

Wolbachia, bottled water, and the dark side of symbiosis

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ABSTRACT Obligate endosymbiosis is operationally defined when loss or removal of the endosymbiont from the host results in the death of both. Whereas these relationships are typically viewed as mutualistic, molecular and cellular analysis reveals numerous instances in which these symbiotic relationships are established by alternative, nonmutualistic strategies. The endosymbiont usurps or integrates into core host processes, creating a need where none previously existed. Here I discuss examples of these addictive symbiotic relationships and how they are a likely outcome of all complex evolving systems.

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Images of a plover bird inside the open jaws of an alligator nonchalantly pecking at the reptile's razor-sharp teeth provide a striking example of a mutualistic form of symbiosis: food for the plover, and dental hygiene for the alligator. Coiled at the other extreme of the symbiosis spectrum is the parasitic tapeworm living in the intestine, getting first dibs on meals and eventually producing anemia in its human host. A third form of symbiosis, commensalism, refers to the situation in which one organism benefits while the other is unaffected. The classic example is the remora fish receiving free transportation and protection through its association with sharks. Often, the nature of the symbiotic relationship is not clear. For example, the remora may provide a net benefit to its host by removing skin parasites. One of the most intimate forms of symbiosis is that in which one organism lives within another. In its most extreme form, known as endosymbiosis, the symbiont lives inside the cells of its host. Endosymbionts also exhibit the spectrum of relationships from parasitism to mutualism. A striking example of the former is *Listeria monocytogenes*, a bacterium that resides inside mammalian cells and is the cause of many food-borne illnesses. *Listeria* is famous for forming a jet-like tail by polymerizing host actin, propelling it within and between cells (Pizarro-Cerda *et al.*, 2017).

Many endosymbionts form an obligate mutualistic relationship with their host. That is, loss or removal of the endosymbiont from

the host results in the death of both. In a number of cases, metabolic complementation is the basis of this obligate relationship. This refers to situations in which the host and its symbiont both lack different steps in a biochemical pathway but together possess the enzymes for the complete pathway. Basically, this is a natural version of Beadle and Tatum's famous metabolic complementation experiments in *Neurospora*. For example, formation of select amino acids in the pea aphid requires intermediate products from its bacterial endosymbiont, *Buchnera* (Gerardo and Wilson, 2011). *Buchnera* carries out the initial steps in the biochemical pathway, and the aphid provides the enzyme to carry out the final transaminase step, resulting in the formation of branched-chain amino acids. In another example, *Wolbachia*, a bacterial endosymbiont, provides vitamin B to its bedbug host, which feeds on mammalian blood (Hosokawa *et al.*, 2010). Although rich in many nutrients, blood is deficient in vitamin B. In accord with this finding, analysis of the bedbug and *Wolbachia* genomes reveals that only the latter contains the genetic pathway required for vitamin B synthesis (Nikoh *et al.*, 2014). There are numerous examples of metabolic complementation, and with the increasing ease of sequencing host and endosymbiont genomes, many more examples of obligate mutualism based on metabolic complementation will be forthcoming.

These examples leave us with a comforting mutualistic *kumbaya* view of obligate endosymbiosis. This perspective is reinforced by the widely accepted idea that mitochondria and chloroplasts originated as endosymbionts establishing a mutualistic relationship with their ancestral host (Martin *et al.*, 2001). Thus it is of interest to ask whether obligate symbiotic relationships might be established by alternative, nonmutualistic strategies. A wonderful series of laboratory experiments probing the molecular basis of endosymbiotic relationships make a compelling case for nonmutualistic origins (Jeon and Ahn, 1978). Free-living, single-celled amoebas were infected with a strain of bacteria that decimated the amoeba population.

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Abbreviations used: A-P, anterior-posterior; Wb, *Wolbachia*.

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However, rare surviving amoebas were recovered. Examination of these survivors revealed that the invading bacteria established a stable presence in their cytoplasm. Particularly striking, during the course of this experiment, the amoebas had become dependent on the bacteria for their survival. This evolution-in-a-test-tube experiment was repeated with the same result: an obligate endosymbiotic relationship was quickly established between the pathogenic bacteria and the amoebas. The invading bacteria resulted in the suppression of the amoeba's S-adenosylmethionine synthetase (SAMS) gene product, and survival of the amoeba now required the bacteria for production of the SAMS gene product (Jeon and Jeon, 2003). Thus the free-living amoebas had become addicted to the presence of originally pathogenic bacteria. It is unlikely that the presence of the bacteria improved the fitness of the amoeba, making it difficult to explain the establishment of this symbiosis from a classic Darwinian perspective. This form of symbiosis has been termed addictive symbiosis (Douglas, 2010).

These experiments raise a series of intriguing questions. Does addictive symbiosis occur in nature? If so, is it frequent or rare? Are there different forms of addictive symbiosis? Can the concept of addictive symbiosis be more broadly applied toward the evolution of core molecular machinery of the cell? Answering the first question is difficult without knowing the functionality of the host immediately before invasion by the symbiont. However, studies exploring the cellular and developmental basis of the obligate mutualism may provide insight. For example, if the symbiont is required for proper functioning of highly conserved core cellular or developmental events, this suggests an addictive symbiotic relationship in which the endosymbiont created a need where none previously existed. That is, conserved host processes rooted deep in the tree of life are likely to have been present before the introduction of the symbiont.

The leafhopper *Euscelis plebejus* and its maternally inherited ball of bacterial endosymbionts provides a wonderful example of an endosymbiont becoming essential for early insect development. Removal of the endosymbiont through antibiotic curing results in the production of embryos lacking abdominal segments (Sander, 1968). In addition, partial reduction of the symbiont ball results in a partial reduction in abdominal segments. Similar results were found with the maternally inherited yeast-like endosymbiont of the brown planthopper: removal of the endosymbiont produces defects in abdominal segmentation (Schwemmler, 1974). Thus these symbionts are essential for normal segmentation in their insect host. It is likely that these highly conserved core mechanisms of axis formation and segmentation were established in the ancestral leafhopper and planthopper well before endosymbiont acquisition. This suggests that during the eons of coexistence, leafhoppers and planthoppers must have compensated for the presence of a large, maternally inherited endosymbiont positioned at a key time and place during insect segment establishment. Consequently, early embryogenesis became dependent on the presence of this endosymbiont. Loss of the endosymbiont disrupts this balance: without the symbiont, the host compensatory

mechanisms disrupt embryonic development. The leafhopper and the endosymbiont exist in an obligate mutualistic relationship, but it is likely based on addiction rather than functional complementation between the two organisms.

Another example of addictive symbiosis occurs between *Wolbachia*, a bacterial endosymbiont that occupies the germline of its filarial nematode host. It is well established that *Wolbachia* is required for the survival and fertility of filarial nematodes, but the basis of the obligate symbiotic relationship remained unresolved (Desjardins et al., 2013). Insight came from antibiotic curing experiments in order to generate nematodes lacking *Wolbachia* (Landmann et al., 2014). Surprisingly, embryos derived from these nematodes exhibit distinct anterior-posterior (A-P) polarity defects identical to the phenotype of *Caenorhabditis elegans* embryos lacking the Par-1 polarity gene product (Figure 1). This result suggests that among the nematode lineages, *Wolbachia* has coevolved with its filarial nematode host such that it has become an essential component of the core conserved machinery driving axis formation.

Wolbachia is also essential for proper regulation of host apoptosis. Loss of *Wolbachia* in the moth *Asobara tabida* results in a dramatic increase in apoptosis and infertility in the host oocytes (Dedeine et al., 2001; Pannebakker et al., 2007). Apoptosis is part of the normal process in insect oogenesis. Through the establishment of *Wolbachia* as an essential component of normal apoptotic regulation in *A. tabida*, an addictive symbiotic relationship has been formed. *Wolbachia* is also essential for normal regulation of apoptosis in filarial nematodes. Loss of *Wolbachia* results in a dramatic increase in apoptosis in both the germline and somatic tissues of the nematode (Landmann et al., 2011). In both organisms, coevolution has resulted in the outsourcing of host apoptosis regulation to the endosymbiont. Remarkably, recent studies demonstrate that embedded in the *Wolbachia* genome is a bacteriophage required for spread of *Wolbachia* through insect populations (Beckmann et al., 2017; LePage et al., 2017). Thus *Wolbachia* itself may be the victim of addictive symbiosis.

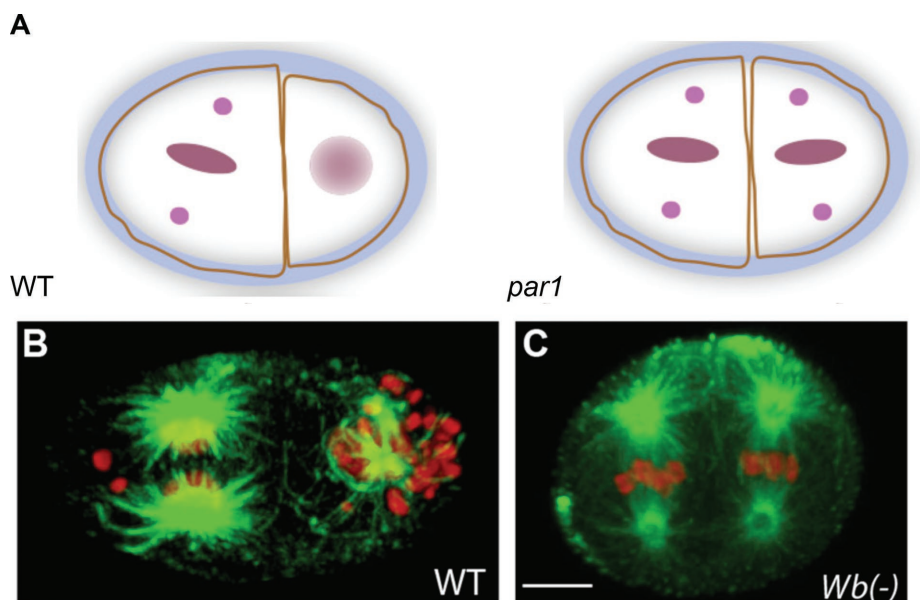


FIGURE 1: *Wolbachia* is required for filarial nematode A-P axis formation. (A) Nematode *par-1* mutants disrupt A-P axis formation, as evidenced by failed spindle rotation at the two-cell stage. (B) Wild-type and (C) *Wolbachia*-depleted filarial nematode two-cell stage embryos exhibit normal and failed spindle rotation, respectively. *Wolbachia* depletion phenocopies *par-1* mutant disruption of A-P axis formation. DNA (red) and tubulin (green). Modified from Landmann et al. (2014).

Addictive symbiosis creates a need where none previously existed. Whereas adaptive processes and selection may have driven initial establishment of the symbiosis, subsequent nonadaptive processes result in the endosymbiont becoming an essential component of core host cellular and developmental processes. As a result, the host and endosymbiont become locked into an obligate mutualistic relationship. Subsequent gene transfer from the endosymbiont to the host nucleus would ensure irreversibility of the symbiosis (Keeling *et al.*, 2015).

One can argue that addictive symbiotic relationships are a likely outcome in all complex evolving systems. In fact, societal systems such as language, business, administration, and technology provide many examples of addictive symbiosis. Although not directly applicable to biological symbiosis, they illustrate the ease in which addictive relationships are established. For example, you may have the impression that there are fewer drinking fountains in public venues. This is not an illusion. The increasing popularity of bottled water largely driven by aggressive industry marketing has lessened the demand for drinking fountains. Whereas drinking fountains were once a signature of an advanced society, the omnipresence of bottled water is making them unnecessary, such that fewer are designed into new buildings and current ones are not being maintained (Pierre-Louis, 2015). The 2015 edition of the plumber's building code has reduced the number of required number of drinking fountains by half. Even more dramatic, Florida Central University recently built a football stadium without fountains. The university saved on construction costs and generated additional income by selling bottled water. Although bottled water clearly has some advantages over fountains, the loss of fountains enforces a lucrative but unnecessary dependence on bottled water. The notorious introduction of baby formula in underdeveloped nations provides a more consequential example of additive symbiosis (Kent, 2015). Nestle's aggressive promotion and marketing of baby formula in place of breast-feeding changed cultural norms, reduced lactation in mothers, and ultimately resulted in an artificial dependence on formula.

Conceptual insight into how an addictive symbiotic relationship might be established is provided by exploring the evolution of core eukaryotic cellular processes. For example, the complexities of RNA processing, splicing, and editing and its associated enzymatic machinery never cease to amaze. The spliceosome alone is endowed with >100 proteins (Papasaiikas and Valcarcel, 2016). Even for RNA-world experts, viewing the evolution of this complexity from a Darwinian adaptive perspective is a daunting task. In addition, although the primary function of prokaryotic and eukaryotic ribosomes is translation, the latter is much more complex, possessing an additional RNA and many more proteins (Ban *et al.*, 2014). Harry Noller (personal communication) likens the prokaryotic ribosome to a Ferrari and the eukaryotic ribosome to a Winnebago lumbering along with assorted camping gear strapped on top. This analogy is in accord with the finding that a number of eukaryotic ribosome proteins provide key functions distinct from their role in the ribosome (Wool, 1996). One could imagine that these proteins originally evolved with functions independent of the ribosome but serendipitously possessed domains that bound ribosomal proteins. As the eukaryotic cell evolved, these proteins became an integral component of the eukaryotic ribosome. Others have made a compelling case that similar nonadaptive "ratchet-like" mechanisms may account for many aspects of cellular complexity (Lynch, 2007; Gray *et al.*, 2010; Koonin, 2016). For example, Gray *et al.* (2010) describe a process in which fortuitous interactions between cellular components led to a functional dependence requiring both

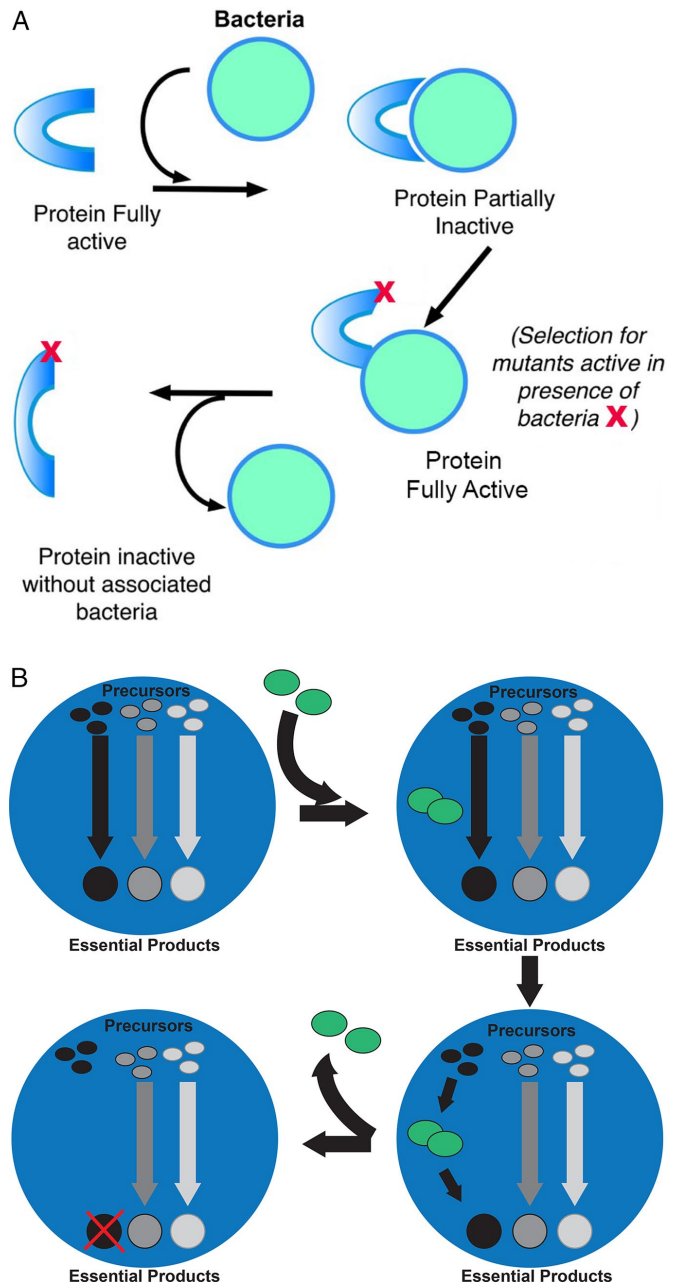


FIGURE 2: Potential origins of addictive symbiosis. (A) Initial interaction: Symbiont compromises function of key host enzyme. Host response: Compensatory host mutations restore the endosymbiont/enzyme complex to full function. However, proper function of the complex now requires the presence of the endosymbiont. This is analogous to the evolutionary ratchet mechanism proposed by Gray *et al.* (2010). (B) Symbiont invades host and is maintained by integrating and becoming essential for a core host cellular or developmental process. By outsourcing essential processes, the host becomes dependent on the presence of the endosymbiont.

components. In addition, studies of the eukaryotic V-ATPase proton pump document how gene duplications followed by mutations that degrade complementary regions of each gene result in increased complexity of a protein complex, with no obvious adaptive advantage (Finnigan *et al.*, 2012). Similarly, it has been shown that complementary patterns of gene inactivation have expanded single obligatory endosymbionts into two distinct lineages, both

essential for host survival (Van Leuven *et al.*, 2014). Again, this reveals increased complexity through nonadaptive processes.

It is possible that obligate functional dependences between an endosymbiont and its host could evolve in a manner analogous to the ratchet-like mechanisms proposed by Gray *et al.* (2010). For example, initial interaction between the endosymbiont and a key host protein complex may slightly compromise the function of the complex. Unable to rid itself of the endosymbiont, a host's compensatory mutations may restore the endosymbiont/protein complex to full function. However, proper function of the complex now requires the presence of the endosymbiont (Figure 2A). A second mechanism by which addictive symbiotic relationships are established is illustrated by the bacterial/ameba experiments described earlier. Whereas the bacterial infection decimated the ameba population, outsourcing of key metabolic functions occurred in the rare ameba that survived infection, resulting in an additive symbiotic relationship. One can imagine a similar scenario playing out in many natural obligate endosymbiont relationships (Figure 2B). That is, initial contact is a prokaryotic invasion of a eukaryote causing significant stress to one or both (Burns *et al.*, 2017). The outcome is uncertain but likely results in the death of either the nascent symbiont or the host (Keeling *et al.*, 2015). In rare survivors, the endosymbiont is maintained by integrating and becoming essential to core host cellular or developmental processes.

I would argue that many obligate endosymbiotic relationships are more akin to a big-box store locating in a town: much outcry and displacement, but ultimately a dependence on its presence. Simply by its presence, the box store created a need where none previously existed. Both in society and in biology, addictive symbiosis may be the rule rather than the exception.

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