In vitro binding of the bacteriophage f1 gene V protein to the gene II RNA-operator and its DNA analog

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ABSTRACT

We have investigated the binding of the f1 single-stranded DNA-binding protein (gene V protein) to DNA oligonucleotides and RNA synthesized in vitro. The first 16 nucleotides of the f1 gene II mRNA leader sequence were previously identified as the gene II RNA-operator; the target to which the gene V protein binds to repress gene II translation. Using a gel retardation assay, we find that the preferential binding of gene V protein to an RNA carrying the gene II RNA-operator sequence is affected by mutations which abolish gene II translational repression in vivo. In vitro, gene V protein also binds preferentially to a DNA oligonucleotide whose sequence is the DNA analog of the wild-type gene II RNA-operator. Therefore, the gene V protein recognizes the gene II mRNA operator sequence when present in either an RNA or DNA context.

INTRODUCTION

The gene V protein of bacteriophage f1 (fd, M13) is a small dimeric single-stranded DNA-binding protein (1). During infection the gene V protein (gpV) increases the intracellular concentration of single-stranded phage DNA by covering newly replicated single-stranded DNA molecules to prevent complementary strand synthesis (2). It also binds to the gene II mRNA leader sequence to repress the translation of the phage encoded replication protein pII (3,4,5). In vitro gpV binds tightly and cooperatively to single but not to double-stranded DNA (6), and with lower affinity to RNA (7,8). The three-dimensional structure of this protein has been determined by X-ray crystallography (9). The amino acids involved in binding (10,11) and the mechanism of binding (8,12−14) have been both investigated by chemical and spectroscopic techniques. These studies have revealed that there are two different binding modes, one for oligonucleotides and another for DNA (12,14). Furthermore, the affinity for homopolymers differs markedly from one homopolymer to another (8,13). Different binding models have been proposed to explain these findings which have however in common a role for the aromatic side chains of gpV which are stack upon the nucleic acid bases and a binding of the DNA phosphate backbone by its lysyl and arginyl side chains (1,8).

We previously investigated by genetic means the target that gpV binds to repress gene II mRNA translation (5). The first 16 nucleotides of the gene II mRNA leader sequence were found to be required for repression in vivo. In vitro, gpV protein binds RNA molecules carrying this sequence with a 10 fold higher affinity than it binds RNA molecules that do not contain this sequence. In this paper we examine in vitro gpV binding to RNAs carrying mutations which impair repression in vivo. Such mutations affect gpV binding in vitro. To further investigate the apparent paradox of both binding without specificity

to single-stranded DNA and binding specifically to an RNA sequence, we studied gpV binding to DNA oligonucleotides. We observed that gpV binds preferentially to an oligodeoxynucleotide whose sequence is the DNA analog of the RNA-operator compared to oligodeoxynucleotides of randomly chosen sequence. Several mutations which impair repression in vivo and RNA binding in vitro also affect the binding of gpV to this operator-analogue DNA.

MATERIALS AND METHODS

1- Plasmids.

For the construction of pTZII-13, pTZII-16 and pTZII-19, the plasmid pTZ18R (Pharmacia) was used as vector. This plasmid was cleaved with *Eco*RI and *Hind*III, gel purified and the oligonucleotides 13, 16, and 19 (nucleotides 5965−5982, 5965−5985 and 5965−5988 of f1 respectively, flanked by *Eco*RI and *Hind*III protruding ends) were inserted. To construct plasmids bearing the mutations shown in fig.1 within the gene II operator, oligonucleotides corresponding to the sequence of oligo-19 with one or two mutations were inserted into plasmid pTZ18R instead. Plasmid DNA was prepared by the alkaline lysis technique (15) or a clear lysate method (16). f1 nucleotides are numbered according to Hill and Petersen (17).

2- RNA synthesis and purification.

To synthesize RNA in vitro, plasmid DNA was cleaved with *Ssp*I restriction enzyme and the larger fragment gel purified by electroelution. Reactions were as described by Uhlenbeck (18) using 1 μg of linear purified template and 60 units of T7 RNA polymerase for two hours at 37°C. RNA was purified by electrophoresis on a 4% acrylamide, 6M urea gel as described (19). The length of the RNA molecules, 207 to 210 nucleotides, is the distance between the T7 promotor and the *Ssp*I restriction site and proved to be experimentally suitable.

3- Oligonucleotide synthesis and purification.

Oligodeoxynucleotides were synthesized on a fully automated Applied Biosystem 380A DNA synthesizer, treated for 6 hours with KOH at 55°C to remove protecting groups, and purified on a 1m long sephadex G75 column. The DNA concentration was determined using a UV spectrophotometer at 260 nm and an extinction coefficient of .025 OD.260/μg. DNA was labelled with T4 polynucleotide kinase as described (20) and then phenol extracted and ethanol precipitated. When analyzed on a 15% acrylamide, 6M urea denaturing gel, all the labelled oligonucleotides migrated as a unique species of the expected size.

4- Gene V protein purification.

Gene V protein was purified as described (6) and visualized in 6M urea, 19% acrylamide, 0.07% bisacrylamide protein gels.

5- Gel shift experiments.

Gel shift of RNA. 2 pmoles of P32-labelled RNA (150 ng) was mixed with increasing amounts of gene V protein in 5% glycerol, 100mM NaCl, 10mM Tris pH 8.0 in the presence of 2 units of RNAsin in a final volume of 10μl (the final concentration of RNA molecules was 0.2 μM). This mixture was incubated for 5 min. at room temperature and then electrophoresed on a 4% polyacrylamide, tris EDTA borate gel (20) at 200 volts at room temperature for two hours. Gels were dried and exposed for 2 to 12 hours.

Gel shift of DNA. The total amount of DNA used was 600 ng in a final volume of 10 μl (11.25 μM for the oligonucleotides 16 nucleotides long). For simple binding experiments 590 ng of unlabelled oligonucleotides were mixed with 10 ng of the same, P32-labelled,

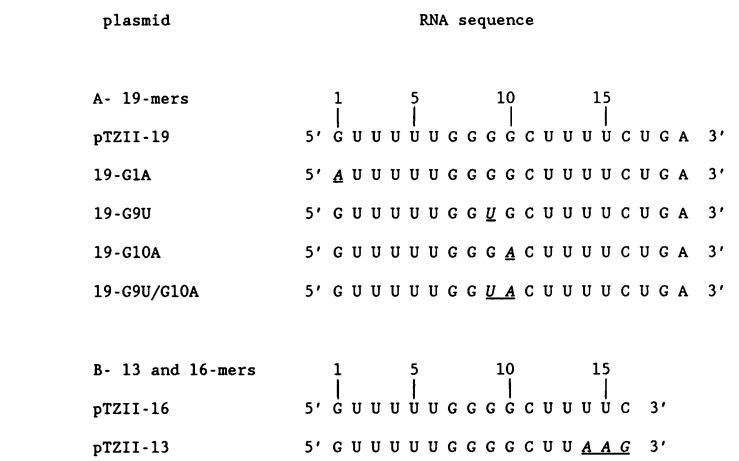

Figure 1. Gene II mRNA sequence carried by the in vitro synthesized RNAs. A- plasmids carrying the DNA sequence encoding the first 19 nucleotides of gene II leader mRNA (pTZII-19) or mutants of this sequence were used as template for RNA synthesis. Mutated nucleotides are underlined and in italics. B- plasmids carrying DNA encoding the first 16 (pTZII-16) or 13 (pTZII-13) nucleotides of gene II mRNA served as template for RNA synthesis. Nucleotides underlined and in italics are those encoded by the 3' adjacent *Hind*III cloning site.

oligonucleotide. For competition experiments 500 ng of unlabelled type-1 oligonucleotide were mixed with 90 ng of unlabelled type-2 oligonucleotide and 10 ng of P32-labelled type-2 oligonucleotide. This constant amount of DNA was then mixed with increasing amounts of gpV in 5% glycerol, 100 mM NaCl and 10mM Tris pH 8.0 (see legends to fig.4 and 5). The mixtures were incubated for 5 min at room temperature and then loaded on a polyacrylamide gel containing Tris, EDTA, borate buffer (20). The bottom and top half of the gel were 12% and 6% acrylamide respectively. Electrophoresis was performed at 200 volts for 2 hours and gels were dried and exposed for 2 to 12 hours.

RESULTS.

1- In vitro binding of gene V protein to RNA molecules carrying wild-type or mutant operator.

We constructed plasmids in which oligonucleotides encoding regions of different lengths of the gene II mRNA leader sequence were cloned downstream from a T7 promotor. Two of the plasmids (pTZII-16 and pTZII-19), when incubated with T7 RNA polymerase, direct the in vitro synthesis of RNA molecules carrying the first 16 or 19 nucleotides of gene II mRNA (see Methods). The gene II mRNA sequences present on pTZII-16 and pTZII-19 are shown in fig.1. These synthetic RNA molecules were 207 and 210 nucleotides long. Due to the sequence requirements of the T7 promotor and to cloning constraints, the first nucleotide of the gene II mRNA corresponds to the eleventh nucleotide of the RNA synthesized. In vitro, binding of gpV protein to P32-labelled RNA molecules can be visualized by a gel retardation assay. In fig 2A we show the binding to pTZII-16 encoded RNA (the same result was obtained with pTZII-19) and to RNA from the vector pTZ18R used as control. As described previously (5), gpV binds with 10-fold higher affinity to an RNA molecule carrying the first 16 nucleotides of the gene II mRNA than to an RNA molecule which lacks this sequence.

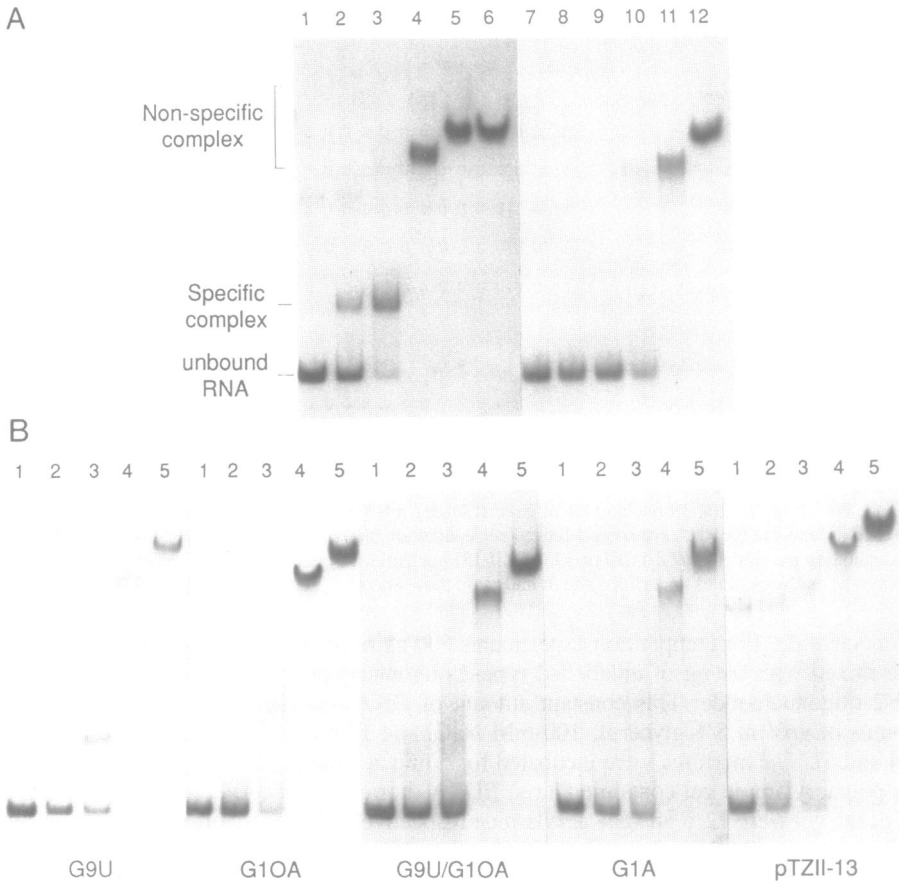

Figure 2. Gel shift experiments with P32-labelled RNA Molecules (0.2 μM) and gpV protein. Panel A, binding to wild-type RNA: lanes 1−6, pTZII-16 encoded RNA; lanes 7−12, pTZ18R encoded RNA (control RNA). Lanes 1−6 and 7−12 correspond to 0, 0.26, 0.78, 2.6, 7.8 and 26 μM gpV respectively. Panel B, binding to mutant RNA: G9U, G10A, G9U/G10A, G1A, and pTZII-13 encoded RNA as indicated. Lanes 1 to 5 correspond respectively to 0, 0.26, 0.78, 2.6 and 7.8 μM gpV added to the RNA.

We characterized mutations which impair the ability of gene V protein to repress translation of gene II mRNA in vivo (5). To study the effect of some of them on gpV binding in vitro, RNAs bearing mutations of the gene II mRNA leader sequence were synthesized. The mutated sequences present on the RNAs tested are shown in fig.1. The G9U and G10A mutations are representative of the two types of mutations obtained in vivo: the G10A mutation eliminates gpV repression (no repression compared with 10 fold for the wild-type operator), whereas some repression still occurs with the G9U mutation (2 fold instead of 10 fold) (5). In the presence of the first 16 or 19 nucleotides of the gene II mRNA, a specific band appears corresponding to gpV binding at low concentration (fig.2A). As shown in fig.2B, in the case of the RNA bearing the G9U mutation, less RNA is retarded at low gpV concentration but the specific complex is still formed. In contrast this band does not appear when gpV binds to RNA bearing the G10A mutation.

A- wild-type sequences

operator-16	G T T T T T G G G G C T T T T C
random-16A	C A C G T T G A A A A T C T C C
random-16B	C C G C C T G T T C C C G A A C
operator-18	T T T T T G G G G C T T T T C T G A
random-18A	G T T T T T A G C G A C G G G G C A
random-18B	T C G T C A G T C T A G T G A A T A
operator-42	AATTCCTGTTTTTGGGGCTTTTCTGATTATCAACCGGGGTCA
random-38	AATTCGGCGGGTGTGGTGGTTACGCGCTACACTTGCCA

B- mutant sequences

16-G1A	*A* T T T T T G G G G C T T T T C
16-G9T	G T T T T T G G *T* G C T T T T C
16-G10A	G T T T T T G G G *A* C T T T T C
16-G9T/G10A	G T T T T T G G *T A* C T T T T C
16-T14A/T15A/C16G	G T T T T T G G G G C T T *A A G*
18-G9T/G10A	T T T T T G G *T A* C T T T T C T G A

Figure 3. DNA oligonucleotide sequences employed for in vitro gpV binding experiments. All sequences are written 5′ to 3′. A: wild-type sequences of different length. B: mutant sequences. Mutant nucleotides are underlined and in italics.

With the double mutant G9U/G10A, the specific band is not formed and the amount of RNA bound is decreased at low gpV concentrations compared to the wild-type operator.

The effect of changing the first G of the gene II mRNA could not be tested in vivo, because this mutation might affect initiation of RNA synthesis as well as repression by gpV. As shown in fig.2B, changing this G to an A impairs gpV binding to the same degree as the G9U/G10A double mutation. The same result was obtained when this change resulted from the cloning of a shorter oligonucleotide lacking this G residue and bringing T2 of gene II mRNA to position 5 of the RNA synthesized in vitro instead of position 12 (data not shown). Therefore, this first G residue could play a significant role in gpV binding to the RNA-operator.

Nucleotides 17 to 23 of the gene II mRNA are not required for in vivo repression or for specific binding by gpV in vitro (Ref.5, fig.2A). In contrast mutations at positions 14, 15 or 16 impair repression in vivo. As shown in fig.2B, the specific band is not formed when these three nucleotides are absent (pTZII-13 encoded RNA). Single point mutations at these positions were not tested.

The gene V protein still binds more efficiently to each of these mutant sequences compared to RNA molecules lacking any gene II mRNA sequence (compare fig.2B to pTZ18R in fig.2A). However, the impaired binding in vitro correlates with the decrease or the absence of repression observed in vivo.

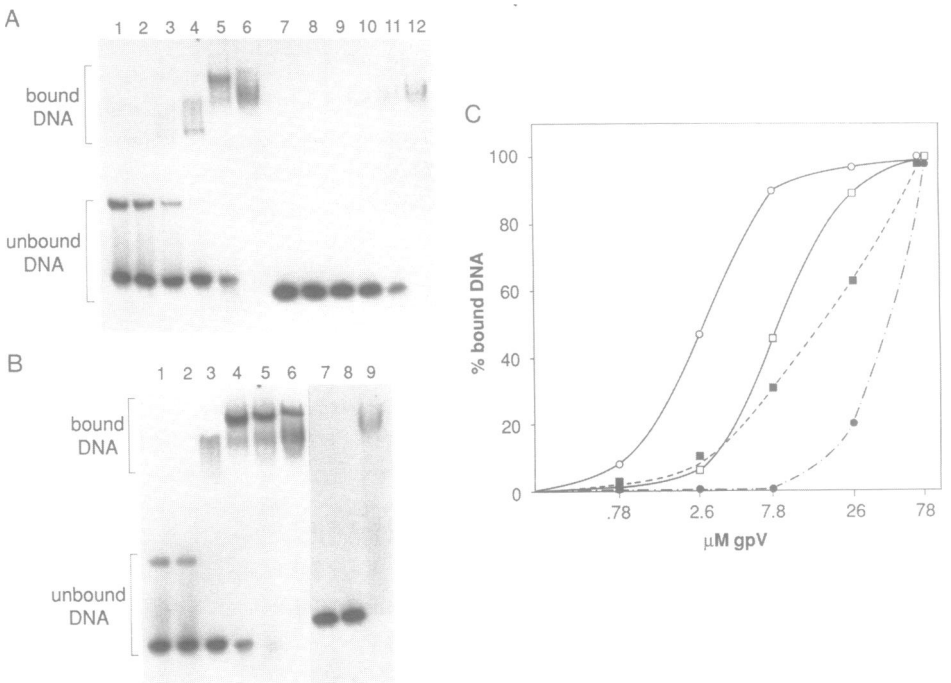

Figure 4. Gel shift experiments with DNA oligonucleotides bearing the sequence analogous to the gene II RNA-operator or random sequences.
A: lanes 1−6, 600 ng (11.25 μM) of operator-16; lanes 7−12, 600 ng of random-16A.lanes 1−6 and 7−12 correspond to 0, 0.78, 2.6, 7.8, 26 and 78 μM gpV respectively.
B: competition experiments: lanes 1−6, 100ng (1.9μM) of P32-labelled operator-16 with a 5 fold excess of unlabelled random-16A; lanes 7−9, 100 ng of P32-labelled random-16A with a 5 fold excess of unlabelled operator-16. Lanes 1−6 correspond to the same amounts of gpV as in panel A, lanes 7−9 are 0, 26 and 78 μM gpV respectively.
C: percentage of labelled oligonucleotides bound as a function of gpV concentration. Binding to operator-16 —□—, random-16A---■---, operator-16 with excess of random-16A —○—, random-16A with excess of operator-16 · —●— ·.

2- In vitro binding of gene V protein to single-stranded DNA oligonucleotides.
We compared the binding of gpV to DNA oligonucleotides composed either of the sequence analogous to nucleotides 1−16 of gene II mRNA or of randomly chosen DNA sequences. These sequences (operator-16, random-16A and random-16B) are shown in fig.3A. Binding was analyzed by the gel retardation assay. In contrast with the single band observed on denaturing gels, the operator oligonucleotide specifically migrated as two bands on non-denaturing gels (fig.4A, lane 1). Although we do not know what the upper band corresponds to, two operator oligonucleotides can anneal to form a partially double-stranded structure through G-T base pairing. Since this structure has single-stranded ends, we expect gpV to melt it.

When a constant amount of P32-labelled oligonucleotide is mixed with increasing amounts of gpV, a band corresponding to a DNA-gpV complex appears. As can be seen in fig.4A, more DNA is retained at the different gpV concentrations tested with the gene II operator sequence than with a random oligonucleotide of the same length. This difference is much

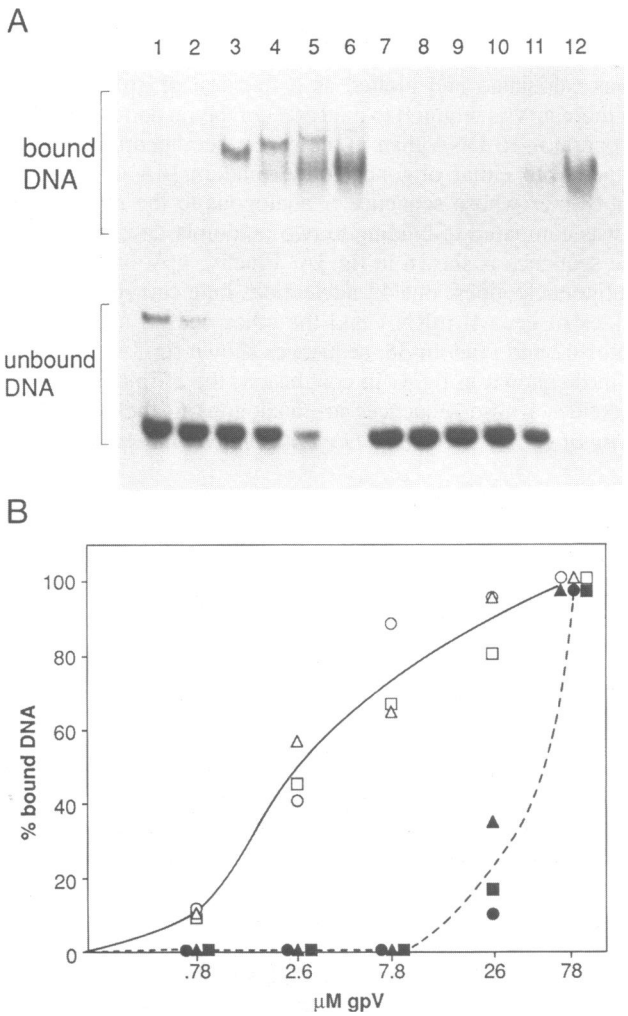

Figure 5. Gel shift experiments with oligonucleotides of mutant sequence.
A: competition experiment between operator-16 and 16-G9T oligonucleotides. Lanes 1−6, 100 ng (1.9μM) of P32-labelled operator-16 and a 5 fold excess of 16-G9T; lanes 7−12, 100 ng of P32-labelled 16-G9T and a 5 fold excess of operator-16. Lanes 1−6 and 7−12 correspond to 0, 0.78, 2.6, 7.8, 26 and 78 μM gpV respectively.
B: percentage of bound labelled oligonucleotides as a function of gpV concentration. 100 ng of labelled operator-16 with 500 ng of unlabelled G9T —□—, G10A —○—,G9T/Gf10A —△—. 100 ng of labelled G9T ---■---, G10A ---●--- G9T/G10A ---▲--- with 500 ng of unlabelled operator-16.

more striking in competition experiments. To keep the total amount of DNA constant, one fifth of the previous amount of P32-labelled operator analogous DNA was mixed with a five fold excess of unlabelled random oligonucleotide. As shown in fig.4B, lanes 1 to 6, under these conditions binding occurs at lower concentrations of gpV. As expected, when the reverse competition experiment was performed, binding to the random 16-mer was prevented by an excess of operator-16 (fig.4B, lanes 7−9). The DNA-protein

complexes were excised from the gel and the amount of labelled DNA migrating in the top part of the gel was counted. The percentage of oligonucleotide bound at each gpV concentration was calculated and plotted as a function of gpV concentration (fig.4C). Roughly 10-fold more gpV is required to bind 50% of the random-16A DNA in the presence of an excess of operator-16 DNA than in the reverse competition experiment. The same result was obtained with either oligonucleotide random-16A or random-16B.

Binding to an 18-mer whose sequence is analogous to the nucleotides 2 to 19 of the gene II mRNA was compared to binding to two randomly chosen oligonucleotides of the same size whose sequence is shown in fig.3A. Finally, gpV binding was also compared for two longer oligonucleotides, one 42 nucleotides long carrying DNA analogous to the first 33 nucleotides of gene II mRNA and the other one 38 nucleotide long of random sequence (operator-42 and random-38, sequences shown fig.3A). In each case the results were similar to those shown in fig.4. In conclusion, the affinity of gpV is higher for the DNA oligonucleotides whose sequences are analogous to the gene II RNA-operator.

3- In vitro binding of gene V protein to DNA oligonucleotides bearing mutated operator sequences.

To determine if those mutations which impair translational repression in vivo and RNA binding in vitro also decrease gpV binding to DNA, gel retardation assays were performed with DNA sequences carrying one or several such mutations. The sequences of the oligonucleotides used in this experiment are shown in fig.3B. In direct binding experiments the amount of mutant oligonucleotides bound by gpV was intermediate between that of operator-16 and random-16 (data not shown). Competition experiments were performed between each of these six mutant oligonucleotides and the wild-type operator sequence of the same length (operator-16). As shown in fig.5A, more DNA is retained with operator-16 than with the mutant G9T at each gpV concentration tested. The same result was obtained in competitions between operator-16 and the mutant G10A or the double mutant 16-G9T/G10A or between operator-18 and 18-G9T/G10A. In fig.5B the percentage of labelled oligonucleotide bound is plotted as a function of gpV concentration. Approximately 10-fold more gpV is required to bind 50% of the mutated oligonucleotides when an excess of wild-type sequence is present compared to the reverse competition experiment. Additional experiments revealed that gpV binds more efficiently to the double mutant 16-G9T/G10A than to random-16A and 16B, and 18-G9T/G10A random-18B, whereas it binds 18-G9T/G10A and random-18A with the same affinity (data not shown). Random-18A shares some sequences with the operator (fig.3). Changing the three 3′ nucleotides of operator-16 also decreased gpV binding. In this case 5-fold more gpV is required to bind 50% of the mutated oligonucleotide in the presence of an excess of wild-type operator than in the reverse competition experiment (data not shown). For the single mutant G1A, no significant difference was detected between the wild-type and the mutant operator DNA. The effect of mutations may not be detectable by this assay when they are at the end of the oligonucleotides. In conclusion, several mutations which affect repression in vivo and RNA binding in vitro also impair the binding of gpV to the analogous DNA sequences of 16 or 18 nucleotides in length.

DISCUSSION

Previously we characterized mutations which impaired translational repression of gene II mRNA by the gene V protein in vivo. These mutations lie within the 16 first nucleotides of gene II mRNA (5,21). In vitro, gpV binds RNA molecules bearing this 16 nucleotide

sequence with at least 10-fold higher affinity than those that lack it (5). Here we show that this preferential RNA binding is affected by mutations which decrease or abolish gpV repression in vivo. Although gene V protein does bind all single-stranded DNA and RNA sequence non-specifically, we conclude that the binding of gpV to the gene II RNA-operator is mediated by sequence specific interactions. We have also examined the binding of gpV to DNA oligonucleotides and find that gpV has a higher affinity for DNA sequences analogous to the RNA operator than for random sequences. In addition, some mutations which impair translational repression in vivo and gpV-RNA binding in vitro also affect gpV binding to the analogous DNA sequence. We therefore suggest that those sequence features which gpV recognizes on RNA are also present on DNA oligonucleotides.

1- Binding to RNA.

Fulford and Model first suggested that gpV recognizes a specific site on the gene II mRNA (22). They showed that the gene 32 protein of the phage T4 and gpV of f1 cannot repress each others target, even though these two single-stranded DNA-binding proteins have similar properties and both act as translational repressors. It was later shown that the gene 32 protein recognizes its mRNA leader by binding to a specific sequence which can fold into a pseudo-knot (23). Initially the gene 32 protein binds to this nucleation site and then cooperatively covers the rest of the mRNA, up to the ribosome binding site, thereby repressing its own translation (23). gpV also binds to a specific sequence to repress translation but this operator is devoid of secondary structure (5).

Binding of gpV protein to homopolymers has been investigated in detail. It binds with higher affinity to poly(rU) than to poly(rA), poly(rC) or 5SRNA for which it has very similar affinities (8). One reason gpV binds the gene II mRNA operator with high affinity could be that it contains 9 uracil residues out of 16 nucleotides, arranged in two groups of 5 and 4 consecutive nucleotides. These uracil residues may be an element of the recognition sequence but they cannot be the sole determinant, since mutations within the row of guanine residues impair in vivo repression and in vitro binding (Ref.5,fig.2, fig.5). Moreover, replacing the C residue adjacent to the 4 consecutive U residues with an additional U reduces in vivo repression (5), and changing the first G of the mRNA to an A affects in vitro binding (fig.2). Thus gpV does not simply recognize the uracil residues of the operator.

A low cooperativity parameter was originally found for gpV binding to phage RNA (7). Bulsink and coworkers reported that the binding of gpV to RNA homopolymers is cooperative, probably due to the absence of secondary structures on these sequences (8). In our gel shift experiments with control RNA a smear is visible at intermediate gpV concentrations which corresponds to RNA molecules bound by varying numbers of gpV molecules, perhaps due to partial dissociation during electrophoresis (fig.4A, lanes 10−11). This suggests that gpV does not bind with high cooperativity to RNA lacking the gene II operator. In addition, only the presence of the RNA operator sequence results in the appearance of a discrete band whose formation is more cooperative (no smear is visible between the bands corresponding to unbound RNA and the specific bound operator RNA in fig.2, even with longer gel exposures). All RNA molecules are converted to this intermediate form before any additional binding occurs. Therefore, this specific binding could be a manifestation of sequence recognition or it may correspond to the absence of structure in this region.

The in vitro binding of gpV and RNA is affected by mutations in two different ways. In one case, G9U, the amount of RNA retained at low gpV concentration is affected but

the cooperative intermediate is still formed (fig.2B); this mutant is repressed 5-fold less in vivo (5). In contrast, with the G10A mutation, the mechanism of binding is modified in that the intermediate complex is no longer formed at any gpV concentration but replaced by a smear (fig.2B). More mutants of each type must be analyzed before the level of repression in vivo and the way the binding is impaired in vitro can be correlated. As expected, the G9T/G10A double mutant combines the defects of each parent mutation and is hence impaired in both binding affinity and mechanism. In addition the G1A single mutant also disrupts both these parameters. Assuming that the nucleotides encoded by the vector sequence flanking the gene II mRNA do not play a role in its formation, the specific intermediate band observed in vitro could correspond to a structure formed in vivo which prevents ribosome binding or later steps of translation initiation.

2- Binding to oligodeoxynucleotides.

Gene V protein binding to oligonucleotide DNA has been investigated by several groups (12,14,24), employing different techniques. From these studies it was concluded that gpV binds to DNA by three different modes: an oligonucleotide mode, a polynucleotide binding mode (12,14), and a polynucleotide binding mode detected only in the absence of salts (24,25). Our experiments were performed under conditions which should not permit this latter mode of binding (presence of 100mM salt).

When gpV binds to (dA)n or (dT)n molecules 8 to 20 nucleotides in length, 3 nucleotides are covered per gpV monomer, the salt dependence of binding is low, and the cooperativity parameter is low ($w=5$). In contrast, in its polynucleotide binding mode, a gpV monomer covers 4 nucleotides, binding is highly salt dependent and the cooperativity parameter varies from 60 to 800 depending on the bound polynucleotide and the method of determination. These two modes may correspond to two different DNA-gpV complex structures (12,14). For oligonucleotides of intermediate size, 25 to 30 nucleotides long, both types of binding mode are observed. At a low protein/DNA ratio the polynucleotide mode occurs while at high protein/DNA ratio gpV binds in its oligonucleotide mode (12,24,25). We have used oligonucleotides 16 and 18 nucleotides long to study gpV binding and have found that the protein preferentially binds DNA sequences analogous to the RNA operator. This was observed at both low and high gpV concentrations. In addition, for a 42-mer carrying the operator, preferential binding was still observed even though gpV should bind to this longer molecule by the polynucleotide binding mode. Therefore, the higher affinity of gpV for the DNA sequence analogous to the gene II RNA-operator is not specific to either DNA binding mode. However gpV binding to different DNA oligonucleotides was highly dependent on their length (not shown). The increase of apparent affinity with length is mainly due to the cooperativity of binding (26) and we therefore always compared short oligonucleotides of the same size. The wild-type operator-analogous DNA sequence is preferentially bound compared to sequences carrying either the point mutations G9T or G10A, a double mutant bearing both these changes, or a triple mutant in which three residues TTC are changed. All of these mutations decrease or suppress repression in vivo and affect RNA binding in vitro. Therefore, even though the uridines are replaced by thymidines residues and the riboses by deoxyriboses, the specific sequence or structural features gpV recognizes on RNA also exist on DNA. However, we found a larger difference between specific and non-specific binding affinities with RNA than with DNA (10-fold for RNA as opposed to 2−3-fold for DNA). This may reflect the primary role of the RNA-operator sequence in vivo. The low level of specificity of the gene II operator recognition compared

to other repressor-operator systems (27) probably reflects the phage requirement for a well-modulated control, where gene II is easily repressed and activated in a low range of gpV concentration.

If the preferential binding to DNA can occur when gpV replaces bound *E.coli* single-stranded DNA-binding protein (SSB) molecules, this specific DNA recognition could play a role in vivo and act as a nucleation site on single-stranded phage DNA. Since gpV binds a DNA and RNA sequence specifically, other single-stranded DNA-binding proteins such as the *E.coli* SSB, the F factor SSF or the phage T4 gene 32 protein could have some DNA sequence specificity as well.

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