

MOLECULAR BASIS OF FOREIGN DNA RECOGNITION BY BREX ANTI-PHAGE IMMUNITY SYSTEM

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[Motivation:]

The Phage: (Bacteriophage)

Natural viral predator of bacteria. Causing 20-40% of daily bacterial mortality!

Phages infecting pathogenic bacteria offer a promising alternative to current (failing) antibiotics.

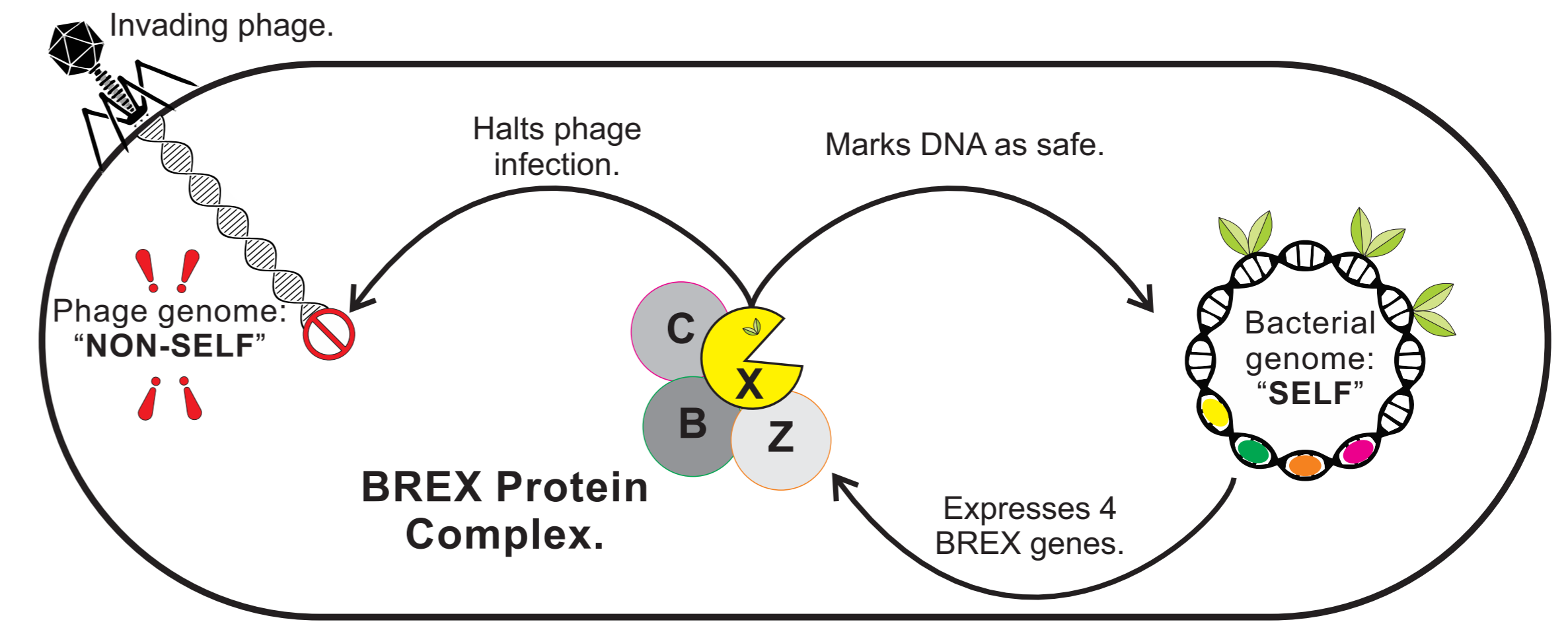
2025 - 2050
An estimated 39 million people could die from Antimicrobial Resistance (AMR).

Phages infecting beneficial bacteria results in big economic losses.

UK Dairy Loss: (failed milk fermentations)
Up to **£0.5 billion/year**

Protect the 'good, eliminate the 'bad' bacteria.
Bacterial defence systems shape the outcome of phage-bacteria encounters.
HOW DO THEY WORK?

[BREX-it.] "BacteRiophage EXclusion System."



□ What do these proteins look like?

□ How is the DNA differentiated?

Understanding the molecular basis of defense mechanisms allows us to leverage them for biotechnology, agriculture and medicine.

[Approaches:] Cryogenic Electron Microscopy (Cryo-EM)

Sample application onto a copper grid.

3mm

ThermoSCIENTIFIC "Titan Krios" microscope. > £6 million!

3m

Proteins flash frozen (-183°C!)

$\lambda = -10^{10} \text{ m}$ (~5000X smaller than visible light!)

Computationally fit single atoms, creating a 3D DNA-bound protein reconstruction.

DNA

BrxX

In vivo Phage Assay

Decreasing phage concentration.

Healthy bacterial growth as a "lawn."

Zones of bacterial death.

[Our findings.]

Our BrxX structure: revealed the molecular details of how the DNA can be marked as "safe."

Our findings enabled atomic resolution engineering of the BrxX protein molecule.

BrxX: "closed" (functional)

BrxX: "open" (non-functional)

Ocr Protein (mimics DNA)

This result explains how phage encoded proteins can overcome the bacterial BREX defense system.

Ocr

BrxX

Broaden Specificity

Reduce Specificity

↑ protein capacity to modify different DNA bases in various contexts.

↑ BREX capacity to defend bacterium against phage *in vivo*.

↓ protein capacity to modify any DNA.

↓ BREX system loses its ability to defend the bacterium against phage attack.

In vivo assays.

Bacteria containing the BREX system.

Bacteria not containing the BREX system.

"Enhanced" BrxX protein.

"Weakened" BrxX protein.

The takeaways.

- ☑ Atomic level resolution of the bacterial protein BrxX.
- ☑ Offers insights into how the BREX system differentiates "self" v. "non-self" DNA.
- ☑ Evolutionary 'arms race' in action demonstrating how phage resist defence systems
- ☑ Reveals a clearer understanding of the "bacterial immune system."

[Impact.]

Development of "new to nature" bespoke molecules for overcoming defences of antibiotic-resistant 'superbugs.'

Cryo-EM facilitates atomic resolution detail to further clarify interactions between bacteria and phages.

Controlling DNA recognition at any level is an enormously powerful tool in biotechnology.

Offers a window into the capabilities of larger scale AI-powered protein engineering.

[Acknowledgements.]

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