The integration of HPV-18 into Hela cells has involved duplication of part of the viral genome as well as human DNA flanking sequences

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We have cloned two restriction enzyme fragments from the Hela cell line S3 which hybridize with the genomes of HPV-6 and 16 under conditions of reduced stringency (T_m -43°C). One of the fragments, a 5.7-kb *Bam* HI fragment, was found to be present within the other, a 15.8-kb *Eco* RI fragment. Restriction enzyme (RE) mapping of this 15.8-kb fragment (see Figure) revealed that the sequence of RE sites at its right hand end is identical to previously proposed maps of the integration site of HPV-18 in the Hela cell genome^{1,2,3,4}. Thus, the 7.0-kb *Eco* RI/*Hind* III and 5.7-kb *Hind* III fragments shown in the Figure below, appear to correspond directly to the 8.4 or 7.9-kb and 5.8-kb *Hind* III fragments identified by Lazo⁴. The RE map also revealed the presence of a 5 to 6-kb region of duplication within the 15.8-kb fragment adjacent to the HPV-18 sequences. Thus, the sequence of RE sites (*Pst* I, *Kpn* I, *Sma* I, *Hind* III) within the 5.7-kb *Bam* HI fragment is repeated exactly in the 6.6-kb *Bam* HI/*Eco* RI fragment. Individual sub-fragments from the 15.8-kb *Eco* RI fragment were therefore isolated and used as probes to investigate the nature of this duplication event. Sub-fragments were also probed with HPV-6 and 16 at low stringency and with human genomic DNA at high stringency (T_m -12°C) to determine the distribution of HPV and human genomic sequences within the 15.8-kb fragment. The results of these experiments are shown diagrammatically in the accompanying figure. The approximate extent of the direct repeat unit is also indicated. When the 2.1-kb *Hind* III fragment and 2.2-kb *Bam* HI/*Hind* III fragment were used to probe *Eco* RI digests of human genomic DNA, hybridization was visible smearing over the entire range of fragment sizes from 0.5- to 23-kb. We interpret this to mean that these fragments contain human repetitive DNA sequences. The 1.35-kb *Pst* I/*Sma* I and 1.0-kb *Sma* I/*Hind* III fragments from both copies of the repeat and the 0.9-kb *Hind* III fragment from the left end of the 15.8-kb fragment, all hybridized to a single 8.1-kb *Eco* RI fragment from the human SiHa cell line under conditions of high stringency (T_m -12°C). Also, the 1.1-kb *Bam* HI fragment (Upstream Regulatory Region/non-coding region) hybridized to the 2.2-kb *Bam* HI/*Hind* III fragment which contains human repetitive DNA sequences. These findings indicate that the integration of HPV-18 into the Hela cell genome not only involved the deletion of viral sequences but also the duplication of viral as well as human repetitive and non-repetitive DNA flanking sequences. The integration of HPV-18 DNA into Hela cells has also been mapped by *in situ* hybridization^{5,6}.

= Human Non-Repetitive Sequences
 = Human Repetitive Sequences
 = HPV 18 Sequences

No RE sites for: Mlu I, Sac I, Sal I, Xho I

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