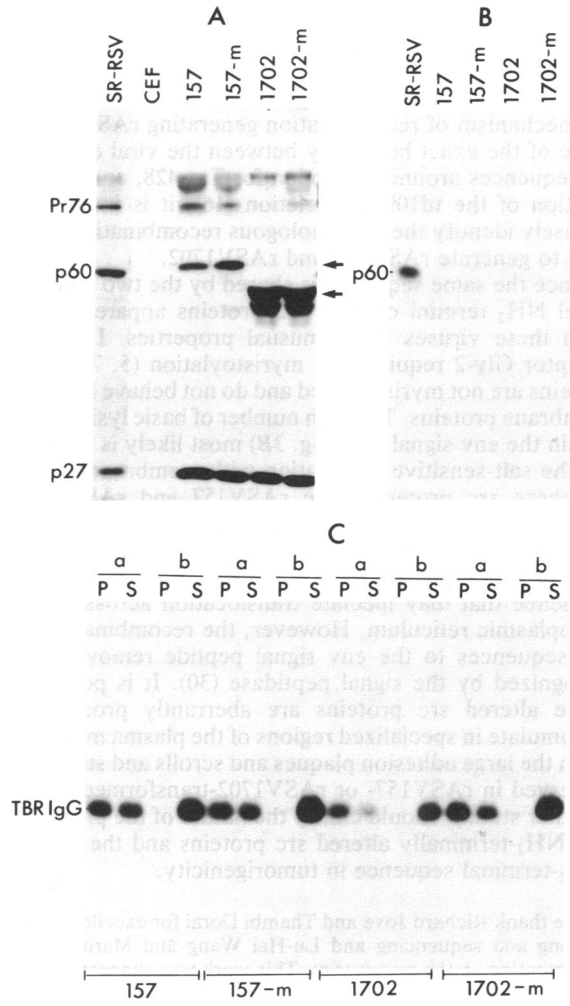


FIG. 5. Analysis of src proteins in infected cells. Uninfected CEF or virus-infected cells were labeled for 4 hr with [$^3$H]leucine (*A*) or [$^3$H]myristic acid (*B*). Cell extracts were immunoprecipitated with TBR serum, and immunoprecipitates were analyzed by NaDodSO$_4$/PAGE and fluorography. Arrows indicate the size-variant 62.5-kDa rASV157 (lanes 157 and 157-m) and 56-kDa rASV1702 (lanes 1702 and 1702-m) src proteins. (The band below rASV1702 src is a proteolytic breakdown product.) (*C*) Salt-sensitive membrane association of rASV src proteins. Homogenates of infected cells were separated by differential centrifugation as described (3, 6) into crude membrane (lanes P) and cytosolic (lanes S) fractions. The salt concentration of the postnuclear supernatant was adjusted to 10 mM (lanes a) or 300 mM (lanes b) NaCl. Fractions were solubilized and immunoprecipitated with TBR serum, and immune complexes were subjected to *in vitro* kinase assay and analyzed by NaDodSO$_4$/PAGE.

subgenomic src mRNAs of these rASVs apparently use the env splice acceptor and are translated into src proteins with novel $NH_2$ termini: the rASV157 src protein $NH_2$ terminus has 30 amino acids of env signal attached to Ser-6 of the src sequence, while the rASV1702 src protein has 45 amino acids of env signal attached to Ala-76 of the src sequence. We verified that the $NH_2$ termini of the src proteins were derived from the $NH_2$ terminus of gag by mRNA splicing by analysis of V8 protease peptides. The $NH_2$-terminal env signal peptide sequence common to the src proteins of rASV157 and rASV1702 gave rise to the previously described unique $NH_2$-terminal tryptic peptides of these src proteins (9). Expression in CEF of the molecularly cloned *src* genes in recombinant constructs produced transforming proteins with properties similar to those of the parental rASVs.

The sequences of the 3′ end of *pol* containing the overlapping *env* signal sequence were identical in the homologous regions of td109 (15), rASV157 and rASV1702. Sequences of homologous regions of rASV157 and rASV1702 and c-*src* (29) and v-*src* (26, 27, 31) were also identical.

The recombinations between td109 and c-*src*, generating rASV157 and rASV1702, apparently occurred downstream of the beginning of the *src* coding sequence. Comparison of the relevant regions of td109 (15) and c-*src* (29) reveals short stretches of homology (underlined),

AGCAAGA** AAGACCCGGAGAAGACACCC in td109
AGCAAGAGCAAG*CCCA*AG**GAC*CCC in c-*src*

(where an asterisk denotes a gap), that may have been involved in the generation of rASV157. The recombination event generated a direct repeat of the 6-bp sequence AGCAAG at the 5′ recombination junction and resulted in the replacement of the last 12 amino acids of pol with 61 new amino acids (Fig. 2). Since the pol protein of rASV157 is complemented by the wild-type td109 pol protein, experi-

ments with nonproducer cells transformed by rASV157 or rASV157-m would be necessary to determine whether the aberrant pol COOH terminus affects any pol function. No immediately obvious homologies could be found to identify the mechanism of recombination generating rASV1702. Because of the exact homology between the viral and cellular *src* sequences around the codon for Trp-428, containing the junction of the td109 *src* deletion (16), it is impossible to precisely identify the 3′ homologous recombination junction used to generate rASV157 and rASV1702.

Since the same sequence is shared by the two rASVs, the novel $NH_2$ termini of their src proteins apparently confer upon these viruses their unusual properties. Lacking the acceptor Gly-2 required for myristoylation (5, 7), their src proteins are not myristoylated and do not behave like integral membrane proteins. The high number of basic lysine residues within the env signal (see Fig. 3*B*) most likely is responsible for the salt-sensitive association with membranes observed for these src proteins. The rASV157 and rASV1702 src proteins could be processed like the env protein, which is translated on membrane-bound polysomes (30). The src protein of rASV1702 even possesses a long hydrophobic sequence that may mediate translocation across the rough endoplasmic reticulum. However, the recombination fusing src sequences to the env signal peptide removes the site recognized by the signal peptidase (30). It is possible that these altered src proteins are aberrantly processed and accumulate in specialized regions of the plasma membrane to form the large adhesion plaques and scrolls and surface blebs observed in rASV157- or rASV1702-transformed cells (11). Further studies should clarify the nature of the processing of the $NH_2$-terminally altered src proteins and the role of the $NH_2$-terminal sequence in tumorigenicity.

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