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 α1 and α2 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 α1 and α2 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 artefacts, 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 HindIII-K/Q region of the AcNPV 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 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 EcoRV–HindIII subfragment of the HindIII-K fragment, labelled at the HindIII site. This probe detects rightward transcripts that cross the 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 z1 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 z1 RNA discovered by Friesen & Miller (1987) started appearing at 1 h p.i., but its accumulation rate was considerably slower than that of the z1 RNA, and seems to be somewhat sensitive to cycloheximide (compare lanes 8 and 3).

To detect leftward transcripts crossing the EcoRV site, we used the same probe as in Fig. 2(a), but labelled it at the EcoRV site. Fig. 2(b) shows that S1 mapping with this probe gave a signal corresponding to the z3 transcript reported by Friesen & Miller (1987) whose 5' end maps 540 bp to the right of the 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 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 z1 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 2 and 6). Accumulation of the z2 transcript (arrow 1 in Fig. 2c) did not seem to be affected one way or the other by cycloheximide added at any of the times in this experiment (compare lanes 2, 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 z1 and z2 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 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 inhibited, at least when the drug is added before 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 trans-activation 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
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 inhibited transcription. However, aphidicolin administration had no effect on the 39K transcript (compare lanes 8 and 9). Furthermore, aphidicolin given beginning at 7.5 h p.i. (lane 13), or 2.5 h p.i. (lane 2), 5 h p.i. (lanes 8 and 12), and 7.5 h p.i. (lane 13) p.i. Aphidicolin treatment was begun at 0 h p.i. (lanes 5 and 9). As a control, cells were not treated with the inhibitors (lanes 1, 3, 6 and 14). Infected cells were harvested at 5 h (lanes 1 to 5) or 10 h (lanes 6 to 14) p.i. for preparing total cytoplasmic RNA. The RNAs were annealed 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 and 2, 3 to 9 and 10 to 14, respectively). Lanes 10 and 11 contained Haell-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.
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 counter-clockwise on the standard map. This transcript first appears about 2 h p.i.; its 5' end maps about 504 bp clockwise from the XhoI site. Beginning at about 8 h p.i., the concentration of this early transcript decreases and a shorter transcript, whose 5' end maps about 80 bp downstream from the first, begins to accumulate. We used S1 mapping to check the sensitivity of the synthesis of these two transcripts to inhibitors. Fig. 5 shows the results. When RNA was isolated from cells 5 h p.i., only the signal corresponding to the early transcript was observed (lanes 1 to 4). In addition, cycloheximide added at 2 h pre-infraction or 2.5 h p.i. had little effect on synthesis of this transcript. If anything, the drug caused extra accumulation of the transcript (compare lanes 2 and 3 with lane 1). The response to aphidicolin was similar (compare lanes 2 and 4). When RNA was isolated from cells 12 h p.i., signals corresponding to both transcripts were detected (lane 7, bands marked A and B). As was the case with 5 h RNA, cycloheximide increased the accumulation of the early transcript; however it completely inhibited accumulation of the late transcript (compare lanes 8, 9 and 10 with lane 7). Aphidicolin had similar effects. It enhanced accumulation of the long transcript, but blocked accumulation of the short transcript (compare lanes 7 and 11). The identification of these two transcripts as early and late therefore seems justified.

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References


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