Regulation of human papillomavirus type 16 (HPV-16) transcription by loci on the short arm of chromosome 11 is mediated by the TATAAAA motif of the HPV-16 promoter

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The human papillomavirus type 16 (HPV-16) enhancer-promoter is virtually inactive in normal human diploid fibroblasts, but active in human fibroblasts with a deletion in the short arm of one chromosome 11 (del-11 cells). Since the HPV-16 enhancer with the simian virus 40 promoter is active in both cell types, the target for chromosome 11-regulated HPV-expression is likely to be located in the HPV-16 early promoter region (nucleotides 57 to 112). We show here that DNA-protein complexes formed with an HPV-16 promoter fragment are quantitatively different in del-11 cell and diploid cell extracts. This quantitative difference detected in band shift experiments disappeared upon mutation of the HPV-16 TATAAAA box to TATTTAT. This mutation also strongly reduced the activity of the HPV-16 enhancer-promoter in del-11 cells. These results indicate that TATA-binding proteins are involved in the chromosome 11-mediated regulation of HPV-16 gene expression.

Specific high-risk types of human papillomavirus (HPV) (e.g. type 16) are thought to be causally involved in the pathogenesis of cervical cancer (de Villiers, 1989; Vousden, 1989; zur Hausen, 1989). The HPV E6/E7 genes appear to play an essential role in the proliferation of cervical tumour cell lines and also in tumour development (Crook et al., 1989; von Knebel Doeberitz et al., 1988). The interaction of the high-risk HPV E6/E7 gene products with cellular tumour suppressor proteins (P53 and pRB) may represent an endogenous factor which induces chromosomal instability (Hurlin et al., 1991; zur Hausen, 1991).

Experimental data point to an important role of chromosome 11 in the pathogenesis of cervical cancer. Cytogenetic studies have shown that in many cervical carcinomas chromosome 11 is affected (Atkin & Baker, 1984, 1988). Furthermore, introduction of a normal chromosome 11 from a human fibroblast into cervical carcinoma-derived cell lines, like HeLa and SiHa, results in suppression of the tumorigenic phenotype of these cells (Kaeling & Klinger, 1986; Koi et al., 1989; Saxon et al., 1986). Loss of chromosome 11 also appears to exert a distinct effect on the transcriptional activity of the HPV-18 enhancer-promoter and thereby on HPV-18 early gene expression. Addition of 5-azacytidine, a demethylating agent, to hybrids between the HPV-18-positive carcinoma cell line HeLa and normal fibroblasts results in the selective suppression of HPV-18 transcription in non-tumorigenic hybrids (Rösl et al., 1988). In tumorigenic segregants, which have lost a normal copy of chromosome 11, HPV-18 expression is not affected.

We have previously shown that in human embryonic fibroblasts the loss of loci [karyotype 46,del(11)-p11.1p15.1] on the short arm of one chromosome 11 (del-11 cells) results in susceptibility to transformation by HPV-16 DNA. The susceptibility of del-11 cells to transformation is the result of the much greater strength of the HPV-16 enhancer-promoter in these cells than in normal diploid fibroblasts (diploid cells) (Smits et al., 1988, 1990). We have also demonstrated that replacement of the promoter in the HPV-16 long control region (LCR) by the simian virus 40 (SV40) promoter overcomes the down-regulatory effect of chromosome 11 in diploid cells (Smits et al., 1990). Therefore, the HPV-16 promoter region located between nucleotides 59 and 112 (Seedorf et al., 1985) is assumed to be involved in down-regulation in diploid cells.

To investigate the involvement of the promoter we performed gel retardation experiments to determine whether the differential regulation of promoter strength in these cell types is reflected in the binding of specific proteins. A 32P-labelled double-stranded synthetic oligonucleotide (HPR; nucleotides 59 to 112) encompassing
the HPV-16 TATA box sequence (see Fig. 1a) was incubated with equal amounts of a nuclear extract from del-11 or diploid cells. These nuclear extracts were prepared as described by Cereghini et al. (1987). Nuclear protein (3 μg) was added to a reaction mixture containing 10 mM-HEPES pH 7.9, 30 mM-KCl, 10% (v/v) glycerol, 0.5 mM-DTT, 10 μg poly(dI-dC), 6 mM-MgCl₂, 5 μg/ml aprotinin, leupeptin and pepstatin, 2 mM-benzamidine and 0.5 mM-PMSF, and the mixture was incubated on ice for 10 min. DNA–protein complexes were separated on a low ionic strength, 6% polyacrylamide gel (30:1; acrylamide:bisacrylamide) containing 0.25xTBE (1 x TBE is 89 mM-Tris-HCl, 89 mM-boric acid, 1 mM-EDTA). After electrophoresis at 12 V/cm for 2 h, the gel was fixed in 10% acetic acid, 10% methanol for 15 min, dried and subjected to autoradiography.

Incubation of the HPR fragment with the nuclear extract from diploid cells (Fig. 1b) resulted in relatively slow (C2) and fast (C1) migrating bands (lane 2). DNA–protein complexes migrating at the same position in the gel but with a different relative abundance were formed by incubation of the promoter fragment with the del-11 cell nuclear extract (lane 8). Although the C2 band was most prominent in the diploid cell nuclear extract, the C1 band was the major band when the del-11 cell nuclear extract was used. This apparent difference in relative abundance was not influenced by the amount of nuclear extract used nor by different preparations of nuclear extract. Excess unlabelled homologous DNA (lanes 3, 4, 5, 9, 10 and 11) competed efficiently with the formation of the C1 and the C2 DNA–protein complexes. With excess unlabelled heterologous DNA consisting of the early promoter fragment of SV40 [AccI–HindIII fragment of pSV2CAT (Gorman et al., 1982)] (Fig. 1b, lanes 6, 7, 12 and 13) or with excess HPR fragment in which the TATAAAA sequence was replaced by its TATTTAT counterpart from the SV40 early promoter (HPM; see Fig. 1c) only partial competition was observed (data not shown).

These results show that two DNA–protein complexes can be formed by a specific interaction between the HPV-16 promoter fragment and nuclear proteins from both diploid and del-11 cells. Furthermore, these results suggest that different amounts of these DNA-binding proteins are present in diploid and del-11 cells. Alternatively, these proteins may be present in equal amounts in both cell types, but have a different affinity for the HPV-16 promoter fragment.

Subsequently we investigated whether the HPV-16 TATA box is involved in the observed quantitative difference in the gel retardation pattern. To this end we
The TATA box sequence of HPV-16 (TATAAAA) and that replacement of TATAAAA by a TATTTAT sequence in diploid cells (see Fig. 2). This analysis suggests that the HPV-16 LCR resulted in the loss of promoter activity in del-11 cells whereas the LCR remained inactive in diploid and del-11 cells. In transient CAT assays we found that with the two probes (HPR and HPM) we ran the migration rates of the C1 and C2 bands were apparent migration rates of the C1 and C2 bands were obtained and no quantitative difference was observed when nuclear extracts from either diploid or del-11 cells were used (compare lanes 1 and 6, Fig. 1d). Excess homologous DNA competed efficiently with the binding of the proteins to the labelled HPM fragment (Fig. 1d, lanes 2, 3, 7 and 8), but excess unlabelled SV40 promoter fragment did not compete efficiently with protein binding (Fig. 1d, lanes 4, 5, 9 and 10). These results indicate that the HPV TATA box (TATAAAA) is involved in the formation of quantitatively different bandshift patterns in nuclear extracts from diploid and del-11 cells, and that for HPM complex formation not only the TATTTAT box but probably also surrounding sequences are essential.

To compare the DNA–protein complexes obtained with the two probes (HPR and HPM) we ran the DNA–protein complexes on the same gel (Fig. 1e). The apparent migration rates of the C1 and C2 bands were different from those of the D1, D2 and D3 bands. These results indicate that mutation of the TATA box leads to the formation of DNA–protein complexes with different migration rates.

Since no cell type difference was observed in the gelshift assay with HPM we tested the effect of this TATA box mutation on the promoter activity in diploid and del-11 cells. In transient CAT assays we found that replacement of TATAAAA by a TATTTAT sequence in the HPV-16 LCR resulted in the loss of promoter activity in del-11 cells whereas the LCR remained inactive in diploid cells (see Fig. 2). This analysis suggests that the TATA box sequence of HPV-16 (TATAAAA) and that of SV40 (TATTTAT) are functionally distinct in terms of their ability to serve as core promoter elements in conjunction with the HPV-16 enhancer.

Mutational analysis has demonstrated that for many promoters the TATA sequence is necessary to initiate transcription efficiently and accurately (Berkhout & Jeang, 1992; Wasylyk et al., 1980; Wefald et al., 1990). One of the earliest steps in the initiation of transcription by RNA polymerase II is binding of the TFIID protein to the TATA box sequence 25 to 30 bp upstream from the transcription initiation site (Nakajima et al., 1988). Upon binding of TFIID to the TATA box, a multiprotein complex is formed containing the TATA-binding protein (TFIID) and several TFIID-associated factors (TAFs). These TAF proteins appear to contain a coactivator function in the regulation of transcription (Dynlacht et al., 1991). The observation that replacement of the HPV-16 TATAAAA sequence by SV40 TATTTAT in the HPV-16 LCR results in a lack of activity of this mutated LCR indicates the specificity of the TATA box sequence. This specificity reflects differential interactions with distinctive TATA box binding factors, only some of which can act cooperatively with proteins binding to the HPV-16 enhancer to generate an active transcription complex.

Our results indicate that loci (p11.11p15.1) on the short arm of chromosome 11 overlapping the Wilms’ tumour suppressor gene (Riccardi et al., 1980) have a distinct effect on proteins complexed to the HPV-16 promoter. We have previously shown that the HPV-16 promoter functions much more strongly in cells lacking these loci (del-11 cells) than in diploid cells, in which the HPV-16 enhancer–promoter is virtually inactive (Smits et al., 1990). We propose a model in which loss of loci on the short arm of chromosome 11 leads to the expression or modification of transcription factors able to activate the HPV-16 promoter. Since only a quantitative difference is present in the bandshift pattern of extracts from diploid and del-11 cells, we suggest that the C2 DNA–protein complex, clearly present in diploid cell extracts and almost absent with del-11 cell extracts, represents an inactive transcription initiation complex containing additional or modified proteins compared to the low M, C1 DNA–protein complex. However it is not known which cellular regulatory mechanism is responsible for the regulation of this factor(s). Neither is it known whether these factors are synthesized de novo or whether transcription factors are activated by protein modification. We have recently demonstrated that the steady-state level of PR55β, a regulatory subunit of protein phosphatase 2A, is much higher in del-11 cells than in diploid cells (P. H. M. Smits, H. L. Smits, R. P. Minnaar, B. A. Hemmings, R. E. Mayer-Jaekel, R. Schuurman, J. van der Noordaa & J. ter Schegget, unpublished results). Therefore, we assume that protein

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<th>Enhancer–promoter construct</th>
<th>Relative CAT expression</th>
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<tr>
<td>1 SV40 enh/Pr CAT</td>
<td>Diploid cells 35.8 Del-11 cells 30.6</td>
</tr>
<tr>
<td>2 TATAAAA CAT</td>
<td>0.8 12.1</td>
</tr>
<tr>
<td>3 TATTTAT CAT</td>
<td>0.3 0.2</td>
</tr>
<tr>
<td>4 CAT</td>
<td>0.1 0.2</td>
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Fig. 2. Activity of the HPV-16 enhancer–promoter with a mutated TATA box in diploid and del-11 cells. Diploid and del-11 cells were transfected with 10 μg plasmid DNA. Relative CAT expression is indicated to the right. Constructs 1, 2 and 4 have been described previously (Smits et al., 1990). In construct 3, containing the HPV-16 enhancer–promoter cloned in front of the CAT gene, the HPV-16 TATAAAA box has been replaced by the TATTTAT sequence.
phosphorylation is involved in the chromosome-11 mediated regulation of HPV-16 transcription.

In the intracellular surveillance hypothesis postulated by zur Hausen (1991), a class of cellular genes called cellular interfering factor (CIF) genes exists in normal cells; these genes can negatively regulate viral oncogenes. Inactivation of these CIF genes is necessary to release the HPV E6/E7 genes from cellular suppression in vivo, thereby leading to the development of cervical carcinoma. In agreement with this hypothesis the constitutive expression of an HPV-18 LCR-chloramphenicol acetyltransferase (CAT) gene construct transfected into a cervical carcinoma cell line is silenced after fusion of these cells with non-malignant cells (Rösl et al., 1991). Suppression occurs at the level of transcription initiation and is eliminated shortly after cycloheximide treatment, implying a short-lived suppressor protein.

These results favour the assumption that a suppressor molecule is present in diploid cells. However, we cannot exclude the possibility that a positive transcription factor is expressed in del-11 cells. Further research will be needed to clarify the nature of the gene products involved and how they regulate the activity of the HPV-16 enhancer–promoter.

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References


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