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The lysis function of RNA bacteriophage Qβ is mediated by the maturation (A_2) protein

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Complete or partial cDNA sequences of the RNA bacteriophage Qβ were cloned in plasmids under the control of the λP_L promoter to allow regulated expression in *Escherichia coli* harbouring the gene for the temperature-sensitive λCI857 repressor. Induction of the complete Qβ sequence leads to a 100-fold increase in phage production, accompanied by cell lysis. Induction of the 5′-terminal sequence containing the intact maturation protein (A_2) cistron also causes cell lysis. Alterations of the A_2 cistron, leading to proteins either devoid of ~20% of the C-terminal region or of six internal amino acids, abolish the lysis function. Expression of other cistrons in addition to the A_2 cistron does not enhance host lysis. Thus, in Qβ, the A_2 protein, in addition to its functions as maturation protein, appears to trigger cell lysis. This contrasts with the situation in the distantly related group I RNA phages such as f2 and MS2 where a small lysis polypeptide is coded for by a region overlapping the end of the coat gene and the beginning of the replicase gene.

Key words: bacteriophage Qβ/cloned sequences/evolution/lysis function/maturation protein

Introduction

Among the four groups of RNA coliphages, representatives of group I (f_2, MS2, R17, fr) and of group III (Qβ) have been studied most intensively (for reviews, see Zinder, 1975; Fiers, 1979). The group I phages served as models to study many aspects of protein synthesis as well as viral regulatory processes, and MS2 was the first virus of which the entire nucleotide sequence of the genome was established (Fiers *et al.*, 1976). Qβ is a suitable model for studying viral RNA replication *in vitro*, and its entire genome sequence has been cloned in plasmids as one cDNA copy (Taniguchi *et al.*, 1978). The nucleotide sequences of the genomes of the two phage groups are very different: no cross-hybridization is detectable and only very short stretches of homology were apparent earlier in the limited regions available for comparison (reviewed by Weissmann *et al.*, 1973). However, from the sequence determination of the entire Qβ cDNA copy, extended regions of homology in the replicase β-subunit with that of MS2 became obvious (Mekler, 1981), definitely implying that the genomes of both phage groups are derived from a common ancestral sequence.

Accordingly, the gene organization of the two phage groups is similar (Figure 1). The main genes coding for the maturation protein (A_2 in Qβ, A in MS2), the coat protein and the replicase β-subunit have similar lengths and are arranged in the same order. However, minor genes are different: in Qβ the readthrough protein A_1, a necessary component of the virus particle (Hofstetter *et al.*, 1974) is formed when ribosomes occasionally readthrough a UGA termination codon (Weiner and Weber, 1971) at position 1743 (Mekler, 1981).

In group I phages, a small polypeptide triggering host cell lysis is coded for by a minor gene overlapping the end of the coat protein gene and the beginning of the replicase β-subunit gene (Model *et al.*, 1979; Beremand and Blumenthal, 1979; Atkins *et al.*, 1979). This polypeptide is formed when ribosomes translating the coat gene occasionally switch the reading frame, terminate and reinitiate translation in the +1 frame (Kastelein *et al.*, 1982).

From its sequence, it became clear that Qβ cannot form a lysis polypeptide in an analogous fashion since the region between A_1 and the replicase gene contains termination codons in all three frames. To localize the region coding for the lysis protein of Qβ, we have cloned full-length and partial Qβ cDNA sequences in an expression plasmid (Remaut *et al.*, 1981) under the control of the λP_L promoter. The same vector has been used before for the expression of MS2 sequences (Remaut *et al.*, 1982; Kastelein *et al.*, 1982).

Results

Cloning of Qβ sequences under control of λP_L

Since expression of the whole cloned Qβ genome or some parts of it is lethal to the bacterial host, it was crucial to use a regulated expression system allowing a relatively tight repression. The λP_L promoter in the plasmid pPLc28 described by Remaut *et al.* (1981) is almost inactive at 28°C in *Escherichia coli* strains producing thermolabile λCI857 repressor, whereas shift-up to 42°C promotes immediate transcription at high rates. We modified the vector for our purposes by eliminating the *Pst*I site [but keeping the ampicillinase (ApR) gene functional], by replacing the *Acc*I by a *Pst*I site and by adding an *Xba*I site to the *Bam*HI recognition sequence (Figure 2A).

To create a series of Qβ sequences with either 5′ or 3′ deletions of different lengths, we first cloned the entire Qβ cDNA flanked by poly(A)-poly(T) tails (Taniguchi *et al.*, 1978) in both orientations into the *Hind*III site of pBR322. From the plasmid with the Qβ insert plus-strand in the counter-clockwise orientation, pBR-Qβ (*Xba-Hin*) was derived (Figure 2A) by replacement of one of the *Hind*III sites by an *Xba*I site. From the plasmid with the clockwise Qβ insert, an analogous plasmid was constructed containing a *Pst*I rather than an *Xba*I site [pBR-Qβ(*Hind-Pst*), not shown]. The former derivative was used to obtain a series of Qβ 3′ deletions adopting a subcloning method (Frischauf *et al.*, 1980), the latter derivative yielded a series of Qβ 5′ deletions. To express these partial Qβ sequences under λP_L control, they were ligated into the appropriately cleaved pPLc28*. Figure 2B shows a number of different Qβ cDNA regions used for insertion into the plasmid vector for expression.

To assay for the lysis function, cultures of *E. coli* strains harbouring various Qβ expression plasmids were heat-induced and the turbidity was monitored as described for the MS2 system (Kastelein *et al.*, 1982). Figure 3, panel A shows

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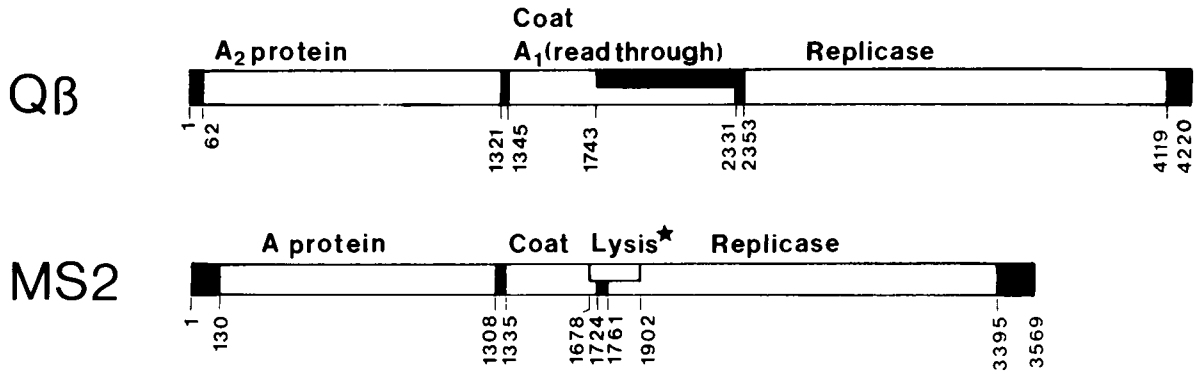

Fig. 1. Maps of the genomes of RNA bacteriphages Qβ and MS2. The white areas indicate translated regions. The nucleotide numbers at initiation and termination are from Mekler (1981) and Fiers *et al.* (1976). In phage Qβ the A_1 protein is produced by occasional readthrough of the termination codon UGA (position 1744) of the coat cistron. *The lysis protein in MS2 is read in the +1 frame relative to the reading frame of coat and replicase.

that with a vector containing the whole Qβ cDNA (pX4220, Figure 2B) the cells continue to grow for ~40 min after heat-induction whereupon the turbidity drops indicating cell lysis, and infectious Qβ phage particles are liberated. A phage titer of $\sim 10^{10}$ p.f.u./ml is obtained in such lysates whereas the total intra- and extracellular phage titer in uninduced cultures is about two orders of magnitude lower. [Note that full-length Qβ cDNA, even if inserted in a non-transcribed region of a plasmid, leads to formation of phage in a small percentage of the cultured *E. coli* cells (Taniguchi *et al.*, 1978). Reinfection by phage cannot occur in the F^- strains used.] In *E. coli* F^+ cells normally infected with Qβ phage, a very similar lysis pattern is observed. In this case the phage yield is $\sim 5 \times 10^{11}$ p.f.u./ml. In contrast, the expression vector without the Qβ DNA insert does not notably affect cell growth after heat induction.

Panel B shows the lysis pattern upon expression of partial Qβ sequences. Deletion of up to 2850 nucleotides from the 3′ end of Qβ DNA (pX1369) does not influence the lysis function whereas the removal of as little as 105 nucleotides from the 5′ end (p105P) completely abolishes lysis. It is, therefore, clear that the region comprising the A_2 cistron contains the information for the lysis function. Other genomic regions, particularly the coat-A_1 cistron, do not code for any lysis function: p105P and pE4220 which express coat protein (see Figure 4A showing the proteins labelled after heat-induction) and thus express presumably also A_1 (as a minor protein not detectable in the protein pattern) but not A_2, do not affect cell growth. Furthermore the presence of the coat-A_1 cistron in addition to A_2 (pX4220, pX2780, pX2605) does not enhance lysis.

To avoid any interaction from other genomic regions, an expression plasmid, pX1340, containing only the A_2 cistron without the strong ribosome binding site of the coat protein cistron, was constructed. This plasmid, as well as pX1888a, which contains similar Qβ sequences to pX1888 (Figure 2B), but does not express coat protein, causes particularly rapid lysis (Figure 3B). This property cannot be explained simply by particularly high production of lysis protein, since plasmids causing slower lysis, e.g., pX1782 also induce high amounts of A_2 protein (cf., Figure 4A; see discussion of the regulation of lysis).

Various small insertions and deletions were introduced into specific regions of the A_2 cistron in pX1340 to test whether the entire A_2 region is required for the lysis function. Figure 2C represents the various constructions and the proteins that are expected to be produced. In all these cases no lysis occurs in heat-induced cells although shortened proteins are produced and are stable. The gel electrophoresis pattern of total proteins labelled *in vivo* in heat-induced cells (Figure 4B) shows that, at least in the case of the two constructions specifying almost full-length A_2 proteins, (the '*Cla*Ifs' fill-up plasmid coding for an altered A_2 protein in which only the C-terminal part is substituted by a small region read in the +1 frame, and the '*Ava*I' deletion plasmid giving rise to a polypeptide which lacks only six amino acids in the middle region) the expected products are clearly visible. In the case of plasmids constructed to specify shorter polypeptides, the expected products were not discernible, probably because of coincident mobility in the gel with cellular proteins, or instability of the shorter polypeptides.

These experiments show that the entire A_2 protein must be synthesized to mediate the lysis function; synthesis of only parts of it or of a protein which is translated from this cistron in a different reading frame either over its full length or only over its C-terminal part is not sufficient. It is most likely that the entire A_2 protein acts to bring about lysis; it cannot be excluded, however, that the correct full-length protein has to be synthesized to form a structure which is then cleaved specifically to give rise to a shorter polypeptide active for cell lysis. Thus, it appears that the Qβ maturation protein, which is present in the Qβ phage particle in one copy (Weiner and Weber, 1971), and which is essential for infectivity, as shown by *in vitro* reconstruction experiments (Hofstetter *et al.*, 1974), fulfils in the virus infection cycle a further, and apparently unrelated, lysis function. It is obvious that for this additional function no association with virus particles is required.

To evaluate the involvement of host protein synthesis in the lysis process, *E. coli* cells infected normally with Qβ were treated with rifampicin to block RNA transcription and thus host protein formation. Figure 5 shows that the drug, present either only early or only late in the infection cycle, strongly inhibited cell lysis. Nevertheless, the formation of phage inside the cells is not inhibited even if the drug is continuously present from 5 min after infection: phage titers in cultures lysed artificially 2 h after infection were 5×10^{11} p.f.u./ml irrespective of the presence or absence of the drug. Since phage formation and infectivity are absolutely dependent on maturation protein (which is thus present in drug-treated cells, presumably in normal quantity) these experiments show that an even temporary block of host protein synthesis almost

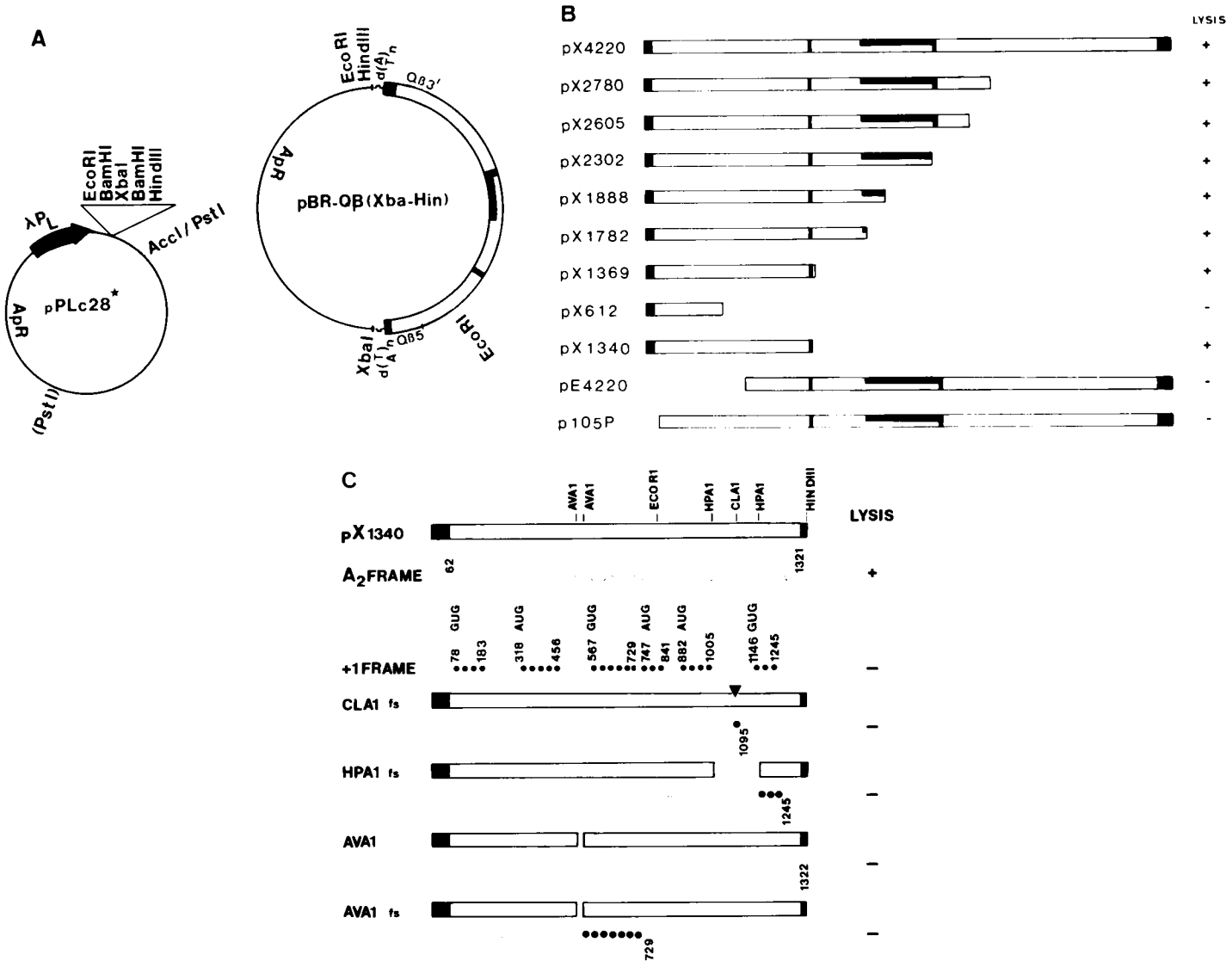

Fig. 2. Plasmids and DNA fragments used for expression of Qβ sequences. **A.** pPLc28* is the expression vector used for insertion of Qβ fragments; pBR-Qβ(*Xba-Hin*) is the parent plasmid used to obtain a series of Qβ sequences with 3′-terminal deletions. **B**: Qβ inserts expressed in the pPLc28* vector. X indicates the *Xba*I site [followed by an oligo(dT) region] preceding the 5′ terminus of Qβ plus strand, P the *Pst*I site [preceded by oligo(dA)] following the Qβ region. E the *Eco*RI site in the Qβ sequence. The numbers describe the nucleotide number of the Qβ genome up to which the cDNA sequence is present. **C**: the upper bar represents the parent insert and the restriction sites used to construct the modified A_2 cistrons. Proteins expected to be produced are represented with circles (normal frame: open circles, +1 frame: filled circles; in the −1 frame only very short oligopeptides could be formed). The modified inserts are identified by the restriction sites used for their construction; fs means that the reading frame is shifted.

completely abolishes cell lysis.

Discussion

From the nucleotide sequence of the Qβ genome (Mekler, 1981), it was clear that a lysis polypeptide could not be coded in a way analogous to the group I RNA phages. We anticipated that the coat-A_1 cistron (Garwes *et al.*, 1969; Horiuchi *et al.*, 1971; Weiner and Weber, 1971) could be coding for this function since it is in a similar position in the genome as the MS2 lysis gene and it is the only major feature distinguishing the genome organizations of the two phage groups (Figure 1). Furthermore, this is the only region of the genome from which theoretically a polypeptide of reasonable size (74 amino acids) could arise by ribosomes reading in a different frame [from nucleotide 1946 (AUG) to 2168 (UAA)]. It was therefore unexpected to find that the maturation protein (or a derivative of the full-length polypeptide) triggers cell lysis.

The involvement of only one virus-coded gene product in cell lysis is similar to the situation in MS2 (Remaut *et al.*, 1982) and in the single-stranded DNA phage φX174 [where only gene E product is needed (Young and Young, 1982)]. It should be mentioned that Horiuchi *et al.* (1971) have noted earlier that amber mutants of the maturation cistron preclude cell lysis in group 3, but not in group 1 phages, an observation readily explained by our results.

Many questions concerning the mechanism and the regulation of phage-induced lysis remain open. Superficially, the process of cell lysis appears very similar in both group 1 and group 3 RNA phages despite the use of completely different lysis proteins. In neither group is a lysozyme-like activity detectable (Model *et al.*, 1979, and our unpublished results), and onset and completeness of lysis are directly related to the

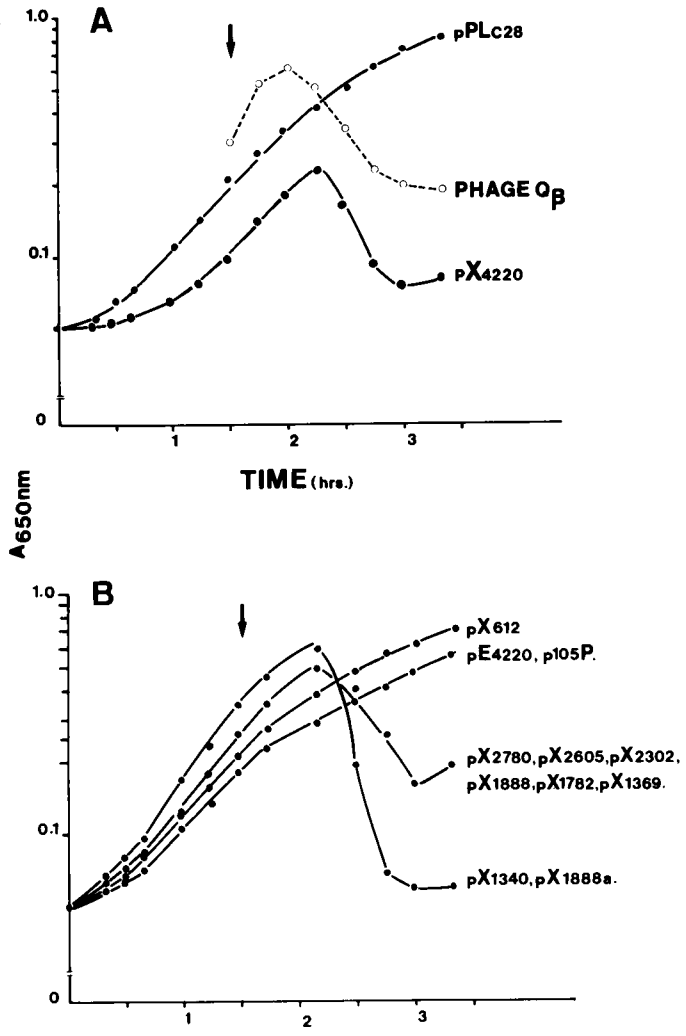

Fig. 3. Cell lysis assay by photometric turbidity measurement. Strains of *E. coli* K-12ΔH1Δtrp containing the plasmids with inserts shown in Figure 2B and *E. coli* Q13 were grown in L-broth at 28°C. At the time indicated by arrows the temperature was shifted to 42°C. *E. coli* Q13 was infected with Qβ at 37°C. An apparent absorption at 650 nm of 0.3 corresponds to 2 x 10^8 cells/ml.

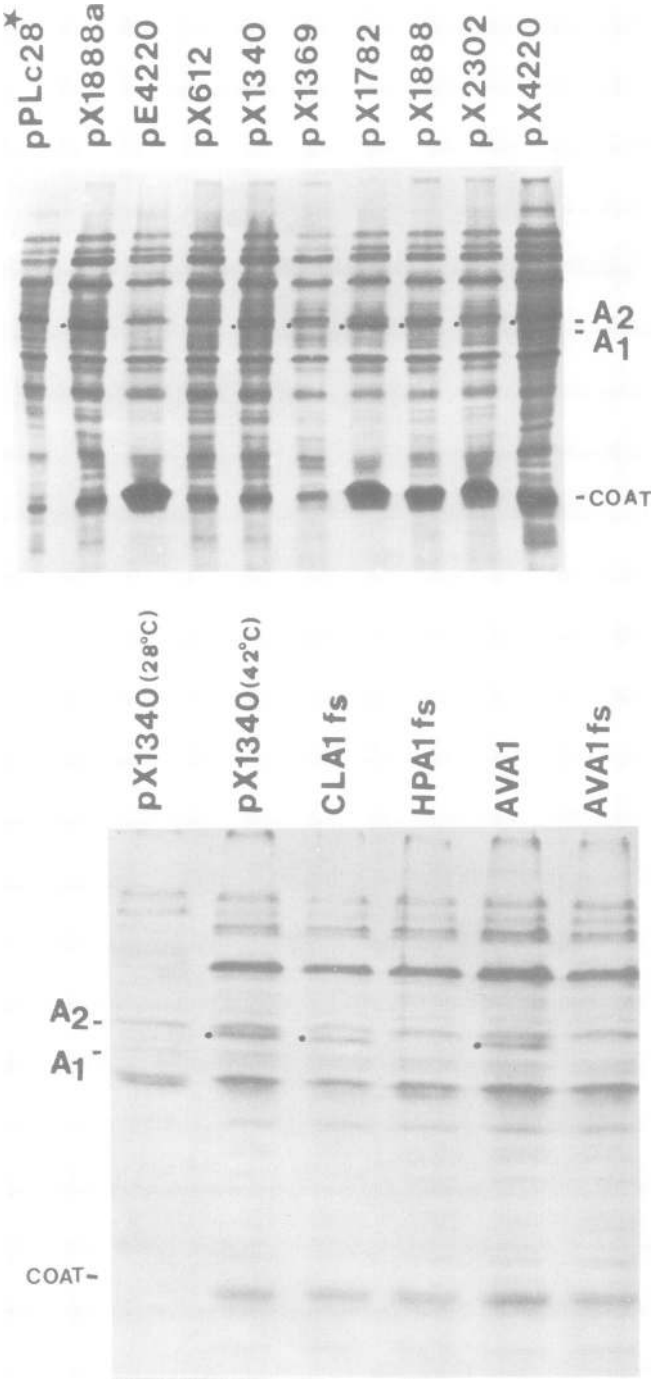

Fig. 4. Analysis of radiolabelled proteins by SDS-polyacrylamide gel electrophoresis (Laemmli, 1970). Proteins of *E. coli* K-12ΔH1Δtrp containing the expression plasmids defined in Figure 2B and C were labelled and analyzed essentially as described (Remaut *et al.*, 1981), but [^{3}H]leucine (50 μ Ci/ml, specific activity 180 Ci/m mol) was incorporated for 25 min after heat induction and the 20% polyacrylamide gels were stained before autoradiography to localize Qβ protein markers (A_2, A_1 and coat protein marked by dashes). The bands corresponding to A_2 and its variants are marked by dots. The truncated proteins produced in strains with plasmids '*Cla*fs' and '*Ava*I' run almost exactly as A_2 protein. (In 15% gels the polypeptide specified by plasmid '*Cla*Ifs' migrated considerably faster; not shown.)

growth rate, both in group 1 phages (Engelberg and Soudry, 1971; Haywood, 1974; Kastelein *et al.*, 1982), and in Qβ (our unpublished observations). Furthermore, lysis is inhibited by drugs such as rifampicin, azide or chloramphenicol which interfere directly or indirectly with protein synthesis (Fromageot and Zinder, 1968; Engelberg and Soudry, 1971; Model *et al.*, 1979).

The target of the lysis-mediating protein is obscure; the murein layer is most likely the ultimate target, but the lysis proteins of both groups of RNA phage [and also that of φX174 (Young and Young, 1982)] contain a high proportion of hydrophobic residues, and therefore interaction with membranes is likely to occur. Furthermore, it is not clear whether the lysis depends in a general way on active cell growth or on specialized interaction with one or a few short-lived proteins produced in sufficient amounts only in rapidly growing cells. It should be mentioned here that cell lysis triggered by β-lactam antibiotics (reviewed by Tomasz, 1979) is also dependent on cell growth.

Our experiments represented in Figure 5 show that even transient inhibition of cellular protein synthesis during phage infection severely impedes lysis. This suggests that the lysis protein has to interact over a long period with freshly synthesized cellular components, possibly by integrating into cellular structures during membrane or cell wall growth, to

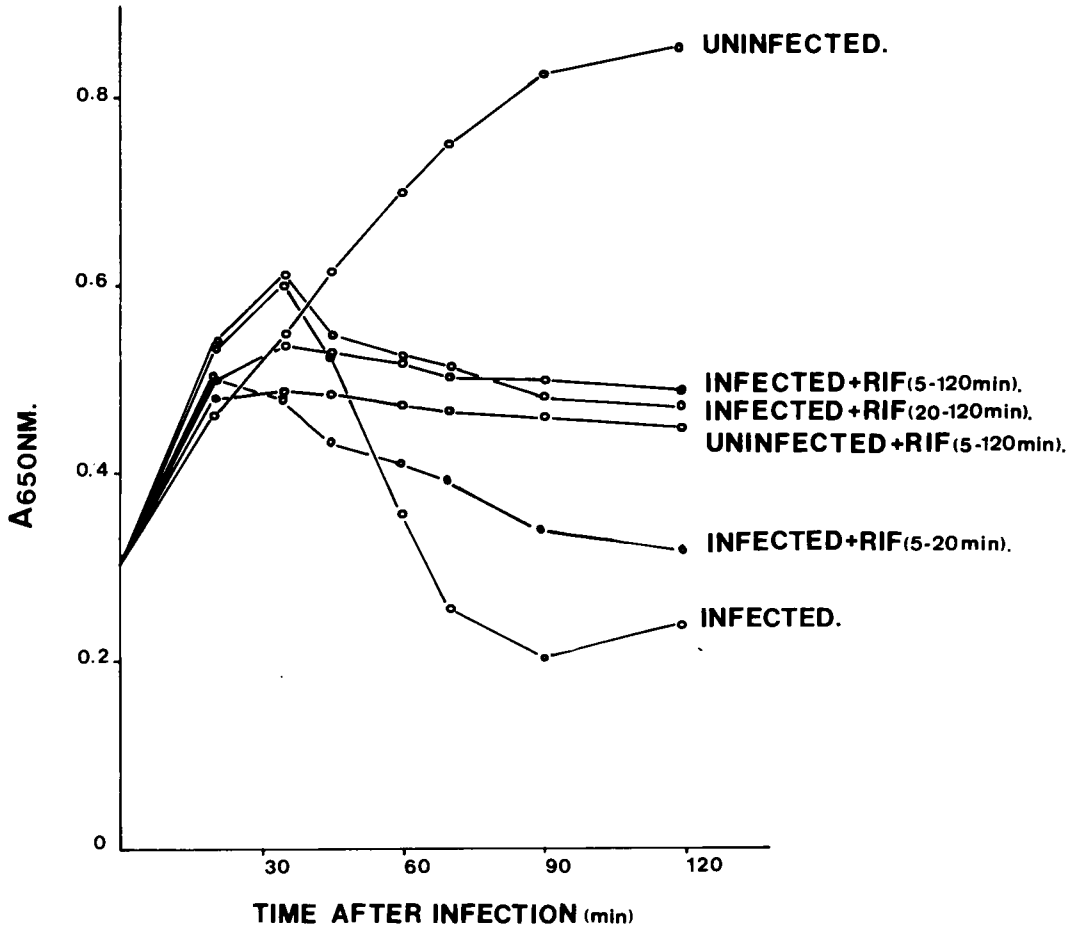

Fig. 5. Effect of rifampicin on cell lysis. *E. coli* Q13 cells growing in L-broth were infected and treated with rifampicin (50 μg/ml) as indicated. To remove the drug, cells were centrifuged (5 min, 5000 *g*) and resuspended in the same volume of fresh medium. Uninfected cells resumed growth very rapidly after this treatment.

mediate lysis. It therefore would appear that it is the availability of host components in addition to that of phage-coded lysis protein which regulates cell lysis. This would also explain why the synthesis of phage-coded lysis proteins does not have to be restricted to late times as in the case of lysis proteins of large phages. In fact RNA phages of both groups seem to synthesize their lysis proteins throughout the infection cycle. In the case of the maturation protein (which only in Qβ is synonymous with lysis-mediating protein), the synthesis early in infection is clearly documented: although it is always formed at a low rate compared with a coat protein, its production is relatively high (1:6) early in infection and drops later [1:30; Viñuela *et al.* (1967); Oeschgen and Nathans (1970)]. This regulation probably comes about as follows. Late in infection the production of replicase is inhibited by coat protein binding to the replicase cistron initiation region in both phage groups (Bernardi and Spahr, 1972; Weber, 1976); therefore replicating RNA is scarce late in infection. On the other hand, only short nascent viral RNA plus strands seem to bind efficiently ribosomes to the initiation site of the maturation cistron, whereas, in mature plus strands, this site is generally poorly accessible (Steitz, 1969). This has been shown independently for both phage groups; for Qβ by ribosome binding to radioactively labelled RNAs of various defined lengths synthesized *in vitro* (Staples *et al.*, 1971) and for group 1 phages by *in vitro* protein synthesis yielding relatively high amounts of maturation protein when partially double-stranded RNA (RI), presumably containing short plus strand tails, were used as mRNA (Robertson and Lodish, 1970).

In the case of the lysis protein of group 1 phage the situation is less clear. It has been reported that lysis protein is not synthesized early in infection (Beremand and Blumenthal, 1979), but we suspect that its detection at early times might be hampered by its generally low expression rate and possibly by sticking to cell components. Its synthesis is thought to come about by ribosomes translating the coat cistron, occasionally switching the reading frame, terminating and reinitiating at the lysis cistron (Kastelein *et al.*, 1982). Thus, synthesis of lysis protein should be coupled to that of coat protein. We therefore must assume that it is produced also early in infection albeit not at such a comparatively high rate as the Qβ lysis-mediating protein.

As mentioned above, the genomes of both phage groups, although very different in their nucleotide sequence in most regions, contain extended segments of rather strong homology in the replicase cistrons implying that they are derived from a common ancestor (Mekler, 1981). It seems likely that the RNA phages were originally without a lysis function. During their evolutionary diversification they developed information for a protein triggering cell lysis in different parts of their genome without expanding it. The recruitment of a protein for an additional function, as it is realized in Qβ, is just another variation of the general feature of multiple utilization of some parts of the genome, first discovered in single-stranded DNA phages (Barrell *et al.*,

1976) and now recognized in different modes in viruses as diverse as, e.g., papova and adenoviruses (reviewed in Tooze, 1980) and influenza virus (Lamb *et al.*, 1981). In the case of the RNA phages, we infer that the two groups have solved the problem of release from the host by the same strategy although using different proteins and thus probably different triggering mechanisms. It could be argued that complete cell lysis is only an extreme outcome of infection, occurring under optimal growth conditions of the host. It has been claimed for the group 1 phage fr that phage-infected hosts do not lyse at low temperature but continue to divide while slowly liberating phage (Hoffmann-Berling and Mazé, 1964). It has also been reported that cells, having extruded under these conditions large numbers of virus particles, appear to have an intact cell wall; this supposed extrusion process is inhibited by rifampicin (Engelberg and Soudry, 1971). However, these claims have been disputed (Lerner and Zinder, 1977). Thus, it remains to be established whether phage extrusion through cell walls occurs and if so, whether the phage lysis proteins are involved in this process.

Materials and methods

Bacterial strains and general techniques

E. coli Q13 [F^+, deficient in RNase I, described by Gesteland (1966)] was used for phage growth and titer determination. *E. coli* K12 strains ΔHΔtrp and M5219 (Remaut *et al.*, 1981) producing tsCI857 repressor were used to express cloned insert sequences under λP_L control. In some cases, cloning was carried out with strain C600 producing wild-type λCI repressor before the characterized plasmids were transferred to the other strains. Transformation was carried out as described by Remaut *et al.* (1981). Preparation of plasmid DNA, cloning of DNA fragments isolated from low melt agarose, introduction of linker fragments and general DNA techniques were carried out according to described procedures (Maniatis *et al.*, 1982).

Construction of plasmids

The expression vector pPLc28* (Figure 2A) was modified from pPLc28 (Remaut *et al.*, 1981): the *Pst*I site in the ApR gene was eliminated (without affecting the antibiotic resistance function) by the analog incorporation technique (Müller *et al.*, 1978), involving partially *Pst*I-cleaved plasmid and HOdCTP; furthermore, an *Xba*I and a *Pst*I site were introduced by the ligation of appropriate linker octanucleotides (Rothstein *et al.*, 1979) into plasmids cleaved with *Bam*HI and *Acc*I, respectively, and made flush-ended by *E. coli* DNA polymerase I (large fragment). pBR322-based plasmids, with the entire Qβ genomic sequences flanked by homopolymer and *Hind*III cleavage sites, were obtained by treating a pCRI-based Qβ hybrid plasmid (Taniguchi *et al.*, 1978) with nuclease S1 under partially denaturing conditions (Hofstetter *et al.*, 1976), then adding poly(A) tails to the isolated Qβ sequence and joining it to *Hind*III-cleaved and poly(T)-tailed pBR322. These plasmids with Qβ inserts in either orientation were modified: one of the two flanking *Hind*III sites was replaced by partial *Hind*III cleavage and insertion of octanucleotide linkers. This yielded the pBR-Qβ (*Xba-Hin*) (Figure 2A) and the analogous pBR-Qβ (*Pst-Hin*) bearing the insert in the opposite orientation, and a *Pst*I site instead of the *Xba*I site. From these plasmids, two series of pBR derivatives with Qβ sequences, having either their 3′ or 5′ region deleted, were derived according to the Lehrach subcloning procedure (Frischauf *et al.*, 1980).

The endpoints of the deletions in these series of plasmids were determined approximately (±10 nucleotides) by cleavage with *Hae*III, end-labelling and 6% polyacrylamide gel electrophoresis. A variety of Qβ inserts (Figure 2B), flanked by either *Xba*I and *Hind*III sites (3′ deletions) or *Hind*III and *Pst*I sites (5′ deletions) were recovered from these plasmids, ligated into appropriately cleaved pPLc28* and transfected into *E. coli* K-12ΔH1Δtrp strain. pX1340 and pE4220 were constructed accordingly from pBR-Qβ (*Pst-Hind*) utilizing known *Bcl*I and *Eco*RI restriction sites, respectively (to the *Bcl*I site made flush-ended by polymerase I a *Hind*III linker was joined). The four expression plasmids containing modified cistron A_2 inserts (Figure 2C) were derived from pX1340. '*Cla*Ifs' was generated by cleaving with *Cla*I, filling up and religating the flush ends, '*Hpa*Ifs' by cleaving with *Hpa*I and religation, '*Ava*I' and '*Ava*Ifs' by cleaving with *Ava*I and religation without and after filling up, respectively.

Acknowledgements

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