Categorizing some early and late transcripts directed by the *Autographa californica* nuclear polyhedrosis virus

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Using an S1 mapping assay on RNA from *Spodoptera frugiperda* cells infected by the *Autographa californica* nuclear polyhedrosis virus in the presence and absence of cycloheximide and aphidicolin, we can distinguish three classes of transcripts. First, there are those whose synthesis is blocked by the DNA synthesis inhibitor aphidicolin and which are therefore late transcripts. These include the late transcript of the 39K gene and a late leftward transcript across the *XhoI* site in the *HindIII-F* region. Second, there are those whose synthesis is not blocked by aphidicolin, but whose accumulation is inhibited by the protein synthesis inhibitor cycloheximide and which are therefore presumably delayed early genes. These include the p26 transcript(s), the early 39K transcript and the z2 transcript in the *HindIII-K/Q* region. Third, there are those whose accumulation is not affected or is enhanced by cycloheximide. These are not necessarily immediate early transcripts, but their response to cycloheximide is clearly different from that of those in the second class. They include the z1 and z3 transcripts in the *HindIII-K/Q* region and the early leftward transcript across the *XhoI* site in the *HindIII-F* region.

The *Autographa californica* nuclear polyhedrosis virus (AcNPV) belongs to the baculovirus family and replicates in lepidopteran insect nuclei. The spatial pattern of transcription in AcNPV is unusually complex. Many regions of the genome are crossed by multiple transcripts (Guarino & Summers, 1986; Lübbert & Doerfler, 1984; Kuzio *et al.*, 1984; Rankin *et al.*, 1986; Friesen & Miller, 1985; Oellig *et al.*, 1987), some running in opposite directions (Friesen & Miller, 1987; Hardin & Weaver, 1990).

The temporal pattern of gene expression in this virus is also complex. Proteins have been classified according to their appearance as follows: α appearing 2 h post-infection (p.i.), β appearing 6 h p.i., γ appearing 12 h p.i. and δ appearing 24 h p.i. (Kelly & Lescott, 1981). Transcripts may also be classified this way, but they do not fall neatly into four classes. Instead, we can identify early transcripts as those which appear before 6 h p.i. (the onset of viral DNA replication) and late transcripts as those which appear after this time. In addition, there are two prominent very late transcripts: those corresponding to the polyhedrin and p10 genes.

The distinction between immediate early and delayed early transcripts has been the most difficult to make. Guarino & Summers (1987) have defined transcripts, including 39K, p26 and the z1 and z2 transcripts in *HindIII-K*, as delayed early because of their dependence on immediate early-1 (IE-1) gene expression in transient assays. On the other hand, Rice & Miller (1986), Friesen & Miller (1987) and Crawford & Miller (1988) have found that synthesis of several early transcripts, including the z1 and z3 transcripts in *HindIII-K*, is not sensitive to the protein synthesis inhibitor cycloheximide. This would normally suggest that these are immediate early transcripts. However, because of the discrepancy between the two sets of data, it is not yet possible to identify immediate early transcripts unequivocally.

On the other hand, delayed early transcripts can be identified by their sensitivity to cycloheximide and late transcripts by their sensitivity to aphidicolin. Although experiments using such inhibitors are subject to artifacts, especially as cycloheximide sometimes fails to block delayed early transcription, positive results (i.e. inhibition) can be valuable. Accordingly, we have performed inhibitor studies similar to those of Rice & Miller (1986), but we have concentrated on the shortest time periods possible to maximize the chance of observing cycloheximide sensitivity. We have also employed a sensitive S1 assay of transcription. Under these conditions, we can distinguish two classes of early transcripts: those whose synthesis is unaffected or stimulated by cycloheximide, and those whose synthesis is inhibited by the drug. The latter are presumably delayed early transcripts. We can also readily distinguish late transcripts by their sensitivity to aphidicolin.

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Friesen & Miller (1985, 1987) have mapped a set of overlapping transcripts in the \textit{HindIII-K/Q} region of the AcNPV (L1 strain) genome. Their S1 mapping and Northern blot analyses identified at least four early transcripts as early as 2 h after infection and 3 late transcripts at 12 h p.i. (Fig. 1a). In order to study transcription in the \textit{HindIII-K} region at even earlier times, S1 mapping analysis was carried out with RNA harvested every 30 min during the first 2 h p.i. RNA was also prepared from cells treated with cycloheximide for 4 h (from 2 h before to 2 h after infection).

Fig. 2(a) shows the results of S1 mapping using the right-hand \textit{EcoRV–HindIII} subfragment of the \textit{HindIII-K} fragment, labelled at the \textit{HindIII} site. This probe detects rightward transcripts that cross the \textit{HindIII-K/Q} boundary. Fig. 2(a), lane 4 demonstrates that no S1 signal was detected when the labelled probe was hybridized to poly(A)+ RNA from cells at 0 h p.i. However, by 30 min after infection, the 430 nucleotide (nt) protected fragment corresponding to ~ RNA was already observed (Fig. 2a, lane 5) and the accumulation of this transcript occurred very rapidly during the next 90 min. Lane 3 shows that cycloheximide did not inhibit the accumulation of this transcript. In fact, the drug appears to stimulate the transcript's accumulation. This experiment also indicated that the 830 nt signal corresponding to \( z_1 \) RNA was detected when the labelled probe was hybridized to poly(A)+ RNA from cells at 0 h p.i. However, by 30 min after infection, the 430 nucleotide (nt) protected fragment corresponding to \( z_1 \) RNA was already observed (Fig. 2a, lane 5) and the accumulation of this transcript occurred very rapidly during the next 90 min. Lane 3 shows that cycloheximide did not inhibit the accumulation of this transcript. In fact, the drug appears to stimulate the transcript's accumulation. This experiment also indicated that the 830 nt signal corresponding to \( z_1 \) RNA was detected when the labelled probe was hybridized to poly(A)+ RNA from cells at 0 h p.i. However, by 30 min after infection, the 430 nucleotide (nt) protected fragment corresponding to \( z_1 \) RNA was already observed (Fig. 2a, lane 5) and the accumulation of this transcript occurred very rapidly during the next 90 min. Lane 3 shows that cycloheximide did not inhibit the accumulation of this transcript.

To detect leftward transcripts crossing the \textit{EcoRV} site, we used the same probe as in Fig. 2(a), but labelled it at the \textit{EcoRV} site. Fig. 2(b) shows that S1 mapping with this probe gave a signal corresponding to the \( z_3 \) transcript reported by Friesen & Miller (1987) whose 5' end maps 540 bp to the right of the \textit{EcoRV} site. This signal was detectable at 1·5 and 2·0 h p.i. (Fig. 2b, lanes 7 and 8) and was markedly enhanced by cycloheximide (compare lanes 3 and 8). We also examined longer term synthesis (12 h) of the rightward transcripts in \textit{HindIII-K}. This allowed us to investigate the effect of adding cycloheximide at different times, as well as the effect of a DNA synthesis inhibitor, aphidicolin. Fig. 2(c) shows that cycloheximide enhanced the accumulation of the \( z_1 \) transcript (arrow C) and that this enhancement increased with increasing length of exposure of cells to cycloheximide (compare lanes 3, 4 and 5 with lane 2). A similar enhancement was observed when cells were exposed to aphidicolin for the full 12 h of the experiment (compare lanes 3, 4 and 5 with lane 2). By contrast, aphidicolin added at the time of infection markedly increased the accumulation of this transcript. In addition to the \( z_1 \) and \( z_2 \) transcripts mapped by Friesen & Miller (1985), this experiment revealed a signal corresponding to a putative third transcript (arrow B in Fig. 2c). This transcript, whose 5' end lies about 530 bp to the left of the \textit{HindIII-K/Q} boundary, appears to be late as its accumulation is blocked by early administration of cycloheximide (compare lanes 2 and 5) and by aphidicolin (compare lanes 2 and 5).
Fig. 2. (a) 5' S1 mapping of RNA transcribed from the HindIII-K fragment during the early phase of infection. Poly(A)+ RNA prepared according to Maniatis et al. (1982) from cells harvested every 30 min from 0 to 2 h p.i. (lanes 4 to 8) or from cells treated with cycloheximide (administration starting at 2 h pre-infection) and harvested at 2 h p.i. (lane 3), were hybridized to the right-hand EcoRI-HindIII segment of HindIII-K (labelled at the HindIII site) and S1 analysis was performed. Labelled size markers included HaeIII-cleaved pBR322 and φX174 DNAs (lanes 1 and 2 respectively). The signals expected for the α1 (430 nt) and α2 (839 nt) transcripts are indicated on the right. (b) S1 nuclease mapping of the 5' ends of the transcripts crossing leftward through the EcoRV site in HindIII-K. Poly(A)+ RNA was hybridized to the right-hand EcoRV-HindIII segment, which was 5' labelled only at the EcoRV site, and S1-mapped. Indicated is the S1-resistant signal expected for the α2 transcript in HindIII (arrow at 540 nt). The 5' end-labelled DNA probe is shown in lane 9. (c) Effect of cycloheximide and aphidicolin on transcription in the HindIII-K region. Cycloheximide and aphidicolin were administered to cells as follows: untreated control (lanes 1 and 2); cycloheximide administered at 7.5 h (lane 3), 5 h (lane 4) and 2.5 h (lane 5) p.i.; aphidicolin added starting at 0 h p.i. (lane 6). At 12 h p.i., these treated, infected cells were harvested to isolate RNA, and poly(A)+ RNA from the inhibitor-untreated cells (lane 1) or total cytoplasmic RNA (lanes 2 to 6) from these cells was S1-mapped using the right-hand EcoRV–HindIII segment of HindIII-K (5' end-labelled at the HindIII site) as the probe. The S1-resistant signals indicated by the arrows were expected for the α2 transcript (arrow A), a late transcript (arrow B) and the α1 transcript (arrow C), respectively. Markers were HaeIII-cleaved φX174 DNA (lane 7) or HaeIII-cleaved pBR322 DNA (lane 8). Spodoptera frugiperda (IPLB-SF-21) cells were cultured in TC-100 medium supplemented with 10% foetal bovine serum. Cells were infected with non-occluded virions of AcNPV strain L1 at a multiplicity of 20 p.f.u. per cell as described previously (Friesen et al., 1985), except that zero time p.i. was defined as the point at which fresh growth medium was added to the cells. S1 mapping was carried out as described by Weaver & Weissmann (1979). Equivalent amounts of RNA were used in each lane within an experiment. Labelled probe was always in excess.
and 6).

Thus, the accumulation of the α1 (p35) and α3 (p94) transcripts in this region appears to be stimulated by cycloheximide, whereas that of the α2 transcript is inhibited, at least when the drug is added before infection begins. The stimulation of transcript accumulation observed here can in principle be explained by either an enhanced synthesis rate or a reduced degradation rate. The former could be due to improved competition for scarce transcription factors when later transcription is blocked by cycloheximide. However, the latter is the more probable explanation in this case and in the case of the p26 transcript to be discussed below, since nuclear run-on experiments showed no increased synthesis of these transcripts in the presence of cycloheximide (Huh & Weaver, 1990). Guarino & Summers (1987) classified the p35 and p94 genes as delayed early based on their dependence on transcription by IE-1 in transient assays. On the other hand, Nissen & Friesen (1989) used a chloramphenicol acetyltransferase-linked transient assay to demonstrate that, although transcription from the p35 promoter is greatly stimulated by IE-1, it occurs at a low level even in the absence of IE-1. It is possible that this basal level has no physiological significance. Unfortunately inhibitor studies cannot accurately identify immediate early AcNPV transcripts and so shed light on these problems but the present data do imply that the α2 RNA is a delayed early transcript.

We have also used the S1 mapping technique to examine the effect of cycloheximide and aphidicolin on transcription of the p26 gene (see Fig. 1a). In this case, because significant p26 transcription is not observed at 2 h p.i. (Rankin et al., 1986), we used longer time periods than in the experiments with the HindIII-K transcripts reported in Fig. 2(a) and (b). Total cytoplasmic RNA isolated from infected cells was analysed by S1 nuclease mapping with the EcoRV–XhoI subfragment of HindIII-Q (5′-labelled at the XhoI site) as the probe (Fig. 3).

The cells used in this experiment were harvested at 10 h p.i., after treating them with cycloheximide or aphidicolin for various times as described in the legend to Fig. 3. In the cells to which cycloheximide was given beginning at 2 h pre-infection, expression of the p26 gene was significantly inhibited (compare lanes 4 and 5). When cycloheximide was administered beginning at 2.5 h p.i., some inhibition of p26 transcription was still observed (compare lanes 8 and 9). However, when cycloheximide was added beginning at 5 h p.i., little inhibition of p26 transcription was detected (compare lanes 4 and 6). This suggests that synthesis of protein factors sufficient for maximal transcription of the p26 gene is not complete until after 2.5 h. p.i. By this criterion p26 behaves as a delayed early gene. Guarino & Summers (1987) have shown that transcription from the p26 promoter in transient assays depends on cotransfection with the trans-activating gene IE-1 and we have confirmed this finding (data not shown). This is consistent with the conclusion that p26 is a delayed early gene.

Aphidicolin added at the time of infection also inhibited p26 transcription considerably (compare lanes 4 and 7). It is unlikely that p26 is really a late gene as Rankin et al. (1986) showed significant accumulation of p26 transcripts at 6 h p.i., implying that this gene is activated prior to this time, in the early phase of infection. Furthermore, transcription of the p26 gene in isolated nuclei appears to be inhibited by α-amanitin, which is characteristic of early transcripts in this virus (Huh & Weaver, 1990). Finally, the inhibition by aphidicolin is far from complete, whereas inhibition of authentic late transcription by this antibiotic seems to be absolute (Rice & Miller, 1986 and see below, Fig. 4 and 5). Why should aphidicolin inhibit the transcription of an early gene? The answer is probably that, although transcription of the p26 gene begins during the early phase, the accumulation of p26 transcripts is most pronounced during the late phase of infection (Rankin et al., 1986). Since aphidicolin prevents late transcription, it may also interfere with p26 transcription during the late phase of infection. Alternatively, by blocking the replication of viral DNA, aphidicolin may simply limit the number of p26 genes available for transcription in the
late phase.

Rice & Miller (1986) used Northern blots to measure transcript accumulation in AcNPV-infected cells in the presence and absence of cycloheximide and aphidicolin. They examined four regions of the viral genome, including two we have studied here by S1 mapping, HindIII-K and HindIII-Q. In general, the Northern blot and S1 results agree, although there are some differences. For example, Rice & Miller (1986) showed strong but not complete inhibition by aphidicolin of accumulation of all transcripts in HindIII-K; the present study shows that accumulation of $a_1$ and $a_2$ transcripts is stimulated by the drug. The latter finding is of course more compatible with the early nature of these transcripts.

A gene coding for a 39K protein is the most abundantly expressed early gene and its transcripts are present until very late in infection (Guarino & Summers, 1986; Vlak et al., 1981). This gene appears to be delayed early because synthesis of the 39K polypeptide is observed first at 3 to 6 h p.i. Furthermore, its expression in transient assays is dependent on trans-activation by the IE-1 gene (Guarino & Summers, 1986). We have used S1 mapping to analyse 39K-specific RNA made in the presence and absence of cycloheximide and aphidicolin. Cells were harvested at 5 h p.i. to study early transcription of the gene (Fig. 4, lanes 1 to 5), or at 10 h p.i. to examine the expression of the gene at a late stage of infection (Fig. 4, lanes 6 to 14). The right-hand HindIII-PstI subfragment of the PstI-K fragment (21.2 to 23.5 map units), 5' end-labelled at the HindIII site, was used as the probe. When the DNA probe was hybridized to RNA harvested at 5 h p.i., a major start site for the early RNA was mapped to a position about 630 bp upstream from the HindIII site (lane 1). When the probe was annealed to RNA isolated at 10 h p.i., the same S1-resistant signal plus another band at 650 nt were observed (lanes 6 and 14, arrows B and A, respectively). Furthermore, the amount of the early transcript had declined to a relatively low level by 10 h p.i. In general, the transcription patterns and transcription initiation sites of the two 39K RNAs in the absence of the inhibitors were consistent with previous reports (Guarino & Summers, 1986; Vlak et al., 1981).

Beginning cycloheximide administration 2 h before infection strongly inhibited accumulation of the early 39K transcript up to 5 h p.i. (compare lanes 3 and 4), but starting to add the inhibitor at 2.5 h p.i. had no such effect (compare lanes 1 and 2). In cells harvested at 10 h p.i., cycloheximide treatment beginning 2 h pre-infection completely inhibited transcription from the late start site (compare lanes 6 and 7, arrow A). Moreover, addition of cycloheximide at 5 h p.i. still strongly inhibits transcription from the early start sites (lanes 5 and 11). This inhibition is alleviated by aphidicolin (lanes 9 and 13) in contrast to the partial inhibition of late transcription seen in the absence of inhibitors (lanes 8 and 12). The RNAs were hybridized to the right-hand HindIII-PstI subfragment of PstI-K (5' end-labelled at the HindIII site), and S1-mapped. These assays were carried out in three different sets of experiments (lanes 1 to 4, 5 to 9 and 10 to 14, respectively). Lanes 10 and 11 contained HaeIII-cleaved φX174 and pBR322 DNAs, respectively. The S1-resistant DNA bands indicated by the arrows on the right were the signals expected for the 39K early (arrow B at 630 bp) and late (arrow A at 650 bp) transcripts, respectively.

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inhibited appearance of the late 39K transcript while having little effect on the level of the early transcript (compare lanes 6 and 8 or lanes 14 and 12). This effect was also observed when cycloheximide was administered as late as 7.5 h p.i. (compare lanes 14 and 13). Starting aphidicolin administration at 0 h p.i. not only completely inhibited accumulation of the late transcript (compare lanes 3 and 5 or lanes 6 and 9), but also allowed the cells to maintain the concentration of the early transcript at the level observed in the control cells harvested at 5 h p.i. (compare lanes 3 and 9). The fact that synthesis of the 39K early transcript is not inhibited by aphidicolin indicates that it is indeed an early transcript. Furthermore, the fact that appearance of this transcript is inhibited by early administration of cycloheximide confirms the report by Guarino & Summers (1986) that it is a delayed early transcript. On the other hand, as expected, the synthesis of the 39K late transcript was completely inhibited by both cycloheximide and aphidicolin.

Hardin & Weaver (1990) have mapped a set of divergent, overlapping transcripts that cross the XhoI site in the HindIII-F fragment (Fig. 1b). One transcript about 2100 nt in length is synthesized clockwise from the XhoI site. Beginning at about 8 h p.i., the concentration of this early transcript decreases and a shorter transcript, corresponding to the early transcript was observed (lanes 7 and 11). The identification of these two transcripts as early and late therefore seems justified.

References


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