Published in final edited form as:

Ann N Y Acad Sci. 2015 April ; 1341: 96–105. doi:10.1111/nyas.12696.

Classification of prokaryotic genetic replicators: between selfishness and altruism

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Abstract

Prokaryotes harbor a variety of genetic replicators, including plasmids, viruses, and chromosomes, each having differing effects on the phenotype of the hosting cell. Here, we propose a classification for replicators of bacteria and archaea on the basis of their horizontal-transfer potential and the type of relationships (mutualistic, symbiotic, commensal, or parasitic) that they have with the host cell vehicle. Horizontal movement of replicators can be either active or passive, reflecting whether or not the replicator encodes the means to mediate its own transfer from one cell to another. Some replicators also have an infectious extracellular state, thus separating viruses from other mobile elements. From the perspective of the cell vehicle, the different types of replicators form a continuum from genuinely mutualistic to completely parasitic replicators. This classification provides a general framework for dissecting prokaryotic systems into evolutionarily meaningful components.

Keywords

bacteria; archaea; prokaryotes; classification; replicators; cell vehicles

Introduction

Prokaryotes encompass bacteria and archaea. A total of about 10^{30} living prokaryotic cells have been estimated to exist in this biosphere at any given moment: these cells make up most of the biomass on our planet.¹ A fraction of prokaryotic cells die every day, whereas others live on to reproduce and fill the remaining void.² Within this constantly renewing prokaryotic biomass, there is a vast number of diverse parasitic or symbiotic genetic elements, such as viruses, plasmids, and chromosomes competing for survival.³ Strikingly, the number of virus particles alone, in several well-characterized environments, such as ocean water, soil or the human gut, typically exceeds the number of cells by at least an order

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Conflicts of interest

The authors declare no conflicts of interest.

of magnitude.^{4–6} The genetic diversity of these elements is enormous and appears to exceed the diversity of cellular life forms.^{6,7} Thus, in terms of both the number of individual molecules and the information content (although not necessarily in terms of mass), these elements dominate the biosphere.⁸ In this article, we examine the relationships between these diverse genetic replicators and their host cells, under the premise that, without at least a conceptual understanding of the genetic juggling occurring within and between prokaryotic cells, we are ill suited to comprehend a major part of our biosphere, and arguably the biosphere itself, as a complex ecosystem.

Cells, or *cell vehicles* (Table S1), obtain from their environment resources that are utilized both to maintain a functional cell and to support the propagation of the genetic replicators within. Some replicators selfishly exploit the cell vehicles, whereas others maintain basic functions that allow them to exist in the first place. Here, we use terms vehicle and replicator according to the definition by Richard Dawkins in *The Extended Phenotype*: “A vehicle is any unit, discrete enough to seem worth naming, which houses a collection of replicators and which works as a unit for the preservation and propagation of those replicators.”⁹ A successful replicator is one that is able to retain and proliferate its genetic information as a part of the living prokaryotic biosphere. However, many of the replicators are not associated with any particular cell and thus their survival does not depend on the long-term survival of the cell vehicle hosting them. Nonetheless, all genetic replicators need cell vehicles for propagation and, at least temporarily, for preservation.

Genetic replicators have widely different relationships with their host cell vehicles. Lytic viruses exploit all the available resources in the cell to produce new virus particles while destroying the host in the process.¹⁰ The relationship between lytic viruses and a cell vehicle is clearly parasitic. However, temperate viruses alternate between the parasitic and commensal life styles, switching to the latter when they integrate with the host chromosome and become proviruses (prophages).¹¹ Moreover, there might even be an aspect of symbiosis between prophages and the host, as suggested by observations that elimination of all prophages in *Escherichia coli* reduces bacterial fitness.¹²

Conjugative plasmids are mobile elements that often induce adaptive phenotypic changes in the cell.¹³ A textbook example is antibiotic resistance, whereby in an environment containing antibiotics, plasmids carrying resistance determinants exist in mutualistic relationships with their hosts.³ A more complex mutualistic or at least symbiotic relationship with the host is also characteristic of a vast class of elements that follow the toxin-antitoxin strategy, including restriction modification systems (RMs) and bona fide toxin-antitoxins (TAs).^{14–19} Typically, these elements consist of two genes, one of which encoding a toxin that, if expressed on its own, kills the cell, and the other one encoding an antitoxin that forms a complex with the toxin and reversibly inactivates it. Clearly, this architecture is beneficial for maintaining element integrity, because inactivation of the antitoxin gene while leaving the toxin intact is fatal to the host. Moreover, typical TA systems are addictive for their hosts. The addiction is achieved through an elegant adaptive strategy. The antitoxin is an unstable protein, whereas the toxin is stable, so continuous production of both proteins keeps the toxin in check, but shortly after synthesis of these proteins stops owing to loss of the element, the toxin is unleashed. Thus, the TA-like elements combine the extremely

selfish strategy of “blackmailing” the host with lending the host a valuable functionality. Apart from their selfish properties, the elements in this class serve as defense and stress-response systems for the host cells. Plasmid-borne and chromosome-borne TA systems provide a mechanism for programmed cell death or dormancy induction that is triggered upon viral infection, thus inducing altruistic behavior within a community of cell vehicles.^{20–23} As the infection deactivates the antitoxin and activates the toxin, the replication of the virus is aborted, and therefore the spread of the virus also ceases. This altruistic behavior benefits the replicator (and not only the cell vehicles) only if there are homologous replicators present in other surrounding vehicles, as the cell going through apoptosis dies.

Other small replicators, such as transposons, appear to exert only minor effects on the phenotype of the host cell. These commensals can move to ectopic locations in the same host genome and hitchhike along with other elements from one cell to another.²⁴ Obviously, every cell vehicle also harbors the ultimate altruistic replicator, the chromosome, which provides all the essential functionalities for maintaining and replicating the cell vehicle itself but also the means for the reproduction of all the other diverse parasitic and symbiotic replicators. Given that the chromosome and the cell vehicle are mutually dependent on each other, their separation might appear farfetched. However, this distinction provides the basis for a meaningful, uniform classification of replicators by allowing consideration of all genetic elements, including chromosomes, as parts of a single, interwoven, dynamic community that exploits cell vehicles for propagation and preservation.

The relationship of replicators with their cell vehicles goes hand in hand with their dependency on the survival of the cell vehicle. Parasites are not strictly dependent on the survival of any particular cell vehicle, whereas altruistic chromosomes are doomed to perish should their host vehicle die. The mobility and within-cell behavior of different types of genetic replicators are far from being spurious or coincidental: they appear to reflect distinct choices of the mode of parasite/symbiont–host interaction and coevolution. We exploit these distinct strategies as the basis to develop a classification of fundamentally analogous, although not necessarily homologous, replicators. We perceive that such a classification is essential for understanding the evolutionary transitions between different classes of replicators within the maze of prokaryotic genomes and for developing explicit models of their evolution.

Basis for classification: mobility and relationships between replicators and cell vehicles

The classification of genetic replicators of prokaryotes proposed here is based on their mobility and the features of their symbiotic or parasitic relationship with the cell vehicle. For the purpose of the present classification, we define a replicator as any replicating genetic entity that forms a genetically coherent unit that is distinguishable from other replicators in the same cell vehicle. The exact mechanism of replication is irrelevant for the classification. Notably, many replicators fuse and separate as part of their life cycle, or do so sporadically.²⁵ However, unless they become defective (as often happens, for example, to

deteriorating prophages²⁶), they retain their identity despite integration with other replicators, and thus they can belong to different groups in the proposed classification.^{27,28}

Replicators are divided into 5 distinct mobility classes: (I) non-mobile (chromosomes), (II) passively mobile (non-conjugative plasmids, transposons, TA-like elements), (III) actively mobile without an extracellular state (conjugative replicators including conjugative plasmids and integrative and conjugative elements), (IV) actively mobile with an extracellular state and a stable within-cell state (temperate viruses), and (V) actively mobile with an extracellular state but without a stable within-cell state (lytic viruses). These classes reflect the typical mobility patterns of the replicators. Clearly, the entire complexity of genome dynamics cannot be captured within a simple classification scheme, and a degree of arbitrariness is inevitable. In particular, non-mobile chromosomal genes and larger fragments of chromosomes are often horizontally transferred between different cell vehicles.²⁹ Moreover, the probabilities of fixation after transfer differ between chromosomal fragments, as reflected, for example, in the selfish-operon concept.³⁰ However, for the purpose of classification, we do not treat genes or operons as replicators, above all, because, however frequent horizontal transfer might be, it is not essential for the propagation of genes and operons that are primarily inherited by dividing cell vehicles as integral parts of the chromosomes.

The relationships between the replicators and the cell vehicle for each mobility class can be characterized as follows: (I) mutualistic (chromosomes); (II) mutualistic, symbiotic, or commensal (non-conjugative plasmids, transposons, TA); (III) mutualistic, commensal, or parasitic (conjugative elements); (IV) mutualistic, commensal, or parasitic (temperate viruses); and (V) parasitic (lytic viruses). The proposed classification is summarized in Table 1 and Figure 1. Clearly, this ecological categorization shows a gradient from pure mutualism to pure parasitism, with all intermediates in between. It should be noted that the discrete modalities of the relationships between replicators and cell vehicles describe only the typical behavior of replicators. For any particular replicator, the position on the Y-axis of the conceptual plot in Figure 1 always depends on the context (i.e. the host vehicle, other replicators it contains, and the community to which the host belongs). Furthermore, replicators, especially those with large genomes, such as chromosomes, are far from being homogeneous, but rather represent a patchwork of genes, only some of which are essential for the reproduction of the cell vehicle,³¹ as well as inserted replicators of other classes. Nevertheless, we define the status of chromosomes as mutualistic because the presence of the chromosome as such is required for the reproduction of the cell vehicle and vice versa. The classification substantially simplifies the actual relationships of replicators with their vehicles, in an attempt to provide an intuitive way to form a picture of prokaryotic systems consisting of multiple different replicators.

Evolution within and between the classes of replicators

The classes of replicators are not separated by impassable barriers. On the contrary, they are connected through interlinked mobility and various aspects of their interactions with the cell vehicles. An individual non-mobile elements is locked within its present host cell, and thus its survival corresponds to that of the cell vehicle. Thus, any trait of a class I replicator that

induces fitness cost to the cell vehicle also decreases the fitness of the replicator. Passively mobile elements of class II can get from one cell vehicle to another either with the help of other mobile replicators (e.g., using a conjugation channel encoded by a class III conjugative plasmid) or by transformation of new cell vehicles by environmental DNA deriving from dead cells.³² Given that both routes provide only sporadic mobility, in general, any fitness cost induced by a class II replicator will also directly decrease its survival. As a consequence, a parasitic class II replicator is likely to evolve strategies to provide benefits to the host cell, to become addictive to the cell, or to induce its own transfer from one host vehicle to another (e.g., evolve into a class IV or even a class V replicator).³³ This multiplicity of survival strategies can be considered to underlie the enormous diversity and versatility of class II replicators.

The lifestyles of replicators from class III and up involve active mobility, and therefore these elements can afford to incur fitness cost on the cell vehicles. However, only purely lytic viruses of class V that kill the host cells as a part of the process allowing efficient transfer of the replicator to the next host are ultimate parasites that do not enter any symbiotic relationships with the host. Outside of class V, mobility does not necessitate parasitism. Replicators of class IV switch from lytic reproduction to lysogeny depending on the state of the host vehicle, and prophages can confer resistance to superinfection and potentially other, still poorly understood, benefits to the host.³⁴ Replicators of class III (i.e., conjugative plasmids) often form mutualistic connections with the host (e.g., by providing antibiotic resistance in antibiotic-containing environments).³ However, if the entire population of cell vehicles harbors the mobile element that confers a gene that is essential for survival, mobility of the element could itself incur a disadvantage,³⁵ such that less mobile replicators would be selected. Under such conditions, class III elements could lose their mobility altogether, thus turning into class II or even class I elements (i.e., segments of chromosomes).³⁶

All classes of replicators are interconnected through distinct evolutionary processes (Fig. 2). Thus, temperate viruses (class IV) can become virulent viruses (class V) by losing the genetic control of their lytic cycles.³⁷ Temperate non-integrative viruses (class IV) may become defective viruses (class II) or plasmids that reproduce only through the division of host cell.³⁸ Integrative temperate viruses (class IV) regularly lose part of their genome and get stuck within the host, thus essentially becoming parts of a class I replicator.¹¹ Some of these defective prophages are recruited for cellular functions in which specific capabilities of parasitic elements are exploited.¹² A notable case in point are gene-transfer Agents (GTAs), defective prophages that retain the ability to form virus particles but, instead of packaging the viral genome, encapsidate random pieces of the host chromosome and apparently function as dedicated devices for horizontal gene transfer.³⁹ In this case, selectively retained capacities of class IV or even class V replicators are utilized by class I replicators (and the cell vehicles). Some class II elements can pick up capsid genes and evolve into viruses.³³ Conjugative plasmids (class III) may lose their ability to conjugate under conditions in which horizontal mobility is evolutionarily unfavorable.³⁶ When changing class, the phenotype of a replicator in a host cell vehicle tends to rapidly evolve to resemble other members of the new class, sometimes because the change of class explicitly involves gene loss or gene gain via recombination. For example, temperate viruses that turn

into virulent viruses immediately excel in virion production,³⁷ and conjugative plasmids that remain in the same cell line for several generations evolve to become beneficial for their host cell vehicle.³⁵ As a general rule of thumb, elements that carry genes that are required or at least beneficial for the survival of the host vehicle are likely to be less mobile compared to elements that carry genes inducing fitness costs to the host vehicle (Fig. 1). The elements of different classes are also linked at a different level: it is common for highly mobile elements, such as viruses and conjugative plasmids, to provide means of transfer for less mobile (class II) elements; conversely, the chromosome (class I element) can provide a persistent and self-sufficient vehicle for elements of classes II, III, and IV.

Discussion

The classification of replicators outlined here provides general categories into which all existing and, probably, yet-to-be-discovered genetic elements can be placed. Although we deliberately limited the discussion to replicators associated with prokaryotic cell vehicles, this classification, perhaps with minor adjustments, should be generally applicable (i.e., it will also encompass eukaryotic replicators, among which diverse elements with varying degrees of dependence on the host are extremely abundant³³). Knowing the class of a genetic replicator gives one the general idea of its within-cell behavior and mobility patterns. Naturally, the exact characteristics of different replicators depend on the respective environmental and ecological contexts. Nevertheless, generalization and simplification can be helpful in reducing the complexity of natural systems into intuitively understandable components.

The classification of well-characterized genetic elements is straightforward. However, sorting replicators into classes in poorly characterized systems can be challenging. For example, marine ecosystems have been recently found to contain numerous membrane vesicles⁴⁰ that might previously have been mistaken for viruses (thus perhaps skewing the estimates of the number of viral particles in the biosphere).⁴¹ These so-called viral membrane vesicles can carry various types of replicators including plasmids, chromosomes, and viruses. Thus, these vesicles could play an important role in horizontal gene transfer,⁴¹ similar to the aforementioned GTAs established to originate from defective prophages. Although the proposed name may be suitable for describing the vesicles themselves, understanding the workings of the evolving community that generates, maintains, and exploits the vesicles requires clear distinction between the ways in which different replicators benefit from the formation of the vesicles. The classification presented here can provide a framework for describing and dissecting such systems.

Hyperthermophilic archaea also have been observed to form membrane vesicles that selectively enclose certain plasmids or other genetic elements and carry them to other cells.⁴² Some of these elements are genetically related to viruses, despite the fact they do not encode a viral capsid: the vesicles are derived from the cellular membrane and only contain proteins encoded in the chromosome.⁴² Because these virus-related genetic elements are transferred via an extracellular state to other host, it should appear reasonable to classify them as viruses (class IV or V replicators). However, in this case, the replicator, although virus-related, requires a host-encoded functionality for mediating horizontal transfer and

thus, according to the proposed classification, is actually a passively mobile replicator (class II). Such classification immediately captures the defining aspects of the behavior of these elements: they depend on specific transfer-mediating functions encoded by other replicators to move between cells and thus are unlikely to be aggressive parasites.

Hallmark genes of different classes of replicators

The classification of replicators presented here is based on their ecological relationships with the cell vehicle. Importantly, there exist also genetic signatures of different replicator classes, as partly captured by the concept of viral hallmark genes.⁴³ For instance, parasitic replicators, namely lytic viruses, possess a distinctive set of hallmark genes that encode capsid proteins as well as some key components of the viral replication apparatus such as fused primases–helicases. Conversely, the mutualistic replicators (the microbial chromosomes) encode their own, much larger set of signature genes, most notably the full complement of translation-system components. The other classes of replicators also have their own hallmarks (e.g., the replication-initiation endonuclease in non-conjugative plasmids and viruses that replicate via the rolling circle mechanism⁴⁴ and the suite of DNA-transfer genes in conjugative plasmids⁴⁵). The mapping between the ecological classification of replicators outlined here and their hallmark genes is highly complex. In particular, the virus state can be supported by several distinct suites of hallmark genes, and, conversely, the same hallmark genes can span more than one class of replicators. For example, homologous capsid proteins are encoded by both lytic and temperate viruses, whereas homologous replication system components, such as the aforementioned primase–helicase, are present in non-conjugative plasmids (class II), temperate viruses (class IV), and lytic viruses (class V). The full characterization and theoretical conceptualization of the relationships between the ecological classification of replicators and the complements of genes supporting distinct lifestyles of genetic elements remain attractive goals for future investigations.

Dissection of novel prokaryotic isolates into components

Only a miniscule fraction of the prokaryotic diversity has been studied under laboratory conditions. Detailed characterization of novel isolates is a common and rapidly growing practice, and it is becoming obvious that most if not all of these environmental isolates encompass multiple replicators of different classes. Identification of distinct replicons in newly isolated microbes is essential for understanding their biology, especially because the salient biological features such as pathogenicity are often, perhaps typically, associated with extrachromosomal elements (free or integrated).⁴⁶ Bacterial virulence determinants often reside in pathogenicity islands, genomic regions that are prone to horizontal transfer and enriched in class II and class IV replicators.⁴⁷ In a well-publicized example, the *E. coli* O104:H4 strain that caused a lethal epidemic in Germany in 2011 contained, along with the bacterial chromosome, several other replicators.⁴⁸ These additional replicators, namely temperate viruses and conjugative plasmids, have been shown to be responsible for the most relevant phenotypic traits of the strain (i.e., deadly virulence factors and resistances). The pathogenic strain emerged when these different replicators ended up in the same bacterial cell vehicle. Another notable example includes drug-resistant “super bugs,” a phenotype that depends on the New Delhi metallo- β -lactamase-1 (NDM-1) that is typically encoded

within class III replicators (mostly conjugative plasmids) and thus rapidly moves between different cell vehicles.⁴⁹ Here, the classification provides an insight: the mobility of this sinister gene indicates that the problem is not limited to any particular organism.

Replicator profile of metagenomes

In recent years, thanks to the progress of sequencing technology, metagenomic sequencing of microbial communities in oceans, soil, the gut, and elsewhere has become an increasingly popular scientific practice.⁵⁰ Metagenomic data can be analyzed in various complementary ways in order to derive cues about the behavior of a system.^{51–54} However, genetic content in itself gives few ideas about the ecology of different genetic replicators within the analyzed system. The classification presented here provides a simple framework to dissect the metagenome into replicator components. Given that most closely related elements belong to the same class in our classification, and many groups within the same class harbor signature genes, metagenomic sequence data can be used to count the genetic elements within each class, thus providing a class profile of the system. Online computational analyses that can be utilized to distinguish temperate viruses (class IV) from lytic viruses (class V) based on the sequence data alone are already available (www.phantome.org). Furthermore, ribosome-encoding replicators (class I chromosomes) are easy to recognize owing to their conserved sequences. Many conjugational transfer systems are also relatively well conserved, and thus automated computational identification of these elements from the sequence data should be feasible. Obviously, similar analyses have been done before, for example, to estimate the amount and types of viruses in various environments.^{2,50,54} However, the proposed classification could provide a uniform framework where class profiles of different systems with essentially unrelated replicators can be compared to one another.

Differences in replicator profiles might reveal system-level changes that are associated with the alternating replicator composition. For example, when a metagenome contains a lot of diverse class V replicators (lytic viruses), it is likely that evolutionary arms races between class I and class V are common. Indeed, avoidance of viral infections is likely to be a dominant selection pressure for class I replicators.⁵⁵ However, if a metagenome encompasses many class IV replicators (temperate viruses), it is more likely that lysogenic conversion provides the resistance to viral infections, and thus evolutionary arms races between hosts and viral parasites could play a less prominent role.^{56,57} As another example, a metagenome that is replete with class III replicators would likely represent a genetically interconnected community in which beneficial genes rapidly disseminate throughout the system.⁵⁸

Conceptual implications of the classification

The organisms that constitute the earth's biosphere have evolved to form various types of mutualistic, symbiotic, and parasitic relationships with one another. Similarly, organisms have adapted to survive in extremely diverse environmental conditions. Replicators are evolving entities: they can form various relationships with one another and adapt to survive in different cellular environments.

The proposed classification forms a continuum from mutualistic to completely parasitic replicators. Each class represents a niche that utilizes cell vehicles in different ways for propagation and (transient) preservation. We argue that, given enough time, any sufficiently large ecosystem with enough productivity will evolve to contain replicators of all classes. In more concrete terms, it appears likely that the presence of replicators of different classes stabilizes the entire replicator network. This hypothesis is amenable to exploration with mathematical models and metagenomic approaches. The absence of replicators belonging to a certain class creates a loophole in the system that is likely to be exploited by other replicators. Indeed, there are numerous examples of replicators changing their class (see e.g. Ref. 33 and above). The present classification puts these changes into a coherent perspective.

This classification can also inform the way in which we view biological phenomena in general. For example, the question of whether viruses should be considered living entities has been the subject of many disputes and has received a lot of attention, even outside of academic literature,^{8,59–63} In the proposed classification, viruses belong to an interconnected system that also includes chromosomes (Figs. 1 and 2). Viruses are just one type of a genetic replicator that utilizes cell vehicles for propagation: they are part of a continuum, nothing more, nothing less. Thus, the classification in itself provides a way both to realize the differences that viruses have from other genetic replicators (namely the extracellular state during horizontal transfer between hosts) and to see them as a natural part of the evolutionary spectrum of entities inhabiting cells.

To conclude, we argue that there is a need for a systematic way to dissect cells (genomes) into the constituent replicators in an evolutionarily relevant manner. Such a framework would have practical applications and help the microbiologist or anyone else interested in gaining an intuitive picture of the genetic reality behind the organization of the prokaryotic cosmos. The classification proposed here provides a way to establish this framework and can stimulate further research in several directions, in particular, explicit mathematical and experimental modeling of the evolution of complex systems that include replicators of different classes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Classes of replicators differ by their intercellular mobility and by the type of relationship with the cell vehicle. The scale is arbitrary.

Figure 2. Transitions between different classes of replicators. Solid arrows denote interconversion of replicators from different classes, and broken arrows denote integration of replicators from different classes such that the recipient class mediates the transmission of the donor class.

Table 1

A classification for the replicators of prokaryotes.

Class number	Mobility class	Type of symbiosis with the host cell vehicle	Example replicators
I	Non-mobile	Mutualistic	Chromosome
II	Passively mobile	Mutualistic, symbiotic, or commensal	Non-conjugative plasmids, transposons, TA-like elements
III	Actively mobile without an extracellular state	Mutualistic, symbiotic, Commensal, or parasitic	Conjugative plasmids, integrative and conjugative elements
IV	Actively mobile with an extracellular state and a stable within-cell state	Symbiotic, commensal, or parasitic	Temperate viruses
V	Actively mobile with an extracellular state but without a stable within-cell state	Parasitic	Lytic viruses