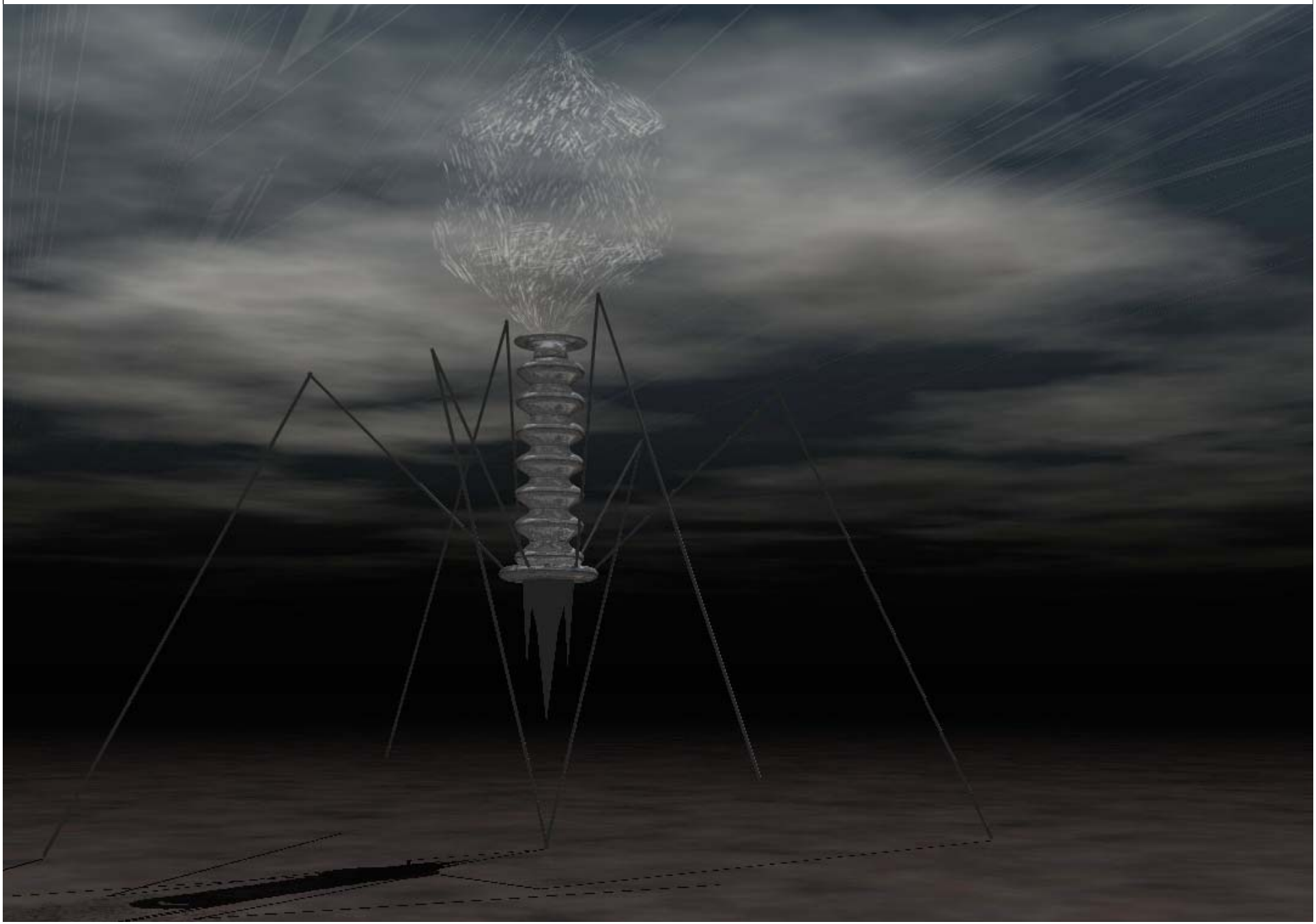
OKLAHOMA PHAGE, just one of the 10^{31}

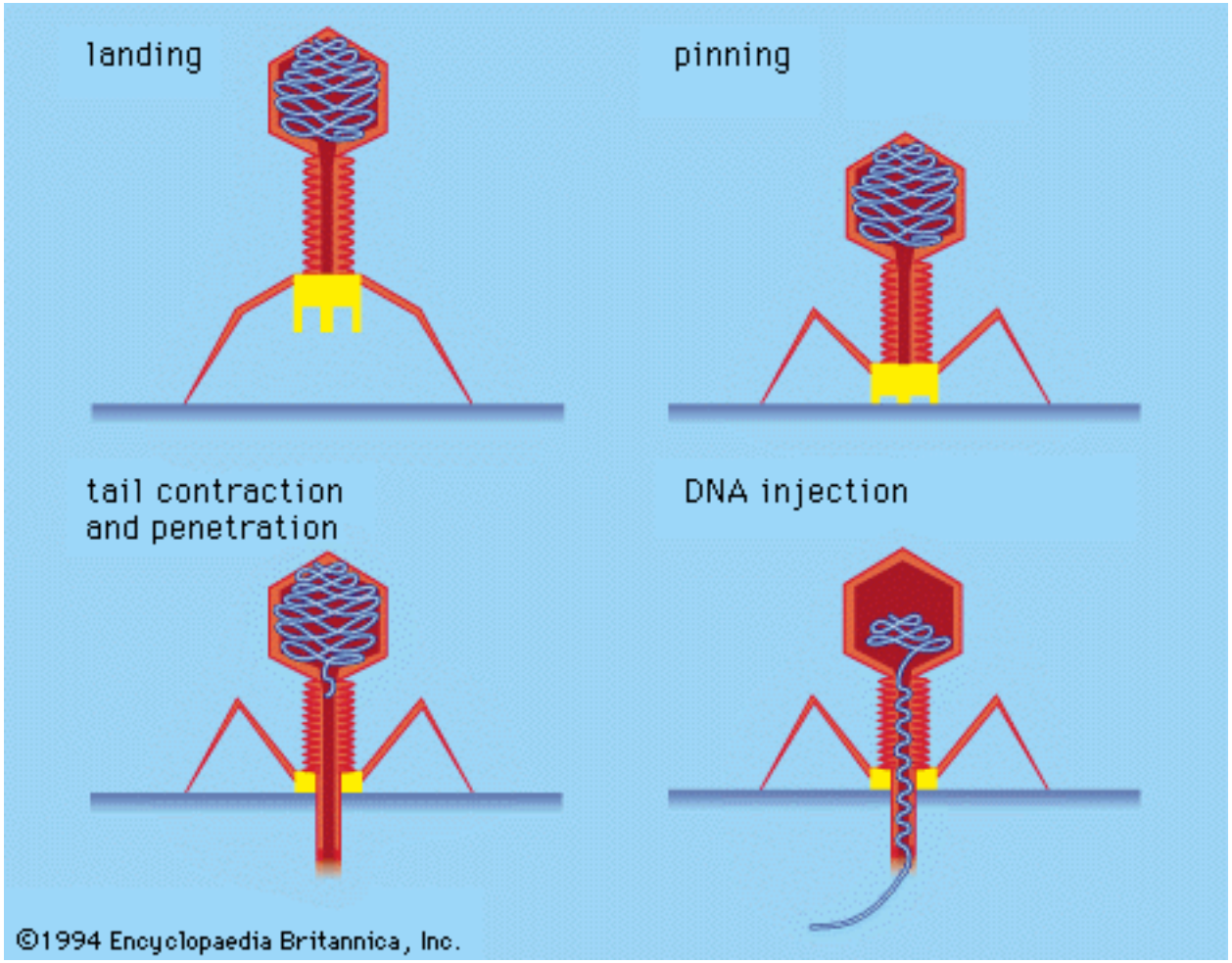


Pressure inside viruses - what's the use?

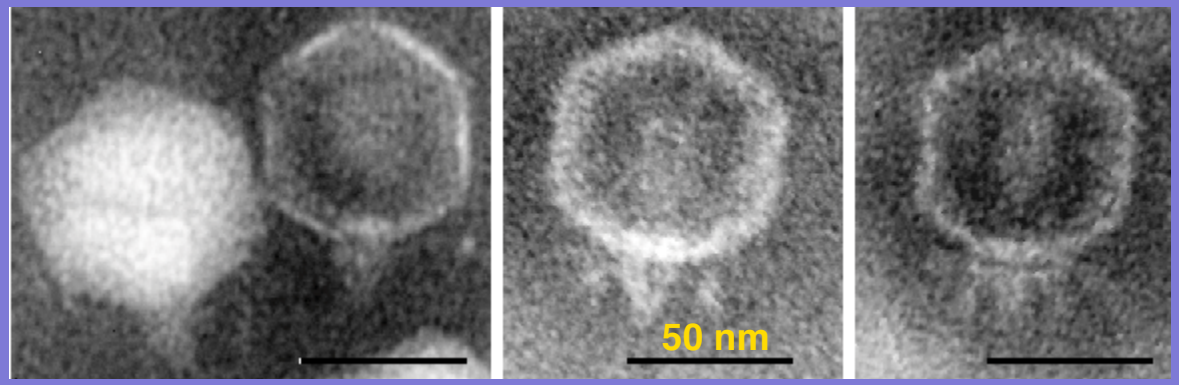
Ian J. Molineux

KITP, June 2006

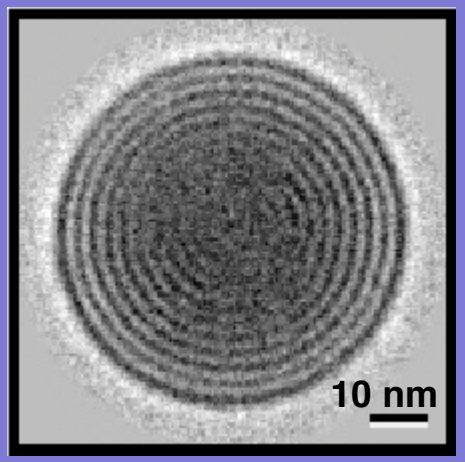
THE mechanism of phage infection

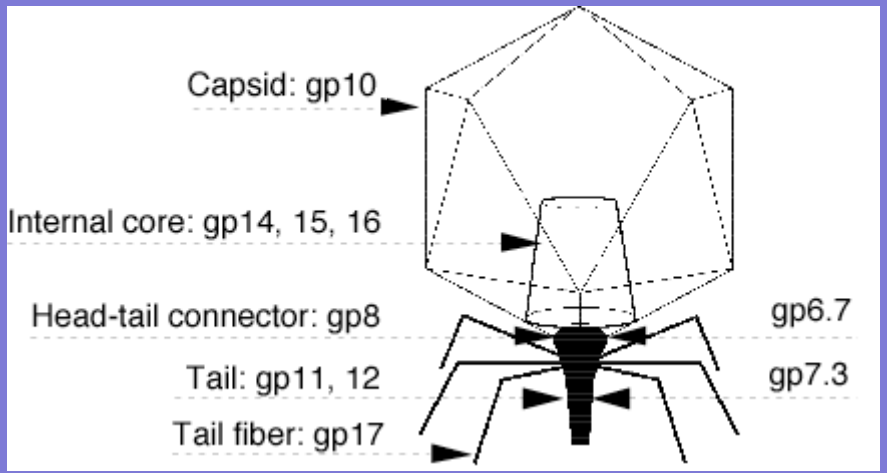


Members of the T7 Family

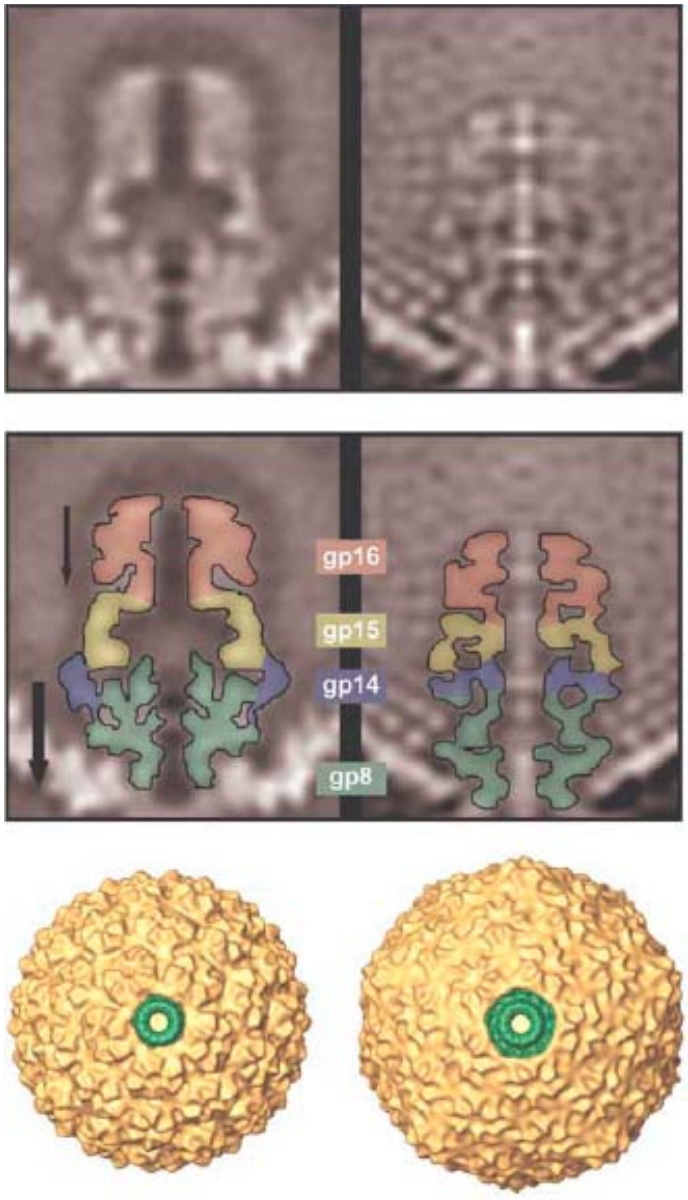


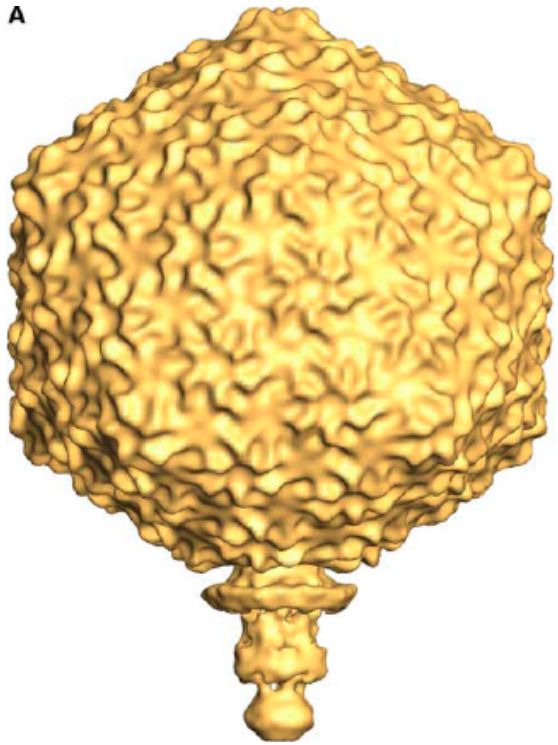
T7





CryoEM reconstruction of T7

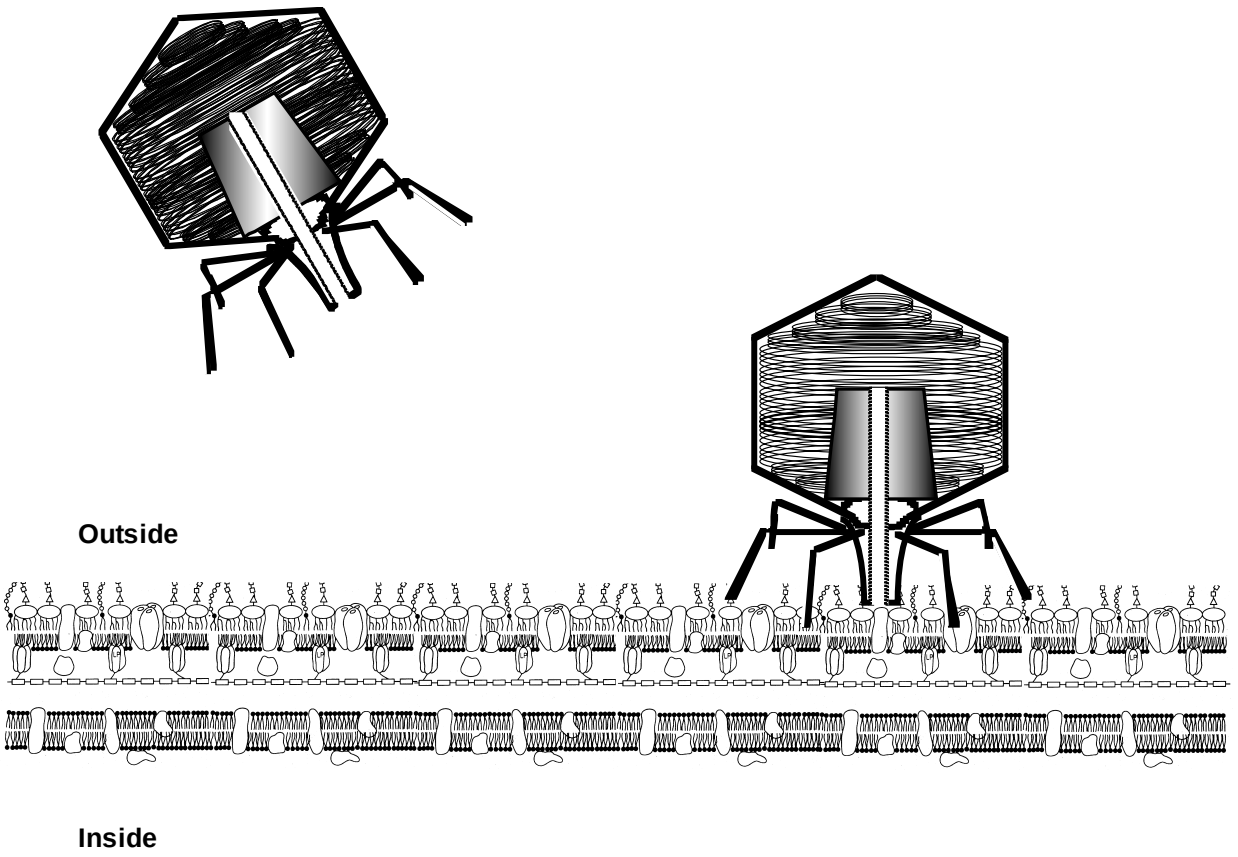


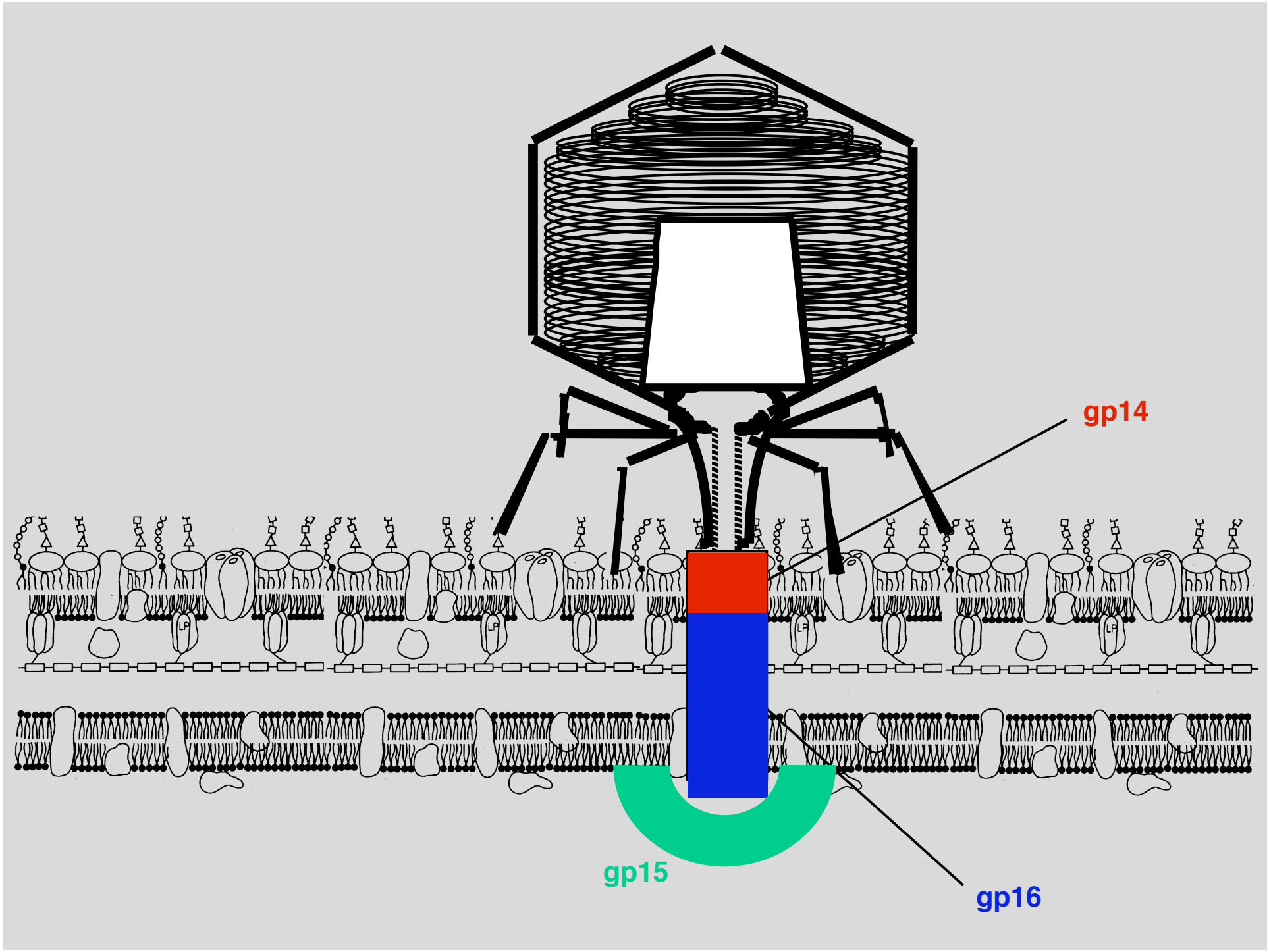


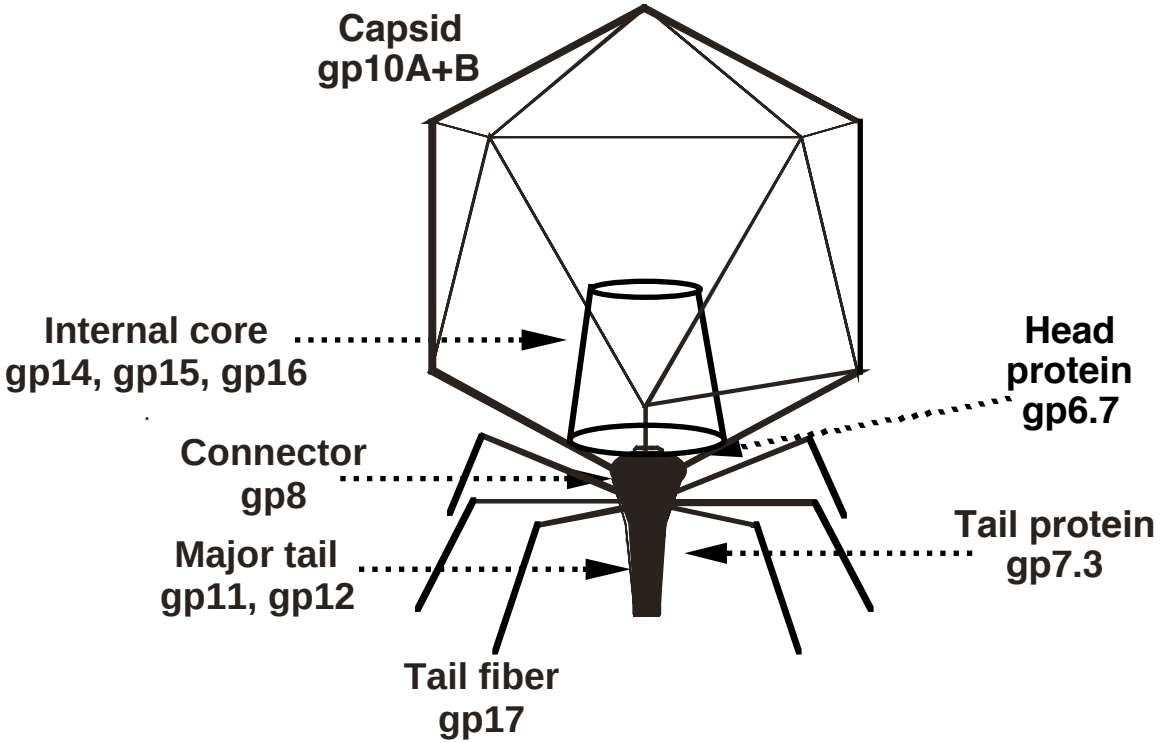
Carrascosa et al., 2005

LENGTH MATTERS!

The T7 tail is too short to penetrate the cell cytoplasm




gp14
LP
LP
LP
LP
gp15
gp16



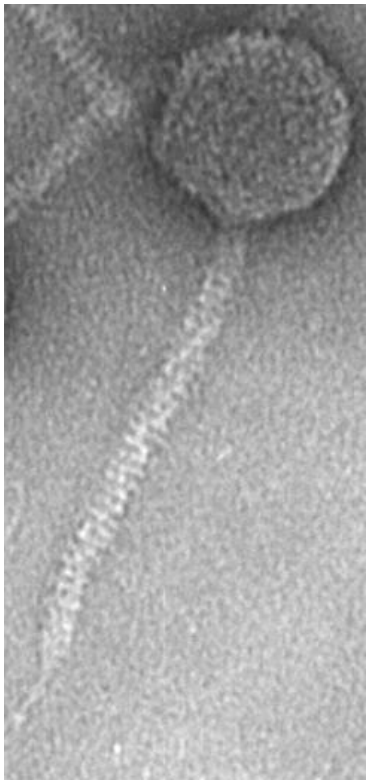
gp6.7, 9.2 kDa	18 copies
gp14, 20.8 kDa	10 copies
gp16, 143.8 kDa	4 copies
gp15, 83.2 kDa	8 copies
gp7.3, 10.0 kDa	33 copies

~75 protein molecules, almost 2 mDa, are ejected from the infecting T7 virion into the cell. ~40 molecules, totaling ~1.6 mDa, must pass through the head-tail connector.

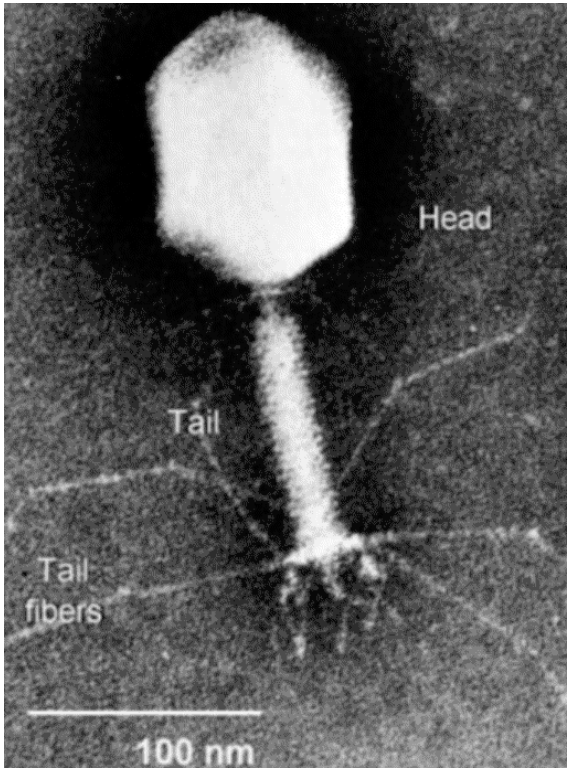
The diameter of the channel through the connector, through which DNA travels into the cell is ~33Å, with a strangling region of ~22Å. There is no information for the extensible T7 tail.

In a mature long-tailed phage, the tail tube is filled with protein, which must be ejected from the infecting particle before DNA can begin to enter the cell

λ



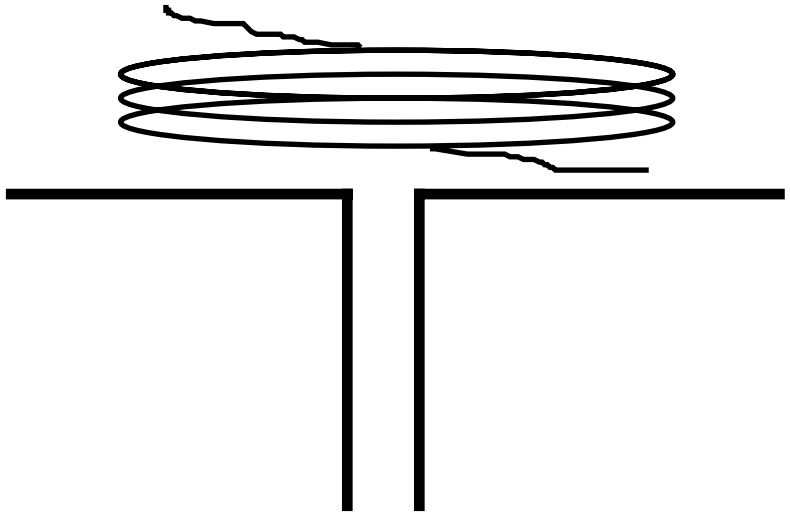
T4



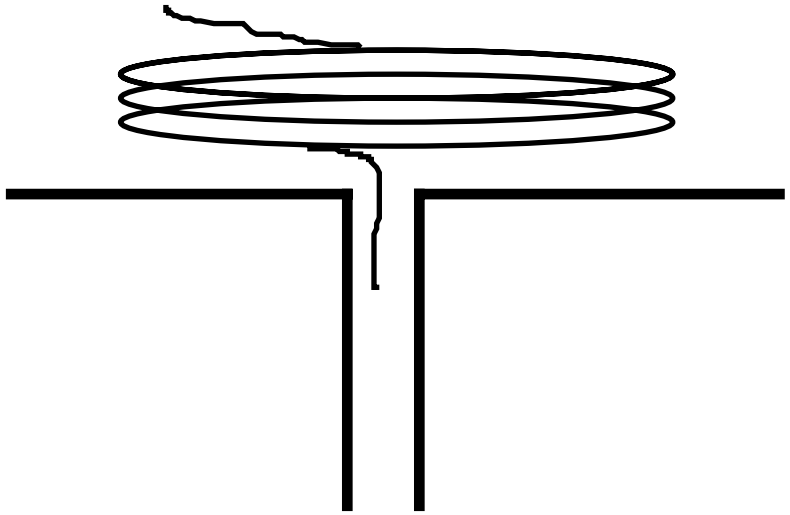
Many phage heads also contain proteins that are ejected from the infecting virion; some proteins must be ejected before the phage DNA, others may follow genome ejection.

~1000 protein molecules inside the T4 head enter the infected cell

How does the DNA exit the head through the tail tube?



Inserting the leading end of the genome into the tail during assembly provides the necessary vectorial parameter



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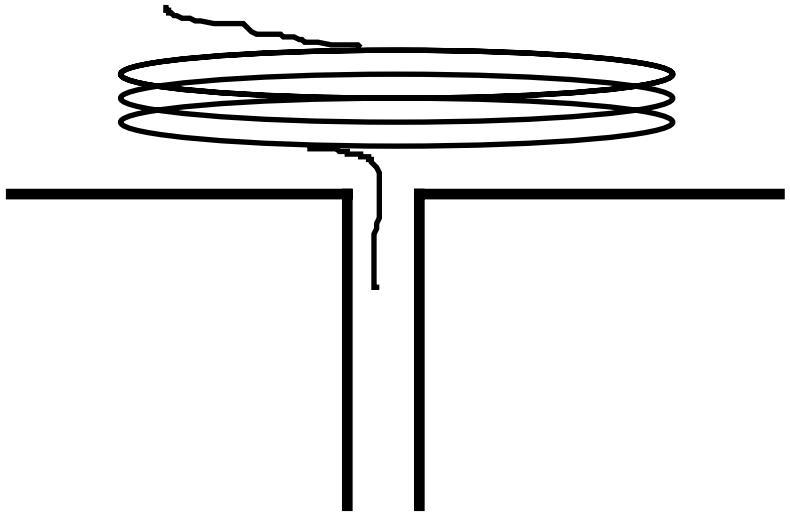
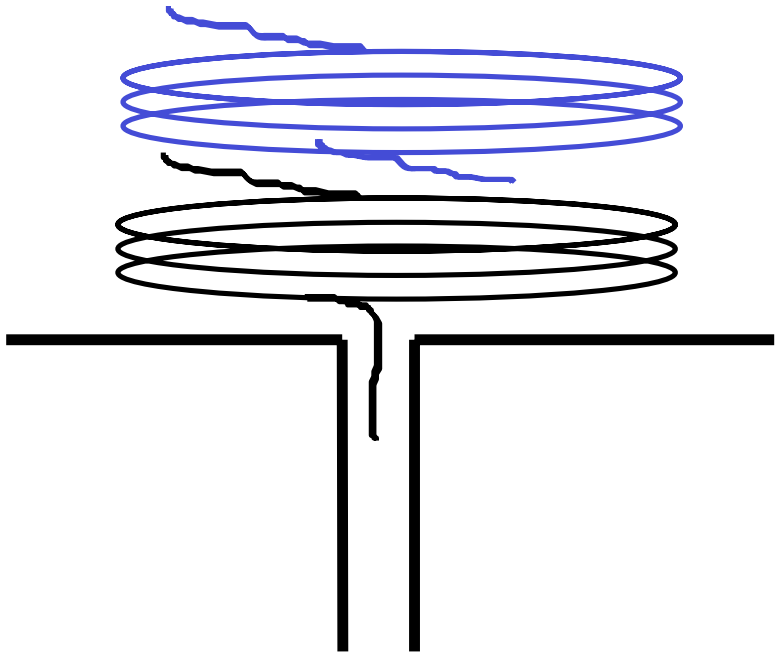


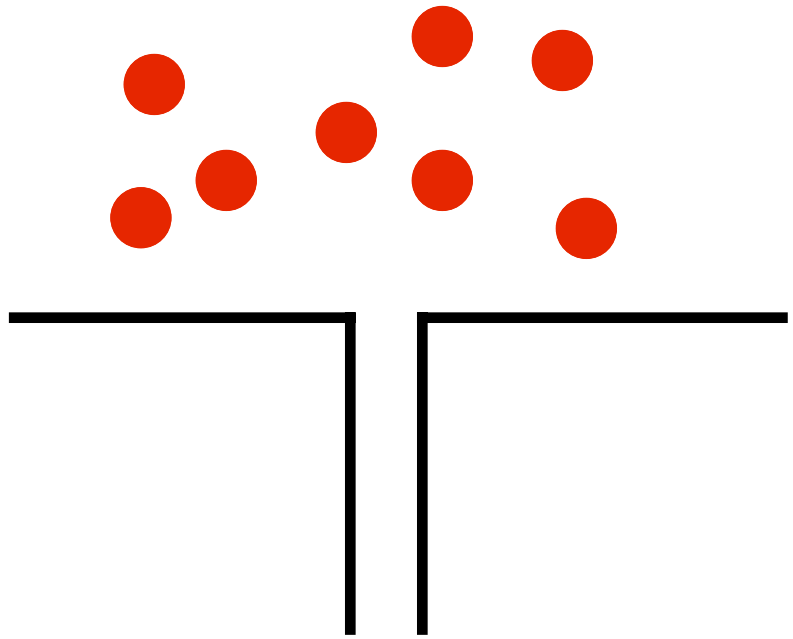
Phages P2, P4, 186, and λ are known by experiment to have this configuration

But multiple DNA molecules can be packaged in - and ejected from - a single phage head. How does the second or subsequent molecules find the exit channel?

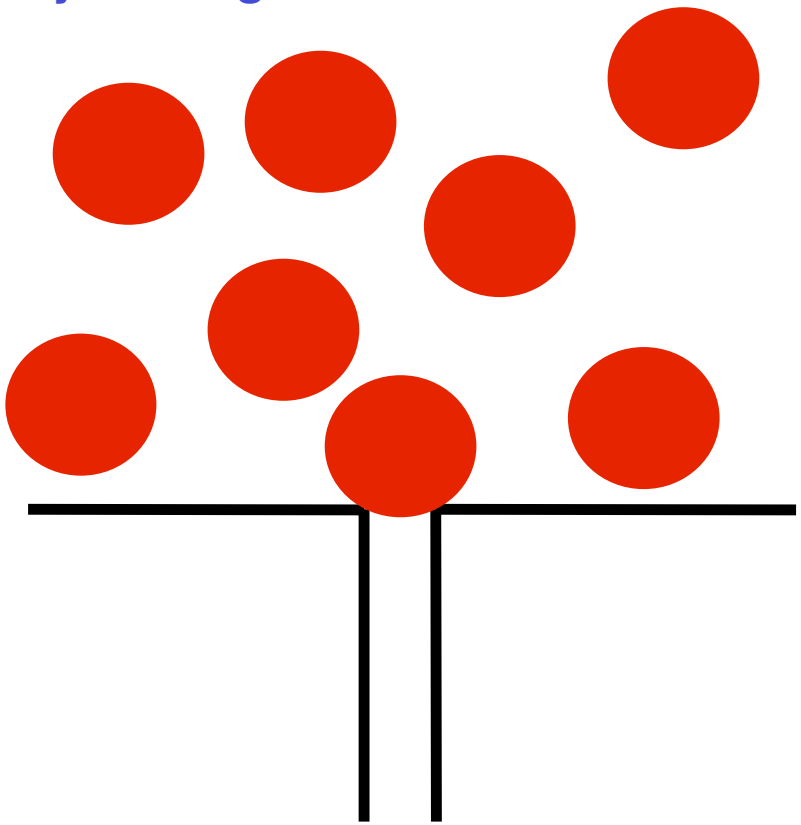


The efficiency of ejection of the second molecule is ~10% that of the first.
This is high unless the end of the second DNA is guided to the exit channel

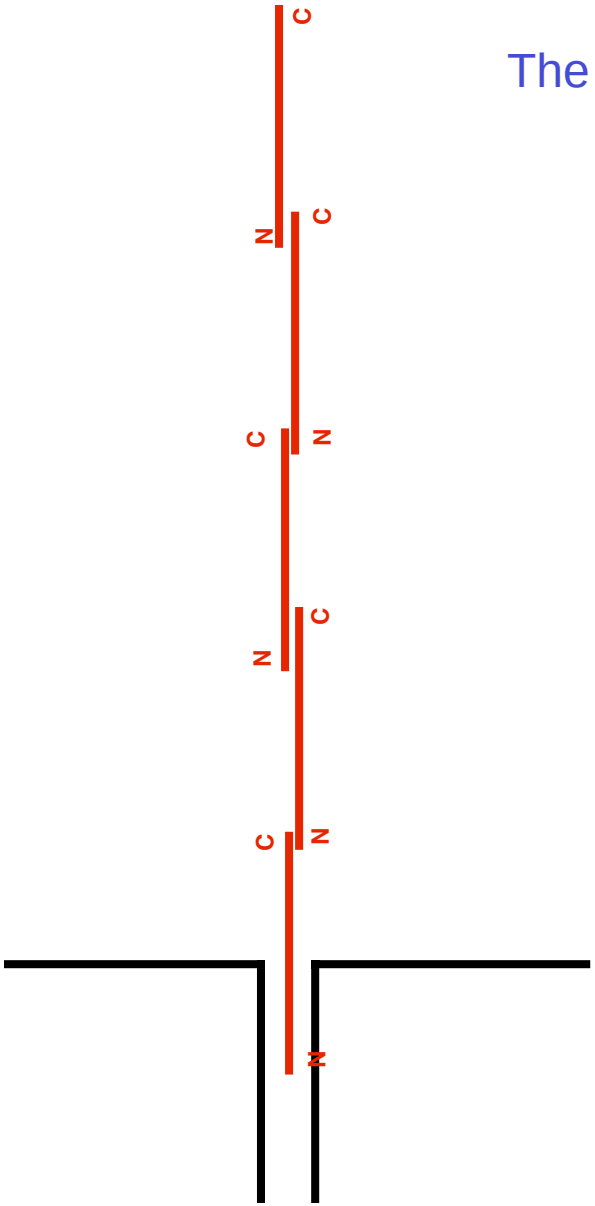
How do multiple protein molecules exit the head in synchrony?

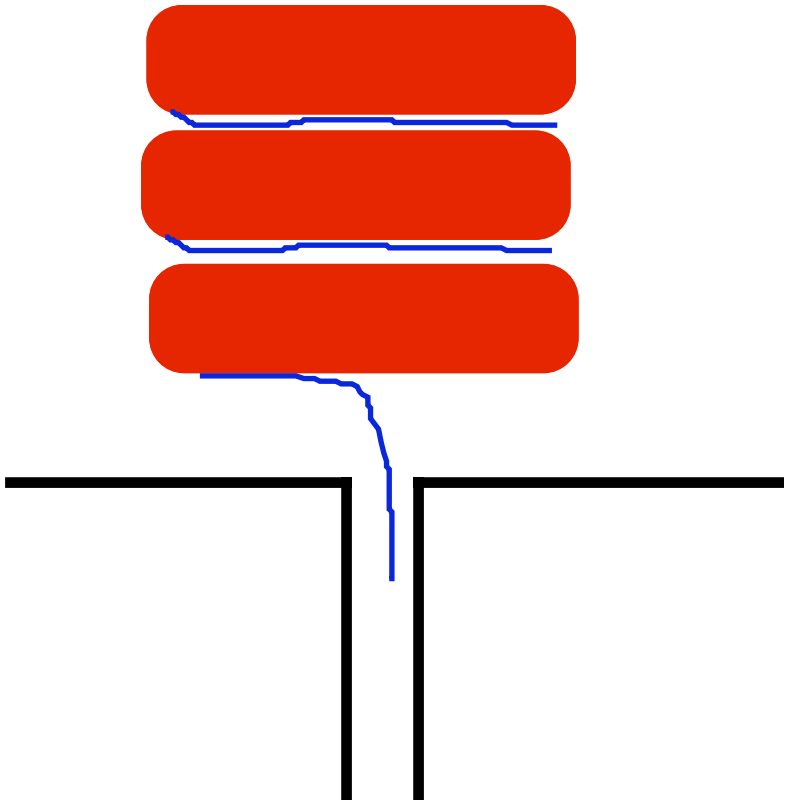


Especially if they are larger than the diameter of the tail tube



The proteins must at least partially unfold. For ejection to be coordinated, proteins must also interact





Any general description of how phages infect cells must account for the fact that protein molecules, and not just DNA, leave the virion

Secondly, not all phage genomes consist of ds DNA. A general description of phage infection must also address how ss DNA or RNA enters the cell

The Initiation of Infection by Bacteriophage T7

Reversible adsorption occurs to the bacterial lipopolysaccharide via gp17 tail fibers and either gp7.3 or the gp11/gp12 tail.

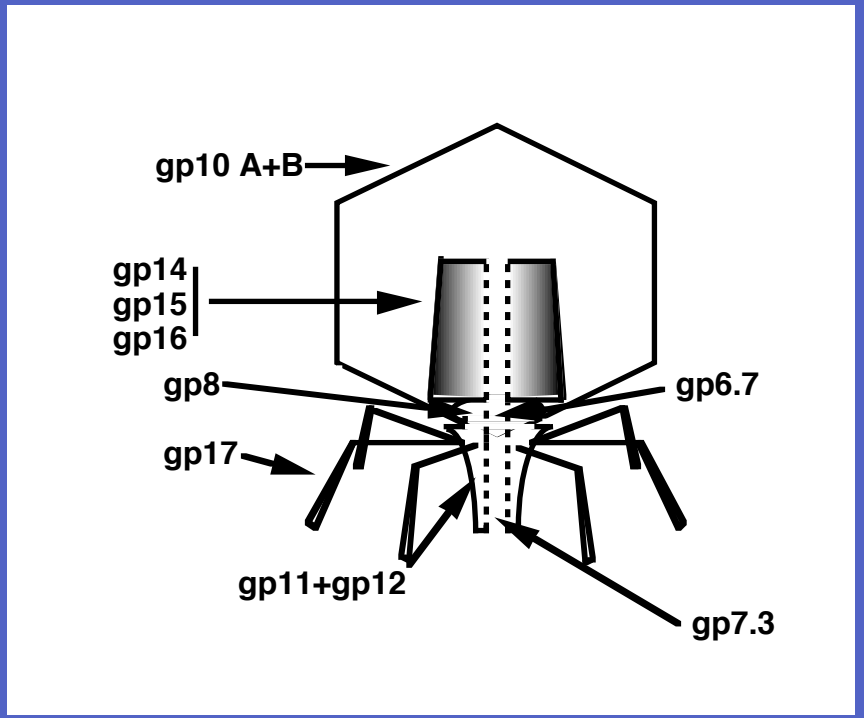
Irreversible adsorption occurs when gp7.3 is ejected from the tail into the outer membrane of the cell, followed by ejection of gp6.7 from the head. Both proteins are degraded by a periplasmic protease.

Ten molecules of gp14 are ejected from the internal core; we think that gp14 forms a DNA translocation channel across the outer membrane. Four gp16 molecules make a channel across the periplasm and form a 2 nm hole in the inner membrane. There is now a channel for DNA transport connecting the phage head and the cell cytoplasm. Eight molecules of gp15 pass through this channel into the bacterial cell cytoplasm.

~850 bp of the 40 kb genome is ratcheted into the cell at 70-75 bp/sec by the gp15/gp16 virion motor.

Transcription by E. coli RNAP internalizes the next ~7 kb of the genome at 40 bp/sec.

Transcription by T7 RNAP internalizes the remaining ~32 kb of the genome at 200-250 bp/sec.

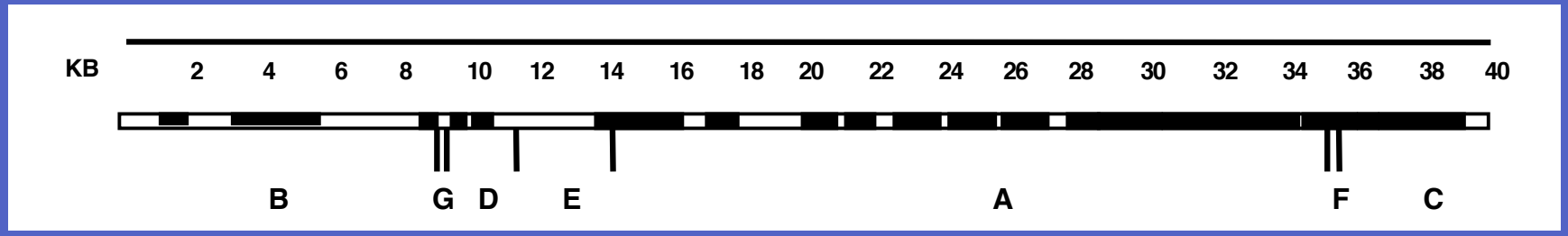


Internalization of the T7 genome takes ~10 min at 30˚C, about one-third of the latent period

Assaying T7 DNA Penetration

1. Infect Dam methylase-overproducing *E. coli* with unmethylated T7.

Dam methylates the A residue in the sequence GATC.



2. At various times after infection, extract DNA from infected cells.

3. Cut all DNA with *Dpn* I

Dpn I only cuts at methylated GATC sites

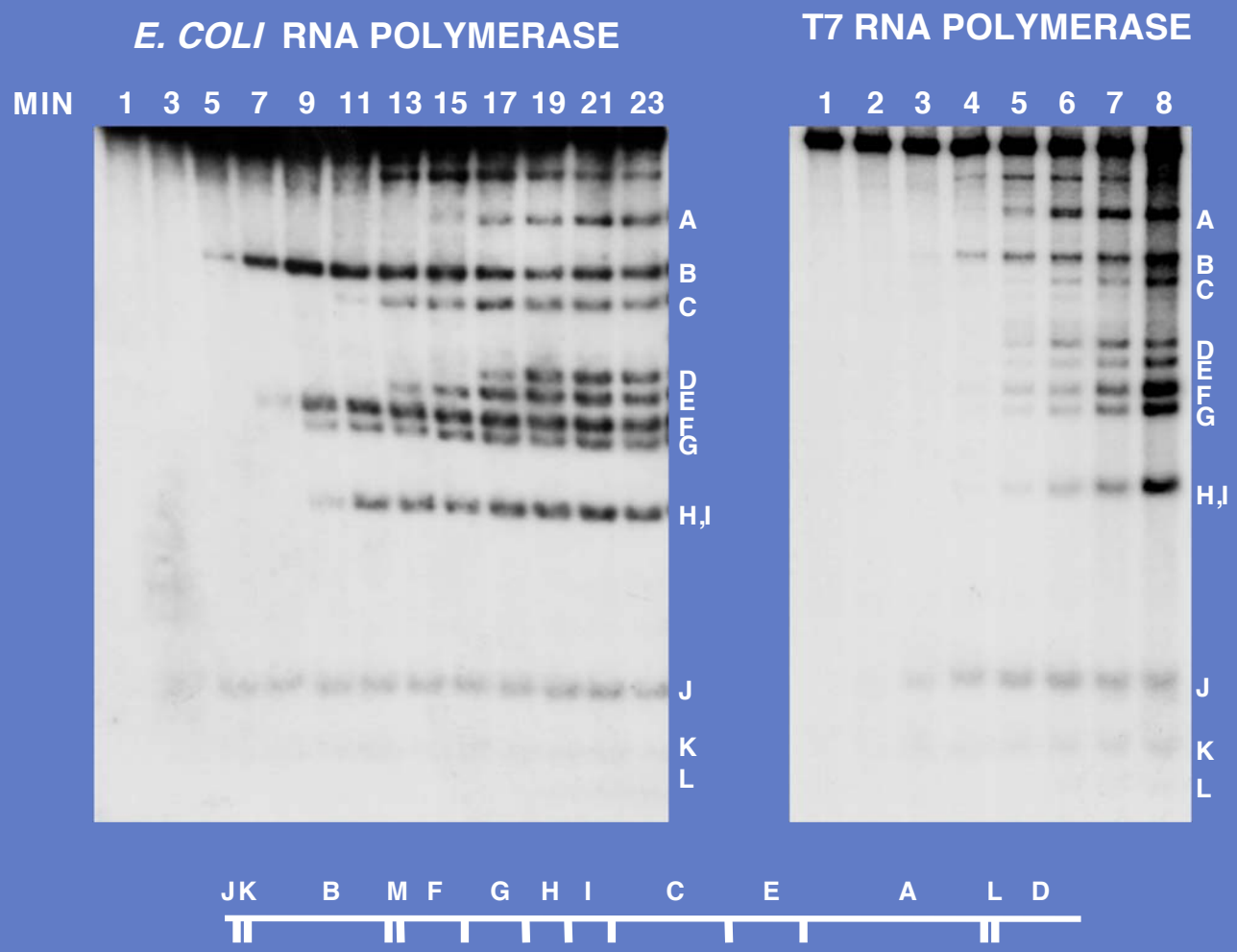
Those parts of the T7 genome that have entered the cell are cut by *Dpn* I

T7 DNA that has not entered the cell is uncut

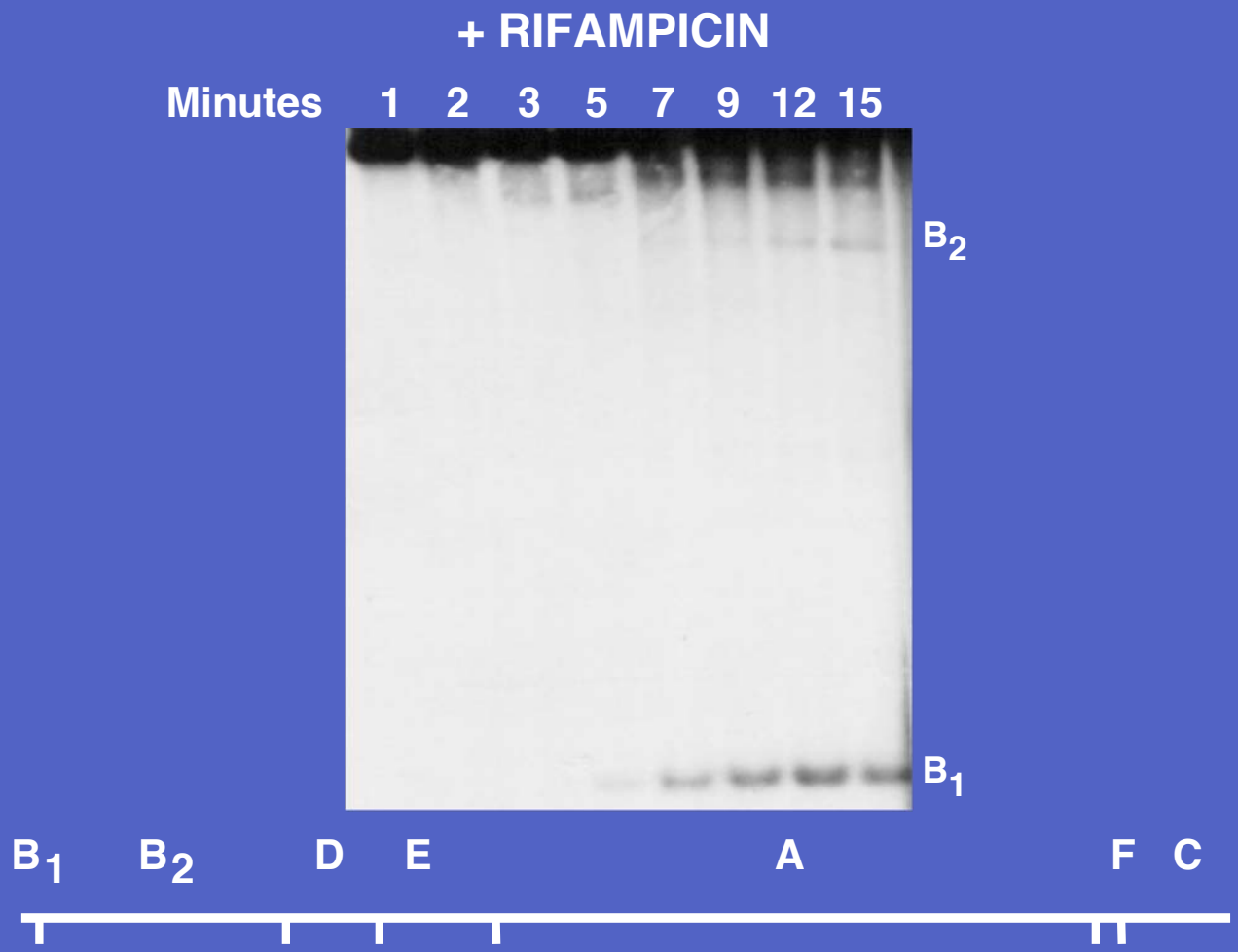
.

4. Separate DNAs by electrophoresis, transfer to a membrane and hybridize, probe with randomly primed ^{32}P T7 DNA.

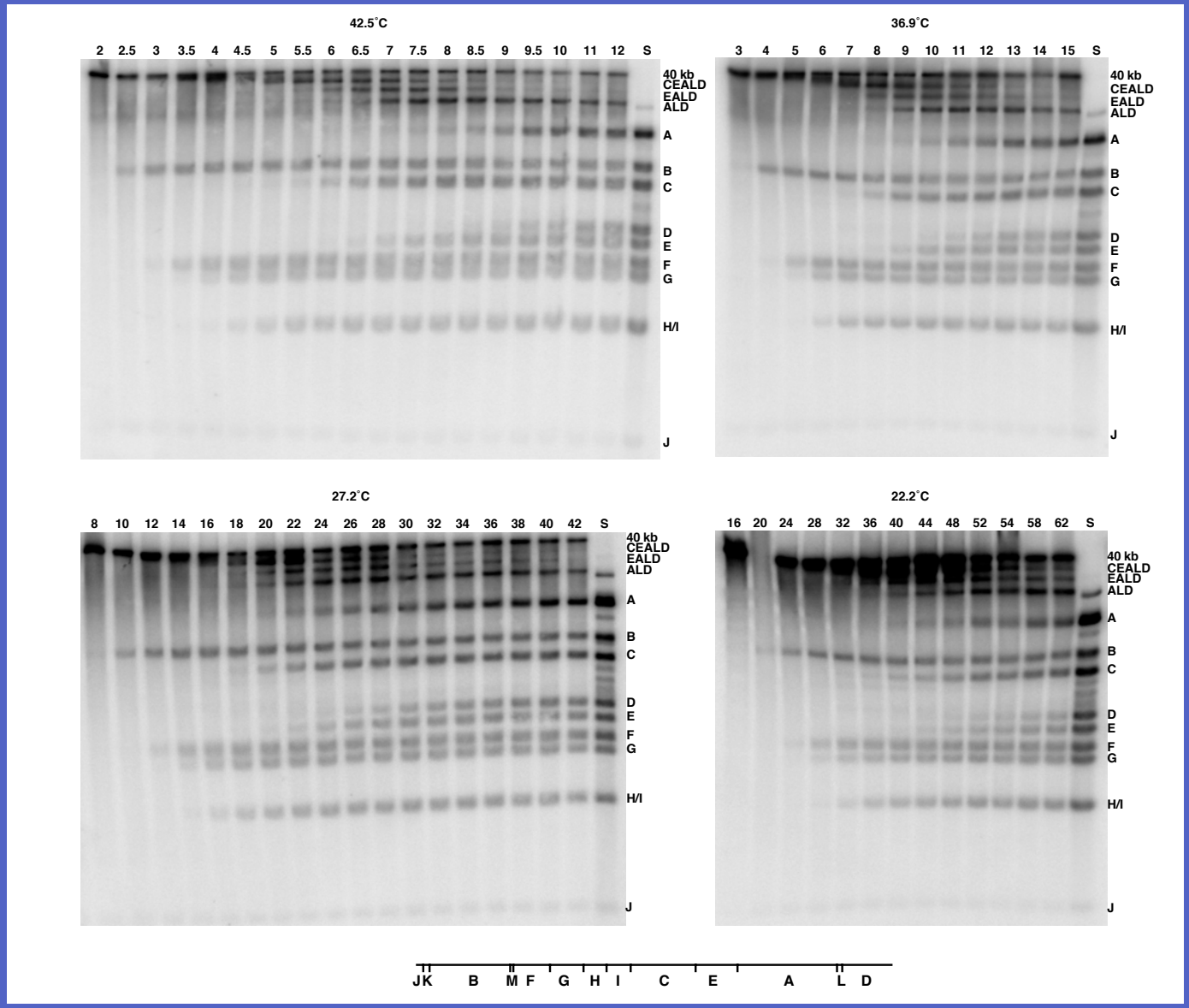
Rates of DNA Entry in Vivo Approximate Rates of Transcription *in vitro* and *in vivo*



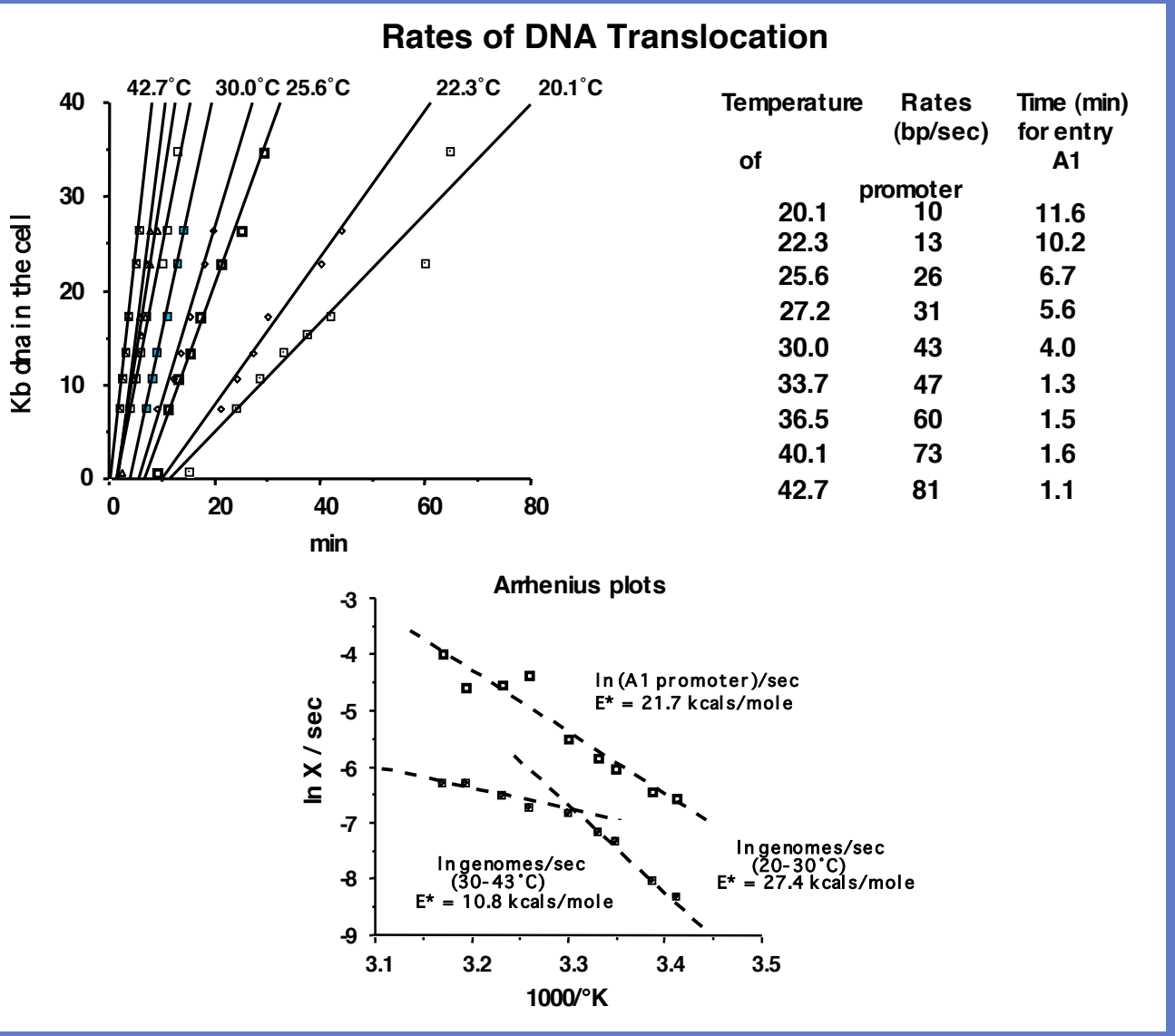
In the absence of transcription by *E. coli* RNA polymerase, only the left ~850 bp of the T7 genome efficiently penetrates the cell



Kinetics of T7 genome internalization by *E. coli* RNA polymerase

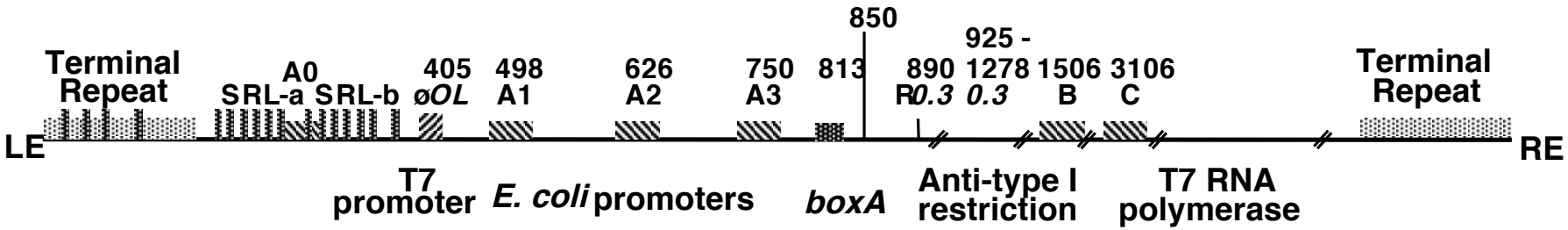


Rates of T7 DNA internalization by *E. coli* RNA polymerase

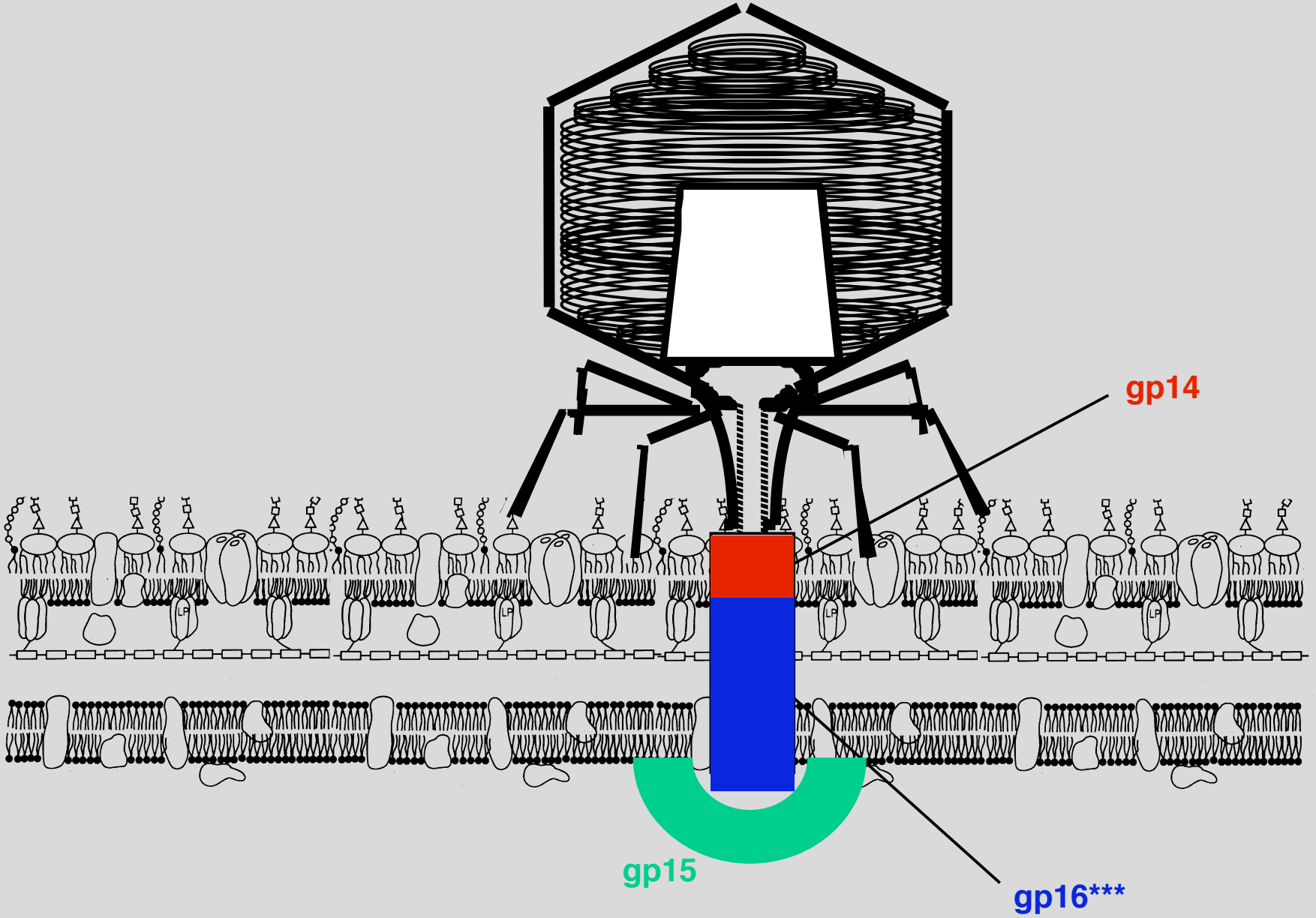


Temperature of promoter	Rates (bp/sec)	Time (min) for entry A1
20.1	10	11.6
22.3	13	10.2
25.6	26	6.7
27.2	31	5.6
30.0	43	4.0
33.7	47	1.3
36.5	60	1.5
40.1	73	1.6
42.7	81	1.1

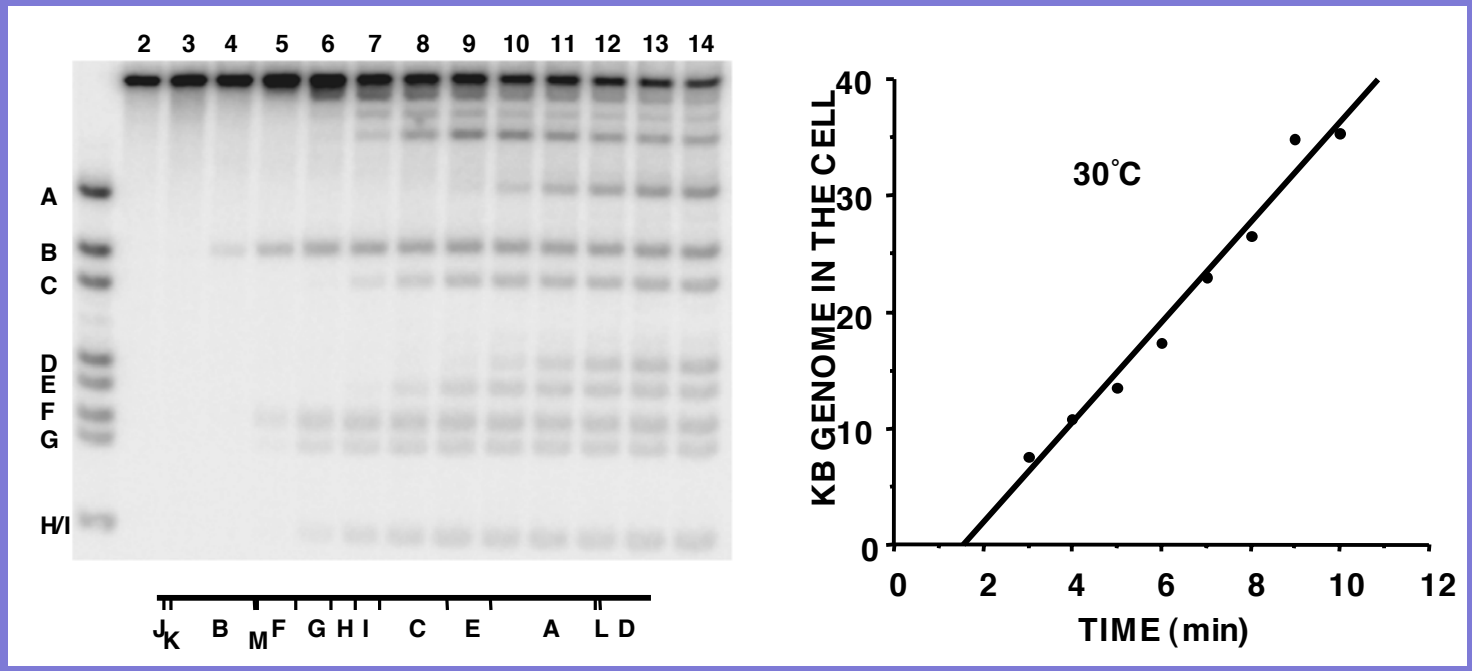
The Genetic Left End of Bacteriophage T7



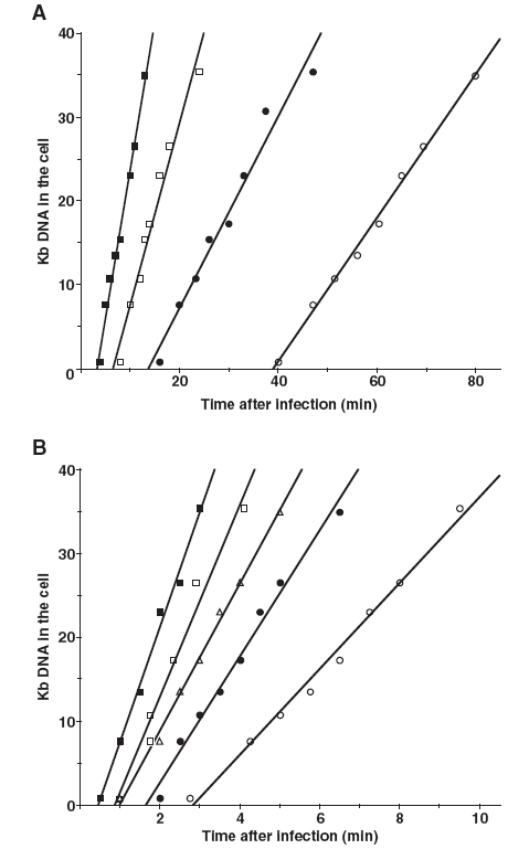
A mutant gp16 (gp16***) allows complete genome internalization in the absence of transcription



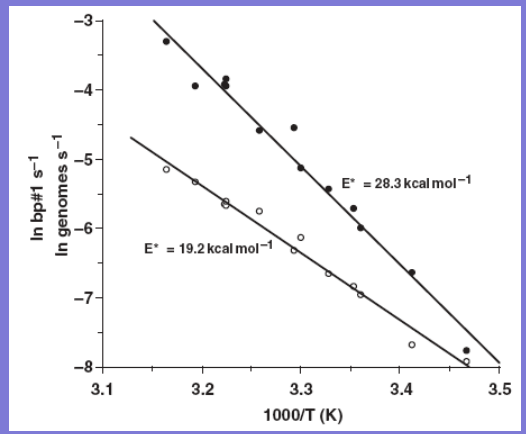
The Rate of Transcription-Independent Translocation is Constant Across the Whole 40 Kb T7 Genome



The rate of transcription-independent internalization of the T7 genome is constant at constant temperature



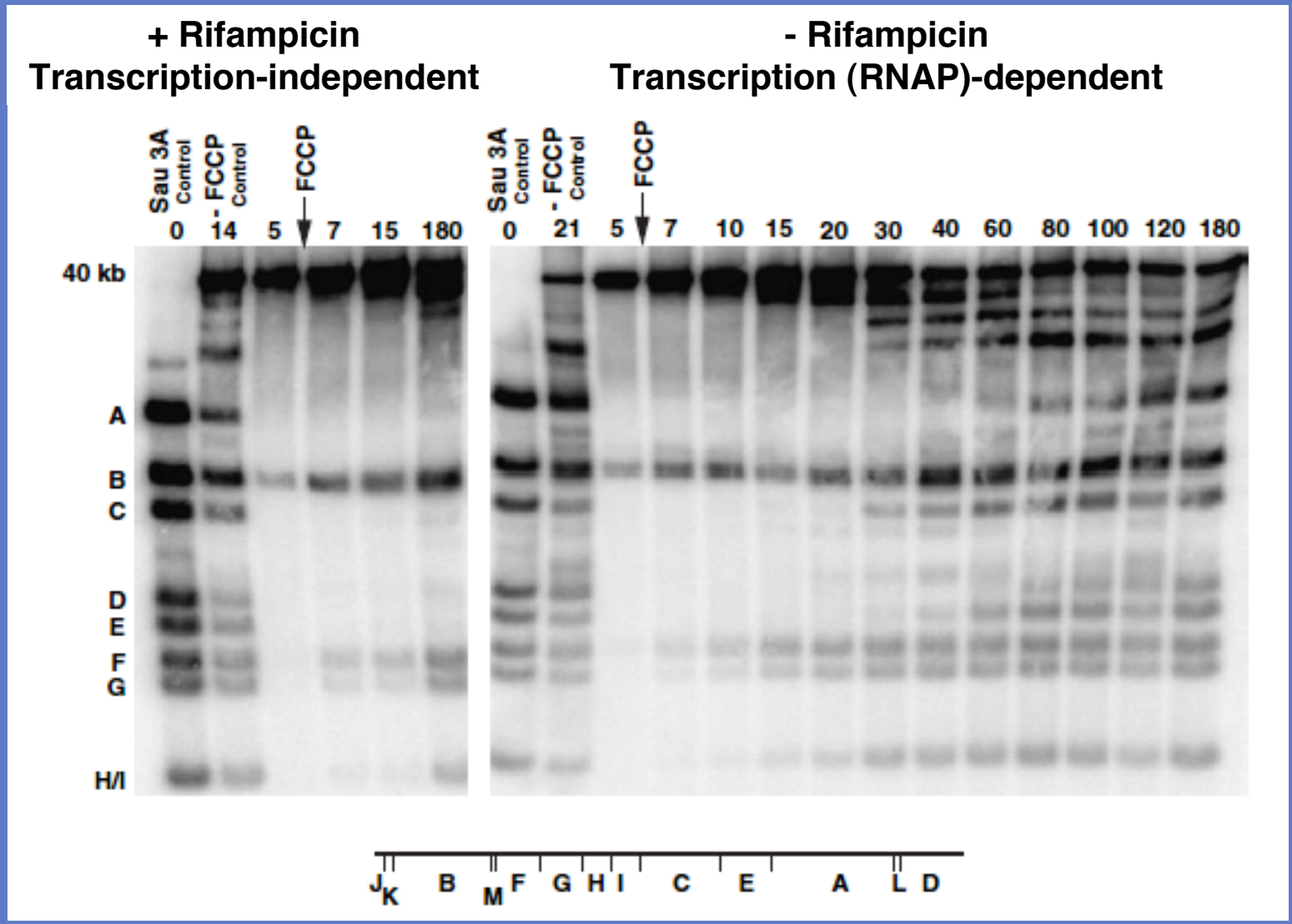
Temperature (˚C)	Rate (bp/sec)	Time, bp#1
15.3	14	39.1
20.1	18	12.6
24.8	40	5.9
27.4	54	3.5
30.5	71	1.6
33.6	105	1.7
37.0	138	0.83
40.0	190	0.86
42.9	228	0.45



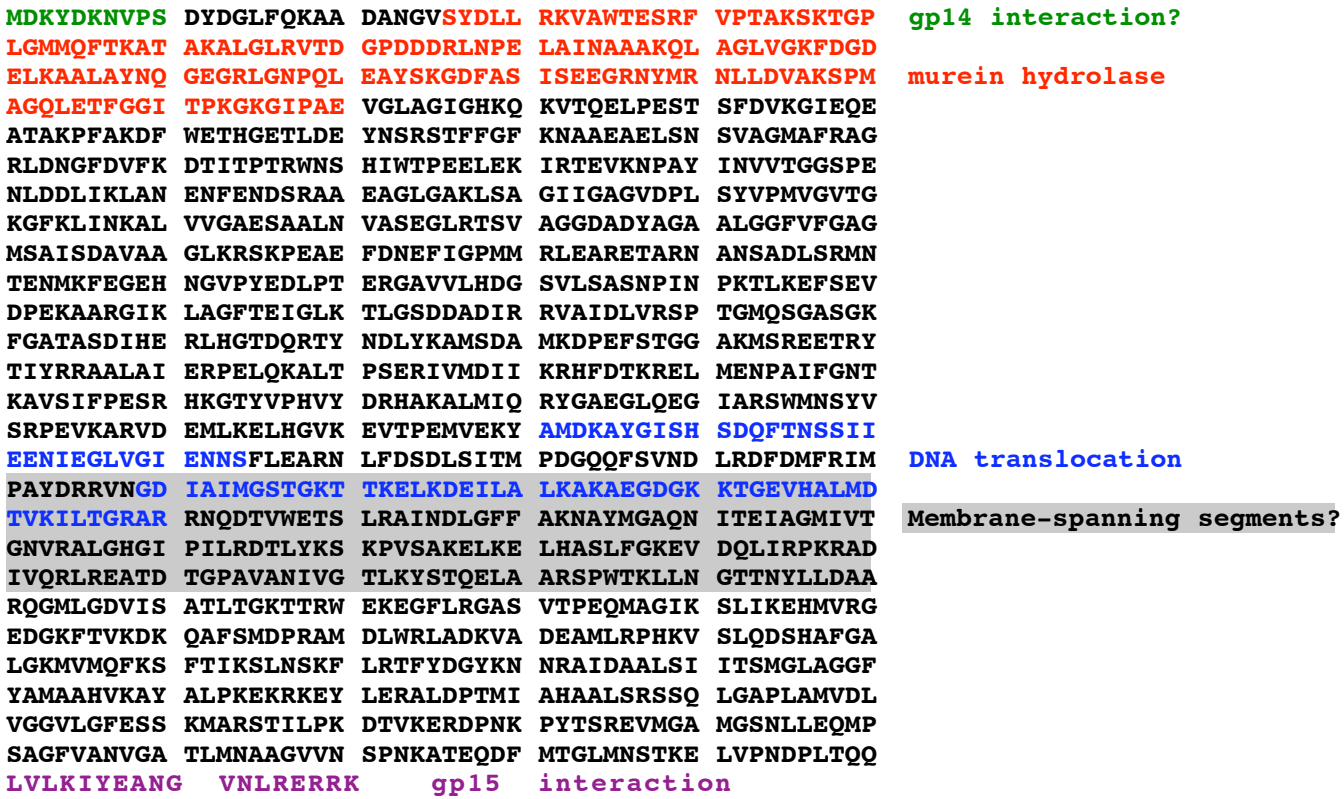
Forces inside the capsid cannot drive T7 DNA internalization!

During infection by wild-type T7, energy is normally provided by the membrane potential for the leading 850 bp of the T7 genome, followed by ATP when RNAP initiates transcription

Internalization of T7 DNA from the phage virion requires the membrane potential



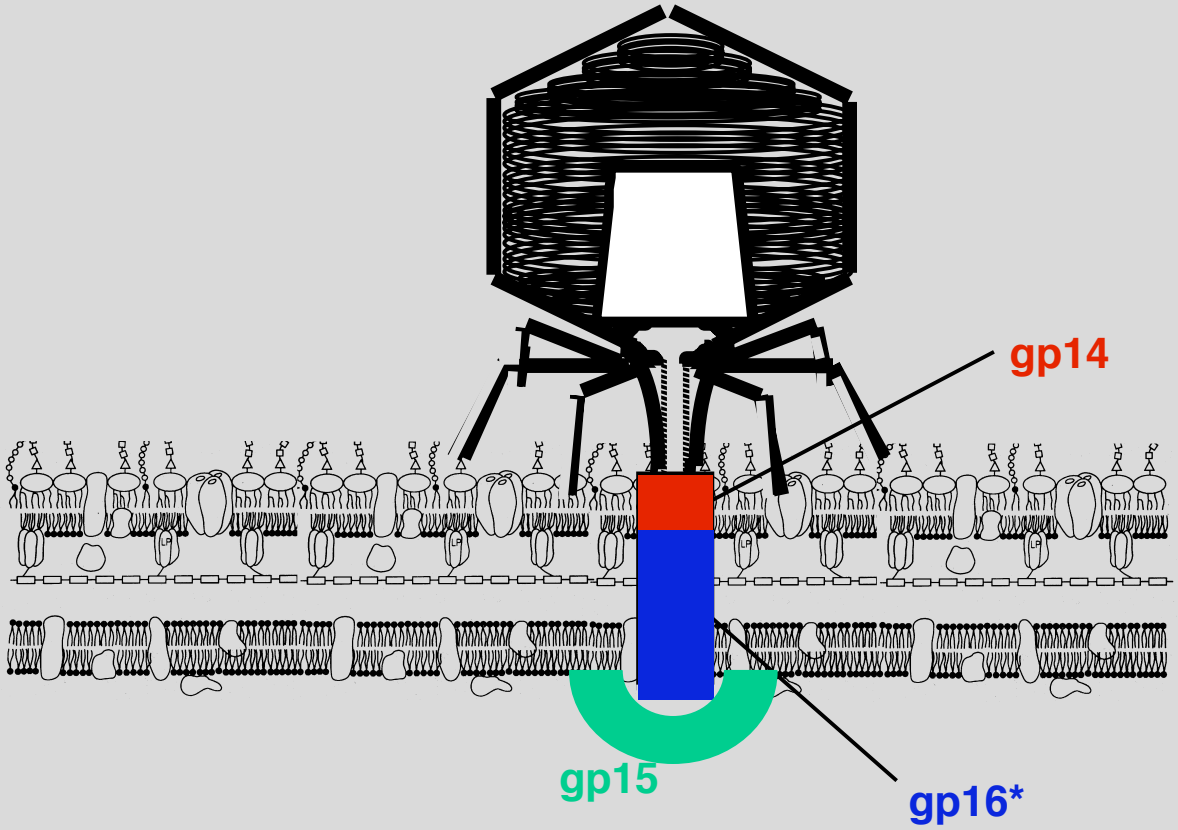
Domains of T7 gp16



Suppressors of T7 gp16 C-terminal truncations affect gp15

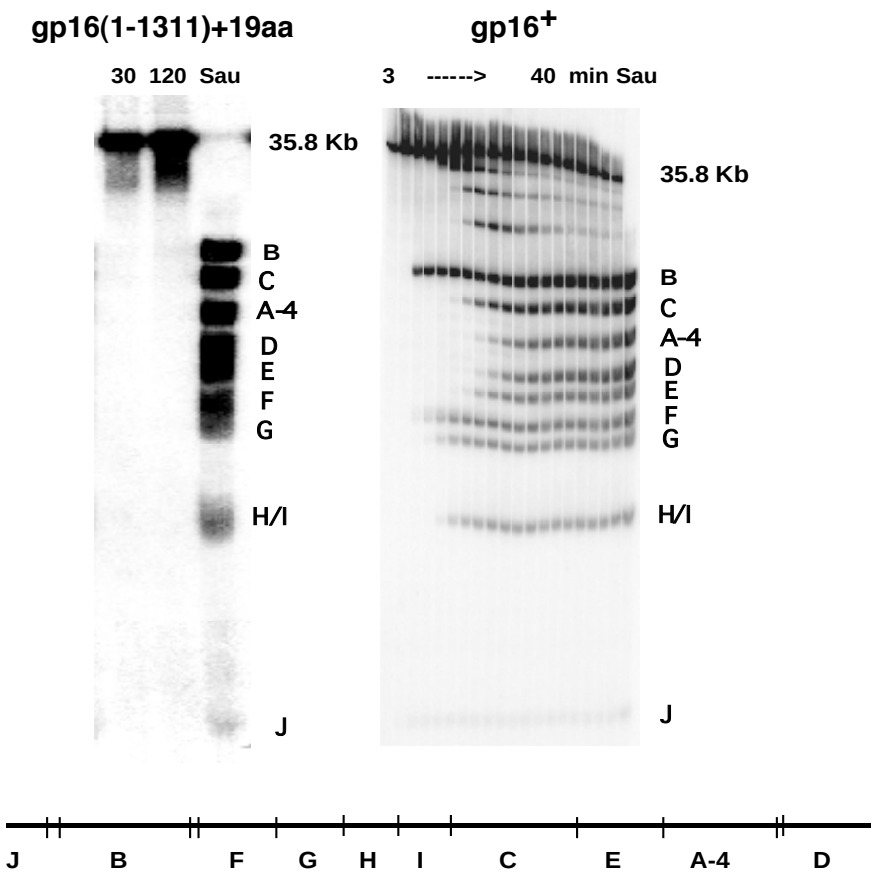
		Virion Assembly	Viability	Extragenic suppressor
gp16+ (1318 aa)	T_{1298} QQLVLKIYEANGVNLRERRK	+	+	
R1317Am	T_{1298} QQLVLKIYEANGVNLRER	+	+	
E1315Am	T_{1298} QQLVLKIYEANGVNLR	+	+	
R1314Am	T_{1298} QQLVLKIYEANGVNL	+	+/-	+
L1313Am	T_{1298} QQLVLKIYEANGVN	+	-	nd
G1310Am	T_{1298} QQLVLKIYEAN	+	-	-
E1307+2	T_{1298} QQLVLKIYE RC	+	-	-
E1307Am	T_{1298} QQLVLKIY	-	-	-
Q1300Am	T_{1298} Q	-	-	-
V1311+19	T_{1298} QQLVLKIYEANGV IKLIDTVDLEGGPGTQFAL	+	-	+
V1311+7	T_{1298} QQLVLKIYEANGV IKLQFAL	+	-	+

When gp16 is defective (gp16*), proteins are ejected normally

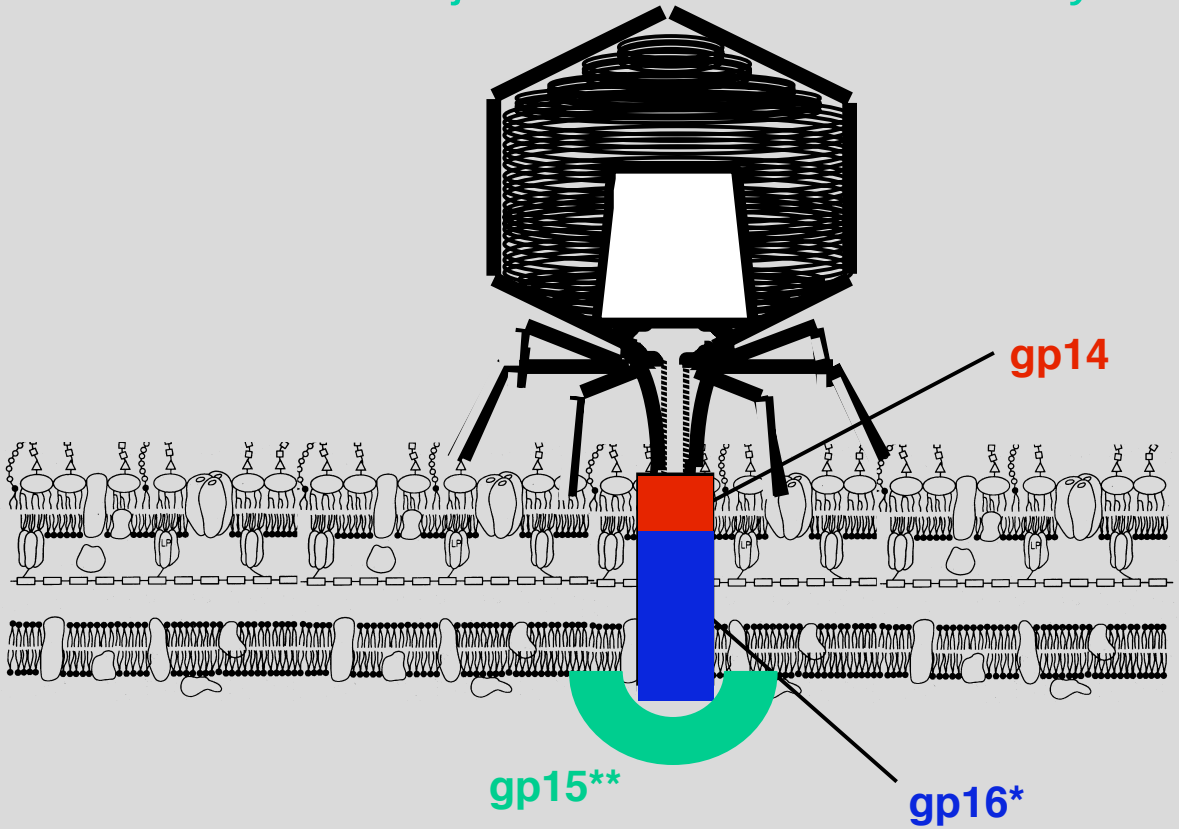


However, the entire T7 genome appears to be stable in the head of the infecting phage!

The C-terminus of gp16 is essential for DNA ejection



A mutant gp15** restores normal infectivity to a virion containing gp16*, and DNA is ejected into the cell normally



Transport of T7 DNA into the infected cell requires energy.

That energy must be supplied by the cell, any forces associated with the encapsidated T7 genome do not cause DNA ejection *in vivo.*

SST of T5 DNA also occurs when the capsid is removed and ~110 kb of the naked genome is in the culture medium

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Are forces associated with the densely packed DNA used in ejecting DNA into a cell?

Transport of T7 DNA into the infected cell requires energy.

That energy must be supplied by the cell, any forces associated with the encapsidated T7 genome do not cause DNA ejection *in vivo.*

But, λ DNA enters a totally poisoned cell (with normal kinetics?)

Both FST and SST of T5 DNA occur in the absence of cellular energy (with normal kinetics?)

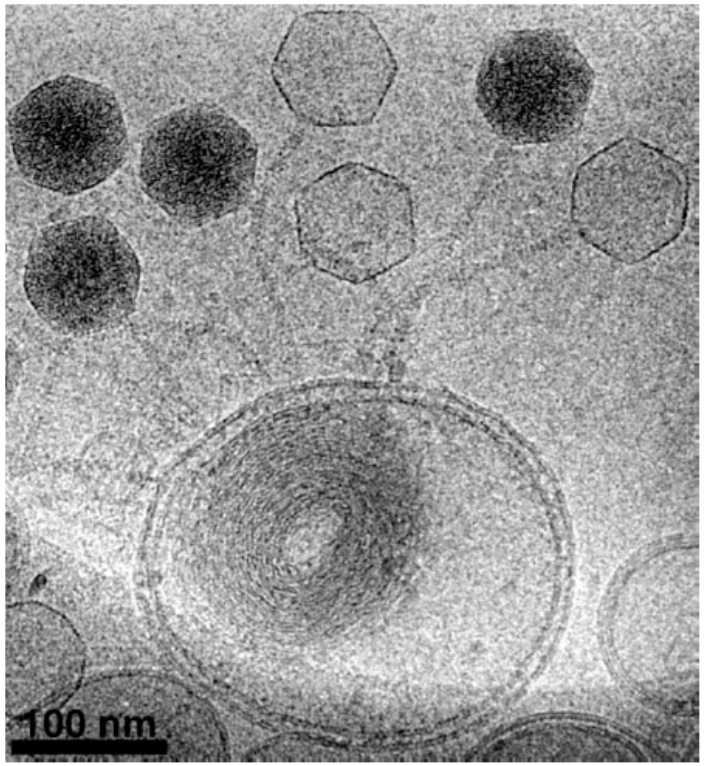
BUT:

λ DNA enters a totally poisoned cell (normal kinetics?)

Both FST and SST of T5 DNA occur in the absence of cellular energy (normal kinetics?)

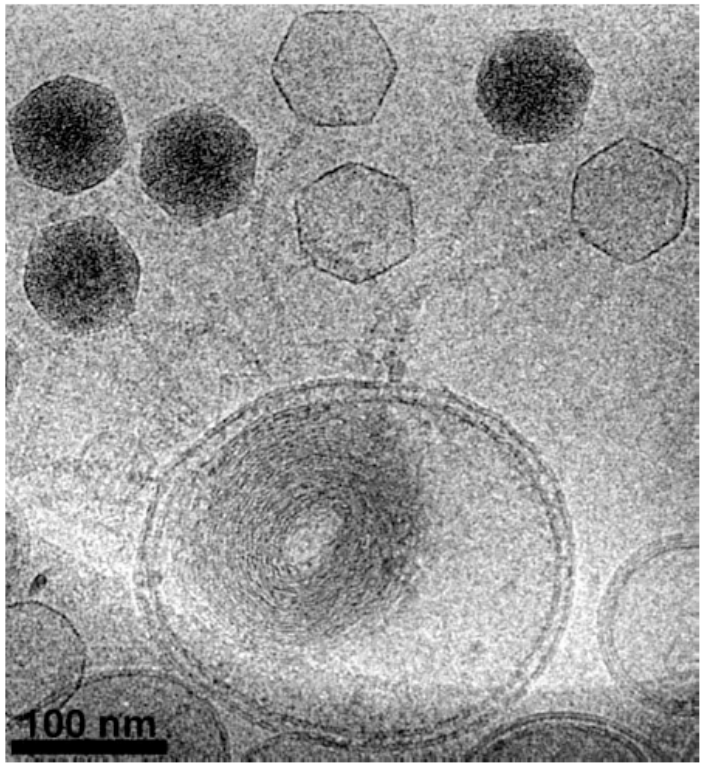
SST of T5 DNA also occurs when the capsid is removed and ~110 kb of the naked genome is in the culture medium

T5 can eject its genome into a FhuA-containing proteoliposome *in vitro*



Letellier et al., Curr. Biol. 2001

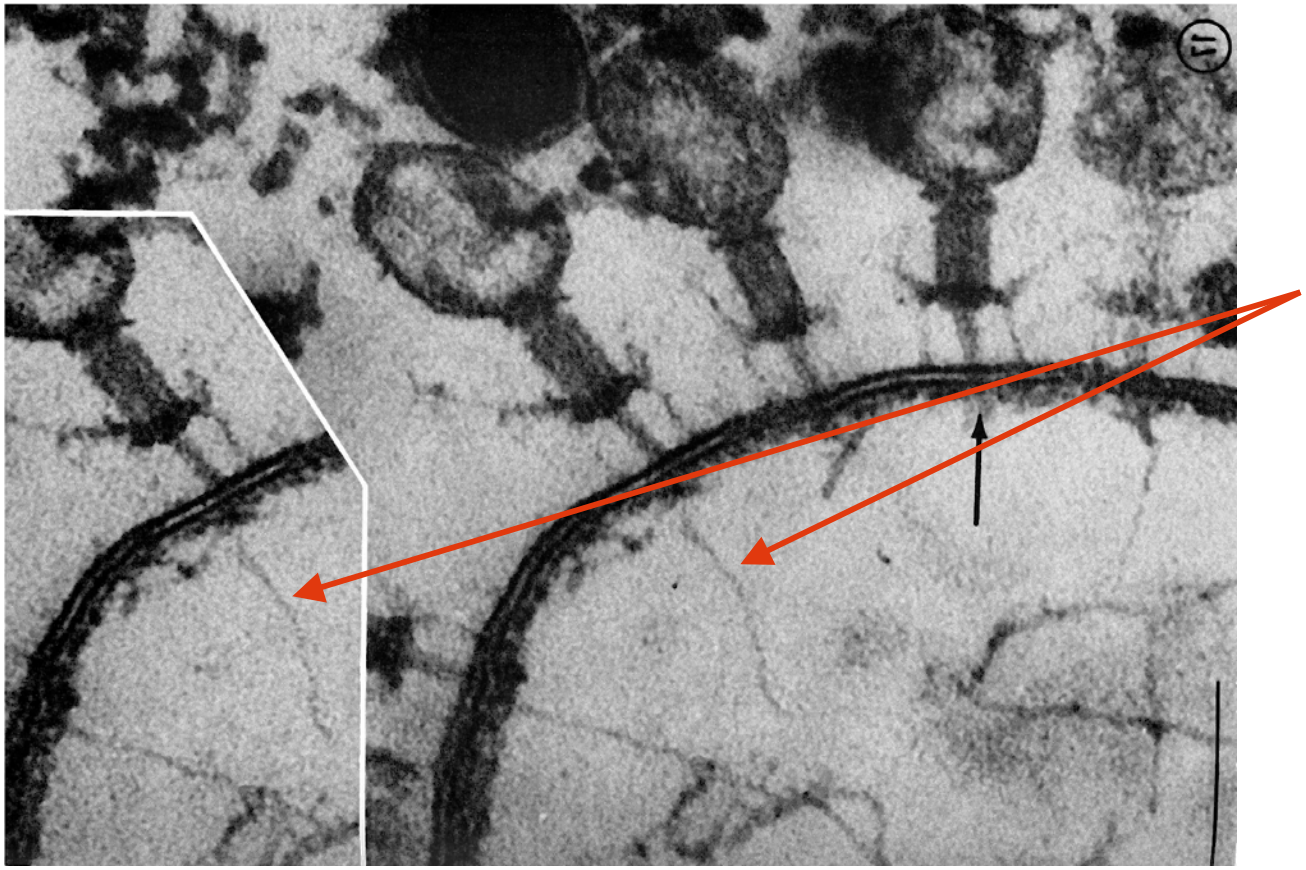
T5 can eject its genome into a FhuA-containing proteoliposome *in vitro*



Letellier et al., Curr. Biol. 2001

And in single molecule experiments *in vitro*,
at ~75 kb/sec at room temp. Letellier et al., Curr Biol. 2005

This remarkable thin section EM by Lee Simon (1967) shows a T4 genome on its way into the infected cell. At least 3 kb DNA is visible



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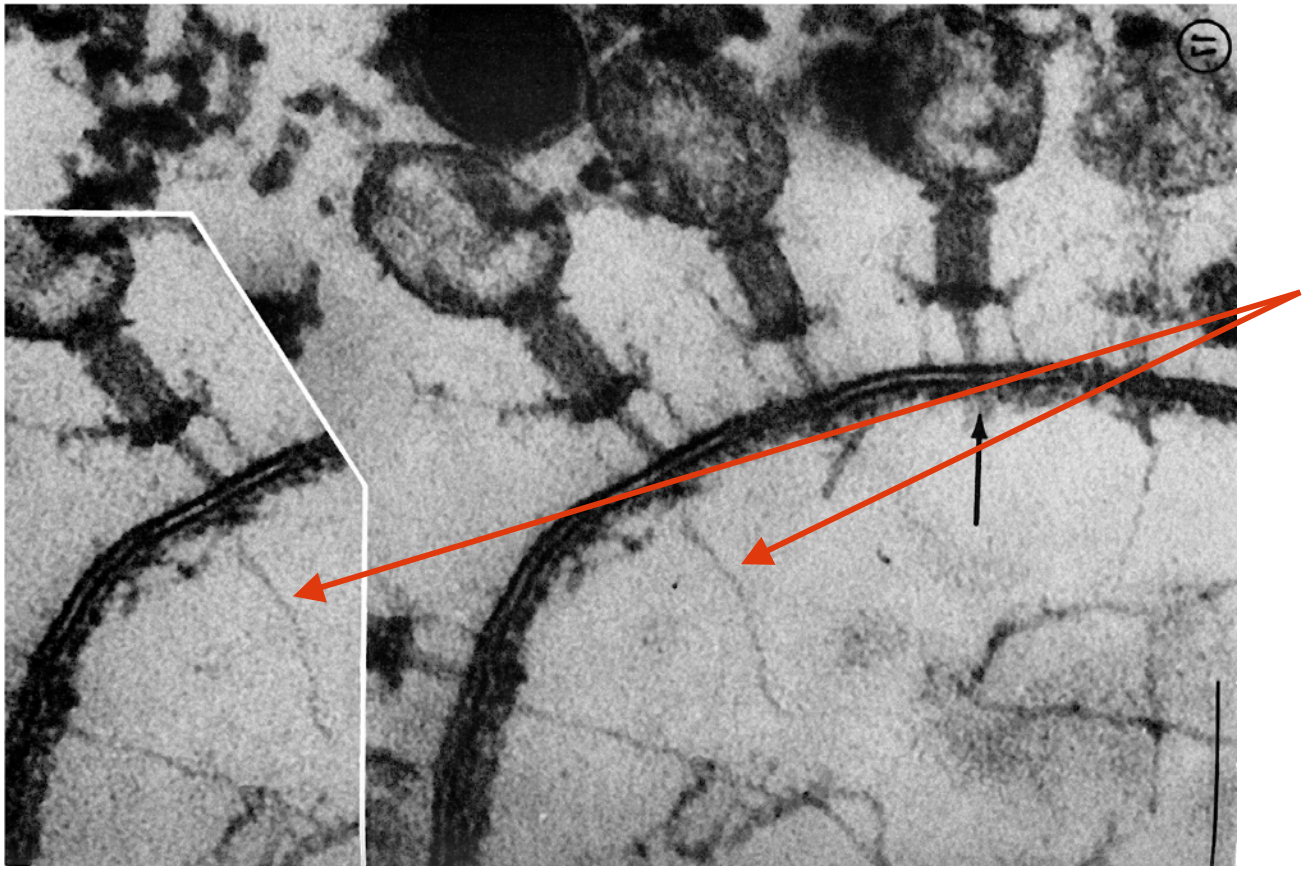
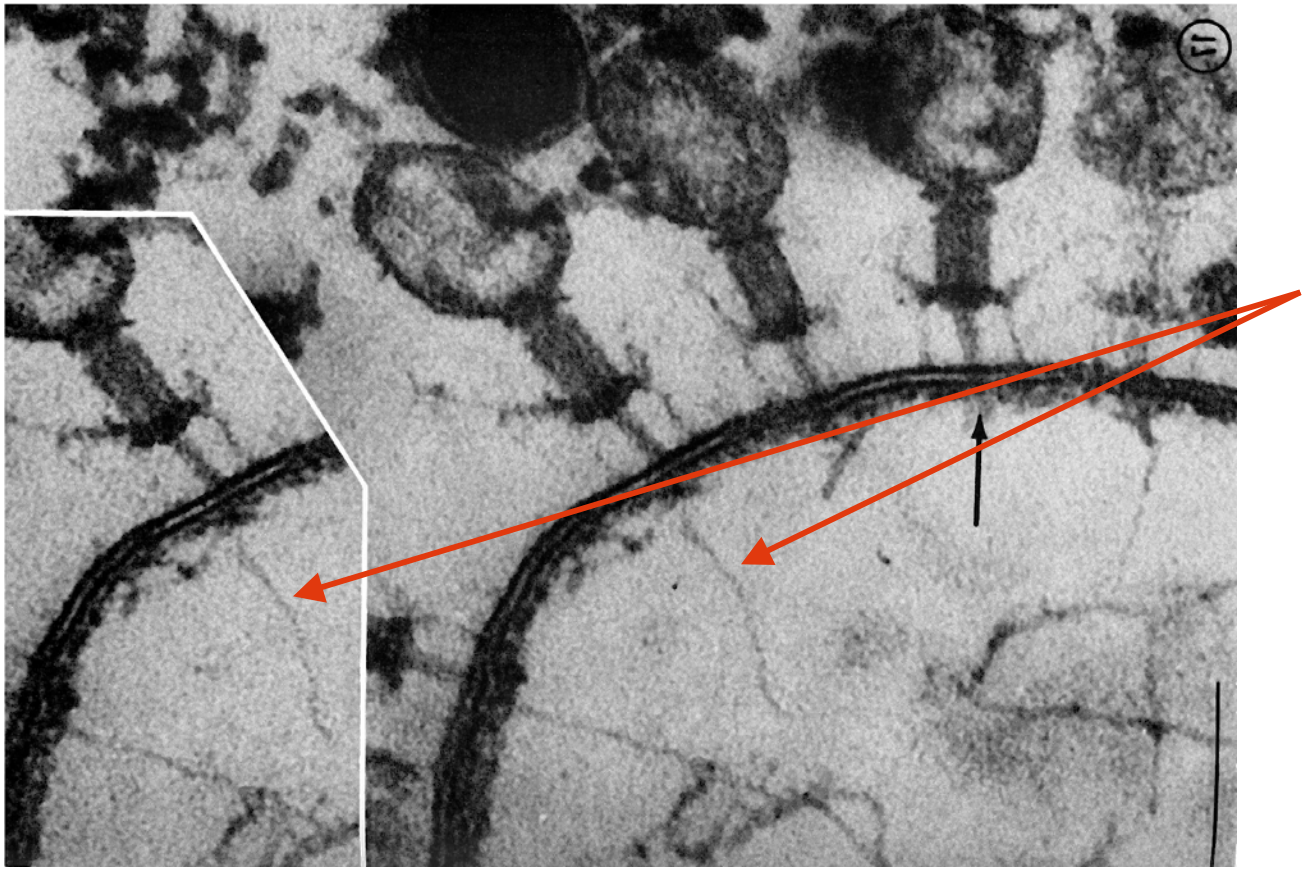
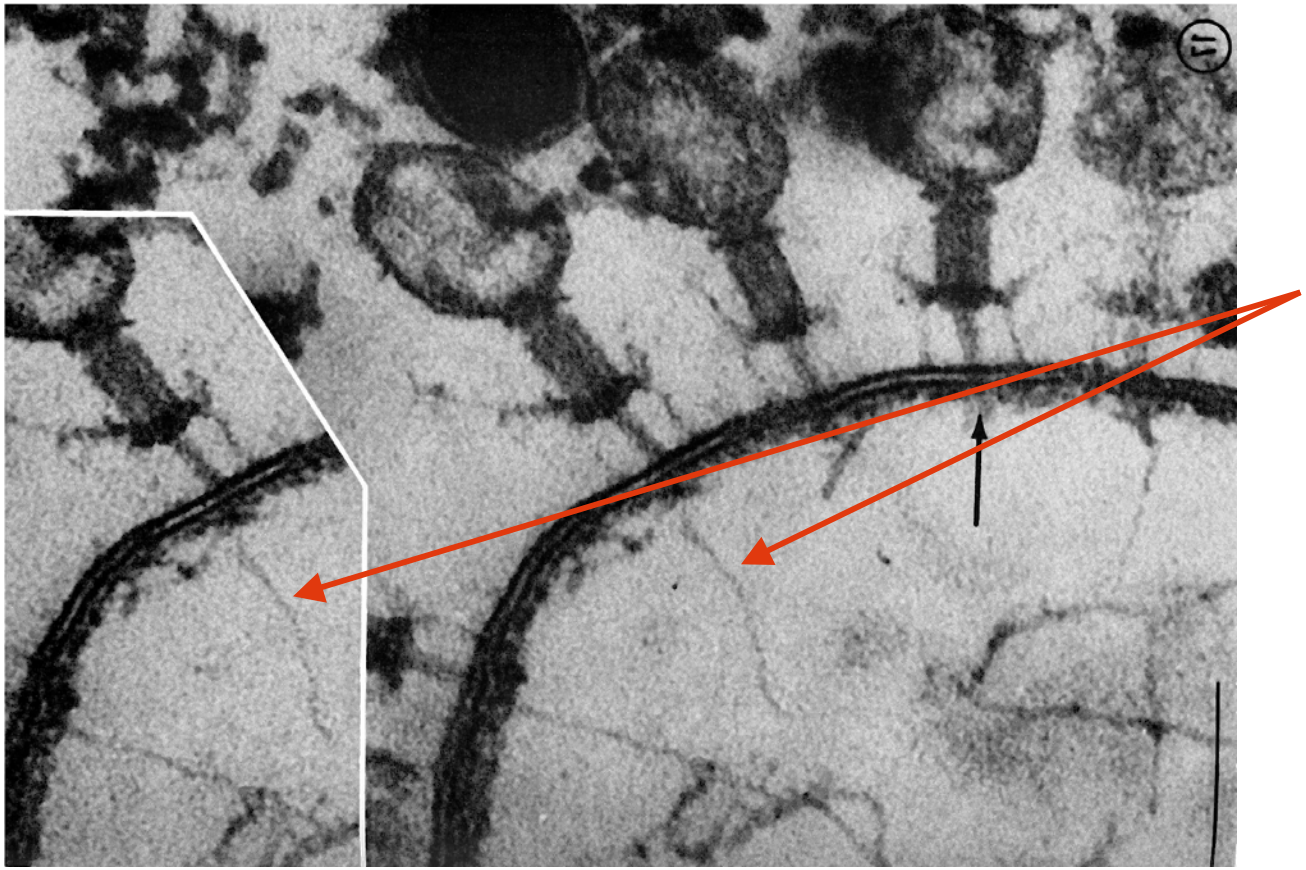


DNA is considered a flexible polymer of persistence length ~150 bp. Yet the entering T4 DNA appears linear and not balled up as it enters the cytoplasm



By comparing the two images, varying by 0.3μ focus, Simon and Anderson concluded that the helical periodicity of the entering DNA was 4 nm, greater than the 3.4 nm of normal B-DNA



By comparing the two images, varying only by 0.3μ focus, Simon and Anderson concluded that the DNA helical periodicity was 4 nm, greater than the 3.4 nm of normal B-DNA

The T4 genome appears that it is being pulled into the cell!

What can be pulling the T4 genome into the cell?

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One clue may stem from the observation that T4, like most phages - although significantly not T7 - causes a transient depolarization of the cell membrane at the initiation of infection. During this period, cytoplasmic ions leak from the infected cell into the external milieu.

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One clue may stem from the observation that T4, like most phages - although significantly not T7 - causes a transient depolarization of the cell membrane at the initiation of infection. During this period, cytoplasmic ions leak from the infected cell into the external milieu.

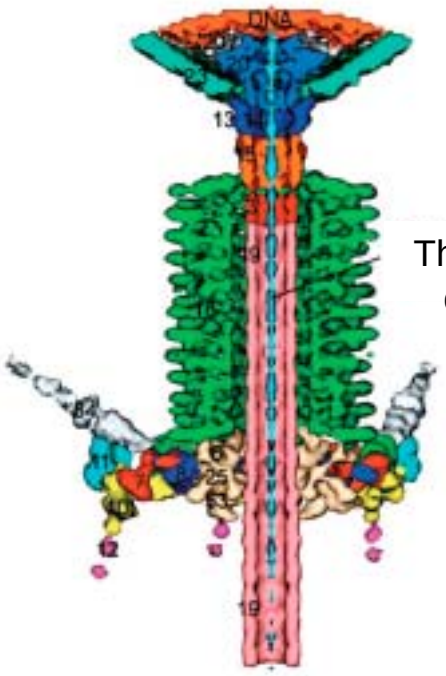
If ions can leak out, perhaps water can flow from the culture medium into the cell, providing a hydrodynamic force that acts on the phage genome.

In other words:

In other words:

BACTERIA SUCK

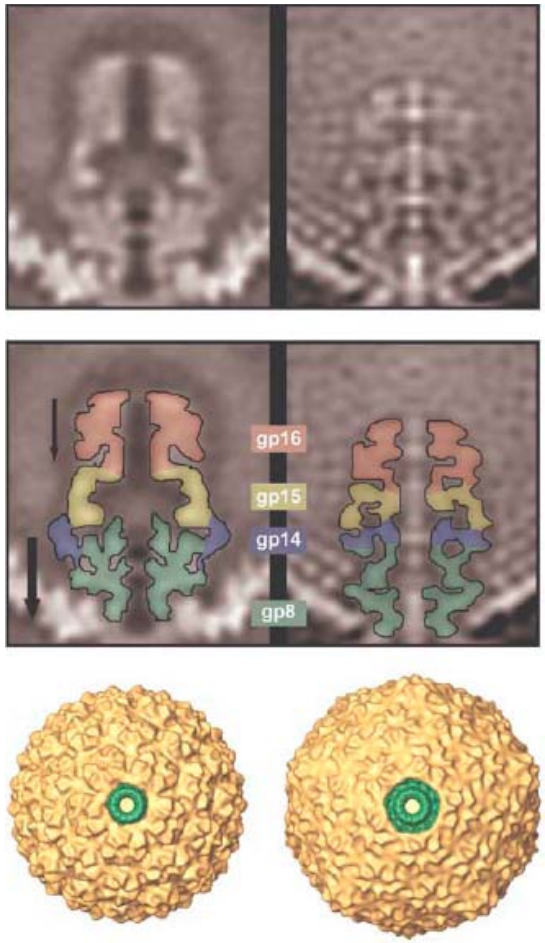
CryoEM reconstruction of contracted T4



The average inner diameter of the T4 tail tube is 43Å

Leiman et al., Biochemistry (Moscow), 2004

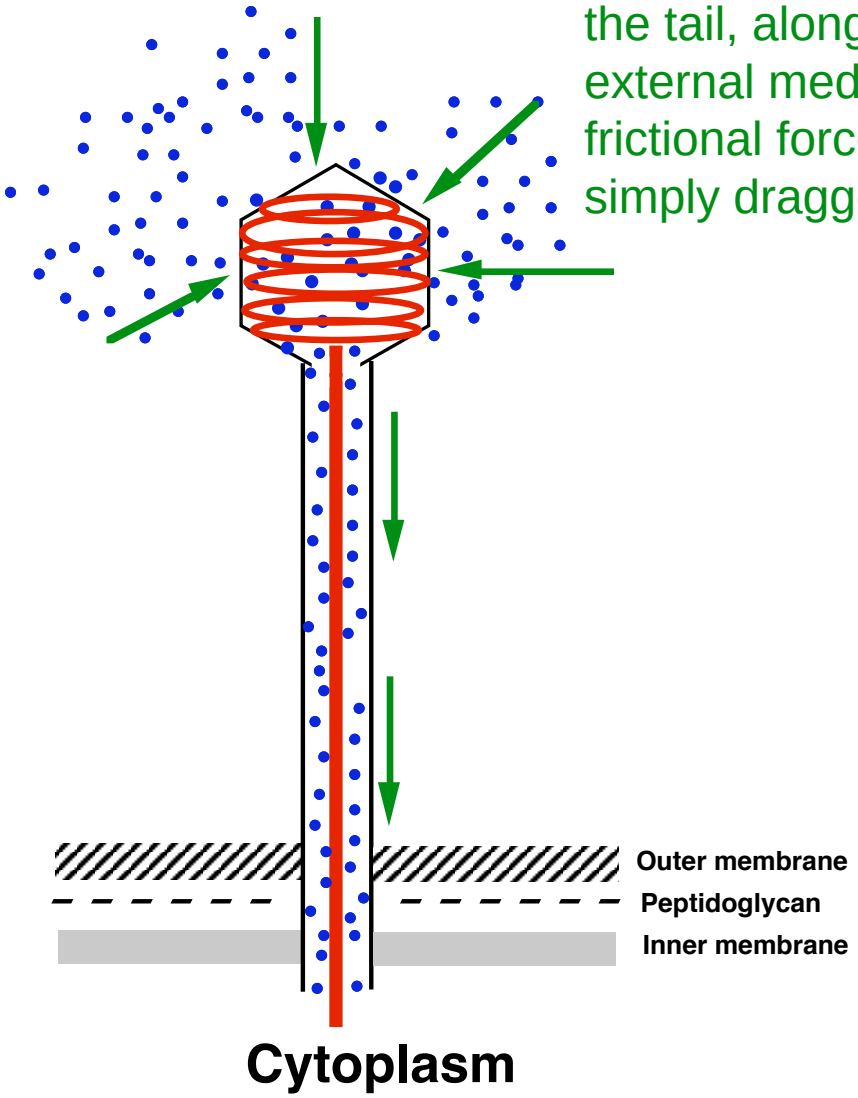
CryoEM reconstruction of T7



Carrascosa et al., 2005

The diameter of the channel through the connector, through which DNA travels into the cell is ~33Å, with a strangling region of ~22Å. There is no information for the extensible T7 tail.

The osmotic pressure differential between the extracellular environment and the cell cytoplasm may provide the force for phage genome ejection



Water molecules moving into the head and through the tail, along the osmotic gradient between the external medium and the cell cytoplasm, exert a frictional force on the infecting genome. DNA is simply dragged into the cell by the moving fluid.

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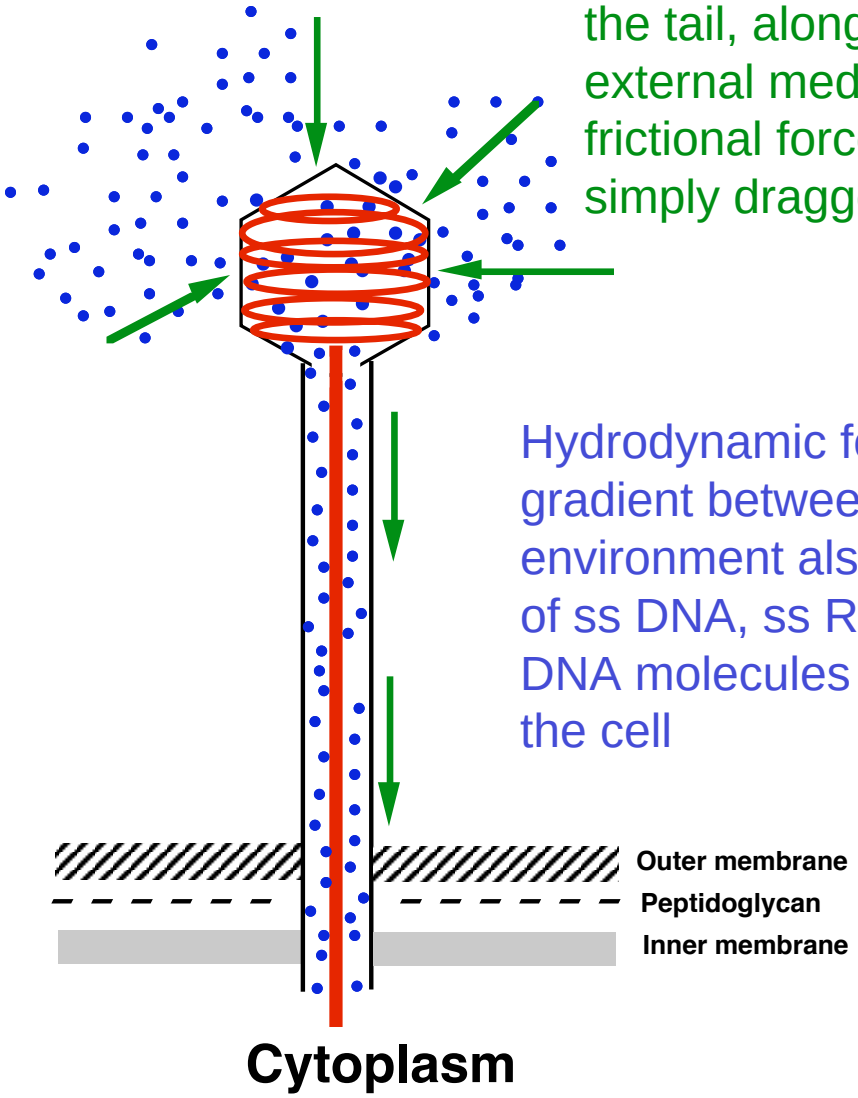

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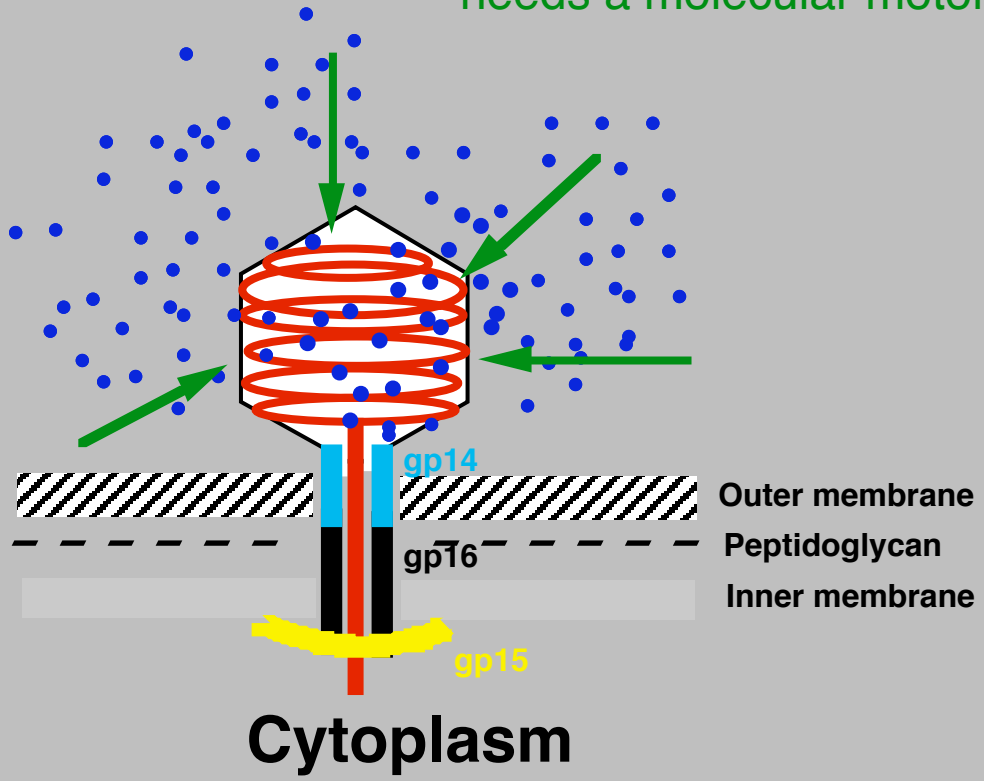
Hydrodynamic forces resulting from an osmotic gradient between the extra- and intra-cellular environment also provide a mechanism for transport of ss DNA, ss RNA, proteins, and even multiple DNA molecules from an infecting phage virion into the cell

Why doesn't T7 eject its DNA in the absence of cellular energy?

The extensible T7 tail is too narrow to allow water molecules to flow past the entering genome. T7 therefore needs a molecular motor to pull its genome into the cell.



Acknowledgments

These people really did the work

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