9.1 Introduction to phage M13

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1) In order to understand the phage display system, I will introduce you to phage M13, which is the most commonly used phage in phage display systems.
2) M13 is a F-specific filamentous phage, which uses E.coli as a host.
   2.1 Typical for F-specific phages is that they infect their hosts by using the hosts F-pilus. This long retractable and extendable structure is normally used for attachment to and getting closer to other bacteria in a mating process called conjugation. It is only present in E.coli cells containing a Fertility plasmid, also called F-plasmid.
   2.2 However in this case the phage makes advantage of it by using it as an attachment point for infection. Here you can see how a filamentous phage like M13 is attached to this pilus.
   2.3 You can also see why they are called filamentous phages. As these phages occur as long flexible filaments.
3) The phage filaments are 7 nm in diameter and about 900 nm long. They exist of a protein mantle that encapsulates the DNA cargo. The mantle exists of 5 different proteins.
   3.1 The most abundant one is the gene 8 protein. About 2800 of these proteins are present along the entire filament. Together they cover nearly the entire phage genome and hence protect it from the environment. Both ends of the filament are decorated with each a different set of proteins.
   3.2 On the distal end, the gene 7 and gene 9 proteins can be found. These are essential when new filamentous virions are formed during infection. They recognize the phage DNA and initiate its packaging.
   3.3 At the proximal end gene 6 and gene 3 proteins are present. While the gene 6 protein mostly provides structural stability of the virion, the gene 3 protein is more exposed and is required for interaction with the F-pilus of target cells. It also aids in release of newly formed virions from the cell.
   3.4 The virions contain the whole phage genome in a single-stranded form. This small genome of around 6400 base pairs encodes all the genes necessary for successful infection.
   3.5 Apart from the structural proteins I just discussed, it also encodes proteins required for DNA replication and virion assembly. Additionally, it contains an intergenic region which does not code for proteins. However, it contains 2 origins of replications (ori), 1 for each possible direction of replication. These are required for replication of the genome. It also contains a packaging signal, which is recognized by the gene 7 and 9 protein complex to initiate the packaging.

   So now that you know what a filament looks like, let's get more into detail on how these filaments are formed.
4) New virions are formed during an infection with M13. A representation of such an infection is given here.

4.1 As mentioned before, M13 phage filaments are only able to infect E.coli which have a F-pilus. This is because they need the pilus recognize and enter the host. In an initial stage, a M13 filament recognizes and binds the pilus by the exposed gene 3 protein. When the pilus retracts, the phage will be dragged towards the cell surface and eventually enter the host. During this entry, the mantle is disassembled and the ssDNA will enter the cell.

4.2 Once inside, the DNA is made double stranded by host enzymes. The genome is then replicated to form new copies. These copies are used both for expression of the encoded genes and formation of additional genome copies.

4.3 Once phage proteins and new genomic copies are present, new viral particles will be formed. Newly formed DNA copies become protected by the gene 5 protein until the packaging signal is recognized by the gene 7 and 9 proteins. This initiates packaging of the phage genome starting from the distal end at a membrane complex consisting of various phage proteins.

4.4 Eventually new phage filaments are released into the environment through this membrane complex. This process does not kill the host cell, but is rather a chronical infection which allows a continuous shedding of new phage particles. These phage particles can then infect other hosts in the environment. Hence a single phage can amplify up to high amounts.

The low complexity, ease of amplification and exposed proteins of this phage make it ideal for the development of a phage display system. This will be discussed in part 2.