Polypeptide Synthesis during Lytic Induction of Phage 11 of
Staphylococcus aureus

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SUMMARY

Staphylococcus aureus phage 11 was induced to replicate by treatment of lysogens with mitomycin C. Lysis was complete in 80 min at 37 °C in complete synthetic medium. Labelling with L-[3H]lysine showed a stimulation of protein synthesis during phage replication, followed after 30 min by shut-down of host protein synthesis while virion protein synthesis continued. The synthesis of approximately 16 virus-specific proteins was detected during replication, with seven of these recovered in purified virions after lysis. The remainder appeared to be synthesized earlier in phage replication and thus may represent precursors of virion proteins, or regulatory proteins, or enzymes associated with phage replication. Protein synthesis during replication of three suppressible morphological (head, tail) mutants of phage 11 did not differ significantly from that seen in the wild-type lysogen, indicating that the mutation in each case affected a protein whose synthesis was not detectable, or which was synthesized but did not fulfil its role in virion maturation or assembly. However, in a suppressible 'early' mutant which did not lyse when treated with mitomycin C, the synthesis of the 'late' (virion) proteins (with one exception) did not occur. 'Early' proteins were apparently made normally in this mutant, and there was no shut-down of host protein synthesis. This mutant phage presumably encodes a defective 'early' protein involved in the regulation of replication, or a key precursor polypeptide of 'late' protein synthesis. The morphological mutants provided a means to analyse and tentatively to allocate six of the virion proteins to head or tail/baseplate structures.

INTRODUCTION

Bacteriophages of Staphylococcus aureus appear to be modulators of chromosomal gene expression in this important opportunistic pathogen. Thus the synthesis of certain enzymes thought to be implicated in determining virulence in this bacterium is subject to lysogenic conversion (de Waart et al., 1962; Duval-Iflah et al., 1977; Jollick, 1972; Kondo, 1973; Kondo & Fujise, 1977; Mason & Allen, 1975; Rosendal et al., 1964).

Lysogenic conversion could be a consequence of virulence genes encoded by the lysogenizing phage, similar to what is known for the diphtheria toxin gene and β-corynephages (Groman, 1984). Alternatively, the phage may act to silence or to activate host chromosomal genes as a result, respectively, of insertion of prophage into the host structural gene concerned, or insertion near the gene so that promoters or other regulatory sequences in the prophage affect the expression of host genes. Recent studies of aureophages have revealed that a phage gene (enterotoxin A; Betley & Mekalanos, 1985) and insertional inactivation (lipase; Lee & Iandolo, 1985) are implicated in aureophage conversions of the host bacterium.

Little is known of the biochemical aspects of aureophage replication, or of the regulatory processes that determine the lytic or lysogenic options for these mostly temperate phages. The option taken by infecting phage between lysogeny and lysis may be important in determining the pathogenic potential of different strains of staphylococci. It was for this reason that, as a first step towards characterizing macromolecular synthesis and assembly during aureophage...
replication, we examined the effects of prophage induction on protein synthesis in *S. aureus* lysogens carrying phage 11, the auerophage about which most is known in genetic and molecular terms. We have included in this analysis representative members of the collection of suppressible mutants of phage 11 originally isolated and described by Kretschmer & Egan (1975).

**METHODS**

*Phage mutants and bacterial strains.* The strains of *S. aureus* and phage 11 used in this study are listed in Table 1.

*Cultures and media.* Overnight cultures of bacteria, grown at 37 °C in trypticase soy broth (TSB; BBL, Cockeysville, Md., U.S.A.), were used to inoculate fresh media with a 1/100 dilution (approx. 10^7 cells/ml). Phage lysates were obtained by treating exponentially growing cultures (approx. 10^8 cells/ml) of the appropriate lysogen with 2 μg/ml mitomycin C. After lysis, cell debris was removed by centrifugation at 10000 g for 10 min. The phage was concentrated by precipitation with polyethylene glycol as described previously (Stewart et al., 1985), and resuspended in synthetic medium (P. A. Pattee, personal communication) with L-lysine omitted. This procedure yielded stocks of 10^9 to 10^11 p.f.u./ml.

Phage were assayed in soft trypticase soy agar (TSA) overlays supplemented with 4 mm-CaCl_2_, using S2 (8325-4 Su^-) or S66 (8325-4 Su^+) as indicator strains. The plates were incubated overnight at 30 °C.

*Radioactive labelling, electrophoresis and autoradiography of proteins.* Synthetic medium was inoculated with approx. 10^6 cells/ml of an overnight TSB culture of the lysogen, and incubated at 37 °C on a rotary shaker at 200 r.p.m. until A_500 reached 0.1 (approx. 10^8 cells/ml). At this point 2 μg/ml mitomycin C was added and incubation was continued until lysis occurred and the cultures cleared.

Pulse labelling was carried out by adding 20 to 50 μCi (40 Ci/mmol) of L-[4,5-^3H]lysine (Amersham) to 10 ml samples of culture at intervals after induction. Incubation of samples was continued with shaking at 37 °C for the required period. The samples were then held at 0 °C until processed.

The labelled samples were centrifuged for 10 min at 12000 g to pellet cells, which were then washed twice in cold TES (30 mM-Tris–HCl, 5 μM-EDTA, 83 mM-NaCl pH 8.0) and resuspended in 50 μl TES containing 20% (w/v) sucrose (freshly prepared). Lysostaphin was then added to a concentration of 200 μg/ml and the cells were incubated at 37 °C for 20 min to digest cell walls. Five μl of 20% SDS was then added to lyse the cells, and the samples were stored at −20 °C until required for electrophoresis.

In some experiments the culture supernatant was examined for the presence of virions or virion substructures by centrifuging 5 ml for 90 min at 150000 g. The pellets were resuspended in 30 μl of 0.1% SDS and stored at −20 °C.

Polypeptides were separated by SDS–polyacrylamide gel electrophoresis as described by Laemmli (1970). Lysed cell or virion suspensions in SDS and mercaptoethanol were heated to 100 °C for 5 min and 20 to 30 μl was applied to 0.8 mm thick slab gels (5% acrylamide in stacking gel and 12% acrylamide in resolving gel).

For identification of protein bands of mature phage 11, samples of a phage 11 preparation purified on a CsCl gradient (Lee & Stewart, 1985), and molecular weight standards were also electrophoresed.

After electrophoresis, polypeptides were visualized by staining with Coomassie Brilliant Blue R. After destaining, the gel was impregnated with 2,5-diphenyloxazole and dried before autoradiography (Bonner & Laskey, 1974). X-ray film was exposed in contact with the dried gels for 7 to 12 days.

*Isolation of S2 lysogens of phage 11 sus mutants.* Phage 11 suppressible (sus) mutants were induced with mitomycin C from their Su^+ lysogens (Table 1), as described previously, and used to infect the non-lysogenic Su^- host S2 at high m.o.i. Lysis of S2 infected in this way was obtained with all sus mutants except susA4, whose lysogen construction is described separately below. Residual viable cells from these infections were allowed to grow out by further incubation of the lysates at 37 °C (4 to 20 h) until they appeared turbid. The lysates were then streaked onto TSA, and single colonies were selected and tested for the presence of mutant prophage. Such strains were immune to superinfection with phage 11 and could be induced to lyse with mitomycin C but yielded less than 1/2 p.f.u./ml of a control phage 11 wild-type lysogen (S80) induced in the same way. As a further check, lysates from mutant phage inductions were examined for the presence of virion substructures (Kretschmer & Egan, 1975) by electron microscopy (Lee & Stewart, 1985).

*Isolation of susA4 lysogen.* The non-lysogenic host strain S2 (Su^-) was not lysed by infection with phage 11 susA4, which appears to carry a mutation in an early gene (Kretschmer & Egan, 1975). For this reason it was impossible to determine directly whether infection and/or lysogenization had occurred. Preliminary tests indicated that the fraction of cells lysogenized by phage was probably small, so the following colony screening method was used.

After infection of S2 (as described previously) with phage susA4, the culture was incubated for 5 h. The culture was then spread on TSA to give approx. 10000 colonies per plate. Non-lysogenic S2 and lysogenic S80 cultures were also plated as negative and positive controls, respectively. Colony replicas were lifted from the plates, using nylon filters (Zeta-Probe from Bio-Rad), and cells lysed by incubating the nylon filters on 3 MM filter paper saturated with 20 μg/ml lysostaphin in TE (10 mM-Tris–HCl, 1 mm-EDTA pH 8) at 37 °C for 2 h. Cell DNA was denatured and fixed to the nylon membranes by placing the membranes on 3 MM filter paper saturated with 0.5 M-
Table 1. Bacterial and phage strains

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Prophage and relevant genotype*</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>S2</td>
<td>8325-4 Su&quot; (non-suppressor host)</td>
<td>Dr J. B. Egan†</td>
</tr>
<tr>
<td>S80</td>
<td>S2(φ11)</td>
<td>Dr J. B. Egan</td>
</tr>
<tr>
<td>S66</td>
<td>8325-4 Su&quot; (suppressor host)</td>
<td>Dr J. B. Egan</td>
</tr>
<tr>
<td>S91</td>
<td>S66(φ11 susA4)</td>
<td>Dr J. B. Egan</td>
</tr>
<tr>
<td>S94</td>
<td>S66(φ11 susM28)</td>
<td>Dr J. B. Egan</td>
</tr>
<tr>
<td>S95</td>
<td>S66(φ11 susO43)</td>
<td>Dr J. B. Egan</td>
</tr>
<tr>
<td>S96</td>
<td>S66(φ11 susP68)</td>
<td>Dr J. B. Egan</td>
</tr>
<tr>
<td>RC-A</td>
<td>S2(φ11 susA4)</td>
<td>This study</td>
</tr>
<tr>
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</tr>
<tr>
<td>RC-O</td>
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<td>This study</td>
</tr>
<tr>
<td>RC-P</td>
<td>S2(φ11 susP68)</td>
<td>This study</td>
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* Designations as described in Kretschmer & Egan (1975).
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NaOH, 0.5 M-NaCl at room temperature for 5 min. The filters were then washed successively for 10 min in 0.5 M-Tris-HCl pH 7.4, 2 M-NaCl and twice in 2 × SSC (0.3 M-NaCl, 30 mM-trisodium citrate pH 7.0). The washed membranes were then probed with 32P-labelled phage 11 DNA using the methods described by Stewart et al. (1985).

Colonies harbouring a susA4 prophage were identified from exposures of the filters to X-ray film, and after purification were checked for immunity to infection by phage 11. These susA4 lysogens did not lyse when treated with mitomycin C.

RESULTS

Induction of phage 11 from strain S80

Mitomycin C (2 μg/ml) induced wild-type phage 11 from the lysogen S80 in synthetic medium, giving maximal lysis in approx. 80 min. Features of this induction are shown in Fig. 1. The number of viable bacteria decreased rapidly after addition of mitomycin C, falling five orders of magnitude in 40 min. During this time phage virions were released from cells into the culture medium, but the total phage titre (free phage plus infected cells which lyse after plating) remained constant. At approx. 45 min after the addition of mitomycin C, both total and free viable phage counts rapidly increased one-hundred-fold, indicating a burst size of this magnitude. At the time of the burst the turbidity of the culture, which had been rising, began to fall and continued to fall for the remainder of the experiment.

Time course of polypeptide synthesis during induction of phage 11

Fig. 2, 3 and 4 show the pattern of polypeptide synthesis in S80 cells after addition of mitomycin C. Protein synthesis increased within 15 min of the addition of mitomycin C to non-lysogenic cells, and slightly later in the lysogen (Fig. 2). Protein synthesis continued to increase in the non-lysogen and in the period 30 to 40 min after addition of mitomycin C had reached approximately six times its initial level, as shown by assaying by scintillation spectrometry the isotope in lanes cut from the dry gel.

In the induced lysogen, however, initial stimulus of protein synthesis was followed by a shutdown of host protein synthesis beginning after approx. 25 min, and becoming most evident at 30 and 40 min (Fig. 3). The synthesis of polypeptides associated with the phage lytic cycle continued, and by 40 min these species predominated. At 40 to 50 min after induction, protein synthesis had fallen to one-third of the initial level.

Fig. 4 is a schematic reconstruction, taken from multiple experiments, of polypeptide synthesis during induction of the wild-type lysogen S80. Approximately seven of the polypeptides associated with phage replication were synthesized in detectable levels for a short time only, generally in the period from 20 to 50 min after induction, though the periods of synthesis of different proteins differed. One transiently synthesized polypeptide (65K) was apparently incorporated as a minor component into the mature virion, but synthesis of the major
virion components generally began approx. 30 min after induction, and became more obvious as host protein synthesis was shut down.

**Protein synthesis during induction of phage 11 sus mutants**

Mitomycin C was used to induce phage 11 sus mutants M28, O43 and P68 from the non-suppressor lysogens as described previously, leading to lysis in approx. 90 min. Samples of the lysate were examined by electron microscopy, which showed the near absence of virion heads from susM28 and susO43, and of tails from susP68; this corresponds to the results described for these mutants by Kretscher & Egan (1975).

Despite these morphological differences, the time course of protein synthesis of these three mutants during induction was identical to that of the wild-type. This was the case both for labelled polypeptides within cells and for labelled polypeptides released into the medium during induction, indicating that all the major virus-encoded proteins were synthesized in normal amounts, including early and late proteins. In addition, host protein synthesis was shut down, as in the wild-type lysogen, 30 to 40 min after induction. Thus, virion structural proteins were apparently synthesized normally but could not be assembled to form mature virions in the case of these mutants.
Protein synthesis during aureophage induction

Fig. 2. Protein synthesis in lysogen S80 during induction of phage 11, and in mock-induced non-lysogen S2. Exponentially growing cultures of S2 and S80 were treated with 2 µg/ml mitomycin C at time zero and 10 ml samples of the culture were pulse-labelled with 40 µCi [3H]lysine at intervals after the addition of mitomycin C. Cells were pelleted by centrifugation and virions and particulate matter in the supernatant were concentrated by ultracentrifugation. Samples were lysed and electrophoresed in 12% acrylamide gels. Gels were dried and exposed to X-ray film for 11 days. Lanes 1 to 3 contain cell samples (one-fifth of total) from the S2 (non-lysogen) control; lanes 4 to 10 are cell samples (one-fifth of total) from the induced lysogen S80; lanes 11 to 13 are pelletable material (half of total) from the supernatant of the S80 induction. Times of labelling after addition of mitomycin C were as follows: lanes 2 and 5, 0 to 15 min; lanes 3 and 6, 15 to 30 min; lane 7, 30 to 40 min; lanes 8 and 11, 40 to 50 min; lanes 9 and 12, 50 to 60 min; lanes 10 and 13, 60 to 70 min. Lanes 1 and 4 are controls of S2 and S80, in which cells were labelled for 15 min in the absence of mitomycin C, then lysed and electrophoresed. The marker bars at the right show the positions and sizes of the major virion proteins.

By contrast, treatment of the susA4 lysogen with mitomycin C showed a pattern of protein synthesis different from that of the wild-type lysogen, and did not lead to lysis. Fig. 5 is a reconstruction of polypeptide synthesis (from multiple experiments) showing that whereas the synthesis of most virus-specific proteins, particularly the early ones, occurred normally, the synthesis of others, particularly the late (virion) proteins, did not (see Fig. 4). One notable exception was the 70K structural protein, whose synthesis was similar to that seen after induction of the wild-type lysogen. The absence of shut-down of host protein synthesis, and the continued synthesis of most of the early viral proteins, are also notable.

Correlation of polypeptides with virion substructures

Although previous attempts to dissociate phage 11 into head, tail and baseplate were unsuccessful (Lee & Stewart, 1985), the perturbation of virion assembly seen in the suppressible mutants examined in the present study provided an opportunity to allocate polypeptides to virion substructures.

Accordingly, lysogens of phages susM28, susO43, susP68 and wild-type were induced, and virions and virion substructures were precipitated with polyethylene glycol and purified by
Fig. 3. Protein synthesis in lysogen S80 during induction of phage 11. Cultures of S80 were induced and 10 ml samples were labelled with 50 μCi [3H]lysine as described in Fig. 2 (sample in lane 2 was labelled with 18 μCi). Cell pellets were lysed and electrophoresed, and the gels dried and exposed to X-ray film for 10 days. Periods of labelling were as follows (time after addition of mitomycin C): lane 1, -5 to 0 min; lane 2, 2 to 17 min; lane 3, 16 to 21 min; lane 4, 21 to 26 min; lane 5, 26 to 31 min; lane 6, 31 to 36 min; lane 7, 36 to 41 min.

centrifugation on CsCl gradients. The virions from susM28 and susO43 lysates, although at a much lower yield, banded at the same density as wild-type virions; electron microscopy showed that they contained mostly intact structures. It seems probable that these intact virions result from the leakiness of the mutations, and that free tails (which would have a lower density than intact virions) were present at the top of the gradient, unresolved from cellular debris. Virions from a susP68 lysate banded at a higher density than wild-type virions, and electron microscopic analysis indicated that this band contained a high proportion of free heads. The proteins from the banded phage were resolved by electrophoresis, and from the relative intensity of staining of the bands (data not shown) it was possible to allocate the 65K and 33K polypeptides (and the 28K species tentatively) to the phage head, and the 31K (and the 72K and 70K polypeptides tentatively) to the tail (or baseplate).

DISCUSSION

The lytic cycle of phage 11 has been shown in this study to involve a gradual shut-down of host protein synthesis and a shift to phage-directed protein synthesis, with considerable overlap of the two. This slow transition in types of proteins synthesized during the lytic cycle of phage 11 is
Protein synthesis during aureophage induction

(a) (b) (c)

Fig. 4. Schematic reconstruction of time course of protein synthesis during induction of phage 11 from lysogen S80. (a) This diagram is derived from a series of experiments similar to those shown in Fig. 2 and 3. Thickness of lines indicates relative amount of isotope incorporation. Proteins which cannot be distinguished from host proteins (seen in mock inductions of S2) are indicated by broken lines; probable virus-associated proteins are shown as solid lines. (b) Major and minor proteins released into the culture supernatant and pelleted by ultracentrifugation after labelling for 50 to 60 min. (c) Labelled proteins (50 to 60 min) from virions purified on a CsCl gradient.

Comparable with that seen in the lytic cycles of other temperate phages, for example Bacillus subtilis phages (Hemphill & Whiteley, 1975; Hawley et al., 1973) and coliphage lambda (Buchwald et al., 1970; Terzi & Levinthal, 1967).

In total, the synthesis of approximately 16 virus-associated proteins could be detected during the phage 11 lytic cycle. These can be divided into two classes, although there is some overlap: those proteins whose synthesis is clearly detected between about 20 and 50 min after induction but then decreases (early proteins); and those whose synthesis is clearly detected from approximately 30 min after induction until cell lysis (late proteins). The late proteins are the more easily detected, probably due to greater amounts synthesized from newly replicated phage DNA, and appear to be predominantly virion proteins. However, one early protein (65K) appears to be a virion protein. A similar phenomenon occurs during phage 29 replication in B. subtilis (Pene et al., 1973).

With the exception of susA4, the suppressor-sensitive mutants used in this study gave patterns of polypeptide synthesis identical to those of the wild-type phage following induction of the suppressor-negative host. This suggests that either the affected proteins were minor ones and changes in their synthesis were not detected, or the mutations were missense rather than
Fig. 5. Schematic reconstruction of time course of protein synthesis in phage 11 susA4 lysogen (suppressor negative) treated with mitomycin C. (a) This diagram is derived from a series of experiments similar to that shown in Fig. 3. Broken and full lines indicate weak and strong isotopic labelling of protein from cell lysates. Arrowheads indicate proteins whose synthesis responds to mitomycin C treatment in both the wild-type and in the susA4 mutant. Bars indicate proteins whose synthesis does not respond to mitomycin C in the mutant as it does in the wild-type. (b) Positions of the major proteins of the mature virion.

nonsense. Genetic evidence is consistent with the latter. Thus, J. B. Egan (personal communication) reports that in the search for suppressor mutants of phage 11, instances occurred in which (i) mutant phage grew on the Su− host at 30 °C but not at 37 °C (suggesting that a thermolabile protein product was present); (ii) mutant phage grew on Su− but not on Su+ strains, again suggesting that an intact protein product was made during mutant phage infections. These two observations could be explained as follows. First, a structural gene containing a missense mutation codes for an incorrect amino acid at that codon, leading to a temperature-dependent change in the three-dimensional structure of the resulting full-length protein. Second, a complementary mutation in a tRNA gene of an Su+ host may result in suppression of the phage mutation, but if such a mutant tRNA species is abundant it may also alter the translation of correct codons at other loci, which could be lethal to phage propagated in Su+ strains. In the case of susA4, the synthesis of early proteins appeared to be normal (including the 65K polypeptide which appears to be a virion component), but the synthesis of late proteins (predominantly virion proteins) was not. This suggests that the mutation is in a gene for a minor early protein concerned either with the timing of gene expression during phage replication, or with virion assembly, the failure of which is fed back to shut down the synthesis of virion polypeptides.

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