Structural Determination of Bacteriophage P22 Genome Ejection with Cryo-Electron Microscopy and UCSF ChimeraX

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Literature Review

- Increasing prevalence of antibiotic-resistance in bacteria (Frieri, et. al)
  - Increases patient mortality, morbidity and length of hospital stays (Frieri, et. al)
- Bacteriophages (phages): viruses that infect bacteria & archaea (McCallin, et. al)
  - High host specificity: phages capable of attaching to only one species or strain of bacteria (McCallin, et. al)
- Bacteriophage therapy: alternative treatment to antibiotic-resistant bacteria (McCallin, et. al)
  - Used as a compassionate treatment (McCallin, et. al)
  - More research needed for bacteriophage therapy to become a commonplace treatment (McCallin et. al)
    - Bacteriophage ecology → infection initiation poorly understood (Hu, et. al)
- Bacteriophage P22: *Salmonella typhimurium* phage used as a model for phage assembly and infection (Wang, et. al)
  - Non-Contractile tail (Wang, et. al)
- Phage P22 Infection Initiation:
  - Attachment to bacterial membrane (Hu, et. al)
  - Pinning of baseplate (Hu, et. al)
  - Needle penetration (Hu, et. al)
  - "Trans-envelope channel" formation (Wang, et. al)
  - Genome Ejection (Hu, et. al)
- Trans-membrane channel formation: bridges inner/outer membranes to phage needle (Wang, et. al)
  - Essential for successful genome ejection (Wang, et. al)
  - Low resolution of images (Wang, et. al)
- Cryo-Electron Microscopy (Cryo-EM): high resolution imaging technique, used for 3-D structure determination of proteins, protein complexes & other cell structures (Turk, et. al)
- Cryo-Electron Tomography (Cryo-ET): imaging technique that constructs 3-D models of proteins, protein complexes & other cell structures with Cryo-EM images of different angles (Turk, et. al)
  - Minicells, specimen thickness: bacterial cells significantly smaller than normal due to aberrant cell division (Farley, et. al)
    - Arabinose: chemical used to induce minicell grow in bacterial colonies (Farley, et. al)
- UCSF ChimeraX: data analysis software used for 3-D visualization of protein structures (Petterson, et. al)

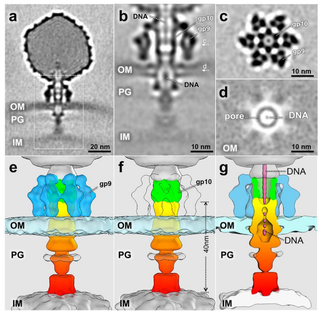

Figure 1

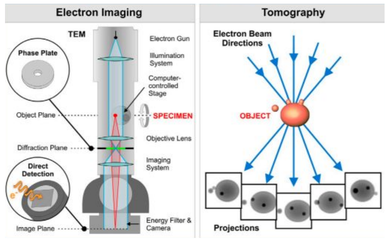

Figure 2

Problem Statement

To determine the structures and proteins that comprise the "extracellular channel" of Bacteriophage P22

Experimental Design

- Independent Variable: *Salmonella typhimurium* samples treated with phage P22
- Dependent Variable: Subsequent phage P22 genome ejection
- Constants: Isolated phage P22

Hypothesis

The protein gp7 aids in forming the extracellular channel of Bacteriophage P22 (Wang, et. al)

Procedure

Salmonella typhimurium grown in-vitro with arabinose and centrifuged, phage P22 amplified & isolated (mentor)

↓

Phage P22 added to *Salmonella typhimurium* minicells then mixture centrifuged to isolate (mentor)

↓

10 nm colloidal gold mixture added to minicells/phages, frozen with liquid ethane, cooled with liquid nitrogen & poured into holey carbon grids (mentor)

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Imaged under cryo-electron microscope (mentor)

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UCSF ChimeraX used to visualize genome ejection nanomachine (mentee)

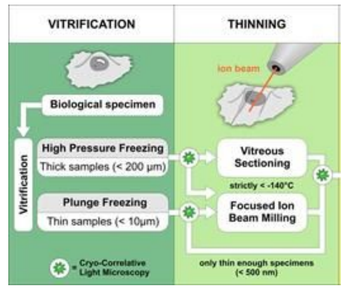

Figure 3

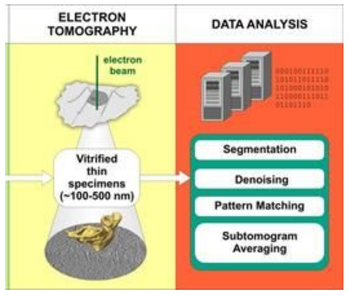

Figure 4

Data

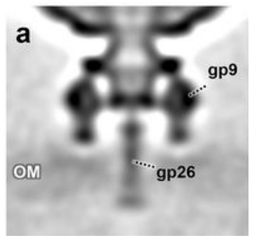

Figure 5

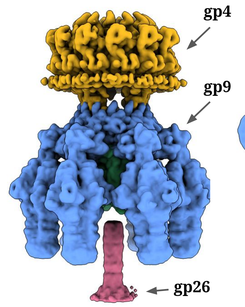

Figure 6

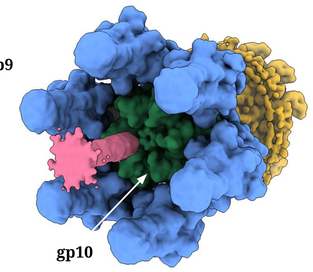

Figure 7

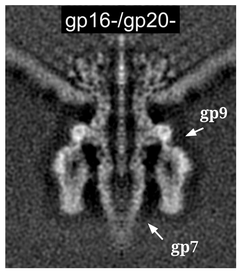

Figure 8

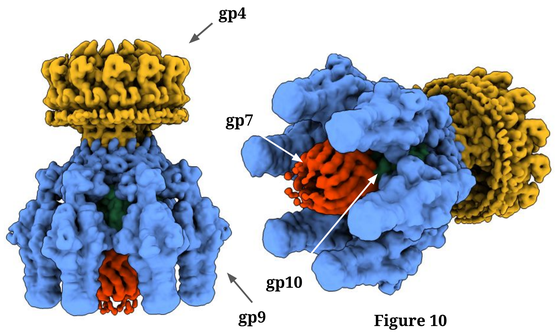

Figure 9

Figure 10

Results

- Figure 5: Cryo-EM map of free bacteriophage P22
- Figure 6: Rendering of free bacteriophage P22
- Figure 7: Rendering of free bacteriophage P22
- Figure 8: Cryo-EM map of bacteriophage P22 in infection initiation
- Figure 9: Rendering of bacteriophage P22 in infection initiation
- Figure 10: Rendering of bacteriophage P22 infection initiation

Discussion

- Results thus far:
  - Hypothesis Supported:
    - Protein gp7 as shown in figure 8, 9, 10 forms the extracellular channel of bacteriophage P22
  - Proteins that make up intracellular complex still unknown

Implications & Limitations

- Implications:
  - More information on the understudied topic of bacteriophage ecology
    - Bacteriophage infection initiation
  - Provide pathway toward approval as commonplace antibacterial treatment
    - Antibiotic-resistant bacteria
    - Compassionate Use
- Limitations:
  - Small sample size
    - 2-3 individual phages imaged
    - Single phage strain

References

Works Cited

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