Ivan Maillard *is at the Life Sciences Institute, the Division of Hematology/Oncology and Department of Internal Medicine, and the Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, Michigan 48109, USA.* **Alan R. Saltiel** *is in the Department of Medicine, University of California, San Diego, La Jolla, California 92093, USA.*
e-mails: imaillar@umich.edu; asaltiel@ucsd.edu

1. Osborn, O. & Olefsky, J. M. *Nature Med.* **18,** 363–374 (2012).
2. Bapat, S. P. *et al. Nature* **528,** 137–141 (2015).
3. Feuerer, M. *et al. Nature Med.* **15,** 930–939 (2009).
4. Winer, S. *et al. Nature Med.* **15,** 921–929 (2009).
5. Kolodin, D. *et al. Cell Metab.* **21,** 543–557 (2015).
6. Cipolletta, D., Cohen, P., Spiegelman, B. M., Benoist, C. & Mathis, D. *Proc. Natl Acad. Sci. USA* **112,** 482–487 (2015).
7. Cipolletta, D. *et al. Nature* **486,** 549–553 (2012).
8. Vasanthakumar, A. *et al. Nature Immunol.* **1w6,** 276–285 (2015).
9. Lumeng, C. N. *et al. J. Immunol.* **187,** 6208–6216 (2011).
10. Brestoff, J. R. *et al. Nature* **519,** 242–246 (2015).
11. Lee, M.-W. *et al. Cell* **160,** 74–87 (2015).
12. Lumeng, C. N. & Saltiel, A. R. *J. Clin. Invest.* **121,** 2111–2117 (2011).
13. Wernstedt Asterholm, I. *et al. Cell Metab.* **20,** 103–118 (2014).

This article was published online on 18 November 2015.

In retrospect

A century of phage lessons

One hundred years after the first description of viruses that infect bacterial cells, the contribution of these bacteriophages to fundamental biology, biotechnology and human health continues unabated and deserves celebration.

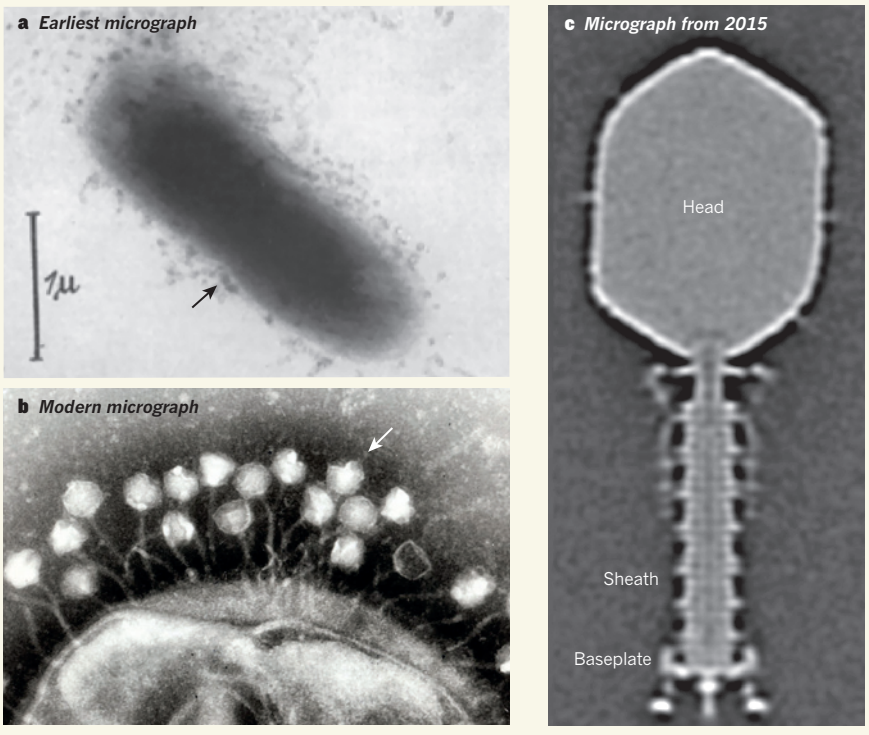
FOREST ROHWER & ANCA M. SEGALL

In 1915, bacteriologist Frederick Twort[1] published the first report of viruses that infect bacteria, replicate there and kill the cells. Since then, studies of these viruses, known as bacteriophages, or more colloquially as phages, have transformed biology. Phages provided the experimental systems and tools for the molecular-biology revolution of the twentieth century, and their rapid growth rates have allowed fundamental principles of ecology and evolution to be tested. We now know that phages are the world's most successful biological entities, being more abundant and genetically diverse than any other life form. Despite their importance, the study of these fascinating entities remains a niche endeavour. Here, we briefly review the history of phage studies, with the hope of inspiring a new generation of phage scientists.

In the early 1900s, most phage scientists were interested in using the viruses as antibacterial agents. This was an era of uncontrolled scientific trials, in which people were injected with phages or the viruses were poured into water wells with the hope of killing pathogenic bacteria, such as those that cause cholera. This line of research dramatically decreased with Alexander Fleming's discovery of antibiotics in 1928. But the concept of 'phage therapy' is currently resurging as antibiotic resistance becomes more of a concern.

Phage science entered the quantitative realm when a network of biologists, biochemists and physicists, known as the Phage Group, used these viruses as models for their pioneering studies of how life works. In 1952, Alfred Hershey and Martha Chase[2] performed a famous experiment in which radiolabelled phages were sheared off bacterial cells using a high-speed blender, helping the researchers to establish that DNA is the genetic material. The discovery of phage-encoded, DNA-manipulating enzymes — such as DNA and RNA polymerases, ligases and endo- and exonucleases — literally catalysed the rise of molecular biology and the biotechnology industry, and phage proteins are now used every day all over the world. Restriction enzymes that protect bacteria from phage infection provided another indispensable tool for molecular biologists. This trend continues today, as can be seen from the revolution in genome editing that has arisen following the discovery of the CRISPR–Cas system, used by bacteria as a defence against phages.

As the genetic code was revealed in the mid-1900s, the sequencing of a complete genome became a major research goal. Phages were attractive targets because of their small genome size and the possibility of making large amounts of DNA for sequencing. Frederick Sanger and colleagues[3] sequenced



A, REF. 15; B, GRAHAM BEARDS/CC-BY-3.0; C, REF. 16

Figure 1 | Bacteriophage in action. Bacterium-infecting viruses were first described[1] in 1915, but it was only in 1940 that the first electron micrographs (**a**) of bacteriophages (arrow) infecting bacteria were published[15]. These early images helped to confirm that the effects attributed to phages were indeed caused by viruses, and not by enzymatic activity. Modern electron microscopy (**b**, **c**) has produced images of phages that reveal details of phage structure and infection processes (see, for example, ref. 16).

© 2015 Macmillan Publishers Limited. All rights reserved

the complete genome of the phage ΦX174 in 1977, decades before any cellular genome was completed. As additional phage genomes accumulated, it became apparent that phages exchange genes and large sections of DNA between individuals[4]. This discovery of horizontal gene transfer changed our understanding of how genetic variability is produced. Marine phage communities were the first to be 'shotgun sequenced', leading to the rise of metagenomics — the sequencing en masse of all members of a community[5].

Understanding phages has contributed to our fundamental understanding of host cells and disease (Fig. 1). When phages integrate into bacterial genomes, they can dramatically change the characteristics of their bacterial hosts — many of the most deadly bacterial pathogens, including *Vibrio cholerae* and *Shigella* and *Salmonella* species, acquire virulence factors through this mechanism. Dissecting the biology of phage replication also uncovered several key host-encoded factors that are needed for the phage life cycle, such as the enzyme DNA gyrase[6] and the 'chaperone' protein complexes GroEL and GroES[7].

When the 'war on cancer' was declared by then US President Richard Nixon in 1971, phage biologists were actively recruited into research on human biology. Building on the knowledge that phages encode some proteins that are similar to those of the host, these scientists looked in the human genome for analogous genes from other viruses. Not only did they find such genes, but they also developed the idea of 'proto-oncogenes' present in our genome that, when mutated, are key drivers of cancer.

Other phage researchers moved into the fields of DNA mutagenesis, repair and recombination, providing the basis for our understanding of cancer today. For example, the understanding[8] that pre-existing mutations can give individual cells growth advantages under different environmental conditions led to the idea that cancer cells harbour dozens of pre-existing mutations that may or may not be related to the actual tumour[9]. With the advent of the AIDS epidemic, phage researchers opened the door to our understanding of how retroviruses integrate into the human genome, and what host proteins are involved[10].

The downside of phage scientists moving into different arenas was a massive decline in phage research from the 1970s onwards. Given that phages are such great anvils for the hammers of biologists, why do many researchers pay them so little attention? One reason might be that, as frequently occurs in any old discipline, the literature is dense and filled with acronyms and a changing nomenclature. To help counter this, we provide some guiding principles on phages.

A first key point is the contribution of phages to biological diversity. There are probably more than 10^{31} phage particles on the planet, with approximately 10 phage particles for every bacterial cell[11]. In humans, the main genetic difference between two individuals is the phages in their gut[12]. Among other roles, these viruses form an adaptable immune system that makes use of hypervariable, immunoglobulin-like protein domains similar to those used by antibodies[13].

The second concept is that phages carry genes encoding proteins that modulate the fundamental physiology of the host, such as metabolism and antibiotic resistance. One fascinating example occurs in photosynthesis by oceanic cyanobacteria[14]. The components of the light-gathering antenna complexes produced by these bacteria are highly labile and decay during phage infection. But the phages can carry genes that encode replacement of the damaged proteins, allowing the bacteria to continue to produce biomass and the phages to produce larger bursts of progeny. Thus, these marine phages contribute to the vast turnover of carbon in the oceans by increasing the efficiency and output of photosynthetic processes.

When phages integrate into bacterial genomes, they can dramatically change the characteristics of their bacterial hosts.

A third phage lesson is that the niche space of any bacterial cell is determined by its phages. The main genomic differences between closely related bacteria derive from integrated phages (prophages) and genomic features, ranging from indels to major rearrangements, that help to guard against phage infection. This never-ending selective pressure exerted on bacteria by their phages is the best-characterized example of the Red Queen hypothesis — that predator and prey species must constantly evolve.

What will the phage future look like? These viruses are relatively easy to synthesize, and their genomes have modular characteristics that appeal to synthetic biologists for engineering biological functions. One hundred years after their discovery, we think that it is time for our fellow biologists to throw off their cell-centric habits and embrace the phage. ■

Forest Rohwer *and* **Anca M. Segall** *are at the Viral Information Institute, Department of Biology, San Diego State University, San Diego, California 92182, USA.*
e-mails: frohwer@gmail.com; asegall@mail.sdsu.edu

1. Twort, F. W. *Lancet* **186,** 1241–1243 (1915).
2. Hershey, A. D. & Chase, M. *J. Gen. Physiol.* **36,** 39–56 (1952).
3. Sanger, F. *et al. J. Mol. Biol.* **125,** 225–246 (1978).
4. Hendrix, R. W., Lawrence, J. G., Hatfull, G. F. & Casjens, S. *Trends Microbiol.* **8,** 504–508 (2000).
5. Breitbart, M. *et al. Proc. Natl Acad. Sci. USA* **99,** 14250–14255 (2002).
6. Nash, H. A. *Annu. Rev. Genet.* **15,** 143–167 (1981).
7. Georgopoulos, C. *Genetics* **174,** 1699–1707 (2006).

50 Years Ago

The Architecture of Molecules. By Prof. Linus Pauling and Roger Hayward — "We are now living in an atomic age. In order to understand the world, every person needs to have some knowledge of atoms and molecules." This is the beginning paragraph of a fascinating work of art … The question as to how some understanding of science, however superficial, can be brought to the man and woman in the street has exercised many organizations as well as individuals. At a practical level, of course, it is unnecessary to know anything about electric currents in order to turn a switch and bring on the light. Babies love to do it before they are one year old. But for all too many people science is still magic even when they are twenty-one … Linus Pauling worries. He thinks, quite rightly, that young people ought to want to know why the 'lead' of a pencil comes off on to the paper, what an atom of hydrogen or uranium 'looks like' … Undoubtedly many an arts sixth-former will pick the book off the school library shelf and will learn a great deal by browsing through it.
From *Nature* 4 December 1965

100 Years Ago

The nation's attitude towards science is, I think, largely due to the popular idea that science is a kind of hobby followed by a certain class of people, instead of the materialisation of the desire experienced in various degrees by every thinking person to learn something about innumerable natural phenomena still unsolved; and, having learned, to control and apply them intelligently for the benefit of the human race … It is to the new generation now being educated that we must look for betterment of our position … We must make all education more scientific.
From *Nature* 2 December 1915

© 2015 Macmillan Publishers Limited. All rights reserved

8. Luria, S. E. & Delbrück, M. *Genetics* **28,** 491–511 (1943).
9. Vogelstein, B. *et al. Science* **339,** 1546–1558 (2013).
10. Craigie, R. & Bushman, F. D. *Cold Spring Harb. Perspect. Med.* **2,** a006890 (2012).
11. Wommack, K. E. & Colwell, R. R. *Microbiol. Mol. Biol. Rev.* **64,** 69–114 (2000).
12. Reyes, A. *et al. Nature* **466,** 334–338 (2010).
13. Barr, J. J. *et al. Proc. Natl Acad. Sci. USA* **110,** 10771–10776 (2013).
14. Sharon, I. N. *et al. ISME J.* **5,** 1178–1190 (2011).
15. Ruska, H. *Naturwissenschaften* **28,** 45–46 (1940).
16. Hu, B., Margolin, W., Molineux, I. J. & Liu, J. *Proc. Natl Acad. Sci. USA* **112,** E4919–E4928 (2015).

QUANTUM PHYSICS

Getting the measure of entanglement

A property called entanglement entropy helps to describe the quantum states of interacting particles, and it has at last been measured. The findings open the door to a deeper understanding of quantum systems. SEE ARTICLE P.77

STEVEN ROLSTON

A puzzling aspect of quantum mechanics is entanglement: the idea that the combined state of two particles can be completely specified, but that the state of each entangled particle is completely random when measured alone. Entanglement entropy marries the concept of entanglement with that of entropy — the degree of randomness of a system — and has become a useful theoretical tool with which to characterize many-body states in condensed-matter physics. On page 77 of this issue, Islam *et al.*[1] report the first experimental measurement of entanglement entropy in a small system of atoms trapped in a lattice of light, a model of a solid-state system.

Quantum mechanics, the theory of the microscopic world, has many features that run counter to our everyday experiences in a classical world. The possibility of entanglement in a quantum system of two or more particles has been a challenging and stimulating idea for many years. Einstein and his colleagues were famously bothered by the idea that measuring one particle of an entangled pair seemingly instantaneously determined the state of its partner — "spooky action at a distance", as they put it[2]. But the existence of entanglement was made concrete through the theoretical work of the physicist John Bell[3], and experimental tests of Bell's inequalities (constraints derived from Bell's work) have unambiguously verified the quantum-mechanical description of the microscopic world (see ref. 4, for example).

Although an understanding of two-particle entanglement is quite well in hand, there is no specific measure of the amount of entanglement in three or more particles. Yet entanglement has become an important tool for understanding the states of many-body systems. When many particles interact with one another, even through simple interactions, the low-energy quantum states can be surprisingly complicated, with lots of entanglement. Entanglement entropy has become a favoured theoretical measure for categorizing such complex states.

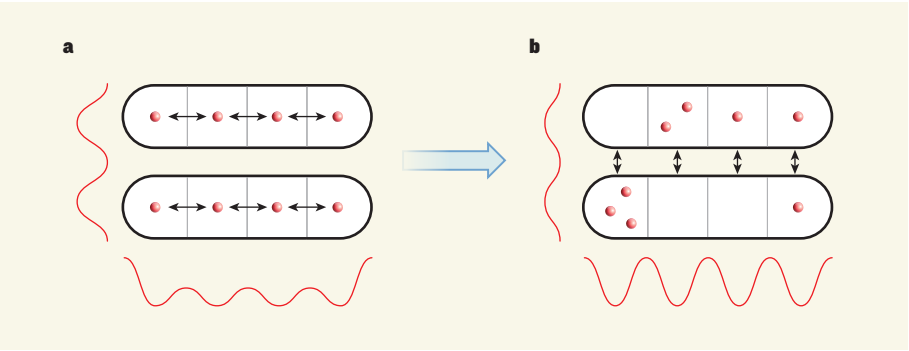


Figure 1 | Probing entropy entanglement in optical lattices. Islam *et al.*[1] report the first experimental measurements of entanglement entropy, a quantity used in theoretical studies to characterize many-body states. **a**, The authors set up two identical systems of four entangled atoms (dots; double-headed arrows indicate horizontal tunnelling), trapped in the potential-energy wells of an optical lattice (an array of interfering laser beams; red lines indicate the optical confining fields). **b**, The confining fields were then adjusted to allow the two four-atom systems to tunnel vertically into one another. The resulting number of atoms in each lattice site contained a signature of each system's state, from which the entanglement entropy can be extracted.

To understand what many-particle entanglement means, let's start by considering a non-entangled system. If I create a system that has N particles, each in an identical state independent of their $N-1$ neighbours, then its many-body description is simple, and measuring one particle or partitioning the sample has little impact on the overall system. Not that such states are uninteresting — this is a good description of a state of matter called a Bose–Einstein condensate, for example. Similarly, if each particle is in its own different state, with no relationship to its neighbours, then measurement or partitioning has no global effect.

But if the particles are entangled with one another, either pairwise or in a more complex fashion, then measurement of one particle affects the state of other particles. Entanglement entropy measures the increase in entropy (which can be thought of as increased randomness) that occurs if we partition such a system[5]. Identifying emergent, complex, lowest-energy states of seemingly simple systems of interacting particles is a particularly challenging task, for which entanglement entropy can be used to understand the nature of the state and to probe its 'quantumness'.

Until now, entanglement entropy has been a purely theoretical construct in condensed-matter physics, because it is difficult to partition a solid-state system and measure its constituents. Islam *et al.* have performed the first such measurements using two identical copies of a small system of four atoms trapped in an optical lattice (an array of interfering laser beams). If the potential-energy 'landscape' of the optical lattice is not too deep, the particles can tunnel from one site to the next and feel the presence of their neighbours. This leads to a many-body state that exhibits entanglement. But if the lattice is deep, the particles act as individuals, and are free of entanglement.

The authors performed their experiment in a quantum gas microscope[6], in which a single layer of an optical lattice is generated just below a high-resolution optical microscope. When Islam *et al.* relaxed some of the optical confining fields, the two copies of the four-atom systems could tunnel into one another and, through quantum interference (the Hong–Ou–Mandel effect[7]), leave a signature of their state in the number of atoms in each lattice site (Fig. 1). The authors simply counted the atoms using the microscope and extracted the entanglement entropy (the second-order Rényi entanglement entropy[5], for those in the know) from the number of atoms. In this way, they show that their four-atom system can have less entropy as a whole than when it is partitioned,

© 2015 Macmillan Publishers Limited. All rights reserved