Involvement of Directly Repeated Sequences in the Generation of Deletions of the Avian Sarcoma Virus src Gene

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Nucleotide sequence analysis of two molecular clones of transformation-defective avian sarcoma virus indicate that direct repeated sequences of 6 and 20 nucleotides are involved in the formation of the src deletions in these clones.

During the replication of avian sarcoma viruses (ASV), transformation-defective variants appear that contain deletions of the transforming gene sequences denoted src (13, 17, 18). These src gene deletions range from those that are 2.0 kilobases in length (5, 13, 15) and appear to have lost most or all of the src gene and are therefore unable to complement point mutations in src (2, 11) to those that are less than 2.0 kilobases in length and are capable of complementing point mutations in the src gene (8, 9, 11). Moreover, the latter deleted transformation-defective ASV are apparently able to recombine with the cellular homolog of src to produce transformation-competent virus (8, 9, 11, 13). In this report, we present nucleotide sequencing data from two molecular clones of transformation-defective ASV that indicate the involvement of direct repeated sequences 6 to 20 nucleotides long in the formation of these src deletions.

DNA sequence analysis was performed on two Prague C (PrC) strains of ASV with src deletions, and the resulting nucleotide sequence information was compared with that for the nondefective PrC strain of ASV. The two src gene deletion sequences were from molecularly cloned circular viral DNAs designated pATV6 and pATV9 as described previously (10). Restriction endonuclease mapping studies indicated that pATV6 and pATV9 contained src gene deletions of approximately 1,700 and 2,000 nucleotides, respectively. Nucleotide sequence analysis of these two clones was performed from a SacI restriction endonuclease site at the C terminus of the envelope (env) gene across the src gene deletion and into the large terminal repeat sequence (Fig. 1). The resulting DNA sequences were compared with the nucleotide sequence of the transformation-competent PrC ASV genome (16). The deletion in pATV9 occurred at a 13- to 14-nucleotide direct repeat, whereas that of pATV6 was at a 5- to 6-nucleotide direct repeat (Fig. 2). The extent of the deletion in pATV6 was 1,679 nucleotides; the deletion in pATV9 was 2,015 nucleotides. The remainder of the nucleotide sequences between the env gene and the large terminal repeat in these two deleted clones were identical to the nucleotide sequence of the nondefective PrC ASV determined previously, except for an adenine-thymine base pair in the noncoding region between src and the large terminal repeat (position 8958 of PrC ASV) (16) in pATV6, which appears as a guanine-cytosine base pair in the nondefective PrC sequence (16). Both of the deleted ASV analyzed lack the entire amino acid coding sequence for src.

When the DNA sequence of the previously described cDNA clone (pSR-2) of the Schmidt-Ruppin A (SRA) strain of ASV (19) is compared with the sequence of the src gene from a nondefective SRA strain of ASV (4), it appears that the deletion occurred at a direct repeat of 17 to 20 nucleotides, resulting in the removal of 2,007 nucleotides (4, 19). The nucleotide sequence of the region contiguous to the src gene in SRA ASV has been previously shown to contain a 126-nucleotide direct repeat exhibiting 79% homology (4). It was suggested that this large direct repeat might be involved in the formation of the src gene deletions present in the transformation-defective ASV (4). In the case of the SRA cDNA clone containing an src deletion, this does appear to be the case (Fig. 1b) since a 17- to 20-nucleotide direct repeat found at the endpoints of the deletion is part of the larger direct repeat. The nucleotide sequences surrounding the src gene in PrC ASV are different from those in SRA ASV. A computer analysis of the nucleotide sequences adjacent to the src

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The structure of the PrC ASV src gene region of two src deletions and the method by which they were sequenced. The size and extent of the deletions in clones pATV6 and pATV9 are shown in comparison with the intact PrC ASV src region (A). The size and extent of the deletion in pSR-2, the SRA ASV cDNA clone of Yamamoto et al., are compared with the intact src region of SRA ASV (4, 19) (B). The deleted regions are shown by the solid black boxes. The large terminal repeat of the virus is designated LTR; the transforming protein and the envelope glycoprotein genes are designated src and env, respectively. Also shown are the direct repeats which surround the src gene regions of PrC ASV and SRA ASV. These direct repeats are indicated by the open boxes with A homologous to A', B to B', and C to C'. The numbering of the PrC sequences is that of the complete PrC ASV sequence (16). The scheme used to sequence the deletions in pATV6 and pATV9 is shown in C. The 5'-end-labeled fragments (●) and 3'-end-labeled fragments (○) were sequenced by the method of Maxam and Gilbert (12, 14).

FIG. 1. DNA sequence at the site of the src deletions and the corresponding sequences in the intact src gene. The DNA sequences determined as shown in Fig. 1C at the deletion points in the src gene are shown for pATV6 (a) and pATV9 (b). They are compared with the corresponding regions from the intact PrC-ASV src gene (16). The DNA sequence from the cDNA clone pSR-2 determined by Yamamoto et al. at the site of deletion is shown and compared with the sequence of the intact SRA ASV src gene (c) (4, 19). The directly repeated sequences from the intact PrC ASV and SRA ASV src genes at the points of deletion are underlined.
observation is consistent with the contention that the shorter deletions occur at short direct repeats present at multiple sites within the src gene region.

A proposed mechanism for deletions facilitated by direct repeats involves slippage of one DNA strand during replication, causing the region between the direct repeats to loop out (1, 6). This results in one of the progeny DNA molecules containing a deletion spanning the region between the direct repeats. Such a mechanism could occur in integrated copies of proviral DNA, thus leading to deleted RNA transcripts being made and packaged into virions. A similar mechanism could also be envisioned to occur during reverse transcription, again resulting in a deleted proviral DNA being integrated into the chromosomes of infected cells and giving rise to deleted virion RNA.

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LITERATURE CITED