3'-Terminal Region of Avian Carcinoma Virus MH2 Shares Sequence Elements with Avian Sarcoma Viruses Y73 and SR-A

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We determined the nucleotide sequence of the acute transforming avian retrovirus MH2 from an HgiAI site within the coding region of its oncogene, v-myc, to the KpnI site within the long terminal repeat. Comparison with published sequences from other retroviruses allowed us to identify all sequence elements in this region. We conclude that MH2 contains a unique assembly of 3'-terminal sequences, which includes part of the helper virus-derived SPC region of avian sarcoma virus Y73 and the complete F3 and F1 segments of Rous sarcoma virus strain SR-A.

MH2 is an avian carcinoma-inducing retrovirus which contains two unrelated and independently expressed oncogenes, v-mil and v-myc (5, 9–11). The v-mil gene is the avian homolog of the previously isolated murine oncogene v-raf (8, 15, 17, 18, 21), and the v-myc gene is shared with the MC29 family of avian acute transforming retroviruses (2). The mode of acquisition of these two oncogenes by the parent virus of MH2 is unclear. The oncogenes may have been combined first in cells by a chromosome rearrangement (4, 14) before retrovirus capture; they may have been transcribed separately, giving rise to independent virus isolates which subsequently recombined; or they may have been acquired from cells in two steps by the same virus. To begin our investigation into the origin of MH2, we sequenced its 3'-terminal region and compared it with those of other avian retroviruses. This portion of the viral genome commonly has a structure that is unique for a given virus strain (20, 22) and is therefore useful for the determination of family relationships.

The complete nucleotide sequence from 55 base pairs downstream of the HgiAI site in v-myc of MH2 to the KpnI site in the U3 region of the long terminal repeat LTR is shown in Fig. 1. The MH2 sequence was compared with the v-myc oncogene in the acute leukemia virus MC29 and in the 3'-terminal regions of two avian sarcoma viruses, Y73 and Rous sarcoma virus (RSV) strain SR-A. Y73 and RSV carry two distinct cell-derived oncogenes, v-yes and v-src, and also differ in their genetic structures in that v-yes was acquired at the expense of the complete viral pol gene and portions of its env gene, whereas v-src is located 3' of the vion structural genes in RSV (Fig. 2). Immediately 3' of the Δenv gene in Y73 is a 161-base sequence termed SPC (12) that is not present in RSV strains PrC and SR-A. The SPC sequence is followed by 121 nucleotides that are common to Y73 and RSV. The MH2 sequence (Fig. 1) from nucleotide positions −78 to −1 was homologous to nucleotides 1241 to 1328 of the v-myc sequence in MC29 (1). This comparison shows that the 3' untranslated region of v-myc in MH2 is 271 base pairs shorter than that reported for v-myc in MC29 (1, 19), which is in excellent agreement with previous oligonucleotide (9) and heteroduplex analyses (10) of the MH2 genome.

Nucleotides 1 to 68 were homologous to nucleotides 3210 to 3277 of the SPC region of avian sarcoma virus Y73 (12). The sequence includes at its 3' end a stretch of 20 nucleotides with homology (18 of 20) to the C terminus of v-src in RSV strains SR-A and PrC. The two single-base changes in this region are in silent third-base positions. There is, however, no splice acceptor nor AUG initiation signal positioned in a way that this sequence could be expressed. The origin of the SPC sequence is unknown, but presumably it was derived from helper virus rather than from cellular sequences (12).

The sequence (nucleotides 69 to 396) following this presumptive helper virus region showed continuous homology to nucleotides 8709 to 9149 of the SR-A strain of RSV (20). The degree of homology was 82%, with a total of 61 single-base changes and 11 small deletions or insertions of 1 to 13 nucleotides. The sequence from nucleotide positions 69 to 182 in MH2 is homologous to a region termed F3 by Takeya and Hanafusa that may have some relatedness to both c-src and a segment of Rous associated virus (RAV) O (22). This region, which is absent in Y73, is followed by the direct repeat sequence (6) termed F1 by Takeya and Hanafusa (22) that occurs 5' and 3' of v-src in RSV strains SR-A and PrC; F1 is also present in the avian sarcoma virus Y73 and in the helper viruses RAV-O and RAV-2 (3, 13).

Figure 2 gives a schematic representation of the relative locations of sequence elements SPC, F1, F2, and F3 in the 3'-terminal regions of avian retroviruses MH2 and RSV strain SR-A. We conclude from this comparison that the 3'-terminal region of MH2 consists of a unique mosaic of sequence elements, all of which are shared with at least one other isolate of avian sarcoma virus.

Because a limited number of sequences were available for comparison, these findings do not establish a clear parentage for MH2. There are some pathways for its formation, however, that appear unlikely. (i) A two-step recombination model in which v-mil was first acquired from cells followed by the incorporation of a partial c-myc gene into its 3'-untranslated region would predict the presence of nonviral (c-mil) sequences 3' of MH2 v-myc, which we did not find. It remains possible, however, that the recombination with c-myc was at the 3' end of mil or that a residual mil sequence was deleted subsequent to this recombination. (ii) A model involving recombination between a v-mil transductant and any of the known v-myc-carrying viruses of the MC29 group (2) appears unlikely because of the unique structure of v-myc in MH2, which contains 172 nucleotides of the first c-myc
intron (21) and has a shorter 3' untranslated region than other isolates, as we show here. We favor instead the possibility that both oncogenes in MH2 were acquired in one step from cells in which they had previously been brought together by a chromosome rearrangement (4, 14).

An emerging pattern suggests that sequence homologies between short segments of virion structural genes and cellular protooncogenes at both the 5' and 3' junctions of viral oncogenes are involved in the mechanism of transduction (23, 24; T. I. Bonner, S. Kirby, P. Sutrave, M. Gunnell, G. E. Mark, and U. R. Rapp, submitted for publication). Such a region of homology is apparent between the human c-raf-1 sequence and the PrC viral gag p12 sequence at the 5' junction of v-mil in MH2 (Bonner et al., submitted for publication). In contrast, no such homology was observed between the SPC sequence (Fig. 2) and the 3' untranslated region of c-myc. However, a 3' junction sequence may have been obliterated by secondary deletion events which are commonly observed in defective retroviruses with a long passage history.

In MH2, 48 nucleotides 3' of myc, there is a relatively long stretch of 20 nucleotides within SPC with homology to the C terminus of v-src. The v-src homologous sequence together with the remainder of the SPC sequence of Y73 have recently also been identified in the 3' noncoding region of RAV-2 (3, 13). From work with recovered avian sarcoma viruses, it was suggested that the 3'-terminal 39 nucleotides of v-src in transformation defective RSV are not sufficient to generate oncogenic virus-c-src recombinants (recovered avian sarcoma viruses) at measurable frequency (24). However, the positions of 20 of the 39 nucleotides at the v-src-virus junction in RSV strains SR-A and PrC and their
presence in cellular DNA downstream from the 12th c-src exon (22) suggest to us that they probably were involved in the original formation of these sarcoma viruses.

The presence of F3, F1, and RF2 in MH2 and RSV strain SR-A may suggest that both viruses as well as RSV strain PrC and avian sarcoma virus strain Y73 were derived from a common helper virus that contained all four elements, SPC, F3, F1, and F2, in their entirety. The current structure of their 3'-terminal regions might then have been formed by secondary deletions in certain of these elements (with the exception of F1, which is uniformly present) which are specific for each virus strain. Alternatively, there might have been different parental helper viruses involved with variable complements of these 3'-terminal sequence elements. So far, the evidence is in favor of this latter possibility, since the two helper viruses for which pertinent sequence data are available, RAV-O and RAV-2 (3, 13) only contain some (F1 for RAV O, F1 and SPC for RAV-2) of these sequences.

Although the high frequency of homologous recombination between different virus strains (7) may make a complete retrospective pedigree analysis impossible, examination of the natural helper viruses of MH2 should provide further insight into their role in the generation of this unique oncogene-transducing avian retrovirus.

LITERATURE CITED


