Fluctuations in an evolutionary process: the Delbrück-Luria experiment

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This painting depicts the fusion of SARS-CoV-2 (magenta) with an endosomal membrane (green), releasing the viral RNA genome into the cell cytoplasm (blue), where it is beginning to be translated by cellular ribosomes to create viral polyproteins. The painting includes speculative elements that are designed highlight the process, most notably, multiple states of the viral spike protein are shown.

Painting by David Goodsell

https://pdb101.rcsb.org/sci-art/goodsell-gallery/sars-cov-2-fusion

See also

http://pdb101.rcsb.org/learn/flyers-posters-and-other-resources/flyer/virus-structures

for more virus structures.

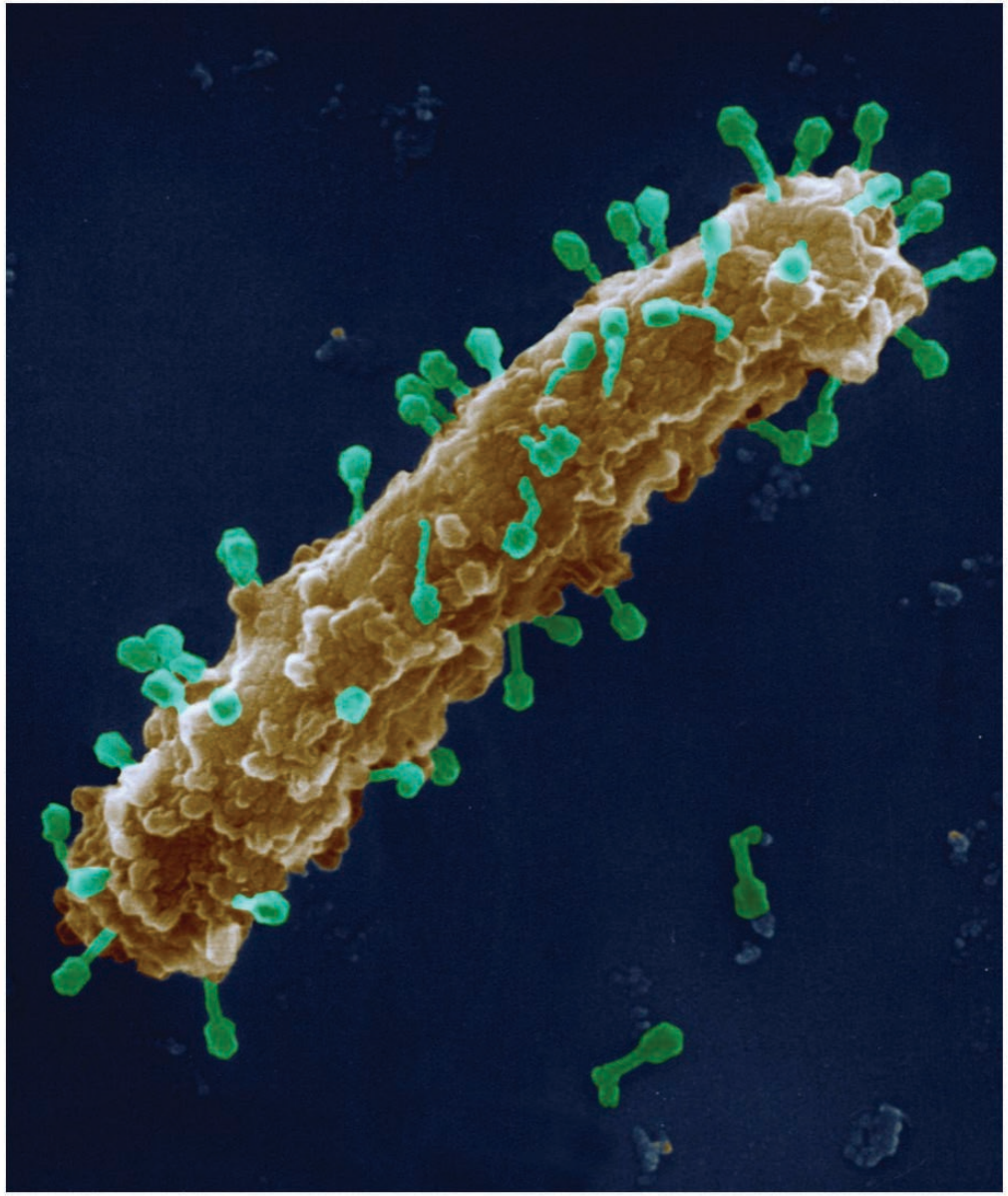

Figure 10.2 Viruses Attached to a Bacterium

The capsule-like coats of these viruses, which are made of protein, remain on the cell surface while the viral DNA enters the bacterium and instructs the cell to make more virus.

From E. O. Wilson's "Life on Earth"

The Nobel Prize in Chemistry 2020

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Emmanuelle Charpentier

Prize share: 1/2

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Jennifer A. Doudna

Prize share: 1/2

The Nobel Prize in Chemistry 2020 was awarded jointly to Emmanuelle Charpentier and Jennifer A. Doudna "for the development of a method for genome editing."

CRISPR

CRISPR stands for "**clustered regularly interspaced short palindromic repeats**", and it is a sort of immune system in bacteria.

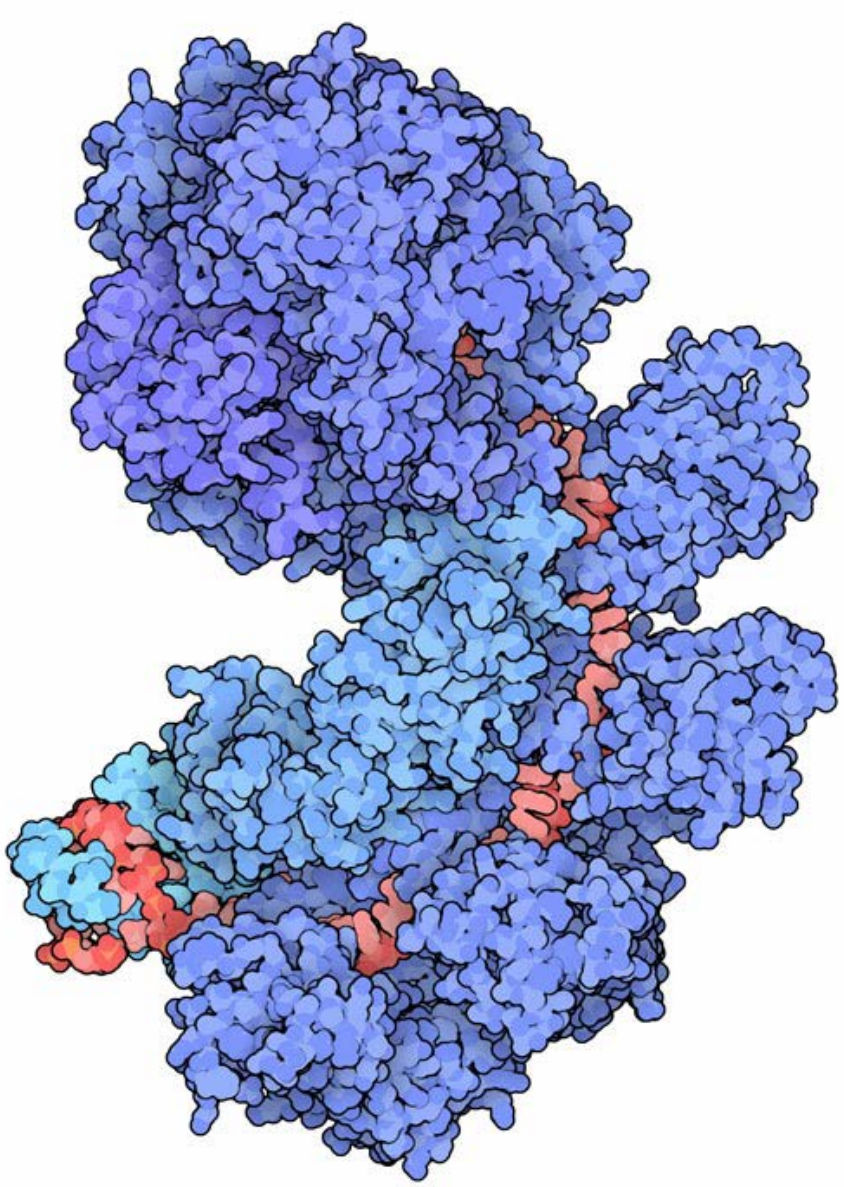
Bacteria use CRISPR sequences, stored in their genome, to identify attacking viruses.

They are composed of many small pieces of viral DNA harvested from viruses that attacked in the past, separated by a distinctive repeated sequence used to create the archive.

Remarkably, new sequences are added at the beginning of this collection, so we can read the CRISPR sequence to get a history of viruses that have attacked the bacterial population in the past.

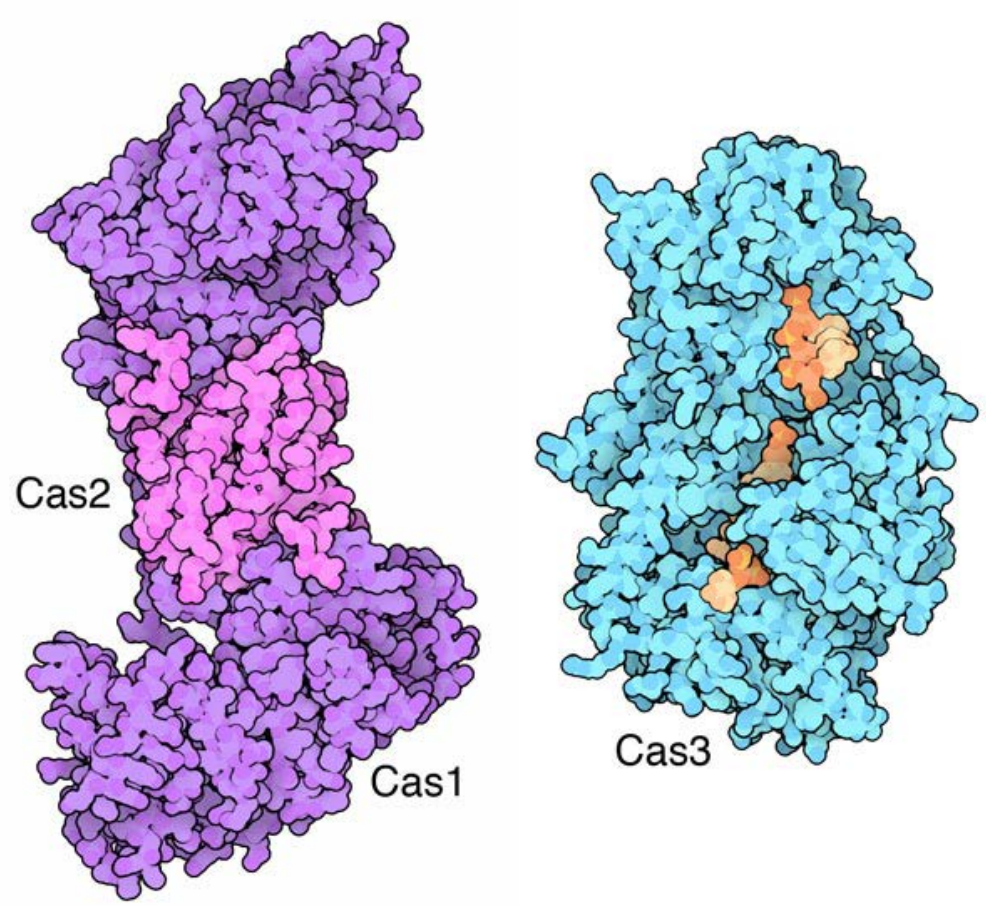
A system of Cas proteins (short for "CRISPR-associated proteins") use this stored information to fight the viruses if they try to infect the bacteria again.

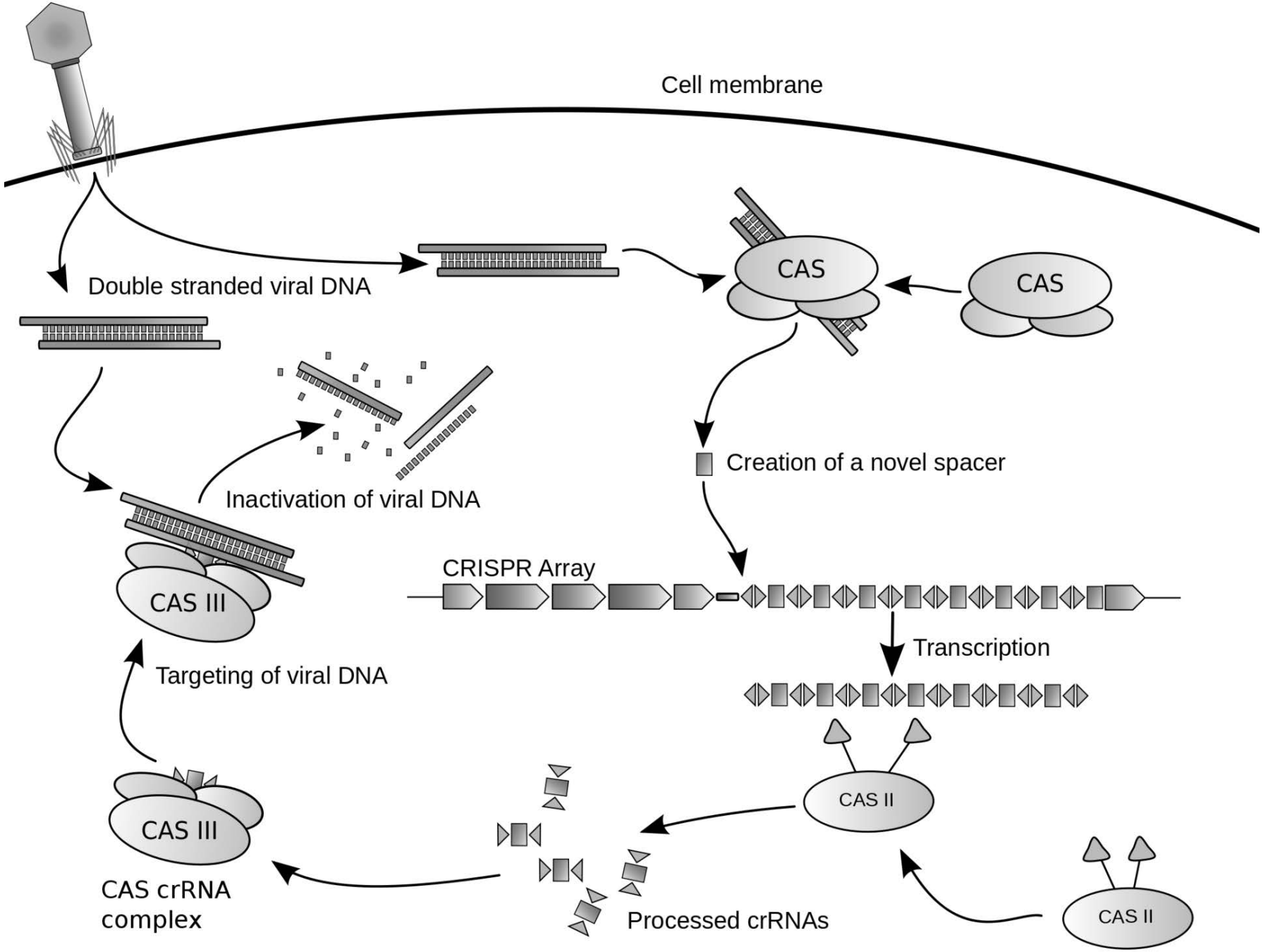
The center of this system is the large complex Cascade. It carries an RNA transcript of the CRISPR sequence and searches through the cell for matching viral DNA from an infection. If it finds viral DNA, it unwinds it and mobilizes nucleases to cut it up. The structure shown here includes the Cascade surveillance complex composed of 6 different types of proteins (in different shades of blue) along with the RNA transcript (red)



Cascade works with a team of proteins to build the CRISPR archive and use it to protect against viruses.

First, recruiting proteins like Cas1 and Cas2 are needed to chop up viruses during an infection and save appropriately-sized bits in the CRISPR. These pieces are then displayed by Cascade to search for later infections by the virus. If the virus is found, Cas3 is the executioner, taking the infecting viral DNA found by Cascade and destroying it.



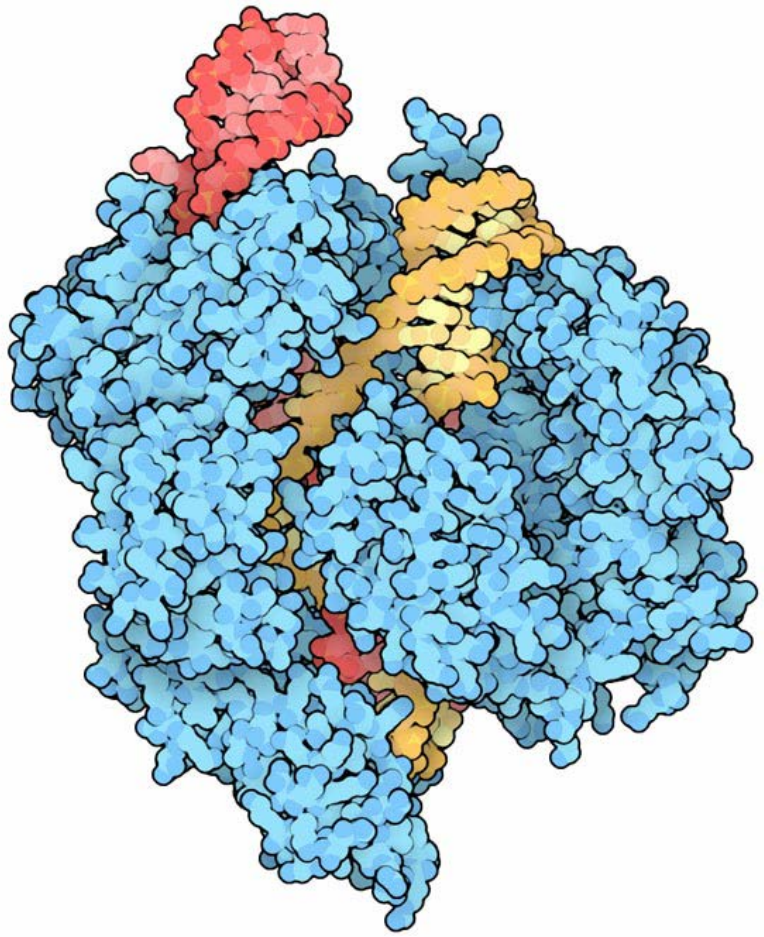

Cell membrane
Double stranded viral DNA
CAS
CAS
Creation of a novel spacer
Inactivation of viral DNA
CRISPR Array
CAS III
Transcription
Targeting of viral DNA
CAS II
CAS III
CAS crRNA
complex
Processed crRNAs
CAS II

Different bacteria and archaea have evolved a number of variations on this general theme.

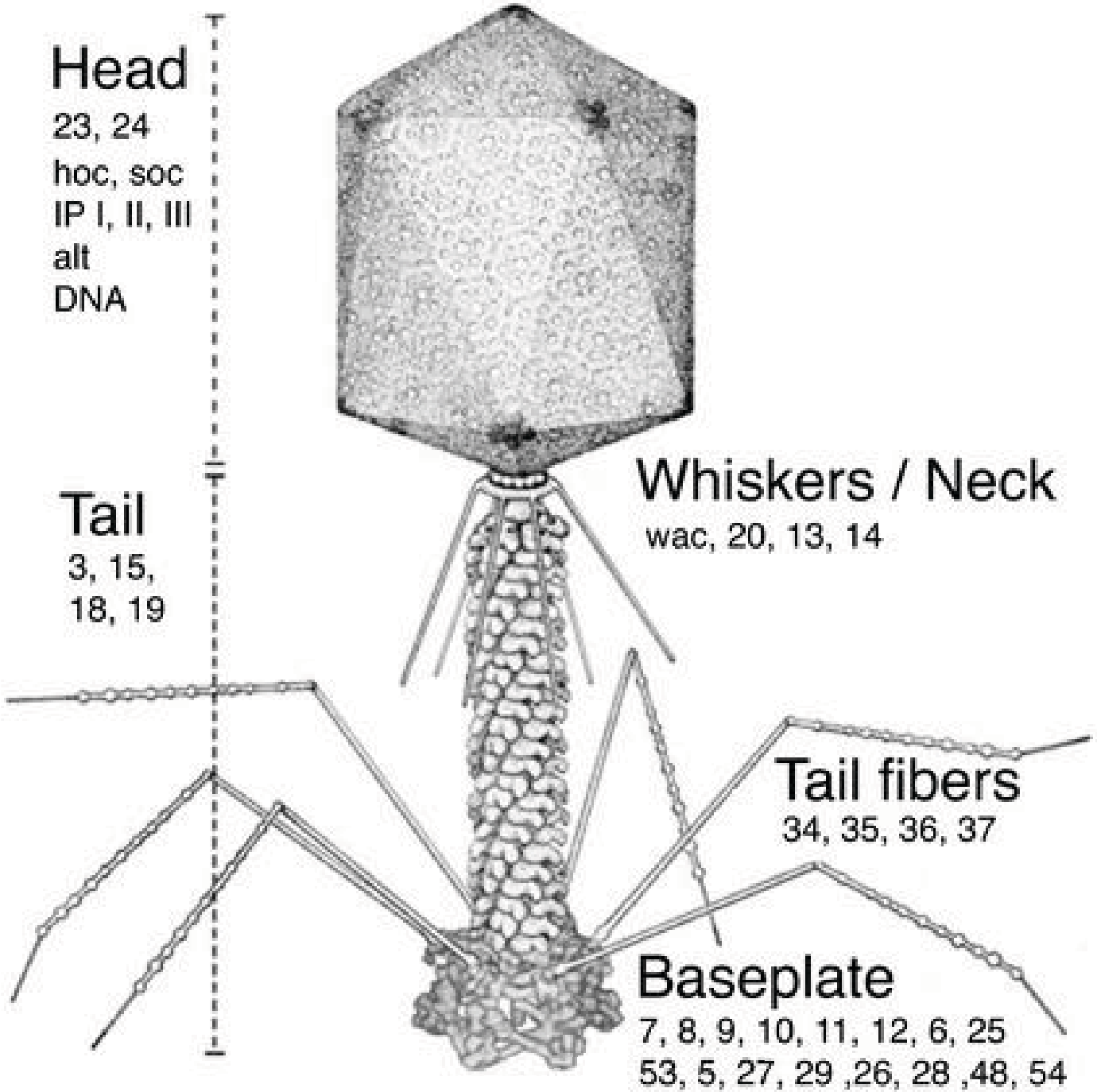
The Cascade complex, termed Type I, displays CRISPR RNA and recruits the executioner Cas3 to chop up the viral DNA.

Type III complexes, on the other hand, have the DNA-cutting enzyme as part of the complex.

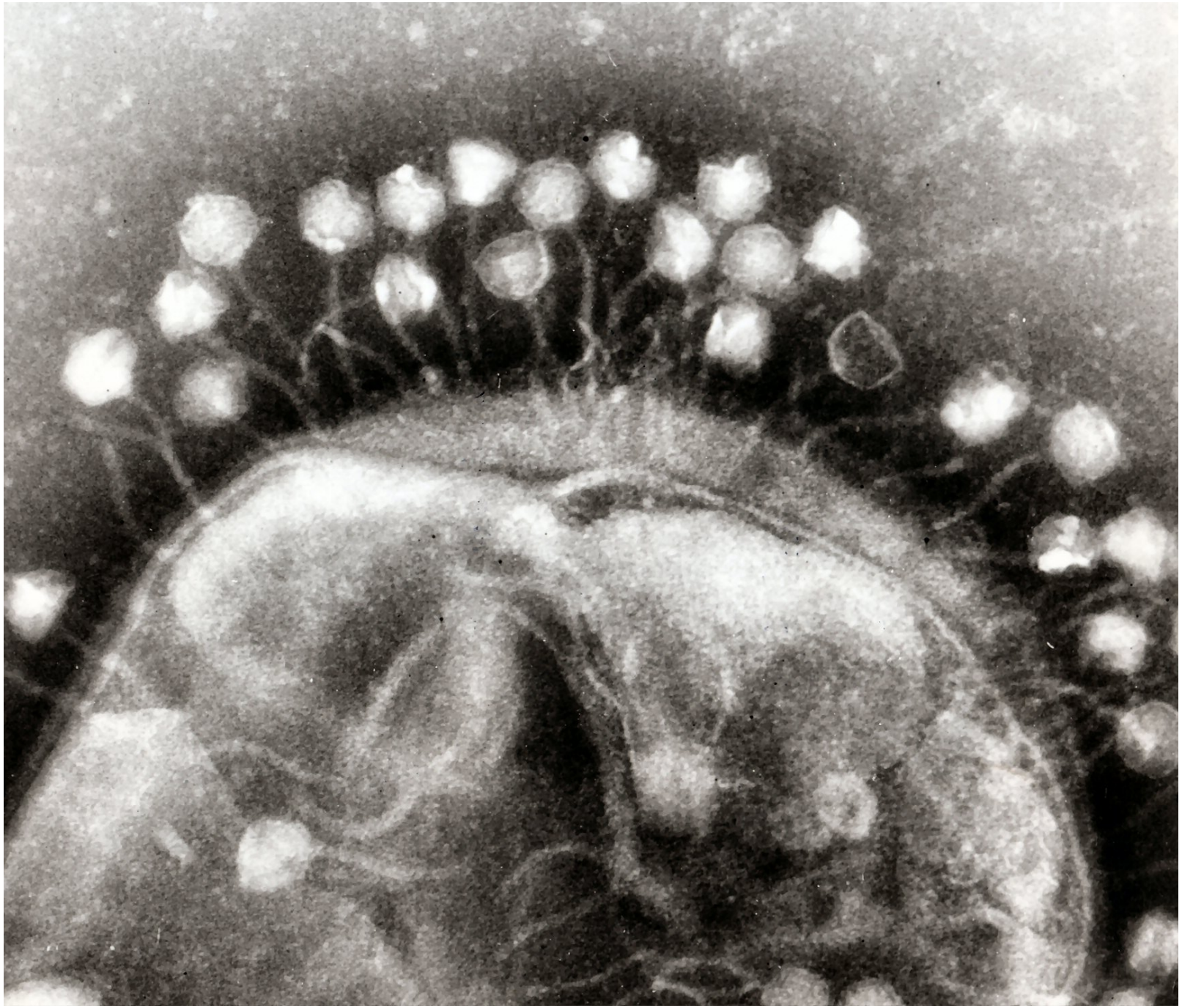
Type II CRISPR systems, such as Cas9 have a surveillance protein and executioner all wrapped up in a single protein chain. The complex shown here includes the CRISPR RNA (red) along with a piece of an attacking viral DNA (yellow).

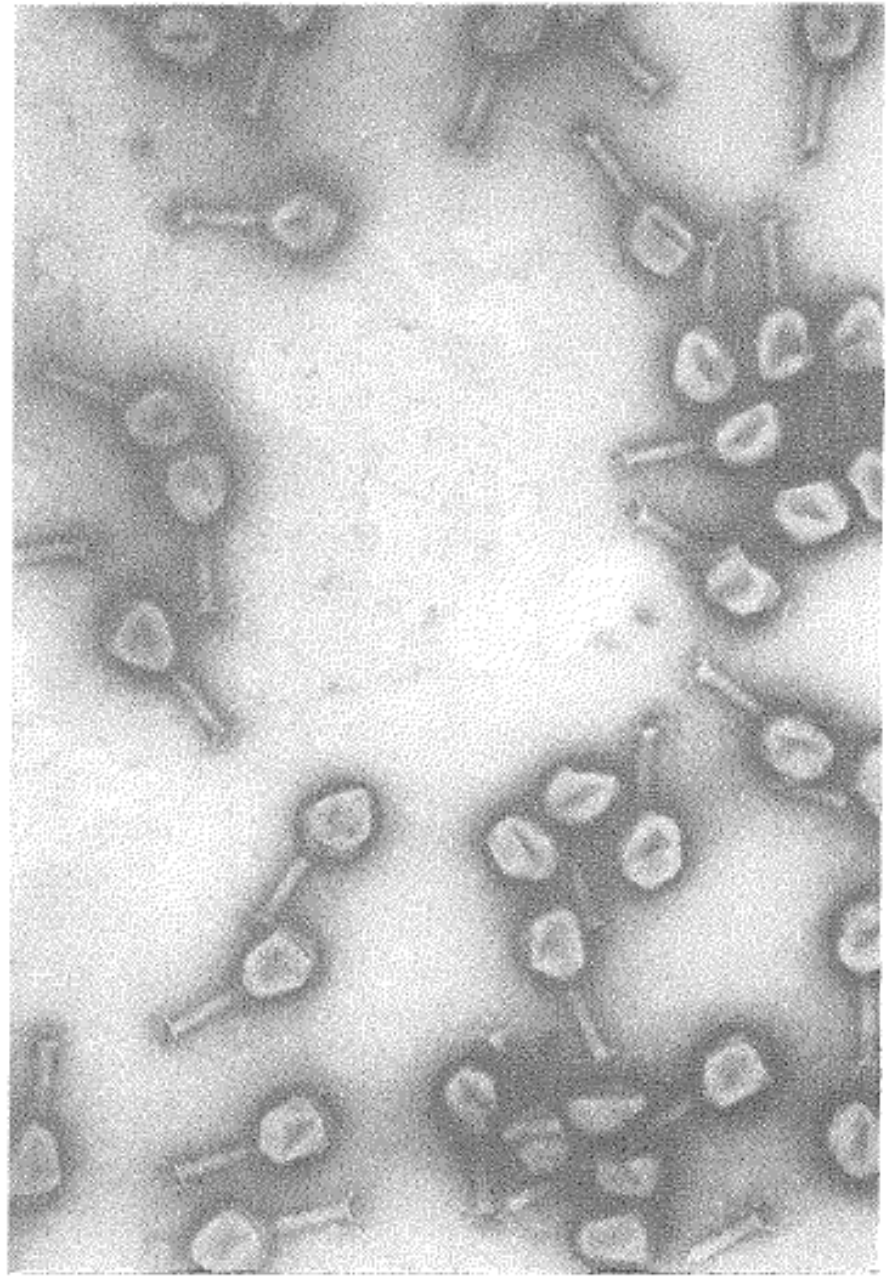


This molecule has recently been used in an experimental approach to curing latent HIV infection. An engineered virus was used to insert Cas9 and an anti-HIV CRISPR into HIV-infected cells, which then chopped up the integrated viral DNA. (from http://pdb101.rcsb.org/motm/181)



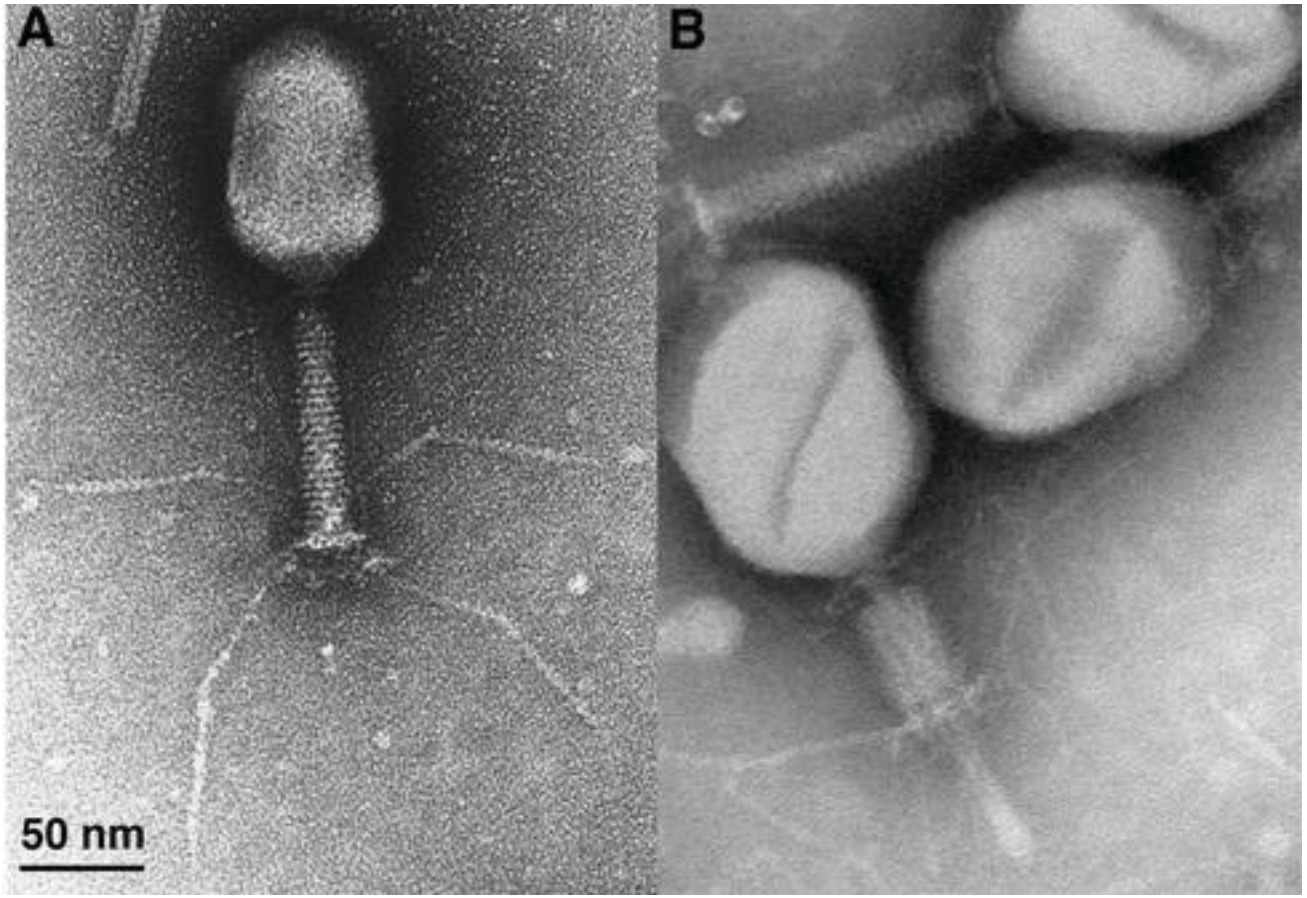
from Karam, J. D., J. W. Drake, K. N. Kreuzer, G. Mosig, D. H. Hall, F. A. Eiserling, L. W. Black, E. K. Spicer, E. Kutter, C. Carlson, and E. S. Miller (ed.). 1994. Molecular biology of bacteriophage T4. American Society for Microbiology, Washington, D.C.





Heads, tails, and tail fibers of T4 bacteriophages can be distinguished in this electron micrograph taken by Ronald Luftig. Magnification is about 150,000 diameters.

from R. S. Edgar and W. B. Wood, "Genetics and Development at the Threshold of Life – The Caltech Phage Group", Engineering and Science **32** (1968) 18



Electron micrographs of bacteriophage T4. (A) Extended tail fibers recognize the bacterial envelope, and its prolate icosahedral head contains the 168,903-bp dsDNA genome. Reprinted with permission of M. Wurtz, Biozentrum, Basel, Switzerland. (B) The DNA genome is delivered into the host through the internal tail tube, which is visible protruding from the end of the contracted tail sheath.

(images and text taken from E. S. Miller et al.: “Bacteriophage T4 genome.”, Microbiol. Mol. Biol. Rev. **67** (2003) 86)

The steps of T4 bacteriophage invasion:

- phage attaches to E. coli using its six fibers at end of tail
- tail enters wall of host (cell wall is degraded using lysozyme from a previous infection)
- viral DNA is injected into the host's cytoplasm (**time: 0**)
- among the **early proteins** produced are a repair enzyme to repair the hole in the bacterial cell wall, a DNAase enzyme that degrades the host DNA into precursors of phage DNA, and a virus specific DNA polymerase that will copy and replicate phage DNA. (**time: +8 min**)
- synthesis of early protein stops and synthesis of **late proteins** starts. The late proteins are mainly structural proteins that make up the capsids and the various components of the tail assembly. (**time: +13 min**)
- first phage particle is complete (**time: +23 min**)
- part of the produced lysozyme (a late protein) is used to destroy the cell wall and the infected cell bursts (**lysis**) and releases more than 100 virus particles (**time: +35 min**)

Max Delbrück

Born: 4 September 1906, Berlin, Germany

Died: 9 March 1981, Pasadena, CA, USA

Nobel Prize in Physiology or Medicine in 1969 (shared with Alfred Hershey and Salvador Luria): "for their discoveries concerning the replication mechanism and the genetic structure of viruses”

Born in Berlin, he had an interest in science since boyhood. He studied astrophysics and then moved to theoretical physics during his graduate studies at Göttingen. During his post-doc in England, Switzerland and Denmark, he contributed to the burgeoning theory of QED, and was in contact with scientific giants such as Pauli and Bohr.

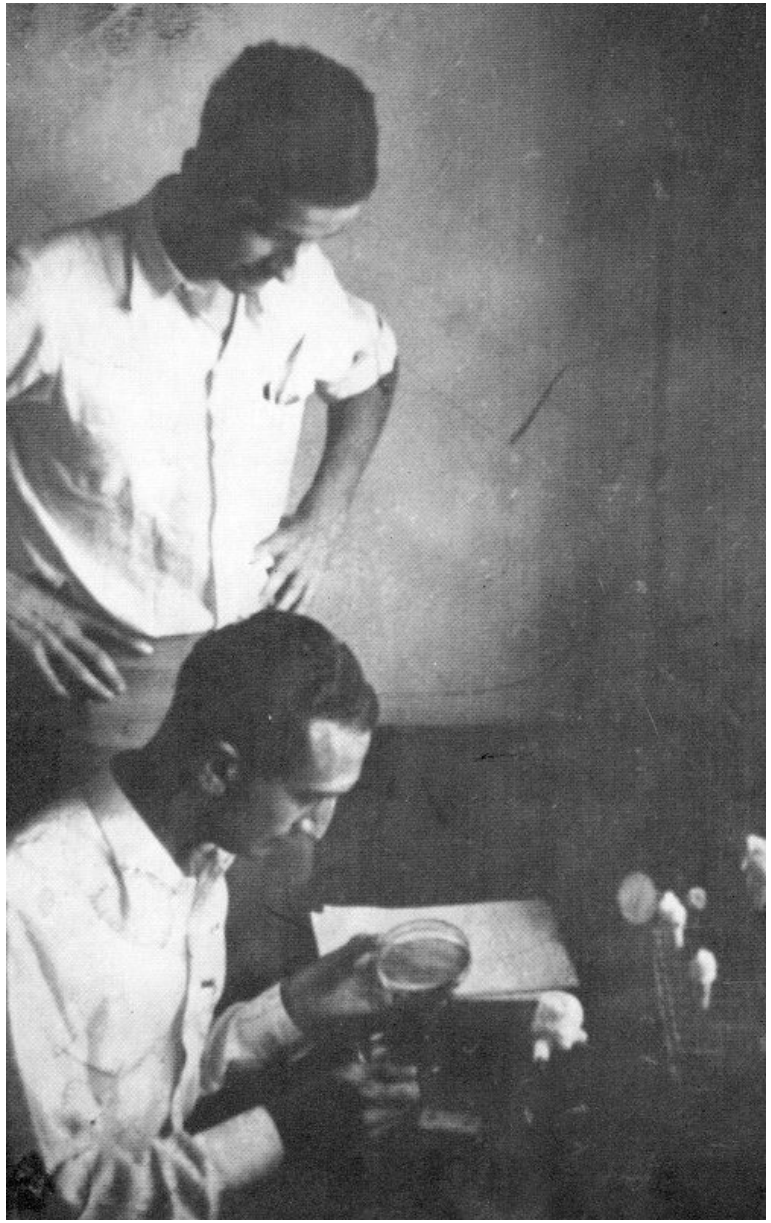
Delbrück moved to Berlin in 1932 where he became assistant to Lise Meitner. He arrived by train on the morning of Bohr’s opening address “Light and Life”, a lecture which changed the course of his life.

In Berlin he had further contacts with the local biologists.

“Paradoxically, this good intention [having contacts with the biologists] was helped by the rise of Nazism which made official seminars less interesting. A small group of physicists and biologists began to meet privately beginning about 1934.
To this group belonged N. W. Timofeeff-Ressovsky (genetics). Out of these meetings grew a paper by Timofeeff, Zimmer, and Delbrück on mutagenesis. A popularization of this paper of 1935 in Schroedinger's little book «What is Life?» (1945) had a curiously strong influence on the development of molecular biology in the late 1940's.” (from the biographical press release for the Nobel prize in Physiology or Medicine awarded to Delbrück in 1969)

Throughout his research Delbrück cultivated the hope of finding some aspect of life not reducible to the basic physico-chemical laws, a kind of “complementarity of life” that would push further the concept put forward by Bohr.

What he left is a lasting contribution to our understanding of the mechanisms of heredity, and – paradoxically indeed – a framework that seems to bar new physics laws, and effectively utilizes the existing ones.



Delbrück (standing)
and Luria in 1941

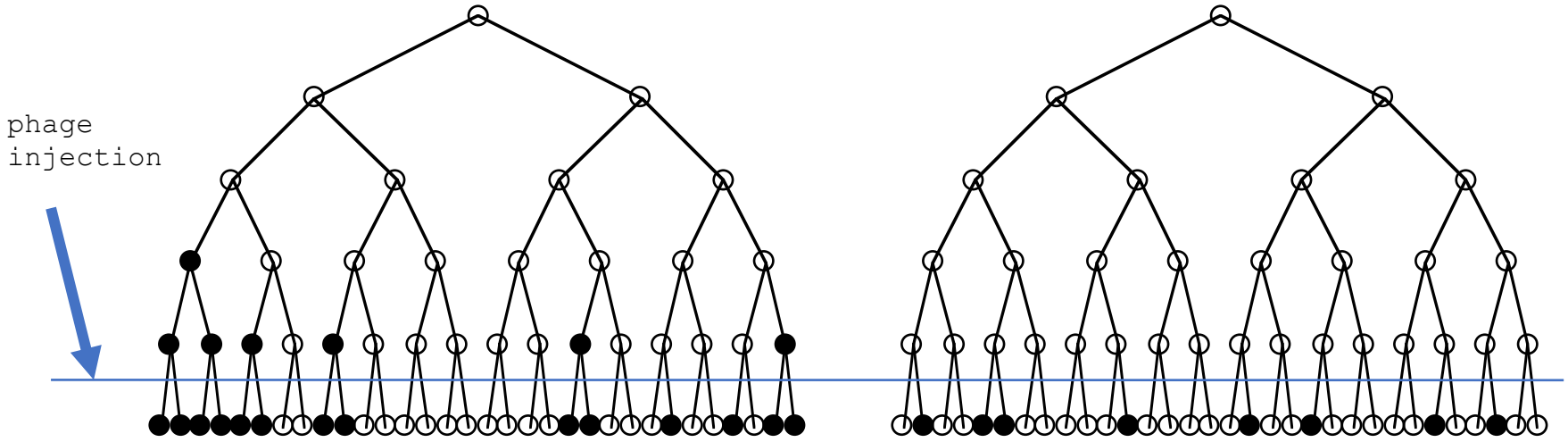
Petri dish with colonies

The experiment of Luria and Delbrück (Genetics **28** (1943) 491).

After exposure to phages only few bacteria survive ...is resistance acquired or does it arise from random mutations?

Random mutations: resistance arises randomly in previous generations

Adaptive heritable resistance: virus action induces resistance which is inherited by offspring



Exponential population growth:

Bacteria proliferate with average duplication time *T*, thus the population size at time *t* is

$$N(t) = 2^{t/T} N_0 = N_0 \exp\left(\frac{t}{T} \ln 2\right)$$

Luria and Delbrück chose time units such that $\frac{T}{\ln 2} = 1$, and therefore

$$N(t) = N_0 e^t \qquad \frac{dN}{dt} = N(t)$$

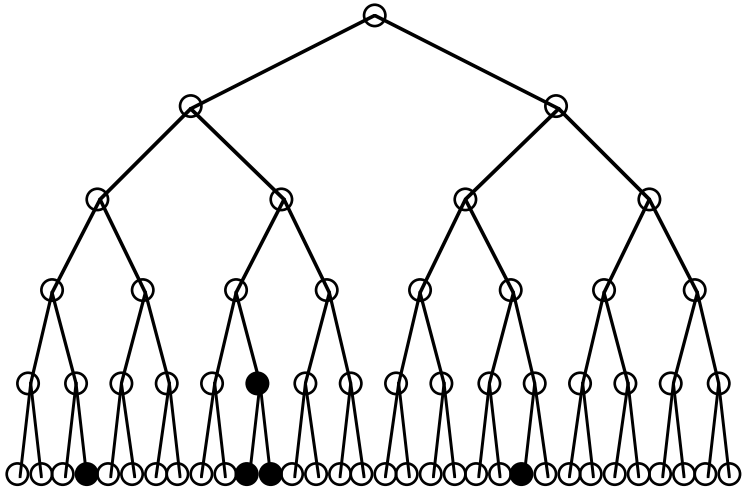
A. Hypothesis of acquired immunity

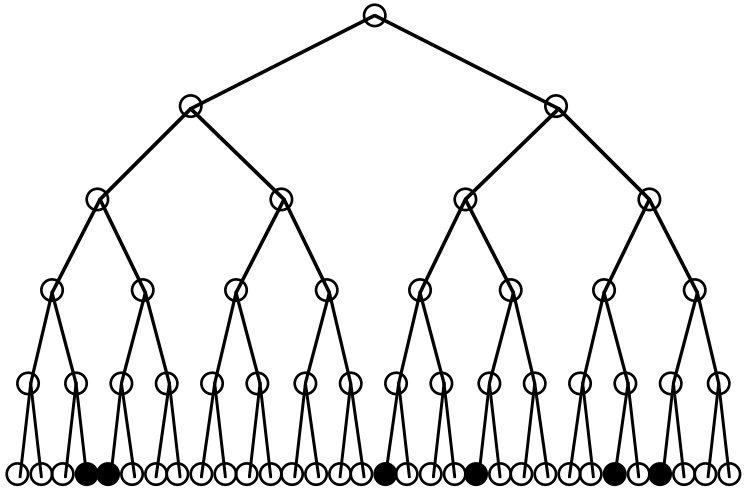
Let *p* be the probability of acquiring immunity after exposure to phages, then the number of bacteria that acquire immunity is a binomial variate.

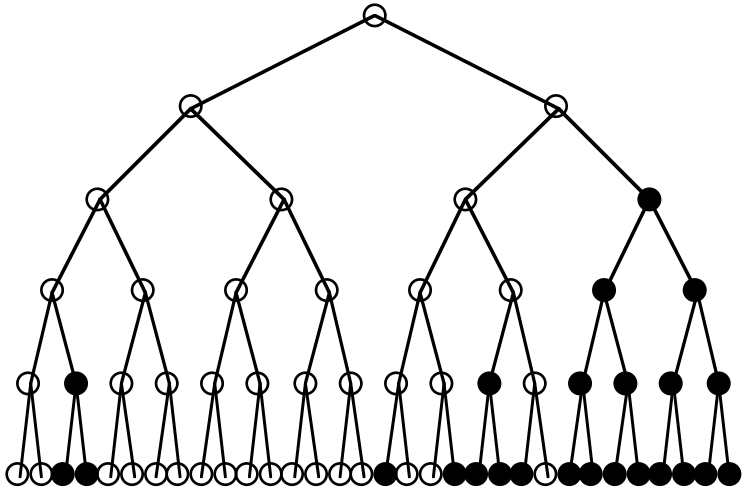
However from experiment we know that few bacteria survive the attack, so *p* must be small and the binomial distribution can be approximated by a Poisson distribution:

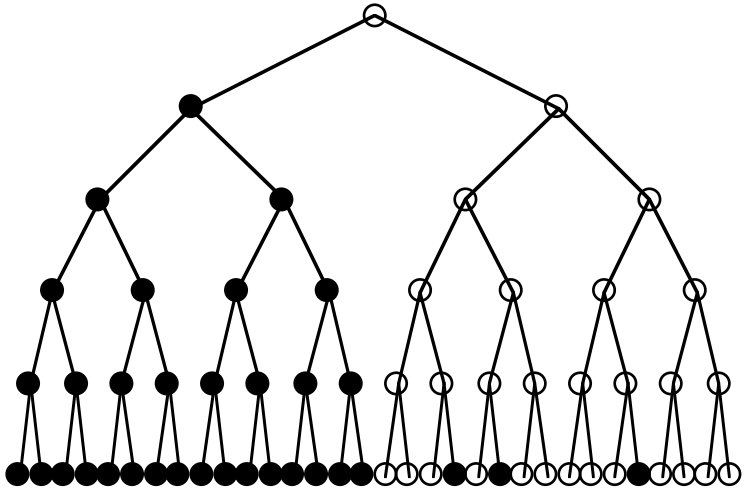
therefore – with this hypothesis – the variance of the number of resistant bacteria is equal to the average.

In the case of **random mutations** we expect a much larger variance

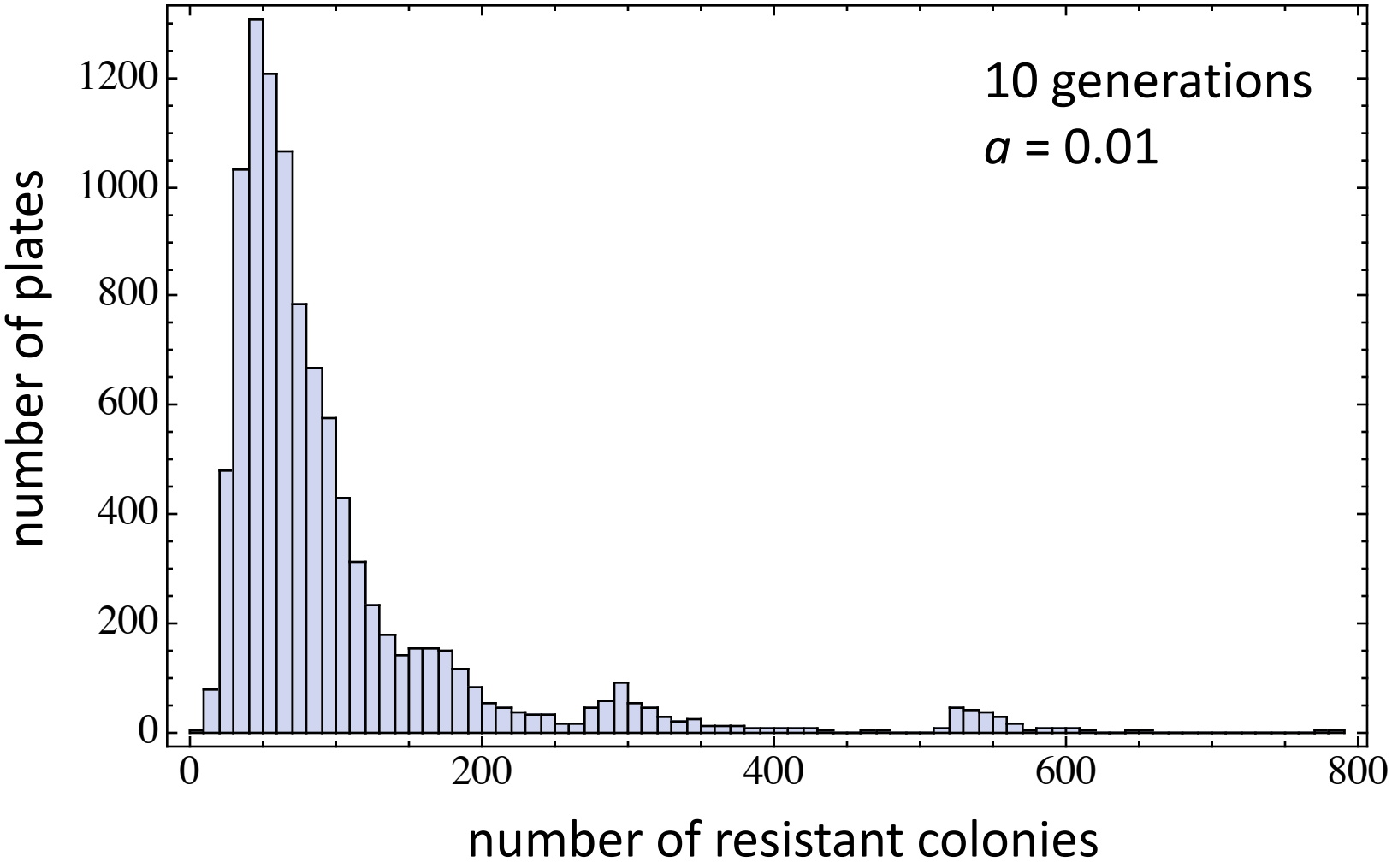








Simulation of Luria-Delbrück experiment



B. Hypothesis of random mutations

Let *a* be the rate of mutation that endows resistance:

$$\Delta p = a\Delta t$$

probability of mutation in time interval Δt

$$\Delta m \approx N(t)\Delta p = N_0 e^t a\Delta t$$

number of mutated cells in the same time interval

The number of mutated cells *in this time interval* is a Poisson variate, therefore

$$\text{var } \Delta m = \Delta m$$

Average population of resistant bacteria

$$d\rho = \left[N(t) - \rho(t)\right]a dt + \rho(t)dt$$

new mutations in the time interval *dt*

exp growth of the population of resistant bacteria

$$d\rho = N(t)a dt + (1-a)\rho(t)dt$$

$$\approx N(t)a dt + \rho(t)dt \qquad (a \ll 1)$$

approximate differential equation

The approximate differential equation

$$\frac{d\rho}{dt} - \rho = aN(t)$$

can be transformed into

$$e^t \frac{d}{dt}\left(e^{-t}\rho\right) = aN_0 e^t$$

and therefore the mean value of mutated cells is

$$\rho(t) = aN_0 t e^t + Ce^t \quad \text{with} \quad \rho(0) = 0$$
$$\Rightarrow \quad \rho(t) = atN(t)$$

Variance of the population of mutated cells:

$$\Delta\rho = \rho(t)\Delta t + aN(t)\Delta t \approx \Delta m$$

$$\text{var}\ \Delta\rho \approx \text{var}\ \Delta m = \Delta m$$

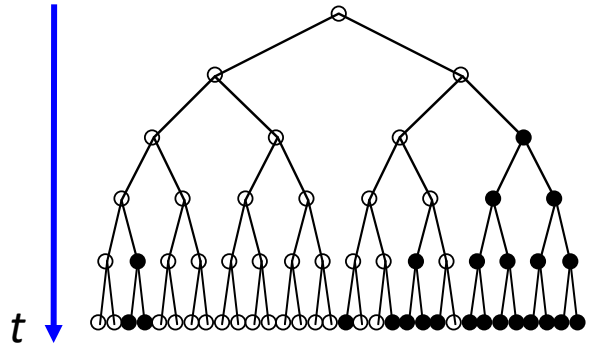
the total variance is the sum of the variances of independent increments

amplification due to population growth

$$\begin{aligned}
\text{var}\ \rho(t) &= \int_0^t \text{var}\left(e^{t-t'} d\rho(t')\right) \approx \int_0^t e^{2(t-t')} dm(t') \\
&= e^{2t} \int_0^t e^{-2t'} aN_0 e^{t'} dt' = aN_0 e^{2t} \int_0^t e^{-t'} dt' \\
&= aN_0 e^{2t}(1 - e^{-t}) \\
&= aN(t)(e^t - 1)
\end{aligned}$$

Variance of the number of resistant bacteria

$$\text{var } \rho(t) \approx aN(t)(e^t - 1) \approx a\frac{[N(t)]^2}{N_0}$$



$$\text{var}\,[\rho(t)] \approx a\frac{[N(t)]^2}{N_0}$$

$$\sqrt{\text{var}\,[\rho(t)]} \approx \left(\frac{a}{N_0}\right)^{1/2} N(t)$$

$$\rho(t) \approx atN(t) = aN(t)\ln\frac{N(t)}{N_0}$$

$$\operatorname{var}\rho(t) \approx a\frac{N(t)^2}{N_0}$$

$$\frac{\sqrt{\operatorname{var}\rho(t)}}{\rho(t)} \sim \frac{1}{\ln N(t)} \neq \frac{1}{\sqrt{\rho(t)}}$$

Luria and Delbrück observed that the variance of the number of resistant bacteria is indeed quite large and confirms the hypothesis of random mutations

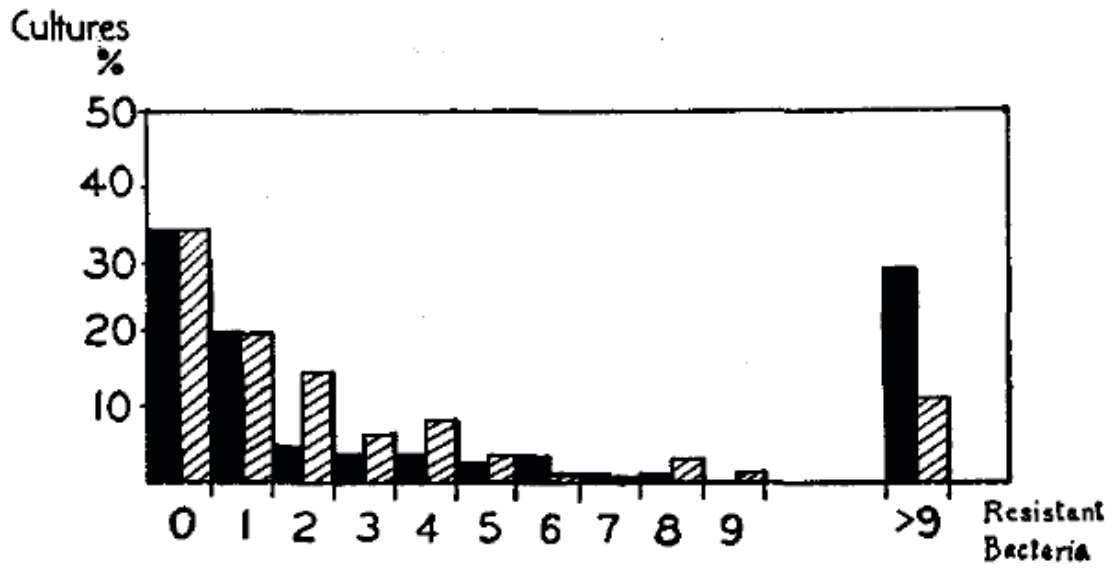


FIGURE 2.—Experimental (Experiment No. 23) and calculated distributions of the numbers of resistant bacteria in a series of similar cultures. Solid columns: experimental. Cross-hatched columns: calculated.

The winners of the 1969 Nobel Prizes stand together before the award presentation by King Gustaf VI Adolf, of Sweden. Left to right are Professor Murray Gell-Mann of the U.S., in physics; Professor Derek H.R. Barton of the U.K., in chemistry; Professor Odd Hassel of Norway, in chemistry; Professor Max Delbruck of the U.S., in medicine; Alfred D. Hershey, of the U.S., in medicine; Salvador E. Luria of the U.S., in medicine; and Professor Jan Tinbergen of the Netherlands, in economics.