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Sequence Homology Between Avian and Human Adenoviruses

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Studies of hybridization between fowl adenovirus type 1 (chicken embryo lethal orphan virus) DNA and human adenovirus type 2 DNA revealed two short but distinct regions which cross-hybridized under stringent conditions. One of the homologous regions was located between map positions 18.1 and 19.3 and did not correspond to any gene recognized so far. The second region mapped in the hexon gene between positions 57 and 58.

The adenovirus family contains a large number of viruses and includes viruses from a number of animal species besides the well-known human serotypes (14). Most members of the adenovirus family share common group-specific antigenic determinants, which together with the capsid morphology are used as criteria for classification. The avian adenoviruses do not share the common group-specific adenovirus antigen (14), and the classification of these viruses as adenoviruses is based primarily on morphological criteria. Ideally, viruses should be classified according to sequence homologies since the evolution of a virus is the result of gradual changes in the nucleotide sequence of the genome. Extensive sequence homology studies of the human adenoviruses have been performed (7, 8, 11), and the results show a remarkably high degree of sequence variation among the different subgroups of human adenoviruses (7, 8). So far, there have been few studies which have examined the relationship between adenoviruses of human and animal origin. Larsen et al. (12) have shown that mouse adenovirus strain FL shares little sequence homology with the human adenoviruses, although clear cross-hybridization was apparent when sensitive methods were used. Studies on the homologies between different adenoviruses are important for several reasons; the sequence relationships between different adenoviruses should provide information about the way in which the viruses evolved from a presumptive common ancestor, and the results are also important for a rational approach to virus taxonomy.

In a comparative hybridization study with avian and human adenoviruses, we used the method of Howley et al. (9), which allows the detection of partially homologous sequences. Since the genomes of the subgroup C human adenoviruses have been mapped in greatest detail, we chose the adenovirus type 2 (Ad2) genome as a reference; Ad2 DNA was cleaved with restriction endonucleases *Hin*dIII and *Xho*I, and the resulting fragments were transferred to nitrocellulose (17). In the hybridization experiments, ^{32}P-labeled chicken embryo lethal orphan (CELO) virus DNA (16) was used as a probe, and hybridizations were carried out at 37°C in 20, 30, 40, and 50% formamide, as described by Howley et al. (9) (Fig. 1). Under the less stringent hybridization conditions (i.e., at concentrations of 20 and 30% formamide), CELO virus DNA hybridized weakly to nearly all fragments, whereas at 50% formamide hybridization was limited to *Xho*I fragments E and F and *Hin*dIII fragments A and B (Fig. 1). *Hin*dIII fragments I and J, which are located between map positions 31.5 and 41.0 in the Ad2 genome, gave particularly weak hybridization signals, even at the lowest formamide concentration.

To map the conserved regions in more detail, we studied hybridization between ^{32}P-labeled CELO virus DNA and different sets of restriction enzyme cleavage fragments of Ad2 DNA. The hybridizations were carried out under standard conditions (i.e., annealing in 6× SSC [1× SSC is 0.15 NaCl plus 0.015 sodium citrate] at 65°C, followed by extensive washing in 2× SSC at the same temperature). The fragments which hybridized are shown in Table 1, and Fig. 2 shows selected autoradiograms from the hybridizations. Taken together, the results suggest that two widely separated regions in the Ad2 genome contain sequences which are homologous to CELO virus DNA. One region maps between positions 18.1 and 21.6, whereas the other maps between positions 57 and 58.5.

For more detailed mapping studies of the

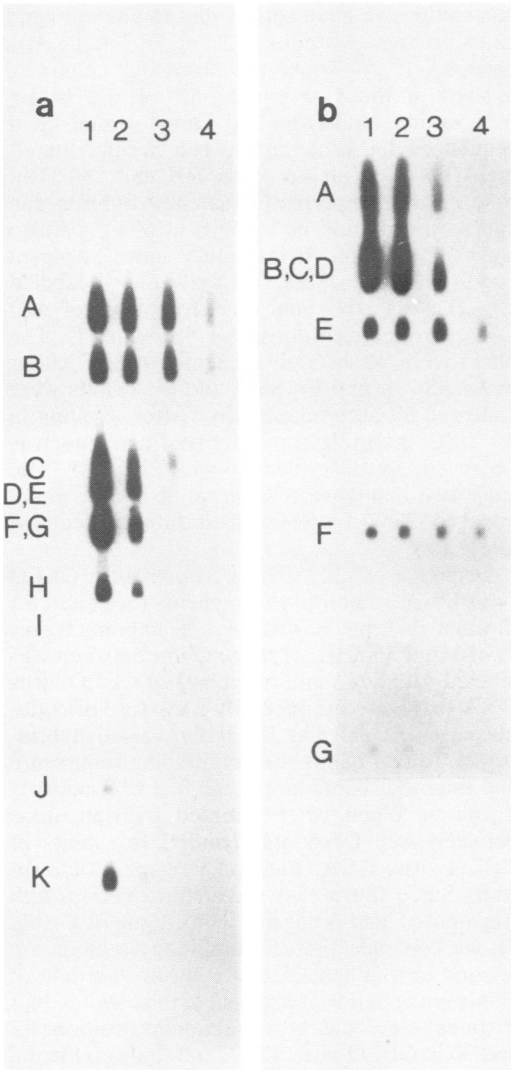

FIG. 1. Hybridization between CELO virus DNA and fragments of Ad2 DNA generated by restriction endonucleases *Hin*dIII (a) and *Xho*I (b). CELO virus was grown as described by Laver et al. (13), and DNA was prepared by the method of Pettersson and Sambrook (15). The hybridizations were carried out at 37°C in 20% (lanes 1), 30% (lanes 2), 40% (lanes 3), or 50% (lanes 4) formamide. The filters were washed in 6× SSC at 45, 50, 55, and 65°C, respectively. Hybridization was visualized by autoradiography, and 250,000 cpm of ^{32}P-labeled CELO virus DNA (16) in a total volume of 2 ml was used as the probe for hybridization.

leftmost homology region, we used a cloned fragment of Ad2 DNA which includes the region between map positions 17.0 and 24.5 (M. Pettersson, unpublished data). DNA from this clone was cleaved with endonuclease *Taq*I, and the resulting fragments were transferred to a nitrocellulose membrane. Subsequent hybridization with ^{32}P-labeled CELO virus DNA as a probe revealed hybridization to a 930-nucleotide fragment (Fig. 2B, fragment Y). From DNA sequencing studies of this region (manuscript in preparation), it has been established that the left border of this *Taq*I fragment is located 20 base pairs to the right of the cleavage site for endonuclease *Sma*I at map position 18.1. To map the leftmost homology region further, we cloned the 930-base pair *Taq*I fragment, and the DNA from the clone was cleaved with either restriction endonuclease *Hin*dII or restriction endonuclease *Sac*II. A subsequent hybridization analysis indicated homology to a fragment located to the left of a *Hin*dII cleavage site at map position 19.3. After *Sac*II cleavage, hybridization to two fragments was observed (Fig. 2C), which indicated that the homology region traverses the *Sac*II cleavage site at map position 18.8. Figure 3 shows a map of the relevant cleavage sites and also indicates the precise location of the homology region.

The mapping of the rightmost homology region was facilitated by the results of our previous studies of the Ad2 hexon gene (1, 2). Several restriction enzyme cleavage sites have been mapped in this region, and consequently it was easy to design experiments which narrowed the limits for the homology region. The most informative results were obtained by using fragments of Ad2 DNA that were generated by endonucleases *Sac*I and *Bal*I. We observed hybridization to *Sac*I fragments E (map positions 16.5 to 21.6) and C (map positions 57 to 70.7), as well as to *Bal*I fragments E (map positions 14.7 to 21.5) and C (map positions 49.6 to 58.0). Taken together, the data show that the rightmost homology region is located between the *Sac*I cleavage site at map position 57 and the *Bal*I cleavage site at position 58. Figure 3 shows a schematic drawing of the homology regions, indicating the positions of the relevant cleavage sites. In previ-

TABLE 1. Restriction enzyme fragments of Ad2 DNA which hybridize to CELO virus DNA under stringent conditions

Fragments of Ad2 DNA which hybridize to CELO virus DNA	Map positions for fragments which give positive hybridization
*Eco*RI-A	0-58.5
*Bam*HI-B, *Bam*HI-C	0-29.0, 42.0-59.5
*Xho*I-F, *Xho*I-E	15.5-22.0, 53.0-66.0
*Hin*dIII-B, *Hin*dIII-A	17.0-31.5, 50.1-73.6
*Sma*I-B, *Sma*I-A	18.1-36.7, 56.9-75.8
*Kpn*I-B, *Kpn*I-D	5.8-23.5, 47.4-61.3
*Sac*I-E,[a] *Sac*I-C	16.5-21.6, 57-70.7

[a] We observed hybridization to a band which contained a mixture of fragments E, F, and G.

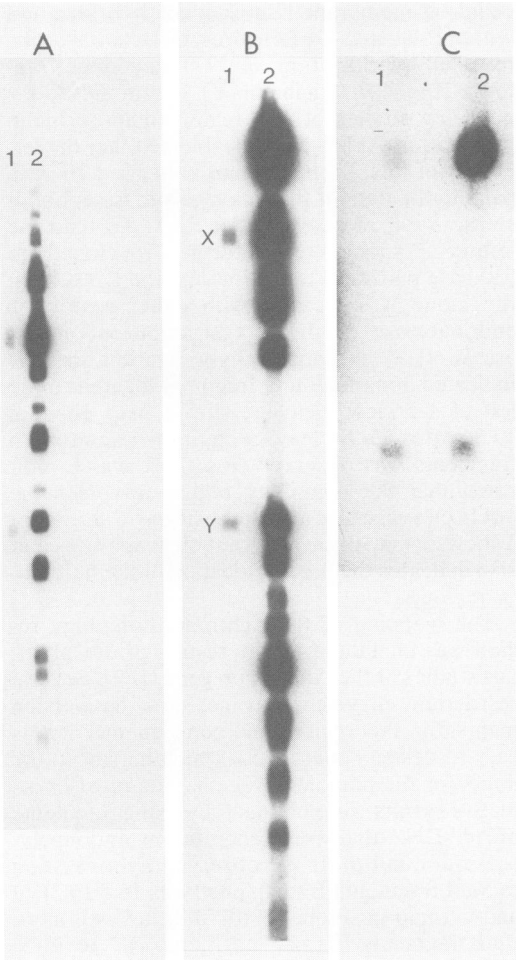

FIG. 2. Hybridization between ^{32}P-labeled CELO virus DNA and fragments of Ad2 DNA under stringent hybridization conditions. The hybridizations were performed in 6× SSC supplemented with 0.2% sodium dodecyl sulfate and 5× Denhardt solution (4) at 65°C for 18 h. The filters were incubated in 6× SSC containing 5× Denhardt solution at 65°C for at least 3 h before hybridization. After hybridization the filters were washed in several changes of 2× SSC at 65°C. Hybridization was detected by autoradiography, using intensifying screens. Fragments generated by a mixture of *Xho*I and *Sma*I (A) or *Taq*I alone (B) were used. In each case a separate filter strip was hybridized to ^{32}P-labeled Ad2 DNA to visualize all fragments (lane 2). Fragments X and Y (B) were 930- and 2,200-base pair *Taq*I fragments which corresponded to the left and the right homology regions. Hybridization was also carried out between ^{32}P-labeled CELO virus DNA and fragments of a plasmid which carries a 930-base pair *Taq*I fragment of Ad2 DNA (C). The fragments were generated with a mixture of endonucleases *Sac*II and *Bam*HI. The results obtained after hybridization with ^{32}P-labeled Ad2 DNA are also shown (lane 2).

ous studies we have shown that the hexon gene maps between positions 51.2 and 59.7 in the Ad2 genome (1, 2). Thus, the homology region is located in the C-terminal part of the hexon polypeptide, and from the known amino acid sequence of the hexon we can predict that it maps between amino acids 609 and 786 (10). Since rather imperfectly matched hybrids can survive incubation in 2× SSC at 65°C, we also washed our filter strips under more stringent conditions. Hybridizations between ^{32}P-labeled CELO virus DNA and *Taq*I fragments of Ad2 DNA were carried out in 6× SSC at 65°C. The filters were washed subsequently at 65°C either in 2× SSC or in 0.1× SSC, and the results were analyzed by autoradiography. After washing in 2× SSC, hybridization to two fragments was observed, as described above (Fig. 2B). The same two bands were apparent also after washing at 65°C in 0.1× SSC, albeit they had reduced intensities.

Denisova et al. (5) have shown that CELO virus DNA is cut into 15 fragments (designated 1 through 15) by a mixture of endonucleases *Eco*RI and *Hin*dIII. Hybridization between ^{32}P-labeled Ad2 DNA and fragments of CELO virus DNA that were produced by a mixture of endonucleases *Eco*RI and *Hin*dIII revealed hybridization to two bands, one containing fragment 6 and the other containing a mixture of fragments 4 and 5. When we performed hybridizations between Ad2 DNA and *Hin*dIII fragments of CELO virus DNA, fragments A and C hybridized. Since these two fragments overlap with fragments 5 and 6 but not with fragment 4 (Fig. 4), we conclude that the homology regions are located in fragments 5 and 6. From the data of Denisova et al. (5), it is possible to calculate that fragment 6 is located between map positions 12 and 20 in CELO virus DNA and that fragment 5 is located between map positions 41 and 49 (Fig. 4). To align the Ad2 and CELO virus genomes with each other, we cloned the fragments of CELO virus DNA which were generated by a mixture of endonucleases *Eco*RI and *Hin*dIII in the *Escherichia coli* plasmid pBR322 by using a previously described procedure (18). Hybridizations between clones containing CELO virus fragments 5 and 6 and *Xho*I fragments of Ad2 DNA showed that CELO virus fragments 5 and 6 correspond to the right and the left homology regions, respectively.

Among the adenoviruses which have been characterized to date, the human and avian adenoviruses ought to occupy the two extreme positions on the evolutionary scale. In fact, the adenoviruses are subdivided into two separate genera, mastadenoviruses and aviadenoviruses. In this study we clearly demonstrated a distinct sequence relationship between the two catego-

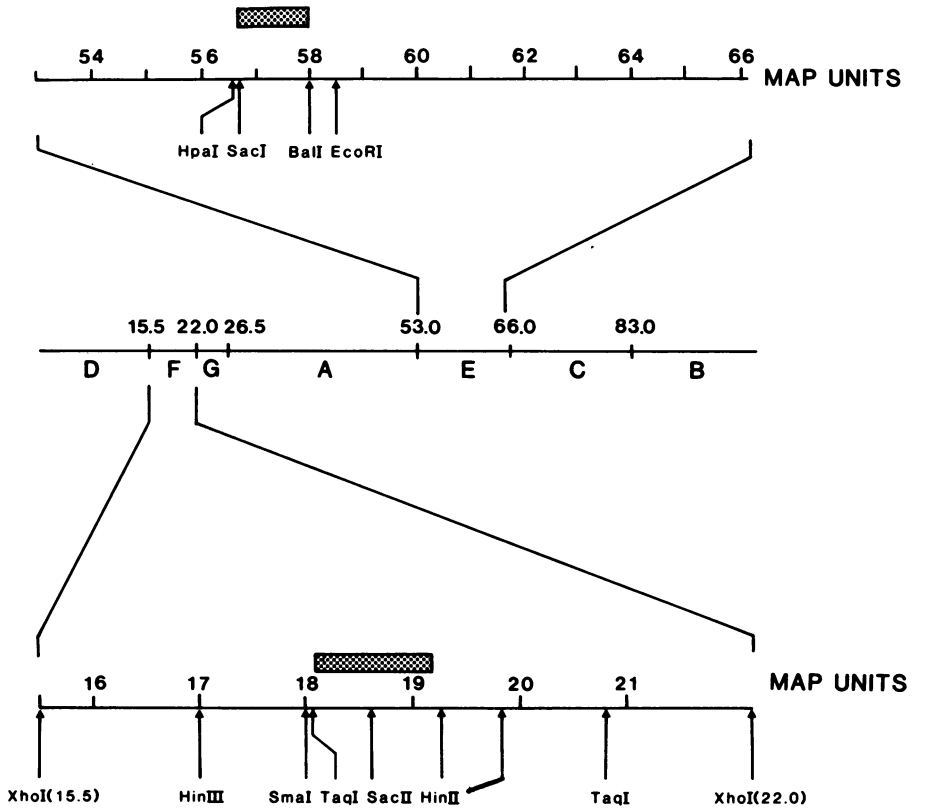

FIG. 3. Schematic drawing indicating the precise locations of the homology regions (stippled areas). The *Xho*I cleavage map of Ad2 DNA is shown, as well as enlargements of *Xho*I fragments F and E with selected restriction enzyme cleavage sites. HinIII, *Hin*dIII.

ries of adenoviruses and found that two regions are highly conserved. Of the two conserved regions, one is located in an expected position; at least part of the hexon gene should be conserved in order to generate a capsid protein which gives rise to the characteristic adenovirus architecture. The second region is located in a somewhat unexpected position. Recently, Stillman et al. (19) and Chow et al. (3) have provided evidence for a transcript coming from the *l*-strand of this region. One of the corresponding mRNAs encodes the terminal protein which is attached to the 5′ termini of adenovirus DNAs (19). However, recent sequence studies (manuscript in preparation) have indicated that the gene for the terminal protein terminates to the

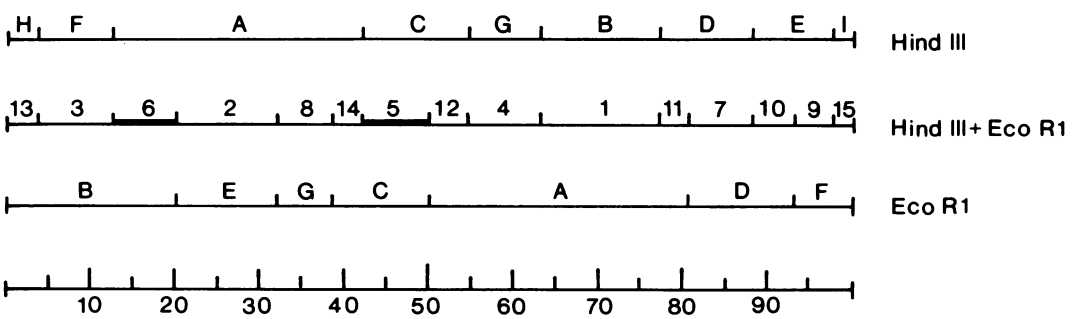

FIG. 4. Restriction enzyme cleavage maps of CELO virus DNA for endonucleases *Eco*RI and *Hin*dIII and a mixture of *Eco*RI and *Hin*dIII. The data are from Denisova et al. (5). The fragments which cross-hybridize with Ad2 DNA are indicated by heavy lines.

right of map position 19.3. Instead, there appears to be an additional open translational reading frame in the region of the Ad2 genome, where we have mapped the homology region (manuscript in preparation). No polypeptide has been assigned yet to this region, although Galos et al. (6) have mapped the so-called N gene close to the position of the homology region.

In eucaryotic cells the histone genes are extremely well conserved in evolution, with only very small differences demonstrable between plants and mammals. The adenoviruses encode their own histone-like proteins. It is surprising that the gene for the major core protein is not included among the highly conserved sequences.

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