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Chapter

Introductory Chapter: Nature's Ancient Nanomachines and Their Synthetic Future

Renos Savva

1. Resurgence

Bacteriophages are generally considered to be the most prevalent biological entities on planet Earth [1], with astronomical estimates of their myriad abundance. These fascinatingly diverse viruses, which infect bacterial cells, were discovered in the second decade of the twentieth century. Under the light microscope, bacterial cells were seen to be apparently eaten away, hence the scientific Greek naming of these viruses meaning literally "bacteria eaters". The middle decades of that century gave us a first glimpse of these viral particles, via their imaging using the technique of negative stain electron microscopy. The morphology of the first studied particles was symbolic of the so-called Space Age in which these discoveries took place: The archetypal Phi X 174 evoked the first artificial satellites, while the T-even phages resembled prototypic lunar landing vehicles.

Such latter-day futuristic symbolism may seem amusingly outdated by the time of writing. However, the undoubtedly ancient bacteriophages are now riding a new wave of technological advancement: synthetic biology. Interestingly, this returns us to the potential revolution in healthcare that surrounded the initial discovery of bacteriophages. The simple fact is that *phages*, which is a common abbreviation used when referring to these viruses, are capable of selective killing of bacterial cells of a given species or strain. In other words, phages are exquisitely specific microbial control agents. In the current antimicrobial resistance era [2], exemplified by the ESKAPE organisms, which is to name only the vanguard of untreatable pathogenic microbes, phages offer a potential panacea for the treatment of pathogenic bacterial infection.

Indeed, deployment of phages to treat bacterial infections in animals and humans was the first exploitative use of these virus entities. Their promise as therapeutic agents was, nevertheless, soon eclipsed by the rise of antibiotics. However, partly by economic necessity, the refinement of such phage-mediated treatments persisted in the former USSR [3]. Today, the fruit of that legacy provides effective alternative treatment in an era of antibiotic-resistant microorganisms [4]. Including the long-standing cases of treatment achieved within the pioneering hospitals in the former Soviet territory of Georgia [5], there have also been recent high-profile cases [6], as well as a rise in commercial offerings based upon sound scientific discovery [7].

2. Repurposed

In a commercial sense, phages are regarded as cheap to manufacture, because they will naturally multiply within their target bacteria. However, process control at

industrial scales presents challenges: most notably in efficiency and reproducibility but also via complications in terms of sterility and purity [7, 8]. Knowing how to keep prevalent diverse phage types out of a production facility will doubtless be as important as manufacturing commercially relevant types efficiently. When considering efficiency of phage production, the ultimate cost depends upon the measures that have to be taken, from plant and tooling to culture volume and starter culture characteristics. Investigations of multiplicity of infection, and economics of scale, plus processing of waste effluent are all factors that will impact costs and affordability [9]. Upscale and purity of phage will have an impact on any type of technology or application that is currently envisaged for phage, from antimicrobial therapy to phage particles as nanomaterials.

We can consider that phage genetic and structural insights are opening doors in nanotechnology and synthetic biology applications, which have a translation potential back into healthcare. That medical relevance is not limited to antimicrobial applications but also encompasses cancer and gene therapy. Indeed, phage display technologies are revolutionising the high-resolution visualisation of metastatic tumours in surgical settings by enabling unambiguous contrast.

Regarding the structure of phage particles, as nanoscale parts assembling into a mechanised vehicle for DNA packaging and delivery, their production as potential therapeutic agents might well be due a synthetic makeover. The concept of a phage cocktail is now very well tested: namely, several phage types recognising the same bacterial species but attacking that cell type in different ways. Phage cocktails are known to make it less likely that the targeted cells can survive via adaptation, as is known to be the case via treatment with a single phage type. Lately, the reprogramming of a phage DNA payload to ensure target cell death rather than phage latent persistence in a population of cells has also been found to be effective [6]. Indeed, such engineering approaches have been used to alter the DNA payload injected by the phage particle in order to make phages more effective at cell killing, rather than infection per se [6, 10].

Nevertheless, from an efficiency and medical regulatory perspective, it could be advantageous to have one or just a few medically approved phage chassis. These might be envisaged as phages known for their low immunogenicity profiles in patients. Perhaps these may be patient-specific, in which the therapeutic DNA payload is installed in a phage chassis matched to the current patient's immune tolerance. Then one can envisage a programmable targeting built in via a synthetic biology approach of re-engineering components of these standardised phages to recognise any desired bacterial target. Demonstrations of swapping out the targeting structures (i.e., tails and adsorption features) have been equally impressive in principle [11, 12]. Thus, furthering a detailed knowledge of phage structure beyond well-studied phage types [13] is incentivised.

3. Reimagined

One of the most fascinating things about phages is that they are hotbeds of molecular adaptation. An eclectic and presently largely arcane phage-encoded protein panoply supports the survival and success of the diverse bacteriophage types. The exploitation of deciphered elements of this phage protein repertoire was central to development of the recombinant biotechnology revolution. Circuitously, these very molecular tools are bringing about the technological revolution that promises to lend a starring role of phage in a biotechnologically repurposed guise. In fact, looking into that mesmerising pool of proteins of unknown function [14], it is easy to believe that the phages' encoded box of tricks isn't yet done revolutionising

applied biotechnology. The numbers of completely sequenced diverse phage genomes expand at a prolific rate, and the mysteries of their encoded protein manifest may yet take a long time and a lot of care to unravel.

Phage biology then, played a central role in the elucidation of some of the major genetic insights of the twentieth century. Other ground-breaking studies in that century concerned phage genome replication, the structures of phage particles and their assembly, and mechanisms of bacterial cell immunity and its viral subversion. Modern techniques in genomics, proteomics, and structural biology are adding novel insights even now [13, 15]. This combination of precision studies illuminates the plasticity of macromolecular and cellular biology in this perennial cauldron of evolutionary cat and mouse.

In terms of replicative strategies, phage activity in a cell involves an interesting sideway taken on biological processes. The enzymatic agents encoded by phages have applications in laboratory research technologies, applied molecular evolution techniques, and also novel ways to make DNA for therapeutic and technological use: free of the heretofore necessity for bacterial cell passage to propagate this DNA and thus of bacterial propagation sequences, including antibiotic resistance genes. The insights from phages therefore bring us to the brink of revolutionising applications as diverse as human gene therapy, cell and regenerative medicine, and DNA as data storage (**Figure 1**).

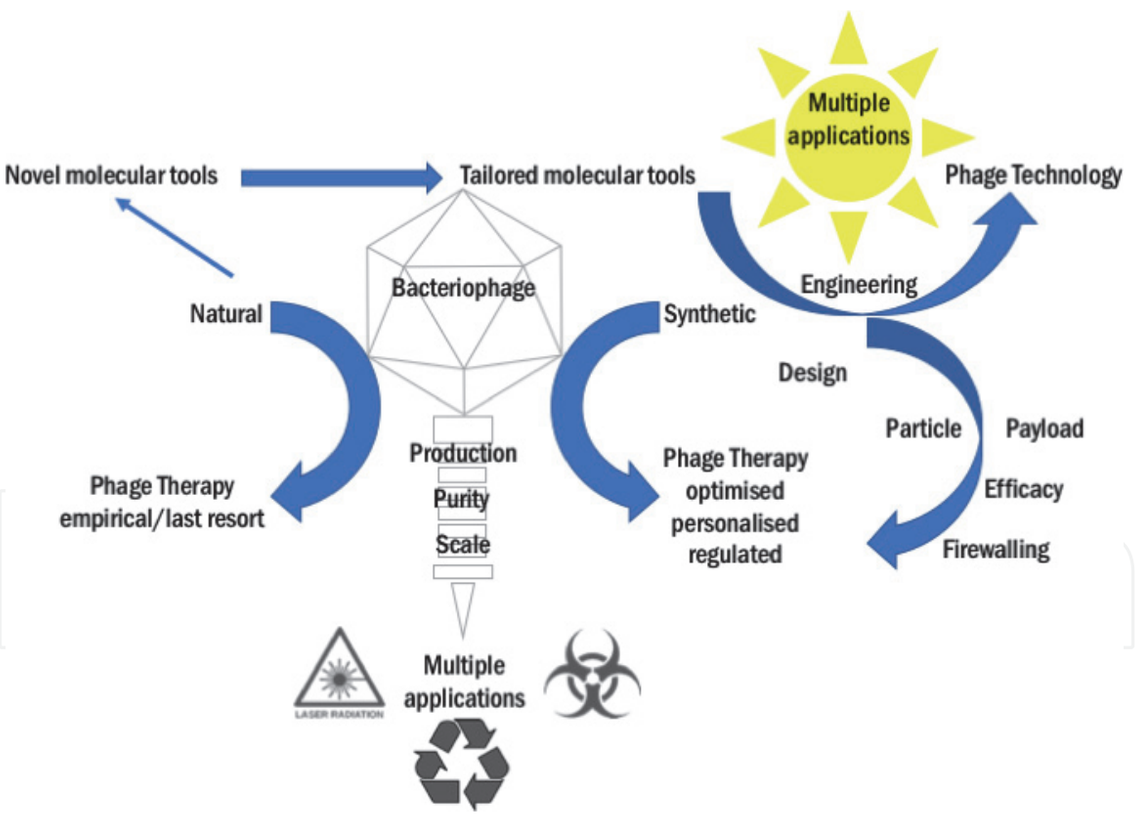

Figure 1.
Bacteriophages can be envisaged as natural microbial control agents, as well as machines for targeted synthetic genetic programming. The encoded proteins, as well as the structures of phages, offer a multitude of possibilities as outlined in this introductory chapter and detailed in the volume.

4. Rebooted

Finally, looking beyond what we have discovered and tested, beyond this volume and into that potential future of synthetically modified bacteriophages with

diverse uses, what might we find? Perhaps we might find nanomachines inspired in their design by the multifarious forms and mechanisms of both known and newly studied types of these viruses, lightning-fast genotyping of bacterial infections at point of care, and efficiently timely synthetic tooling of medically approved phages to rapidly quell those infections, even on the scale of a few hours. We might also find synthetic phages reprogramming the microbiome, both by selective population control and by targeted and firewalled genetic modification in situ, and even the possibility of tooling wild species of bacteria for bioremediation purposes via reversible and firewalled genetic modification. There could be phage-encoded elements brought together in new and as yet unimagined combinations to effect all manner of building and alteration performed at the macromolecular scale. The future is always imagined yet unseen: with phages in mind that refers both to its dazzling scale of possibility and in its infinitesimal scale of operation. The bacteriophages will be just as much a major part of that synthetic future, as they have ever seemingly been in nature to this day.

Conflict of interest

Author Sophie E. Knott is a co-author of the included chapter *The unusual linear plasmid generating systems of prokaryotes* and has recently started a collaborative research partly funded by Touchlight Genetics Ltd., towards a PhD in the editor's laboratory: This fact presents no conflicts of interest to the presently published work.

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