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To be or not to be alive: How recent discoveries challenge the traditional definitions of viruses and life

Patrick Forterre

Institut Pasteur, Unité de Biologie Moléculaire du Gène chez les Extrêmophiles, Département de Microbiologie, F-75015, Paris, France

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ABSTRACT

Three major discoveries have recently profoundly modified our perception of the viral world: molecular ecologists have shown that viral particles are more abundant than cells in natural environments; structural biologists have shown that some viruses from the three domains of life, Bacteria, Eukarya and Archaea, are evolutionarily related, and microbiologists have discovered giant viruses that rival with cells in terms of size and gene content. I discuss here the scientific and philosophical impact of these discoveries on the debates over the definition, nature (living or not), and origin of viruses. I suggest that viruses have often been considered non-living, because they are traditionally assimilated to their virions. However, the term virus describes a biological process and should integrate all aspects of the viral reproduction cycle. It is especially important to focus on the intracellular part of this cycle, the virocell, when viral information is actively expressed and reproduced, allowing the emergence of new viral genes. The virocell concept theoretically removes roadblocks that prevent defining viruses as living organisms. However, defining a "living organism" remains challenging, as indicated by the case of organelles that evolved from intracellular bacteria. To bypass this problem, I suggest considering that all biological entities that actively participate in the process of life are living.

1. Introduction: how recent discoveries impact the debate over the living/non-living status of viruses

Scientists often describe the material world using concepts first developed by human beings to describe their environment and mode of existence. However, these concepts may take on very different meanings when translated into a world far beyond human experience. For example, the concepts of space and time have different meanings for us, in our daily life, and for astrophysicists dealing with general relativity. Quantum mechanics also highlight major confrontations between human experience and reality at the ultramicroscopic level. Similar problems came to the forefront in biology when scientists began to try to apply concepts such as "life" and "organism", to the world of microbes (Dupre & O'Malley, 2009, Pradeu, 2010). The case of viruses is especially interesting because biologists have argued for more than one century about their living and organismal status (Helvoort, 1994, Kostyrka, 2016, Méthot, 2016).

Viruses use the same macromolecules (proteins and nucleic acids) as cellular organisms for the reproduction and expression of genetic information. This indicates that viruses and cells fit into the same historical process that we call "life". Viral genomes may consist of RNA (a situation encountered only in viruses) or DNA. They have a reproductive cycle with two characteristic phases. In the extracellular phase, the viral genome remains inactive within a viral particle, also known as a virion, until it encounters a susceptible cell that can be infected. In the intracellular phase, the viral genome may temporarily remain silent (as a free chromosome or integrated into the cellular chromosome) or be actively expressed and replicated in the infected cell. When activated, these coupled processes lead to the production of infectious virions (viral particles), which serve as vehicles for the dissemination of viral genomes. In virions, the viral genome is encased within a protein coat, which may differ in complexity

E-mail address: forterre@pasteur.fr.

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between viruses, with some containing or surrounded by a lipid envelope and/or decorated with polysaccharides.[1]

The nature and definition of viruses, especially their "living" status, have been the focus of heated debates among biologists for decades (Helvoort, 1994, see Kostyrka, 2016, and Méthot, 2016). Recently, these debates have become more acute following three startling discoveries. First, it has been demonstrated that viral particles outnumber cells by one or two degrees of magnitude and that viral genes greatly outnumber cellular genes in most environments (Kristensen, Mushegian, Dolja, & Koonin, 2010, Suttle, 2013). Viral genes also massively integrate into cellular genomes, greatly influencing cellular evolution (Forterre & Prangishvili, 2013). Second, the sharp distinction between viruses infecting prokaryotes (bacteriophages) and eukaryotes was put upside down by the discovery of evolutionarily related viruses infecting cells from the three domains of life, Bacteria, Eukarya and Archaea (Abrescia, Bamford, Grimes, & Stuart, 2012). Finally, the traditional view of viruses as submicroscopic entities has been challenged by the discovery of giant viruses infecting Amoeba, such as Mimivirus and Pandoravirus (Philippe et al., 2013; Raoult et al., 2004; see also Claverie and Abergel, 2016). Here, I critically review how these discoveries have led to new proposals about the nature, definition, and origin of viruses, trying to emphasize the philosophical aspects of these debates. I will argue that we should probably modify the meaning of common concepts, such as life or organisms, when applied to biology, in order to make them useful for an objective description of nature, and introduce new concepts (such as the virocell concept) to prevent some of the ambiguities inherent to current paradigms.

2. The traditional view of viruses assimilated to their virions

2.1. The "virus/virion" paradigm

The name "virus" was traditionally used both as a concept and as a general term to name concrete objects (viral particles) within the material world. This often led to a narrow concept of virus assimilated to viral particle (also called virion). This assimilation is general and pervasive for both historical and practical reasons (Forterre, 2012b) and will be referred to as the "virus/virion" paradigm hereafter.[2] Historically, the origin of the "virus/virion" paradigm can be traced back to the discovery of viruses, because the term "virus" was first used to describe the infectious entities able to pass through a Chamberland porcelain filter that was known to retain bacteria (Bos, 1999). Practically, virions can be isolated and purified, allowing their biochemical analysis and their observation. As a consequence, they can be visualised and used to illustrate and popularize the virus concept with pictures in publications, textbooks and conferences.

By contrast, viruses have no specific form in the intracellular phase, with their components being dispersed among those of the infected cell. As a consequence, the intracellular phase has been largely excluded from traditional virus definitions. For example, Jacob and Wollman (1961) defined a virus as "*a genetic element enclosed in a protein coat*". The "virus/virion" paradigm has indeed influenced most definitions of a virus. For example, Lwoff (1957) claimed that viruses carry only one type of nucleic acid (either RNA or DNA), whereas cells carry two types: DNA for information storage, and RNA for gene expression. However, this affirmation is correct only in the framework of the virus/virion paradigm, because, like cells, DNA viruses undergo transcription to generate viral messenger RNA! The resulting viral mRNAs belong to the virus just as much as cellular mRNAs belong to the cell. Thus, all DNA viruses actually possess both types of nucleic acid, DNA and RNA. Most virologists would deny that they identify the virus with the virion, but in fact they are constantly liable to do this implicitly. The best example is provided by the work of environmental virologists who have traditionally determined the number of viruses in a given environment by counting the number of viral-like particles (assimilated to viruses) by epifluorescence microscopy (Forterre, 2013).

Originally, the virus/virion paradigm was not contradictory with the idea that viruses are living because the mysterious entities that crossed the Chamberland filters displayed all the classical properties of life: reproduction, multiplication and evolution by natural selection. However, once it was realized that virions are not tiny cells but giant macromolecular complexes, viruses were frequently considered to be simple biological "objects", intermediate between living and non-living entities, existing "*at the threshold of life*" (Bos, 2000) or not living at all (Morange, 2011; Moreira and Lopez-Garcia, 2009; Van Regenmortel, 2003).

2.2. A special case of the virus/virion paradigm: viruses as replicators

In apparent contradiction with the "virus/virion" paradigm, viruses have been traditionally classified according to the nature of their nucleic acid (Baltimore, 1971). Several authors indeed used to define viruses primarily on the basis of their genomes. This happens, for instance, when viruses are considered to be "pure genetic information" (Rohwer & Barott, 2013) or when they are primarily defined as "parasitic genetic elements" (Koonin and Wolf, 2013) or replicators (Jalasvuori & Koonin, 2015; see also Koonin and Starokadomskyy, 2016). These definitions can be viewed as particular forms of the "virus/virion" paradigm in which the virion is assimilated to the viral genome, located within the capsid. This is well illustrated by the fact that naked RNA molecules infecting plants are recognized as viruses (e.g. Narnaviruses) by the International Committee on the Taxonomy of Viruses, ICTV.[3] These infectious RNA have been called recently "*capsidless viruses*" by Dolja and Koonin (2012). In that case, the viral genome is implicitly assimilated to a "virion", since it corresponds to the "infectious element" triggering the infection.

Historically, the view that confuses virus and their genomes probably explains why the "escape theory" became the dominant explanation for the origin of viruses in the second half of the last century (see section 2.5). Hence, in this theory, the origin of viruses is linked to the autonomization of some part of cellular chromosomes (prokaryote or eukaryote) that becomes a selfish replicator, the acquisition of a proteinic capsid to form a virion being a secondary event.

2.3. The "virus/virion" paradigm minimizes the role of viruses in biological evolution

A significant consequence of the "virus/virion" paradigm is that most biologists profoundly underestimate viral "creativity" (i.e. the opportunity for emergence and selection of novel traits encoded by viral genomes). This is probably because viruses, confounded with their virions, are assimilated to passive, inert objects (Forterre, 2011). As a consequence, it is often assumed that all (or almost all) viral genes are derived from the cellular hosts (the "viral pickpocket"

[1] The different types of virion morphologies, which can be quite diverse, are illustrated on the ViralZone website (Hulo de Castro et al., 2011).

[2] For previous critiques of the "virus/virion" paradigm, see Bandea, 1983, Claverie, 2006, Forterre, 2011, Van Regenmortel, 2010.

[3] These infectious RNA molecules only encode an RNA replicase homologous to that of RNA viruses.

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paradigm) (Moreira & Lopez-Garcia, 2009). In contradiction with this view, genomics analyses have revealed that most viral genes have no cellular homologues (Cortez, Forterre, & Gribaldo, 2009, Fisher et al., 2010, Ogata & Claverie, 2007, Prangishvili, Garrett, & Koonin, 2006, Yin & Fisher, 2008). Proponents of the virus pickpocket paradigm often explain this observation by the fast evolution of viral genes that became rapidly so divergent of their cellular ancestors that their homology cannot be recognized anymore. This phenomenon has indeed probably happened for some viral genes. However, it is reasonable to assume that most viral genes without cellular homologues have simply originated in viral genomes (during intracellular replication) by the same mechanisms that produce novel genes in cellular genomes (Forterre, 2011). It has been estimated that 10^{30} base pairs of viral DNA are made each second on planet Earth (Rohwer & Barott, 2013), providing unlimited opportunity for the emergence of new genes and explaining why viruses are an unlimited reservoir of genetic diversity. Emergence of new genes *de novo* overlapping ancient genes has been nicely demonstrated in the case of RNA viruses (Rancurel, Khosravi, Dunker, Romero, & Karlin, 2009). In viral DNA genomes, new genes should mostly occur by recruitment and extension of intergenic open reading frames (protogenes), as recently shown for cellular genomes (Carvunis et al., 2012, Zhao, Saelao, Jones, & Begun, 2014).

The "viral pickpocket" paradigm has led to a profound underestimation of the role of viruses in the history of life. This underestimation has been pointed out by Koonin and Wolf (2012) who correctly noticed that: "*viruses are no part of the modern synthesis or more generally the traditional narrative of evolutionary biology*". Many evolutionists have recognized for a long time that viruses are interesting models for analyses of micro-evolutionary processes, but most of them have failed to recognize viruses as major actors in the history of life. They emphasize the role of viruses as passive vehicles of cellular genes in horizontal gene transfer and as strong selection agents. However, they do not acknowledge their major role as cradles of new genes. Importantly, these viral genes can become cellular following the integration of viral genomes into cellular chromosomes, providing sometimes critical novelties to the benefit of the cells, and profoundly modifying the evolution of cellular lineages. This phenomenon is often minimized or ignored by proponents of the traditional view. For instance, Moreira and Lopez-Garcia (2009) stated that: "*viruses have played only a minor role in shaping the gene content of cells*" because "*the cell-to-virus gene flux is quantitatively overwhelming if compared with the opposite event*". In fact, it is probably the other way around (Forterre & Prangishvili, 2013). This is because the amount of cellular DNA that a virus can take up is limited by the size of the virion, whereas viral genomes can integrate into cellular genomes with few constraints in term of size (particularly in eukaryotes). Furthermore, considering the astronomical number of viral genes in the biosphere, viral genomes represent an unlimited reservoir of new genes that continuously integrate into cellular genomes (Cortez et al., 2009, Daubin & Ochman, 2004, Feschotte, Jiang, & Wessler, 2002, Forterre & Prangishvili, 2013).

Beside introducing new genes in cellular genomes, viral infections are well known to promote genome rearrangements and force cells to invent new defence mechanisms (Comeau & Krisch, 2005, Makarova, Wolf, & Koonin, 2013, Stern & Sorek, 2011). Several authors thus conclude now that viruses have been major actors in biological evolution (Brüssow, 2009, Forterre, 2006a,b, Forterre & Prangishvili, 2009a, 2013, Koonin & Dolja, 2013, Koonin, Senkevich, & Dolja, 2006, Nasir, Forterre, Kim, & Caetano-Anollés, 2014, Ryan, 2009, Villarreal & Witzany, 2010, see Pradeu, 2016, and several papers in the volume edited by Witzany, 2012). I consider myself that viruses are the major actors of both variation and selection, the two pillars of Darwinism (Forterre, 2012a).

2.4. A network of paradigms

The dominant theory on virus origin in the second half of the last century was that viruses originated from genes that had escaped from cellular chromosomes (Lwoff, 1953). Unfortunately, this hypothesis, which was in line with the virus pickpocket paradigm, become popular at the same time as the "eukaryote/prokaryote" dichotomy was proposed (Stanier & Van Niel, 1962) and was interpreted accordingly. This led to an evolutionary scenario in which viruses infecting bacteria [called bacteriophages (meaning "bacteria eaters") before their viral nature was fully recognised, or simply phages, and viruses infecting eukaryotes, simply called "viruses", had different origins. In this scenario, phages evolved from "prophages" (meaning "before phages"), which were parts of ancient bacterial genomes (Lwoff, 1953), whereas viruses infecting eukaryotes evolved from "protoviruses" which were parts of ancient eukaryotic genomes (Temin, 1971). This erroneous view (see below) has been used to perpetuate the semantic distinction between "viruses" and "phages" (hereafter called the "phage/virus" paradigm), which itself led to a schism in the virology community, with phages studied by microbiologists and viruses by cellular biologists (cells being frequently assimilated to eukaryotic cells). The phage/virus paradigm persists because the "eukaryote/prokaryote" dichotomy became itself a paradigm that has remained dominant in cell biology until now (Pace, 2006, Sapp, 2005). Together with the virus/virion paradigm, they form a network in which these three paradigms strengthen each other, making difficult their global refutation, despite scientific evidence.

2.5. Refutation of the "phage/virus" dichotomy: viruses originated before modern cells

The "eukaryote/prokaryote" dichotomy, in which classification of living organisms was based on cellular structure, has been refuted by the work of Carl Woese and his colleagues (Pace, 2006, Sapp, 2005). They have clearly demonstrated that cellular organisms correspond to three distinct evolutionary lineages or "domains" — Archaea, Bacteria and Eukarya — that can be identified on the basis of the heritable information present in ribosomal RNA (Woese, Kandler, & Wheelis, 1990) but also in universal proteins (Forterre, 2015 and references therein). Although Archaea share with Bacteria the prokaryotic cellular structure (mainly the absence of a nucleus and the coupling between transcription and translation), all their informational molecular mechanisms (DNA replication, transcription and translation) are much more closely related to those of Eukarya (Forterre, 2015, Woese et al., 1990 and references therein). Comparative molecular biology indeed suggests that Archaea and Eukarya are sister groups (Forterre, 2015).[4] The term "Procaryotes" thus probably does not correspond to a to a valid taxonomic unit, since it probably does not correspond to a monophyletic assemblage.

Notably, the three cellular domains are associated with three different ensembles of virus families corresponding to archaeoviruses, bacterioviruses (phages) and eukaryoviruses, respectively (Prangishvili, 2013, Raoult & Forterre, 2008). However, comparative analyses have shown that some key proteins encoded by viruses belonging to different ensembles (for instance bacterioviruses and eukaryoviruses) are evolutionarily related (Abrescia et al., 2012 and

[4] Recent phylogenetic analyses have suggested that Eukarya emerged from within a particular archaeal phylum reconstructed from metagenomic analyses and called Lokiarchaeota (Spang et al., 2015). However, these analyses are biased by the presence in the datasets of fast evolving archaeal and eukaryotic sequences (Forterre, unpublished observations).

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references therein). These observations, which refute the "phage/virus" dichotomy, were first reported for viral proteins involved in DNA replication (Forterre, 1992, 2002, Koonin et al., 2006 and references therein) and later on for proteins involved in the formation of virions, such as major capsid proteins and packaging ATPases (Abrescia et al., 2012 and references therein). Two major universal lineages of double-stranded DNA viruses have been identified up to now. The first one, called the Adenovirus/PRD1 lineage, includes several families of eukaryoviruses, such as *Adenoviridae*, NucleoCytoplasmicLarge DNA Viruses (NCLDV) and satellite virophages, together with bacterial *Tectiviridae* (PRD1) and archaeal *Turriviridae*. The second one, called the HK97 lineage,[5] groups head and tailed bacterioviruses and archaeoviruses together with *Herpesviridae* infecting Eukarya. All viral members of the same lineage share homologous major capsid proteins and packaging ATPases. This suggests that all viruses from the same lineage descend from a common ancestral virus that existed at the time of the Last Universal Common Ancestor (LUCA). Importantly, the major capsid proteins and packaging ATPases characteristic of the Adenovirus/PRD1 lineage are not homologous to those of HK97 lineage, confirming that viruses are polyphyletic, i.e. virions and mechanisms for their formation and dissemination have been "invented" (by natural selection) several times independently, some of them before LUCA.

This does not, of course, mean that viruses originated before cells, because the LUCA itself had many cellular ancestors and viruses need ribosome-containing cells for their multiplication (see section 3.4). The confusion between "cells" and "modern cells" (the descendants of LUCA) is another major drawback in discussions about the origin of viruses. This confusion may account for the rejection, by some authors, of evidence suggesting that viruses are very ancient, and their claims that "*there are no ancestral viral lineages*" (Moreira and Lopez-Garcia, 2009). These authors suggest that the homologous traits in viruses infecting members of different domains of life result from convergent evolution. This possibility can be ruled out from three lines of evidence: 1) the similarities in protein structures and sequences among viruses of the same lineage infecting different domains are too great to be explained by convergence, 2) viruses from the same lineage infecting different domains have several independent homologous features in common, including similar major capsid proteins and ATPase packaging, which could not have converged independently by chance in different domains, and 3) completely different protein folds can produce similar capsid structures, so the similar folds of the major capsid proteins of viruses infecting different domains provides real evidence for an evolutionary link between them, rather than for convergence to produce a particular structure (e.g. an icosahedral capsid). The resistance to the idea of "*ancestral viral lineages*" actually provides an additional illustration of the way in which the traditional view of viruses prevents some biologists from accepting new data not consistent with accepted paradigms.

3. The co-dependence of virus definitions and the views on virus nature and origin

3.1. Viruses as capsid (virion)-encoding organisms

The "virus/virion" paradigm remains the backbone of several new definitions of viruses that have recently been proposed. Some highlight opposing views of the virion, focussing either on the capsid or on the viral genome. Hence, Bamford (2003) has suggested considering the capsid as the virus "*self*", whereas Jalasvuori and Koonin (2015) primarily defined viruses as one class of replicators (viral genomes) (see also Koonin and Starokadomskyy, 2016). As discussed in section 2.3, these two contradictory views are two faces of the same coin. In the "*virus self*" concept, viruses are assimilated to their capsid, whereas as "replicators", viruses are assimilated to their genomes, the two major components of the virion.

A few years ago, Didier Raoult and I suggested defining viruses as "*capsid-encoding organisms*" by opposition to cellular organisms (Archaea, Bacteria, Eukarya) defined as "*ribosome-encoding organisms*" (Raoult & Forterre, 2008). In the same paper, we proposed to group plasmids and transposons under the umbrella "*orphan replicons*", because these replicators encode neither capsid nor ribosomal protein genes. Koonin and Dolja (2013, 2014) criticized the definition of viruses as capsid-encoding organisms because it precisely excludes these orphan replicons from the definition of a viruse. However, including "capsidless viruses" in the definition of a virus entertains the confusion between viruses and their genomes. In particular, considering the capsid to be the hallmark (and not the self) of the virus is essential to distinguish viruses from plasmids.[6] This has been nicely illustrated by Krupovič and Bamford (2010) who compared the smallest known plasmid, encoding one protein (a replication protein), to the smallest known virus, encoding two proteins (a replication protein and a capsid protein). The presence, in the viral genome, of a gene encoding a capsid protein is the only difference distinguishing the virus from the plasmid. In my opinion, the confusion between viruses and plasmids fundamentally underestimates the importance of the mechanism of virion production as a major biological process, the emergence of which introduced a new way of propagating genomes in the biosphere.

The definition of viruses as "*capsid-encoding organisms*" is still plagued with some ambiguities if taken literally. This is because some cells harbour genes encoding capsid proteins in their genomes that may, in some cases, produce empty "capsids" (Akita et al., 2007). These cells are thus, strictly speaking, "*capsid-encoding organisms*"! Other types of biological entities that are strictly speaking "*capsid-encoding organisms*", but not viruses, correspond to the recently described "viral membrane vesicles" (Gaudin et al., 2014). These vesicles contain a defective viral genome encoding a capsid protein. However, they are not *bona fide* virions, because the capsid protein is not present in the vesicle envelope. For these reasons, viruses are now best defined as "*virion-producing organisms*" (Forterre, Krupovic, & Prangishvili, 2014). To prevent further ambiguities, virions should be precisely defined as particles containing at least one protein that interacts with the viral genome (i.e. a naked nucleic acid is not a virion) (On all these definitions, see Box 1).

3.2. The hypotheses about the origin of viruses are influenced by virus definitions

The decision as to whether or not limit the concept of viruses to entities producing capsids has important consequences for the debate about the origin of viruses. If viruses are assimilated to their genomes, then the origin of viruses becomes the origin of parasitic RNA in the RNA world. In scenarios in which RNA replicators (naked or within vesicles) predate cells (molecular RNA world), this implies that viruses actually originated before cells. For instance, Koonin et al. (2006) imagine an "ancient virus world" that first evolved within mineral cages of a hydrothermal chimney, infecting acellular macromolecular RNA complexes, whereas Jalasvuori and Bamford

[5] From a bacteriovirus isolated in Hong-Kong.

[6] The prokaryote/eukaryote paradigm probably explains why "capsidless DNA viruses" are called plasmids (not viruses) in Archaea and Bacteria (prokaryotes) whereas "capsidless RNA viruses" are called viruses (not plasmids) in Eukaryotes!.

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Box 1
Definitions:

Virus: an organism producing virions
Virocell: a virus-infected cell that will not divide anymore but produces virions.
Ribocell: a cell that encodes ribosomes and produces two cells upon cell division.
Ribovirocell: a virus-infected cell that can still divide but also produces virions
Virion (viral particle): a particle that protects viral genome during the extracellular phase and allows viruses to infect new ribocells. The virion contains at least one protein interacting with the viral genome but some of them could contain hundred of proteins.
Life: The mode of existence of living individuals.

(2008) proposed a scenario in which empty lipid vesicles were progressively invaded by infectious RNA vesicles, assimilated to "proto-viruses" thriving in a pre-cellular world. Confusion between viruses and their genomes thus end up usually promoting "*virus first views*" in origin of life scenario, a position correctly criticized in that case by Lopez-Garcia and Moreira (Lopez-Garcia, 2012, Lopez-Garcia & Moreira, 2012).

By contrast, viruses cannot have originated before cells if we define viruses as "virion-encoding organisms", virions being characterized by the presence of at least one protein associated to the viral genome. Indeed, in that case, the emergence of the first *bona fide* viruses could have only occurred after the emergence of cell sufficiently sophisticated to produce ribosomes synthesizing already rather complex proteins. If we adopt this view, then the infectious lipid vesicles containing RNA present in the early RNA world or the first parasitic selfish replicators cannot be considered to be *bona fide* viruses, but possibly "protoviruses" by analogy.

A new hypothesis for virus origin, "*the ancient escape hypothesis*", was recently proposed (Forterre & Krupovic, 2012). In this scenario, the first viruses originated during the second age of the RNA world (defined as a world of cells with RNA genomes producing ribosomes), i.e., the period between the emergence of ribosomes and the origin of DNA (Forterre, 2005). The RNA/protein cells of the time probably harboured different types of RNA replicons, including parasitic ones competing with each other. As the mechanisms of cell division coupled to RNA replication were probably not very sophisticated at the time, there was probably strong selection pressure in favour of replicators that could bypass the cell division process by using protein-based vehicles (virions) to penetrate and replicate in new cells. Ancient structures present in and/or produced by these RNA/protein cells, such as membrane vesicles, icosahedral intracellular compartments, or primitive chromosome scaffolds, may have provided the basis for the emergence of different types of simple virions (pleomorphic vesicle-like virions, icosahedral capsids and nucleocapsids). Virions may subsequently have increased in size and structural complexity in some lineages of DNA viruses and new groups of viruses have emerged (Filée, 2013, Forterre, 2010, Krupovic, 2013). For instance, eukaryotic single-stranded DNA viruses probably originated from recombination between DNA plasmids and RNA viruses (Krupovic, 2013).

This "ancient escape" scenario contrasts strongly with hypotheses according to which viruses (assimilated to their virions) originated by regressive evolution from ancient cells that lost their translation apparatus (Bandea, 1983, 2009, Forterre, 1991, 2005). These "regression hypotheses", have recently become popular, following the discovery of giant viruses (Claverie & Abergel, 2013, Nasir, Kim, & Caetano-Anollés, 2012). It has been suggested that these viruses originated from cells from an ancient cellular domain (the fourth domain hypothesis). However, these regression hypotheses appear unlikely because it is difficult to imagine an evolutionary pathway explaining how a cell (even a primitive one) can be transformed into a virion! This transformation is particularly difficult to imagine for minimalist virions, such as nucleocapsids. The regression hypothesis, when generalised to the whole virosphere, implies that, ultimately, an ancient cell could be transformed into a single protein, the major capsid protein of the smallest virus! Proponents of the regression hypothesis have suggested that we should distinguish between viruses of different sizes, with the regression hypothesis valid only for large DNA viruses (Claverie & Abergel, 2013). However, there is a continuum of genome sizes from the smallest to the largest viruses, and any division of the virosphere on the basis of genome sizes would necessarily be arbitrary (Forterre et al., 2014).

Proponents of regression hypotheses, such as Bandea (1983) and Claverie (2006), who have strongly opposed the "virion/virus" paradigm, possibly do not realize that regression hypotheses function in the framework of this paradigm, the ancient cell being transformed into a virion. I myself supported such regression hypotheses in the 1980s, when I first realised that viruses encode very ancient DNA replication proteins, I concluded that these proteins originated from extinct lineages of ancient cells that subsequently became viruses (Forterre, 1992). Having fallen under the spell of the "virus/virion" paradigm, I did not consider the possibility that these proteins might have originated directly in ancient viral lineages. I think the same sort of rationale explains why scientists impressed by the huge numbers of genes without cellular homologues present in the genome of giant viruses conclude that these genes must have originated in an extinct cellular domain (Boyer, Madoui, Gimenez, La Scola, & Raoult, 2010, Claverie & Abergel, 2013; Nasir et al., 2012). One should get rid of the virus/virion paradigm and focus instead on the intracellular step of the virus reproductive cycle to realize that most of these proteins could have also arose directly in viral lineages (see section 2.3). A few years ago, I proposed the "virocell concept", to precisely focus attention on the active stage of the viral reproduction cycle, when new viral proteins can emerge during replication and/or recombination processes (Forterre, 2011, 2012b, 2013).

4. The various forms of viruses and the virocell concept

4.1. The virocell concept and the virocell as a concrete entity

During the infection process, viral genetic information progressively transforms the cell — a bacterium, an archaeon or a eukaryotic cell — into a new type of cellular entity (a virus/virion factory, *sensu* Lwoff, 1966) that I suggested calling a "virocell" (Forterre, 2011, 2012b, 2013). At the beginning of the infection, the gene expression pattern of the infected cell is indeed drastically modified (for an example, see Quax et al., 2013). At a later stage of viral infection, the expression of the host genome is completely repressed (with sometimes its complete destruction) and the only active genome in the virocell is the viral one. In some cases, the viral genome encodes proteins that interfere with cellular metabolism, but in other cases, it encodes viral enzymes with metabolic activities complementary to or replacing those of the host cell (Thompson et al., 2011). It is not just a *modification* of the cell's metabolism, but the emergence of a completely different metabolism with a different function that provides autonomy for the virus. To paraphrase the metaphor from François Jacob: "*the dream of a cell is to produce two cells*", one can say: "*the dream of a virocell is to produce as much virions as possible*".

The virocell concept is complementary to the definition of viruses as "*virion producing organisms*" since it allows refuting the

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idea that viruses are not organisms because, as stated by Lwoff (1966): "*an organism is constituted of cells*". This criticism is of course only valid in the framework of the virus/virion paradigm since the virocell concept tells us that viruses are also cellular organisms during the virocell stage of their life cycle.

Focussing on the virocell, it is also possible to refute another classical argument used to suggest that viruses should not be considered to be living: their supposed lack of inherent metabolic activity. Van Regenmortel (2003) thus wrote that: "*viruses do not possess many of the essential attributes of living organisms, such as the ability to capture and store free energy and they lack the characteristic autonomy arising from the presence of integrated, metabolic activities*". This is true for virions, but not for virocells which need to capture and store free energy for the production of virions. The virocell concept thus emphasizes that viruses, like other cellular organisms, cannot exist without both a genomic and a metabolic component. In the framework of the virocell concept, viruses are thus living entities because they are both *genetic* and *metabolic* entities.

It is interesting to compare the term "virocell" with the other names that have been proposed for the intracellular phase of the virus reproductive cycle, before virions start to accumulate. This phase has been called the "*eclipse*" phase, due to the impossibility of observing viral particles within the infected cell. This again highlights the tenacity of the virus/virion paradigm, because the absence of visible virions was interpreted as a disappearance (eclipse) of the virus itself. Jacob and Wollman (1961) used the term "*vegetative state*" for the intracellular phase. This clearly had a negative connotation, because, in French language, an individual in a vegetative state is not really considered to be living, but *at the threshold of life*. It was particularly unfortunate to give the name "vegetative phase" to the period in which viruses express and replicate their genomes.

4.2. Virocells and ribocells

The term "virocell" is not the equivalent of the term "infected cell". The latter, commonly used in virology, is ambiguous because the infected cell may be considered to be the bacterium, the archaeon or the eukaryotic cell infected with a particular virus, or the virocell itself. By analogy with the term virocell, I have proposed using the term "ribocells", for cells encoding ribosomes to clarify the nature of the infected cell (Forterre, 2011). For instance, the virocell of a lytic archaeal virus is no more an infected archaeon but a new type of biological entity, the active form of the viral organism. However, In many cases, the viral infection does not lead to the death of the ribocell, but to coexistence of the virus and its host, a phenomenon sometimes called "persistence", corresponding to a form of symbiosis (Ryan, 2007, Villarreal, 2007). The virus can persist in a carrier state, in which the cell continuously produces small numbers of virions, or a lysogenic state, in which the viral genome is present but silent, either free or integrated into the cellular genome. In the lysogenic state, the cell clearly remains a *bona fide* ribocell, whereas in the carrier state, when virions are produced but the cell carrying the viral genome continues to divide, the same cell is both a ribocell and a virocell, i.e. a ribovirocell (Forterre, 2011).

During infections, the ribocell becomes progressively a virocell or a ribovirocell. The transition is gradual and the two organisms (for instance a bacterium and a virus) coexist in the same cell for a short time (in the case of lytic infection) or for a much longer time (in the case of persistence). Interestingly, during this stage, it is impossible to determine to which organism some of the molecular organs present in the cell (such as the membrane or the ribosomes) belong. This is in agreement with the fact that: "*an organism is made of constituents that do not need to have originated in it*" (Pradeu, 2010). The coexistence of several organisms in the same cell is not a situation unique in biology. In many cases, a cell can be infected by several viruses and/or other cells. For instance, some amoeba can be infected by a bacterium and a giant virus; the giant virus itself being infected by a "virus of a virus" (Moliner, Fournier, & Raoult, 2010). In that case, one can consider that four organisms corresponding to different evolutionary lineages and different genetic information are present in the same cell (Forterre, 2010).

4.3. Viruses are evolved by virocells, not by ribocells

Notably, the distinction between the ribocell and the virocell allows to refute another argument proposed against viruses being considered to be living, i.e. "*Viruses do not replicate or self-replicate themselves, but are 'being replicated' (i.e. passively rather than actively) through the metabolic activities of the cells they have infected*" (Moreira and Lopez-Garcia, 2009, Van Regenmortel, 2010). This argument has been summarised in the sentence "*viruses are evolved by cells*" (Moreira and Lopez-Garcia, 2009). In that case, the authors tacitly confuse cells and ribocells and assume that viruses are evolved by the ribocell. However, it seems difficult for me to claim that "*viruses are evolved by cells*" (in the sense of a ribocell), when the genome of the ribocell is either destroyed or inactivated in the first step of viral infection. This would mean that cells without a genome are "living" and could play an active role in virus evolution!.

In fact, neither viruses nor cells (ribocells) can evolve something. The sentence "*viruses are evolved by cells*" is a metaphor that sounds valid at first sight because we are used to think that cells are "living". In contrast, metaphors in which viruses "evolve cells", "invent","-create" or "dream" sounds odd to scientists who consider that viruses are not living (van Regenmortel, 2016). For instance, the metaphor "*the dream of a cell is to produce two cells*" proposed by François Jacob, is acceptable for biologists, whereas the metaphor "*the dream of the virocell is to produce as many virocells as possible*" is not acceptable for some (see van Regenmortel, 2016).

4.4. Critiques and discussion of the virocell concept

The virocell concept has provoked violent reactions. In two papers, it has been stigmatised as a "*conceptual trick*" or "*artifice*", and I have been accused of "*epistemological cheating*" (Lopez-Garcia, 2012, Lopez-Garcia & Moreira, 2012). The use of such pejorative language, with moral implications, is unusual in a scientific article and reflects the passion surrounding the debate concerning the living nature of viruses[7] (see also Morange, 2011). Lopez-Garcia and Moreira (2012) derided the virocell concept, saying that, by analogy to this concept, remora (a parasitic fish) becomes a remora/shark when the remora attaches itself to the shark! This argument is not appropriate because the remora does not penetrate the body of the shark, dissolving its own structure to reproduce! The virocell concept is not even applicable to intracellular organisms that are themselves "ribosome-encoding organisms", such as bacteria "living" within eukaryotic cells (Forterre, 2012b). Within infected cells, the bacterial parasites retain their own distinct cellular structure, remaining separate (surrounded by their own membranes); they divide by binary fission and use their own ribosomes to produce proteins. Viruses are completely different, because they highjack the structure of the infected cell, using its ribosomes for the synthesis of viral proteins and the production of infectious virions.

Lopez-Garcia (2012) also took a more philosophical stance to refute the virocell concept, stating that: "*defining an entity (a virus)*

[7] The reasons for this passion remain to be studied. I suspect that it could be related to the human desire to restrict the usage of the term "life", which has been for a long time considered as a sacred concept.

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in terms of itself plus a portion of another entity (a cell) is alien to logic and can be viewed as epistemological cheating". In this sentence, the author clearly equates the virus "entity" to the virion, in line with the "virus/virion" paradigm. I myself was wrong when, proposing the virocell concept for the first time, I wrote that: *"the infected cell (*the virocell*) is the real viral organism*" (Forterre, 2011). This formulation was misleading, suggesting that the virus can be assimilated to the virocell. However, in the same paper, I also wrote that: "*the virocell represents the cellular phase of the capsid-encoding organism"*. Two kinds of situations well illustrate why viruses cannot be practically confused with virocells (Forterre, 2012b). Some viruses can infect multiple species. For example, arboviruses (such as the yellow fever virus) transmitted by mosquitos can infect both insect and human cells. These and other viruses can also infect various types of cells in multicellular organisms. In these cases, the same virus produces different types of virocells, depending on the type of cell infected. Finally, it has been shown that poliovirus genome can be expressed and replicated in a lysate of human cells, producing infective virions (Cello, Paul, & Wimmer, 2002, Wimmer, 2006). This result shows that the virocell itself is not an obligatory step in the viral reproductive cycle, as long as a scientist is available to prepare a cell lysate! Therefore, we should not replace the "virus/virion" paradigm by a "virus/virocell" paradigm. The term virus should describe a biological process and integrate all aspects of the viral reproduction cycle (the virion, the virocells, and genomic integrated forms) (see Dupré and Guttinger, 2016, and Claverie and Abergel, 2016).

5. To be or not to be alive: when it is not possible to define the border between living and non-living

The virocell concept removes all roadblocks preventing to consider viruses as living. Interestingly, this raises new challenging questions. One can ask for instance if plasmids should be also considered to be living? Plasmid and viral genomes are indeed evolutionary related. A virus can be easily transformed into a plasmid if it loses its capacity to produce virions. Conversely, a plasmid can be transformed into a virus if it acquires a capsid-encoding gene. Hence, new families of single-stranded DNA viruses have originated from plasmids that acquired capsid genes from RNA viruses (Krupovic, 2013). As previously mentioned, the only difference between the smallest virus and the smallest plasmid is the presence of a capsid gene in the viral genome but not in the plasmid (Krupovič & Bamford, 2010). Plasmids are usually considered to be non-living because they are pure chemicals (macromolecules). However, if we consider that viruses are living but plasmids are not, one should conclude that a single gene encoding a capsid protein is sufficient to confer the living status to a biological entity! Should we conclude that plasmids are finally living, when their genes are expressed and their genomes replicated in a living cell?

In fact, as previously noticed by Dupre and O'Malley (2009), there are many situations in the living world – similar to the plasmid/virus case - where there is " *no sharp distinction between life and non life*". One of them refers to intracellular organisms and organelles, such as mitochondria and chloroplasts. These organelles originated from ancient parasitic bacteria that lived as endosymbionts within ancient eukaryotic cells. These bacteria were living organisms, raising the question, are mitochondria and chloroplasts still living? Most biologists would say that mitochondria and chloroplasts are not living because they are under the complete control of the eukaryotic cells. For example, Van Regenmortel (2010) concluded that organelles are not living because "*they lack autonomy and a life cycle*". However, considering organelles as non-living raises a critical question: when did the transition from living to non-living occur in the evolutionary pathway leading from intracellular bacteria to organelles? In fact, there is no possible good answer to this question, because the relative degree of autonomy of an endosymbiont towards its host decreases in a continuous manner in the course of reductive evolution. Endosymbionts with reduced genomes that are intermediate between a bacterium and an organelle have been discovered (Nakabachi et al., 2006), and in some eukaryotic lineages, mitochondria evolved toward organelles lacking DNA altogether (mitosomes) (Dyall & Johnson, 2000). Many parasitic organisms considered by most biologists as "living" lack one or more essential genes that are provided by their "hosts" (and *vice versa*). It is thus hopeless to search for the good gene or the good gene set that define life or that qualify a cellular organism as autonomous or not.

It is thus impossible to define a border between living and non-living biological entities based on quantitative (number of genes) or qualitative (autonomy) features. The situation is not dissimilar to that of determining when a foetus passes from being just a cluster of cells that can be discarded to being a human being that should be protected. These are political and/or judicial rather than scientific questions. We are used to the idea of there being a clear line separating living from non-living, because, as humans, we are ourselves either alive or dead. This makes it harder for us to accept that the transition between life and non life cannot be determined during an evolutionary process. How can we solve this philosophical impasse? One possibility would be to exclude the terms "life" and "living" from the biological literature all together, considering that they cannot be rigorously defined in scientific terms. However, this position would reflect a mechanistic view of the world, i.e. a view in which historical objects or processes are not considered. Precisely, life could be viewed as an "historical process" that began on our planet, and has created various transient biological entities at different levels of complexity (molecules, organisms, populations). These entities evolved by diversification and selection, continuously interacting with each other and their environment. One possibility would thus be to consider all these entities to be living as long as — being part of the process of "life" — they remain operational in the context of this process. For instance, a protein is a "living entity" as long as it performs its function in a living biological organism (defined in that case by opposition to a dead organism). The same protein, once isolated from the living organism, and working in a test tube (with the assistance of a biochemist) is still a biological entity but is no more living and could be compared to a human maintained into an artificial life in a hospital! A plasmid is living when it replicates in a cellular organism but not when it is manipulated in a test tube by a molecular biologists constructing a shuttle vector. Does this mean that all biological entities are living? Is a gene living? In my opinion, the answer is no and one could distinguish between living and non living entities depending of their nature. I propose that, an organism, a cell, a virus, a plasmid or a protein can be defined as "living" because these biological entities are "individuals", i.e. they are "*separable*, *countable and have acceptable clear-cut spatial boundaries*" (Chauvier, 2008, Pradeu, 2010). On the opposite, biological entities that are only "particulars", i.e. "*everything that can be designed through a demonstrative reference*" (Pradeu, 2010) cannot be defined as living (e.g. a gene or a protein fold that are not separable and/or have no clear-cut spatial boundaries are not living).

Interestingly, this view would be in agreement with a recent definition of life proposed by Dupre and O'Malley (2009) stating that "*Life arises when lineage forming entities collaborate in metabolism"*. In our broad definition of living entities, proteins and plasmids also define lineages that are products of Darwinian evolution. They are living when they collaborate with metabolism to perform their function in a living organism. Paradoxically, and

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counter intuitively, such a broad usage of the term "living" would be less affected by remnants of vitalism or anthropocentric usage, because the term "living" would then only mean that the living entity is part of the material process of "life" viewed as an historical process of matter development. Notably, many biologists already tacitly assume this position in their publications as a metaphor. For instance, describing the outcome of infection by a lytic virus, Jalasvuori and Koonin (2015) wrote that "*the altruistic chromosome chromosomes are doomed to perish*" implying that this chromosome was previously "living"! In the same publication they confirm this viewpoint by discussing the "lifestyle" of replicators.

Looking for a materialist definition of life, I previously referred to the definition proposed by Frederich Engels in the 19th century "*life is the mode of existence of an albuminoid body*" (Engels, 2006 [1883]) and suggested an updated version: "*life is the mode of existence of living organisms*" (Forterre & Prangishvili, 2009a,b). If we adopt the view that I now propose here, a better definition could be that: "*life is the mode of existence of living biological individuals*".

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Appendices.

Traditional view	View presented here
A "virus" is defined as a nucleic acid enclosed in a protein coat, i.e. the virus is assimilated to the virion	The virus is the whole process integrating all aspects of the viral reproduction cycle
Viruses are mainly pickpockets that recruit their genes from cells	Viruses are cradles of new genes that are often transferred and domesticated by cells. Cells are the true pickpockets
Viruses are byproducts of viral evolution	Viruses have played a major role in biological evolution
Viruses are not living entities because they do not display a metabolism of their own	Viruses are living entities because they display a metabolism of their own during the virocell stage of their reproductive cycle
Viruses originated after cells. Eukaryotic viruses and bacterial ones (bacteriophages) are unrelated	The first viruses originated after ancient cells but before modern cells (the descendents of LUCA). Some viruses from the three domains of life (archaeoviruses, bacterioviruses and eukaryoviruses) are evolutionarily related
The virus disappears during the "eclipse phase" of its reproduction cycle	The virus is "living" during the intracellular phase of its reproduction cycle
Viruses are evolved by cells	Viruses are evolved by virocells
Viruses are not cellular organisms	Viruses are cellular organisms during the virocell stage
Only cellular organisms are living	All biological entities are living when they are actively involved in a living process

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