Mechanism of translation of the bicistronic mRNA encoding human papillomavirus type 16 E6–E7 genes

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The transforming genes E6 and E7 of human papillomavirus (HPV) type 16 and other HPV types are expressed from a bicistronic mRNA with a characteristic spacing of 3 to 6 bp between the termination codon of E6 and the initiation codon of E7. Plasmid pSP64E6E7 which contains the reading frames of both E6 and E7 was constructed in order to study the expression of both proteins in a coupled transcription/rabbit reticulocyte translation system. Both E6 and E7 proteins were expressed simultaneously. This translation could be interfered with by antisense oligonucleotides corresponding to various regions of the transcript. Antisense oligonucleotides targeted at sequences flanking either side of the translation initiation codon of the E6 open reading frame were effective in inhibiting the synthesis of both proteins, whereas oligonucleotides complementary to the coding regions downstream of the first start codon showed either a considerably reduced effect or none at all. In particular, there was limited inhibition of E7 translation by antisense oligonucleotides flanking the translation start region of the E7 gene. In the presence of RNase H, it was possible to selectively inhibit the synthesis of either E6 or E7 by several gene-internal antisense oligonucleotides. We conclude that HPV16 E6–E7 bicistronic mRNA is fully functional and that both proteins are translated with equal efficiency via the scanning mechanisms with reinitiation at the second open reading frame. In addition, both AE6 and AE7 may have therapeutical potential as they are capable of inhibiting the proliferation of CaSki cells which contain the HPV16 genome.

Introduction

Antisense oligonucleotides are short segments of single stranded RNA or DNA with nucleotide sequences complementary to a specific gene or its mRNA. When targeted at mRNA or its precursor (pre-mRNA), antisense oligonucleotides hybridize and form duplexes which prevent the translation of the message into the encoded protein (Paterson et al., 1977; Izant & Weintraub, 1985; Melton, 1985; Blake et al., 1985). A refined understanding of this inhibitory mechanism could lead to using it as a tool for interfering with pathogenic processes. To study this mechanism we used the transforming genes of the human papillomavirus.

Papillomaviruses have a circular DNA genome of approximately 8 kb. To date, more than 60 human papillomavirus types (HPV) have been identified. Among these a subgroup, including HPV16 and HPV18, is frequently detected in cervical cancer (De Villiers, 1989). The transforming properties of HPV16 and HPV18 are encoded in the E6 and E7 genes whose products interfere with the functions of the p53 and the Rb tumour suppressor genes (Hudson et al., 1990; Münger et al., 1989; Hawley-Nelson et al., 1989; Werness et al., 1990; Dyson et al., 1989). The fact that the E6 and E7 proteins are consistently expressed in HPV-positive cervical cancers and in cell lines derived from this cancer (Schwarz et al., 1985; Schneider-Gädicke & Schwarz, 1986; Smotkin & Wettstein, 1986; Androphy et al., 1987; Bedell et al., 1989; Cullen et al., 1991) supports the hypothesis that expression of E6 and E7 plays a role in cervical carcinogenesis. The HPV16 E6 and E7 proteins are transcribed from the same promoter, P97, in the form of a bicistronic message (Schneider-Gädicke & Schwarz, 1986; Smotkin & Wettstein, 1986).

This study examines whether the E6–E7 message allows E7 to be translated efficiently. The use of antisense oligonucleotides to elucidate the mechanism for translation of this bicistronic message will be described. The implications of the results for understanding the mechanism of translation of this bicistronic message and for the design of specific and effective antisense oligonucleotides will be discussed.
Methods

Synthesis of oligonucleotides. Oligonucleotides were synthesized by B. Li at the Institute of Molecular and Cell Biology or custom-made by New England Biolabs. The oligonucleotides were purified by HPLC. All oligonucleotides were dried and resuspended at a concentration of 1 mM in water. The sequence of the oligonucleotides and their expected positions upon hybridization are shown in Fig. 1. Phosphorothioate analogs were custom-made by Oligos Etc and resuspended at a concentration of 2 mM in water.

Plasmids. Segments of the HPV-16 genome that either encoded the E6 protein, E7 protein or both proteins together as a bicistronic sequence were obtained from a HPV-16 clone using the PCR. The forward PCR primer contained a HindIII restriction site whereas the reverse primer had a PstI site (Table 1). After cleavage with these enzymes, each of the three PCR products were ligated into the plasmid pSP64. The recombinant vectors will be referred to as pSP64E6, pSP64E7 and pSP64E6E7 (Fig. 1).

In vitro protein translation. The TNT SP6 coupled reticulocyte lysate system (Promega) was used for the coupled transcription-translation reactions. The pre-mix contained 52 μl lysate, 4 μl reaction buffer, 4 μl SP6 polymerase, 2 μl amino acid mixture without cysteine (all the above reagents were supplied with the system), 4 μl recombinant RNasin ribonuclease inhibitor (40 U/μl; Promega), 6 μl of H2O and 8 μl of 35S-labelled l-cysteine containing 74 pmol (NEN, DuPont). The specific activities of the four batches of 35S-labelled l-cysteine ranged from 1150 to 1220 Ci/mmol. Pre-mix (8 μl) was added to 1 μg of recombinant vector DNA and the reaction volume was adjusted to 10 μl before incubation at 37 °C for 120 min.

Hybrid arrested in vitro translation. The 10 μl reaction mixture containing 1 μg of pSP64E6E7 was similar to that described above except for the presence of the antisense oligonucleotides at a final concentration of 50, 100 or 200 μM. Translation of pSP64E7 was also performed in the presence of 50 and 100 μM of antisense oligonucleotides.

Inhibition of translation by RNase H and antisense oligonucleotides. The 10 μl reaction mixture containing 1 μg of pSP64E6E7 was similar to that described above except for the presence of 25 μM of the antisense oligonucleotide and 1-5 U of RNase H (Promega). For reactions conducted in the presence of 25 μM of AE6 or the control oligonucleotide, 0.25 μg of a luciferase expression plasmid (provided as a positive control in the TNT SP6 coupled reticulocyte lysate system) was added as a control.

Denaturing gel analysis of translation products. After 120 min of incubation at 37 °C, 2 μl samples were removed from each reaction mixture for analysis on 12.5% SDS-PAGE. The electrophoresis was stopped when the bromophenol blue dye had run off the bottom of the gel. The gel was fixed in acetic acid:isopropylalcohol:water (10: 25 : 65) for 20 min, soaked in Amplify (Amersham) for 10 min followed by immersion in 5% glycerol for 20 min before being dried and then fluorographed overnight at 70 °C. The bands were quantified with the

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Table 1. Primers used for obtaining the E6, E7 and E6–E7 open reading frames

<table>
<thead>
<tr>
<th>Oligonucleotides</th>
<th>Sequences (5'-3')</th>
<th>Position targeted</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASP6</td>
<td>AGTTAAGCTGTGATATCC</td>
<td>SP6 TS</td>
</tr>
<tr>
<td>AE6</td>
<td>TTGGTGTCTGGAAACAT</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>TATTGCTGTTCTAATG</td>
<td>363-378</td>
</tr>
<tr>
<td>B</td>
<td>GATGATCTGCAACGAAAG</td>
<td>516-531</td>
</tr>
<tr>
<td>AB7</td>
<td>GTGATCTCCATGCTGATG</td>
<td>562-577</td>
</tr>
<tr>
<td>C</td>
<td>TGGTTTCTGAGAACAG</td>
<td>840-855</td>
</tr>
<tr>
<td>D</td>
<td>CTGCCTGTCGACGCTGG</td>
<td>682-697 (455-469)</td>
</tr>
<tr>
<td>Control</td>
<td>TTGCCCCTGCATATGCC</td>
<td></td>
</tr>
</tbody>
</table>

* The underlined bases in the forward primers show the HindIII restriction site whereas those on the reverse primers show the PstI site. Bases in bold indicate either the start codon (ATG) or the termination codon (TTA).

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Denaturing gel analysis of translation products. After 120 min of incubation at 37 °C, 2 μl samples were removed from each reaction mixture for analysis on 12.5% SDS-PAGE. The electrophoresis was stopped when the bromophenol blue dye had run off the bottom of the gel. The gel was fixed in acetic acid:isopropylalcohol:water (10: 25 : 65) for 20 min, soaked in Amplify (Amersham) for 10 min followed by immersion in 5% glycerol for 20 min before being dried and then fluorographed overnight at 70 °C. The bands were quantified with the
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Cell proliferation as determined by tetrazolium reduction. An established cervical epithelial tumour cell line, CaSki, containing HPV16 was used in this study. The cells were grown in MEM supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin and 2 mM-glutamine. Another cervical tumour cell line, C-33A, lacking the HPV genome was used as a control cell line. The C-33A cells were grown in medium similar to that used for CaSki cells with the addition of 0.8 mg/l of MEM non-essential amino acids and 1 mM-pyruvate. Cells (5 x 10⁶) were seeded in each well of the 96-well plate (Nunc) and allowed to recover for 48 h prior to the treatment with phosphorothioate oligonucleotides. Fresh medium containing 5 μg/ml of the transfection reagent, DOTAP (Boehringer-Mannheim) and 20 μM of phosphorothioate analogs was then added to the cells and the cells were treated for 48 h with a change of medium after 24 h. Twenty μl of freshly prepared MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium]PMS (phenazine methosulphate) was then added to each well. (MTS and PMS were supplied as solutions of 2 mg/ml and 0.92 mg/ml respectively in the CellTitre 96 AQueous Non-radioactive cell proliferation assay kit from Promega). After incubation at 37 °C for 3 h, the A₄₉₀ was read using an ELISA plate reader. Assays were performed in triplicate and the results were expressed as percentage inhibition of the untreated culture. This was determined by finding the difference in A₄₉₀ values between the untreated and oligonucleotide-treated cultures, and subsequently by calculating the ratio between this and the A₄₉₀ value of the untreated culture as a percentage.

Results

Synthesis of E6 and E7

Our initial aim was to identify the HPV16 proteins E6 and E7 after expression in a coupled in vitro transcription–rabbit reticulocyte translation system, and to monitor their expression from a bicistronic mRNA. The plasmids pSP64E6 and pSP64E7 were used to confirm the identities of the E6 and E7 translation products and pSP64E6E7 was constructed as a source for the bicistronic mRNA (Fig. 1). The products were analysed by SDS–PAGE and fluorography (Fig. 2). The E6 protein migrated as predicted as a 17K protein. By contrast, the HPV16 E7 protein migrated slowly to an abnormal extent, with a Mᵣ of 19K instead of the expected 12K value. This aberrant migration behaviour of HPV 16 E7 has been previously described (Münger et al., 1991; Scheffner et al., 1992). The bicistronic mRNA transcribed from pSP64E6E7 resulted in the synthesis of both proteins. The intensity of the E7 signal was determined to be 51.9% ± 3.7% (N = 4) of the E6 signal.

Effects of antisense oligonucleotides on E6 and E7 translation

To study the effects of interference by antisense oligonucleotides on the translation of E6 and E7 from the bicistronic E6–E7 mRNA, protein synthesis was carried out in a combined transcription–translation system with pSP64E6E7 as described above but with antisense oligonucleotides added at the start of the reaction. Oligonucleotides AE6 and ASP6, which were complementary to sequences flanking the E6 start codon or overlapping the start of the SP6 transcript respectively, were effective in inhibiting both E6 and E7 protein synthesis. Inhibition levels were greater than 90% with

<table>
<thead>
<tr>
<th>Antisense oligonucleotides</th>
<th>Concentrations (μM)</th>
<th>Percent inhibition of translation of E6</th>
<th>Percent inhibition of translation of E7</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>50</td>
<td>38.7%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>37.8%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>60.5%</td>
<td>28.2%</td>
</tr>
<tr>
<td>B</td>
<td>50</td>
<td>1.7%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>5.9%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>5.0%</td>
<td>10.6%</td>
</tr>
<tr>
<td>C</td>
<td>50</td>
<td>0.0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.0%</td>
<td>0%</td>
</tr>
<tr>
<td>D</td>
<td>50</td>
<td>7.6%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.0%</td>
<td>0%</td>
</tr>
<tr>
<td>ASP6</td>
<td>50</td>
<td>27.9%</td>
<td>52.1%</td>
</tr>
<tr>
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<td>100</td>
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<td>AE7</td>
<td>50</td>
<td>5.6%</td>
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<tr>
<td></td>
<td>200</td>
<td>65.0%</td>
<td>66.3%</td>
</tr>
</tbody>
</table>
Fig. 3. Inhibition of E7 translation by antisense oligonucleotides AE6 and AE7. Lane 1 is a negative control without any plasmid. Lanes 2 and 7 show the translation of E7 from pSP64E7. Lane 3 has 100 μM of AE7 and lane 4 has 50 μM of AE7. Lane 5 has 100 μM of AE6 and lane 6 has 50 μM of AE6. The positions of Mr markers are indicated on the right and the position of E7 is indicated by the arrowhead on the left.

Fig. 4. Inhibition of E6/E7 translation by antisense oligonucleotides AE6 and AE7 in the presence of RNase H. A control protein, luciferase (62K) was simultaneously translated. The translation reactions were similar to those described in Methods with the exception of the addition of 0.25 μg of the luciferase plasmid (provided as a positive control with the Promega kit). Lanes with (+) indicate the addition of RNase H. Lane 1 is without any plasmid. Lane 2 shows the translation of L, E6 and E7 in the presence of RNase H. Lane 3 is a negative control without any plasmid. Lanes 4 and 5 have unrelated control oligonucleotide (5' TTGGCCGCTGCC-ATCC 3') and lanes 6 and 7 have oligonucleotide AE6. Lane 7 shows the normal translation of all three proteins. The concentration of all oligonucleotides was 25 μM. The positions of Mr markers are indicated on the right and the positions of luciferase (L), E6 and E7 are indicated by arrowheads on the left.

100 or 200 μM of either oligonucleotide for E6, as well as for the downstream E7 gene (Table 2). In the case of the oligonucleotide AE7, which is complementary to sequences flanking the E7 initiation codon, only approx. 40% and 65% inhibition levels were observed at 100 and 200 μM-AE7. AE7 also had some effect on the expression of the E6 gene especially when added in amounts of 200 μM. This non-specific inhibition is due to the partial identity between oligonucleotides AE6 and AE7 (nine out of 16 bp). Both 100 μM-AE6 and -AE7 did not affect the transcription of pSP64E6E7 (data not shown). Oligonucleotides A, B, C and D, which were complementary to sequences within the E6 or the E7 gene or to the sequences at the 3' terminus of the genes, had little or no effect on E6 or E7 translation (Table 2).

In addition, the effect of AE6 and AE7 on the translation of E7 from pSP64E7 was also examined. AE6 did not inhibit the synthesis of E7 when added at the 50 μM level but there was some inhibition at the 100 μM level which was due to the partial identity between AE6 and AE7. In contrast, AE7 affected E7 synthesis more significantly when added in 50 μM amounts and there was almost complete inhibition when added at 100 μM amounts (Fig. 3).

**RNase H and antisense inhibition of E6 and E7 translation**

Exogenous RNase H cleaves RNA–DNA hybrids and it has been demonstrated that its addition to *in vitro* translating systems can increase the extent of hybrid-arrest translation of mRNAs (Minshull & Hunt, 1986). In this study, we examined the effects of RNase H on the
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expression of E6 and E7 proteins from the bicistronic mRNA. Fig. 4 illustrates the effects of RNase H in combination with 25 μM of oligonucleotides A, B, C or D. In the absence of RNase H, these oligonucleotides had little or no effect (Table 2). However, together with RNase H, oligonucleotides A and B could selectively inhibit the synthesis of E6 (Fig. 4). Oligonucleotide C in the absence or presence of RNase H had little effect, whereas oligonucleotide D caused the complete inhibition of E7 synthesis in the presence of RNase H and also a significant inhibition (77.6%) of E6 synthesis. The inhibition in E6 expression is due to the 75% identity between the oligonucleotide and the E6 coding region.

As a means of examining the specificity of the RNase H and antisense oligonucleotide combined inhibitory effects, a series of experiments using a luciferase expression plasmid as the control were carried out. Fig. 5 (lane 2) shows the synthesis of E6, E7 and the 62K luciferase protein in the presence of RNase H. The synthesis of all three proteins was slightly decreased in the presence of RNase H and this is because of the effects of minimal unspecific degradation of the luciferase and bicistronic E6–E7 RNAs. When 25 μM of oligonucleotide AE6 and RNase H were used it was possible to selectively inhibit E6 synthesis without significant effect on the synthesis of luciferase and E7 (Fig. 5). The unrelated control oligonucleotide also had no effect on the expression of the three proteins.

Effect of the antisense oligonucleotides on cell proliferation

Treatment of the CaSki cell cultures for 48 h with either AE6 or AE7 phosphorothioate analogs, resulted in the inhibition of proliferation of CaSki cells by 38.6% and 52.6%, respectively. In contrast, treatment of C-33A cell cultures only led to slight inhibition levels of 6.6% and 18.2% by AE6 and AE7, respectively. Treatment of both cell lines with the unrelated control oligonucleotide also resulted in minimal inhibition.

Discussion

Our research examined the efficiency of translation of both proteins from a bicistronic viral mRNA, and the possibility of interfering differentially with the expression of both genes by antisense oligonucleotides. In cervical cancers and cervical carcinoma-derived cell lines containing HPV16, mRNAs encoding E6 and E7 proteins are transcribed from the same promoter, P97 (Schneider-Gädicke & Schwarz, 1986; Smotkin & Wettstein, 1986), in the form of a bicistronic mRNA. The unspliced E6–E7 mRNA encodes the full-length E6 as well as E7 proteins. In addition, two spliced transcripts E6*I–E7 and E6*II–E7 whose biological functions are unknown are usually detected (Shirasawa et al., 1991). It has been proposed that it is a function of these spliced transcripts to stimulate E7 translation (Schneider-Gädicke & Schwarz, 1986; Smotkin et al., 1989). The unspliced transcripts of all HPV types that infect genital epithelia, have a short spacing region (5 bp in the case of HPV16) between the termination codon of E6 and the initiation codon of E7. As it is not possible to examine the translation of the bicistronic HPV16 E6–E7 in cultures using cell lines containing the HPV16 genome since splicing events occur, we examined the translation in vitro using an eukaryotic-derived rabbit reticulocyte system which should mimic the intracellular conditions that are present for translation of HPV mRNAs.

Most mRNAs of eukaryotes and their viruses are monocistronic but in the case of those mRNAs which are bicistronic, the ribosomes can reinitiate translation at the next AUG codon downstream (Kozak, 1984; Liu et al., 1984; Peabody et al., 1986). The efficiency of reinitiation in such cases is usually low (Kozak, 1984; Liu et al., 1984) but this progressively improves as the intercistronic sequence lengths (Kozak, 1987). This reinitiation only occurs when the upstream open reading frame (ORF) is a 'minicistron' and encodes for a small
peptide. For plant and animal virus mRNAs that are structurally bicistronic, encoding two full-length proteins, only the 5'-proximal end ORF is usually translated, i.e. they are functionally monocistronic (Kozak, 1986). This study demonstrates that despite the close proximity of the ORFs of E6 and E7 and the fact that the message codes for two full-length proteins, the translation of the protein E7 is as efficient as that of E6 in vitro. This quantification was done by comparing the E7 and E6 signals in radiolabelling experiments using [35S]cysteine. The E7 signal was expected to be half that of E6 as there are seven cysteine residues in E7 as compared with the 14 cysteine residues in E6. In our experiments the E7 signals had an intensity which was 51% of that of E6, leading us to conclude that both proteins are translated with almost equal efficiency. Similar effective expression of both proteins was also observed when E6 and E7 were translated in a rabbit reticulocyte lysate system using RNAs transcribed from pSP64E6E7 (data not shown). The efficient translation of the E6 and E7 proteins of other HPVs has also been reported (Roggenbuck et al., 1991).

For antisense inhibition studies, a series of antisense oligonucleotides was constructed. The AE6 and AE7 oligonucleotides were constructed to include sequences flanking the start codons of E6 and E7 respectively as these regions are probably most sensitive to antisense inhibition (Gupta, 1987; Maher & Dolnick, 1987). As such, there is partial identity between AE6 and AE7 (nine out of 16 bp). However, at concentrations of 50 μM or less, AE6 could selectively bind to sequences flanking the E6 start codon and did not inhibit the translation of E7 from pSP64E7 which encodes for E7 alone. In contrast, 50 μM of AE6 inhibited the synthesis of both E6 and E7 from pSP64E6E7 which encodes the bicistronic E6–E7 message whereas AE7 did not affect this synthesis. At high concentrations, especially at 200 μM levels, there were some unspecific effects from both oligonucleotides.

Our data on antisense inhibition also indicate that there is efficient translation of E6 and E7 from the bicistronic message. Oligonucleotides ASP6 and AE6 targeted at sequences around the E6 initiation codon can inhibit translation of both proteins efficiently at 100 and 50 μM levels respectively. This observation fully supports the scanning process model. In its simplest form, this model (Kozak, 1989) postulates that a 40S ribosomal subunit and an imperfectly defined set of initiation factors (Pain, 1986) enters at the 5' end of the mRNA and migrates along the mRNA until it reaches the first start codon, whereupon a 60S ribosomal subunit joins it and the first peptide bond is formed. In line with this model, the inhibition of expression of E6 by ASP6 is probably due to the interference with the binding of the 40S ribosomal subunit and the initiation factors or their passage to the start codon, whereas AE6 probably interferes with the binding of the 60S subunit.

Oligonucleotides B and AE7 that are targeted at sequences flanking the E7 initiation codon, however, are less effective in reducing E7 translation that arises from the bicistronic message, although partial inhibition was observed at 200 μM levels of these oligonucleotides. These observations together with the inhibition by ASP6 and AE6, strengthen the hypothesis that E6 and E7 are translated from a bicistronic sequence and that the ribosomes reinitiate translation efficiently after termination of E6 translation. The hindrance caused by the antisense oligonucleotides B and AE7 is not sufficient to dislodge the ribosomes and prevent E7 synthesis. Alternatively, a mechanism whereby the ribosomes bind mRNA internally at the initiation AUG of E7 could be operating. Our data do not support this possibility since oligonucleotide AE7 would then have blocked translation of E7 more effectively. In addition, the ratio of the E7:E6 [35S]cysteine signal would not be 51% if E7 was translated by the internal ribosome-binding mechanism since one would not expect equal molecules of both proteins to be translated. The coding region-specific antisense oligonucleotides also did not prevent protein synthesis. This again is probably due to the fact that the translating ribosome can dislodge the oligonucleotide from the RNA template (Sankar et al., 1989).

RNase H was used to examine whether the genes' internal binding antisense oligonucleotides which did not lead to a significant inhibition of E6 and E7 expression were interacting specifically with the targeted regions under the experimental conditions used. We observed that RNase H has a dramatic effect on translation inhibition even in the case of oligonucleotides other than AE6 and ASP6. Also, the concentration of oligonucleotide AE6, which is effective without RNase H at a concentration of 50 μM, can be reduced to 25 μM in the presence of RNase H. The interaction of the oligonucleotide AE6 with the mRNA was observed to be highly specific since the translation of an unrelated protein, luciferase, was not affected at all. Taken together, these results are in agreement with published data demonstrating that oligonucleotides directed against gene-internal sequences form heteroduplexes with mRNA efficiently. These heteroduplexes can be detected due to their destruction by RNase H, but they are unstable during the translation process (Minshull & Hunt, 1986; Walder & Walder, 1988; Gagnor et al., 1987).

Therefore, for effective interference of translation from such bicistronic messages, one should employ antisense oligonucleotides that are complementary to regions at the 5' end or at the initiation codon of the first open reading frame. Hence, antisense inhibition of translation from such a bicistronic message closely
resembles that of monocistronic messages in that regions flanking the first initiation codon at the 5' end are most sensitive to antisense oligonucleotide inhibition of translation (Sankar et al., 1989; Maher & Dolnick, 1987; Gupta, 1987).

In vivo, a large fraction of the E6–E7 transcript is spliced to produce two shorter transcripts, E6*I-E7 and E6*II-E7. Since these two shorter transcripts have the same 5' oligonucleotide sequence in common with the full-length E6–E7 transcript, one would assume that the effects of the AE6 oligonucleotide would be on all three transcripts. To monitor this possibility, we studied the growth inhibitory effects of AE6 and AE7 on CaSki and C-33A cells. It is well documented that the expression of the HPV E6 and E7 genes is directly linked to the proliferative capacity of cervical cancer cells (von Kenbel Doeberitz et al., 1988, 1990; Watanabe et al., 1993). Interference with the expression of these proteins in CaSki cells by the phosphorothioate analog of AE6 is sufficient to inhibit cell proliferation. The AE7 oligonucleotide was also effective in inhibiting cell proliferation. This inhibition of cell proliferation is specific since both antisense oligonucleotides did not affect C-33A cells that do not harbour HPV genome. These results suggest that these oligonucleotides may be useful therapeutically.

In conclusion, our research provides evidence for the mechanism of translation of a bicistronic mRNA of a human virus. The use of antisense oligonucleotides as a means of dissecting the mechanism of translation provides an useful method for analysing protein synthesis. This type of analysis is an important prerequisite for studies conducted in vivo and to predict optimal targets in research that aims to use antisense oligonucleotides as pharmaceutical substances.

References


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