DNA ejection from bacteriophage T5 analysed by cryo-electron microscopy

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Tailed bacteriophages are complex macromolecular machineries that deliver their genome into the host cytoplasm while their capsid and tail remain bound to the cell surface. The ejection is triggered by the interaction of the phage tail with a specific bacterial receptor. For some species, like T5, the DNA ejection can be reconstituted *in vitro* by adsoption of the phage onto its purified receptor (FhuA for T5) [1]. Light scattering and fluorescence microscopy analyses of the T5 ejection process *in vitro* have revealed an original stepwise process [2, 3].

Here we will show how the ejection can be followed by cryo-electron microscopy, and analyse how this process reacts to an opposing external pressure. In the phage capsid, DNA is indeed highly pressurized, due to the confinement of the molecule into the small volume of the capsid. It has been hypothesised that this internal pressure is responsible for DNA release [4]. This hypothesis has been confirmed in the case of phage λ, showing that DNA ejection is directly related to the difference between osmotic pressure inside and outside the phage [5, 6].

Our results confirm the stepwise ejection of bacteriophage T5. We will analyse the intermediate steps of the process and discuss their possible origin. When the ejection is triggered under external pressure, we evidence an unexpected behaviour: within a wide range of pressure, the phage population is heterogeneous and the ejection leads to the transfer of s 0%, 60%, 90 % or 100% of the genome, regardless of the applied pressure (Figure 1).

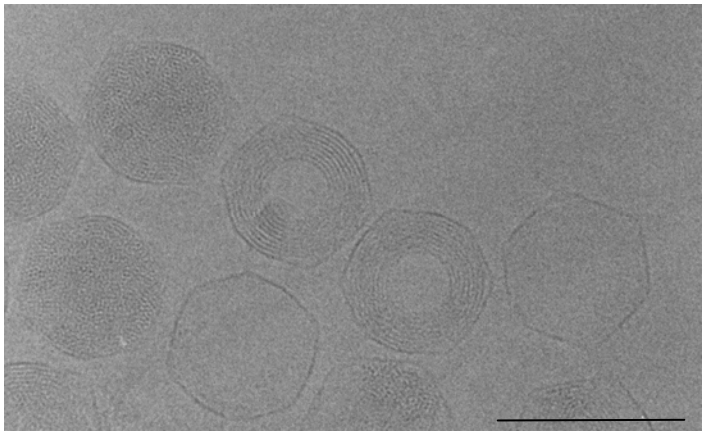

Figure 1 - T5 bacteriophages observed by cryo-electron microscopy after ejection under pressure (6.2 Atm): empty phages, full phages and phages containing intermediate amounts of DNA coexist in the specimen. Scale bar 100 nm.

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These results illustrate the complexity of DNA ejection in bacteriophages, even in a simplified *in vitro* system. We will discuss the possible mechanisms involved in DNA transfer.

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