Progress in Biophysics and Molecular Biology 114 (2014) 80–122

Contents lists available at ScienceDirect

Progress in Biophysics and Molecular Biology

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

Review

Structure and assembly of filamentous bacteriophages

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ARTICLE INFO

Article history:
Available online 28 February 2014

Keywords:
Fibre diffraction
Inovirus
Membrane transport
Phage display
Phyllotaxis
Solid-state NMR

ABSTRACT

Filamentous bacteriophages are interesting paradigms in structural molecular biology, in part because of the unusual mechanism of filamentous phage assembly. During assembly, several thousand copies of an intracellular DNA-binding protein bind to each copy of the replicating phage DNA, and are then displaced by membrane-spanning phage coat proteins as the nascent phage is extruded through the bacterial plasma membrane. This complicated process takes place without killing the host bacterium.

The bacteriophage is a semi-flexible worm-like nucleoprotein filament. The virion comprises a tube of several thousand identical major coat protein subunits around a core of single-stranded circular DNA. Each protein subunit is a polymer of about 50 amino-acid residues, largely arranged in an α-helix. The subunits assemble into a helical sheath, with each subunit oriented at a small angle to the virion axis and interdigitated with neighbouring subunits. A few copies of "minor" phage proteins necessary for infection and/or extrusion of the virion are located at each end of the completed virion.

Here we review both the structure of the virion and aspects of its function, such as the way the virion enters the host, multiplies, and exits to prey on further hosts. In particular we focus on our understanding of the way the components of the virion come together during assembly at the membrane. We try to follow a basic rule of empirical science, that one should chose the simplest theoretical explanation for experiments, but be prepared to modify or even abandon this explanation as new experiments add more detail.

Contents

Abbreviations: CS, chemical shift; MAS, magic angle spinning; PISEMA, polarization inversion spin exchange at the magic angle; PISA, polarity index slant angle; nt/su, DNA nucleotides per protein subunit; ORF, open reading frame; TB, TolA-C binding domain of p3 protein; PB, pilus-binding domain of p3 protein; LC, low-complexity domain of p3 protein; ICS, infection-competence segment of p3 protein; Tol, TolA-C; K69H, residue K at position 69 changed to H, etc.; FTIR, Fourier-transform infra-red; UVRR, ultraviolet resonance-Raman; CD, circular dichroism; FRET, Förster (or fluorescence) resonance energy transfer; IHRSR, iterative helical real space reconstruction; RF, replicative form; rmsd, root-mean-square deviation; OB-fold, oligomer-binding-fold; RPA70, human replication protein A.

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http://dx.doi.org/10.1016/j.pbiomolbio.2014.02.003
0079-6107/

1. Introduction

1.1. Early days

The filamentous bacteriophage has only a few genes and is one of the simplest biological systems known. In the half-century since it was first identified, there has been an explosion in understanding the molecular basis of the phage life cycle; and a parallel explosion in applications of the phage system to biotechnology and nanotechnology. In this extended Introduction we outline the development of the field, including a few examples of the false starts and accidental discoveries that are not usually discussed in a formal review and that illustrate Max Delbrück's "principle of controlled sloppiness", sometimes phrased as the motto "fail early to succeed sooner". We also illustrate in passing how the experimental and computational techniques of fibre diffraction and solid-state NMR have improved over the last 50 years.

In the late 1950s and early 1960s, interest developed in "small" phages, so-called because their genome is an order of magnitude smaller than the classical tadpole-shaped T2 and T4 phages that were then widely studied: it was thought that the small phages might be simpler and easier to understand (Sinsheimer, 1966). A few of these small phages were characterized as isometric (roughly spherical) in shape, with a diameter of about 250 Å. They attracted interest not only because of their small size, but also because some of them had a single-stranded DNA genome (for instance φX174); and some of them had a single-stranded RNA genome (for instance MS2 and f2) and were specific for male (F+ or Hfr) strains of bacteria, which can transfer DNA to other bacteria. Several labs at this time isolated small male-specific phages without characterizing them other than to show that they had only a few genes.

The first phage characterized as filamentous, rather than isometric, was fd. Hartmut Hoffmann-Berling at the Max-Planck Institute in Heidelberg became interested in small male-specific phages, and on a family outing to a favourite country restaurant, the Bierhelder Hof, he collected from the dung heap on the adjacent cattle farm a small sample which he took back to his lab. He selected for further study two plaque-formers on Hfr *Escherichia coli*: one, containing DNA, he named fd, and the other, containing RNA, he named fr. The fr phage was structurally similar to previously characterized isometric RNA phages. But fd phage preparations showed mixtures of elongated filamentous particles and isometric particles in electron micrographs, and this mixture was initially interpreted as contaminating bacterial pili among putative isometric phage. However, infectivity of these preparations was far more sensitive to ultrasonication than control preparations of similar-sized isometric phage, as expected if the elongated particles are in fact the phage (Fig. 1). Physical–chemical characterization confirmed the general features of filamentous fd phage and its DNA (Marvin and Hoffmann-Berling, 1963a,b). Other researchers looked at their own small phages, which they had thought to be isometric, and some of these turned out to be filamentous phages as well, notably f1, which was isolated at the same time as the isometric RNA phage f2, but not further characterized at the time (Loeb and

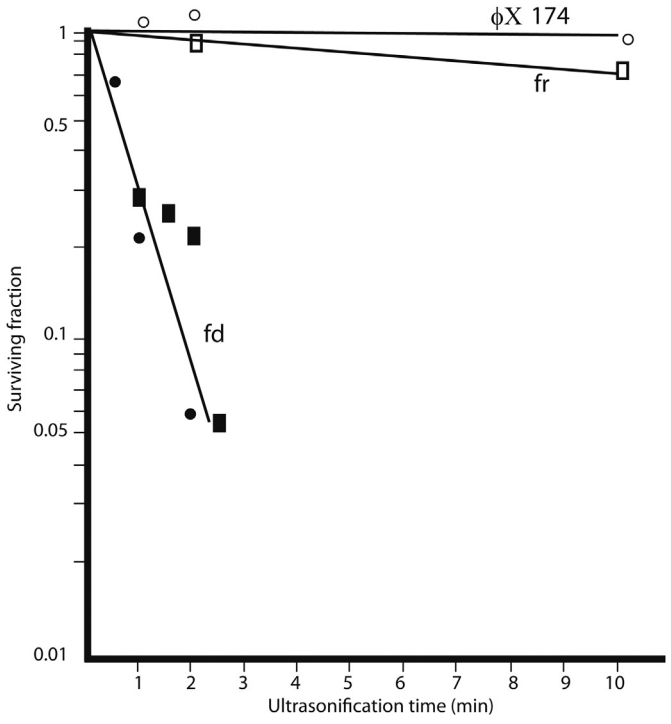

Fig. 1. Inactivation of fd filamentous phage by ultrasonication, relative to isometric phage of similar size. Mixtures of fd with control phage (either fr or φX174), 10^{11} to 10^{12} plaque-forming units/ml in each case, were sonicated for the indicated time and plated on an F+ strain of *E. coli* C (which is sensitive to all three phages). The different phages could be distinguished by their different plaque types. Circles, fd mixed with φX174; rectangles, fd mixed with fr. Filled symbols, fd; open symbols, control phage. First reported in *Nature* on 2 February 1963 (Marvin and Hoffmann-Berling, 1963a).