

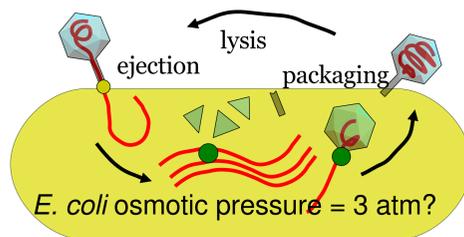
The role of pressure in DNA ejection from bacteriophage λ

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Pressure in the bacteriophage lifecycle

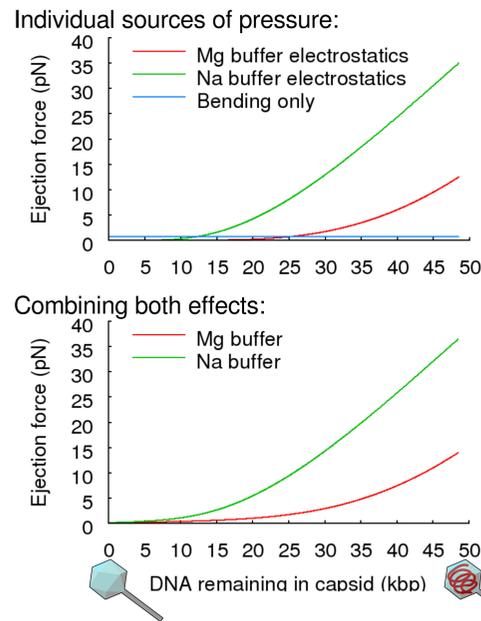
The textbook model of a bacteriophage shows that pressure is built up within the capsid by a strong molecule motor during packaging, and that the pressure is used to forcibly inject the DNA into the cell. Our goal is to analyze this inherently mechanical method of gene transfer, to quantitatively understand the origin of the internal pressure and its relation to the kinetics of the ejection process.



Modeling the pressure

Our model for the pressure within a bacteriophage capsid takes into account two factors: the interactions between neighboring strands and the bending stiffness of the DNA. The interstrand interactions are strongly dependent on the ionic conditions; we use data from Rau *et al.* (1984) to get these forces for Mg²⁺/Na⁺ buffer. For the bending forces, we simply assume $\xi=50$ nm. Minimizing the energy gives us the interstrand spacing in the capsid and the force on the DNA.

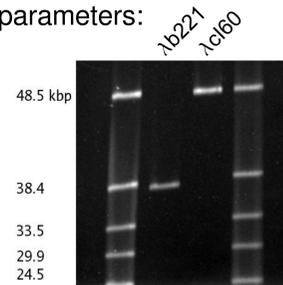
- The forces are testable predictions with no fitting parameters (see Purohit *et al.*, 2005, Grayson *et al.*, 2006)
- Bending appears negligible, but it is important below 50% packaging and essential for determining the shape of the DNA, which will affect dynamics.
- This method alone does not tell us anything at all about kinetics!



Predictions of the theory

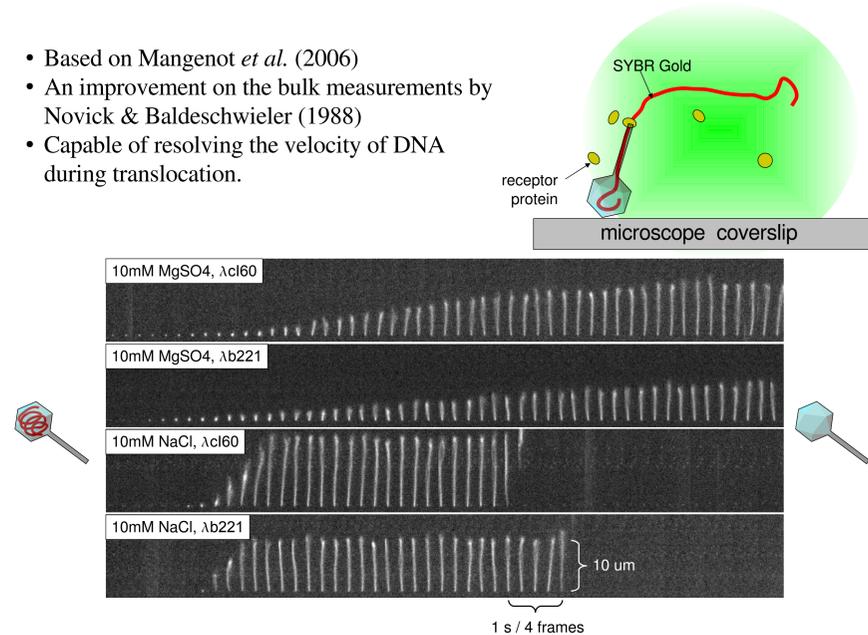
- High force: ejection proceeds quickly & smoothly
- Ejection *in vitro* depends only on internal DNA
- Highly ion-dependent forces & dynamics

Plan of action: test by varying parameters:



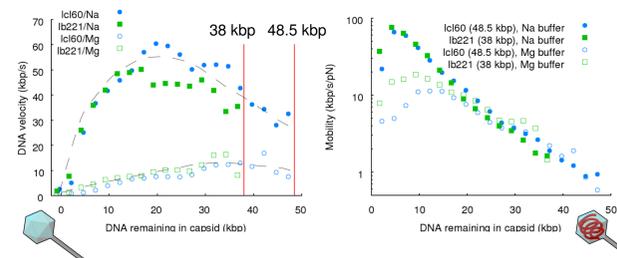
Single-molecule ejection measurements

- Based on Mangenot *et al.* (2006)
- An improvement on the bulk measurements by Novick & Baldeschiwiler (1988)
- Capable of resolving the velocity of DNA during translocation.



- Reproducible within exp. error
- Smooth motion
- Strong effect of ions in buffer
- Shorter phage slightly faster
- Long time at max. extension

Quantification of ejection kinetics

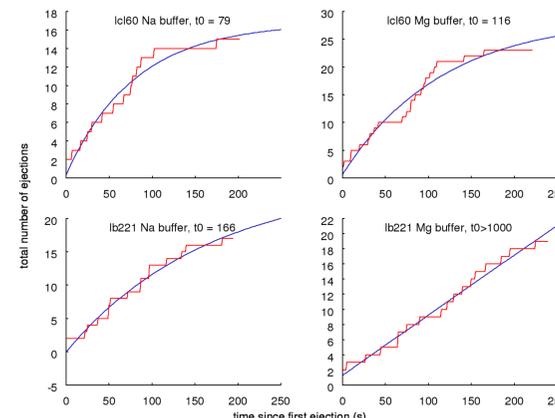


- Velocity only depends on genome length
- Na⁺ produces max. translocation velocity 4-5 times higher than Mg²⁺
- Mobility = v/F is independent of salt over most of the ejection
- Mobility decreases with the DNA density: hydrodynamic drag?

A plot of the ejection times reveals the force dependence of the triggering event. We expect

$$t_0 \propto \exp(-A/kT)$$

where A is the energy barrier of ejection. Increasing the force by changing ionic conditions or genome length reduces A .

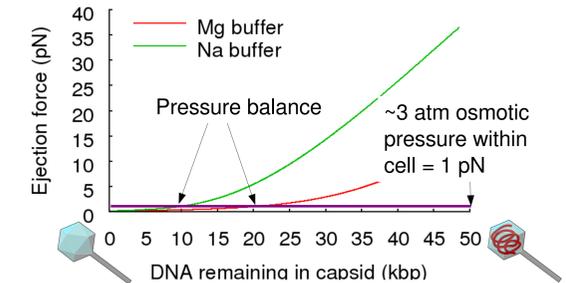


Current work: observing ejection *in vivo*

Pressure can only be responsible for 50-80% of the ejection:

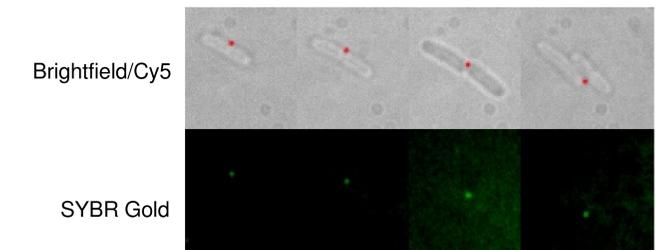
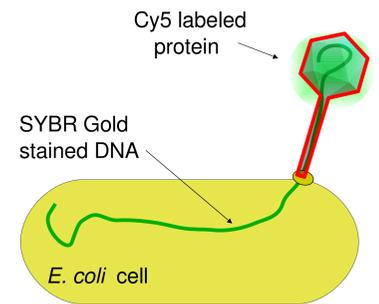
What is the second step?

- RNAp – T7 (Kemp, Garcia, Molineux, *et al.*)
- Specific motor protein – $\phi 29$ (Gonzalez-Huici *et al.*)
- Water flow through tail?

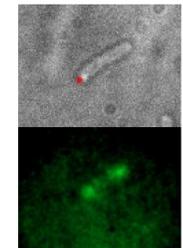


Two-color observations of capsid & DNA:

- DNA stained with SYBR Gold (green)
- Capsid protein conjugated to Cy5 (red)
- Observation possible for >1h at 1 Hz



If left for ~1h before observation, some cells are found with DNA inside; this has not yet been seen in real time. Leakage through external solution?



Conclusions and questions

- Ion and genome length dependences consistent with theory.
- What determines the dynamics?
- What other forces are at play *in vivo*?
- Relationship to DNA/RNA transfer in Eukaryotic viruses?

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