Making temporal maps using bacterial luciferase: Bacteriophage λ

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ABSTRACT

A method for making temporal maps in bacteria, plasmids and bacteriophages is described. A cassette containing both the genes for bacterial luciferase and kanamycin resistance can be introduced at precise sites. The technique involves cloning followed by genetic recombination. The result is formation of structures that have the luciferase genes in place of the normal DNA and this allows the very precise measurement of transcription/translation of the substituted regions. Very low levels of transcription as well as the kinetics of induction can be easily ascertained. As a specific demonstration of this general method, the technique was used with bacteriophage λ, one of the best known organisms. By measuring light emission, the expression of luciferase was followed after induction for both early and late genes. The exact timing of initial expression of genes was also determined by sampling at very short intervals. The results show that the early genes express almost without delay implying that the function of the N antitermination system is not temporal regulation.

Keywords: Temporal maps, bacteriophage λ, gene N function

1. INTRODUCTION

The measurement of gene expression is central to biology. In all organisms, many genes are expressed continuously throughout the life cycle. Other genes are particularly or specifically expressed at certain times. For example, in higher organisms some genes are only expressed in particular tissues. Although unicellular, the prokaryotes also have some similar systems which react to certain stresses or a change in the environment. In *Escherichia coli*, a specific battery of genes is turned on after heat shock[1], a different battery when the cell is exposed to ultraviolet light[2] and yet another as the cell carries out its transition from the growth stage to the resting one[3]. Bacterial viruses, known as bacteriophage or simply phage, often offer dramatic examples of temporal regulation as they progress from replication of their genetic material to assembly of the mature virion and the release of progeny by lysis of the host cell. We have investigated the cascade of events during the life cycle of one of these phages, namely phage λ that grows on *Escherichia coli*.

Gene expression can be determined by assays of the gene product or by substituting the gene with one whose product is easily measured. The former method involves either direct or, when specific antibodies are used, indirect measurement. However, by its nature the use of antibodies is specific and each different gene product requires its own quantification. Gene substitution by genes whose products are easily determined is a more general way. Among the possibilities are genes that specify flourescent proteins or luciferase. Bacterial luciferase[4] requires oxygen, $FMNH_2$ and a long chain aldehyde to convert chemical energy to visible light. Higher organisms lack or nearly lack $FMNH_2$ and bacterial luciferase is not particularly useful for these. However, prokaryotes all contain $FMNH_2$ and aldehyde readily enters the cell by diffusion which permits the *in vivo* measurement of luciferase on a large number samples at a time. If the substituted gene is essential for a process, its homolog supplies the product in diploid organisms; in bacteria and phages this can be done by making the cells merodiploid for the region under study.

Bacteriophage λ can grow either lytically or form a lysogen in which its genome is integrated into the chromosome of its host[5]. In lysogens most λ genes are not expressed because the λ repressor shuts off the two early promoters. As diagrammed in Figure 1, when repression is removed, two main gene products are made: N and cro[6]. Cro acts to prevent further repressor synthesis and at later times also represses the early promoters. N acts to antiterminate[7] the transcripts

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emanating from the early promoters and allows RNA polymerase to read through normal terminators. In the presence of N, genes for recombination, excision of the λ genome from the chromosome, DNA replication and Q are made among others. After Q is synthesized, the cascade continues with the expression of all the late genes of the phage and these include the structural proteins for its head and tail and proteins that cause bacterial lysis after which the mature progeny are released. Q acts similarly to N in that it allows transcription that began at $p_{R'}$, the late promoter, to read past its normal termination sequence[8].

In the present research, we have made a temporal map of λ using luciferase to measure gene expression. Previous investigators have examined particular proteins[9] or mRNA[10] of λ. Such measurements are relatively crude and cannot measure either low levels or the precise kinetics of expression. The method presented herein is general and can be used for different phages and bacterial genes that are expressed during a differentiation process or after certain environmental shocks.

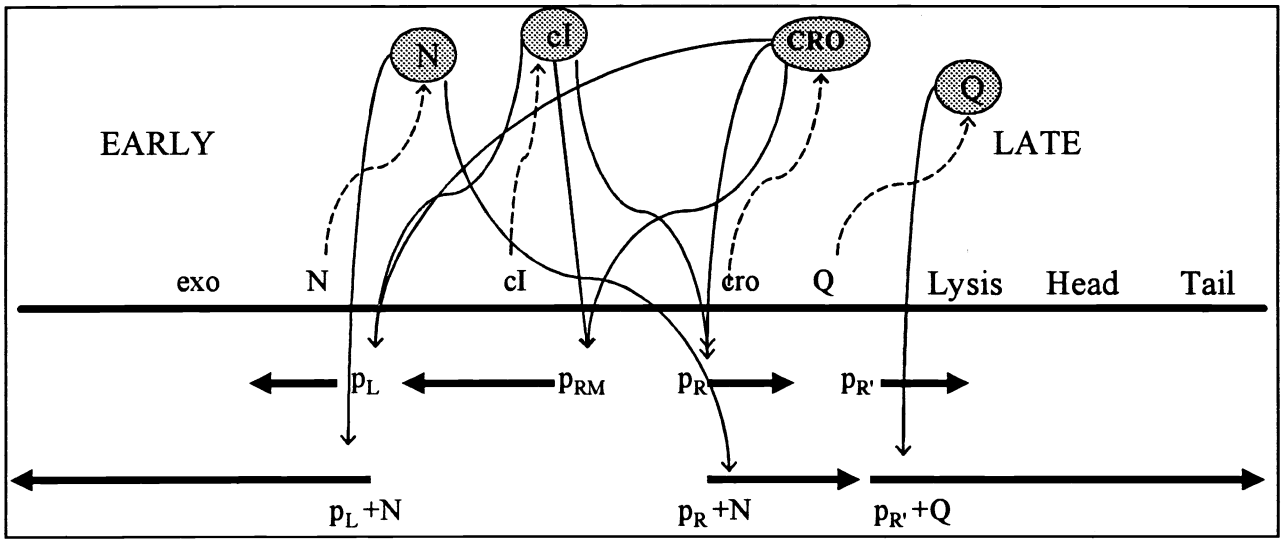

Figure 1. A schematic representation of the main features of λ regulation. The *cI* product, the repressor, blocks transcription from p_L and p_R and at higher concentrations blocks its own synthesis from p_{RM}. The *cro* protein blocks repressor synthesis and at higher concentrations prevents transcription from p_L and p_R. The *N* protein causes antitermination of transcripts initiating at p_L and p_R and leads to the synthesis of all the early proteins and the *Q* protein. The *Q* protein antiterminates transcripts initiated at $p_{R'}$ and allows transcription of the late genes.

2. METHODOLOGY

2.1 Overview

The method involves 4 stages. The first of these is the cloning of a section of DNA that encodes the gene of interest. The second is the preparation of a source of the *lux* genes without a promoter that includes an adjacent gene whose presence can be selected for. The third is the insertion of the cassette into the desired gene such that the orientation of the *lux* genes is the same as that of the unsubstituted gene. The fourth, if necessary, is the transfer of the cassette insert into the entire genome of the phage, plasmid or bacterium. The entire scheme is shown diagrammatically in Figure 2.

2.2 Cloning of λ segments

The entire sequence of the λ genome is known[11] and hence the precise cleavage sites for restriction endonucleases. By making use of this knowledge most of the λ genome was cloned as relatively small fragments into cloning vectors such as pHG171, pHG327 and pHG329[12].

2.3 The *lux-kan*^{R} cassette

The cloned *lux* operon[13] from *Vibrio fischeri* was subcloned to give a smaller piece containing part of the *luxC* gene, all of the *luxA*, *luxB* and *luxE* genes. Using Bal31 exonuclease digestion, the *luxC* DNA was removed and only 23 base pairs before the start of the *luxA* gene remained and these base pairs are those of the ribosomal binding site. Most of the *luxE*

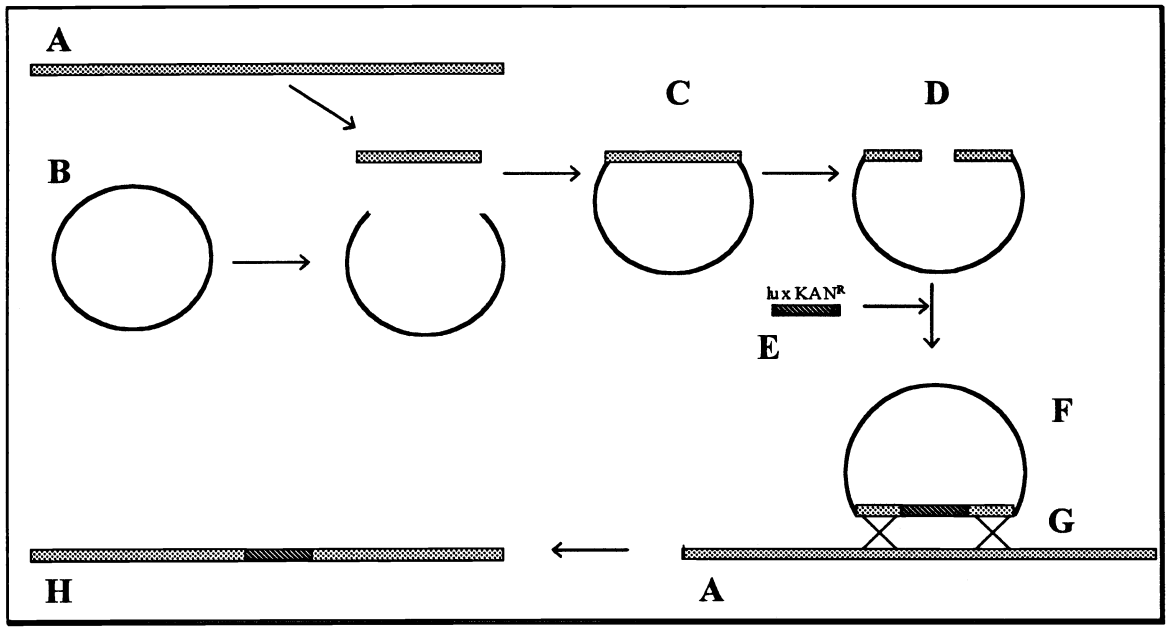

Figure 2. The procedure for introducing the light cassette into λ or other DNA.
A: The λ chromosome which is cleaved by a restriction enzyme. **B:** Plasmid DNA which is then cut by a restriction enzyme or enzymes and combined with the fragments from **A** to yield **C:** A fragment of λ has been cloned into the plasmid vector. **D:** The plasmid is opened by cleavage with a restriction enzyme. **E:** The light cassette fragment containing the *lux* genes and a gene for kanamycin resistance. **F:** The light cassette has been introduced to a specific site within λ DNA. **G:** The recombinant plasmid is in a lysogenic cell containing the entire λ genome. Recombination between them can occur to yield **H:** The λ genome containing the *lux* cassette at a specific predetermined site.

gene was removed in the same way to leave all of the *luxB* gene and a small segment of the *luxE* gene. The *kan*^{R} gene from Tn903[14] conferring kanamycin resistance and its promoter were then placed downstream of the *luxA* and *luxB* genes and the *kan*^{R} gene was in the same orientation as the *lux* genes and its transcription is away from the *lux* genes. To the ends of this *lux-kan*^{R} segment were added DNA linkers that include a site for restriction endonuclease SnaBI. The *lux-kan*^{R} segment does not include a SnaBI site. The SnaBI endonuclease cuts the sequence TAC↓GTA and leaves blunt ends. The cassette can therefore be isolated in quantity after SnaBI digestion and agarose gel electrophoresis.

2.4 Insertion of the *lux-kan*^{R} cassette into cloned λ segments

The *lux-kan*^{R} cassette was cloned into the λ segments by standard techniques[15]. Unique restriction enzyme sites within the λ piece were chosen and cleaved with the appropriate enzyme. When there were 5' protruding ends, these were made blunt by filling them in using *E. coli* DNA polymerase (Klenow fragment) and the appropriate triphosphate deoxynucleotides[15]. The isolated *lux-kan*^{R} cassette was then joined to these ends with T4 DNA ligase. Restriction enzyme mapping was used to determine the orientation of the *lux-kan*^{R} cassette in relation to the λ segment. The wrong orientation provides a background control while the right orientation allows the expression of the inserted cassette to be followed.

2.5 Transfer of the *lux-kan*^{R} insert to the complete λ genome

The plasmid containing a segment of λ with an inserted *lux-kan*^{R} cassette was transferred to a strain lysogenic for λ that carries a mutation in its repressor gene, *cI*. The mutation leads to the synthesis of a thermolabile repressor that is fully active at 30°C or below but inactive above 40°C. As needed, the prophage can carry other mutations. When the additional mutations were of the amber nonsense type, strain C600 of *E. coli* was used which carries a suppressor of amber mutations. The lysogenic strain carrying the introduced plasmid was grown at 28°C and then shifted to 43°C to inactivate the λ repressor and initiate the lytic cycle. After 30 minutes, the culture was place at 37°C and incubation continued until lysis occurred. Chloroform was then added to a final concentration of 1% and the culture centrifuged at 6000 rpm to remove bacterial debris. The resulting lysate contains, in the main, normal λ and some rare particles that are due to genetic recombination between the phage genome and the phage DNA of the plasmid. These recombinant phage have picked up the *lux-kan*^{R} insert. The λ DNA flanking the cassette provides DNA sequences that have allowed the homologous regions to undergo recombination[16,17]. The lysate was used to infect *E. coli* W3110 which is a prototrophic strain[18] that doesn't suppress amber mutations. After infection, the growth of the infected cells was continued for 2 hours at 28°C and then the culture was spread on rich plates containing kanamycin (30 μg/ml) to select for lysogens that had received the *kan*^{R} gene. These lysogens were checked for ampicillin resistance: the desired lysogens should not have picked up the *bla* (ampicillin resistance) gene from the plasmid if a double crossover had taken place in the formation of the recombinant λ phage. Lysogens were also checked for growth at 42°C: lysogens that carry the cI mutation should die at this temperature. Some recombinant phage were checked for the site of the *lux-kan*^{R} insert. All were found to contain the insert at the expected site.

2.6 Measurement of light emission during the growth cycle

The strain to be examined was grown overnight at 30°C in Lennox broth[19] without glucose (i.e. Luria broth[15] without neutralization) containing 10 mM $MgSO_4$. The culture was diluted 1:50 in the same medium and grown in a shaking water bath at 28°C until mid log-phase. The density of the cells was adjusted to A_{600} = 0.2 and the culture was incubated for several minutes at 28°C to ensure that the correct temperature had again been attained. An equal amount of the same medium at 58°C was added and this brought the temperature immediately to 43°C. The culture was place in a shaking water bath at 43°C for three minutes. This treatment inactivates the phage repressor. Then the same medium at 0°C (10.7 ml of ice cold medium/20 ml of culture at 43°C) was added to immediately reduce the temperature of the culture to 28°C and incubation was continued at 28°C in a shaking water bath at this temperature. It should be mentioned that bacterial luciferase is denatured at 43°C but is stable and maximally active at 28°C.

At various times during the life cycle, samples of 1ml were taken into scintillation vials immersed in an ice-water bath. The vials contained 100μl of chloramphenicol (1mg/ml) and thus have 100μg of this antibiotic. The cold stops further synthesis of luciferase within a short time and the chloramphenicol prevents synthesis during re-warming. A control culture that had never been subjected to the higher temperature was also sampled and its density initially was A_{600} = 0.2 (i.e. 3 times that of the treated cultures). After 30 minutes or more in the 0°C bath the vials were placed in an incubator (10-15 minutes) at 30°C to bring them to room temperature. Light was measured in a Packard Scintillation counter with the coincidence off and with settings for visible light. Dodecyl aldehyde is made up in ethanol to a final concentration (v/v) of 0.1%. 20μl of aldehyde is added per vial and each vial is read exactly after four minutes at which time the aldehyde has diffused into the cell. This amount of time has been determined as giving the maximum emitted light. Samples are counted for 12 seconds and the light emitted recorded as counts per minute (cpm). In most cases samples were taken every 10 minutes. After about 90 minutes the amount of light emitted often begins to decrease. This is probably due to a loss of $FMNH_2$ by the cells as the onset of lysis begins rather than inactivation of accumulated luciferase.

2.7 Measurement of the time of the initial wave of gene expression

To measure the initial expression of various genes after induction of the prophage and its switch to the lytic cycle, a different protocol is required since induction of gene expression can already commence during the first three minutes. Experiments showed that the time of induction can be shortened to 1 minute when 47°C rather than 43°C is used. The cultures were grown in the same way as above and then adjusted to an A_{600} = 0.3. The heat shock was begun by adding 10ml of medium at 58°C to 5.8ml of cell culture at 28°C. The temperature was thereby immediately raised to 47°C and

the culture flask was put into a shaking water bath at 47°C for 60 seconds. Exactly at 60 seconds, 10.7ml of 0°C medium was added to return the temperature to 28°C. Samples were taken every 20 seconds for genes that are expressed almost immediately (the early genes) and every minute for those strains with the *lux-kan*^{R} insert in the late genes. Induction as a function of different lengths of exposure to 47°C are shown in Figure 3 and the Figure demonstrates that 60 seconds is required for adequate induction.

For measurement at shorter time intervals, the amount of time required before luciferase synthesis stops when samples are put into vials in an ice-water bath is also important. Figure 4 shows the difference in light output as a function of time for vials stopped in the way described above and that for samples that were put into tubes containing crushed ice. The difference between them indicates that in the normal protocol, it takes about 40 to 60 seconds before cessation of *de novo* luciferase synthesis occurs.

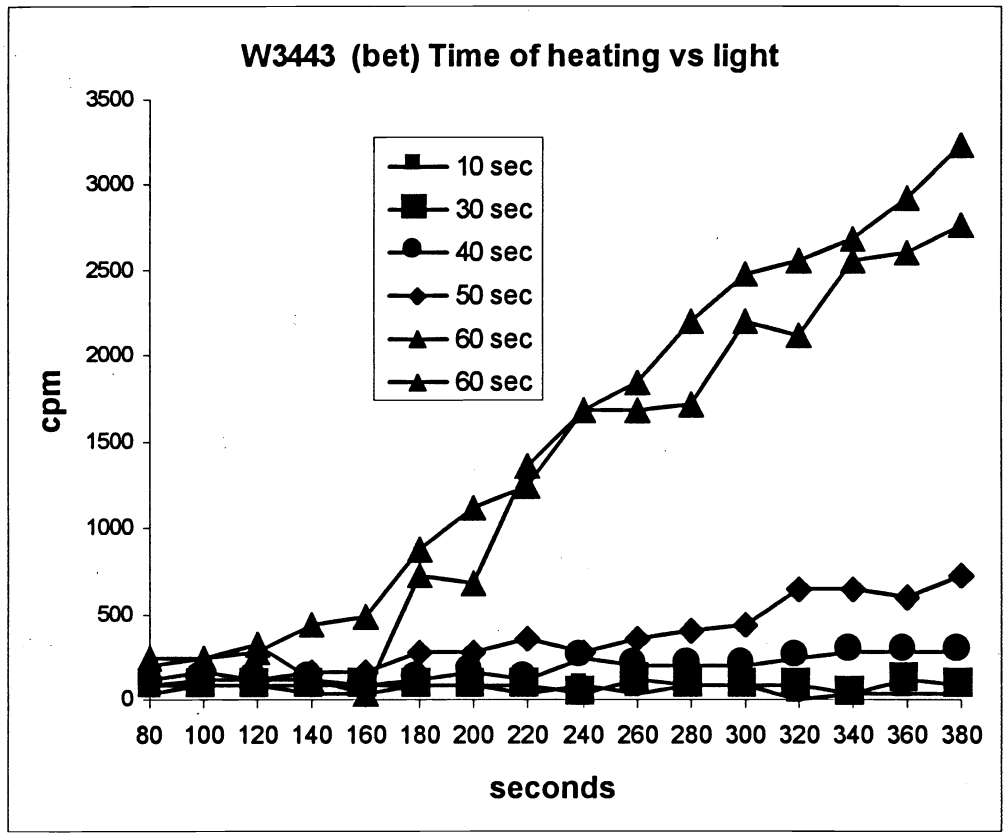

Figure 3. Light emission as a function of the time of heating.
A culture of a lysogen with a temperature sensitive repressor was grown at 28°C and then its temperature was immediately brought to 47°C for the indicated times. The culture was immediately cooled to 28°C as described in Methodology and samples taken at short intervals and their efflux measured.

3. RESULTS

3.1 Expression of λ genes during the lytic cycle

The integrated λ prophage can be induced to enter the lytic cycle. Normally, this event is caused by environmental factors such as exposure to ultraviolet light, mutagens or certain antibiotics[20]. In contrast in the laboratory using a phage mutant that specifies a thermolabile repressor, this induction can be brought on by exposing such a lysogen to an elevated temperature (40°C to 45°C). The repressor is thereby inactivated and the λ lytic cycle commences in most or all of the bacterial cells. Transcription of the phage genes begins and shortly thereafter the phage DNA is replicated. In

addition the phage chromosome is excised from that of the bacterium. By putting the *lux-kan*^{R} cassette at different locations, one can easily measure the relative extent of transcription of a given region and its pattern of expression during the phage's life cycle.

Figure 5 shows the pattern of induction for one of the early genes of λ, namely the *exo* gene involved in generalized recombination. This gene is non-essential in the laboratory and its inactivation or removal does not prevent the virus from completing a normal life cycle that leads to plaque formation. The figure shows that this gene is expressed quite strongly at an early time. The other genes examined in the p_L operon (*ssb, ral-git, ssb-cIII, bet, Ea22,* and *Ea8.5*) behaved similarly except for *int* which has its own promoter and is transcribed constitutively. For most *E. coli* genes there is almost no time differential between that needed for transcription and the appearance of the gene product since the translating ribosomes follow closely after RNA polymerase. At about 25 to 30 minutes after induction, the expression of the gene ceases. This is caused by repression due to the product of the *cro* gene. It is somewhat surprising that this cessation of expression is so abrupt and complete. The expression of the *exo* gene depends on the presence of a functional *N* gene. The Figure shows that when no *N* product is made, there is essentially no transcription of the *exo* gene.

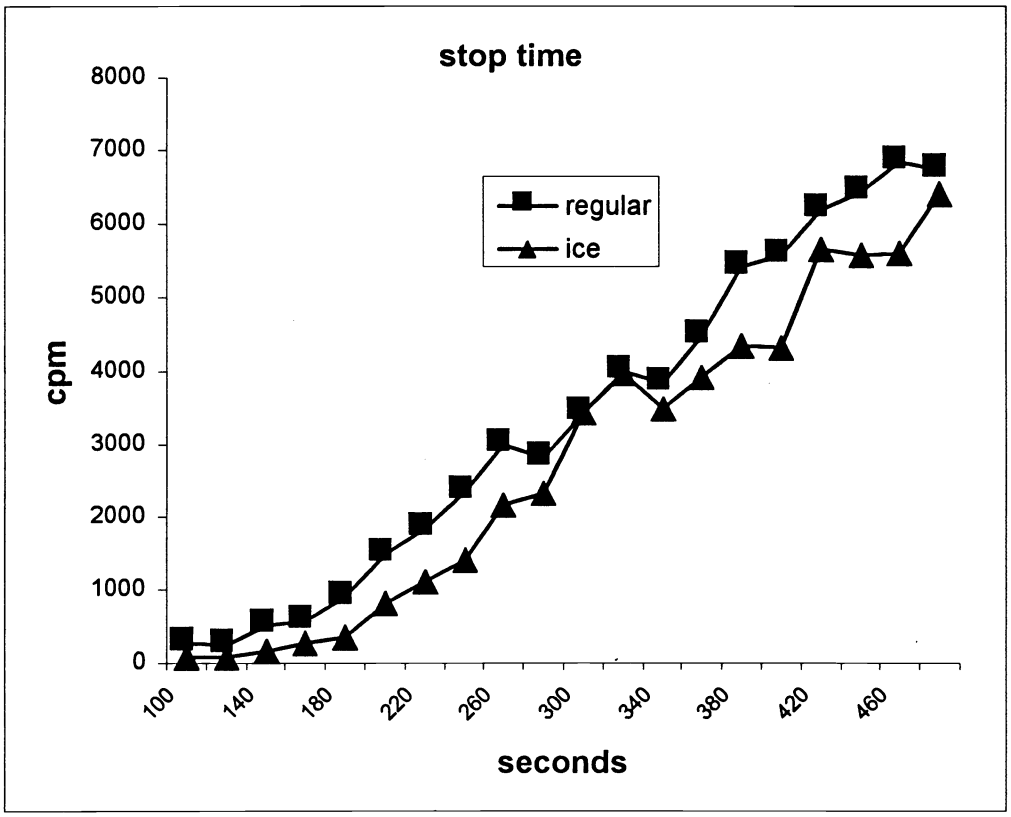

Figure 4. Cessation of luciferase synthesis.
Samples were taken as described in Methodology into cold scintillation vials containing chloramphenicol or taken onto crushed ice with chloramphenicol. The latter samples were then concentrated by centrifugation and resuspended with 1.1 ml of cold medium containing chloramphenicol.

In Figure 6, the results of a similar experiment in which the *lux-kan*^{R} insert is in a late gene are shown. Transcription of the gene begins somewhat later and continues almost linearly throughout the life cycle. Similar results were found for genes *D, E, H, L* and *J*. Near the end of the cycle (lysis and release of phage occurs at about 120 minutes under the conditions used) transcription seems to stop and the level of luciferases seems to decrease. This is probably an artifact in the sense that light emission depends on the presence of $FMNH_2$ which may be lost in some of the cells as they begin to lyse.

3.2 Measurement of the initiation of transcription

Due to the ease of directly taking samples, the initial time of transcription after induction of various genes can be quite accurately measured. Samples can be taken at very short intervals and the appearance of the first wave of transcription/translation followed. As described in Methodology the cells are exposed to a short period of high temperature which is enough to cause some induction. Induction is not complete under these conditions but this is irrelevant since one is only interested in the time of first appearance of luciferase activity. For late genes, samples were taken every minute. Figure 7 shows an induction curve for gene H, a late gene. Induction begins at about 10 minutes for this gene and continues to rise linearly during the time course of the experiment.

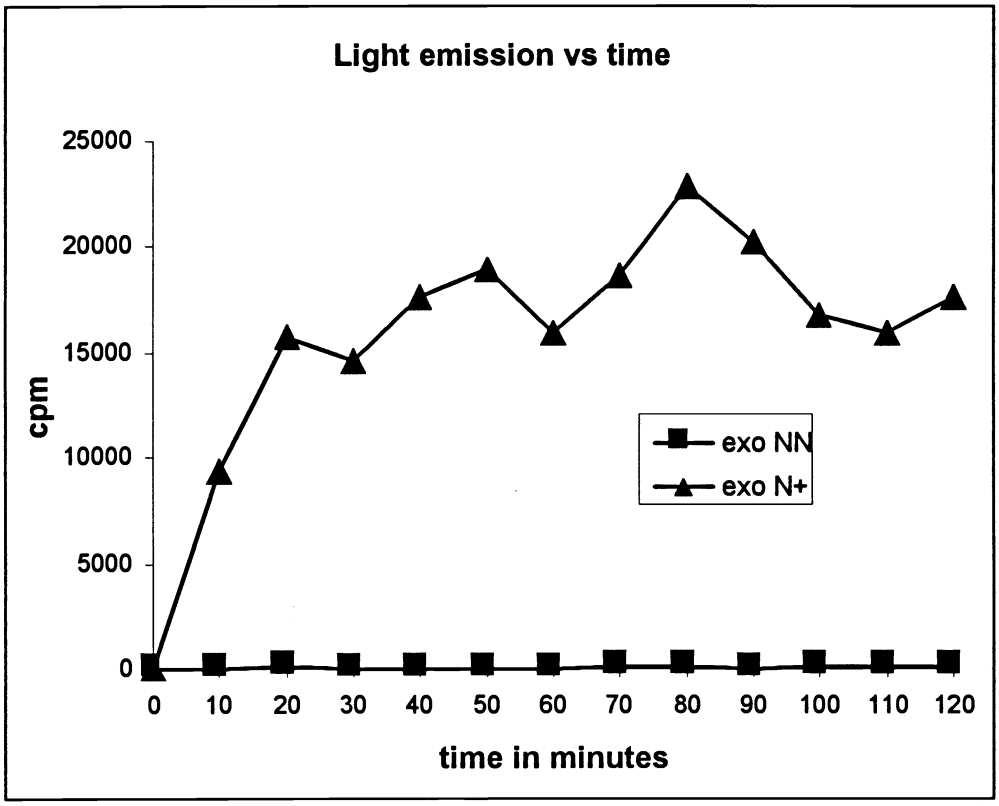

Figure 5. Light emission by a lysogen carrying the lux genes in the λ exo gene.
The cultures were induced by exposure to 43°C for 3 minutes as detailed in Methodology. In both cultures, the *lux-kan*^{R} cassette was in the *exo* gene. One of the lysogens carried two amber mutations (N_7N_{53}) in the *N* gene and no functional *N* protein is made.

The results of a similar experiment for an early gene, *exo*, are shown in Figure 8. The interval between samples is 20 seconds which is about as fast as samples can be accurately taken by hand. The triangles in the Figure are for the same strain lacking an active *N* gene and show that the rise in light emission is not a general effect caused by the heat treatment but rather that the increase in light emission by the N^+ strain (squares) is due to transcription that depends on the presence of an active *N* gene. The *exo* gene region begins to be expressed after about 180 seconds. When this is corrected for the time necessary to stop the reaction (20 to 40 seconds), the time elapsed before expression is not very dissimilar to that required by RNA polymerase to transcribe from p_L to the *exo* gene. That is, the expression of the *exo* gene is found to be close the minimum needed for transcription and there is a very small time delay, if any, in its expression. Similar results were found for the other genes in the early p_L operon.

4. CONCLUSIONS

4.1 Making temporal maps

The techniques described above can be used for most bacteriophage. In the case of temperate phages, the method using an antibiotic resistance gene allows the selection of rare recombinant phage carrying the cassette with the resistance gene and the associated promoterless *luxA* and *luxB* genes. For virulent phages, this method would be very difficult to use as there would be, at most, a small window during the life cycle in which the antibiotic could be applied. A better way to insert a cassette is the use of phage nonsense mutations, and in particular those that are amber. The cassette is then changed to carry an amber suppressor and the *lux* genes. Again recombinants that have incorporated the cassette can be selected by their ability to grow on a strain lacking a suppressor. Of course, for essential genes the insertion inactivates the gene and its product must be supplied *in trans*. The use of a suppressor-*lux* cassette has been detailed by us in previous communications, albeit for a different purpose[16,17].

Incorporation of a cassette into a plasmid by recombination presents a different type of problem, namely that the cassette donor must be removed from the cell. For transmissible plasmids this is not a problem because, after mating, many or most cells receiving the desired plasmid will not have received the donating plasmid. In the case of non-transmissible plasmids another way must be found and it would seem best to simply separate the donor from the recipient plasmid by agarose gel electrophoresis followed by transformation and selection of those cells with antibiotic resistance and which also emit light.

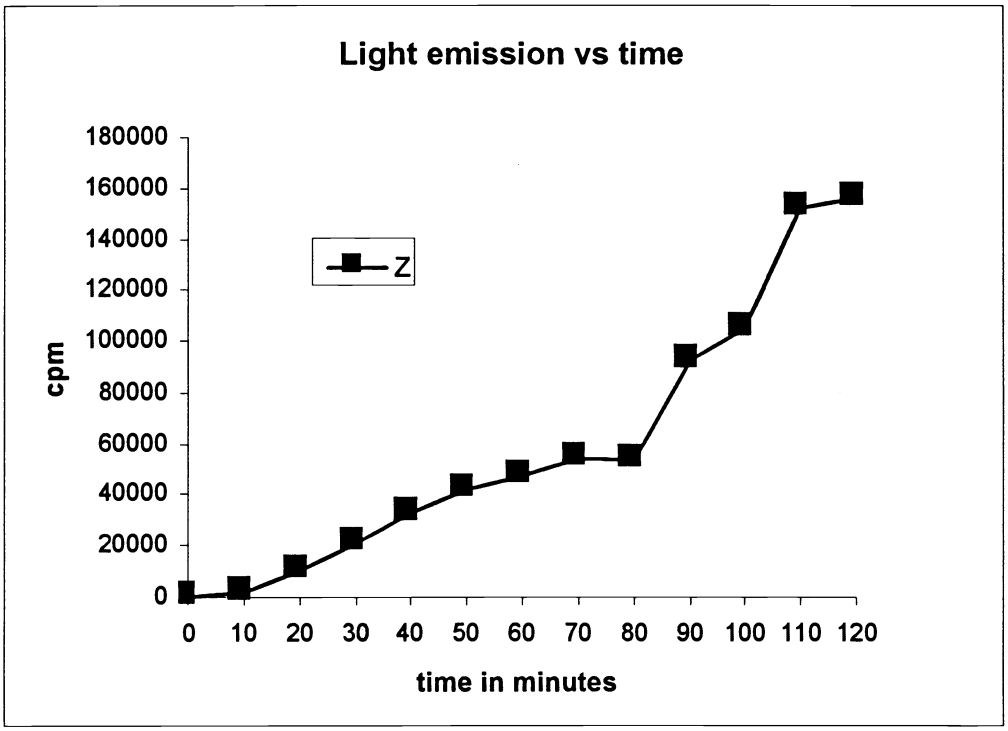

Figure 6. Light emission by an induced lysogen with the *lux* genes in the λ Z gene.
The conditions used and sampling were the same as those described in Figure 5.

The hardest target for inserting the *lux* genes at specific sites and generating a temporal map is undoubtedly the bacterial chromosome. Still a variety of methods are open to the investigator. For bacteria that have natural transformation systems, transformation by linear DNA containing the cassette at a specific site should yield the desired recombinants. In those that do not have such a system, strains that don't allow the donor plasmid to replicate or plasmids whose replication is defective at higher temperatures can be used. A different method has been devised for *Escherichia coli* using special

strains that allow transformation of linear DNA. When none of the above is possible, transposition may be the only course left to follow. This creates a major problem because of the lack of site specificity which leads to the insertion of the transposed genes at many sites and makes it necessary to genetically map each strain that has the proper phenotype, i.e. an insertion in a gene in the temporal pathway.

The end result in all cases is the same: the expression of a particular gene can be easily measured by determining the amount of light emitted at different times. This is very important for those genes which make proteins for which no easy assay exists. Even for those that can be assayed, it is often very difficult to accurately measure them when their levels are low.

4.2 The importance of temporal maps

When one looks at a bacterial process that is temporal, it is not immediately obvious whether the timing of any or all of the steps is critical. That is, if some stage is blocked, does the process simply halt and wait for the missing protein or are side pathways then followed that lead to a non-productive result. It must be assumed that in nature the timing that we see has evolved to be optimal and is important for the organism's success against other competitors. However, in the laboratory these competitors are absent. Therefore, we consider a "critical temporal function" one that must occur at the

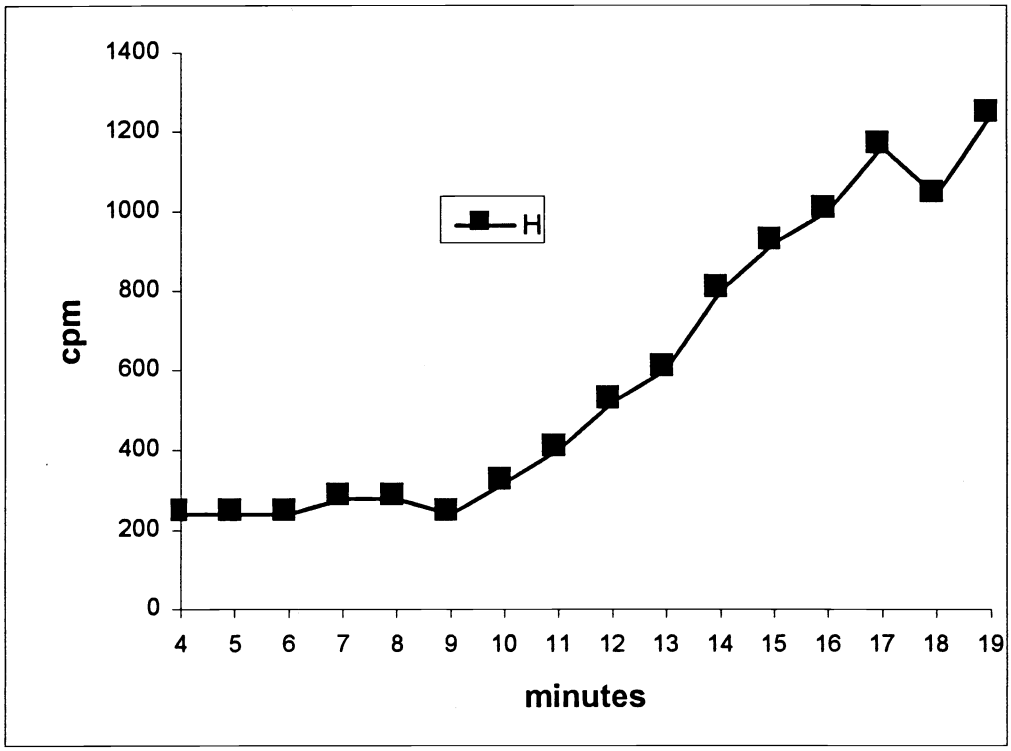

Figure 7. Initial induction of gene *H*.
A lysogenic culture whose λ prophage contains the *lux-kan*^{R} cassette in the *H* gene was induced for 1 minute as described in Methodology. Samples were taken every minute and the time of raising the temperature to 47°C was t = 0. Induction is expected to be only partial under this regimen.

proper time. If expressing the function too early or too late causes disruption of the temporal cascade and leads to non-productive side pathways, then it should be termed critical. This is in contrast to the usual classification of genes as essential (prevent growth, e.g. no plaques are formed) and non-essential. On the other end of the scale are those functions which may be expressed during the cascade but whose presence is not necessary for the process to be normally completed under laboratory conditions. Such functions may be auxiliary in nature.

In λ both the N and Q gene products are essential for development and mutants that lack either one do not form plaques. However, this does not answer the question of whether the timing of their expression is critical. As will be discussed in a subsequent publication, it turns out that Q can be expressed over a wide range of times without affecting the amount of progeny formed and thus its time of expression is not critical. When Q is expressed at times much later than usual, the developmental pathway simply stops and waits for its appearance. As shown above and for many other genes of the p_L operon the expression of these is very rapid. It can be estimated that the N protein on whose presence their expression depends only leads to a delay of about a minute or less. This raises the question of whether the function of N with regard to the p_L operon is in fact to cause a delay of the expression of genes in this operon. In another publication, it will be shown that the function of N is not related to temporal regulation but rather to strengthening repression during lysogeny.

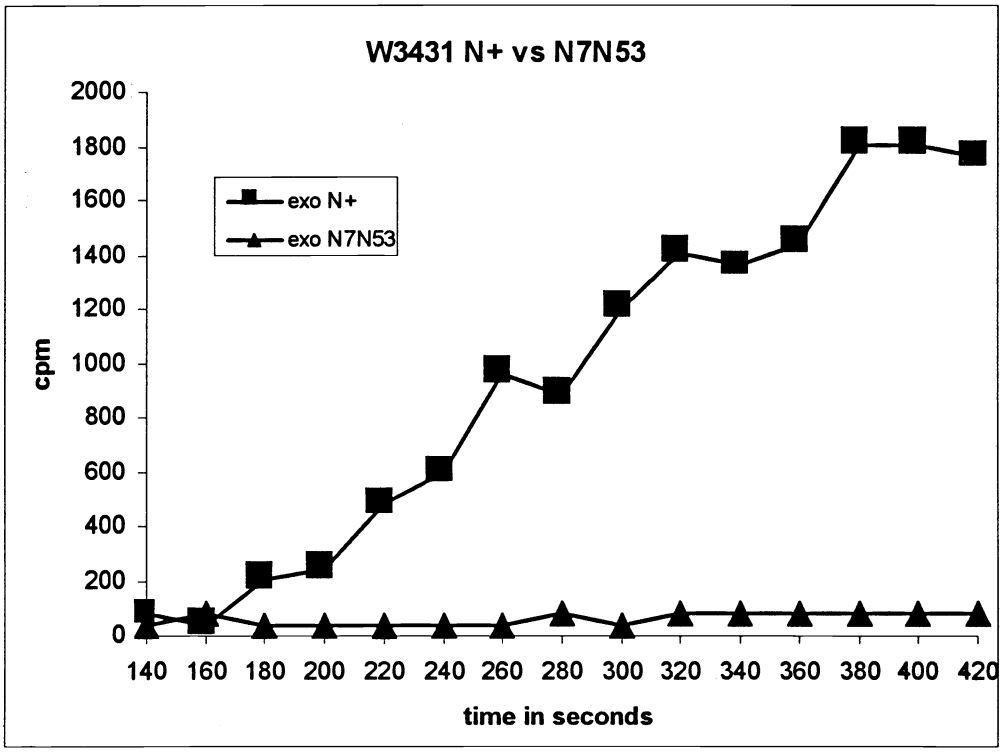

Figure 8. Initial expression of the *exo* gene of λ.
The experimental conditions were the same as those described in Figure 7 except samples were taken at 20 second intervals. A second culture with the *lux-kan*^{R} cassette in the *exo* gene also carried an inactive *N* gene.

4.3 The λ life cycle

In the present study, the transcription/translation of a large number of regions of the λ genome has been studied. Most of the early genes of the p_L operon are expressed with little delay. The delay is mostly caused by their relative positions with respect to their promoter, i.e. those that are closest to the promoter express before those more distal. There may be a slight delay in their expression because all depend on the presence of the N protein. Since this latter delay is very minimal it can be concluded that the N protein exerts its effect without delay. That is, the concentration of the N protein necessary to cause antitermination would seem to be quite low and the protein need not accumulate before it exerts its activity. The p_L operon is turned off quite abruptly between 25 and 30 minutes after induction. This is somewhat surprising since a more gradual shutoff would be expected.

A previous study on the expression of the late genes of λ has shown that vastly different amounts of their proteins are made during the life cycle[21]. This differential expression is not due to the relative position of a gene. It was concluded that the amount of protein product synthesized by a given gene depends on the efficiency of translation of its mRNA rather than the relative amount of mRNA made. The amount of mRNA made from $p_{R'}$ should be fairly similar for all the

late genes although the degradation rate of mRNA might be different for different regions or genes. However, experiments have shown that there is no great differences in the rate of m RNA degradation in this region. When an mRNA is present, the initiation of its translation depends on the binding of a ribosome to the ribosomal binding site which is directly upstream of each coding region in mRNA. The amount of protein made could then depend on the sequence of the each specific ribosomal binding site and whether this site is relatively available or sequestered. Our results entirely support the idea that it is ribosomal binding that leads to differential synthesis of the gene products rather than the relative amounts of mRNA synthesis or degradation. No real difference in light emission was seen in this region regardless of the position of the insert. While it could be argued that the presence of the cassette has altered mRNA degradation or prevented the sequestration of the ribosomal binding site of the cassette by changing the conformation of the mRNA molecule, this seems unlikely because a number of different sites in the $p_{R'}$ operon were examined yet no such effect was found.

4.4 Practical applications of the technique

The techniques detailed above can be used for the study of basic and applied biological systems and as described below also directly for applied purposes. In the present communication a basic study of the life cycle of λ is presented. Previously we have looked at promoters that are activated during the sporulation of *Bacillus megaterium*[22].

A related method which we patented[23] has been used by us to develop diagnostic tests for the pathogenic bacterial genus, *Salmonella*[16]. By implanting light genes in the genome of a phage species specific for 98% of all *Salmonella* strains, a phage reagent was developed that can specifically identify the presence of such strains in mixed cultures. When *Salmonella* is present, light is emitted because the phage carrying the *lux* genes infects it. When there is no *Salmonella*, no signal is generated because phage have no metabolism of their own and all the bacteria of medical or industrial importance are dark. We are presently developing a similar test for *Escherichia coli* O157:H7, a food borne pathogen that causes very unpleasant syndromes. Other groups are attempting to make practical use of this idea for *Mycobacterium tuberculosis* and other pathogenic species although in some cases other reporter genes, such as ice nucleation, have been chosen.

We believe that the general technique described here should find application in understanding the cascade of events during bacterial pathogenesis. By knowing the developmental pathway of pathogens and those triggered by interaction with their host, it is hoped that antibiotics or other substances can be found that will block particular steps and abort such infections.

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