Virology ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Review

Bacteriophage lambda: Early pioneer and still relevant

Sherwood R. Casjens [a,b,*], Roger W. Hendrix [c]

[a] Division of Microbiology and Immunology, Pathology Department, University of Utah School of Medicine, Emma Eccles Jones Medical Research Building, 15 North Medical Drive East, Salt Lake City, UT 84112, USA
[b] Biology Department, University of Utah, Salt Lake City, UT 84112, USA
[c] Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, USA

ARTICLE INFO

Article history:
Received 9 December 2014
Returned to author for revisions
13 January 2015
Accepted 5 February 2015

Keywords:
Bacteriophage
Lambda
Lambdoid phages

ABSTRACT

Molecular genetic research on bacteriophage lambda carried out during its golden age from the mid-1950s to mid-1980s was critically important in the attainment of our current understanding of the sophisticated and complex mechanisms by which the expression of genes is controlled, of DNA virus assembly and of the molecular nature of lysogeny. The development of molecular cloning techniques, ironically instigated largely by phage lambda researchers, allowed many phage workers to switch their efforts to other biological systems. Nonetheless, since that time the ongoing study of lambda and its relatives has continued to give important new insights. In this review we give some relevant early history and describe recent developments in understanding the molecular biology of lambda's life cycle.

Contents

Introduction

From the earliest studies on lambda genetics by Jean Weigle (1953), Francois Jacob and Elie Wollman (1953, 1954) and Dale Kaiser (1955 in volume number 1 of VIROLOGY) in the 1950s until it helped usher in the age of genetic engineering in the late 1970s and early 1980s, phage lambda was in its golden age at the center of the

* Corresponding author at: Division of Microbiology and Immunology, Pathology Department, University of Utah School of Medicine, Emma Eccles Jones Medical Research Building, 15 North Medical Drive East, Salt Lake City, UT 84112, USA. Tel.: +1 801 581 5980.
E-mail address: sherwood.casjens@path.utah.edu (S.R. Casjens).

http://dx.doi.org/10.1016/j.virol.2015.02.010
0042-6822/

molecular genetic universe. During those decades lambda was one of the few experimental "organisms" that was sufficiently experimentally accessible to be viewed as potentially completely understandable. Its genome was small enough not to be overwhelming, yet it was capable of making a decision of whether to lysogenize or grow lytically and was clearly complex enough to be interesting and informative on many fronts. In particular it was unique in that a genetic and molecular understanding of the control of gene expression seemed to be within almost immediate reach. Those of us lucky enough to be involved in the study of phage lambda during this time were tremendously excited about future possibilities and threw ourselves into the work with all the energy we had.

Lambda was originally discovered in 1951 by Esther Lederberg (1951) at the University of Wisconsin (Madison), when she serendipitously found it was released from the laboratory *Escherichia coli* strain K-12 after ultraviolet irradiation. Since that time all aspects of lambda's lytic and lysogenic lifestyles have been studied. Lambda became a more important and approachable experimental system when Allan Campbell (1961) isolated a set of suppressible nonsense mutants of lambda (or *amber* mutations, originally called suppressor sensitive or *sus* mutations) that identified genes essential for its lytic growth. Additional *amber* mutations isolated by Sandy Parkinson (1968) and Goldberg and Howe (1969) and prophage mutations isolated by Clarence Fuerst (Mount et al., 1968) that were lethal for lambda lytic growth identified a few more essential genes. Genetic complementation tests of these mutations succeeded in defining all but one of lambda's 29 genes that are essential for plaque formation under laboratory conditions (this includes the frameshifted *G* and *G–T* gene pair and the alternate starts of the *S105* and *S107* genes as well as the *C* and *Nu3* genes as two genes each; see below). We note that two additional genes, *Rz* and *Rz1* should probably also be considered essential genes even though plaques often form in their absence. They are absolutely required for disruption of the outer membrane and lysis unless external physical solution forces that are often present during laboratory growth help to destabilize the outer membrane of infected cells (Berry et al., 2012). Only the small *cro* gene was not found by the above random mutant hunts. It was discovered during the genetic analysis of the control of lysogeny (Eisen et al., 1970), and its absence leads to the lysogenic state and hence no lytic growth. Recombination frequency and deletion mapping of these and various promoter and operator mutations isolated by others during this time allowed the construction of a detailed genetic map. Analysis of the phage DNA molecule led to the most detailed physical map of any organism's genome at the time. Detailed study of the phenotypes of phage mutants defective in known, mapped genes gave an early overall picture of the lambda life cycle. Indeed, the careers of both authors here started at about the same time with the analysis of lambda virion morphogenesis by utilizing the various *amber* mutants to identify the proteins expressed from the lambda morphogenetic genes and determining the nature of the defects in their absence – S.R.C. in the laboratory of Dale Kaiser at Stanford University and R.W.H. in the laboratory of Jim Watson at Harvard University (Happily, rather than generating animosity, our early competition in this arena led to mutual respect and a lifelong friendship). We believe that the clever use of forward genetic selections and screens to isolate informative mutations and combinations of mutations and the use of these molecular genetic experiments to deduce the underlying molecular mechanisms reached its zenith in the study of phage lambda during this period. Those were heady days with lambda running at the front of the scientific pack.

We discuss here early experiments that led to our current understanding of phage lambda and molecular biological processes in general. During this period VIROLOGY published many of these important findings. We also mention recent developments to show how far the field has come in the past 60 years (we assume a basic knowledge of the phage lambda life cycle; see Hendrix et al., 1983; Hendrix and Casjens, 2006). We include discussion of phages related to lambda, which are commonly referred to as "lambdoid" phages. This ill-defined (and often incorrectly used) term refers phages with very similar lifestyles to lambda and genomes that are mosaically related to lambda. This definition included the notion that a lambdoid phage is capable of recombination with lambda itself to produce a functional hybrid phage, as was first shown with phage 434 by Kaiser and Jacob (1957). Recent phage genome sequences have caused an expansion of this term to mean a phage with the same functional gene order as lambda and that carries patches of nucleotide sequence homology with lambda or another lambdoid phage. Thus, in theory a single recombination event between lambdoid phages could give rise to a fully functional phage that has all the necessary genes (Hendrix, 2002; Casjens, 2005; Hendrix and Casjens, 2006; Grose and Casjens, 2014). In this discussion we use lambdoid to include for example, the three phages HK97, P22 and N15, which typify three of the best-studied lambdoid groups that have significant differences from lambda. Citations are in general not meant to bestow credit for the original discoveries but to allow the reader access to the literature. Other more detailed historical treatments of some of the topics covered below can be found in the books *Bacteriophage lambda* (Hershey, 1971) and *Lambda II* (Hendrix et al., 1983) (and in Gottesman and Weisberg, 2004; Stahl, 2005; Georgopoulos, 2006; Campbell, 2007; Court et al., 2007a).

Historical importance and recent progress

The lambda genome

Phage lambda DNA was one of the earliest model systems for studying the physical nature of DNA and genes, and a substantial amount of early research examined the hydrodynamic properties of the lambda chromosome with the goal of, among other things, determining its absolute molecular weight. At the same time methods (now considered to be primitive) were devised for separating the two halves of the lambda chromosome and for mapping genes identified genetically to these halves and to smaller physical intervals (reviewed by Davidson and Szyblaski, 1971). Soon thereafter electron microscopic analysis of absolute length and of lambda DNA in heteroduplex with various altered lambda DNAs gave rise to a major step forward, the construction of a detailed physical map of lambda DNA – a gene map in base pairs (bps) rather than recombination frequency units (Fiandt et al., 1971; Simon et al., 1971). The lambda virion chromosome was also among the first natural linear DNAs whose end structure was understood in detail; it was found not to have blunt ends, but to have complementary 12 bp protruding 5′-ends called cohesive (or sticky) ends, since the two single-strand ends can pair and thus bind to each other (Hershey et al., 1963; Hershey and Burgi, 1965). Meanwhile, lambda contributed greatly to the ultimate mapping of DNA, the complete nucleotide sequence. It is not generally appreciated that the 12 bp lambda cohesive ends were the subject of the first direct nucleotide sequencing of a biological DNA. Ray Wu devised methodology for using DNA polymerase to fill in the cohesive ends with specifically labeled nucleotides, followed by analysis of the product to determine this exact 12 bp nucleotide sequence (Wu and Kaiser, 1968; Onaga, 2014). This sequencing was not a trivial undertaking, as determining this 0.012 kbp of sequence required several years of painstaking work. Fourteen years later, Fred Sanger et al. (1982) used lambda as the subject for the first determination of the complete genome sequence of a dsDNA virus – 48,502 bp – using his dideoxynucleotide chain termination

Please cite this article as: Casjens, S.R., Hendrix, R.W., Bacteriophage lambda: Early pioneer and still relevant. Virology (2015), http://dx.doi.org/10.1016/j.virol.2015.02.010