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Bacterial sensing of bacteriophages in communities: the search for the Rosetta stone

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Billions of years of evolution have resulted in microbial viruses and their hosts communicating in such a way that neither of these antagonists can dominate the other definitively. Studies of the molecular mechanisms underlying this dialog, initially in bacteriophages, rapidly identified several of the ways in which bacteria resist bacteriophage infections and bacteriophages defeat bacterial defenses. From an ecological perspective, recent data have raised many questions about the dynamic interactions between bacteria and bacteriophages, the densities of which, in complex microbial populations, are only beginning to be investigated. The next challenge will be determining how the dialog between microbial viruses and their hosts modulates complex ecosystems, such as those found in healthy humans or infected patients.

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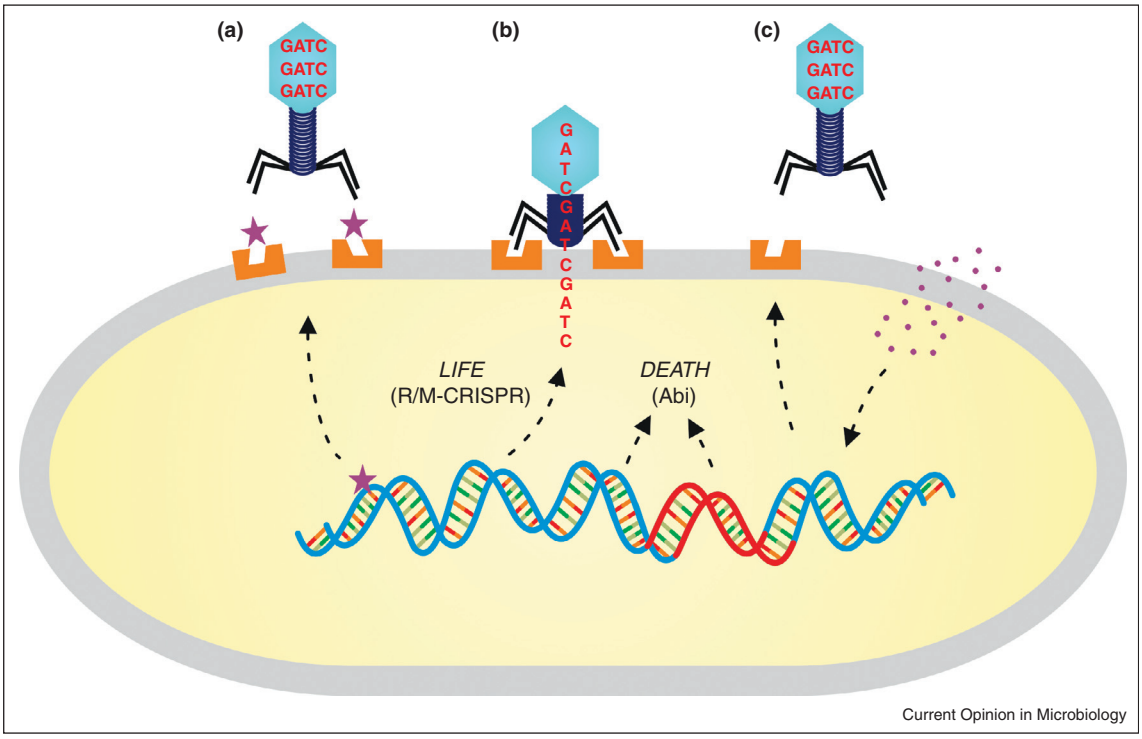
Introduction

Viruses infecting microbes (including those infecting Archaea, bacteria, fungi and protists) are considered to be the most abundant biological entity on Earth, with an estimated 10^{31} particles. They play a major biogeochemical role, by releasing material from the hosts they infect, but they also have a potentially useful but as yet untapped ecological impact on cellular populations. Bacteriophages, the most widely known and well-studied of these viruses, are predominantly virulent, their infectious cycle ending with the destruction of the bacterial host to release progeny. A minority of free bacteriophages is temperate and may, in some situations, initiate a lysogenic cycle rather than a lytic cycle, by integrating their genome into the bacterial chromosome to form a prophage. Bacteria have developed several molecular defenses against viral infection (bacteriophage resistance mechanisms have been described in detail elsewhere [1,2]). Instead this opinion focuses on recent publications related to bacterial sensing of bacteriophages from simple models to more complex situations such as microbial communities of mammals.

Binding to host receptor as first signal

Preceding resistance, bacterial sensing of bacteriophages operates at various stages (Figure 1). The first signal involves detection of the binding of a particular bacteriophage protein to a defined molecular structure present on the cell surface. Only for a few model bacteriophages have host and viral partners involved in this binding been identified [3]. Indeed, several genetic mutations were shown to interfere with bacteriophage binding, but no signaling-based mechanism has yet been identified. Recently, cutting-edge electron microscopy studies revealed how the T7 bacteriophage finds the most appropriate site for starting an infection [4••]. The bacteriophage fibers, which remain bound to the capsid, function primarily to facilitate the interaction of the bacteriophage tail with its specific receptor. Binding remains reversible until the fibers identify a suitable site. Infection begins only after stable adsorption of the bacteriophage into the bacterium, with (i) the injection of the internal core proteins into the cell; (ii) the formation of an extended tail and (iii) injection of the viral genome into the cell. During this scanning process, which is also thought to occur for bacteriophage SPP1 and might be widely spread amongst tailed bacteriophages, the rate of successful fiber binding to bacterial receptors may be limited by the host, through a signal initiated in response to the first molecular contact. This signal may be propagated to neighboring receptors via conformational changes, decreasing availability for irreversible binding. Another possible mechanism can be extrapolated from the recent identification of the molecular mechanism underlying the binding of the filamentous bacteriophage fd to the bacterial pilus, coupling unfolding and the prolyl isomerization of viral protein Gp3 [5]. The partially unfolded Gp3 uncovers the binding site for bacterial protein TolA, the secondary receptor. As a defense signal, binding to the pilus may trigger signaling to the cell membrane, to decrease TolA availability. Such signals, which may be the least costly defense solution for hosts, have not yet been demonstrated in practice. They may be irrelevant in test tubes, due to the high frequency of contacts, but play a more important role in mixed bacterial

Figure 1



Bacterial defense systems against bacteriophages. **(a)** Modifications of bacterial genetic information (mutations of genes encoding bacterial receptors, indicated by a star) can alter bacteriophage adsorption. **(b)** The modification of viral genetic information by restriction-modification (R/M) or CRISPR systems and self-suicide by abortive infection (Abi) prevent completion of the bacteriophage infectious cycle. **(c)** The modification of bacterial gene expression by diffusible molecules (purple dots) alter bacteriophage infection (by decreasing receptor synthesis, for example), leading to the spread of resistance phenotypes in the host population. The bacterial genome is shown as a blue DNA molecule, with a red part corresponding to a prophage element. Arrows highlight the flow of information.

populations, in which the frequency of contact is lower, and may then increase the threshold value for bacteriophage amplification.

Targeting the viral information

The second step, the injection of viral molecules (DNA and proteins) into the cytoplasm, is the last chance for bacteria to counteract viral infection. Once this process has begun, the host has a limited amount of time to react before the virus highjacks the functions of the cell to transform it into a viral factory. Studies *in vitro* and *in vivo* led to the identification of two possible mechanisms for the physical ejection of viral information from the capsid and its injection into the cell [6]. The ejection of lambda bacteriophage genetic material and its entry into *Escherichia coli* cells are estimated to take about five minutes, on the basis of single-cell fluorescence microscopy observations inspired by the famous Hershey and Chase experiment [7•]. However, this time varies between cells, consistent with a mechanism driven by internal cell processes as opposed to a repulsive mechanism originating in the viral capsid [6]. The host must then respond to bacteriophage infection within these five minutes, targeting the viral information.

Restriction modification and CRISPR (clustered regularly interspaced short palindromic repeats)/Cas protein (CRISPR-associated protein) systems are the two major mechanisms by which bacteria interfere with viral genetic information (Figure 1). CRISPR/Cas, the most recently discovered bacteriophage resistance mechanism, has been found in many bacteria and archaea. Detailed studies revealed that several CRISPR/Cas systems fulfill various functions, from defense against virulent bacteriophage infection to bacterial pathogenesis [8–10]. Once DNA ejection from the capsid has begun, the CRISPR/Cas system provides the bacterium with a means of interfering with the viral cycle and disrupt the integrity of the viral information, by making use of a short nucleotide sequence present in the host genome, that matches a sequence in the bacteriophage genome. However, it remains unclear how this sequence is integrated into the host genome in the first place. It is possible that defective bacteriophages, unable to complete the viral cycle, provide the bacteria with an opportunity to acquire sequences for the development of immunity. The molecular dissection of CRISPR systems is currently underway and several examples of bacteriophages carrying anti-CRISPR systems are being discovered, suggesting that