Global transcription pattern of φC31 after induction of a Streptomyces coelicolor lysogen at different growth stages

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Using two complementary strategies for low-resolution S1 mapping, the global pattern of φC31 transcription was studied after induction of thermoinducible φC31 lysogens of Streptomyces coelicolor A3(2). A complex pattern of early transcripts was seen, with a peak of abundance at about 10 min post-induction. Nearly all of these transcripts were from DNA located to the right of the c (repressor) gene and to the left of the attP site: a region of about 14 kb. Early transcription was also observed immediately to the left of the c gene. The c gene itself was also induced, with an earlier expression peak (about 5 min post-induction). Primary late transcripts were generally relatively long, but degraded. They apparently corresponded to most of the 18 kb region to the left of the c gene. Some shorter and more persistent late transcripts corresponded to DNA close to or overlapping the cos site. Large late transcripts from a region close to the left-hand end of the φC31 genome showed evidence of processing to more stable, smaller RNA species. A failure of older cultures (more than 12 h old) to be induced productively was correlated with a much longer period of early transcription, reduced late transcription, failure to synthesize a major virion protein, and failure to package φC31 DNA. Moreover, heat treatment of the older lyogenic cultures did not result in the φC31-dependent shut-down of host rRNA transcription previously observed for young cultures (Rodríguez et al., Journal of General Microbiology (1986) 132, 1695-1701; Clayton & Bibb, Molecular Microbiology (1990) 4, 2179-2185).

Introduction

Most detailed studies of temperate bacteriophages have focused on lambdoid coliphages. Studies of temperate phages of bacteria phylogenetically distant from Escherichia coli, such as the industrially important streptomycetes, would usefully extend our general understanding of phage biology and lysogeny, as well as providing insights into the molecular biology of the hosts. Temperate phages of streptomycetes have been studied for the last 20 or so years. Probably the most characterized is φC31, a wide-host-range phage morphologically resembling coliphage λ (Lomovskaya et al., 1980; Suárez et al., 1984; Chater, 1986). φC31 can infect and lysogenize Streptomyces coelicolor A3(2), the streptomycete favoured for genetic studies. Like λ for E. coli, φC31 has provided useful cloning vectors and tools for molecular genetic studies in streptomycetes (Hopwood et al., 1987; Bruton et al., 1991; Kobler et al., 1991; Kuhstoss et al., 1991).

The genome of φC31 is a 41.2 kb double-stranded DNA molecule (Chater, 1986) with 10 nucleotide complementary single-stranded 3' extensions at its ends (Kobler et al., 1991). A single gene (c) located near the centre of the DNA molecule controls lysogeny (Lomovskaya et al., 1980). This gene has been sequenced and its expression studied at the mRNA and protein levels (Sinclair & Bibb, 1988, 1989; Smith & Owen, 1991). Late-expressed temperature-sensitive (ts) mutations and host-range mutations all map to the left of c, and the single characterized early ts mutation (L24) maps to the...
right of c (Lomovskaya et al., 1980). This suggests that the c gene may separate the late genes (on the left) from the early genes (on the right), a possibility recently qualified by the discovery that a gene immediately to the left of c, encoding a nucleotide kinase-like product, is also transcribed early (Smith et al., 1992; Smith & Owen, 1991). Close to the right-hand end of φC31 DNA is a long region (~8 kb) dispensable for plaque formation, and containing determinants for prophage integration and for interaction of φC31 with restriction-modification systems (Kuhstoss et al., 1991; Chater, 1986).

In this paper, we describe the global transcription of φC31 during the lytic cycle after induction of a heat-inducible prophage. The information is used to clarify the reason(s) why induction is productive only in young cultures.

**Results**

**Strategy for mapping of φC31 transcripts at different times after prophage induction**

SphI fragments of φC31 c1 DNA were cloned into pBR322 (to give the pKC series of plasmids), and EcoRI fragments were cloned into pBR329 (the pUO series) (Fig. 1). The resulting plasmids were used in low-resolution S1 mapping of RNA obtained at different time intervals (10, 20, 30, 40 and 50 min) after initiating induction of a young exponential-phase culture of a heat-inducible φC31 lysogen (S. coelicolor 01, 7 h after inoculating the culture with spores).

Two S1 mapping strategies were adopted (Fig. 2). In type A experiments, full-length φC31 virion DNA was protected by mRNA. Southern blots of the protected fragments were then probed with radiolabelled preparations of each of the plasmid subclones. This showed the sizes of transcripts present in the induced culture, their approximate origins, and the time course of their accumulation. In the type B experiments, the DNA to be protected was from individual plasmid subclones, and Southern blots of protected fragments were probed with radiolabelled total φC31 c1 DNA. This showed the lengths of the larger overlaps between the subcloned φC31 DNA fragments and the phage transcripts (in general, protected DNA of less than 0.5 kb might not have been seen in these experiments). In relating type A and B experiments, comparable size was generally taken to indicate transcript identity. It is important to note that these experiments do not distinguish between rightward and leftward transcription. No type B experiments were done for the fragments EcoRI-E or EcoRI-G. In three autoradiographs, all derived from a single type A S1 nuclease digestion (Fig. 3), an intense smear was obtained with a 30 min sample, yet no equivalent signal was obtained in the type B experiment; this was attributed to a presumptive failure to expose some of the φC31 DNA to the nuclease in this one digestion mixture, through inadequate solubilization or mixing.

**Overall summary of S1 mapping results**

The data are shown in Fig. 3 and are summarized in Fig. 1. No transcription (even of the c gene – see next section) was detected before induction in this first series of experiments. At least 13 different transcripts, ranging from 0.5 kb to 4.5 kb, were detected from the approximately 18 kb region between SphI site 8-3 and EcoRI site 19. These were all early transcripts, since they were most abundant in the earliest sample (10 min post-induction). In most cases, their levels at later time-points declined dramatically. There was no evidence of comparably

**Methods**

Strains, plasmids, phages and culture conditions. Two independently constructed lysogens of S. coelicolor A3(2) derivatives were used: S. coelicolor 01 (hisA1 strA1 strA1 Pgl' SCP1-; Rodríguez et al., 1986) and S. coelicolor TMC1 (wild-type: Clayton & Bibb, 1990). Both contain the thermoinducible φC31 ets1 prophage (Novikova et al., 1973). This phage has a 1.72 kb deletion (AM) in its EcoRI-C fragment (Chater et al., 1981). Conditions for growth and thermal induction of Streptomyces cultures were as described by Rodríguez et al. (1986) and Clayton & Bibb (1990). Standard recombinant DNA techniques were used to subclone fragments of φC31 c1 DNA into pBR322, pBR329, and M13mp9 and to prepare plasmid and replicative-form DNA (Sambrook et al., 1989). Conditions for φC31 c1 propagation were as in Hopwood et al. (1985).

Extraction of nucleic acids from mycelial cultures. For cultures of S. coelicolor 01, the extraction procedures were as in Rodríguez et al. (1986); for S. coelicolor TMC1, procedures were as in Clayton & Bibb (1990).

S1 mapping. For low-resolution global mapping of transcripts, the procedures were essentially those of Smith & Chater (1988). Unless stated otherwise, each reaction used 2 μg RNA and 100 ng DNA. In brief, the mixture was incubated in formamide-containing buffer at 85 °C for 10 min, then at 64 °C for 3.5 h, before adding S1 nuclease (871 units, 37 °C, 1 h). After precipitation, alkaline denaturation and electrophoresis in alkaline 1%/w/v agarose gels, the protected DNA was visualized by probing Southern blots with suitable 32P-nick translated DNA probes (Sambrook et al., 1989). S1 mapping analysis of rrnD transcription was done with a 32P-end-labelled 570 bp DNA probe, as described by Clayton & Bibb (1990).

Dot-blot analysis of RNA. RNA samples (1 μg) were spotted on nitrocellulose filters (pretreated in 20 × SSC and then dried). The filters were dried and processed for hybridization as for Southern blots (Sambrook et al., 1989).

Analysis of proteins by SDS-polyacrylamide gel electrophoresis (SDSPAGE). Mycelial suspensions were washed in ice-cold 50 mM-Tris/HCl, 10 mM-EDTA, pH 8.0, and lysed by sonication. After centrifugation (15 min, 4 °C, Eppendorf microcentrifuge) the supernatants were analysed by SDS-PAGE as in Laemmli (1970) (see Suárez et al., 1984, for precise details).
abundant very long early transcripts from this region. In contrast, RNA accumulating late in infection (from 20 min onwards) mainly protected DNA to the left of the c region. Thus, the leftmost approximately 18 kb of φC31 DNA, extending a short distance into the right-hand end (these ends are presumably joined during the replicative cycle), is the late region. In contrast to the discrete and generally fairly short bands protected with early mRNA, the DNA protected by late mRNA ran as smears on the autoradiographs, consistent with the occurrence of a small number of relatively long, and significantly unstable, late mRNA species.

**Early transcription of the repressor gene**

Transcription of the c region in uninduced cultures was analysed by using radiolabelled Sphl-G fragment to probe the fragments resulting from protection of φC31 DNA. In spite of the high amounts of RNA used (up to 100 µg per reaction) no hybridization was found, though other experiments did detect transcription (see below, and Sinclair & Bibb, 1989; Smith & Owen, 1991) (Fig. 3). In contrast, 10 min after the start of induction three DNA fragments of approximately 1.35, 1.5 and 2.1 kb were protected, identifying mRNA species a, b and c, respectively. All three were apparently internal to the Sphl-G segment, because they were not detectably changed when that fragment was used in the protection. Smith & Owen (1991) reported RNAs of 2070, 1560 and 1270 nucleotides in similar experiments on a φC31 cts1 lysogen, and located them precisely in relation to the nucleotide sequence. The latter information is incorporated into Fig. 1. These RNA molecules were almost absent by 20 min, and undetectable in later time samples.
Transcription of early genes to the right of the repressor gene

For ease of presentation, each apparent mRNA species is dealt with separately, working rightwards from the c region.

A relatively abundant and persistent early RNA (d; 1-4 kb) was detected in a type A experiment with the Spht-C probe, but not with probes for the flanking Spht-G or EcoRI-E fragments. A similarly sized band was detected in the type B experiment, though it was of comparatively low abundance and persistence.

An approximately 2-6 kb mRNA (e) is transcribed from sequences entirely located between Spht sites 8-6 and 9-1, and overlapping EcoRI site 9. It was detected in type A experiments with the Spht-C and EcoRI-E probes, and was unchanged in the type B experiment done with Spht-C. This mRNA was abundant at 10 min post-induction, but it was undetectable in the later samples.

An approximately 2-2 kb mRNA (f) traverses Spht site 9-1, and could have its leftmost end close to EcoRI site 9. In type A experiments it gave a weak signal with Spht-C as probe, a strong signal with EcoRI-E, and no signal with EcoRI-G. A 1-4 kb fragment (f') detected in the type B experiment with Spht-C probably resulted from overlap of mRNA f with the right-hand end of Spht-C. Very little mRNA f was detectable at 20 min post-induction, and none in later samples.

An approximately 4-5 kb mRNA (g) has one end to the left of Spht site 9-1 and extends to the right of EcoRI site 11, being detected in type A experiments by the Spht-C+I, EcoRI-E and EcoRI-G probes. It may be coterminal at its left end with mRNA f. It is of low abundance, perhaps because of a short half-life: a smear extended from the front of the 4-5 kb band, and no signal was detected at 20 min or later. In type A experiments, the band was strongest with EcoRI-E as probe, even weaker signals being obtained with Spht-C+I and EcoRI-G probes.

Fairly abundant mRNAs of approximately 1-6 kb (h) and approximately 1-0 kb (i) have most if not all of their lengths between EcoRI sites 11 and 13. They were detected in type A experiments only with EcoRI-G, and were virtually undetectable by 20 min post-induction.

An approximately 2-5 kb mRNA (j), also fairly abundant but short-lived, has one end close to EcoRI site 11, and the other end about 1 kb to the right of EcoRI site 13. The 2-5 kb band was detected in type A experiments with EcoRI-G and EcoRI-C, giving a stronger signal with EcoRI-G. Probably, this mRNA gave rise to a 1-0 kb band (j') observed with type B experiments using EcoRI-C.

Several mRNA species appeared to be transcribed entirely from sequences between EcoRI sites 13 and 19. In type A experiments, bands k (0-8 kb, strong), l (2-0 kb, weak), m (1-6 kb, strong), and n (0-5 kb, weak) were relatively persistent: they were still detectable with RNA isolated 20 min post-induction, using EcoRI-C as probe, though they were undetectable by 40 min post-induction. A type B experiment with EcoRI-C as the protected DNA fragment gave relatively weak hybridization, but bands apparently corresponding to mRNA species l and
Fig. 3. RNA-protected fragments of φC31 DNA obtained with RNA made during the lytic development of the phage. The larger panels are the results of 'type A' experiments (Fig. 2). In each of these panels, RNA extracted from lysogens at the indicated times after heat induction was used to protect φC31 DNA against S1 nuclease digestion. The protected DNA was detected by alkaline agarose gel electrophoresis and Southern blotting, followed by hybridization with the indicated 32P-labelled probes (see also Fig. 1), and autoradiography. For the EcoRI-E and F panels, longer exposures are also shown. For all the other regions, a small second panel shows the result of a 'type B' experiment (Fig. 2), in which the RNA was used to protect the appropriate fragment of φC31 DNA, and the eventual Southern blot was probed with 32P-labelled φC31 DNA. Each 'type B' panel represents that RNA time sample giving the strongest signal. In some cases, a series of pBR327-derived size markers (provided by D. J. Lydiate: Lydiate et al., 1986) was probed with pBR327. In others, a similar (but not identical) series was provided by F. Malpartida (John Innes Institute). The markers MM are readily seen only with the early time samples, in which a longer exposure was used to detect the protected DNA fragments. Tracks marked with black crosses are blank, and in tracks marked with white crosses a presumptive fault in the S1 digestion has given a very strong false signal (the latter three marked tracks were from a single S1 nuclease digest).
were detected, as well as the 1.0 kb putative j' band. The 0.8 kb band (k) was not detected in the type B experiment. Any of these RNA species that traverses the ΔM deletion present in the ϕC31 cts1 prophage would give rise to an S1-sensitive D-loop when ϕC31c1 DNA or pUO25 (both containing the undeleted EcoRI-C fragment) was used in S1 mapping, so their true lengths may be longer than estimated in this work.

Late mRNA from the region to the left of the c gene

A diffuse smear was seen in type A experiments with Sphl-D, -B or -[F + I] (Fig. 3, right). The smear was first visible at 20 min and increased in intensity in later samples. When the Sphl-D fragment (3.32 kb) was used in a type A experiment, bands of about 7, 5, 3-2, 2-7, 2-0, 1-4 and 1 kb became increasingly obvious from 20 to 50 min, against a comparably intensifying background smear (9 kb–1 kb). A type B experiment with Sphl-D gave relatively weak bands, one of about 4-5 kb (perhaps indicating full-length protection) and others of about 3-8, 3-2, 2-7 and 2 kb in a faint background smear. In contrast, the smear was featureless when Sphl-B or -[F + I] were the probes in type A or type B experiments, with virtually no full-length protection in the type B experiment. Interestingly, the Sphl-[F + I] probe also gave two faint but definite discrete bands of about 1.6 kb and 0.8 kb, with RNA from 10 min post-induction. This indicates that part of the region between Sphl sites 8.0-1 and 8.3 is transcribed early, in agreement with the finding by Smith & Owen (1991) of early transcription from the region immediately to the left of the c gene. The simplest interpretation of these results is that from about 20 min post-induction one or a small number of relatively long mRNA species are made, between them traversing most of the approximately 17 kb between Sphl sites 2-1 and 8-2. This RNA is rapidly degraded, with some of the degradation leading to the accumulation of more stable forms with endpoints in preferred positions.

Small late transcripts that increase in amount throughout the late stages of the lytic cycle

With samples from 20 min onwards, bands of 0.8 kb (o'), 1.3 kb (p) and 2.2 kb (q) steadily increased in amount in type A experiments with Sphl-E+H (a probe that encompasses the cos site). A faint background smear including higher molecular mass material also increased coordinately with these bands. In a type B experiment a 1.3 kb band (p') was again seen, but a 1.4 kb band (o) replaced the 0.8 kb o' band and the 2.2 kb band q was apparently shortened to 2.1 kb. Possibly, the 1.4 kb band (o) includes the cos site, which is continuous in the Sphl-

E+H probe but discontinuous in ϕC31c1 DNA. The bands p and q do not include the cos site, since they were not reduced in size in the type A experiments. If the apparent size differences between bands q and q' were correct, then q would overlap Sphl site 16-2 (q and q' must fall mainly within the Sphl-E fragment, since they are larger than the Sphl-H fragment). However, differences in molecular mass standards and electrophoresis conditions mean that we are not confident that q and q' differ in size. It is not excluded that these RNA species (o, p, q) are relatively stable products from processing of the major late mRNA.

RNA dot-blot analysis of the accumulation of mRNA corresponding to selected regions of ϕC31

To extend some aspects of the transcription analysis described above, comparable RNA samples were made from an induced 7 h culture of an independently prepared ϕC31 cts1 lysogen, TMC1. These were analysed by RNA dot-blot experiments (Fig. 4). Selected regions of ϕC31 c1 DNA were radiolabelled for use as probes, as follows (see Fig. 1): the cos region (probe C); a region just to the right of cos (probe D); the Sphl-I fragment immediately to the left of the c gene (probe E); a fragment internal to the c gene itself (probe A); and a fragment located about 7 kb to the right of the c gene (probe B). RNA samples were isolated 5, 10, 15 and 20 min after the start of the 5 min heat induction period.
These experiments showed that c gene transcription was high at 5 min, and was already declining by 10 min (Fig. 4, probe A). In contrast, the other probe for early transcription (probe B) revealed little transcription at 5 min, but a maximum at 10 min. This demonstrated the occurrence of two classes – 'immediate' and 'delayed' – of early transcript. Late transcripts, detected with probes C and D, were increasing by 10 min and continued to accumulate markedly through the later time points. Probe E, the SphI-I fragment, revealed marked increases in transcription at 10 min and 15 min, and little further increase. The pattern of transcription in this region was not clear in the S1 mapping experiments, in which the SphI-I fragment was used only in combination with other larger fragments, but it seems possible that the 1-6 kb and/or 0-8 kb transcripts detected at 10 min with the SphI-[F + I] probe in Fig. 3 may correspond to the signal detected with probe E and to the early transcript traversing part or all of SphI-I that was reported by Smith & Owen (1991).

**The effect of culture age on heat induction of a φC31 lysogen**

For biochemical studies of the lytic cycle of φC31, it would be convenient to induce cultures relatively late in growth to increase the quantities of material readily available for analysis. However, previous results (Rodríguez et al., 1986; Sinclair, 1987) showed that productive induction was obtained only for the few hours after germ tube emergence. By 10–12 h, yields of φC31 after induction were negligible. This corresponded approximately with the cessation of rapid growth. It was important to establish whether this failure in induction was due to a block early in the lytic cycle, preventing the normal course of φC31 transcription, or to a late block, perhaps affecting lysis of the host and release of progeny virions. To examine this question, suitable sets of RNA samples were analysed by dot-blotting using the probes described in the preceding section. The RNA samples were made 20 min after the start of induction, from TMC1 cultures aged 7, 10, 15, 20 and 40 h. Rapid growth occurred until about 14 h, after which little further increase in optical density was observed. Control RNA samples were made from uninduced mycelium of the same age from the same culture vessel. In interpreting the data, it is necessary to compare results from the induced and control cultures, because the controls themselves showed an interesting progression with time; it should also be remembered that by 20 min after induction in the earlier experiments, the early transcripts had declined significantly, while the late transcripts were...
accumulating rapidly (the 7 h samples of Fig. 4 illustrate this).

After 10 h and 15 h of culture, there seemed to be a slowing in the progression of induced lytic transcription, in that 'delayed early' mRNA was more abundant 20 min after induction than in 7 h samples at the same interval after induction (Fig. 5, probe B). By 15 h of culture, two of the late transcripts were greatly reduced in abundance 20 min after induction, as if late transcription were either delayed, or absent altogether (Fig. 5, probes D and E). By 20 h, there was no evidence at all of heat induction of \( \phi C31 \) early or late transcription over background spontaneous events.

By 10 h, the level of \( c \) gene transcripts had increased markedly in the uninduced control culture, and no significant further increment was observed in response to induction (Fig. 5, probe A). In a very old culture (40 h), the \( c \) gene transcripts were virtually absent with or without induction (Fig. 5, probe A). Surprisingly, transcripts corresponding to the \( cos \) region (Fig. 5, probe C) were also more abundant in older uninduced cultures, in this case being present even at 40 h. This may indicate a specialized stationary-phase mode of transcription, or an increased stability of this part of the late mRNA. The possibility of fortuitous hybridization of the \( cos \) probe to host RNA was ruled out by the absence of any signal with RNA from an equivalent non-lysogenic culture.

In these experiments, RNA was extracted only up to 20 min after the start of induction. To investigate further the apparent delay in transcription in older cultures,
RNA was extracted from a 19 h culture of strain 01 both before induction and at 35, 65 and 95 min after the start of induction, and analysed by type A S1 mapping as described earlier (Fig. 6). Transcription in the c gene region (SphI-G probe) was detectable in the uninduced 19 h culture, and in substantially increased amounts at all times after induction, giving the same S1 mapping pattern as at 7 h. No transcription of the remainder of the early region (probes SphI-[C+I] and EcoRI-E, -G and -C) nor any late transcription (probes SphI-[E+H], -D, -B and -[F+I]) was seen in the uninduced 19 h culture. Early mRNA was abundant at 35 min post-induction in the 19 h culture; but little was detectable after 65 min. Late mRNA was also detected at 35 min post-induction in the 19 h culture, though it was not very abundant (except the RNA corresponding to SphI-D). It was much less abundant by 65 min. In contrast to the dot-blot experiments with independent RNA preparations, no significant transcription in the cos region (probe SphI-[E+H]) was detected in this 19 h culture before induction. This disparity might be due to the lower sensitivity of the S1 mapping experiments and their inability to detect very short transcripts in the experimental conditions used.

In summary, these results all suggest that, in older cultures, transcription of the early genes to the right of the c region is greatly delayed, and there is a consequent reduction in the extent of late transcription. This reduction was less marked for the SphI-D region, perhaps indicating that RNA for this region is relatively stable.

A major φC31 virion protein is readily detectable in a young culture late in the lytic cycle, but is not made in an older culture after induction.

The relative deficiency of late mRNA synthesis observed after induction of old cultures of φC31 cts1 lysogens suggested that there should be a deficiency of virion...
structural proteins, the probable major products of the late genes. SDS-PAGE analysis of cell-free extracts of 7 h and 24 h cultures, taken at intervals after induction, revealed at least one apparently phage-specific protein that accumulated late in lytic development in the young culture (Fig. 7). This protein, of about 29-5 kDa, had the same mobility as the major protein detectable in purified φC31 virion preparations (Suárez et al., 1984). There was no detectable induction of this protein in the 24 h cultures, even 185 min after induction (Fig. 7). It is unlikely that the 29-5 kDa protein is a host heat-shock protein, since no protein of this size was reported in a survey of the heat-shock response in several Streptomyces spp. (Guglielma et al., 1991).

**φC31 DNA that accumulates late in the induction cycle is processed to linear monomers in young, but not old, cultures**

DNA was extracted from a 7 h culture of the 01 lysogen at different times after induction, then digested with EcoRI and subjected to agarose gel electrophoresis (Fig. 8). Faint traces of φC31-specific bands were detectable in the uninduced and 15 min post-induction samples,
possibly reflecting a low proportion of spontaneously induced cells in the population. Samples taken at 30 min or later showed conspicuous ϕC31-specific bands. The autonomous state of this DNA is revealed by the equimolar quantity of the EcoRI-CAM fragment in relation to other fragments. This fragment is interrupted in the prophage state because it contains the attP site involved in crossing-over with the host chromosome. During replication, the phage DNA is expected to be circular and/or concatameric, whereas packaging into virion particles is expected to involve cleavage at the cos site into linear monomers. This cleavage results in the conversion of a 12.5 kb EcoRI fragment into the 9.6 kb (B) and 2.96 kb (F) fragments that make up the left and right ends of DNA extracted from virions. The latter fragments were fairly abundant in the DNA samples taken late in the induction cycle (55 min onwards), though barely detectable at 30 min, when the phage DNA was less abundant.

In samples taken from an older culture (15 h), ϕC31 DNA was amplified even in the uninduced control, and little, if any, further amplification could be seen at various times after induction. The EcoRI-C fragment was fully represented, indicating that the DNA was extrachromosomal, but the EcoRI-B or -F fragments were not detectable (even after heating and rapid cooling of the DNA to separate any hydrogen-bonded cohesive ends). Thus, none of the DNA was packaged. The low level (if any) of replication after induction, and the absence of packaging, are compatible with the relatively low levels of late transcription and the absence of a major virion protein, as discussed in the previous section.

The ability of ϕC31 to inhibit transcription of host rRNA genes is lost with increasing culture age

Induction of a ϕC31 prophage leads to inhibition of host rRNA transcription (Rodriguez et al., 1986; Clayton & Bibb, 1990). The effect of culture age on this inhibition was analysed by S1 mapping of transcripts from the rRNA gene cluster using the TMC1 RNA samples, isolated at different stages of culture, with and without prophage induction. A dramatic reduction in rRNA transcription occurred 20 min after induction of a 7 h culture (Fig. 9); however, inhibition declined with increasing culture age, and by 20 h the S1 mapping profiles with or without prophage induction were extremely similar. The ability to inhibit transcription from host rRNA promoters appears to be an early phage function, beginning about 5 min after induction (Clayton & Bibb, 1990); in cultures older than 10 h, either this phage early function is delayed beyond 20 min or it is unable to take place at all.

Discussion

Four temporal classes of ϕC31 mRNA have now been described. Other data (Sinclair & Bibb, 1989; Smith & Owen, 1991) showed that the c gene is transcribed during uninduced growth of lysogens ('prophage transcription'). Increased transcript accumulation for the c region immediately after repressor inactivation, reported by Sinclair & Bibb (1989) and Smith & Owen (1991), was also seen in the present work, and defines an 'immediate early' phase of lytic cycle transcription. Probably, this is a simple consequence of negative autoregulation of c transcription. Higher abundance of c gene transcripts in older uninduced cultures of a ctsl lysogen may indicate a more rapid turnover of the repressor in cultures entering stationary phase, reducing the effective repressor concentration, and allowing increased c transcription to occur. It would be interesting to know whether c gene transcription in a wild-type (c+) lysogen showed the same dependence on culture age.

'Delayed early' transcription, reaching a peak of RNA accumulation 10 min after induction, was detected by dot-blot analysis (Fig. 4) in a region about 6 kb to the right of the c gene. The distinction between immediate early and delayed early transcription was not made for the remainder of the early region. Smith & Owen (1991) also reported an early transcript reading leftwards through SphI site 8-3, which may be related to one or both of the faint early transcripts that we detected with the SphI-[F+I] probe (Fig. 3). Overall, the early region extends between a position close to SphI site 8-2 and an undefined position in the EcoRI-C fragment—a length of 15–17 kb. Genetic evidence, from studies with temperature-sensitive mutants, had already indicated that an early gene was to the right of c, but most ts mutants (among those that were sufficiently stable to allow analysis) defined late genes which mapped to the left of the c region (Chinenova & Lomovskaya, 1975; Lomovskaya et al., 1980; Sladkova & Rebentish, 1980). Detailed deletion analyses have revealed only two extensive regions of ϕC31 that are dispensable for plaque formation: the c region, and a region of about 8 kb including restriction sites 13–19. Thus, about 10 kb of DNA between these regions fulfils essential early functions. It is not obvious why stable ts mutants in this early region should be relatively rare.

The low-resolution S1 mapping of this region does not suggest that a single major early mRNA is sequentially transcribed and translated across the whole region, though it is not excluded that such a primary transcript may be formed and very rapidly processed into the series of relatively short RNA species detected both in our work and in a more detailed transcription analysis of part of the early region (SphI site 8-6 to EcoRI site 11) carried
out in parallel by Ingham & Smith (1992). (We note that the latter experiments detected additional transcripts to those that we observed.) The apparent pattern of early transcription in φC31 contrasts with observations on coliphage λ, in which only two major promoters (P1 and P2) are used for early transcription. Multiple RNA species are generated from these λ promoters mainly by control of termination, though processing close to the 5' end of the λ leftward transcript also occurs (Friedman & Gottesman, 1983). The essential early region of φC31 is currently being subjected to further detailed transcription analysis and DNA sequencing (C. Ingham & M. C. M. Smith, personal communication; C. J. Bruton, personal communication; G. J. P. Murphy, N. Hartley & K. F. Chater, unpublished).

The right-hand dispensable region has also proved to contain early genes. This is consistent with results indicating that at least two different functions that interact with host-controlled restriction-modification systems are in this region (Chater, 1986). Our S1 mapping analysis in this region is incomplete, because the ctsl prophage carries a deletion (AM) of 1.72 kb from the EcoRI-C fragment (Chater et al., 1981).

Late transcription extends over an approximately 18 kb segment including most of the Sphl-F, B, D, H and E fragments. Possibly, there is a single major mRNA covering all this region even including cos, though it is equally possible that several fairly large transcription units are involved. A single RNA species of this length would probably take as much as 5–10 min to transcribe (Bremer & Yuan, 1968). Not surprisingly, considering that φC31 mRNA has a half-life of only 1–1.5 min (Rodriguez et al., 1986), only degraded molecules were detectable. Some apparently more stable species are generated during degradation, giving perceptible striations in some of the tracks in S1 mapping. Transcripts of sequence does not give any clues about possible gene generation during degradation, giving perceptible striations covering all this region even including cos has been sequenced (Kobler et al., 1991), and unpublished sequence features.

The construction of integrating vectors carrying the φC31 att region (Kuhstoss et al., 1991), and unpublished nucleotide sequencing studies by S. Kuhstoss (personal communication) and H. Rausch (personal communication), suggest that attP, together with integration and possibly excision functions, is in the Sphl-E fragment. Possibly, the transcripts q and/or p specify components of the site-specific recombination system that would be needed for prophage excision. If so, it is surprising that these transcripts are abundant late in infection.

Cultures of a heat-inducible lysogen could be induced to release φC31 only during the early stages of rapid growth (Rodriguez et al., 1986). This effect is also seen at the transcription level. As culture age increases, early transcription seems to be delayed, and early transcripts are abundant for much longer (as if early transcription were not properly shut off). Both late transcription and DNA synthesis are greatly reduced, and at least one — perhaps all — of the major late proteins is not made. There is little or no production of mature φC31 particles inside the cells, as judged by the absence of cleavage at the cos site of amplified φC31 DNA. The deficiencies in the induction cycle in old cultures are mirrored by observations on the shut-off of host rRNA gene transcription: in young cultures, induction is rapidly followed by rRNA shut-off, but in older cultures, heat treatment has no effect. These results suggest changes in host factor abundance at different stages of growth, such that either required host factors decrease in amount, or inhibitory host factors increase, as culture age increases. In any case, it is clear that biochemical studies of events during the lytic cycle can at present be done only with young, rapidly growing cultures.

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References


Transcription of Streptomyces phage 4C31


