Bacteriophages in Human Gut Microbiome

Student: Kevin S Lam, Mphil candidate
Supervisor: Prof. Paul KS Chan, Prof. Siew C Ng

Joint Graduate Seminar

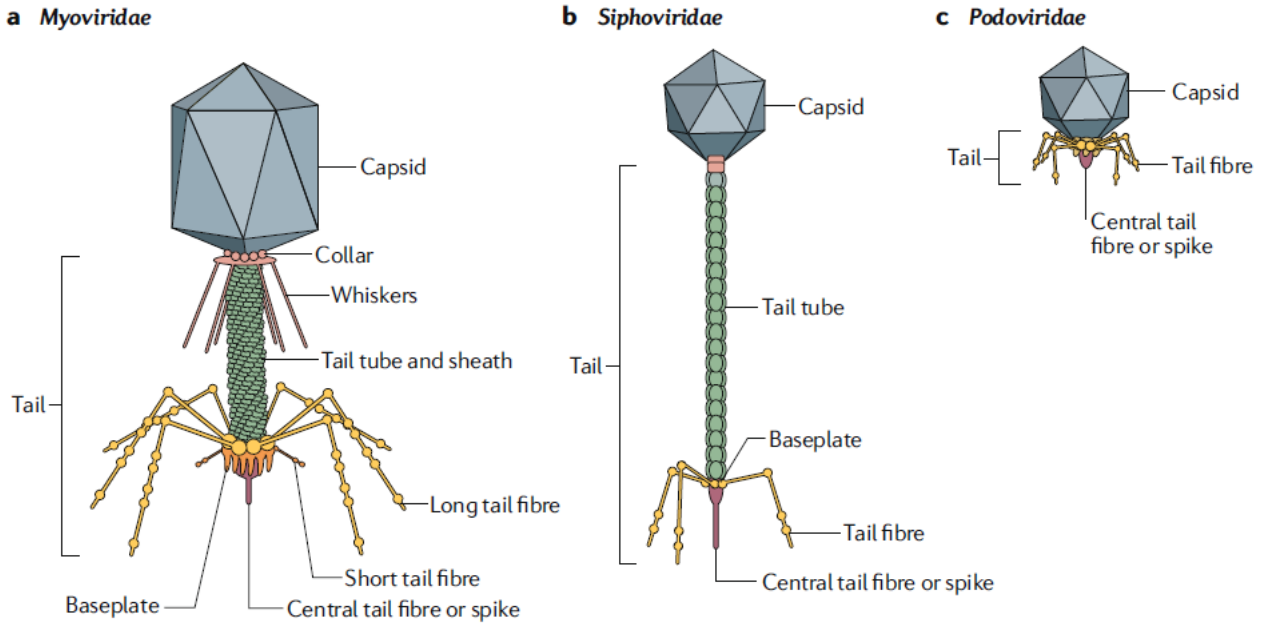
Department of Microbiology, CUHK Date: 13th Dec, 2018

香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

Table of Content

What are bacteriophages?

- Bacteriophages (phages) are viruses that can attack and kill a target prokaryotes within minutes of infection.
- Double stranded DNA, single stranded DNA, RNA (3k-500kbp)

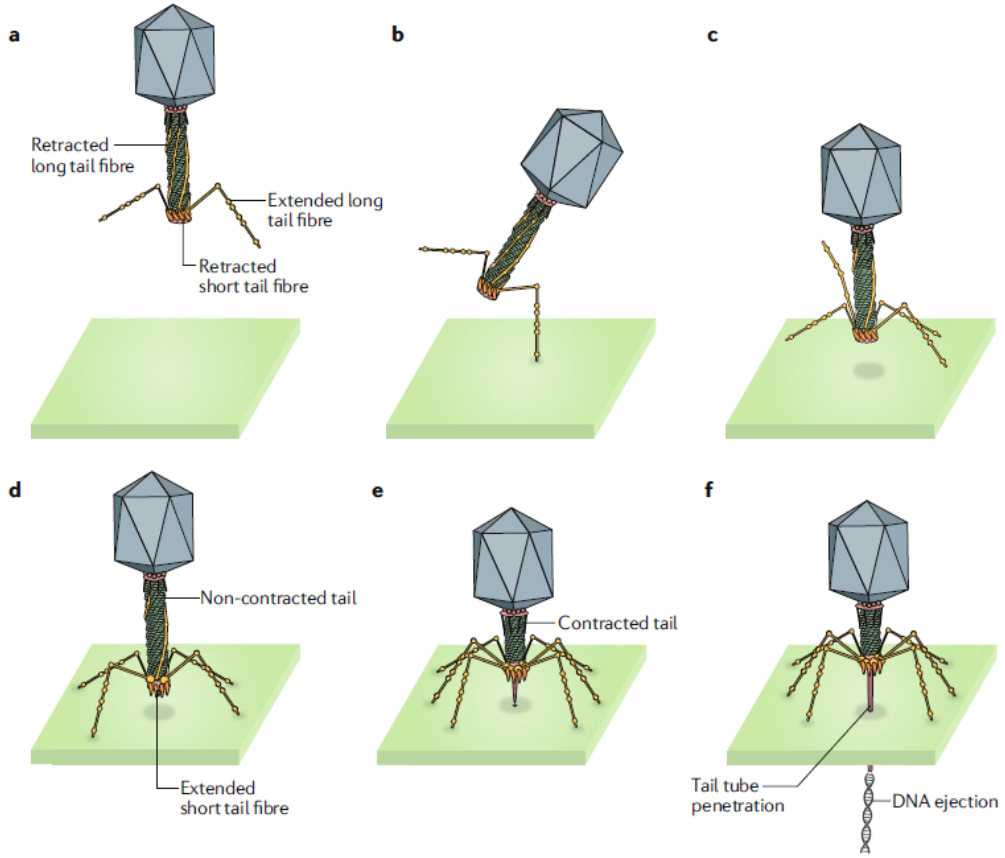


香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

(Nobrega et al., 2018)

Infecting target bacteria

- Specifically bind to the bacterial cell glycoprotein
- Normally find its target by passive diffusion in human gut
- Individual phage targets a narrow range of bacteria based on its specificity



香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

(Nobrega et al., 2018)

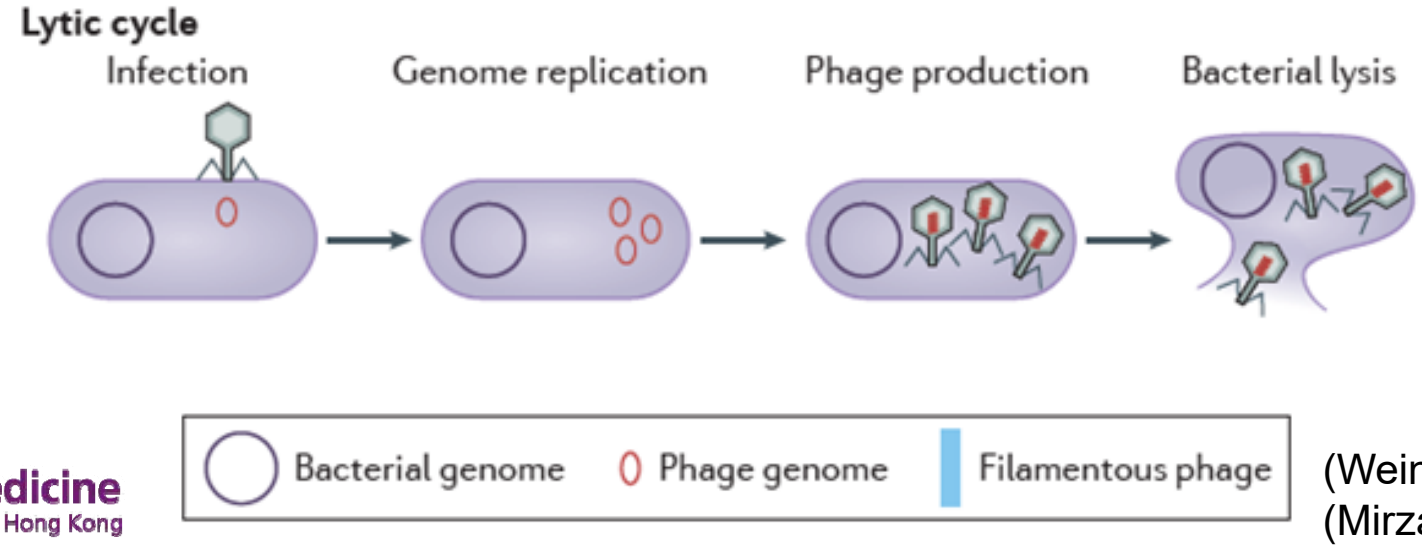
Bacteriophage life cycles

Bacteriophages undergo replications through:

- Lytic cycles
- Lysogenic cycles
- Chronic cycles
- Pseudolysogenic cycles

Lytic Cycle

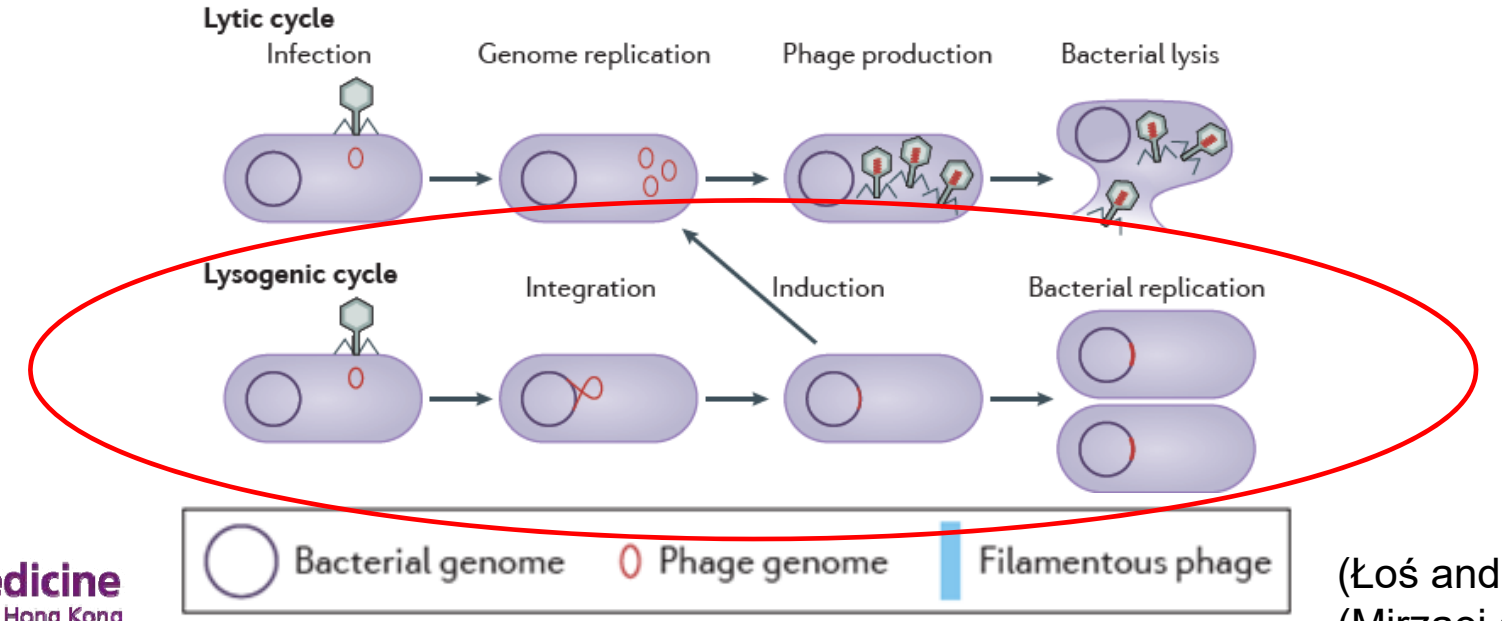
- Specifically target and infect the bacterial cell
- Undergoing replications via redirecting the cell metabolism to produce new phage particles
- Released during programmed cell lysis



(Weinbauer, 2004)
(Mirzaei and Maurice, 2017)

Lysogenic (or temperate) Cycle

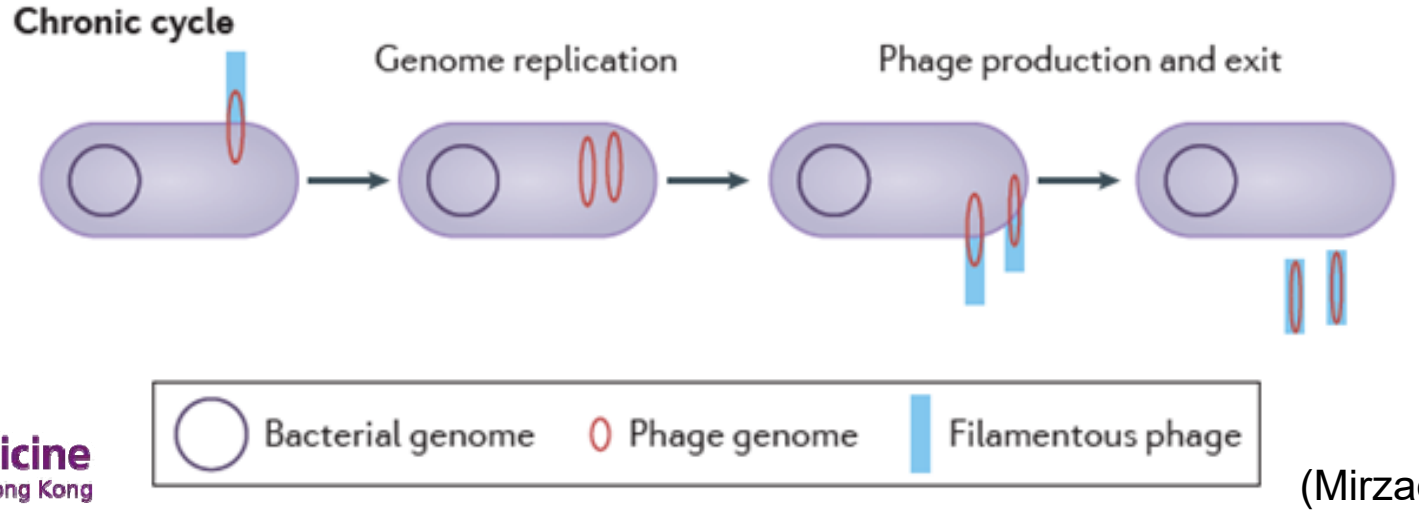
- Phage DNA integrates into the bacterial genome **WITHOUT inducing cell lysis**.
- The phage genome (termed a prophage) can then replicate in concert with the host chromosome until such time that a lysis event is induced.



(Łoś and Wegrzyn, 2012)
(Mirzaei and Maurice, 2017)

Chronic Cycle

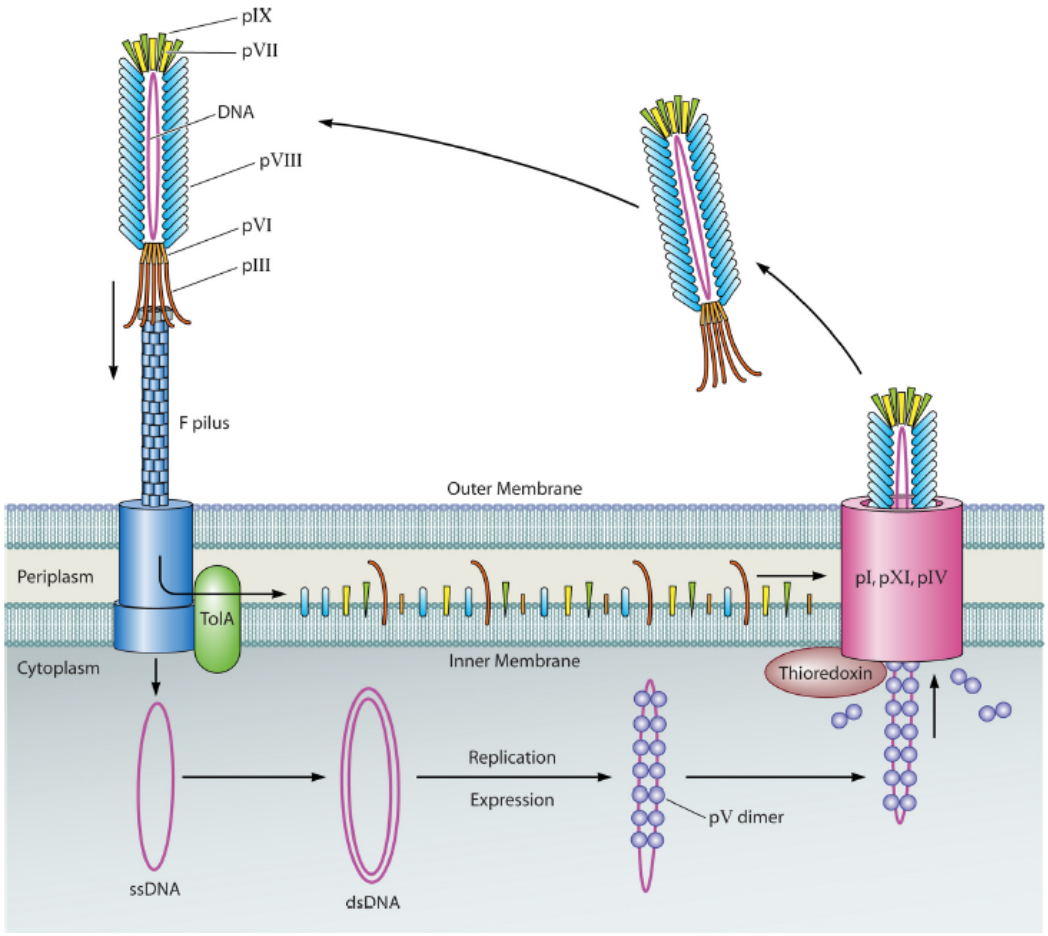
- Remain poorly defined.
- Mainly by filamentous bacteriophage (a type of bacteriophage defined by its filament-like or rod-like shape.
- As seen for phage M13, which is characterized by a ‘budding’ mechanism
- Replicating and being released without killing the host



(Mirzaei and Maurice, 2017)

Budding Mechanism of a Chronic Cycle

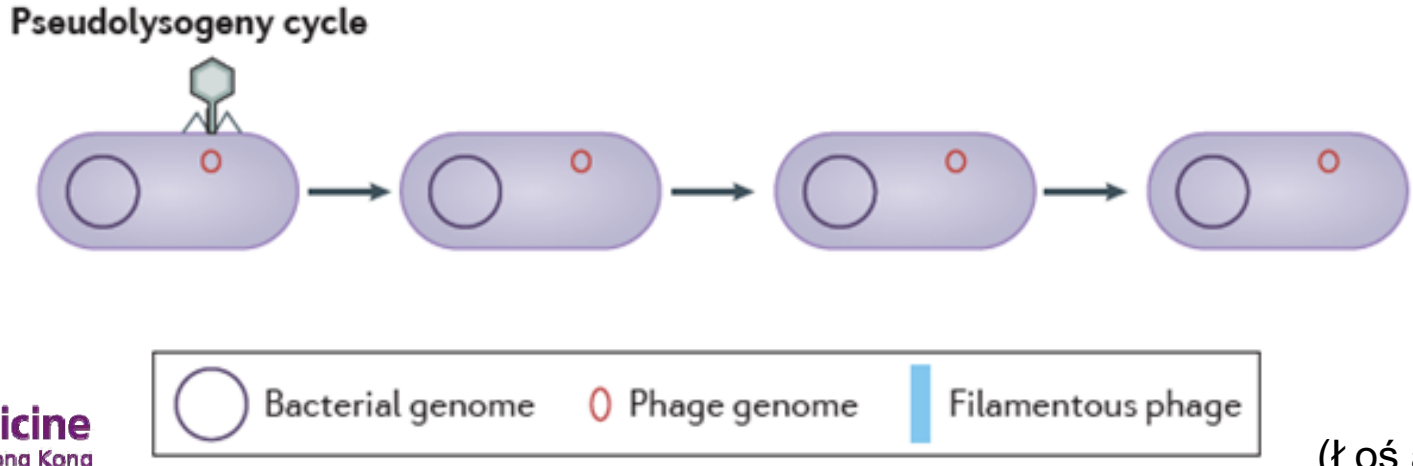
- Filamentous phage binds to F pilus of a host *single* cell through pIII
- Host **TolA protein** **depolymerize the phage coat proteins, which remain in the inner membrane for recycling**
- ssDNA of the phage enters into the cytoplasm, converts into dsDNA, and starts replication
- ssDNA and coated pV protein dimers form the precursors of the phage
- Then pV is replaced by pVIII in the channel formed by pI, pXI, pIV, and host thioredoxin



(Huang et al., 2018)

Pseudolysogenic cycles

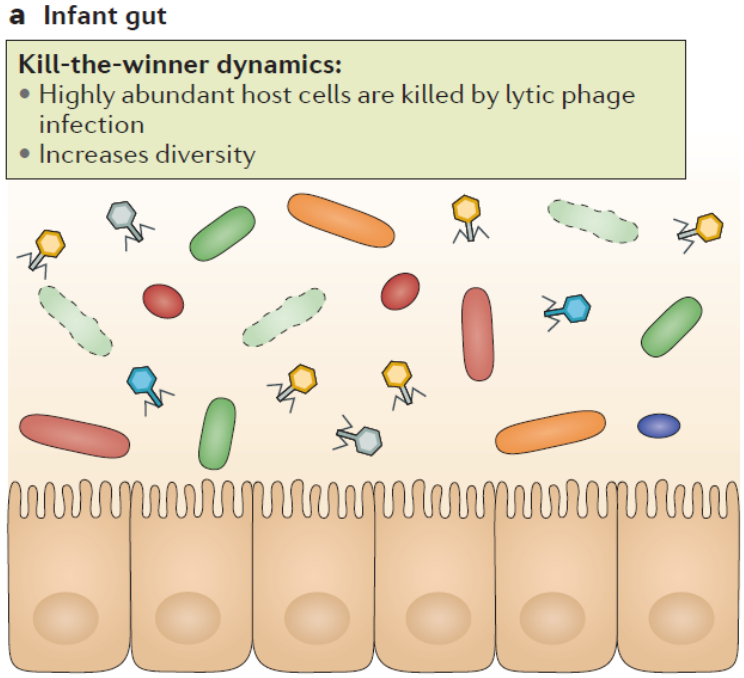
- Poorly understood
- The phage genome **neither integrates nor propagates**
- Observed in nutritional conditions that limit bacterial DNA replication or protein synthesis
- Phage exists as a plasmid-like prophage
- Without inducing a lytic cycle or integrating into the bacterial chromosome



(Łoś and Wegrzyn, 2012)

When do we recognize our gut phageome?

- Since the early 20th century
- Human gut contains ~10^{15} bacteriophages (the 'phageome')
- Probably the richest concentration of biological entities on earth
- Recognized that phages can play a role in human health
- Taking advantage of their ability to destroy pathogens



香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

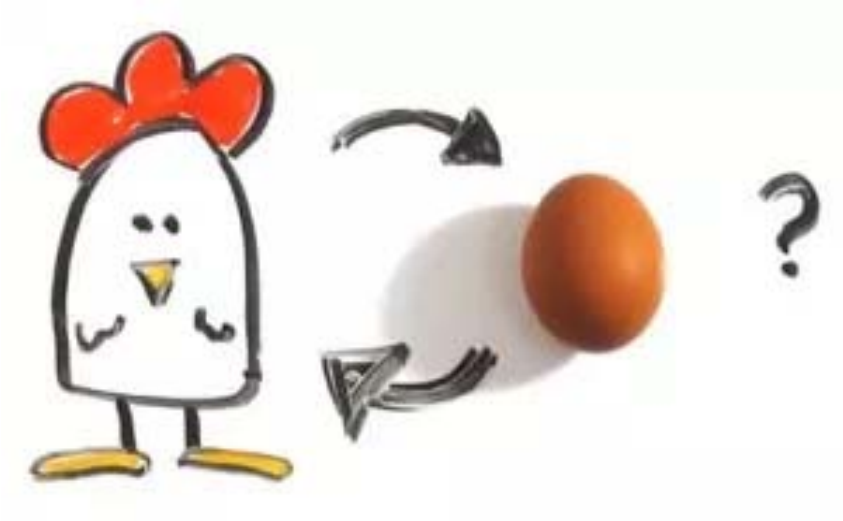
(Wittebole et al., 2014)
(Dalmasso et al., 2014)

Meconium – the earliest infant stool

- Infant stool is sterile
- No microorganisms could be detected by direct epifluorescent microscopy examination

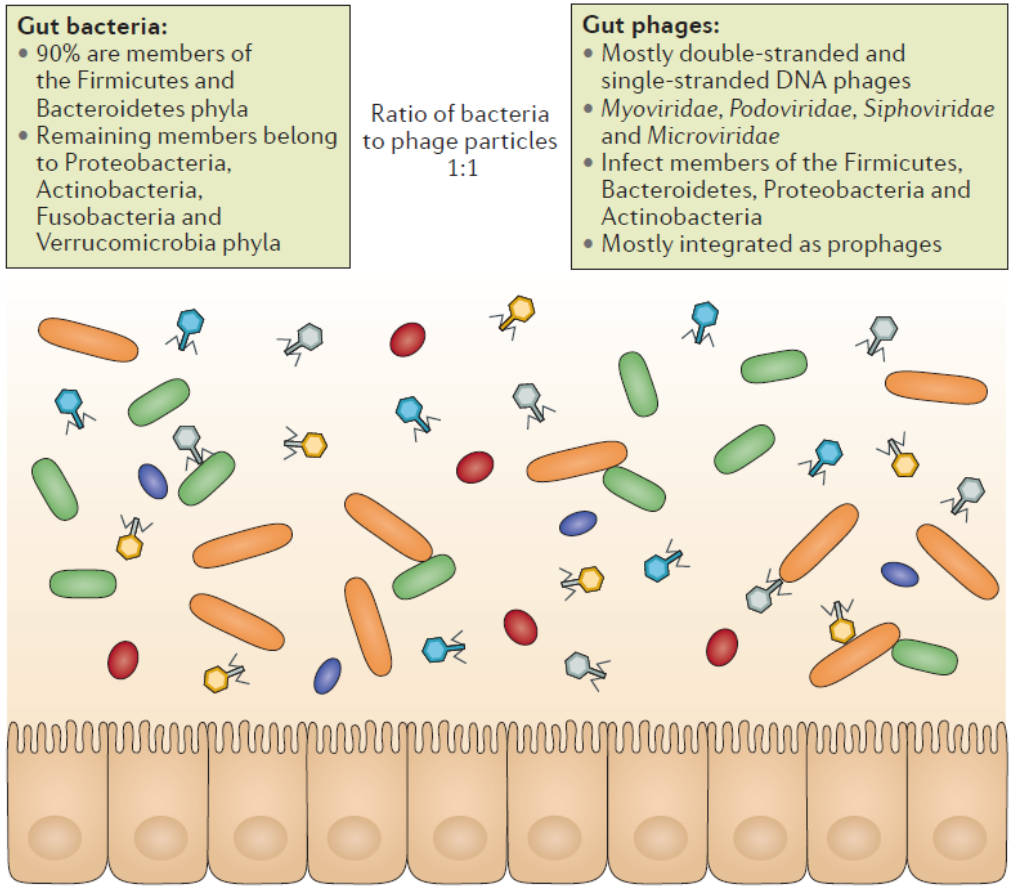
A question raised

- Which came first? Bacteria or Phages?



Gut development and maturation along with Phages colonisation

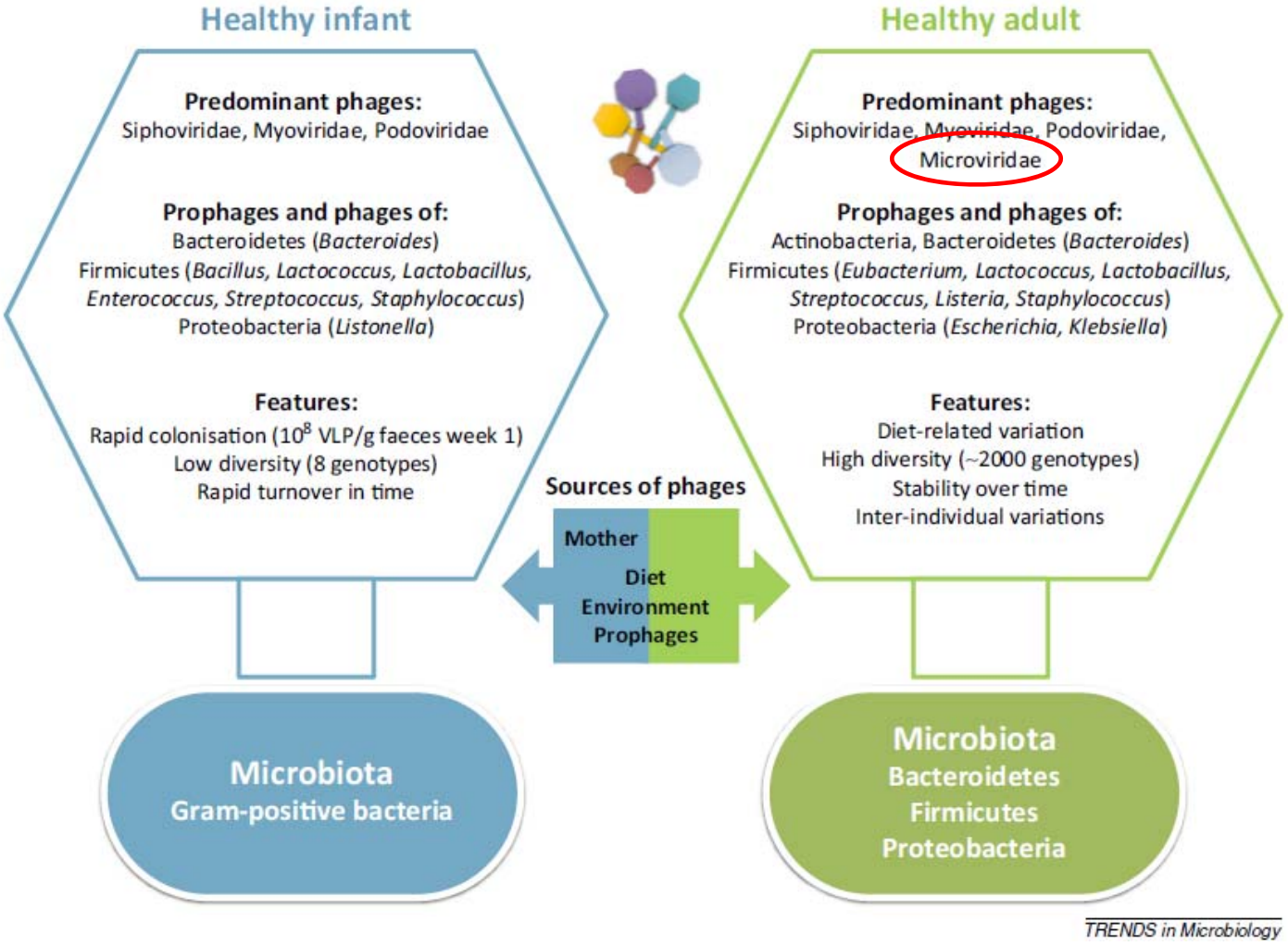
- Phages rapidly appear after earlier bacterial colonisation
- Reports of 10^8 virus-like particles (VLP) per gram of faeces 1 week after birth
- Stabilised with aging
- Still less diverse than the healthy adult phageome



(Breitbart et al., 2008)

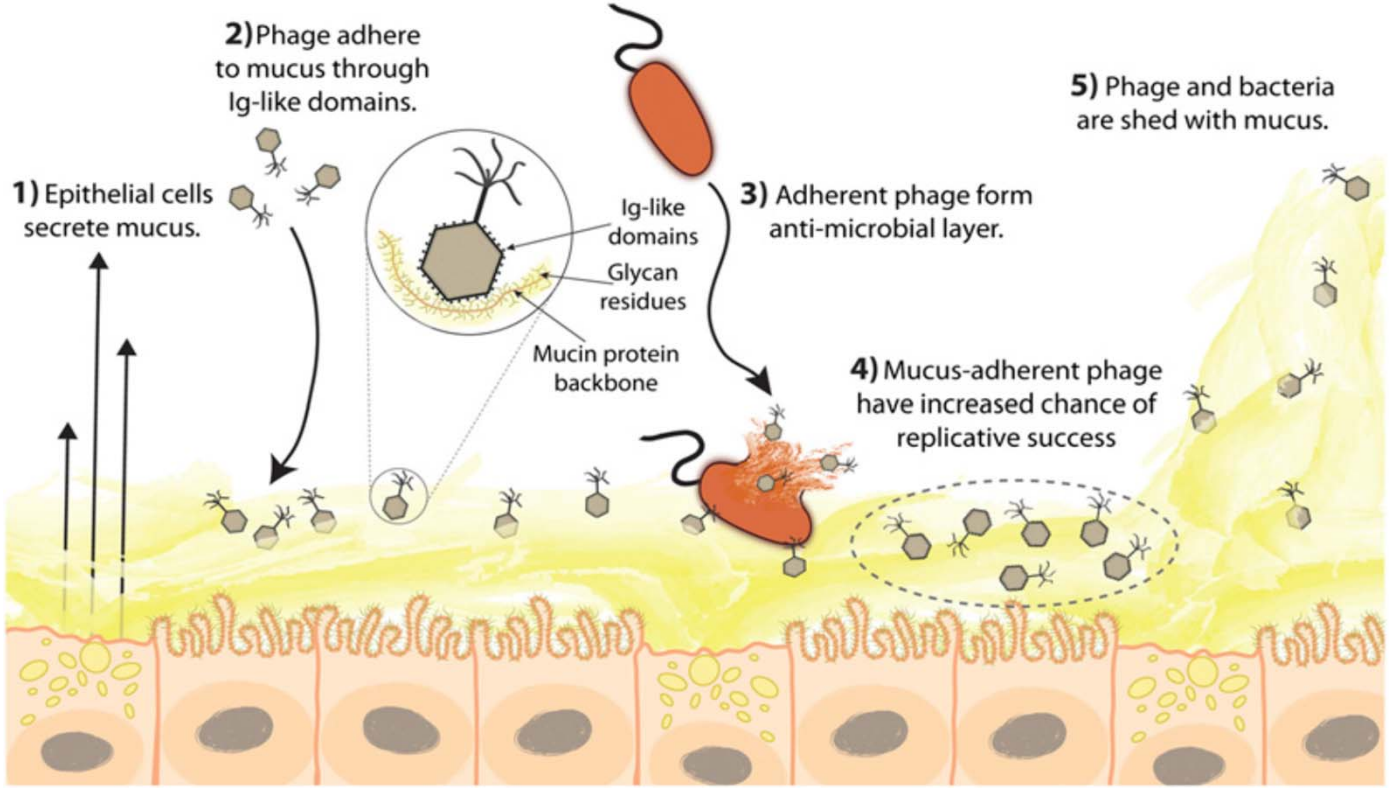
香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

Gut development and maturation along with Phages colonisation



(Breitbart et al., 2008)

Phage – Host Interactions



- Conferring protections in healthy individuals
- Protecting the underlying epithelium from bacterial infection.
- A non-host-derived immunity to the human gut.

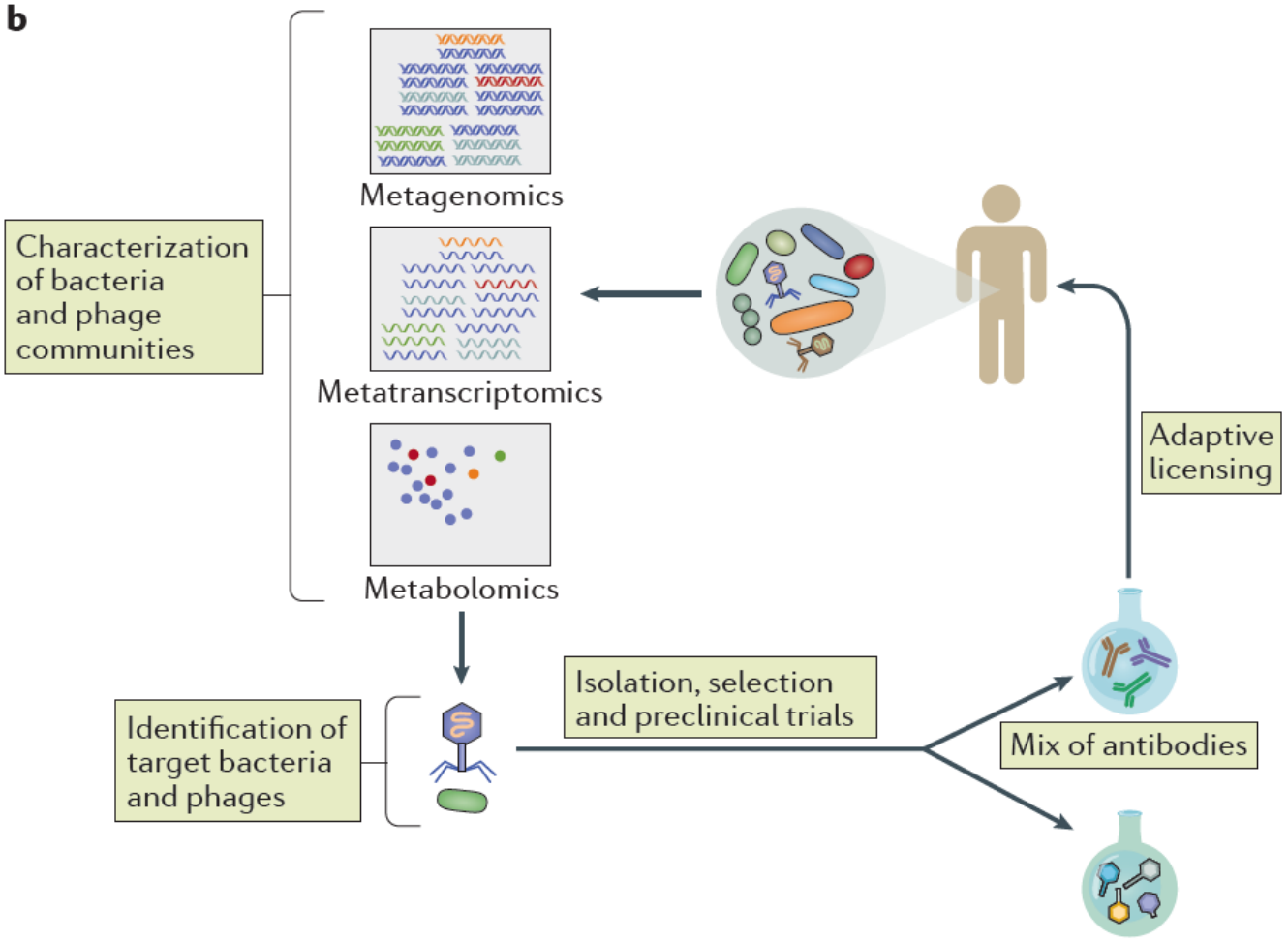
(Barr et al., 2013)

Evaluating Gut Phages Compositions

To interrogate whole-community metagenomes and access subliminal phage sequences:

- Metagenomic sequencing
- CRISPR analysis
- genome signature-based approaches

Evaluating Gut Phages Compositions

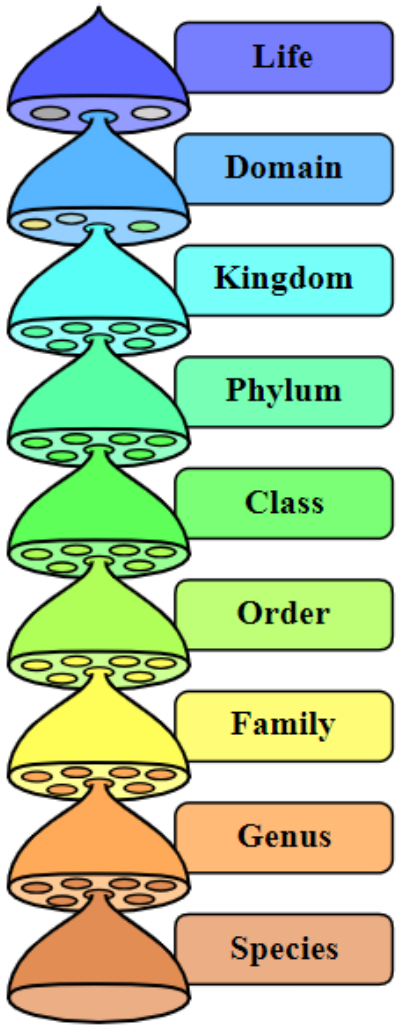


香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

(Mirzaei and Maurice, 2017)

Classification of Gut Phages

- Classified at family level
- Families identified are Siphoviridae,Myoviridae, Podoviridae, and Microviridae
- Identified viral sequences in the human gut represented <0.02% of the RNA viruses (only 7 of a total of clones matched bacteriophage sequences in GenBank)
- Rare occurrence of RNA phages responsible for lacking attempts for further analysis



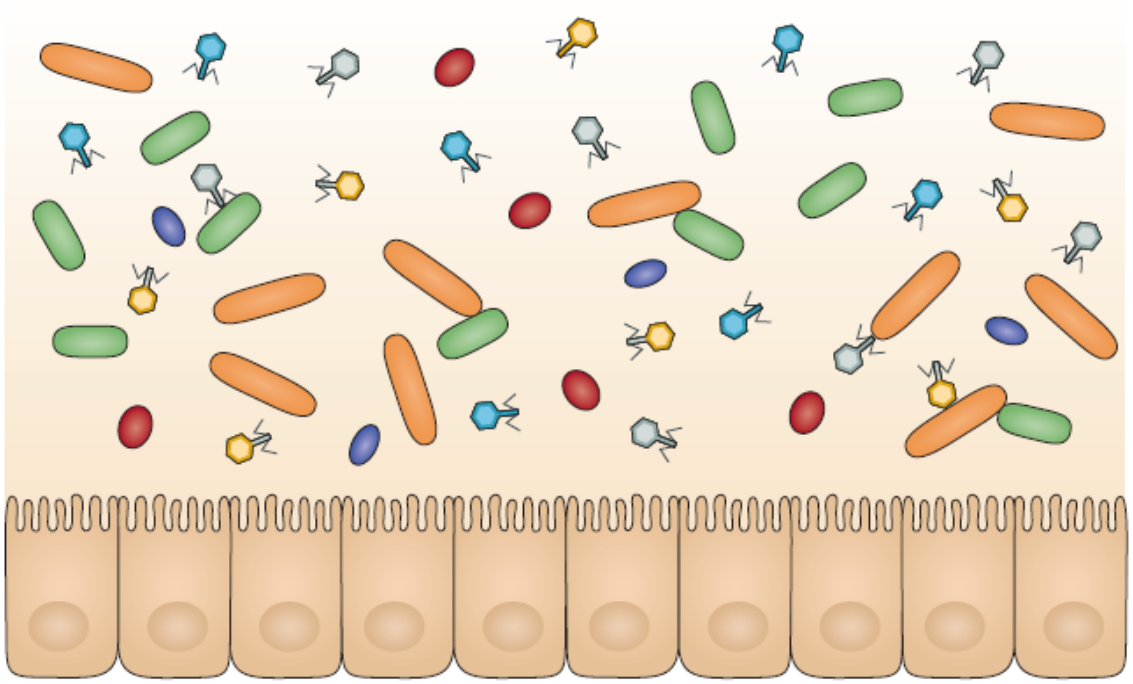
(Breitbart *et al.*, 2008)
(Zhang *et al.*, 2006)

Gut phages and bacteria

Gut bacteria:
- 90% are members of the Firmicutes and Bacteroidetes phyla
- Remaining members belong to Proteobacteria, Actinobacteria, Fusobacteria and Verrucomicrobia phyla

Ratio of bacteria to phage particles 1:1

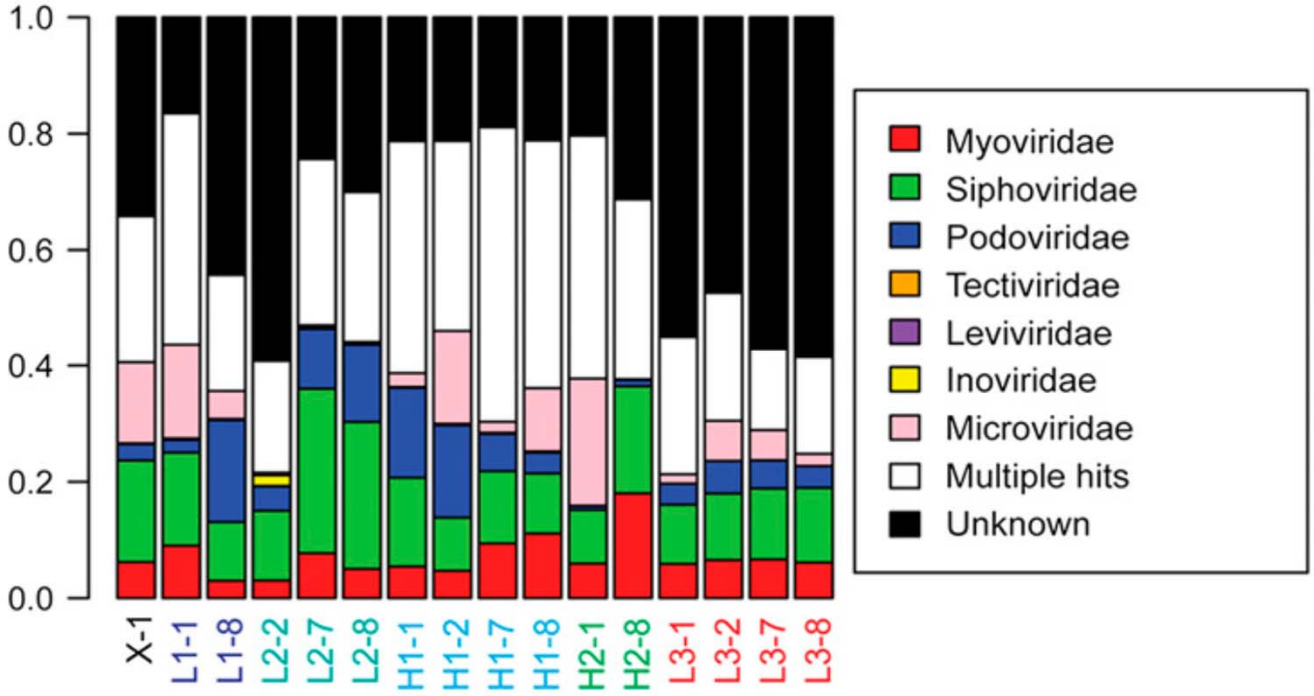
Gut phages:
- Mostly double-stranded and single-stranded DNA phages
- *Myoviridae*, *Podoviridae*, *Siphoviridae* and *Microviridae*
- Infect members of the Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria
- Mostly integrated as prophages



(Mirzaei and Maurice, 2017)

Environmental stress – Diet

- Change of diet triggers dynamic fluctuations across phageome compositions

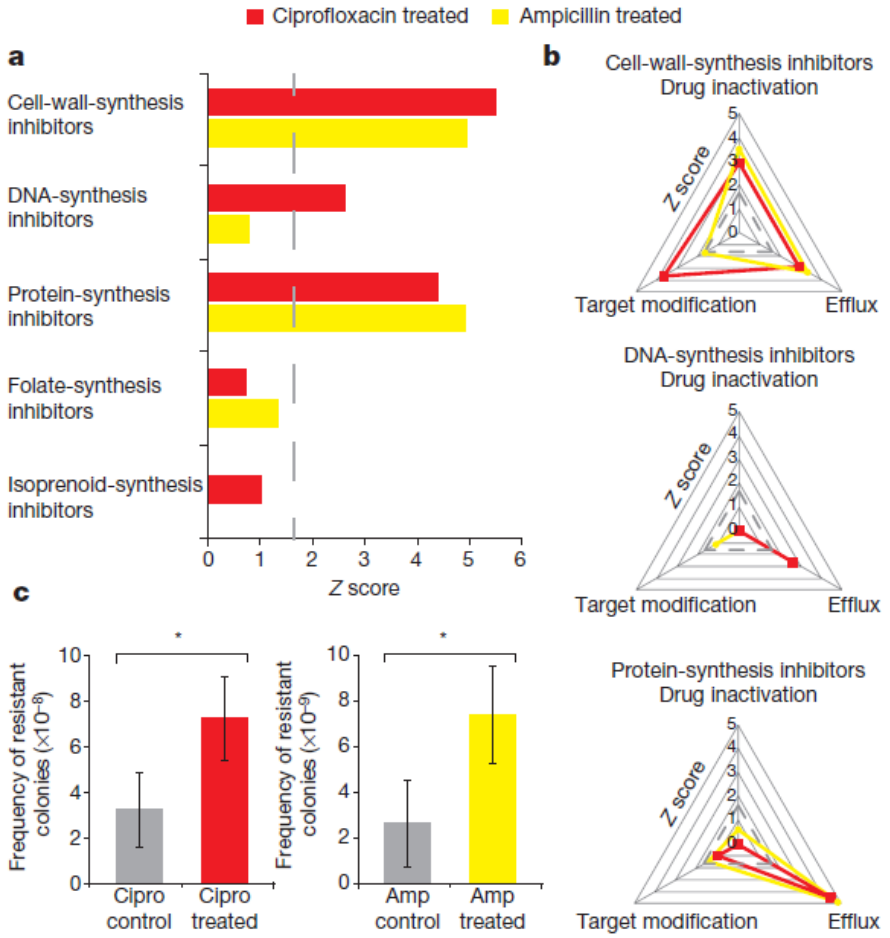


Samples are labeled by subject according to diet: high-fat (H1,H2), low-fat (L1, L2, L3), and baseline (X), as well as day of dietary intervention (days 1, 2, 7, or 8)

(Minot et al., 2011)

Environmental stress – Antibiotics

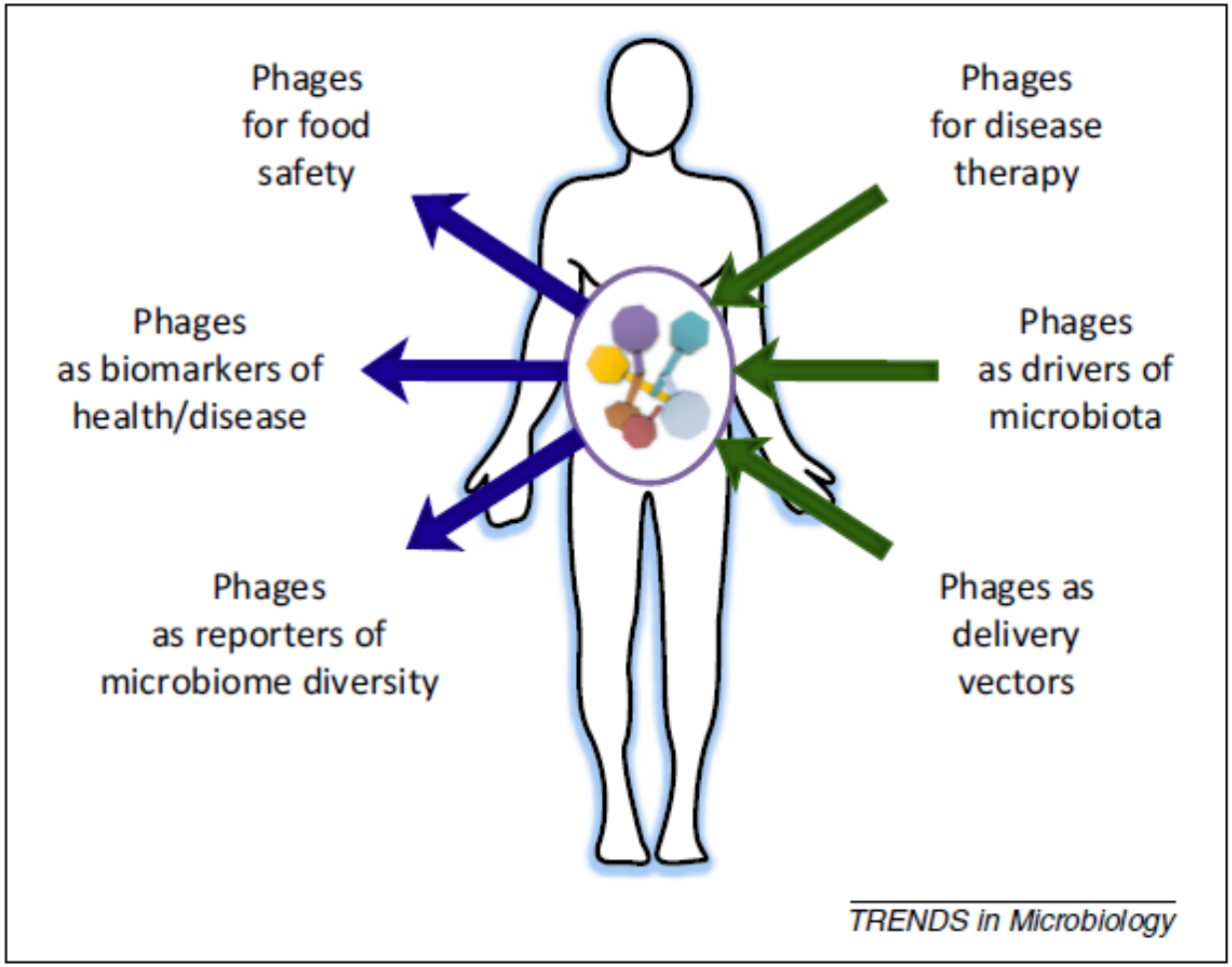
- Antibiotic treatment **expands the resistance reservoir** and ecological network of the phage metagenome.
- **a, b**, Z scores are shown for sequencing reads annotated as antibiotic-resistance genes in phages from ciprofloxacin-treated (red) and ampicillin-treated (yellow) mice in comparison with respective control mice.
- Dashed lines correspond to a Z score of 1.65 (P=0.05).



Antibiotic resistance is enriched in phage metagenomes following drug perturbation in mice.

香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

Significance of gut phages



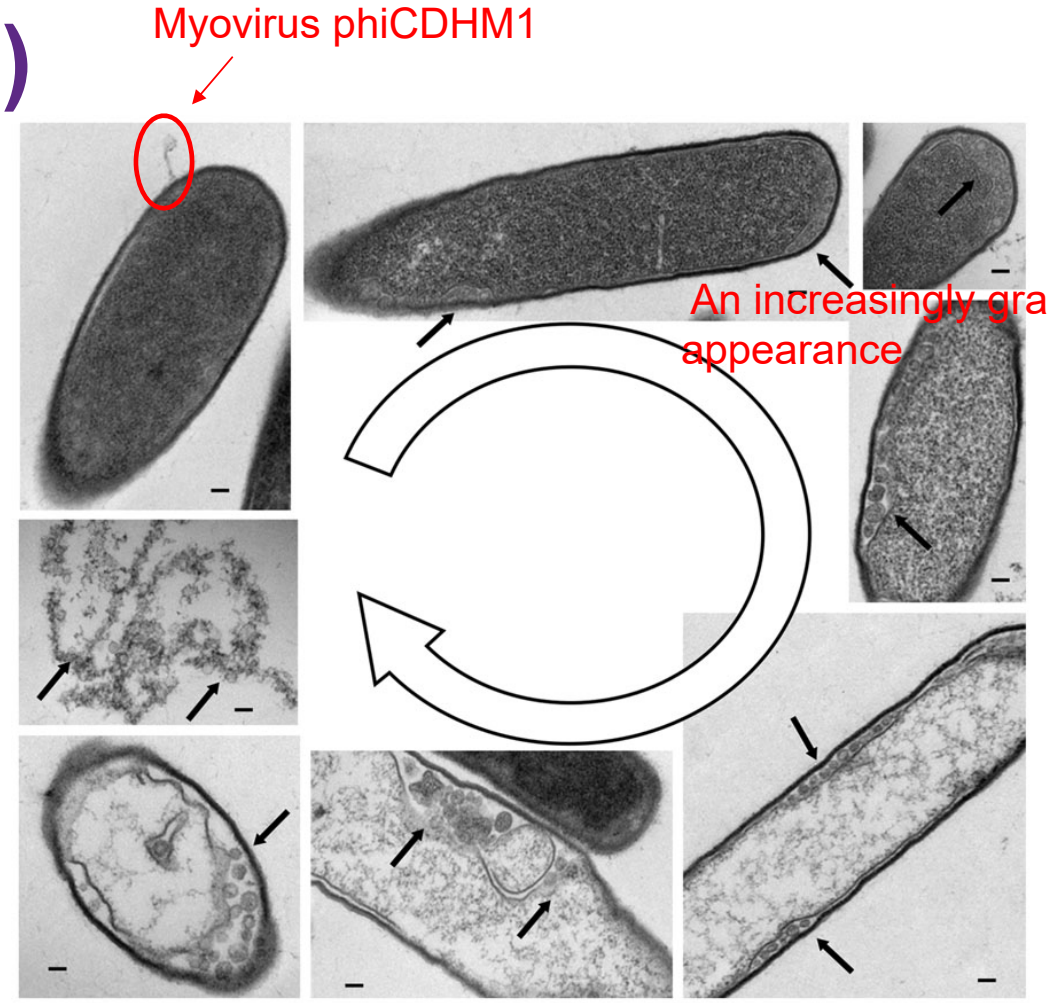
香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

(Dalmasso et al., 2014)

Exploiting lytic property to cure *Clostridium difficile* infection (CDI)

Lysis of *C. difficile* cells by myovirus phiCDHM1 using transmission electron microscopy. An increasingly granular appearance inside the cell, and the formation of putative capsid structures at the outermost edges of the cell as indicated by arrows.

Scale bars represent 10 nm.

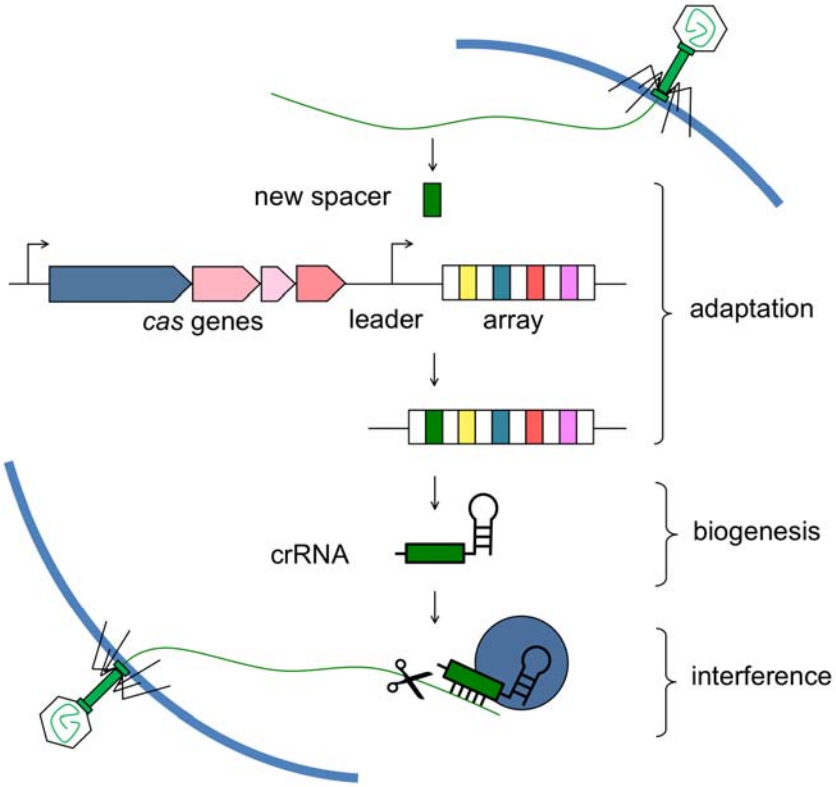


TEM photomicrographs courtesy of Katherine Hargreaves and Natalie Allcock, the Electron Microscopy Facility, University of Leicester.

香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

CRISPRs (clustered regularly interspaced short palindromic repeats)

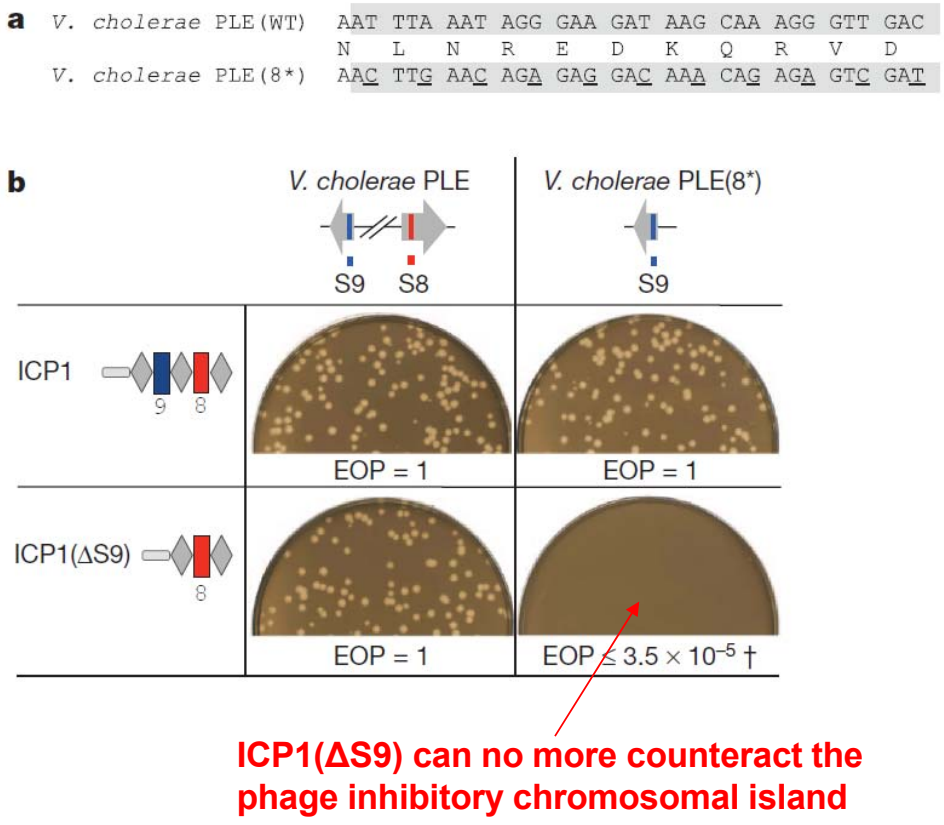
- Highly conserved short DNA repeat sequences interspaced by stretches of variable sequences (spacers) originating from phages or plasmids
- As a ‘bacterial immune system’
- Inducing sequence-specific cleavage at foreign genetic elements



Three stages of CRISPR-Cas immunity within a bacterium

Bacteriophage encodes its own CRISPR/Cas adaptive response

- ICP1 (for the International Centre for Diarrhoeal Disease Research, Bangladesh cholera phage 1)-related, *V. cholera* O1-specific virulent myoviruses
- Used to **counteract a phage inhibitory chromosomal island** of the bacterial host
- ICP1 has two CRISPR spacers (8 and 9) that have 100% identity to sequences within the V. cholerae PLE
- PLE(8*) was infected with a spontaneous ICP1 spacer 9 deletion mutant, referred to as ICP1(DS9).
- **ICP1(DS9) was blocked for plaque formation on V. cholerae PLE(8*)**



ICP1(ΔS9) can no more counteract the phage inhibitory chromosomal island

(Seed et al., 2013)

Summary

- Advancement in metagenomics sequencing and CRISPR analysis are crucial for deeper gut phageome evaluation
- The earliest infant stool is sterile
- Phages rapidly appear after earlier bacterial colonisation
- Environmental factors, diet and antibiotic administration impact on the gut phageome stability significantly
- Phage therapy will become a new trend to ease the burden of antibiotic-resistant bacteria
- Phage to host interaction will be clearly defined in the future

Thank you