The influence of antisense RNA on transcriptional mapping of the 5' terminus of a baculovirus RNA

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S1 nuclease protection and primer extension analyses were used to determine the 5' end of the Autographa californica nuclear polyhedrosis virus 603 open reading frame (ORF) transcript upstream of and on the opposite strand to the polyhedrin gene. These analyses suggested that the 5' end of the 603 ORF was located near the initiation site for polyhedrin gene transcription. Primer extension products of reverse transcription of 603 RNA were dependent on the presence of the TAAG sequence, an essential polyhedrin promoter element. The results could be interpreted as indicating bidirectional transcription from the TAAG element. However, the data could also be due to an artefact of antisense transcripts and RNA duplex formation in this region. Since the bidirectional transcriptional model is not consistent with other mapping data and Northern blot analysis, we conclude that the presence of antisense RNA can result in mapping artefacts and that caution must be taken in interpreting data where overlapping sense and antisense RNAs are present.

Introduction

Transcription of the circular, 128 kbp DNA genome of the baculovirus, Autographa californica nuclear polyhedrosis virus (AcMNPV), often involves multiple, unspliced, overlapping RNAs of both sense and antisense transcripts (Friesen & Miller, 1985, 1986, 1987; Lubbert & Doerfler, 1984a, b; Mainprize et al., 1986). Antisense transcription was proposed to play a regulatory role in baculovirus gene expression (Friesen & Miller, 1987) and evidence of a regulatory role for transcripts in the polyhedrin gene region was provided recently (Ooi & Miller, 1990).

The polyhedrin gene is flanked by two open reading frames (ORFs), the upstream 603 ORF (Gearing & Possee, 1990) and the downstream 1629 ORF (R. D. Possee & D. H. L. Bishop, unpublished results), which are encoded by the DNA strand opposite the one encoding polyhedrin (see Fig. 1). Initiation of transcription of the 603 and 1629 ORFs precedes that of the polyhedrin gene; the 603 and 1629 ORFs are transcribed primarily as 'late' genes with steady state levels reaching a maximum at 12 h post-infection (p.i.), although they also have an 'early' transcriptional component (Ooi & Miller, 1990). In contrast, polyhedrin transcription initiates at 12 to 18 h p.i. and reaches a maximum very late in infection (Friesen & Miller, 1985; Ooi et al., 1989).

The predominant transcript of the 603 ORF is a 3-2 kb RNA which initiates near the start site of the 1629 ORF, traverses the 1629 ORF and terminates just downstream of the 603 ORF (Ooi & Miller, 1990; Fig. 1). Steady state levels of this 3-2 kb RNA decline rapidly at 18 h p.i. when polyhedrin gene transcription increases. This decline is dependent on polyhedrin gene transcription, as shown by the prolonged presence of the 3-2 kb RNA at 18 and 24 h p.i. in cells infected with a mutant virus, VXpoly, which differs from wild-type virus only by a linker replacement mutation in the essential TAAG sequence of the polyhedrin promoter. The precise mechanism by which the steady state levels of the 3-2 kb RNA are regulated by polyhedrin gene transcription is not yet known. Likely mechanisms are accelerated turnover of the 3-2 kb RNA stemming from duplex formation with antisense RNA and/or the turn-off of 3-2 kb RNA initiation. The latter mechanism might involve disruption of 3-2 kb RNA promoter complexes by polyhedrin-specific transcriptional complexes across the initiation site in the opposite direction.

In this manuscript we describe some of our early attempts to map the 5' end of the 603 ORF transcript. Both S1 nuclease protection and primer extension mapping techniques were compromised severely by...
artefacts probably produced by RNA duplex formation between sense and antisense RNAs. We present these results primarily as cautionary examples of the effects that antisense RNAs may exert on transcriptional mapping and as an alternative interpretation of similar data presented in a previous report by another laboratory (Gearing & Possee, 1990).

Methods

Cells and viruses. Spodoptera frugiperda IPLB-SF21 cells (Vaughn et al., 1977) were maintained in TC100 medium (Gibco) supplemented with 10% foetal calf serum and 0.25% tryptose broth (Gibco) at 27°C. Wild-type AcMNPV L-1 (Lee & Miller, 1978) and isogenic recombinant linker scan mutants (the vhcLS series; Ooi et al., 1989) were described previously. To construct the vXpoly recombinant virus carrying the LSX linker mutation only, the 0.7 kb MluI-BglII fragment was isolated from the phcLSX transplacement plasmid used previously for vhcLSX construction (Ooi et al., 1989). In the vhcLS series, the BglII site is located in place of the original polyhedrin ATG. The MluI site is found in wild-type AcMNPV within the 603 ORF (Fig. 1). The BglII site was blunt-ended with mung bean nuclease before MluI digestion in order to remove the 5' overhang of the BglII site and hence remove the mismatch with wild-type polyhedrin gene sequences. This MluI/BglII blunt-ended fragment was cotransfected with wild-type AcMNPV (L-1) viral DNA into S. frugiperda cells for allelic replacement of this region of the genome. A recombinant virus carrying the LSX mutation in the polyhedrin promoter region was identified by its predicted occlusion-negative plaque phenotype and initially replacement of this region of the genome. A recombinant virus carrying AcMNPV (L-l) viral DNA into

Digestion in order to remove the 5' overhang of the BglII site is found in wild-type AcMNPV within the 603 ORF (Fig. 1).

The map and ORFs in the region of the AcMNPV genome that encodes the polyhedrin gene. The region shown lies within the 7.3 kb EcoRI fragment which maps from the origin (0.0 map units; m.u.) of the 128 kb AcMNPV DNA genome to 5.7 m.u. The position of the polyhedrin ORF is shown by the slashed arrow in the central section and the positions and directions of the 603 and 1629 ORFs, which flank the polyhedrin gene, are shown as speckled arrows. The positions of key restriction sites are shown. The precise or approximate nucleotide number at the centre of the site is provided; these numbers are based on the coordinates and sequence of Matsuura et al. (1987). Above the central ORF, arrows show the direction and approximate length of the polyhedrin sense transcripts (Friesen & Miller, 1983). The arrow below the central ORFs shows the position of the 3.2 kb RNA covering the 603 and 1629 ORFs in the sense direction and the polyhedrin gene in the antisense direction.

Results

Previous Northern blot analyses of RNA isolated at representative times during AcMNPV infection revealed that the 603 ORF is transcribed as a large 3-2 kb to 3.7 kb RNA with maximum levels occurring at 12 h p.i. (Gearing & Possee, 1990; Ooi & Miller, 1990). Using S1 nuclease analysis, we mapped the 5' end of this 3-2 kb RNA (Ooi & Miller, 1990) to a point which is approximately 10 nucleotides downstream of a polyadenylation signal (A(2)U(3)) which overlaps the 603 ORF translation termination codon (UAA; Gearing & Possee, 1990). Gearing & Possee (1990) reported that S1 nuclease
Fig. 2. Location of an apparent 5' end of a 603 ORF-specific transcript by S1 nuclease protection and primer extension analyses. (a) The AcMNPV region containing the 603 and polyhedrin ORFs with relevant restriction sites and translation initiation codons (ATGs) noted. The numbers refer to the nucleotide sequence coordinates of Matsuura et al. (1987). Above the restriction sites is an arrow indicating the 5' to 3' direction of the probe used in (b) for S1 nuclease analysis; the asterisk indicates the position of the 5' radiolabel. Below the ORFs is a short heavy arrow showing the 5' to 3' direction and position of the primer used for primer extension analyses shown in (c). (b) S1 nuclease analysis of the 5' ends of RNAs traversing the MluI site of the 603 ORF using probe 'B' shown in (a). Lanes 2 to 7 show fragments protected by RNA isolated 0, 3, 6, 12, 24 and 48 h after infection with wild-type AcMNPV. Lanes 8 and 9 are the results of using RNA isolated 12 h after infection in the presence of either aphidicolin (lane 8) or cycloheximide (lane 9). Lanes 10 to 12 are dilutions (1:4, 1:16 and 1:64, respectively) of the uninhibited 12 h RNA sample to provide relative quantification of lane 5. Lane 13 contains Mr markers, lane 1 contains untreated probe DNA. The sizes of major protected fragments are indicated on the left. (c) Results of a primer extension analysis of the 5' end of 603 ORF-specific RNA using the FI-r primer (shown in a) to prime reverse transcription of total RNA isolated from mock-infected, wild-type AcMNPV (L1)-infected, vXpoly-infected, vhcLSX-infected or vhcwt-infected cells (lanes 1 to 5) 12 h p.i. On the left and right are dideoxyribonucleotide sequencing ladders using FI-r as a primer and either pGP-IF, a plasmid containing the wild-type polyhedrin promoter sequence, or pXpoly, a plasmid containing the LSX mutant promoter region, as a template. The sequence on the left of the panel is the wild-type promoter sequence with the start site(s) of the 603 ORF sequence indicated. On the right is the sequence of the LSX mutant promoter surrounding the same start site(s) in vXpoly and vhcLSX mutant viruses.
5' end mapping studies revealed the presence of a protected fragment mapping to the essential TAAG site at the polyhedrin transcription initiation point, suggesting bidirectional transcription from this site. However, because primer extension data and 3' end data were not obtained, their identification of the 5' end of the 603 ORF was tentative (Gearing & Possee, 1990).

We also observed a similar protected fragment mapping to the polyhedrin transcription start site (TAAG) using S1 nuclease protection analysis (Fig. 2). A 5' end-labelled probe, extending in the 5' to 3' direction from the MluI site within the 603 ORF, crossing the EcoRV site and terminating at a HindIII site (nucleotide 2436) within the polyhedrin ORF (Fig. 2a), was used in S1 nuclease protection assays with RNA isolated at various times p.i. (Fig. 2b). Protected DNA fragments were not observed in mock-infected or RNA samples analysed at 0 h p.i. At early times (3 and 6 h p.i.), protection of the full-length DNA probe and a 0-6 kb DNA fragment were observed. These protected fragments were observed also using 12 h p.i. RNA samples from infected cells treated continuously with either aphidicolin, a DNA synthesis inhibitor known to block late AcMNPV gene transcription, or cycloheximide. Thus, there appeared to be two early transcripts of the 603 ORF, one initiating near the translational start site of the 603 ORF and one initiating beyond the HindIII site; the latter is consistent with the 5' end of the major 3-2 kb RNA mapping far upstream (Ooi & Miller, 1990). Two fragments would also be consistent with the presence of two early RNAs of approximately 1 kb and 3-2 kb, observed in Northern blots of cycloheximide-treated RNA hybridized to the 603 ORF probe (Ooi & Miller, 1990). (The 1 kb RNA is somewhat large for the 0-6 kb protected fragment but polyadenylation at the 3' end and/or splicing at the 5' end might explain the additional length of the observed RNA.)

At 12 h p.i. a new, more abundant, 0-7 kb protected fragment was observed in addition to more abundant levels of the full-length protected probe (Fig. 2b). The 0-7 kb and full-length protected fragments were observed maximally at 12 h p.i. and in reduced levels at 24 and 48 h p.i. The 0-7 kb fragment is not protected by 12 h RNAs from aphidicolin- or cycloheximide-treated cells, indicating that the presence of this fragment depends on DNA replication. The regulation of the 0-7 kb RNA is not fully consistent with either of the two RNAs (1 kb and 3-2 kb) observed in this region by Northern blot analysis (Ooi & Miller, 1990).

The nature of the RNA indicated by the predominant late 0-7 kb protected fragment was also curious with regard to its predicted size. The size of the protected fragment would place the 5' end of the corresponding RNA near the TAAG site of the polyhedrin promoter. However, the 3' end of RNAs traversing the EcoRV site map to the end of the 603 ORF and the predicted size of such an RNA would thus be approximately 730 bp. Northern blots of the 603 ORF region did not reflect the presence of a late, approximately 0-7 kb to 1 kb RNA, although the blots did reveal a 1 kb RNA regulated as an early RNA (Ooi & Miller, 1990). We refer to the 'late' RNA reflected by the 0-7 kb protected fragment as the 603 ORF-specific late RNA or 603SL RNA.

**Primer extension analysis of the 5' end of the 603SL RNA**

Using an oligonucleotide (FI-r) located near the 5'-terminus of the 603 ORF to prime reverse transcription of RNA isolated 12 h p.i. from cells infected with wild-type AcMNPV, a primer extension product was found in infected but not mock-infected cells and was observed to map to three nucleotides (2145 to 2147; sequence coordinates of Matsuura et al., 1987) at approximate position −40 relative to the polyhedrin ATG (positions +1, +2 and +3). Thus, the 5' end mapped by primer extension differed from the S1 nuclease protection localization of Gearing & Possee (1990) by approximately 12 nucleotides. From primer extension analysis, the 5' ends of 603SL and polyhedrin RNAs would be expected to overlap by approximately 10 nucleotides. The same extension product was observed also for RNA from vhcwt-infected cells as was found for RNA isolated from wild-type virus-infected cells (Fig. 2c); vhcwt is a recombinant virus containing a chloramphenicol acetyltransferase (CAT) gene in place of the polyhedrin gene, with a wild-type polyhedrin promoter region controlling CAT gene expression.

There were two curious aspects of this putative 603SL RNA start site. First, the corresponding primer extension product was not observed for RNA isolated from cells infected with the linker scan mutant virus vhcLSX, a vhcwt analogue containing the CAT gene but having a linker mutation at the TAAG site of the polyhedrin promoter (Fig. 2c). Furthermore, the primer extension product was not found in cells infected with vXpoly (Fig. 2c, lane 3). The vXpoly mutant virus is identical to wild-type AcMNPV except that it has a linker mutation within the TAAG site of the polyhedrin promoter that blocks transcription initiation of polyhedrin RNA. Although the lack of 603SL RNA in vXpoly-infected cells could be explained by invoking a requirement for the TAAG site for bidirectional transcription of the late 603 ORF RNA, the vXpoly mutant was shown previously to produce quite normal levels of the 603 ORF RNA at all times p.i., including normal levels of the 3-2 kb RNA at12 h p.i. (Ooi & Miller, 1990). Thus, there is no evidence for a TAAG-promoted 603 ORF RNA in Northern blots.
The effect of the linker scan mutations on 603 RNA primer extension products

In the course of our studies on the cis-acting elements of the polyhedrin promoter we had constructed a series of recombinant viruses in which the polyhedrin gene was replaced by the CAT gene and the region containing the polyhedrin promoter contained one of a series of 10-nucleotide linker substitutions (Rankin et al., 1988; Ooi et al., 1989). The only difference among members of this series of recombinant viruses is in the 93 nucleotide region between nucleotides +1 and −92 (relative to the original polyhedrin ATG). The 5′ start site of the polyhedrin RNA maps to position −50 within this region and the putative 5′ start site of the 603SL RNA mapped by primer extension to approximate position −40 within this region.

It was of interest to determine how these linker scan mutations influenced primer extension products specific for the 603 ORF RNAs. The substitution of the CAT gene for the polyhedrin gene in these recombinant viruses did not affect the putative initiation site, as shown previously by primer extension analysis with vhcwt (Fig. 2c).

Transcripts from cells infected with the vhc series of viruses bearing selected linker mutations in the polyhedrin promoter were monitored by primer extension analyses (Fig. 3) and by S1 nuclease analysis (data not shown). (S1 nuclease protection assays were generally not as informative for these recombinant viruses as primer extension assays because each mutant virus has a sequence discontinuity in the promoter region giving rise to protected fragments mapping to the sequence discontinuity rather than the ‘5′ end’. The S1 nuclease protection assays do, however, provide information on the levels of RNAs crossing the sequence discontinuity.) Control viruses in these experiments included wild-type AcMNPV (L-1), vhcwt, vhcnp and vhcTAC. The ‘no promoter’ mutant, vhcnp, lacks the entire polyhedrin promoter region between nucleotides −1 and −92. This mutant showed no extension products (Fig. 3), as expected owing to the requirement for TAAG. However, vhcnp did show similar levels of 603 ORF RNA to wild-type AcMNPV (data not shown). The recombinant virus (vhcTAC), in which the CAT gene as well as the polyhedrin promoter region (to the EcoRV site) are reversed in their genomic orientation from the rest of the vhc series, showed different apparent start sites. Since the oligonucleotide used as a primer lies outside the reversed region in this virus, the products observed must reflect sites within the C-terminal portion of the CAT gene which serve as cryptic initiation sites or primer extension blocks.

Linker mutations at the TAAG site that eliminate polyhedrin gene transcription (i.e. vhcLSX and vhcLSXI; see Ooi et al., 1989) lack primer extension products in this region (Fig. 3, lanes 9 and 8), consistent with our observation of the requirement for TAAG (Fig. 2). However, S1 nuclease analysis of these mutants showed that 603 ORF RNA levels were similar to that of wild-type AcMNPV (data not shown). Two linker scan mutations (vhcLSXVI and vhcLSXIV) between 12 and 30 nucleotides upstream of the TAAG, and five mutations between five and 40 nucleotides downstream of the TAAG site, showed the same primer extension products as vhcwt and wild-type virus.

Mutations immediately flanking TAAG, those of the vhcLSXIII and vhcLSVIII mutants, have unique effects. The LSVIII mutation, which replaces 10 nucleotides on the 5′ side of the TAAG sequence and thus replaces the putative transcriptional start point of the 603SL RNA mapped by primer extension, resulted in a shift in the transcriptional start site by two to three nucleotides towards the polyhedrin start site. This mutation could be viewed as either shifting the start point of the 603SL RNA or as shifting the point at which the primer
extension reaction can no longer proceed (see Discussion below on duplex RNA formation). The LSVIII mutation does not affect the initiation point of polyhedrin sense RNA (Ooi et al., 1989). The LSXIII mutation, which replaces the 10 nucleotides immediately 5' of TAAG, resulted in a severe reduction of the 603 ORF primer extension product. This particular mutation was noted previously as a unique mutation with regard to its temporal regulation; CAT expression is reduced significantly at 12 h p.i. relative to vhcwt or the other LS series viruses but, by 48 h p.i., the level of CAT in vhcLSXIII-infected cells is approximately one-third that of wild-type infected cells and higher than many of the other LS series-infected cells (Ooi et al., 1989; especially note the inset in Fig. 2 of this reference). Thus the absence of a primer extension product for LSXIII in these assays using 12 h RNA also correlates with the absence of polyhedrin gene transcription in this mutant at this time.

The putative 5' end of 603SL RNA appears to be an artefact of the mapping techniques

Our data led us to consider seriously the possibility that the 603SL extension product mapping near the polyhedrin gene start site was an artefact of the mapping techniques, probably due to interference by antisense RNA. If one considers that polyhedrin RNA can form a stable duplex with the 3.2 kb 603/1629 ORF RNA, then it could be possible that primer extension is blocked when it attempts to displace the 5' end of polyhedrin RNA in the duplex RNA. Furthermore, the stability of RNA:RNA duplexes is greater than RNA:DNA duplexes and one would expect that S1 nuclease analysis would also reveal the presence of a protected fragment near the start point of the RNA:RNA duplex (i.e. the polyhedrin 5' end). This point might differ slightly from the point detected by primer extension depending on the ability of reverse transcriptase to displace the 5' end of the polyhedrin RNA.

To examine further the possibility that antisense RNA creates an artefact, we performed an additional experiment using a synthetic antisense RNA to show that the same primer extension products can arise simply as a result of the presence of antisense RNA. At 48 h p.i. the 3.2 kb RNA levels are extremely low but the polyhedrin RNA levels represent a substantial proportion of the total cellular RNA. Using the FI-r oligonucleotide as a primer for reverse transcription of 40 μg of total RNA isolated 48 h p.i., no 603 ORF-specific extension product was observed. Upon addition of a synthetic antisense RNA mimicking the 3.2 kb RNA, a 603 ORF-specific RNA extension product was observed. We conclude that antisense RNA can give rise to artefacts in primer extension analyses.

Discussion

Data from both primer extension and S1 nuclease protection analyses, which were conducted originally to map the 5' end of the major late transcript of the 603 ORF of AcMNPV, suggested that the 5' end of the 603 ORF RNA mapped to a position near the TAAG sequence, the initiation site of polyhedrin RNA and a sequence essential for polyhedrin RNA initiation. Gearing & Possee (1990), using S1 nuclease protection analysis of the 5' end alone, tentatively concluded from similar results that the 5' end of the major late 603 ORF RNA is at the TAAG site. They proposed that this site can promote bidirectional transcription. Bidirectional transcription from a TAAG site of a retroposon (TE-D) inserted into the AcMNPV genome has been reported previously (Friesen et al., 1986). The two situations differ substantially, however. In the case of TE-D, the 5' RNA ends did not overlap and each 5' end mapped to symmetrical 5' TAAG 3' sequences within an imperfect palindrome. The 5' ends of all characterized late and very late RNAs of AcMNPV map to a TAAG sequence read in the 5' to 3' direction.

We consider another interpretation of the mapping data for the 603 ORF RNA to be more likely. Specifically, we think that the 5' end mapping data presented herein and by Gearing & Possee (1990) are compromised severely by the presence of polyhedrin RNA from the opposite strands which can form a duplex structure with the 3.2 kb transcript of the 603 ORF. Transcriptional mapping artefacts arising in S1 nuclease protection studies with sense/antisense RNA duplexes have been described previously (Favoloro et al., 1980). The mapping artefact is termed a 'shadow' and is known to confound transcriptional mapping by the nuclease protection method. Since RNA:RNA duplexes are more stable than RNA:DNA duplexes, the polyhedrin RNA competes effectively with the S1 nuclease DNA probe for hybrid formation with the 3.2 kb RNA. The 3.2 kb RNA can form a duplex structure with both the polyhedrin RNA and the DNA probe used in the S1 nuclease protection assays. The polyhedrin RNA would be expected to displace the 3' end of the S1 nuclease probe DNA at the common point of hybrid formation (i.e. at the TAAG site). The result would be an S1 nuclease-cleavable site near the 5' end of the polyhedrin sense RNA which would appear to mark the 5' end of the late 603 ORF RNA. This is the equivalent of the 'shadow' artefact described by Favoloro et al. (1980); in this case we are observing the 'shadow' of the 5' end of the polyhedrin RNA(s).

This duplex of polyhedrin sense RNA and the 3.2 kb RNA, composed of 603 ORF/polyhedrin antisense/1629 ORF information, would be expected also to block
extension of a primer originating from the 603 ORF and extending toward the 1629 ORF. A model experiment showing that antisense RNA can result in artefactual bands supports this interpretation.

The key facts that indicate that the 5' end of the 603 ORF late RNA is not near the polyhedrin promoter TAAG site are (i) the major late RNA of the 603 ORF is 3.2-3.7 kb in length (Gearing & Possee, 1990); (ii) the 3' end of the major late 603 ORF RNA is just beyond an A2UA3 signal at the 3' terminus of the 603 ORF, only 0.7-3 kb from the TAAG site (Ooi & Miller, 1990); (iii) the 3-2 kb RNA extends beyond this site, as shown by S1 nuclease protection analyses and Northern blot analyses, and the 5' ends of this 3.2 kb RNA map near the 5' terminus of the 1629 ORF (Ooi & Miller, 1990); and (iv) a mutation of the polyhedrin promoter TAAG sequence eliminates the 603 ORF primer extension product mapping to this site but does not affect the presence of the 3-2 kb 603 ORF RNA.

The nuclease protection assays (Fig. 2) revealed the presence of three major protected fragments. The 0-7 kb fragment corresponds to the putative 603 ORF RNA start site at the TAAG sequence and is not found before 12 h p.i. The larger 0-95 kb fragment, which corresponds to protection across the entire length of the probe, is observed earlier in infection and in the presence of cycloheximide. The 3-2 kb RNA is present under these conditions but polyhedrin RNA is not synthesized significantly until 12 h p.i. Thus, although the 3-2 kb RNA is present at this time, one would not expect a 0-7 kb primer extension product until 12 h p.i., when polyhedrin RNA is initiated. The amount of 3-2 kb RNA present falls dramatically between 18 and 24 h p.i. in a wild-type infection so that the amount of the 0-7 kb RNA would be expected to drop as that of the 3-2 kb RNA declined. The 0-95 kb protected fragment would be expected to decline even more rapidly because polyhedrin RNA is in vast excess of the 3-2 kb RNA by 18 h p.i. All of the 3-2 kb RNAs would be expected to form duplexes with the polyhedrin RNA so that no full-length protection would be expected to be observed by 18 h p.i. The amount of 0-7 kb protected fragment present does not appear to decline as rapidly at 24 h p.i. (Fig. 2b) as that of the 3-2 kb RNA observed in Northern blots (Ooi & Miller, 1990). This may be due to a rapid degradation of duplexed RNA at the 5' end of the 3-2 kb but less rapid degradation of the 3' end, which contains the 603 ORF. In this case, one would predict that the 3-2 kb RNA would appear as a smear in the 24 h lanes of the Northern blots, in the 0-8 to 2 kb range, when probed with the 603 ORF sequences. The Northern blots which have been presented could be interpreted in this manner (Gearing & Possee, 1990; Ooi & Miller, 1990). Further analysis of the mechanism by which the decline in 3-2 kb RNA occurs should clarify this aspect.

The effects of linker scan mutations in this critical region revealed that the presence of protected fragments and/or primer extension products mapping to TAAG depended on polyhedrin gene transcription. These results could be interpreted as evidence for the essential nature of TAAG in bidirectional transcription resulting in 603 ORF RNA. However, the effects of the LSVIII and LSXIII mutations are more easily explicable by the hypothesis of interference from duplex RNA than by the bidirectional promoter hypothesis. In the former hypothesis, the effect of the LSVIII mutation would be to displace the position at which reverse transcriptase is blocked. This displacement would be expected to be sequence dependent; the higher (G + C) content of the linker would influence (stabilize) the duplex RNA structure making it more difficult to displace the 5' end. Indeed, the LSVIII mutation is the only linker scan mutation mapping to this overlap region (other than those mutations affecting the essential TAAG sequence). This mutation confers a higher (G + C) content to the overlap region and the primer extension product is a few nucleotides shorter, as would be predicted from the model. In the case of the LSXIII mutation, the lack of an extension product for RNA from vhcLSXIII-infected cells probably reflects the lack of polyhedrin promoter-initiated CAT RNA; its production is delayed for this mutant at 12 h p.i. (Ooi & Miller, 1990; see inset of Fig. 2 of this reference).

In summary, transcriptional mapping techniques must be used and interpreted very carefully when mapping antisense RNAs. Artefacts can arise from the presence of antisense RNA and the distinction between actual RNA termini and artefacts resulting from duplex formation may be extremely difficult.

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References


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