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Review on the Similarities between Virus and Plasmid and its Movement between the Cells Membrane.

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ABSTARCT

Viruses are the most abundant biological entities on earth and show remarkable diversity of genome sequences, replication and expression strategies, and virion structures. Evolutionary genomics of viruses revealed many unexpected connections but the general scenario(s) for the evolution of the virosphere remains a matter of intense debate among proponents of the cellular regression, escaped genes, and primordial virus world hypotheses. A comprehensive sequence and structure analysis of major virion proteins indicates that they evolved on about 20 independent occasions, and in some of these cases likely ancestors are identifiable among the proteins of cellular organisms. Virus genomes typically consist of distinct structural and replication modules that recombine frequently and can have different evolutionary trajectories. The present analysis suggests that, although the replication modules of at least some classes of viruses might descend from primordial selfish genetic elements, bona fide viruses evolved on multiple, independent occasions throughout the course of evolution by the recruitment of diverse host proteins that became major virion components. Plasmids on the other hand are extra pieces of genetic material found in many cells that usually confer a specific property to the cell. These properties include antibiotic resistance, toxin production, and many other features. Plasmids are used in genetic engineering to generate recombinant DNAs and as a mechanism to transfer genes between organisms. They are often very similar in their mode of invading the cell membranes, also some viruses evolved from them.
Keywords: Virus, Plasmid, virions, DNA, RNA and cell Membrane.

INTRODUCTION

Plasmids are extra pieces of genetic material found in many cells that usually confer a specific property to the cell. These properties include antibiotic resistance, toxin production, and many other features. Plasmids are used in genetic engineering to generate recombinant DNAs and as a mechanism to transfer genes between organisms [1]. Plasmids are “extra” self-replicating genetic elements found in cells. They are distinct from chromosomes in being non-essential. Although plasmids are self-replicating molecules (replicons) that reside within host cells, they are not considered part of the cell’s genome for two reasons. First, the same plasmid may exist in two different species and be transferred between these species. Second, some members of the same species have plasmids, while others do not. Although plasmids carry useful genes, they are not absolutely necessary under most growth conditions [2]. As replicons they are DNA or RNA molecules with their own origin of replication. A replicon is a general term to describe any nucleic acid that contains its own origin of replication and can direct its own replication. This means that the enzymes needed for replication may not be encoded within the DNA or RNA molecule. Instead, by definition, it is the simple possession of an origin of replication that determines whether the molecule is a replicon or not. Plasmids are replicons, and so are viral genomes, viroids, and chromosomes [3]. Plasmids usually time their replication with the cell division of the host cell so that each daughter cell receives a copy of the plasmid [3].

Viruses are small obligate intracellular parasites, which by definition contain either a RNA or DNA genome surrounded by a protective, virus-coded protein coat. Viruses may be viewed as mobile genetic elements, most probably of cellular origin and characterized by a long co-evolution of virus and host [4]. For propagation viruses depend on specialized host cells supplying the complex metabolic and biosynthetic machinery of eukaryotic or prokaryotic cells. A complete virus particle is called a virion. The main function of the virion is to deliver its DNA or RNA genome into the host cell so that the genome can be expressed (transcribed and translated) by the host cell [4]. The viral genome, often with associated basic proteins, is packaged inside a symmetric protein capsid. The nucleic acid-associated protein, called nucleoprotein, together with the genome, forms the nucleocapsid. In enveloped viruses, the nucleocapsid is surrounded by a lipid bilayer derived from the modified host cell membrane and studded with an outer layer of virus envelope glycoproteins.

Therefore in this review articles, we will discuss the similarity between virus and plasmid during cell invasion through the cell membrane.

Replication and Transfer Mechanisms of Plasmid

In plasmids, replication occurs at a specific site known as the origin of vegetative replication (oriV). Well-known replication systems of circular plasmids include theta-type replication, rolling-circle replication, and strand displacement-type replication [5]. Many theta-type replicating plasmids contain repeated DNA sequences, or iterons, which bind the replication initiation protein. The mechanisms of replication control have been extensively studied in two iteron-containing plasmids, pPS10 and R6K. ColE1-family plasmids are another group of theta-type replicating plasmids whose replication is strictly controlled by an antisense RNA. The rolling-circle replication mechanism is found in many small multi-copy plasmids. The representative plasmids using rolling-circle replication are pT181, pC194 (Inc8), pMV158 (Inc11), and pUB110 (Inc13), all identified in staphylococci. Similar plasmids are also found in Bacillus and several genera in Actinobacteria. [6] recently reviewed the replication mechanisms of a prophage of Escherichia coli, N15, which was the first linear plasmid identified with covalently closed ends [7]. Most linear plasmids have conserved “telomeres” containing inverted repeat sequences [7]. The 5′ telomeric ends are blocked by covalently attached telomere terminal proteins [7]. Linear plasmids have sets of conserved telomere replication genes known as tpg and tap [8]. Many linear type plasmids have been found in Actinobacteria, especially in the genera Mycobacteria, Rhodococcus, and Streptomyces. Conjugative transfer is another important mechanism by which plasmids spread DNA among different bacteria. Self-transmissible plasmids in Gram-negative bacteria generally carry complete sets of genes required for transfer, the origin of transfer (oriT), the relaxase protein, the type IV coupling protein (T4CP), and the type IV secretion system (T4SS). [9] classified the conjugative, or mobilizable, plasmids in the GenBank database into six mobility (MOB) types (MOBC, MOBF, MOBH, MOBP, MOBQ, and MOBV) according to the amino acid sequences of their relaxase proteins. An additional classification was performed based on the plasmids' T4SS involved in mating pair formation (MPF) during conjugation. Smillie et al. proposed four classes of MPF (MPFF, MPFG, MPFI, and MPFT) according to the T4SS amino acid sequences. They also investigated the presence of two key elements of plasmid mobility, type IV pili coupling protein (T4CP) and the ATPase VirB4 [10]. During conjugation, double-stranded plasmid DNA is cleaved at the oriT site by a relaxase protein, which then covalently binds to the oriT DNA. The resultant DNA-protein complex is transported to the recipient cell by T4SS. This single-stranded DNA is transferred into the recipient cell by the T4CP. The mobilizable plasmids only have oriT,

relaxase, and sometimes T4CP. Gram-positive bacteria transfer plasmids by two methods, although the detailed mechanisms are not well understood. First, a single strand of plasmid DNA is transported via a T4SS, which seems to be widely used as a means for transferring plasmids in Gram positive bacteria. Several plasmids of the order Actinomycetales have conjugative systems that function in a manner similar to the segregation of chromosomal DNA during bacterial cell division and sporulation. The translocation of double-stranded DNA to the recipient cell is mediated by an FtsK-homologous protein [11]. As for archaeal plasmids, only the plasmids in Sulfolobales are known to be transferred; however, the mechanisms are still not well understood.

Replication and Transfer Mechanisms of Plasmid

Viruses are perfect parasites. It has been known for decades that once a virus gets inside a cell, it hijacks the cellular processes to produce virally encoded protein that will replicate the virus's genetic material [12]. Viral mechanisms are capable of translocating proteins and genetic material from the cell and assembling them into new virus particles. Contemporary research has revealed specific mechanisms viruses use to get inside cells and infect them. An individual viral particle, called a virion, is a far simpler structure than a bacterium. It has often been questioned whether a virus is alive. It is certainly not living in the everyday sense of the word. Virions consist of genetic material DNA or RNA enclosed in a protein coating [13]. Many viruses, called enveloped viruses, have an additional outer membrane that encloses the protein coat. This membrane envelope is material co-opted from the cell's own membrane. As the new virion buds out from an infected host cell, it is wrapped by the cell's bilayer membrane and carries with it any protein that happens to be embedded in the membrane at the budding site [14]. Enveloped viruses are then free to begin a new cycle of infection by fusing their cell-derived envelope with the cellular membrane of an uninfected cell. Some types of enveloped virus fuse directly to the cell's outer (plasma) membrane, whereas others are engulfed whole by endocytosis or similar processes and then fuse their envelope with the membrane of the engulfing internal organelle (e.g., an endosome) to gain access to the interior of the cell. In either case, the genetic material of the virus has invaded the cell through the barrier of its membrane, and infection will inevitably follow (Fig. 1) [15]. Infection can be prevented if fusion of the viral envelope with the cell or endosomal membrane can be blocked. Similarly, if a vaccine can be directed against the viral fusion protein, infection can be prevented. Vaccines against the influenza virus, for example, target the fusion proteins of the virus.

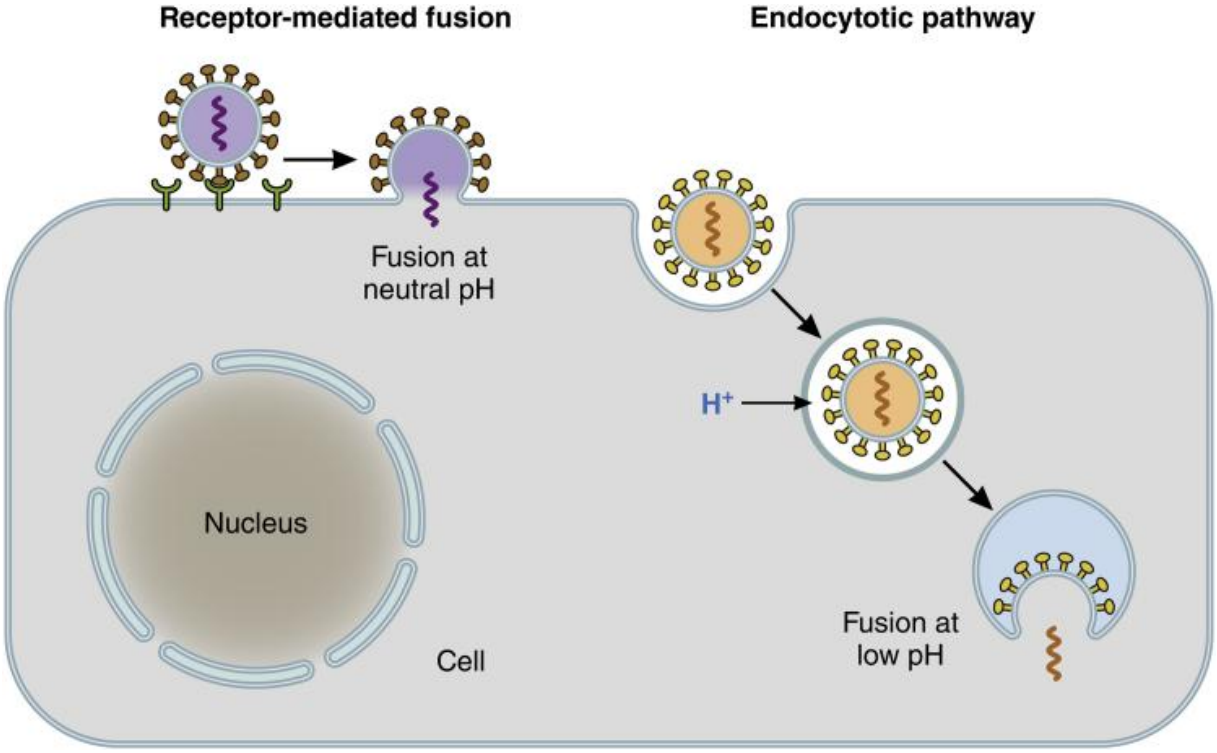

Figure 1: Viral entry pathways. Virus can fuse either directly to the plasma membrane (receptor-mediated fusion) or after being swallowed into an endosome. Which of these routes is followed depends on the type of virus. In fusion with the plasma membrane, the virus binds to a protein in the cell membrane. The function of this cellular protein (a receptor for the virus, shown in *green*) is perverted to induce a conformational change in the viral fusion protein, leading to fusion. For virus that is triggered within an endosome, the endosome's acidic conditions induce fusion. In either case, the viral genome passes through a fusion pore into cytosol, and infection is initiated [15].

Viral genetic material is relatively small, encoding only a few proteins. All enveloped viruses contain fusion proteins, which are the molecules responsible for fusing the envelope to a cellular membrane. These proteins are derived from the virion's genetic sequence [16]. The precise genetic material, the amino acid sequence, and details in structure of a fusion protein are unique for each type of virus. Consequently, broad-spectrum antiviral drugs do not exist, and specific vaccines and drugs typically need to be developed for each virus type. The viral surface of an individual virion contains multiple copies of its fusion protein. Influenza virus, for example, typically contains 500–1000 copies, whereas HIV contains only about a dozen copies (1, 2). A virion's machinery is so efficient that each cell infected by even a single virion can produce about a million new virions [17]. Because enveloped viruses use similar mechanisms for delivery of genetic material into cells, there may be ways to prevent infection before viral entry that would be effective for large numbers of different viruses.

The membrane that is the skin of a cell and an enveloped virion, and is the gateway of viral entry, consists of lipids and proteins. Lipids are roughly linear molecules of fat that are attached at one end to a water-soluble headgroup. Lipids provide the cohesion that keeps biological membranes intact [18]. They spontaneously arrange themselves into a lipid bilayer because oily fat does not mix with water. The headgroups of one monolayer face an external aqueous solution, whereas the headgroups of the other monolayer face the interior of the cell. Integral membrane proteins, such as viral fusion proteins, are inserted into the bilayer and project out from the lipid

surface into the external solution-like icebergs. Membranes are generally 50% lipids and 50% proteins by weight, but proteins are much heavier than lipids, and so there are about a hundred times more lipids than proteins in a membrane. Membranes are able to fuse to each other because they are fluid, and the lipids provide fluidity to the membrane. Viruses initially stick to cell membranes through interactions unrelated to fusion proteins [18]. The virus surfs along the fluid surface of the cell and eventually the viral fusion proteins bind to receptor molecules on the cell membrane. If only binding occurred, the two membranes would remain distinct. Fusion does not happen spontaneously because bilayers are stable. Fusion proteins do the work of prodding lipids from their initial bilayer configuration. These proteins cause discontinuities in the bilayers that induce the lipids of one membrane (e.g., the viral envelope) to connect with lipids of another (e.g., a cellular membrane), converting two bilayers into one. Fusion proceeds in two major steps (Fig. 2) [19]. First, the two monolayers from opposite membranes that touches each other merge, a process known as “hemifusion.” The two unmerged monolayers collapse onto each other to create a single bilayer, known as a hemifusion diaphragm, which continues to prevent the viral genome from entering cytosol. In the second step, the fusion proteins disrupt this single bilayer to create a pore that provides an aqueous pathway between the virus and the cell interior. It is through this fusion pore that the viral genome gains entry into a cell and begins infection.

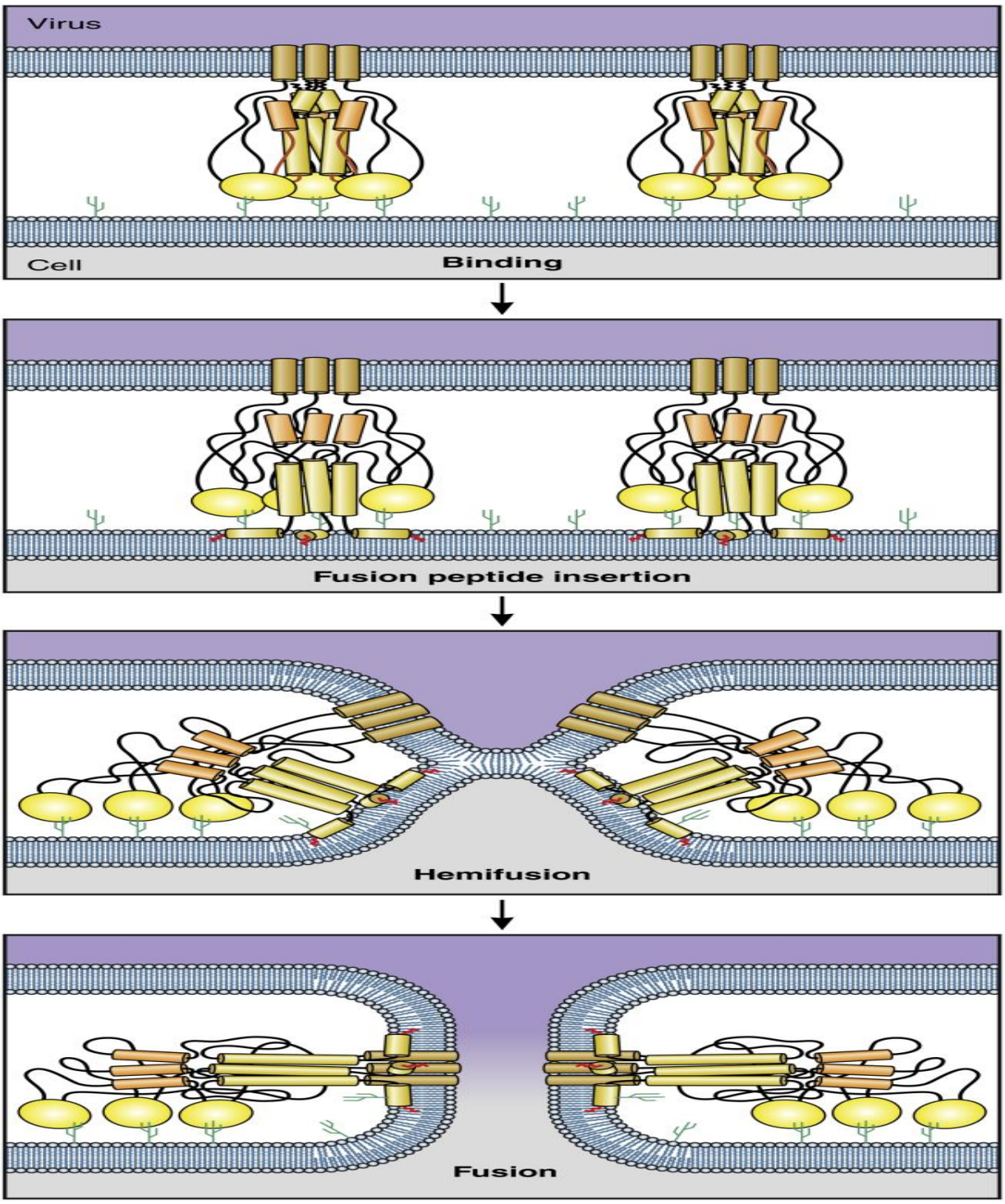

Figure 2: The steps of fusion. Virus binds to specific receptors (each illustrated as a small *cactus*) on a cell membrane. Initially, four monolayers (in *blue*) separate the two interior aqueous compartments. After fusion peptides insert into the target membrane, monolayers that face each other merge and clear from the merged region. The noncontacting monolayers bend into the cleared region and come into contact with each other, forming a new bilayer membrane known as a hemifusion diaphragm. At this point (hemifusion), only two monolayers separate the compartments. The fusion protein acts as a nutcracker to force the formation of a pore within the hemifusion diaphragm. This establishes continuity between the two aqueous compartments and fusion is complete [19].

Hemifusion and pore formation appear to require comparable amounts of work, but the exact amount of energy needed for each step is not yet known [19]. These energetic details may be important because the more work required to achieve a step, the easier it may be to pharmacologically block that step. These energies are supplied by the viral fusion proteins, which are essentially molecular machines. Some of their parts move long distances during the steps of fusion. Fusion proteins can be thought of as a complex assembly of wrenches, pliers, drills, and other mechanical tools [20]. Because fusion is not spontaneous, discontinuities must be transiently created within the bilayer that allows water to reach the fatty, oily interior of the membrane. Even a short-lived exposure of a small patch of the fatty interior to water is energetically costly. Similarly, creating a pore in a hemifusion diaphragm requires exposure of the bilayer interior to water [20]. In contrast, pore enlargement needs no such exposure. Nevertheless, pore enlargement requires the most amount of work in the fusion process. Energy is also needed because of another fundamental property of bilayer membranes. Though bilayers are fluid, they don't entirely behave like water or oil, in that they do not assume the shape of their container. Biological membranes have shapes that are determined by their precise lipids and the proteins associated with them [21]. Work is required to force membranes out of their spontaneous shape, which is the shape of lowest energy. The fusion pore that connects the virus and cell is roughly an hourglass shape. The wall of a fusion pore is a membrane with components that are a mixture of the two original membranes. An hourglass shape deviates significantly from the spontaneous shape of the initial membranes that constitute the pore. The greater the diameter of the pore, the greater is the area of the lining membrane, and so pore expansion is a highly energy consuming process [22]. Viral genetic material, the genome, is rather large, on the order of ~100 nm. The initial fusion pore is only ~1 nm, so considerably more membrane must line a pore as it enlarges to a size sufficient to allow passage of a viral genome from a virus to a cell interior. In fact, it appears that more energy is required for pore expansion than for hemifusion or pore formation. All viral fusion proteins contain a greasy segment of amino acids, referred to as a fusion peptide or fusion loop. Soon after activation of the fusion protein, the fusion peptide inserts into the target membrane (either plasma or endosomal) [23]. At this point, two extended segments of amino acids are anchored to the membranes: the fusion peptides in the target membrane and the membrane-spanning domains of the fusion proteins in the viral envelope (Fig. 2). The fusion proteins continue to reconfigure, causing the two membrane-anchored domains to come toward each other. This pulls the viral envelope and cellular membrane closely together [24]. The fusion proteins exert additional forces, but exactly what these forces are and how they promote fusion remains unknown. An initial step in a cell's digestive system is to internalize extracellular materials through engulfment by endosomes. A virion engulfed into an endosome is like a Trojan horse, because the cell perceives the virus particle as food. Endosomes become increasingly acidified as they move from the cell surface further into the cell's interior. Fusion of viruses within endosomes depends critically on the acidic environment. By breaking molecular bonds, acid triggers the conformational changes in the fusion protein that lead to the sequential steps of membrane fusion.

The hemifusion diaphragm is a bilayer membrane that is unusual in that each of its lipid monolayers is derived from different membranes, and it does not contain any membrane-spanning proteins [24]. Several copies of the fusion protein within a virus are required to induce both hemifusion and pore formation. During hemifusion, the proteins form a ring just outside the diaphragm and act cooperatively to create stresses that lead to a local

rupture in the diaphragm, thereby creating the initial fusion pore. The universality of this mechanism is remarkable when one considers that the primary amino acid sequences and structures of fusion proteins are quite diverse.

Differences between a plasmid and a virus

A plasmid is very similar to some types of viruses but it doesn't have, or code for a protein coat. Plasmids are short, double-stranded circular DNA molecules. Almost certainly some viruses evolved from them [25]. But there is nearly every possible permutation represented by some virus or another, so they are not as restrictive in structure as plasmids. This includes differences in protein coat (including lipid layers), number of strands (1, 2 or mixed), RNA or DNA, circular linear or mixed, etc. There are also circular single stranded RNA viroids that infect plants that are certainly related to some RNA viruses, but without protein coats [25].

A plasmid on the road to becoming a virus

Plasmids have been discovered that can move from cell to cell within membrane vesicles in a species of Archaea. They provide clues about the origin of virus particles. Electron microscope analysis of the culture medium from *Halobrum lacusprofu*ndi R1S1, an Archaeal strain from Antarctica, revealed spherical particles which were subsequently shown to contain a 50,000 base pair circular double-stranded DNA molecule [26]. When added to *H. lacusprofundi*, the purified membrane vesicles entered the cells and the DNA replicated. Nucleotide sequence analysis of the plasmid within the membrane vesicles revealed 48 potential protein coding regions and an origin of DNA replication. None of these proteins showed any similarity to viral stuctural proteins, leading the authors to conclude that these particles are not viruses. Many of the proteins encoded in the plasmid DNA were found in the membrane vesicles [26]. Some of these are similar to cell proteins known to be involved in the generation of membrane vesicles. However no DNA polymerase-like proteins are encoded in the plasmid. These data suggest that the plasmid encodes proteins that generate, from the membranes of the cell, the vesicles needed for their transport to other cells. However, replication of the plasmid is carried out by cellular DNA polymerases. It is likely that the plasmid-containing membrane vesicles are precursors of what we know today as virus particles [27]. It is thought that viruses originated from selfish genetic elements such as plasmids and transposons when these nucleic acids acquired structural proteins. Phylogenetic analyses of the structural proteins of many enveloped and naked viruses reveal that they likely originated from cell proteins on multiple occasions [28]. The membrane-encased Archaeal plasmid seems well on its way to becoming a virus, pending acquisition of viral structural proteins. Such an early precursor of virus particles has never been seen before, emphasizing that science should not be conducted only under the streetlight [29].

CONCLUSION

The most reliable way to prevent infection caused by any virus is to eliminate entry in the first place. Intellectual and technological progress has been great, but recurrent viral outbreaks highlight the need for more innovative approaches. In addition to the proteins responsible for viral entry, many other targets are being explored, including genetic variations that increase susceptibility to infection, proteins that bind to viral proteins, and host immunity proteins. Genomic and proteomic analysis of cellular factors and their interactions, manipulation of experimental animals, live cell and molecular imaging, and analysis and integration of protein and gene data sets will identify host factors that viruses exploit in their life cycle. Because viruses make use of cellular machinery and invariably do so in a streamlined and robust manner future viral studies will provide new understandings that will

apply not only to virally induced diseases but to other diseases as well. A plasmid is very similar to some types of viruses but it doesn't have, or code for a protein coat. Plasmids are short, double-stranded circular DNA molecules. Almost certainly some viruses evolved from them. But there is nearly every possible permutation represented by some virus or another, so they are not as restrictive in structure as plasmids. Most viral and plasmid-encoded proteins involved in central information-processing systems, such as polymerases, ligases and topoisomerases, are very different in terms of sequence similarity from their cellular functional analogues. Recent advances in the identification of novel types of plasmids and plasmid transfer by culture-independent methods using samples from natural environments is obtained.

REFERENCES

1. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z. and Miller, W. (2016). Gapped BLAST and PSI-BLAST: a new generation of proteins database search programs. *Nucleic Acids Res* 25: 3389– 3402.
2. Aravind, L., Walker, D. R. and Koonin, E. (2015). Conserved domains in DNA repair proteins and evolution of repair systems. *Nucleic Acids Res* 2:1223– 1242.
3. Bergerat, A., De Massy, B., Gadelle, D., Varoutas, P.C., Nicolas, A., Forterre, P. (2019). An atypical type II DNA topoisomerase from Archaea with implication for meiotic recombination. *Nature* 386: 414–417.
4. Bernander, R. (2017). Archaea and the cell cycle. *Mol Microbiol* 29: 955– 961.
5. Bernstein, H. and Bernstein, C. (2013). Bacteriophage T4 genetic homologies with Bacteria and Eukarya. J *Bacteriol* 171: 2265–2270.
6. Bidnenko, E., Erlich, S.D. and Chopin, M. C. (2017). Lactococcus lactis phage operon coding for an endonuclease homologous to RuvC. *Mol Microbio*l 28: 823– 834.
7. Bonner, C. A., Stukenberg, P. T., Rajagopolan, M., Eritja, R., O'Donnell, M. and McEntee, K. (2012). Processive DNA synthesis by DNA polymerase II mediated by DNA polymerase III accessory proteins. *J Biol Chem* 267: 11431–11438.
8. Bouché, J. P., Béjar, S. and Cam, K. (2016). Cooption of prophage genes: new data on the Kim prophage region of the Escherichia coli chromosome. In The Bacterial Chromosome. Drlica, K., and Riley, M. (eds). Washington, DC: *American Society for Microbiology Press*, pp. 373– 378.
9. Brinkmann, H. and Phillippe, H. (2011). Archaea sister group of Bacteria? Indications from tree reconstruction artefacts in ancient phylogenies. Mol Biol Evol (in press).
10. Brown, J. R. and Doolittle, W. F. (2017). Archaea and the prokaryote-to-eukaryote transition. *Microbiol Mol Biol Rev* 61: 456– 502.
11. Bruand, C., Erlich, S. D. and Jannière, L. (2011). Primosome assembly site in Bacillus subtilis. *EMBO J* 14: 2642– 2650.
12. Chernomordik, L.V., Zimmerberg, J. and Kozlov, M. M. (2016). Membranes of the world unite. *J. Cell Biol.*175:201–207.
13. Cohen, F. S. and Melikyan, G. B. (2014). The energetics of membrane fusion from binding, through hemifusion, pore formation, and pore enlargement. *J. Membr. Biol.*199:1–14.
14. Dickerson, R. E. (2019). The structure of cytochrome c and the rates of molecular evolution. *J Mol Evol* 1: 26– 45.
15. Doolittle, R.F. (2010). Microbial genomes opened up. *Nature*. 392: 339– 342.

16. Doolittle, W. F. (2010) You are what you eat: a gene transfer ratchet could account for bacterial genes in eukaryotic nuclear genomes. *Trends Genet* 14: 307– 311.
17. Edgell, D. R. and Doolittle, W. F. (2017). Archaea and the origin(s) of DNA replication proteins. *Cell* 89: 995– 998.
18. Fuller, T. L., Gilbert, M. and Smith, T. B. (2013). Predicting hotspots for influenza virus reassortment. *Emerg. Infect. Dis.* 19:581–588.
19. Harris, A., Cardone, G. and Steven, A.C. (2016). Influenza virus pleiomorphy characterized by cryoelectron tomography. *Proc. Natl. Acad. Sci.* USA.103:19123–19127.
20. Kim, J. H., Resende, R. and Withers, S. G. (2013). Mechanism-based covalent neuraminidase inhibitors with broad-spectrum influenza antiviral activity. *Science*. 340:71–75.
21. Kozlov, M. M., Campelo, F. and McMahon, H. T. (2014). Mechanisms shaping cell membranes. *Curr. Opin. Cell Biol.* 29:53–60.
22. Kuzmin, P. I., Zimmerberg, J., Cohen F.S. A quantitative model for membrane fusion based on low-energy intermediates. *Proc. Natl. Acad. Sci*. USA. 98:7235–7240.
23. Lehmann, M. J., Sherer, N. M. and Mothes, W. (2015). Actin- and myosin-driven movement of viruses along filopodia precedes their entry into cells. *J. Cell Biol*. 170:317–325.
24. Melikyan, G. B., White, J. M. and Cohen, F.S. (2017). GPI-anchored influenza hemagglutinin induces hemifusion to both red blood cell and planar bilayer membranes. *J. Cell Biol.* 131:679–691.
25. Nelson, M. I. and Holmes, E.C. (2014). The evolution of epidemic influenza. *Nat. Rev. Genet*. 8:196–205.
26. Ryham, R. J., Ward, M. A. and Cohen, F. S. (2013). Teardrop shapes minimize bending energy of fusion pores connecting planar bilayers. *Phys. Rev. E Stat. Nonlin. Soft Matter Phys*. 88:062701.
27. Sharp, P. M., Hahn, B. H. (2010). The evolution of HIV-1 and the origin of AIDS. Philos. Trans. *R. Soc. Lond. B Biol. Sci*. 365:2487–2494.
28. White, J. M., Delos, S. E. and Schornberg, K. (2018). Structures and mechanisms of viral membrane fusion proteins: multiple variations on a common theme. *Crit. Rev. Biochem. Mol. Biol.* 43:189–219.
29. Zhu, P., Liu, J. and Roux, K.H. (2016). Distribution and three-dimensional structure of AIDS virus envelope spikes. *Nature*. 441:847–852.