[Frontiers in Bioscience E4, 1823-1829, January 1, 2012]

Cell biology perspectives in phage biology

Mireille Ansaldi [1]

[1]*Laboratoire de Chimie Bacterienne, CNRS-UPR9043, Institut de Microbiologie de la Mediterranee, Aix-Marseille Universite, 31 Chemin Joseph Aiguier,13402, Marseille cedex 20, France*

TABLE OF CONTENTS

1. ABSTRACT

Cellular biology has long been restricted to large cellular organisms. However, as the resolution of microscopic methods increased, it became possible to study smaller cells, in particular bacterial cells. Bacteriophage biology is one aspect of bacterial cell biology that has recently gained insight from cell biology. Despite their small size, bacteriophages could be successfully labeled and their cycle studied in the host cells. This review aims to put together, although non-extensively, several cell biology studies that recently pushed the elucidation of key mechanisms in phage biology, such as the lysis-lysogeny decision in temperate phages or genome replication and transcription, one step further.

2. INTRODUCTION

Being able to look at the processes occurring in living organisms is a challenge for modern biology. Cell biology enabled researchers to understand some key cellular processes, such as cytoskeleton organization, chromosome and plasmid segregation, or cell motility (for recent reviews see (1–5)). Bacteriophages develop within bacterial cells and, as a result, depend entirely on their host to produce newly synthesized virions. Most key cellular functions are high jacked by infecting viruses. This is typical of chaperone and chaperonine proteins, which were discovered through their role in the lambda phage development in *Escherichia coli* (6). Sometimes, phage genomes encode host-like proteins to fulfill similar roles;

these viral proteins interact with, and in some cases, substitute host proteins (7).

For decades, numerous studies have looked at phage-host interactions by using various approaches and techniques ranging from genetics to structural biology. However, bacterial cell biology has recently provided alternative methods for studying virus-host interaction and shed light on long studied bacteriophage biology aspects, such as the lysogeny decision in lambda phage, the replication linked to the cell architecture in Phi29, and the transcription of segmented genome viruses. Some connections between phage infection and cell architecture and metabolism are highlighted in this review.

3. LYSIS-LYSOGENY DECISION IN LAMBDA PHAGE

3.1. Phage adsorption and DNA injection

An important step in being able to visualize infecting bacteriophages was to develop new labeling methods since, until recently, infecting phages could only be observed by electron microscopy of stained preparations (8). Recently, a step forward was made by Edgar and collaborators who described labeling techniques that allowed the decoration of infectious phage particles with covalently linked biotin conjugated to streptavidin-coated QDots (9,10). As a result, they were able to visualize QDots labeled lambda and T7 phages and follow phage adsorption to living *E. coli* cells without affecting the ability of the labeled phages to form plaques. An important finding of the later study was that, at low multiplicity of infection (MOI), phages were found preferentially attached to the cell poles, either actual poles or at mid-cells where new poles are being made (9). This finding is not restricted to the lambda phage since the authors were able to observe similar attachment patterns with virulent coliphages T4 and T7, as well as with virulent phages infecting other bacterial species, such as *Yersinia pseudotuberculosis* and *Vibrio cholerae*. However, it is noteworthy that this study focused on phages infecting Gram positive bacteria. The question raised hereafter is the localization of phage receptors to cell poles. The LamB protein, which is the maltose high-affinity receptor involved in maltodextrine transport, is also used as a receptor by the lambda phage (11,12). A previous study showed that the LamB protein forms spirals extending from pole to pole (13), which is consistent with phage binding all around the cell at high MOI (9). The model postulated by these authors is that the lambda phage can bind to LamB at any position of the cell, and then ride the LamB spiral to the cell pole where DNA is delivered in a ManY dependant manner. This is in agreement with the dynamics of LamB that shows a highly mobile subpopulation which may be responsible for lambda phage riding along the bacterial cell although no mechanism has been proposed to explain LamB mobility (13). A conclusive experiment would be to perform time-lapse microscopy to follow phage lambda migration together with mobile LamB molecules to the cell pole until DNA enters the cell.

The next question Edgar and collaborators addressed was the localization of lambda DNA delivery. As a consequence of the lateral movement of lambda particles to the cell pole, lambda DNA uptake should occur at the cell pole as well. The ManY protein, which is part of the mannose phosphotransferase system (PTS), is required for lambda DNA injection (14–17). Consistently, a ManY-GFP fusion was found co-localized with QDots labeled phages at low MOI, which suggests that DNA injection occurs preferentially at the poles (9).

3.2. Lysis-lysogeny decision

The lysis-lysogeny genetic switch of the lambda phage has been the subject of extensive genetics studies for several decades (for recent reviews see (8,18,19)). However, cell biology helped to understand how this switch takes place in individual host cells. Following phage attachment and DNA release into the host cell, the lysis-lysogeny decision should be somehow influenced by the fact that lambda phage infection occurs at the cell poles. Indeed, Edgar *et al.* also showed that FtsH (or HflB) was localized mainly at the cell poles, which increased its local concentration (9). FtsH is an essential membrane-bound zinc-dependent metalloendoprotease involved in septation (20,21). Besides its role in cellular proteins degradation, including the heat shock promoter protein Sigma 32 (22), FtsH controls the abundance of several lambda phage proteins involved either in the early decision of lysis versus lysogeny mode of development (cIII, cII) or in the lytic development (Xis) (23–25). The localization of FtsH at the pole led Edgar and collaborators to propose that at low MOI, the CII protein is totally degraded as a consequence of the higher local concentration of FtsH, and the phage undergoes a lytic mode of development (9). This is in agreement with lysis being the preferential mode observed at low MOI (26–28).

As mentioned above, in the early seventies, it was observed and documented that the lysis versus lysogeny decision was dependant on two main infection conditions: the MOI and the physiological state of the host (26). Indeed, at low MOI and when the host is metabolically active, the lytic development is preferred; whereas, at a high MOI and in the presence of slowly dividing cells, the decision tends to be a lysogenic development. Host cell fate upon lambda phage infection has been recently re-investigated by St-Pierre and Endy at the level of individual cells, and the lysis-lysogeny decision was correlated with infected cell size (29). In this paper, the authors refer to the assumption made by Arkin and collaborators that if cells are believed to be identical at the genetic level, then they nevertheless may randomly accumulate different contents in regulatory molecules, which stochastically determines cell fate upon lambda infection (30). However, they provide an alternative model that involves an intrinsic and preexisting variation within the cell population and propose to challenge it by measuring individual cell sizes, which are then correlated to cell fate upon lambda phage infection. *E. coli* cells were separated according to their size and volume and each fraction was infected separately. The authors observed a strong correlation between the cell size and the phage developmental mode: large cells became lysogenic, whereas small ones were prompted to lysis (29). In order to look at individual cells, a fluorescent version of the lambda

phage was then used (lambda *A*am19 *b*::*GFP c*I857) and the probability of lysogeny (or lysis) was determined as a function of the volume of individual wild-type cells grown to stationary phase (small cells) or of a high frequency of lysogeny mutant grown in a defined M9-based medium and infected during exponential growth phase (large cells). As before, a strong correlation was observed between cell size and fate shorter cells becoming lysogens with a higher frequency than longer cells. It remains however unclear what is the parameter important for the lysis-lysogeny decision: the volume of the cell, and thus the local concentration of cellular factors important for the decision, or the fact that large cells have a higher metabolic activity which could represent favorable conditions for virion synthesis. However, as mentioned by the authors, the correlation is not absolute and some cells divided giving rise to two cells with different fate indicating that preexisting variations are not sufficient to explain the post-infection decision. Therefore, space is left for some intrinsic noise in the decision (29).

Elucidation of the lysis-lysogeny decision was recently further developed by L. Zeng and collaborators. They analyzed the decision making at the level of individual phages rather than individual cells (31). In this work, the authors look at individual phages by using mosaic phages expressing a gpD-EYFP protein variant. Phages expressing the fluorescent gpD protein are able to propagate just as regular phages (32), and the enrichment in fluorescent molecules per phage particle increased the resolution sensitivity of epifluorescence detection allowing to observe single phage particles. Importantly, the lysis-lysogeny decision was also unaffected (31). The results obtained by Zeng and collaborators are slightly divergent compared to the studies mentioned above. Indeed, they confirm that the host cell size is important in the whole cell decision making; however, they conclude that location of phage infection is important for the success rate of infection rather than for cell fate. Indeed, if infection occurs elsewhere than at the cell pole, phage DNA is not efficiently translocated into the cell probably because the ManY protein is localizes preferentially at the cell pole. More importantly, in this paper, a new model is being proposed that implies that decision making is made at the subcellular level and that individual phages independently choose between lysis and lysogeny, a decision that is dependent on the viral concentration inside the cell. A straightforward demonstration involves the integration of individual sharp decisions through an "AND" gate, meaning that lysogeny is possible only when all viruses that have entered the cell choose the lysogenic way, which is a condition more difficult to reach when the intracellular phage content increases. Another implication of this study is that subcellular decision making is most probably affected by cellular factors local concentrations, as it is the case for ManY and FtsH, thus linking the physical parameters (volume of the host cell and localization of infection) to the individual decision of viruses into the cell.

4. REPLICATION AND TRANSCRIPTION

4.1. Phi29 replication

Phage Phi29, which infects *Bacillus subtilis,* is the paradigm of double-stranded DNA viruses infecting Gram + bacteria (33–35). Replication of Phi29 involves a protein primed mechanism extensively studied by M. Salas and collaborators (36–39). An important question that was addressed by using epifluorescence techniques concerns the association of Phi29 replication with the host cell replication factories. Indeed, it was found that replication sites of the viral genome are different from the host replication sites and that Phi29 replication sites are redistributed at different stages of the infection cycle (40). Post-injection, Phi29 DNA is located at one end of the nucleoid where the first rounds of replication occur. Later on, during the infection cycle, Phi29 DNA replication expands to occupy multiple sites around the nucleoid. This process involves the viral gene 16.7, which is transcribed earlier and encodes a membrane localized DNA binding protein (41). Indeed, phage DNA replication is delayed in *B. subtilis* cells that do not express gene 16.7 (40). When Phi29 DNA replication ends, phage DNA accumulates within the bulk of the host cell's nucleoid.

The combination of host cell biology studies to Phi29 cycle also pointed to relationships between Phi29 replication and the host cytoskeleton. MreB, the bacterial actine-like protein discovered in *B. subtilis* (42–44), was found to organize Phi29 replication at the membrane through a direct interaction with p16.7 (45). Indeed, Phi29 DNA replication requires a functional MreB and Phi29 DNA polymerase localizes in a helix-like pattern that is MreB dependant (Figure 1A). Phage DNA replication and thus progeny synthesis is totally abolished in an *mreB* mutant, while the early steps of phage infection such as phage adsorption and DNA entrance are unaffected. It was found that MreB colocalizes and interacts with p16.7. Therefore, this study demonstrates that Phi29 phage exploits the MreB cytoskeleton in order to replicate its genome. The terminal protein TP is associated to the membrane and is required for efficient Phi29 replication. TP recruits Phi29 DNA polymerase to the bacterial nucleoid, and subsequently, both proteins relocalize to a peripheral helix-like structure that is MreB dependant. TP binds directly to the bacterial nucleoid via its N-terminal moiety. However, no sequence specificity was observed since TP can bind any DNA sequence (46). This study suggests that Phi29 DNA attaches to the host nucleoid and uses the MreB cytoskeleton as a driving force to redistribute at peripheral sites.

4.2. *Cystoviridae* transcription

Phi6 phage and related phages that belong to the *Cystoviridae* family have segmented genomes composed of three molecules of double-stranded RNA (S, M, and L dsRNA) (47,48). Phi6 propagates in *Pseudomonas syringae*, a well-described plant pathogen; an interesting particularity of this phage and its relatives is the ordered process of packaging of the three moieties of the genome (49).

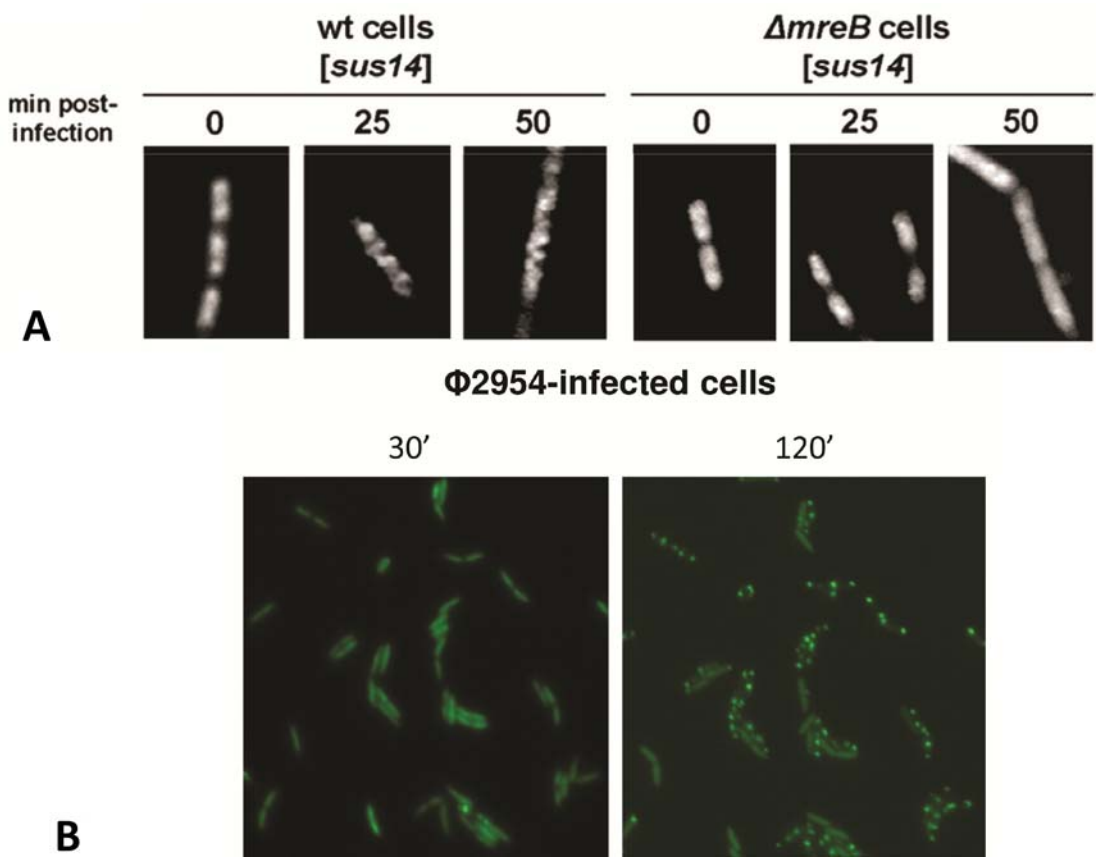

Figure 1. (A), Replication of Phi29 phage, adapted with permission from (45). *B. subtilis* strains (wild-type and *mreB*) expressing YFP-TP infected with a delayed-lysis mutant of Phi29 (*sus14*). The TP protein recruits the phage DNA polymerase and localizes in a helix-like shape that is MreB dependant. (B), Control of transcription of bacteriophage Phi2954, adapted with permission from (52). Relocalization of the host protein GrxC-GFP in *Pseudomonas syringae pv. Phaseolicola* HB10Y upon Phi2954 infection (30' and 120' post-infection).

The L fragment encodes earlier expressed core particle proteins, whereas the S and M segment contain genes expressed later during the infection process (47). Transcription of the L fragment and productive infection are dependent on the host protein YajQ, which binds to the major nucleocapsid protein P1 and modify the transcriptional activity of the polymerase located inside the virion (50). When the phage genome enters the host cell, the core polymerase covered by YajQ transcribes the genomic segment L. A surprising finding is that YajQ-dependant replication of phage Phi6 provokes a punctuate fluorescence pattern of YajQ upon phage infection (51). In this case, GFP fusions showed that YajQ is associated with virions through capsid component binding from the incoming step until lysis, which is consistent with the recycling process of parts of the phage virions. Indeed, YajQ binds to P1 the major structural protein of the inner core, and this interaction is not disrupted during the synthesis of newly made phage particles, indicating that P1 is recycled into the progeny.

This result has to be correlated with the recent finding that GrxC is involved in transcription regulation of the L segment of Phi2954, a Phi6 distantly related phage, by removing the shell formed by the P8 protein from the infectious particle (52). As demonstrated *in vitro*, incubation of GrxC with the nucleocapsid, results in the loss of the P8 protein and subsequent binding of GrxC to core particles. As a result, the GrxC protein exhibits a punctuate fluorescence pattern upon Phi2954 infection (Figure 1B), as is the case for YajQ when cells are infected with Phi6. Interestingly, Phi2954 mutants that are independent of GrxC *in vivo* show undifferentiated transcription of all three DNA segments. One such mutant contains a single base change at the 5' end of the L segment (ACAAA to GCAAA) that leads to L segment transcription at the same level than S and M and independently of GrxC, probably allowing the polymerase to bind to this segment with a higher affinity (52).

Thus, in both phages, transcription of the L segment relies on the presence of host proteins, YajQ for Phi6 and GrxC for Phi2954, which remain associated to the core virion throughout the virus cycle. Newly assembled particles do not bind YajQ or GrxC and consequently only S and M segments are transcribed. It is worthnoting that Phi6 genome transcription is independent of GrxC, and in turn Phi2954 does not require YajQ. Although the cellular

function of YajQ remains unknown, the GrxC protein is glutaredoxine C, which harbors ribonucleotide reductase activity, however it remains unclear if this activity is involved in Phi2954 transcription. In both systems, the specific interaction of the nucleocapsid with a host protein results in specific L segment transcription, which is correlated with a punctuated pattern location of the host proteins.

5. PERSPECTIVES

Since their discovery in the 1920s, phages have been used as tools. Phages have several direct applications, such as therapy (more widely used in the former Eastern bloc but this has recently begun to change), recombination in prokaryotic and eukaryotic cells, as well as phage display (for review see (53)). However, being able to visualize individual phages permits researchers to look at and to discriminate the host cell. Indeed, several attempts have been made to produce phage-based detectors, mostly for the purpose of pathogenic strain identification. In particular, phage-based detectors directed against *E. coli* O157:H7 and several species of mycobacteria, including TB, have been developed recently (54–56). The advantages of these techniques are their rapidity and specificity, as well as the detection of living bacteria that can support phage adsorption and progeny synthesis. It is likely that this kind of tool will develop in the near future to detect pathogens.

Phage-host interaction studies benefited from the implementation of cell biology techniques in several ways. Indeed, several long studied aspects of phage biology gained insight from these studies; the lysis-lysogeny decision of temperate phages exemplified by lambda is one of them. However, new questions can now be addressed, such as the localization of phage factories inside the host cell. It is noteworthy that phages, such as Phi29, develop with respect to host cell architecture and organization. As genomics have led to the third age of the phage (57), it is likely that the combination of phage genomics with cell biology may lead to a fourth one. An important implication of the studies described in this review is that cell biology of the host enable to understand phage biology, and in turn sometimes shed light on host proteins whose function in the host is poorly understood such as GrxC. Other phage related functions may beneficiate from cell biology studies, in particular, it has been shown for decades that the GroE chaperonine plays a key role in lambda virions assembly, and cell biology may give new insight on the lambda capsid assembly in association with the GroES proteins (6). Since phages rely on their host to replicate, either passively for lysogenic phages or actively when lysis is the outcome, the relationship between host proteins localization and dynamics and virus cycle should be further studied in the future.

6. ACKNOWLEDGMENTS

The author thanks all present and past members of the phage group for stimulating discussions and CNRS, French Research Ministry and ANR (ANR-08-BLAN-0122-01) grants for supporting our research.

7. REFERENCES

1. M. Thanbichler and L. Shapiro: Getting organized--how bacterial cells move proteins and DNA. *Nat.Rev.Microbiol.* 6, 28-40 (2008)

2. S. Jun and A. Wright: Entropy as the driver of chromosome segregation. *Nat.Rev.Microbiol.* 8, 600-607 (2010)

3. J. Salje, P. Gayathri and J. Lowe: The ParMRC system: molecular mechanisms of plasmid segregation by actin-like filaments. *Nat.Rev.Microbiol.* 8, 683-692 (2010)

4. K. Gerdes, M. Howard and F. Szardenings: Pushing and pulling in prokaryotic DNA segregation. *Cell* 141, 927-942 (2010)

5. E. M. Mauriello, T. Mignot, Z. Yang and D. R. Zusman: Gliding motility revisited: how do the myxobacteria move without flagella? *Microbiol.Mol.Biol.Rev.* 74, 229-249 (2010)

6. C. Georgopoulos: Toothpicks, serendipity and the emergence of the *Escherichia coli* DnaK (Hsp70) and GroEL (Hsp60) chaperone machines. *Genetics* 174, 1699-1707 (2006)

7. K. Hertveldt, R. Lavigne, E. Pleteneva, N. Sernova, L. Kurochkina, R. Korchevskii, J. Robben, V. Mesyanzhinov, V. N. Krylov and G. Volckaert: Genome comparison of Pseudomonas aeruginosa large phages. *J.Mol.Biol.* 354, 536-545 (2005)

8. M. Ptashne - A genetic switch: phage lambda revisited.Cold Spring Harbor Laboratory Press Cold Spring Harbor (2004)

9. R. Edgar, A. Rokney, M. Feeney, S. Semsey, M. Kessel, M. B. Goldberg, S. Adhya and A. B. Oppenheim: Bacteriophage infection is targeted to cellular poles. *Mol.Microbiol.* 68, 1107-1116 (2008)

10. R. Edgar, M. McKinstry, J. Hwang, A. B. Oppenheim, R. A. Fekete, G. Giulian, C. Merril, K. Nagashima and S. Adhya: High-sensitivity bacterial detection using biotin-tagged phage and quantum-dot nanocomplexes. *Proc.Natl.Acad.Sci.U.S.A* 103, 4841-4845 (2006)

11. L. Randall-Hazelbauer and M. Schwartz: Isolation of the bacteriophage lambda receptor from *Escherichia coli*. *J.Bacteriol.* 116, 1436-1446 (1973)

12. T. Schirmer, T. A. Keller, Y. F. Wang and J. P. Rosenbusch: Structural basis for sugar translocation through maltoporin channels at 3.1 A resolution. *Science* 267, 512-514 (1995)

13. K. A. Gibbs, D. D. Isaac, J. Xu, R. W. Hendrix, T. J. Silhavy and J. A. Theriot: Complex spatial distribution and dynamics of an abundant *Escherichia coli* outer membrane protein, LamB. *Mol.Microbiol.* 53, 1771-1783 (2004)

14. D. Scandella and W. Arber: An *Escherichia coli* mutant which inhibits the injection of phage lambda DNA. *Virology* 58, 504-513 (1974)

15. J. Elliott and W. Arber: *E. coli* K-12 *pel* mutants, which block phage lambda DNA injection, coincide with *ptsM*, which determines a component of a sugar transport system. *Mol.Gen.Genet.* 161, 1-8 (1978)

16. N. Williams, D. K. Fox, C. Shea and S. Roseman: Pel, the protein that permits lambda DNA penetration of *Escherichia coli*, is encoded by a gene in *ptsM* and is required for mannose utilization by the phosphotransferase system. *Proc.Natl.Acad.Sci.U.S.A* 83, 8934-8938 (1986)

17. F. Huber and B. Erni: Membrane topology of the mannose transporter of *Escherichia coli* K12. *Eur.J.Biochem.* 239, 810-817 (1996)

18. M. E. Gottesman and R. A. Weisberg: Little lambda, who made thee? *Microbiol.Mol.Biol.Rev.* 68, 796-813 (2004)

19. A. B. Oppenheim, O. Kobiler, J. Stavans, Court DL and S. Adhya: Switches in bacteriophage lambda development. *Annu.Rev.Genet.* 39, 409-429 (2005)

20. D. Santos and D. F. De Almeida: Isolation and characterization of a new temperature-sensitive cell division mutant of *Escherichia coli* K-12. *J.Bacteriol.* 124, 1502-1507 (1975)

21. M. M. Jayasekera, S. K. Foltin, E. R. Olson and T. P. Holler: *Escherichia coli* requires the protease activity of FtsH for growth. *Arch.Biochem.Biophys.* 380, 103-107 (2000)

22. C. Herman, D. Thevenet, R. D'Ari and P. Bouloc: Degradation of sigma 32, the heat shock regulator in *Escherichia coli*, is governed by HflB. *Proc.Natl.Acad.Sci.U.S.A* 92, 3516-3520 (1995)

23. C. Herman, D. Thevenet, R. D'Ari and P. Bouloc: The HflB protease of *Escherichia coli* degrades its inhibitor lambda cIII. *J.Bacteriol.* 179, 358-363 (1997)

24. Y. Shotland, A. Shifrin, T. Ziv, D. Teff, S. Koby, O. Kobiler and A. B. Oppenheim: Proteolysis of bacteriophage lambda CII by Escherichia coli FtsH (HflB). *J.Bacteriol.* 182, 3111-3116 (2000)

25. G. G. Leffers, Jr. and S. Gottesman: Lambda Xis degradation *in vivo* by Lon and FtsH. *J.Bacteriol.* 180, 1573-1577 (1998)

26. P. Kourilsky: Lysogenization by bacteriophage lambda. I. Multiple infection and the lysogenic response. *Mol.Gen.Genet.* 122, 183-195 (1973)

27. P. Kourilsky: Lysogenization by bacteriophage lambda. II. Identification of genes involved in the multiplicity dependent processes. *Biochimie* 56, 1511-1516 (1974)

28. P. Kourilsky and A. Knapp: Lysogenization by bacteriophage lambda. III. Multiplicity dependent phenomena occuring upon infection by lambda. *Biochimie* 56, 1517-1523 (1974)

29. F. St-Pierre and D. Endy: Determination of cell fate selection during phage lambda infection. *Proc.Natl.Acad.Sci.U.S.A* 105, 20705-20710 (2008)

30. A. Arkin, J. Ross and H. H. McAdams: Stochastic kinetic analysis of developmental pathway bifurcation in phage lambda-infected *Escherichia coli* cells. *Genetics* 149, 1633-1648 (1998)

31. L. Zeng, S. O. Skinner, C. Zong, J. Sippy, M. Feiss and I. Golding: Decision making at a subcellular level determines the outcome of bacteriophage infection. *Cell* 141, 682-691 (2010)

32. L. J. Alvarez, P. Thomen, T. Makushok and D. Chatenay: Propagation of fluorescent viruses in growing plaques. *Biotechnol.Bioeng.* 96, 615-621 (2007)

33. W. J. Meijer, J. A. Horcajadas and M. Salas: Phi29 family of phages. *Microbiol.Mol.Biol.Rev.* 65, 261-287 (2001)

34. J. L. Carrascosa, E. Vinuela and M. Salas: Proteins induced in *Bacillus subtilis* infected with bacteriophage phi29. *Virology* 56, 291-299 (1973)

35. M. R. Inciarte, J. M. Lazaro, M. Salas and E. Vinuela: Physical map of bacteriophage phi29 DNA. *Virology* 74, 314-323 (1976)

36. M. Salas: A new mechanism for the initiation of replication of phi 29 and adenovirus DNA: priming by the terminal protein. *Curr.Top.Microbiol.Immunol.* 109, 89-106 (1984)

37. M. Salas: Initiation of DNA replication by primer proteins: bacteriophage phi 29 and its relatives. *Curr.Top.Microbiol.Immunol.* 136, 71-88 (1988)

38. M. Salas: Protein-priming of DNA replication. *Annu.Rev.Biochem.* 60, 39-71 (1991)

39. L. Blanco, J. M. Lazaro, V. M. de, A. Bonnin and M. Salas: Terminal protein-primed DNA amplification. *Proc.Natl.Acad.Sci.U.S.A* 91, 12198-12202 (1994)

40. W. J. Meijer, P. J. Lewis, J. Errington and M. Salas: Dynamic relocalization of phage phi 29 DNA during replication and the role of the viral protein p16.7. *EMBO J.* 19, 4182-4190 (2000)

41. W. J. Meijer, A. Serna-Rico and M. Salas: Characterization of the bacteriophage phi29-encoded protein p16.7: a membrane protein involved in phage DNA replication. *Mol.Microbiol.* 39, 731-746 (2001)

42. R. Carballido-Lopez and J. Errington: A dynamic bacterial cytoskeleton. *Trends Cell Biol.* 13, 577-583 (2003)

43. L. J. Jones, R. Carballido-Lopez and J. Errington: Control of cell shape in bacteria: helical, actin-like filaments in Bacillus subtilis. *Cell* 104, 913-922 (2001)

44. R. A. Daniel and J. Errington: Control of cell morphogenesis in bacteria: two distinct ways to make a rod-shaped cell. *Cell* 113, 767-776 (2003)

45. D. Munoz-Espin, R. Daniel, Y. Kawai, R. Carballido-Lopez, V. Castilla-Llorente, J. Errington, W. J. Meijer and M. Salas: The actin-like MreB cytoskeleton organizes viral DNA replication in bacteria. *Proc.Natl.Acad.Sci.U.S.A* 106, 13347-13352 (2009)

46. D. Munoz-Espin, I. Holguera, D. Ballesteros-Plaza, R. Carballido-Lopez and M. Salas: Viral terminal protein directs early organization of phage DNA replication at the bacterial nucleoid. *Proc.Natl.Acad.Sci.U.S.A* 107, 16548-16553 (2010)

47. L. Mindich: Bacteriophage phi 6: a unique virus having a lipid-containing membrane and a genome composed of three dsRNA segments. *Adv.Virus Res.* 35, 137-176 (1988)

48. L. Mindich, X. Qiao, J. Qiao, S. Onodera, M. Romantschuk and D. Hoogstraten: Isolation of additional bacteriophages with genomes of segmented double-stranded RNA. *J.Bacteriol.* 181, 4505-4508 (1999)

49. L. Mindich: Packaging, replication and recombination of the segmented genome of bacteriophage Phi6 and its relatives. *Virus Res.* 101, 83-92 (2004)

50. X. Qiao, Y. Sun, J. Qiao and L. Mindich: The role of host protein YajQ in the temporal control of transcription in bacteriophage Phi6. *Proc.Natl.Acad.Sci.U.S.A* 105, 15956-15960 (2008)

51. X. Qiao, Y. Sun, J. Qiao and L. Mindich: Interaction of a host protein with core complexes of bacteriophage phi6 to control transcription. *J.Virol.* 84, 4821-4825 (2010)

52. J. Qiao, X. Qiao, Y. Sun and L. Mindich: Role of host protein glutaredoxin 3 in the control of transcription during bacteriophage Phi2954 infection. *Proc.Natl.Acad.Sci.U.S.A* 107, 6000-6004 (2010)

53. Phages, their role in bacterial pathogenesis and biotechnology.Waldor, M. K., Friedman, D. I., and Adhya, S. ASM Press Washington, DC (2005)

54. T. Funatsu, T. Taniyama, T. Tajima, H. Tadakuma and H. Namiki: Rapid and sensitive detection method of a bacterium by using a GFP reporter phage. *Microbiol.Immunol.* 46, 365-369 (2002)

55. Bardarov S Jr, H. Dou, K. Eisenach, N. Banaiee, S. Ya, J. Chan, W. R. Jacobs, Jr. and P. F. Riska: Detection and drug-susceptibility testing of M. tuberculosis from sputum samples using luciferase reporter phage: comparison with the Mycobacteria Growth Indicator Tube (MGIT) system. *Diagn.Microbiol.Infect.Dis.* 45, 53-61 (2003)

56. S. Ripp: Bacteriophage-based pathogen detection. *Adv.Biochem.Eng Biotechnol.* 118, 65-83 (2010)

57. N. H. Mann: The third age of phage. *PLoS.Biol.* 3, e182 (2005)

Key Words: Bacteriophage cycle, Cell Biology, Lysogeny, Viral Replication, Viral Transcription, Review

Send correspondence to: Mireille Ansaldi, Laboratoire de Chimie Bacterienne, CNRS-UPR9043, Institut de Microbiologie de la Mediterranee, Aix-Marseille Universite, 31 Chemin Joseph Aiguier, 13402, Marseille cedex 20, France, Tel 33-4-91164585, Fax : 33-4-91718914, E-mail: ansaldi@ifr88.cnrs-mrs.fr

http://www.bioscience.org/current/vol4E.htm