ANNUAL REVIEWS

Annual Review of Cell and Developmental Biology

The Genomics and Cell Biology of Host-Beneficial Intracellular Infections

John P. McCutcheon

Biodesign Center for Mechanisms of Evolution, School of Life Sciences, Arizona State University, Tempe, Arizona 85287, USA; email: john.mccutcheon@asu.edu

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

Annu. Rev. Cell Dev. Biol. 2021. 37:115–42

First published as a Review in Advance on July 9, 2021

The *Annual Review of Cell and Developmental Biology* is online at cellbio.annualreviews.org

https://doi.org/10.1146/annurev-cellbio-120219-024122

Copyright © 2021 by Annual Reviews.
All rights reserved

ANNUAL REVIEWS CONNECT

www.annualreviews.org

- Download figures
- Navigate cited references
- Keyword search
- Explore related articles
- Share via email or social media

Keywords

endosymbiosis, organelles, intracellular pathogens, endocytosis, phagocytosis, exocytosis

Abstract

Microbes gain access to eukaryotic cells as food for bacteria-grazing protists, for host protection by microbe-killing immune cells, or for microbial benefit when pathogens enter host cells to replicate. But microbes can also gain access to a host cell and become an important—often required—beneficial partner. The oldest beneficial microbial infections are the ancient eukaryotic organelles now called the mitochondrion and plastid. But numerous other host-beneficial intracellular infections occur throughout eukaryotes. Here I review the genomics and cell biology of these interactions with a focus on intracellular bacteria. The genomes of host-beneficial intracellular bacteria have features that span a previously unfilled gap between pathogens and organelles. Host cell adaptations to allow the intracellular persistence of beneficial bacteria are found along with evidence for the microbial manipulation of host cells, but the cellular mechanisms of beneficial bacterial infections are not well understood.

Contents

1. INTRODUCTION: THE OUTCOMES OF BACTERIAL INFECTIONS 116
2. BECOMING AN ORGANELLE: GENETIC AND GENOMIC CONVERGENCE IN ENDOSYMBIOSIS 117
   2.1. Eukaryotic Organelles of Bacterial Origin 117
   2.2. Genome Reduction in Endosymbiotic Bacteria 118
   2.3. Losing Genes, Moving Genes, and Importing Proteins 120
3. CELLS GETTING INTO CELLS: THE MECHANISMS AND OUTCOMES OF PHAGOCYTOSIS 121
   3.1. Eukaryotic Phagocytosis: A Mechanism for Food and Defense 121
   3.2. Evasion and Exploitation of Eukaryotic Phagocytosis by Intracellular Pathogens 122
   3.3. Escape Strategies for Intracellular Pathogens 124
4. HOST-BENEFICIAL BACTERIAL ENDOSYMBIONTS: FROM PATHOGENS TO ORGANELLES 125
   4.1. Case Study: Aphids and Their Nutritional Host-Beneficial Endosymbiont *Buchnera* 126
   4.2. Case Study: Pathogens as the Source of Host-Beneficial Endosymbionts 128
   4.3. Case Study: Nitrogen-Fixing Host-Beneficial Endosymbiont Bacteria in Leguminous Plants 129
   4.4. Case Study: Intracytoplasmic Host-Beneficial Endosymbionts in Insects 129
   4.5. Case Study: Photosynthetic Host-Beneficial Endosymbionts 130
5. CONCLUSIONS 131

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

1. INTRODUCTION: THE OUTCOMES OF BACTERIAL INFECTIONS

Interactions between eukaryotes and bacteria occur on a complex and dynamic continuum of type and duration. Most interactions are probably transient and meaningless—just cells or organisms bouncing off each other as they go about their business. But some interactions matter to one or both of the organisms, and, in these situations, we say that a symbiosis has formed. In this review, I use symbiosis in its original formulation (De Bary 1879, Oulhen et al. 2016), in which it simply means dissimilar organisms living closely together in a sustained interaction, without judgment of whether that interaction is good or bad for the participants. Symbioses that develop between plants and animals but that occur outside of host cells, or ectosymbioses, such as those found on the leaves of plants (Koskella 2020) or in the gut lumen of animals (Sommer & Bäckhed 2013) are not discussed further in this review. Here I focus on symbioses that occur inside of host cells, or endosymbioses.

It is difficult to overstate the impact that endosymbioses have had on the evolution of life. The endosymbiosis of an alphaproteobacterial cell into an archaeal cell predated the diversification of all known eukaryotes (Embley & Martin 2006, Zaremba-Niedzwiedzka et al. 2017), and the establishment of a cyanobacterial cell into an ancient single-celled eukaryote gave rise to the photosynthetic eukaryotes (Gould et al. 2008, Keeling 2010). Plants, animals, fungi, and protists would not exist without endosymbiosis. But these same eukaryotic organisms, born of intracellular symbioses, are now plagued by a huge diversity of pathogens that live out some or all of their life cycle

inside eukaryotic cells. Eukaryotic organisms have evolved sophisticated mechanisms to deal with microbial intruders, such as the cell-autonomous innate immunity of plants and animals (Dodds & Rathjen 2010, Randow et al. 2013, Tam & Jacques 2014) and the professional and adaptable immune system of vertebrate animals (Hirano et al. 2011, Hoebe et al. 2004). Intracellular microbial pathogens have evolved equally complex mechanisms to minimize or evade these eukaryotic cellular defenses (Cossart et al. 2019). These interaction types—the formation of an organelle or the establishment of an infection by an intracellular pathogen—give rise to three well-known outcomes of endosymbiosis: A cell or organism is infected by a microbe and defeats it (the host wins; see **Figure 1*d***), a cell or organism is infected by a pathogen and is killed (the microbe wins; see **Figure 1*f***), or a host cell is infected by a bacterium and an organelle is established (**Figure 1*k***).

Host-beneficial endosymbiont (HBE): a beneficial microbe that lives most of its life cycle inside a host cell

Here I review some of the genomic and cell biological features of these three endosymbiotic outcomes but only as a prelude to discussing the fourth possible outcome of infection, in which a microbe enters a host cell and neither it nor the host is destroyed, and in which the presence of an endosymbiont that is not quite an organelle becomes required by the host cell (**Figure 1*b***). I call these microbes host-beneficial endosymbionts (HBEs) (McCutcheon et al. 2019). HBEs are interesting because their interactions with host cells are a bit pathogen-like and a bit organelle-like, but in general they are not well understood at the cell biological level.

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

2. BECOMING AN ORGANELLE: GENETIC AND GENOMIC CONVERGENCE IN ENDOSYMBIOSIS

2.1. Eukaryotic Organelles of Bacterial Origin

While many host–pathogen relationships experience coevolution over long periods of time, most individual host–pathogen interactions tend to occur in acute bouts because these relationships result in the sickening or death of one of the participants. In contrast, the establishment of the mitochondrion and plastid happened deep in evolutionary history and has resulted in near-permanent establishment inside of host cells. The mitochondrion and plastid are so stable that they are no longer considered extrinsic to their hosts—they are bacterial infections that have become parts of their host cells, or organelles.

The nature and origin of mitochondria and plastids were debated for decades before DNA sequencing, phylogenetics, and biochemical analysis definitively proved their bacterial origins (Archibald 2014, Sapp 1994). While the bacterial provenance of mitochondria and plastids is no longer seriously questioned, the nature of the interaction between the ancestral host cell and the proto-organelle, along with the timing of integration relative to the evolution of other eukaryotic organelles, is currently a matter of vigorous debate (Booth & Doolittle 2015, Lane & Martin 2015). For my purposes, simply saying that mitochondria and plastids are the two oldest intracellular bacterial infections known to science is sufficient.

The bacterial nature of mitochondria and plastids is reflected in the structures of their genomes (Smith & Keeling 2015); the organization, biosynthesis, and composition of their membranes and other biochemical pathways (Kořený et al. 2013); and the phylogenetic signatures of the genes that support their function (Keeling 2010, Roger et al. 2017). Because these organelles were established in the common ancestors of eukaryotes and photosynthetic eukaryotes before the host lineages started to diversify, transitional forms of early mitochondrial and plastid evolution do not exist (Poole & Gribaldo 2014). But it is generally accepted that mitochondria and plastids did not arise fully formed: They were once free-living bacteria that transitioned to an endosymbiotic state (**Figure 1*i,j***), and, in turn, these endosymbionts transitioned to the organellar state (**Figure 1*j,k***).

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

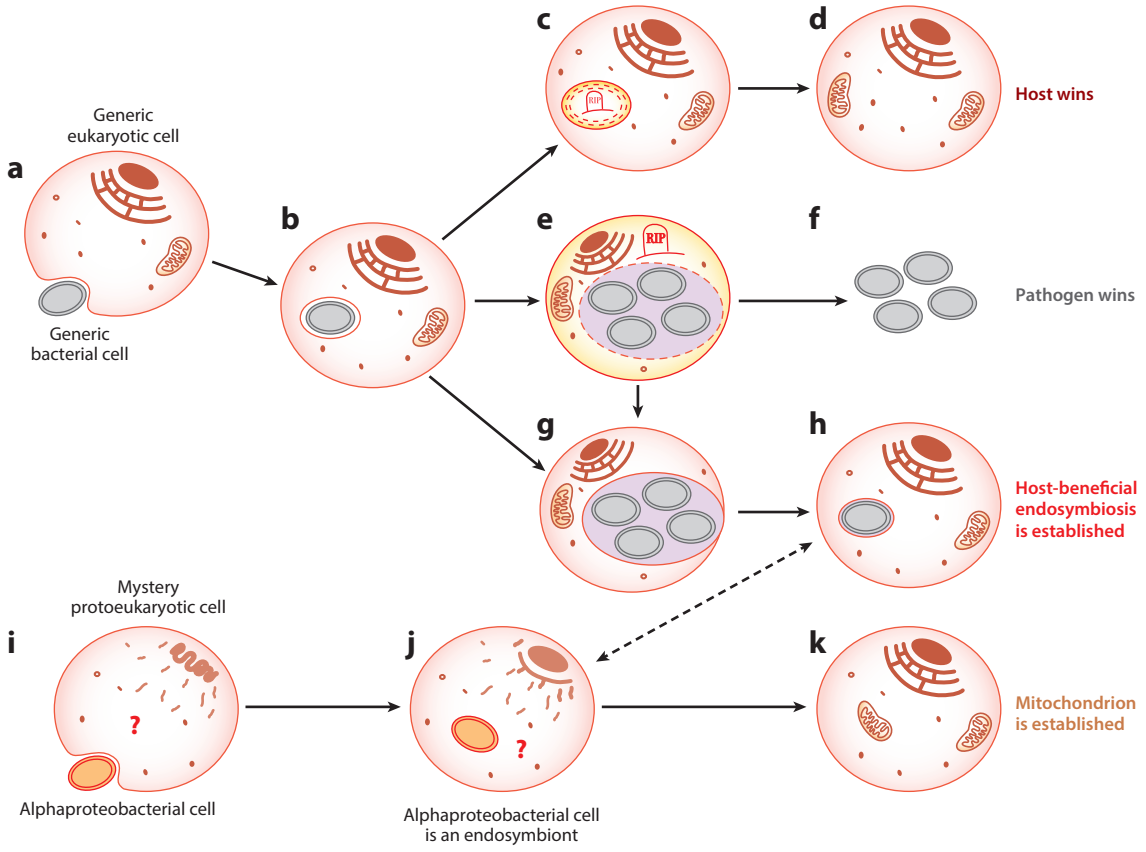

Figure 1

The possible outcomes of a bacterium infecting a host cell. Images in panels *a–h* show the outcomes of intracellular associations between bacterial and eukaryotic cells. Images in panels *i–k* show some of the possible historical steps in the formation of the mitochondrion-bearing eukaryotic cell. (*a*) A generic eukaryotic cell engulfs a generic gram-negative bacterial cell containing two lipid bilayers (*double gray ovals*). (*b*) The bacterial cell is enveloped in a host-derived vacuole (*red oval*). (*c*) The bacterial cell is successfully destabilized (*yellow*) by the host by vacuolar fusion with lysosomes and (*d*) eliminated. (*e*) The bacterial cell is a successful pathogen and modifies its host vacuolar membrane to enable bacterial persistence and replication, which sickens (*yellow*) and eventually (*f*) kills the host cell. (*g*) The benefits of a bacterial infection outweigh its costs, the vacuolar membrane is stabilized, and eventually (*h*) an endosymbiosis is established with a bacterium containing three lipid bilayers. This outcome could originate from a host-benign situation, such as the ingestion of bacterial food (shown as transitions from panels *b* to *g*) or from a mild pathogenic situation (shown as transitions from panels *e* to *g*). (*i*) A host cell of unknown origin, likely an archaeal cell, somehow begins to house an alphaproteobacterial cell. The alphaproteobacterial cell loses (or never gained) its host vacuolar membrane and (*j*) goes through a period of endosymbiosis of unknown similarity to modern endosymbioses (*dashed two-way arrow*). (*k*) The alphaproteobacterial endosymbiont becomes the mitochondrion of the modern eukaryotic cell.

2.2. Genome Reduction in Endosymbiotic Bacteria

From a genomic perspective, the outcome of bacterial endosymbiosis is highly reproducible: The genomes of bacteria that take up residence inside host cells lose genes and get smaller as the bacterium spends more time in the endosymbiotic state (Casadevall 2008, George et al. 2020, Husnik & Keeling 2019, McCutcheon & Moran 2012, Moran 2002, Moran & Bennett 2014, Nowack et al. 2016, Ochman & Davalos 2006). Bacteria along this continuum can be divided into four categories (**Figure 2**). The first category is the free-living state (**Figure 2*a***), in which

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

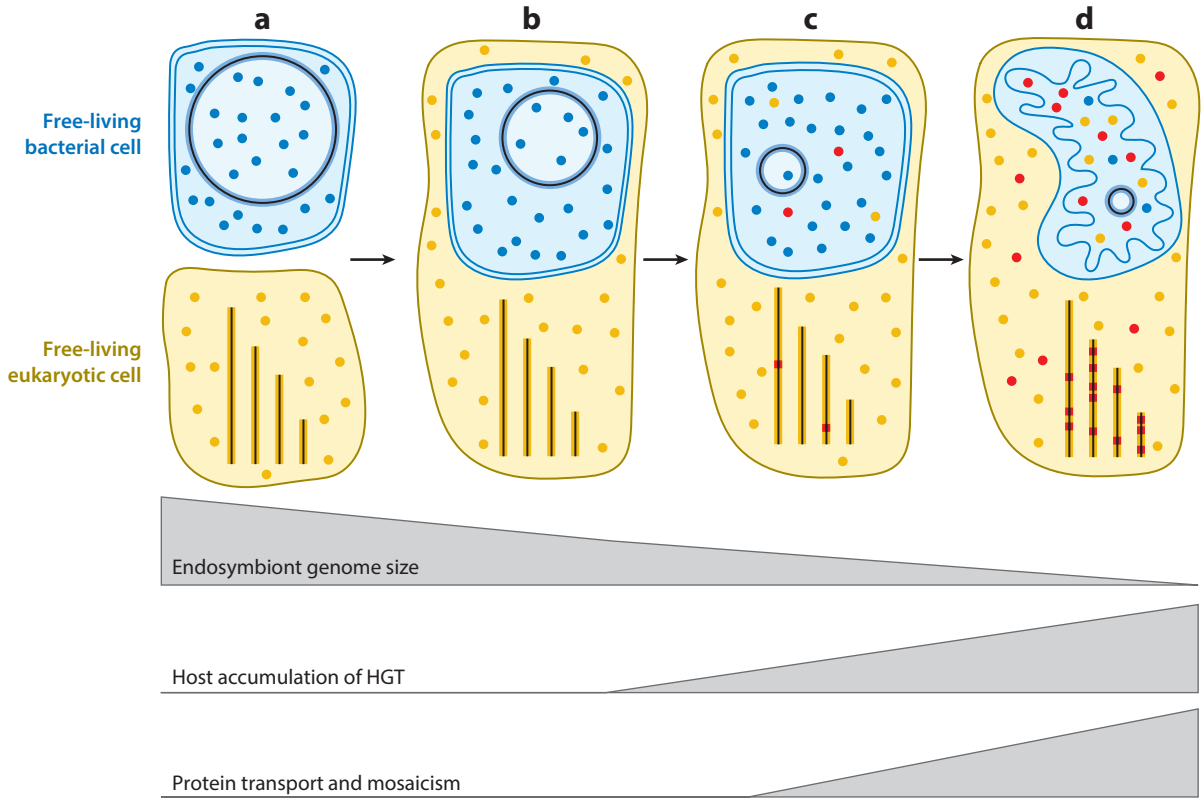

Figure 2

Genome reduction and genetic integration in endosymbiosis. (*a*, *top*) A free-living bacterial cell is shown in blue, in which the circular chromosome is represented by a circular wide blue line marked with a thin black line. Bacterial proteins made from that genome are shown as blue dots. (*Bottom*) A free-living eukaryotic cell is shown in gold, in which the multiple linear chromosomes of the genome are represented by wide gold lines marked with thin black lines. Eukaryotic proteins made from this genome are shown as gold dots. (*b*) In the early stages of endosymbiosis—no matter whether a host-beneficial endosymbiont (HBE) or a pathogen—the bacterial genome loses genes and becomes smaller. This genome-reductive process is represented by a somewhat smaller circular wide blue line marked with a thin black line. (*c*) If the bacterial endosymbiont is beneficial to the host and remains sequestered in host cells for long periods of time, the genome of the bacterium reduces further. In some cases, the host genome acquires bacterial genes through horizontal gene transfer (HGT; *red squares* on host genome) that compensate for genes lost from the endosymbiont genome. A few proteins made from these HGTs (*red dots*) or native host genes (*gold dots*) have been shown to be transported into endosymbiont cells. Some bacterial endosymbionts of sap-feeding insects and protists are found in this state. (*d*) In organelles, the bacterial genome has become extremely reduced and encodes few genes. The host genome has acquired numerous HGTs (*red squares*) that compensate for lost endosymbiont genes. Numerous proteins of both native host (*gold dots*) and HGT (*red dots*) origin are transported into the organelle, and in some cases HGTs of bacterial origin function in the cytoplasm of the host. Gray bars at the bottom of the figure represent trends for genome size, HGT, and protein transport across the levels of integration.

the bacterium and host are either unaffiliated or the bacterium is only a facultative or part-time intracellular resident. The genomes of these bacteria tend to be larger in size (3–10 Mb) and coding capacity (3,000–10,000 genes). The second category comprises bacteria that have recently transitioned to an endosymbiotic state (**Figure 2*b***). These bacterial genomes are smaller (1–3 Mb) and encode fewer genes (500–3,000) than their free-living relatives and can contain many recently broken genes, or pseudogenes. Both pathogens and host-beneficial bacteria are found in this category. Bacteria in the third category (**Figure 2*c***) are typically HBEs that have been restricted

to their host cells for long periods of time but include some intracellular pathogens. Bacteria in this state tend to have very small genomes (0.1–1 Mb) encoding few (150–500) genes—the tiniest HBE genomes can be less than 5% of the size of those of their free-living relatives. Notably, the most-reduced HBE genomes in this category are smaller than some of the larger organellar genomes. The fourth category includes only two endosymbionts: the mitochondrion and the plastid (**Figure 2*d***). The genomes of these organelles are diverse in size and structure, but the coding capacity of mitochondria ranges from ~100 genes to zero genes in mitochondria-derived organelles that completely lack a genome. The range in plastids is about 250 genes to less than 20.

The evolutionary and mechanistic forces driving genome reduction in endosymbiotic bacteria have been reviewed in detail elsewhere (Batut et al. 2014, Kirchberger et al. 2020, McCutcheon & Moran 2012, McCutcheon et al. 2019, Moran & Bennett 2014), but here I highlight a few general trends. The genes retained most consistently across the four categories of bacterial genomes shown in **Figure 4** are those involved in the core informational processing activities of bacteria: genome replication, transcription, and translation. Outside of these core genetic processes, retained genes reflect the role of the bacterium in the symbiosis and the age of the association. Intracellular pathogens retain genes involved in infecting, manipulating, and escaping the host cell (Lawrence 2005), while losing or modifying genes involved in immune recognition by the host such as the lipopolysaccharide (LPS) layer in the outer membrane of gram-negative bacteria (Needham & Trent 2013). HBEs with relatively large genomes (**Figure 2*b***) also often retain genes to infect and manipulate host cells (such as the secretion systems discussed in Section 3.2) and often completely lose the pathways to build LPS and peptidoglycan (Moran & Bennett 2014). What most strongly differentiates the gene inventories of pathogens and HBEs are genes involved in producing energy and nutrients such as vitamins, amino acids, and ATP. The genomes of intracellular pathogens tend to encode few pathways to build these metabolites because they steal them from their hosts, while HBEs tend to retain these pathways because they are often the reason for the HBEs' existence (from the perspective of the host). In the HBEs with the smallest genomes (**Figure 2*c***), such as in the tiny bacterial genomes from insect nutritional endosymbionts, their genomes consist of little else besides genes devoted to genome replication, transcription, translation, and nutrient provisioning for the host (McCutcheon 2010, McCutcheon & Moran 2012, Moran & Bennett 2014). The most-reduced HBE genomes—the most organelle-like—encode no genes to build lipid bilayers or cell walls, encode few or no transmembrane proteins, and have even lost numerous so-called essential genes involved in translation such as amino acyl-tRNA synthetases and numerous ribosomal proteins (Galperin et al. 2021, McCutcheon & Moran 2012, Moran & Bennett 2014).

2.3. Losing Genes, Moving Genes, and Importing Proteins

The progression of genome reduction shown in **Figure 2** is accompanied by a progression of genetic and cell biological integration through horizontal gene transfer (HGT) and protein import. The genomes and proteomes of bacteria that are free-living (**Figure 1*a***) or that have recently transitioned to the intracellular state (**Figure 1*b***) are independent from their host from the perspective of vertical transmission. The genomes of hosts and endosymbionts in these two categories may evolve in response to one another, such as when a host and a pathogen engage in tit-for-tat Red Queen evolution (McLaughlin & Malik 2017), and the proteomes may even physically interact in some cases, such as when a bacterial pathogen injects effector proteins into the cytoplasm of a host cell (Omotade & Roy 2019), but genes that directly support the interaction are typically not transferred between host and symbiont in these early stages of association.

Genetic integration starts to occur in the endosymbioses shown in **Figure 1*c***. For example, in some insects that host long-term HBEs with small genomes, bacterial genes have been transferred

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

to the host insect genome to support the functioning of the endosymbionts (Husnik et al. 2013, Luan et al. 2015, Nikoh & Nakabachi 2009, Nikoh et al. 2010, Sloan et al. 2014). In most cases, these transferred genes are not from the genomes of the nutritional endosymbionts themselves but from reproductive manipulators such as *Wolbachia*. This makes sense because bacteria such as *Wolbachia* reside within the germ cells of insects (Serbus & Sullivan 2007) and have been shown to be frequent sources of bacterial DNA in arthropod genomes (Brelsfoard et al. 2014, Dunning Hotopp et al. 2007, Hamilton et al. 2018, Leclercq et al. 2016). Similarly, in the example of the amoeba *Paulinella chromatophora* and its cyanobacterial endosymbiont (highlighted in Section 4.5), hundreds of bacterial HGTs are found in the amoeba genome, but only a minority of them seem to have been transferred from the cyanobacterial endosymbiont (Nowack et al. 2016). Finally, in the case of the ancient mitochondrial and plastid endosymbioses (**Figure 1*d***), the organellar genomes encode few genes, and the host genome encodes a complex mix of genes of various taxonomic origins. Genes on the nuclear genomes of eukaryotes reflect the native genome of the host, the bacterial ancestors of the organelle (that is, genes with alphaproteobacterial and cyanobacterial origins), but also various other bacterial sources (Gray 2015, Ku et al. 2015, Qiu et al. 2013, Stairs et al. 2020).

This progression of gene loss in endosymbionts and gene gain through HGT in the host is coincident with a progression of biochemical mosaicism due to the transport of proteins to compartments unrelated to the genome on which they are encoded. In insects, two proteins produced from bacteria-to-insect HGTs have been shown to be transported into the cytoplasm of the nutritional endosymbionts by unknown mechanisms (Bublitz et al. 2019, Nakabachi et al. 2014). In the amoeba *Paulinella*, hundreds of host proteins, some encoded by HGTs, are transported into the cyanobacterial endosymbiont cells (Nowack & Grossman 2012, Singer et al. 2017). Finally, in mitochondria and plastids, most of the proteins that function in the organelles are encoded in the nuclear genome and are transported into the organelle by specialized protein import systems (Pfanner & Meijer 1997, Reumann et al. 2005, Schmidt et al. 2010, Whelan 1999). In organelles, the taxonomic origin and genome location of a gene are often unlinked to the compartment where the protein encoded by that gene ends up functioning (Duchêne et al. 2005, Kořený et al. 2013, Pagliarini et al. 2008).

The progression of gene loss on endosymbiont genomes, gene gain on host genomes, and protein import of host genes into endosymbiont and organellar compartments shown in **Figure 2** leads to mosaic genomes and proteomes in endosymbiotic systems. While mitochondria and plastids used to seem somewhat distinct in this progression—of bacterial origin, of course, but so unique that they require their own names—we now have many insect and protist examples that span the genomic gap that once existed between bacterium and organelle (Graf et al. 2021, Husnik & Keeling 2019, McCutcheon et al. 2019, Nowack & Melkonian 2010). This repeatable and convergent genomic response to sustained endosymbiosis is likely to have emerged from shared and repeatable cell biological mechanisms of integration. Because these symbioses involve cells living within cells, I next review some of the mechanisms used by microbes to enter into and persist within eukaryotic cells.

3. CELLS GETTING INTO CELLS: THE MECHANISMS AND OUTCOMES OF PHAGOCYTOSIS

3.1. Eukaryotic Phagocytosis: A Mechanism for Food and Defense

While the processes by which the ancestors of the mitochondrion and plastid entered their ancient host cells are unknown, microbes do not gain access to modern eukaryotic cells by accident. With rare exceptions (Morisaki et al. 1995), microbes enter eukaryotic cells through the controlled

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

Phagocytosis: in eukaryotic cells, the endocytosis of large particles such as bacteria, fungi, and amoebas

Phagosomal membrane: the internalized host-derived membrane that results from the phagocytosis of an extracellular microbe

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

production and internalization of the plasma membrane, or endocytosis (Doherty & McMahon 2009). Bacteria use many different endocytic mechanisms to get inside of cells, which at the highest level can be broken down into passive or active entry from the bacterial perspective. Passive bacterial entry occurs in eukaryotic cells that are specialized in phagocytosis, a form of endocytosis restricted to large particles greater than 0.5 μm (Fairn & Grinstein 2012, Lancaster et al. 2019, Russell 2007). Active bacterial entry can occur in almost any eukaryotic cell type, in which pathogens induce their entry into host cells by exploiting numerous different types of endocytic mechanisms (Cossart & Sansonetti 2004, Veiga et al. 2007, White et al. 2017). All microbes that persist and replicate inside of eukaryotic cells use some idiosyncratic combination of factors to manipulate their hosts, but here I focus on microbial internalization into a newly formed phagocytic vacuole that interacts with the classic endocytic pathway (Omotade & Roy 2019).

Because bacteria-eating phagotrophic protists occur in all of the major eukaryotic supergroups, it seems reasonable to conclude that phagocytosis as a form of bacterial cell uptake and destruction happened early in eukaryote evolution (Burki et al. 2020, Cavalier-Smith 2002, Koumandou et al. 2013, Mills 2020, Simpson & Eglit 2016). The cell biology of food ingestion by heterotrophic protists shares deep homology with the ingestion and destruction of pathogenic microbes by animal immune cells such as neutrophils and macrophages (Boulais et al. 2010, Bozzaro et al. 2008, Lancaster et al. 2019, Steenbergen et al. 2001). This similarity has motived the hypothesis that heterotrophic protists serve as training cells for microbes that become intracellular animal pathogens (Barker & Brown 1994, Casadevall 2012, Erken et al. 2013, Harb et al. 2000, Matz & Kjelleberg 2005, Molmeret et al. 2005). Consistent with the ancient origin of phagocytosis and its conserved molecular mechanisms in eukaryotes, many animal cell types other than professional immune cells can internalize and destroy bacterial cells in a process called cell-autonomous immunity (Gaudet et al. 2016, Randow et al. 2013).

The process of eukaryotic phagocytosis is schematically illustrated in **Figure 3**. Briefly, after the plasma membrane internalizes a microbe (here, a gram-negative bacterium), the maturation of a nascent phagosome occurs through a complex process involving cell signaling, actin polymerization, lipid remodeling, vesicle trafficking, phagosome acidification, and lysosomal fusion (Flannagan et al. 2012). The process starts with bacterial recognition by receptors on the cell surface (**Figure 3*a***), followed by plasma membrane deformation around the bacterial cell in an actin-dependent process, with an internal vacuole (a phagosome) being formed through membrane scission (**Figure 3*b***). The phagosomal membrane is rapidly remodeled by interaction with the endocytic pathway, eventually transforming the vacuole into a potent bacteria-killing phagolysosome (Fairn & Grinstein 2012, Flannagan et al. 2012) (**Figure 3*c–e***). In some cases, cellular debris is eliminated from the cell through exocytosis (**Figure 3*f***). For my simplified purposes in this review, phagosome maturation can be broken down into three phases. These three phases are marked by the presence of different Rab GTPases—key regulators and organizers of vesicular traffic in eukaryotic cells (Stenmark 2009)—on the vacuolar membrane (Rink et al. 2005). Newly formed phagosomes quickly become enriched in Rab5, and as such Rab5 is commonly used as a marker for early phagosomes (**Figure 3*b***). As the phagosome matures, Rab5 is replaced by Rab7 (**Figure 3*c***), the latter of which is a commonly used marker for late endosomes (**Figure 3*d***). Late endosomes eventually fuse with lysosomes to become phagolysosomes (**Figure 3*e***), in which the invading microbe is killed by low pH and a potent cocktail of degradative acid hydrolases.

3.2. Evasion and Exploitation of Eukaryotic Phagocytosis by Intracellular Pathogens

Intracellular pathogens have developed numerous strategies to escape or block the process of phagolysosome formation (Cornejo et al. 2017, Omotade & Roy 2019). Setting aside the

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

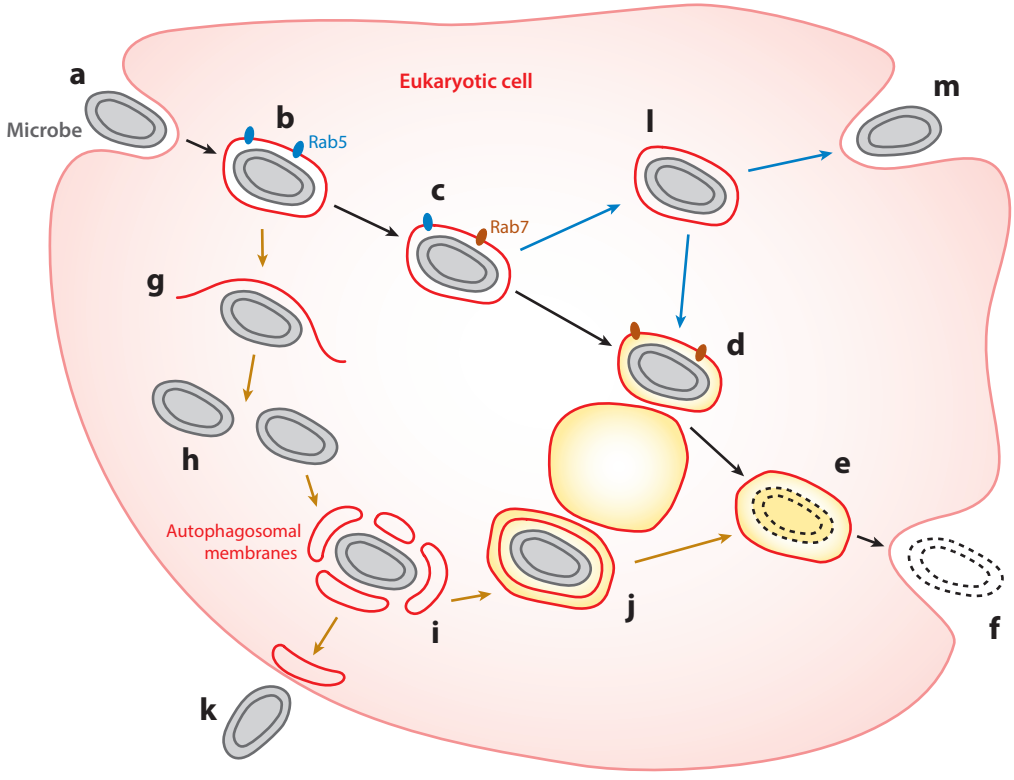

Figure 3

Routes of passage and persistence of microbes through host cells. (*a*) A microbe (*gray*) is recognized by a eukaryotic cell (*red*). (*b*) The nascent phagosome acquires Rab5 (*blue ovals*) on the surface of the vesicle. (*c*) Rab5 is replaced by Rab7 (*brown oval*) during maturation to a late phagosome. (*d*) Lysosomal fusion occurs and (*e*) destroys the phagocytosed microbe. (*f*) In some cell types, unused cell debris is exocytosed. (*g*) Some intracellular microbes escape the phagosome to (*h*) replicate in the host cytoplasm. (*i*) These microbes can be enveloped by autophagosomal membranes and (*j*) delivered to lysosomes in double-membrane-bound vesicles for destruction. (*k*) Some microbes can hijack the autophagy machinery to force their exit from the host cell without killing it. (*l*) Many intracellular microbes remodel the phagosome to avoid phagolysosome formation. (*m*) In some cases, intracellular microbes can be released intact from the host cell in a process called vomocytosis.

mechanisms used by bacteria to form long-term chronic infections (see the sidebar titled Chronic Bacterial Infections), these avoidance strategies can be broken down into two main types depending on their cellular location: cytoplasmic and vacuolar. Cytoplasmic microbes such as *Shigella flexneri* and *Listeria monocytogenes* escape the phagosomal vacuole to replicate free in the cytoplasm (Deretic & Levine 2009, Ray et al. 2009) (**Figure 3*g,h***). After vacuolar escape, cytoplasmic pathogens must also evade the autophagy system of the host to avoid destruction in autolysosomes (Jo et al. 2013) (**Figure 3*i,j***). But most intracellular pathogens, such as *Salmonella enterica* (Steele-Mortimer 2008), *Brucella* spp. (Celli 2019), *Chlamydia trachomatis* (Rother et al. 2019), and *Mycobacterium tuberculosis* (Russell 2001), spend all or most of their intracellular life inside of a modified host vacuole (**Figure 3*l***). No matter their primary cellular location, intracellular pathogens are unified by their manipulation of conserved eukaryotic factors involved in phagosomal maturation and stability, autophagy, and the secretory pathway (Anand et al. 2020, Case et al. 2016, Fairn & Grinstein 2012, Omotade & Roy 2019).

Autophagy: the process by which eukaryotic cells internalize organelles and microbes in membranous compartments for destruction and recycling

CHRONIC BACTERIAL INFECTIONS

Because host-beneficial endosymbionts (HBEs) form long-term intracellular infections, it is tempting to look for similarities between HBEs and pathogenic bacteria that form chronic, long-term infections in hosts. Bacteria such as *Mycobacterium tuberculosis* and *Salmonella typhi* can form chronic intracellular infections in humans by—among other tricks to evade the host immune system such as modifying their cellular envelope—hiding out in macrophages where the bacteria seem to replicate slowly or perhaps not at all (Monack et al. 2004, Pieters 2008, Ruby et al. 2012, Young et al. 2002). In the case of *M. tuberculosis*, infected macrophages are often sequestered into specialized tissues called granulomas where they can persist for up to the lifetime of the individual (Pieters 2008). While these long-term infections share some features with HBEs, such as the ability to persist inside of host phagosomes, they differ in that hosts require HBEs to be intracellularly active so that the HBE can perform their host-beneficial functions. Whereas bacteria that form chronic infections use the intracellular niche of a macrophage to stealthily hide from the host in near-dormant states, hosts that maintain HBEs require robust growth of their intracellular microbes to extract their beneficial goods.

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

Intracellular pathogens manipulate their host cells through the production and secretion of effector proteins (Alix et al. 2011, Green & Mecsas 2016). Effectors are injected into the host cytoplasm using several types of syringe-like secretion systems that are classified into different types based on their structure, taxonomic distribution, and cargo specificity (Green & Mecsas 2016). Here I briefly highlight the type III secretion system (T3SS) as an example, because it is found in a wide variety of both pathogenic and host-beneficial intracellular bacteria (Büttner 2012, Dale et al. 2002, de Souza Santos & Orth 2020, Degnan et al. 2009). T3SSs are typically encoded by a conserved cluster of 20 to 30 genes that likely originated from genes used to build the flagellar apparatus in bacteria (Abby & Rocha 2012). The T3SS complex spans the inner and outer membranes of gram-negative bacteria and selectively injects tens to hundreds of proteins into the cytoplasm of its target eukaryotic cell to alter or kill it (Galán & Waksman 2018).

Because intracellular pathogens manipulate the ancient and conserved phagocytic pathway, a single type of pathogenic bacterium can often infect multiple host cell types in a single animal or even infect cells of completely different eukaryotic hosts. For example, *Legionella pneumophila* can kill both amoebas and human monocytes by remodeling its phagosomal vacuole to appear more endoplasmic reticulum–like and avoid lysosome fusion (Prashar & Terebiznik 2015, Swanson & Hammer 2000). Similarly, fundamental cell biological mechanisms involved in intracellular infection of the human pathogens *M. tuberculosis* and *L. monocytogenes* were discovered using genetic screens in *Drosophila* cell lines (Cheng et al. 2005, Philips et al. 2005).

3.3. Escape Strategies for Intracellular Pathogens

A successful pathogen must have a way to escape its host cell so that it can continue to infect new cells. The intracellular escape strategies of microbial pathogens fall into two main types (Flieger et al. 2018, Hybiske & Stephens 2008). The first strategy overwhelms and kills the host cell, via either the induction of programmed cell death or other indirect means. The second strategy does not kill the host cell but rather involves the ejection of the microbe by some form of extrusion or budding (which leaves the microbe in at least one host-derived membrane) or by ejection, exocytosis, or other nonlytic exocytosis-like mechanisms (Alvarez & Casadevall 2006, Di Venanzio et al. 2017, Jacobovitz et al. 2021, Ma et al. 2006, Seoane & May 2020). For my purposes here, I focus on pathogen escape strategies that leave both the host cell and microbe alive, because I suspect these nonlytic strategies are the most likely to be used by the HBEs I outline in Section 4. Known

pathogenic strategies for nonlytic host cell escape can be broken down into two types, depending on whether the pathogen replicates in the cytoplasm or in a host-derived vacuole. *M. tuberculosis* is predominantly a vacuolar resident but cytoplasmic growth has been observed (Jamwal et al. 2016, van der Wel et al. 2007). Cytoplasmic *Mycobacterium* have been shown to use the autophagic pathway to nonlytically escape their host cell in a structure called the injectosome (Gerstenmaier et al. 2015, Hagedorn et al. 2009) (**Figure 3*k***). Pathogens that reside in host vacuoles can use exocytic pathways for nonlytic escape from host cells, a process sometimes referred to as vomocytosis (Seoane & May 2020) (**Figure 3*m***). While vomocytosis is often used to describe the escape of fungal pathogens (Alvarez & Casadevall 2006), it—or a process like it—also occurs with intracellular bacterial pathogens (Chen et al. 2004, Di Venanzio et al. 2017, White et al. 2017). For the purpose of this review, I call any nonlytic escape by a vacuolar microbe vomocytosis, even though the mechanisms of escape through conventional exocytosis and vomocytosis differ across eukaryotic cell types (Watkins et al. 2018).

Vomocytosis: a type of exocytosis of microbial cells out of eukaryotic cells that leaves both host and microbe intact and alive

4. HOST-BENEFICIAL BACTERIAL ENDOSYMBIONTS: FROM PATHOGENS TO ORGANELLES

The reproducible genomic patterns observed in bacterial endosymbionts of various ages (**Figure 2**) suggest the potential for convergent cellular mechanisms of HBE integration. The limited number of mechanisms available for microbial entry into, persistence within, and escape from eukaryotic cells (**Figure 3**) suggest that the manipulation of endocytic and exocytic processes is the mechanistic funnel through which convergent HBE evolution is expressed. While the general mechanisms used by pathogens, mitochondria, and plastids to allow persistence in host cells are understood, relatively little is known about how bacteria that are not classified as pathogens or organelles persist in host cells. Like pathogens, HBEs interact with eukaryotic phagocytosis and autophagy systems but differ in that their hosts are strongly inclined to retain—rather than destroy—their endosymbiotic microbes. Like mitochondria and plastids, some HBEs are very old, genetically integrated with their hosts, and faithfully transmitted from mother to offspring every generation, but they have not yet quite become permanent parts of their host cells.

The range of eukaryotic organisms that host HBEs, and the functions that these HBEs provide to their hosts, is enormous. The diversity of host–HBE interaction types, outcomes, durations, and cellular locations is so large that trying to establish themes or rules for these interactions is difficult. While I nevertheless try to extract themes and rules, be aware that a counterpoint exists for almost every declarative state I make here.

Putting aside mitochondria and plastids, compiling a list of eukaryotic groups that do not host HBEs is easier than making one of those that do: Vertebrates, by and large, do not seem to establish intracellular relationships with microbes that benefit them. However, HBEs can be found in nearly every other eukaryotic group. Leguminous plants host intracellular bacteria that fix nitrogen (Young & Johnston 1989). Protists—which represent the bulk of eukaryotic diversity but are also the least-studied type of eukaryote (Keeling & Burki 2019)—have numerous and diverse HBEs (George et al. 2020, Graf et al. 2021, Nowack & Melkonian 2010). Plant and animal parasitic nematodes host bacterial HBEs that enable their parasitism (Brown et al. 2015, Kozek 1977). Corals host photosynthetic endosymbiotic algae that help power their reef building (Muscatine & Porter 1977). Insects, which represent the bulk of animal species, host a proportionately gigantic variety of microbial HBEs (Baumann 2005, Buchner 1965, Moran et al. 2008). Some fungi have even been shown to host intracellular bacteria (Bianciotto et al. 1996, Pawlowska et al. 2018). Because of this immense diversity, I do not attempt to review every type of host–microbe endosymbiosis. Rather, I focus on several systems that highlight one or more of the key steps in

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

HBE biology. My examples are biased toward insect–bacterial systems because their incredible diversity of HBE interaction types range in age from those that have been established extremely recently (and reflect possible pathogenic origins) to those that are hundreds of millions of years old (and reflect possible organellar ends).

Symbiosomal membrane: a host-derived membrane that surrounds vacuolar host-beneficial endosymbionts and regulates molecular exchange between the host and symbiont

Bacteriocyte: a specialized cell in an animal that houses host-beneficial endosymbionts

Bacteriome: a specialized tissue in an animal composed of bacteriocytes and other supporting cells

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

4.1. Case Study: Aphids and Their Nutritional Host-Beneficial Endosymbiont *Buchnera*

Aphids are sap-feeding insects that house intracellular bacteria to supplement their nutrient-poor diets (Lamelas et al. 2011, Moran et al. 2003, Shigenobu et al. 2000). Like all animals, aphids cannot make half of the amino acids—the essential amino acids—and these essential amino acids are not present at high levels in their plant sap diets. In pea aphids, essential amino acids are synthesized by an HBE of gammaproteobacterial origin called *Buchnera aphidicola* (Shigenobu et al. 2000). Each *Buchnera* cell retains the two membranes of its ancestral gram-negative envelope and is surrounded by a third host-derived symbiosomal membrane in adult aphids (Baumann et al. 1995). This symbiosomal membrane is known to be important in the regulation and exchange of nutrients between the host and symbiont (Wilson 2020). *Buchnera* are not found in all aphid cells but are restricted to specialized cells called bacteriocytes, which aggregate to form specialized tissues called bacteriomes (**Figure 4**).

The symbiosomal membrane is not present at all stages of the aphid life cycle. [The aphid life cycle is complex, containing an asexual phase in which females produce genetic clones of themselves and a sexual phase more typical of animal reproduction (Moran 1992). While the transmission of *Buchnera* differs between the asexual and sexual life cycles (Braendle et al. 2003, Szklarzewicz & Michalik 2017, Wilkinson et al. 2003), here I outline the sexual phase because it is more representative of HBE transmission in sap-feeding insects in general.] In adult aphids, *Buchnera* cells are surrounded by the three lipid bilayers (Baumann et al. 1995). As female aphids age, *Buchnera* cells are released from bacteriocytes in a vomocytosis-like process (Koga et al. 2008) that results in the loss of the symbiosomal membrane. These *Buchnera* cells then migrate to openings at the posterior end of eggs, where they are held in a tightly clustered ball of cells (Buchner 1965, Koga et al. 2008, Szklarzewicz & Michalik 2017) (**Figure 4*b,c,e***). (*Buchnera* cells in the symbiont ball are once again free-living with respect to their ancestral gram-negative diderm structure.) As aphid embryos develop, the cells that are fated to become new bacteriocytes endocytose *Buchnera* cells, whereby they regain their symbiosomal membrane (**Figure 4*f***). However, not all *Buchnera* cells are transmitted to the next generation through migration to eggs (Wilkinson et al. 2003). Excess *Buchnera* cells in aging aphids are destroyed by the host in a complex process culminating in Rab7 recruitment to the symbiosomal membrane and the destruction of *Buchnera* through lysosomal activity (Hinde 1971, Nishikori et al. 2009, Simonet et al. 2018).

The life cycle of *Buchnera* shares many similarities to the intracellular biology of pathogens, and so it seems worthwhile to review the process from a pathogen-centric view (**Figure 3**). In a new aphid embryo, *Buchnera* cells are endocytosed into bacteriocytes and gain a vacuolar membrane (e.g., **Figure 3*a,b***). At some unknown point and in some unknown way, the normal process of phagolysosome formation is diverted or stalled, and *Buchnera* resides safely in the symbiosomal membrane (**Figure 3*l***) for most of the life cycle of the insect. When *Buchnera* cells are needed for transmission to the next generation, some *Buchnera* cells are vomocytosed (**Figure 3*m***). Excess *Buchnera* cells that are not needed for transmission to eggs gain Rab7 markers on their symbiosomal membrane and rejoin the process of phagolysosome maturation for destruction and recycling (**Figure 3*d,e***). The main difference between an HBE such as *Buchnera* and a pathogen is that in aphids the host is now incentivized (indeed, required) to remodel the phagosomal membrane to

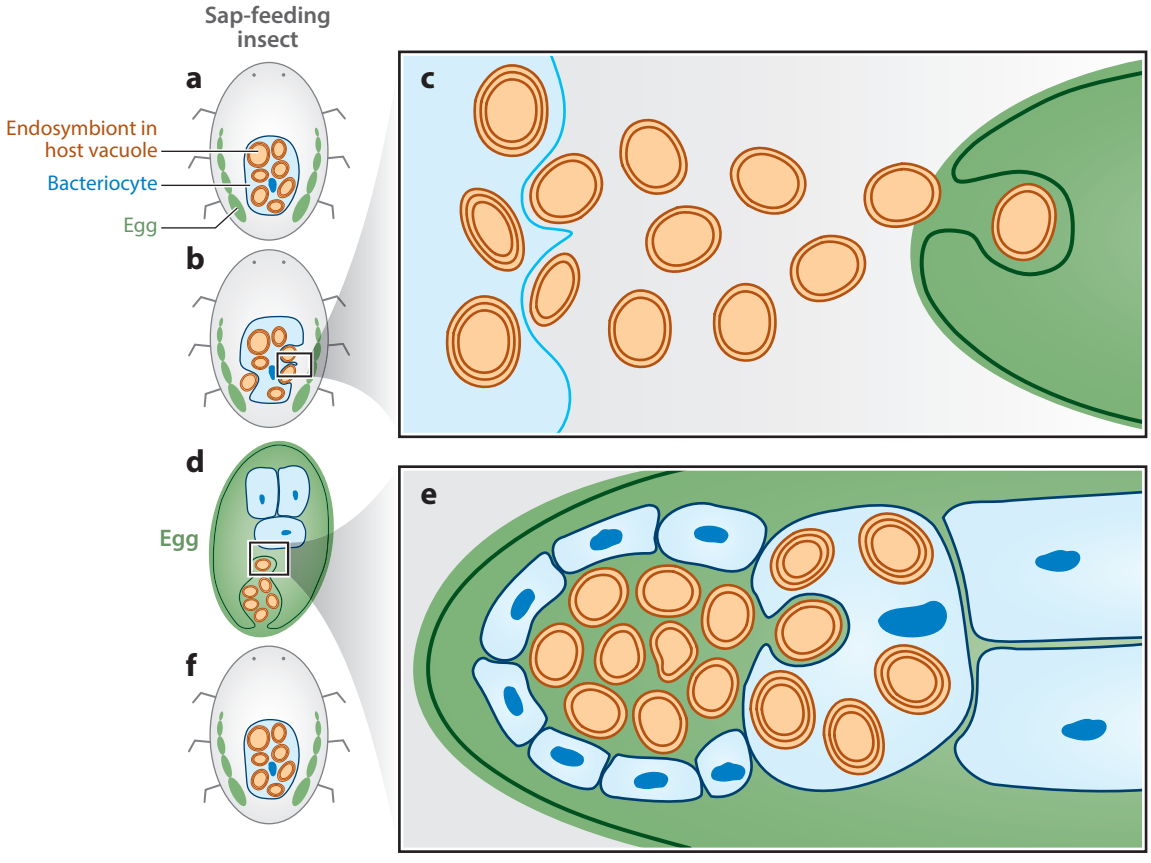

Figure 4

The transmission of host-beneficial endosymbionts (HBEs) in sap-feeding insects. (*a*) A female adult sap-feeding insect (*gray*) contains a bacteriocyte (*blue*) with HBEs contained within a host vacuole (*orange*). (*b*) At the appropriate developmental stage, HBEs are vomocytosed out of the bacteriocyte, lose their host membrane, and migrate to the (*c*) egg (*green*) to be held in an extracellular symbiont ball. (*d*) During embryo development, (*e*) HBEs are phagocytosed into the cell fated to become the new bacteriocyte and regain the host vacuolar (or symbiosomal) membrane again as (*f*) adult insects.

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

avoid HBE destruction, at least until the host is old enough that the bacteria are no longer required. Because the aphid host seems to have the ability to destroy most of its *Buchnera* population, and because *Buchnera* has lost most of its traditional pathogenic pathways during genome reduction, the remodeling of HBE-containing vacuoles may be more of a host-dominated process than an act of microbial manipulation (of course, *Buchnera* cells likely still possess molecular signals indicating their beneficial nature). In this view, the delay of microbial destruction in phagolysosomes is not a trick played on the host but rather a deliberate act by the host to exploit its microbe for nutrients. No matter the evolutionary forces at play, overall these limited data suggest that the end result of *Buchnera* infection is modification and subversion of the classic endocytic pathway.

The transmission strategy outlined in **Figure 4** is not unique to aphids. While the details of timing and cell migration patterns vary widely in sap-feeding insects, the vast majority of their HBEs go through at least one cycle of endocytosis and exocytosis during transmission (Szklarzewicz & Michalik 2017). Indeed, the pattern of somatic to germline cell transfer seems to be a widely conserved mechanism across vertically transmitted endosymbionts (Russell et al.

2019). Even *Wolbachia*, the famous germline-associated reproductive manipulator of invertebrates (Werren et al. 2008), undergoes cycles of exocytosis and endocytosis as it moves from somatic to germline cells in insects and nematodes (Landmann 2019, Landmann et al. 2012, White et al. 2017).

4.2. Case Study: Pathogens as the Source of Host-Beneficial Endosymbionts

Although insect HBEs have long been known to come from groups of bacteria that include animal pathogens (Baumann et al. 2006, Moran et al. 2005, Unterman et al. 1989), the sometimes extreme levels of genome reduction in host-associated bacteria (**Figure 2*c***) have made the precise nature of the free-living antecedents of HBEs difficult to infer. Genes retained in old HBEs with small genomes tell you what they do for their host, but not much about how they got to be that way (McCutcheon & Moran 2012, Moran & Bennett 2014). From a comparative perspective, understanding the transition from free-living bacterium to HBE requires catching the process in the early stages of establishment.

Several genera of insect-associated bacteria have members that have been isolated across the spectrum of integration shown in **Figure 2**: *Arsenophonus* (Bressan 2014, Nováková et al. 2009), *Sodalis* (Clayton et al. 2012, McCutcheon et al. 2019), *Serratia* (Moran et al. 2005, Petersen & Tisa 2013), and *Symbiopectobacterium* (Martinson et al. 2020) (hereinafter collectively referred to as the ASSS bacteria). ASSS bacteria are all in the Enterobacteriaceae family within Gammaproteobacteria, which also contains pathogens such as *Escherichia*, *Salmonella*, *Klebsiella*, and *Pantoea* (Hauben et al. 1998, Husnik et al. 2011, Williams et al. 2010). Bacteria in the Enterobacteriaceae are usually associated with plants and animals, often as facultative intracellular pathogens or HBEs, and the genomes of free-living, pathogenic, or newly host-restricted strains often encode one or more types of secretion system (e.g., T3SS) to manipulate host cells (Bengoechea & Sa Pessoa 2019, Dale et al. 2002, Degnan et al. 2009, Husnik et al. 2011, Nováková et al. 2009, Ohl & Miller 2001, Petersen & Tisa 2013, Walterson & Stavrinides 2015). ASSS bacteria are known to be pathogens of plants, vectored by insects (Bressan 2014); to be pathogens of insects, vectored by plants (Pons et al. 2019a); or to have gene inventories and mechanisms suggesting they may be both (Clayton et al. 2012, Munoz et al. 2020).

One of the clearest examples of a very recent transition from a free-living state to a host-restricted state comes from the *Sodalis* genus. Members of this genus have been identified as HBEs in a huge diversity of insects and have been found across the integration spectrum shown in **Figure 2** (McCutcheon et al. 2019). The size of *Sodalis* HBE genomes ranges from the tiny 0.35 Mb genome of *Mikella endobia* (Husnik & McCutcheon 2016) to the 4.5 Mb genome of *Sodalis* SOPE (Oakeson et al. 2014). Remarkably, a free-living version of *Sodalis* called *Sodalis praecaptivus* was serendipitously isolated from a nonhealing wound in a man who had been impaled with a dead crab apple tree branch (Clayton et al. 2012). A comparison of the *S. praecaptivus* and *Sodalis* SOPE genomes revealed that over 3 Mb of these two genomes were alignable at >99% sequence identity (Clayton et al. 2012). This suggests an extremely recent transition of a *S. praecaptivus*–like ancestor into an insect, an event that likely occurred on the order of tens of thousands of years ago (Clayton et al. 2012). What sort of lifestyle does *S. praecaptivus* have before it transitions to an HBE? Like many pathogens, its genome encodes T3SS systems and has other genes indicative of an ability to be at least a facultative pathogen (Clayton et al. 2012). *S. praecaptivus* contains a quorum-sensing system that turns off virulence genes when the bacterium reaches high titers in insects, suggesting a mechanism that *S. praecaptivus* may use to infect insects without killing or overwhelming them (Enomoto et al. 2017). Consistent with this idea, quorum-sensing mutant strains of *S. praecaptivus* make infected insects sicker than does the native *S. praecaptivus* strain (Munoz et al. 2020).

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

A similar situation has been described for bacteria in the *Serratia* genus (Manzano-Marín & Latorre 2016, Petersen & Tisa 2013). *Serratia* relatives have been isolated across a spectrum from human pathogens (Iguchi et al. 2014) to nutritional HBEs with reduced genomes (Lamelas et al. 2011, Manzano-Marín & Latorre 2014, Perreau et al. 2021). Some insect pathogenic strains infect the guts of insects and are culturable in the lab (Pons et al. 2019b, Renoz et al. 2019). Recently, pathogenic strains of *Serratia* have been shown to be endocytosed into aphid embryos and later migrate into specialized cells meant for HBEs, but *Escherichia coli* cells are not (Perreau et al. 2021). However, while the aphid–*Serratia* interaction is specific in the sense of cellular recognition and endocytosis, insects that take up pathogenic *Serratia* strains are eventually killed by the infection (Perreau et al. 2021). This suggests that other, less pathogenic strains may be the source of *Serratia* HBEs, or that the routes of intracellular infection in nature are not as detrimental to the insects in some contexts.

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

4.3. Case Study: Nitrogen-Fixing Host-Beneficial Endosymbiont Bacteria in Leguminous Plants

Legumes are important plants for humans as a source of nutrient-rich foods (soybeans, beans, chickpeas, and others) and for their ability to grow in nitrogen-poor soil (Zahran 1999). Their ability to fix atmospheric N_2 into biologically accessible nitrogen compounds comes from an endosymbiosis between plant root cells and rhizobial bacteria (Oldroyd et al. 2011). The plant–rhizobia endosymbiosis is a well-understood host–HBE relationship at the cell biological level and is notable for the clear links it provides between the pathogenic and host-beneficial lifestyles.

Soil is teeming with bacteria and other microbes (Fierer 2017), but only a small number of bacteria from the classes Alphaproteobacteria and Betaproteobacteria are able to form endosymbioses with legume root cells. This symbiosis is established through a complex interplay of host and symbiont signals; successful endosymbiosis results in bacterial cells that are enclosed in host-derived membranes (called the symbiosomal membrane, as in aphids) where N_2 fixation takes place (Jones et al. 2007, Oke & Long 1999, Oldroyd et al. 2011, Sachs et al. 2018, Young & Johnston 1989). Cell biological parallels between rhizobial bacteria and pathogens have long been noted (Deakin & Broughton 2009, Djordjevic et al. 1987, Jones et al. 2007, Kambara et al. 2009, Soto et al. 2006). In fact, rhizobia have been called the "sophisticated parasite" by authors (Djordjevic et al. 1987, p. 146) noting the shared strategies that rhizobia and pathogens take to infect cells. These shared strategies include the use of protein secretion systems (including T3SS and T4SS) to alter the biology of infected cells (Kambara et al. 2009), the modification of bacterial surface LPSs to evade the immune system and allow chronic infections of rhizobia in host cells (Ferguson et al. 2004, Glazebrook et al. 1993), and the use of quorum sensing to coordinate bacterial growth and gene expression (Loh et al. 2002, Soto et al. 2006). Finally, a close phylogenetic relationship between rhizobia and intracellular pathogens such as *Brucella abortus* has been noted (Jones et al. 2007, Leclercq et al. 2019).

4.4. Case Study: Intracytoplasmic Host-Beneficial Endosymbionts in Insects

Just as pathogens exist as vacuolar or cytoplasmic forms (**Figure 3**), different HBEs are found in host-derived vacuoles (**Figure 3*l***) or free in the cytoplasm (**Figure 3*b***). Rhizobia, *Buchnera*, and *Wolbachia* all reside in host-derived symbiosomal membranes, but many other insects host HBEs that primarily exist as cytoplasmic associates. Weevils have cytoplasmic HBEs that provide key nutrients involved in cuticle production (Nardon et al. 2003), and, in some weevil species, this HBE is the *Sodalis* SOPE strain introduced in Section 4.2 (Oakeson et al. 2014, Toju et al. 2013). Weevils retain a somatic cell population of bacteria that grows quickly to produce nutrients in young insects

when cuticle production and hardening is needed, and a germline cell population that is used to transmit bacteria to the next generation (Vigneron et al. 2014). Somatic *Sodalis* cells are destroyed by autophagy (e.g., **Figure 3*i,j***) when no longer needed by their hosts, but germline bacteria are retained by the weevil (Masson et al. 2015, Vigneron et al. 2014). Retained bacteria need to leave host cells and infect new ones to be transmitted to the next generation, but how this happens is unclear. Transcriptome experiments show that *Sodalis* SOPE express T3SS genes when infecting new cells, suggesting that they retain a pathogen-like ability to remodel new host cells upon infection (Maire et al. 2020). Similarly, carpenter ants have a nutritional HBE called *Blochmannia* that primarily exists as a cytoplasmic resident (Schröder et al. 1996). Like *Sodalis* SOPE in weevils, *Blochmannia* levels have been proposed to be regulated by cell-specific host autophagy (Gonçalves et al. 2020, Kupper et al. 2016, Stoll et al. 2010). The mechanism of *Blochmannia* cell-to-cell spread is also unknown, but these bacteria are seen in vacuoles during oocyte transfer, and host endocytic and exocytic pathways have been proposed as possible mechanisms of host cell transfer (Kupper et al. 2016).

Trogocytosis: the process of sharing membrane or cytoplasmic material, including microbes, between two temporarily adjoined eukaryotic cells

The mechanisms used by cytoplasmic HBEs to escape donor cells and infect recipient host cells are unknown. In some cases, the host donor cell might be destroyed or ruptured by apoptotic mechanisms (Lawen 2003) to allow bacterial escape and phagocytosis by the recipient host cell, although this approach seems a sloppy and inelegant solution in the otherwise tightly regulated process of HBE transmission. Some cytoplasmic bacterial pathogens such as *Listeria* and *Shigella* use actin-based motility to force pathogen-containing protrusions to be internalized by neighboring cells (Ireton 2013). Other cytoplasmic and vacuolar pathogens might use autophagy-related mechanisms to enable their exocytosis (Checroun et al. 2006, Starr et al. 2012) or ejection (Gerstenmaier et al. 2015) from donor cells (e.g., **Figure 3*i,k***). Finally, direct cell-to-cell bacterial spread has been observed in the cytoplasmic pathogen *Francisella tularensis* in a process called trogocytosis (Steele et al. 2016). In trogocytosis, components of the plasma membrane or cytoplasm are transferred between neighboring cells through direct but temporary cell membrane fusion (Dance 2019, Joly & Hudrisier 2003). Trogocytosis may be a way for cytoplasmic (or vacuolar) HBEs to move from cell to cell when host donor and recipient cells are able to physically touch one another.

4.5. Case Study: Photosynthetic Host-Beneficial Endosymbionts

Cyanobacteria were the first organisms to evolve the ability to fix CO_2 into organic molecules using light energy (Soo et al. 2017). The first plastid-bearing eukaryotes harnessed this ability by acquiring a cyanobacterium as an HBE, which over time evolved into the plastid (Rodríguez-Ezpeleta et al. 2005). *P. chromatophora* is an amoeba that has secondarily acquired photosynthesis in an event that strongly parallels the original plastid acquisition (Bhattacharya et al. 2012, Marin et al. 2005). This endosymbiotic cyanobacterium, now called the chromatophore, was established about 100 million years ago (Delaye et al. 2016) and has a genome that has experienced a reduction in size compared with those of free-living cyanobacteria (Nowack et al. 2008). Like mitochondria and plastids, large numbers of host proteins are transported into the chromatophore to compensate for HBE gene loss (Nowack & Weber 2018, Nowack et al. 2016, Singer et al. 2017). While the precise organization of the chromatophore cell envelope is not entirely clear, it seems to be surrounded by a host-derived (presumably phagocytic) membrane (Bhattacharya et al. 2012, Kies 1974, Sato et al. 2020). This is in contrast to the plastid resulting from the endosymbiosis of the ancestor of plants (the chloroplast), which is cytoplasmic and not in a vestigial phagocytic membrane (McFadden 2001).

Paramecium bursaria is a ciliate that, similar to *Paulinella*, has secondarily acquired photosynthetic capabilities through endosymbiosis, except in this case it was the acquisition of a green alga

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

rather than a cyanobacterium that endowed the host with light-harvesting abilities (Siegel 1960). Also differentiating this symbiosis from that of *Paulinella* is the ability of both host *Paramecium* and its endosymbiont [usually a species of *Chlorella* (Hoshina & Imamura 2008)] to grow independently of each other. This symbiosis can thus be considered facultative rather than obligate from the host perspective: Many algae are digested (rather than retained as a photosymbiont) when the symbiosis is reestablished in the lab (Karakashian & Karakashian 1973), and the host is able to control the algal symbiont load depending on how much light is available (Lowe et al. 2016). Successful endosymbionts are engulfed by phagocytosis, but the hosts are somehow able to remodel alga-containing vacuoles into stable compartments that evade fusion with lysosomes (Kodama & Fujishima 2005, 2010).

Cnidarians (corals, anemones, and jellyfish) establish intravacuolar symbioses with photosynthetic algae in the genus *Symbiodinium* for the provisioning of photosynthate and other nutrients (Kazandjian et al. 2008). The engulfment of *Symbiodinium* by its host occurs through phagocytosis, in which the HBE is retained in a stalled phagosome that has somehow remodeled its host-derived membrane to evade phagolysosome formation (Davy et al. 2012, Mohamed et al. 2016). Anemones that phagocytose *Symbiodinium* create a phagocytic vacuole that rapidly becomes Rab5-positive but excludes Rab7, a process that is disrupted with heat-killed *Symbiodinium*, suggesting that vacuole remodeling is at least in part an active process by the endosymbiont (Chen et al. 2003). Recent work has shown that discrimination between beneficial and pathogenic microbes occurs after phagocytosis, in which nonbeneficial microbes are expelled from anemone cells by vomocytosis rather than being destroyed in phagolysosomes (Jacobovitz et al. 2021).

The *Paulinella*, *Paramecium*, and cnidarian examples all use (or used) phagocytosis to internalize their endosymbionts. In contrast to the insect and plant examples described above, however, there are no known pathogenic close relatives to the cyanobacterium or algae that become HBEs in these systems. This suggests that the signals produced by the nascent photosynthesizing HBEs during establishment to avoid destruction in phagolysosomes may be different from those produced by pathogens, such as effectors secreted by secretion systems. The pathogenic abilities of these photosynthesizing microbes could be presently underappreciated, or the engulfed microbes could use other indirect mechanisms to punish overly ambitious host digestion (Jenkins et al. 2021). However, the hosts of these photosynthesizing HBEs may have also established surveillance systems to screen for endocytosed microbes that produce a benefit, and this signal may in part be used to stall phagolysosome formation.

5. CONCLUSIONS

The idea that host-beneficial bacteria often have pathogenic origins, and that both HBEs and pathogens may use common molecular mechanisms to interact with their host cells, is not new (Dale et al. 2002, Ewald 1987, Goebel & Gross 2001, Hentschel et al. 2000, Jones et al. 2007, Kambara et al. 2009, Sachs et al. 2011). Much of the previous work, especially in insects, has focused on the similarities between the microbial players: that both HBEs and pathogens encode secretion systems, that they both encode quorum-sensing systems, that their genomes show shared and reproducible patterns, and that they often group together in phylogenetic trees. What I have tried to highlight here is that the pathogen-to-HBE transition is not distinct from but is rather continuous with the HBE-to-organelle transition at the genomic level, and that the host processes of endocytosis, exocytosis, and autophagy are likely central to understanding how HBEs become established in, persist in, and are transmitted between host cells. In all of these systems, a key outstanding point to address is how cell-in-cell relationships often arising from unstable antagonisms shift toward contexts that allow HBE formation. While this transition probably occurs rapidly in evolutionary time and likely involves complex combinations of ecological factors that flip the

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

cost-benefit ratio toward one favoring infection, it must take place inside of a host cell and so must involve host cellular processes. Increased attention on HBE biology may therefore reveal general cell biological mechanisms of interest to both pathogen and organelle biologists.

SUMMARY POINTS

1. Bacteria that adopt intracellular lifestyles experience genome reduction. Genes that are retained reflect the bacterium's role in the endosymbiosis: Pathogens retain genes to replicate themselves and infect and manipulate hosts, while host-beneficial endosymbionts (HBEs) retain genes to replicate themselves and provide host services. In some cases, the retained gene sets overlap (e.g., type III secretion systems).
2. HBEs that remain in host cells for long periods of time can lose more than 95% of their genes compared with free-living relatives. The genome sizes of some long-established HBEs overlap with mitochondria and plastids in terms of genome size, gene number, and cellular and genetic integration with their host cells.
3. While HBEs and pathogens both require and exploit the same host cell biological pathways (endocytosis, exocytosis, and others) to complete their life cycles, they differ in outcome. Host cells fight pathogens but enable HBEs. That they both use the same host cell biological pathways suggests that transitions between the pathogen and HBE states could be rapid and driven by subtle changes in host needs, host environment, and the signals sent from the infecting microbe to the host.
4. Phagocytosis first evolved in eukaryotes as a mechanism to gain nutrients through microbial digestion, but, in some cases, eukaryotic cells can domesticate phagocytosed microbes to create a type of live nutritional farming. The ability of eukaryotes to survey phagocytotic prey for benefit and to stall prey digestion in phagolysosomes if a benefit is sensed seems to play an important role in photosymbioses but may also be important in other systems.
5. Phagocytosis was secondarily adopted for host protection through the evolution of professional microbe-digesting immune cells such as macrophages and neutrophils. Microbes digested in professional immune cells have mechanisms, such as the production of host-modifying effector proteins, to block their destruction in phagolysosomes, and these stalling mechanisms may also be used in the establishment of HBEs.

FUTURE ISSUES

1. How do the mechanisms of intracellular persistence—such as escaping the host vesicle and stalling phagolysosome formation—differ between pathogens and HBEs?
2. How do the mechanisms of cellular integration—such as the transferral of genes and the importation of host proteins—differ between HBEs and organelles?
3. What are the lifestyles of microbes that are prone to becoming HBEs before they are HBEs? Why do some groups of microbes, such as bacteria from the *Sodalis* clade, form HBE relationships with eukaryotes over and over again, while others rarely form these types of relationships?

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

4. How do hosts differentiate between harmful and beneficial intracellular microbes? How is the balance of microbial farming versus microbial digestion determined in endosymbiosis?
5. What is the balance between intracellular HBE persistence through classical pathogenic means (e.g., secretion of effector proteins) versus persistence by sending their hosts signals of benefit (e.g., production of a nutrient)?
6. Could some pathogens delay host digestion by sending false signals (e.g., production of a nutrient), in the same way that some HBEs avoid digestion and promote farming through the use of classically pathogenic mechanisms?

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

I thank DeAnna Bublitz, Paul Caccamo, Colin Dale, Arkadiy Garber, Aziz Heddi, Patrick Keeling, Maria Kupper, Anna Michalik, Ewa Nowack, Tom Richards, Alastair Simpson, Joan Strassmann, and Bill Sullivan for interesting and helpful discussions on various aspects of this review. All errors of omission and interpretation are mine. I gratefully acknowledge support from the National Science Foundation (grant IOS-1553529), the Gordon and Betty Moore Foundation (grant GBMF5602), and the National Aeronautics and Space Administration Astrobiology Institute (grant NNA15BB04A) during the writing of this review.

LITERATURE CITED

Abby SS, Rocha EPC. 2012. The non-flagellar type III secretion system evolved from the bacterial flagellum and diversified into host-cell adapted systems. *PLOS Genet*. 8(9):e1002983

Alix E, Mukherjee S, Roy CR. 2011. Subversion of membrane transport pathways by vacuolar pathogens. *J. Cell Biol.* 195(6):943–52

Alvarez M, Casadevall A. 2006. Phagosome extrusion and host-cell survival after *Cryptococcus neoformans* phagocytosis by macrophages. *Curr. Biol.* 16(21):2161–65

Anand I, Choi W, Isberg RR. 2020. The vacuole guard hypothesis: how intravacuolar pathogens fight to maintain the integrity of their beloved home. *Curr. Opin. Microbiol.* 54:51–58

Archibald J. 2014. *One Plus One Equals One: Symbiosis and the Evolution of Complex Life*. Oxford, UK: Oxford Univ. Press

Barker J, Brown MR. 1994. Trojan horses of the microbial world: protozoa and the survival of bacterial pathogens in the environment. *Microbiology* 140(Part 6):1253–59

Batut B, Knibbe C, Marais G, Daubin V. 2014. Reductive genome evolution at both ends of the bacterial population size spectrum. *Nat. Rev. Microbiol.* 12(12):841–50

Baumann P. 2005. Biology of bacteriocyte-associated endosymbionts of plant sap-sucking insects. *Annu. Rev. Microbiol.* 59:155–89

Baumann P, Baumann L, Lai CY, Rouhbakhsh D, Moran NA, Clark MA. 1995. Genetics, physiology, and evolutionary relationships of the genus *Buchnera*: intracellular symbionts of aphids. *Annu. Rev. Microbiol.* 49:55–94

Baumann P, Moran NA, Baumann L. 2006. Bacteriocyte-associated endosymbionts of insects. In *The Prokaryotes*, Vol. 1: *Symbiotic Associations, Biotechnology, Applied Microbiology*, ed. M Dworkin, S Falkow, E Rosenberg, KH Schleifer, E Stackebrandt, pp. 403–38. New York: Springer. 3rd ed.

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

Bengoechea JA, Sa Pessoa J. 2019. *Klebsiella pneumoniae* infection biology: living to counteract host defences. *FEMS Microbiol. Rev.* 43(2):123–44

Bhattacharya D, Price DC, Yoon HS, Yang EC, Poulton NJ, et al. 2012. Single cell genome analysis supports a link between phagotrophy and primary plastid endosymbiosis. *Sci. Rep.* 2:356

Bianciotto V, Bandi C, Minerdi D, Sironi M, Tichy HV, Bonfante P. 1996. An obligately endosymbiotic mycorrhizal fungus itself harbors obligately intracellular bacteria. *Appl. Environ. Microbiol.* 62(8):3005–10

Booth A, Doolittle WF. 2015. Eukaryogenesis, how special really? *PNAS* 112(33):10278–85

Boulais J, Trost M, Landry CR, Dieckmann R, Levy ED, et al. 2010. Molecular characterization of the evolution of phagosomes. *Mol. Syst. Biol.* 6:423

Bozzaro S, Bucci C, Steinert M. 2008. Chapter 6 Phagocytosis and host–pathogen interactions in *Dictyostelium* with a look at macrophages. *Int. Rev. Cell Mol. Biol.* 271:253–300

Braendle C, Miura T, Bickel R, Shingleton AW, Kambhampati S, Stern DL. 2003. Developmental origin and evolution of bacteriocytes in the aphid-Buchnera symbiosis. *PLOS Biol.* 1(1):e21

Brelsfoard C, Tsiamis G, Falchetto M, Gomulski LM, Telleria E, et al. 2014. Presence of extensive *Wolbachia* symbiont insertions discovered in the genome of its host *Glossina morsitans morsitans*. *PLOS Negl. Trop. Dis.* 8(4):e2728

Bressan A. 2014. Emergence and evolution of *Arsenophonus* bacteria as insect-vectored plant pathogens. *Infect. Genet. Evol.* 22:81–90

Brown AMV, Howe DK, Wasala SK, Peetz AB, Zasada IA, Denver DR. 2015. Comparative genomics of a plant-parasitic nematode endosymbiont suggest a role in nutritional symbiosis. *Genome Biol. Evol.* 7(9):2727–46

Bublitz DC, Chadwick GL, Magyar JS, Sandoz KM, Brooks DM, et al. 2019. Peptidoglycan production by an insect-bacterial mosaic. *Cell* 179(3):703–12.e7

Buchner P. 1965. *Endosymbiosis of Animals with Plant Microorganisms*. New York: Intersci. Publ.

Burki F, Roger AJ, Brown MW, Simpson AGB. 2020. The new tree of eukaryotes. *Trends Ecol. Evol.* 35(1):43–55

Büttner D. 2012. Protein export according to schedule: architecture, assembly, and regulation of type III secretion systems from plant- and animal-pathogenic bacteria. *Microbiol. Mol. Biol. Rev.* 76(2):262–310

Casadevall A. 2008. Evolution of intracellular pathogens. *Annu. Rev. Microbiol.* 62:19–33

Casadevall A. 2012. Amoeba provide insight into the origin of virulence in pathogenic fungi. *Adv. Exp. Med. Biol.* 710:1–10

Case EDR, Smith JA, Ficht TA, Samuel JE, de Figueiredo P. 2016. Space: a final frontier for vacuolar pathogens. *Traffic* 17(5):461–74

Cavalier-Smith T. 2002. The phagotrophic origin of eukaryotes and phylogenetic classification of Protozoa. *Int. J. Syst. Evol. Microbiol.* 52(Part 2):297–354

Celli J. 2019. The intracellular life cycle of *Brucella* spp. *Microbiol. Spectr.* 7(2). **https://doi.org/10.1128/microbiolspec.BAI-0006-2019**

Checroun C, Wehrly TD, Fischer ER, Hayes SF, Celli J. 2006. Autophagy-mediated reentry of *Francisella tularensis* into the endocytic compartment after cytoplasmic replication. *PNAS* 103(39):14578–83

Chen J, de Felipe KS, Clarke M, Lu H, Anderson OR, et al. 2004. Legionella effectors that promote nonlytic release from protozoa. *Science* 303(5662):1358–61

Chen M-C, Cheng Y-M, Sung P-J, Kuo C-E, Fang L-S. 2003. Molecular identification of Rab7 (ApRab7) in *Aiptasia pulchella* and its exclusion from phagosomes harboring zooxanthellae. *Biochem. Biophys. Res. Commun.* 308(3):586–95

Cheng LW, Viala JPM, Stuurman N, Wiedemann U, Vale RD, Portnoy DA. 2005. Use of RNA interference in *Drosophila* S2 cells to identify host pathways controlling compartmentalization of an intracellular pathogen. *PNAS* 102(38):13646–51

Clayton AL, Oakeson KF, Gutin M, Pontes A, Dunn DM, et al. 2012. A novel human-infection-derived bacterium provides insights into the evolutionary origins of mutualistic insect-bacterial symbioses. *PLOS Genet.* 8(11):e1002990

Cornejo E, Schlaermann P, Mukherjee S. 2017. How to rewire the host cell: a home improvement guide for intracellular bacteria. *J. Cell Biol.* 216(12):3931–48

Cossart P, Roy CR, Sansonetti P. 2019. *Bacteria and Intracellularity*. Hoboken, NJ: John Wiley & Sons

Cossart P, Sansonetti PJ. 2004. Bacterial invasion: the paradigms of enteroinvasive pathogens. *Science* 304(5668):242–48

Dale C, Plague GR, Wang B, Ochman H, Moran NA. 2002. Type III secretion systems and the evolution of mutualistic endosymbiosis. *PNAS* 99(19):12397–402

Dance A. 2019. Core Concept: Cells nibble one another via the under-appreciated process of trogocytosis. *PNAS* 116(36):17608–10

Davy SK, Allemand D, Weis VM. 2012. Cell biology of cnidarian-dinoflagellate symbiosis. *Microbiol. Mol. Biol. Rev.* 76(2):229–61

De Bary A. 1879. *Die Erscheinung Der Symbiose* [The Phenomenon of Symbiosis]. Strasbourg, Fr.: Karl J. Triibner

de Souza Santos M, Orth K. 2020. The role of the type III secretion system in the intracellular lifestyle of enteric pathogens. In *Bacteria and Intracellularity*, ed. P Cossart, CR Roy, P Sansonetti, pp. 197–214. Hoboken, NJ: John Wiley & Sons

Deakin WJ, Broughton WJ. 2009. Symbiotic use of pathogenic strategies: rhizobial protein secretion systems. *Nat. Rev. Microbiol.* 7(4):312–20

Degnan PH, Yu Y, Sisneros N, Wing RA, Moran NA. 2009. *Hamiltonella defensa*, genome evolution of protective bacterial endosymbiont from pathogenic ancestors. *PNAS* 106(22):9063–68

Delaye L, Valadez-Cano C, Pérez-Zamorano B. 2016. How really ancient is *Paulinella chromatophora*? *PLOS Curr.* 8:ecurrents.tol.e68a099364bb1a1e129a17b4e06b0c6b

Deretic V, Levine B. 2009. Autophagy, immunity, and microbial adaptations. *Cell Host Microbe* 5(6):527–49

Di Venanzio G, Lazzaro M, Morales ES, Krapf D, Véscovi EG. 2017. A pore-forming toxin enables *Serratia* a nonlytic egress from host cells. *Cell. Microbiol.* 19(2):e12656

Djordjevic MA, Gabriel DW, Rolfe BG. 1987. Rhizobium—the refined parasite of legumes. *Annu. Rev. Phytopathol.* 25:145–68

Dodds PN, Rathjen JP. 2010. Plant immunity: towards an integrated view of plant-pathogen interactions. *Nat. Rev. Genet.* 11(8):539–48

Doherty GJ, McMahon HT. 2009. Mechanisms of endocytosis. *Annu. Rev. Biochem.* 78:857–902

Duchêne A-M, Giritch A, Hoffmann B, Cognat V, Lancelin D, et al. 2005. Dual targeting is the rule for organellar aminoacyl-tRNA synthetases in *Arabidopsis thaliana*. *PNAS* 102(45):16484–89

Dunning Hotopp JC, Clark ME, Oliveira DCSG, Foster JM, Fischer P, et al. 2007. Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. *Science* 317(5845):1753–56

Embley TM, Martin W. 2006. Eukaryotic evolution, changes and challenges. *Nature* 440(7084):623–30

Enomoto S, Chari A, Clayton AL, Dale C. 2017. Quorum sensing attenuates virulence in *Sodalis praecaptivus*. *Cell Host Microbe* 21(5):629–36.e5

Erken M, Lutz C, McDougald D. 2013. The rise of pathogens: predation as a factor driving the evolution of human pathogens in the environment. *Microb. Ecol.* 65(4):860–68

Ewald PW. 1987. Transmission modes and evolution of the parasitism-mutualism continuum. *Ann. N. Y. Acad. Sci.* 503:295–306

Fairn GD, Grinstein S. 2012. How nascent phagosomes mature to become phagolysosomes. *Trends Immunol.* 33(8):397–405

Ferguson GP, Datta A, Baumgartner J, Roop RM II, Carlson RW, Walker GC. 2004. Similarity to peroxisomal-membrane protein family reveals that *Sinorhizobium* and *Brucella* BacA affect lipid-A fatty acids. *PNAS* 101(14):5012–17

Fierer N. 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. *Nat. Rev. Microbiol.* 15(10):579–90

Flannagan RS, Jaumouillé V, Grinstein S. 2012. The cell biology of phagocytosis. *Annu. Rev. Pathol.* 7:61–98

Flieger A, Frischknecht F, Häcker G, Hornef MW, Pradel G. 2018. Pathways of host cell exit by intracellular pathogens. *Microb. Cell Fact.* 5(12):525–44

Galán JE, Waksman G. 2018. Protein-injection machines in bacteria. *Cell* 172(6):1306–18

Galperin MY, Wolf YI, Garushyants SK, Vera Alvarez R, Koonin EV. 2021. Non-essential ribosomal proteins in bacteria and archaea identified using COGs. *J. Bacteriol.* 3:JB.00058-21

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

Gaudet RG, Bradfield CJ, MacMicking JD. 2016. Evolution of cell-autonomous effector mechanisms in macrophages versus non-immune cells. *Microbiol. Spectr.* 4(6). **https://journals.asm.org/doi/10.1128/microbiolspec.MCHD-0050-2016**

George EE, Husnik F, Tashyreva D, Prokopchuk G, Horák A, et al. 2020. Highly reduced genomes of protist endosymbionts show evolutionary convergence. *Curr. Biol.* 30(5):925–33.e3

Gerstenmaier L, Pilla R, Herrmann L, Herrmann H, Prado M, et al. 2015. The autophagic machinery ensures nonlytic transmission of mycobacteria. *PNAS* 112(7):E687–92

Glazebrook J, Ichige A, Walker GC. 1993. A *Rhizobium meliloti* homolog of the *Escherichia coli* peptide-antibiotic transport protein SbmA is essential for bacteroid development. *Genes Dev.* 7(8):1485–97

Goebel W, Gross R. 2001. Intracellular survival strategies of mutualistic and parasitic prokaryotes. *Trends Microbiol.* 9(6):267–73

Gonçalves WG, Fernandes KM, Silva APA, Gonçalves DG, Fiaz M, Serrão JE. 2020. Ultrastructure of the bacteriocytes in the midgut of the carpenter ant *Camponotus rufipes*: endosymbiont control by autophagy. *Microsc. Microanal.* 26(6):1236–44

Gould SB, Waller RF, McFadden GI. 2008. Plastid evolution. *Annu. Rev. Plant Biol.* 59:491–517

Graf JS, Schorn S, Kitzinger K, Ahmerkamp S, Woehle C, et al. 2021. Anaerobic endosymbiont generates energy for ciliate host by denitrification. *Nature* 591:445–50

Gray MW. 2015. Mosaic nature of the mitochondrial proteome: implications for the origin and evolution of mitochondria. *PNAS* 112(33):10133–38

Green ER, Mecsas J. 2016. Bacterial secretion systems: an overview. In *Virulence Mechanisms of Bacterial Pathogens*, ed. IT Kudva, pp. 213–39. Washington, DC: ASM Press

Hagedorn M, Rohde KH, Russell DG, Soldati T. 2009. Infection by tubercular mycobacteria is spread by nonlytic ejection from their amoeba hosts. *Science* 323(5922):1729–33

Hamilton PT, Hodson CN, Curtis CI, Perlman SJ. 2018. Genetics and genomics of an unusual selfish sex ratio distortion in an insect. *Curr. Biol.* 28(23):3864–70.e4

Harb OS, Gao L-Y, Kwaik YA. 2000. From protozoa to mammalian cells: a new paradigm in the life cycle of intracellular bacterial pathogens. *Environ. Microbiol.* 2(3):251–65

Hauben L, Moore ER, Vauterin L, Steenackers M, Mergaert J, et al. 1998. Phylogenetic position of phytopathogens within the Enterobacteriaceae. *Syst. Appl. Microbiol.* 21(3):384–97

Hentschel U, Steinert M, Hacker J. 2000. Common molecular mechanisms of symbiosis and pathogenesis. *Trends Microbiol.* 8(5):226–31

Hinde R. 1971. The control of the mycetome symbiotes of the aphids *Brevicoryne brassicae*, *Myzus persicae*, and *Macrosiphum rosae*. *J. Insect Physiol.* 17(9):1791–1800

Hirano M, Das S, Guo P, Cooper MD. 2011. The evolution of adaptive immunity in vertebrates. *Adv. Immunol.* 109:125–57

Hoebe K, Janssen E, Beutler B. 2004. The interface between innate and adaptive immunity. *Nat. Immunol.* 5(10):971–74

Hoshina R, Imamura N. 2008. Multiple origins of the symbioses in *Paramecium bursaria*. *Protist* 159(1):53–63

Husnik F, Chrudimský T, Hypša V. 2011. Multiple origins of endosymbiosis within the Enterobacteriaceae (γ-Proteobacteria): convergence of complex phylogenetic approaches. *BMC Biol.* 9:87

Husnik F, Keeling PJ. 2019. The fate of obligate endosymbionts: reduction, integration, or extinction. *Curr. Opin. Genet. Dev.* 58–59:1–8

Husnik F, McCutcheon JP. 2016. Repeated replacement of an intrabacterial symbiont in the tripartite nested mealybug symbiosis. *PNAS* 113(37):E5416–24

Husnik F, Nikoh N, Koga R, Ross L, Duncan RP, et al. 2013. Horizontal gene transfer from diverse bacteria to an insect genome enables a tripartite nested mealybug symbiosis. *Cell* 153(7):1567–78

Hybiske K, Stephens RS. 2008. Exit strategies of intracellular pathogens. *Nat. Rev. Microbiol.* 6(2):99–110

Iguchi A, Nagaya Y, Pradel E, Ooka T, Ogura Y, et al. 2014. Genome evolution and plasticity of *Serratia marcescens*, an important multidrug-resistant nosocomial pathogen. *Genome Biol. Evol.* 6(8):2096–110

Ireton K. 2013. Molecular mechanisms of cell-cell spread of intracellular bacterial pathogens. *Open Biol.* 3(7):130079

Jacobovitz MR, Rupp S, Voss PA, Maegele I, Gornik SG, Guse A. 2021. Dinoflagellate symbionts escape vomocytosis by host cell immune suppression. *Nat. Microbiol.* 6:769–82

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

Jamwal SV, Mehrotra P, Singh A, Siddiqui Z, Basu A, Rao KVS. 2016. Mycobacterial escape from macrophage phagosomes to the cytoplasm represents an alternate adaptation mechanism. *Sci. Rep.* 6:23089

Jenkins BH, Maguire F, Leonard G, Eaton JD, West S, et al. 2021. Emergent RNA-RNA interactions can promote stability in a nascent phototrophic endosymbiosis. bioRxiv 439338. **https://doi.org/10.1101/2021.04.11.439338**

Jo E-K, Yuk J-M, Shin D-M, Sasakawa C. 2013. Roles of autophagy in elimination of intracellular bacterial pathogens. *Front. Immunol.* 4:97

Joly E, Hudrisier D. 2003. What is trogocytosis and what is its purpose? *Nat. Immunol.* 4(9):815

Jones KM, Kobayashi H, Davies BW, Taga ME, Walker GC. 2007. How rhizobial symbionts invade plants: the Sinorhizobium-Medicago model. *Nat. Rev. Microbiol.* 5(8):619–33

Kambara K, Ardissone S, Kobayashi H, Saad MM, Schumpp O, et al. 2009. Rhizobia utilize pathogen-like effector proteins during symbiosis. *Mol. Microbiol.* 71(1):92–106

Karakashian MW, Karakashian SJ. 1973. Intracellular digestion and symbiosis in *Paramecium bursaria*. *Exp. Cell Res.* 81(1):111–19

Kazandjian A, Shepherd VA, Rodriguez-Lanetty M, Nordemeier W, Larkum AWD, Quinnell RG. 2008. Isolation of symbiosomes and the symbiosome membrane complex from the zoanthid *Zoanthus robustus*. *Phycologia* 47(3):294–306

Keeling PJ. 2010. The endosymbiotic origin, diversification and fate of plastids. *Philos. Trans. R. Soc. B* 365(1541):729–48

Keeling PJ, Burki F. 2019. Progress towards the tree of eukaryotes. *Curr. Biol.* 29(16):R808–17

Kies L. 1974. Elektronenmikroskopische Untersuchungen an Paulinella chromatophora Lauterborn, einer Thekamfbe mit blau-griinen Endosymbionten (Cyanellen) [Electron microscopic examinations of *Paulinella chromatophore Lauterborn*, a thecamoeba with blue-green endosymbionts (Glaucophyta)]. *Protoplasma* 80:69–89

Kirchberger PC, Schmidt ML, Ochman H. 2020. The ingenuity of bacterial genomes. *Annu. Rev. Microbiol.* 74:815–34

Kodama Y, Fujishima M. 2005. Symbiotic Chlorella sp. of the ciliate *Paramecium bursaria* do not prevent acidification and lysosomal fusion of host digestive vacuoles during infection. *Protoplasma* 225(3–4):191–203

Kodama Y, Fujishima M. 2010. Secondary symbiosis between *Paramecium* and *Chlorella* cells. *Int. Rev. Cell Mol. Biol.* 279:33–77

Koga R, Meng X-Y, Tsuchida T, Fukatsu T. 2008. Cellular mechanism for selective vertical transmission of an obligate insect symbiont at the bacteriocyte-embryo interface. *PNAS* 109(20):E1230–37

Kořený L, Oborník M, Lukeš J. 2013. Make it, take it, or leave it: heme metabolism of parasites. *PLOS Pathog.* 9(1):e1003088

Koskella B. 2020. The phyllosphere. *Curr. Biol.* 30(19):PR1143–46

Koumandou VL, Wickstead B, Ginger ML, van der Giezen M, Dacks JB, Field MC. 2013. Molecular paleontology and complexity in the last eukaryotic common ancestor. *Crit. Rev. Biochem. Mol. Biol.* 48(4):373–96

Kozek WJ. 1977. Transovarially-transmitted intracellular microorganisms in adult and larval stages of *Brugia malayi*. *J. Parasitol.* 63(6):992–1000

Ku C, Nelson-Sathi S, Roettger M, Garg S, Hazkani-Covo E, Martin WF. 2015. Endosymbiotic gene transfer from prokaryotic pangenomes: inherited chimerism in eukaryotes. *PNAS* 112(33):10139–46

Kupper M, Stigloher C, Feldhaar H, Gross R. 2016. Distribution of the obligate endosymbiont *Blochmannia floridanus* and expression analysis of putative immune genes in ovaries of the carpenter ant *Camponotus floridanus*. *Arthropod Struct. Dev.* 45(5):475–87

Lamelas A, Gosalbes MJ, Manzano-Marín A, Peretó J, Moya A, Latorre A. 2011. *Serratia symbiotica* from the aphid *Cinara cedri*: a missing link from facultative to obligate insect endosymbiont. *PLOS Genet.* 7(11):e1002357

Lancaster CE, Ho CY, Hipolito VEB, Botelho RJ, Terebiznik MR. 2019. Phagocytosis: what's on the menu? *Biochem. Cell Biol.* 97(1):21–29

Landmann F. 2019. The *Wolbachia* endosymbionts. *Microbiol. Spectr.* 7(2). **https://doi.org/10.1128/9781683670261.ch10**

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

Landmann F, Bain O, Martin C, Uni S, Taylor MJ, Sullivan W. 2012. Both asymmetric mitotic segregation and cell-to-cell invasion are required for stable germline transmission of *Wolbachia* in filarial nematodes. *Biol. Open* 1(6):536–47

Lane N, Martin WF. 2015. Eukaryotes really are special, and mitochondria are why. *PNAS* 112(35):E4823

Lawen A. 2003. Apoptosis—an introduction. *Bioessays* 25(9):888–96

Lawrence JG. 2005. Common themes in the genome strategies of pathogens. *Curr. Opin. Genet. Dev.* 15(6):584–88

Leclercq S, Cloeckaert A, Zygmunt MS. 2019. Taxonomic organization of the family Brucellaceae based on a phylogenomic approach. *Front. Microbiol.* 10:3083

Leclercq S, Thézé J, Chebbi MA, Giraud I, Moumen B, et al. 2016. Birth of a W sex chromosome by horizontal transfer of *Wolbachia* bacterial symbiont genome. *PNAS* 113(52):15036–41

Loh J, Pierson EA, Pierson LS III, Stacey G, Chatterjee A. 2002. Quorum sensing in plant-associated bacteria. *Curr. Opin. Plant Biol.* 5(4):285–90

Lowe CD, Minter EJ, Cameron DD, Brockhurst MA. 2016. Shining a light on exploitative host control in a photosynthetic endosymbiosis. *Curr. Biol.* 26(2):207–11

Luan J-B, Chen W, Hasegawa DK, Simmons AM, Wintermantel WM, et al. 2015. Metabolic coevolution in the bacterial symbiosis of whiteflies and related plant sap-feeding insects. *Genome Biol. Evol.* 7(9):2635–47

Ma H, Croudace JE, Lammas DA, May RC. 2006. Expulsion of live pathogenic yeast by macrophages. *Curr. Biol.* 16(21):2156–60

Maire J, Parisot N, Galvao Ferrarini M, Vallier A, Gillet B, et al. 2020. Spatial and morphological reorganization of endosymbiosis during metamorphosis accommodates adult metabolic requirements in a weevil. *PNAS* 117(32):19347–58

Manzano-Marín A, Latorre A. 2014. Settling down: the genome of *Serratia symbiotica* from the aphid *Cinara tujafilina* zooms in on the process of accommodation to a cooperative intracellular life. *Genome Biol. Evol.* 6(7):1683–98

Manzano-Marín A, Latorre A. 2016. Snapshots of a shrinking partner: genome reduction in *Serratia symbiotica*. *Sci. Rep.* 6:32590

Marin B, Nowack ECM, Melkonian M. 2005. A plastid in the making: evidence for a second primary endosymbiosis. *Protist* 156(4):425–32

Martinson VG, Gawryluk RMR, Gowen BE, Curtis CI, Jaenike J, Perlman SJ. 2020. Multiple origins of obligate nematode and insect symbionts by a clade of bacteria closely related to plant pathogens. *PNAS* 117(50):31979–86

Masson F, Moné Y, Vigneron A, Vallier A, Parisot N, et al. 2015. Weevil endosymbiont dynamics is associated with a clamping of immunity. *BMC Genom.* 16:819

Matz C, Kjelleberg S. 2005. Off the hook – how bacteria survive protozoan grazing. *Trends Microbiol.* 13(7):302–7

McCutcheon JP. 2010. The bacterial essence of tiny symbiont genomes. *Curr. Opin. Microbiol.* 13(1):73–78

McCutcheon JP, Boyd BM, Dale C. 2019. The life of an insect endosymbiont from the cradle to the grave. *Curr. Biol.* 29(11):R485–95

McCutcheon JP, Moran NA. 2012. Extreme genome reduction in symbiotic bacteria. *Nat. Rev. Microbiol.* 10(1):13–26

McFadden GI. 2001. Primary and secondary endosymbiosis and the origin of plastids. *J. Phycol.* 37(6):951–59

McLaughlin RN Jr., Malik HS. 2017. Genetic conflicts: the usual suspects and beyond. *J. Exp. Biol.* 220(Part 1):6–17

Mills DB. 2020. The origin of phagocytosis in Earth history. *Interface Focus* 10(4):20200019

Mohamed AR, Cumbo V, Harii S, Shinzato C, Chan CX, et al. 2016. The transcriptomic response of the coral *Acropora digitifera* to a competent *Symbiodinium* strain: the symbiosome as an arrested early phagosome. *Mol. Ecol.* 25(13):3127–41

Molmeret M, Horn M, Wagner M, Santic M, Abu Kwaik Y. 2005. Amoebae as training grounds for intracellular bacterial pathogens. *Appl. Environ. Microbiol.* 71(1):20–28

Monack DM, Mueller A, Falkow S. 2004. Persistent bacterial infections: the interface of the pathogen and the host immune system. *Nat. Rev. Microbiol.* 2(9):747–65

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

Moran NA. 1992. The evolution of aphid life cycles. *Annu. Rev. Entomol.* 37:321–48
Moran NA. 2002. Microbial minimalism: genome reduction in bacterial pathogens. *Cell* 108(5):583–86
Moran NA, Bennett GM. 2014. The tiniest tiny genomes. *Annu. Rev. Microbiol.* 68:195–215
Moran NA, McCutcheon JP, Nakabachi A. 2008. Genomics and evolution of heritable bacterial symbionts. *Annu. Rev. Genet.* 42:165–90
Moran NA, Plague GR, Sandström JP, Wilcox JL. 2003. A genomic perspective on nutrient provisioning by bacterial symbionts of insects. *PNAS* 100(Suppl. 2):14543–48
Moran NA, Russell JA, Koga R, Fukatsu T. 2005. Evolutionary relationships of three new species of Enterobacteriaceae living as symbionts of aphids and other insects. *Appl. Environ. Microbiol.* 71(6):3302–10
Morisaki JH, Heuser JE, Sibley LD. 1995. Invasion of *Toxoplasma gondii* occurs by active penetration of the host cell. *J. Cell Sci.* 108(Part 6):2457–64
Munoz MM, Spencer N, Enomoto S, Dale C, Rio RVM. 2020. Quorum sensing sets the stage for the establishment and vertical transmission of *Sodalis praecaptivus* in tsetse flies. *PLOS Genet*. 16(8):e1008992
Muscatine L, Porter JW. 1977. Reef corals: mutualistic symbioses adapted to nutrient-poor environments. *Bioscience* 27(7):454–60
Nakabachi A, Ishida K, Hongoh Y, Ohkuma M, Miyagishima S-Y. 2014. Aphid gene of bacterial origin encodes a protein transported to an obligate endosymbiont. *Curr. Biol.* 24(14):R640–41
Nardon P, Charles H, Delobel B, Lefevre C, Heddi A. 2003. Symbiosis in the dryophthoridae weevils (Coleoptera, Curculionoidea): morphological variability of symbiotic intracellular bacteria. *Symbiosis* 34(3):231–51
Needham BD, Trent MS. 2013. Fortifying the barrier: the impact of lipid A remodelling on bacterial pathogenesis. *Nat. Rev. Microbiol.* 11(7):467–81
Nikoh N, McCutcheon JP, Kudo T, Miyagishima S-Y, Moran NA, Nakabachi A. 2010. Bacterial genes in the aphid genome: absence of functional gene transfer from *Buchnera* to its host. *PLOS Genet*. 6(2):e1000827
Nikoh N, Nakabachi A. 2009. Aphids acquired symbiotic genes via lateral gene transfer. *BMC Biol*. 7:12
Nishikori K, Morioka K, Kubo T, Morioka M. 2009. Age- and morph-dependent activation of the lysosomal system and Buchnera degradation in aphid endosymbiosis. *J. Insect Physiol.* 55(4):351–57
Nováková E, Hypsa V, Moran NA. 2009. Arsenophonus, an emerging clade of intracellular symbionts with a broad host distribution. *BMC Microbiol*. 9:143
Nowack ECM, Grossman AR. 2012. Trafficking of protein into the recently established photosynthetic organelles of *Paulinella chromatophora*. *PNAS* 109(14):5340–45
Nowack ECM, Melkonian M. 2010. Endosymbiotic associations within protists. *Philos. Trans. R. Soc. B* 365(1541):699–712
Nowack ECM, Melkonian M, Glöckner G. 2008. Chromatophore genome sequence of *Paulinella* sheds light on acquisition of photosynthesis by eukaryotes. *Curr. Biol.* 18(6):410–18
Nowack ECM, Price DC, Bhattacharya D, Singer A, Melkonian M, Grossman AR. 2016. Gene transfers from diverse bacteria compensate for reductive genome evolution in the chromatophore of *Paulinella chromatophora*. *PNAS* 113(43):12214–19
Nowack ECM, Weber APM. 2018. Genomics-informed insights into endosymbiotic organelle evolution in photosynthetic eukaryotes. *Annu. Rev. Plant Biol.* 69:51–84
Oakeson KF, Gil R, Clayton AL, Dunn DM, von Niederhausern AC, et al. 2014. Genome degeneration and adaptation in a nascent stage of symbiosis. *Genome Biol. Evol.* 6(1):76–93
Ochman H, Davalos LM. 2006. The nature and dynamics of bacterial genomes. *Science* 311(5768):1730–33
Ohl ME, Miller SI. 2001. Salmonella: a model for bacterial pathogenesis. *Annu. Rev. Med.* 52:259–74
Oke V, Long SR. 1999. Bacteroid formation in the *Rhizobium*-legume symbiosis. *Curr. Opin. Microbiol.* 2(6):641–46
Oldroyd GED, Murray JD, Poole PS, Downie JA. 2011. The rules of engagement in the legume-rhizobial symbiosis. *Annu. Rev. Genet.* 45:119–44
Omotade TO, Roy CR. 2019. Manipulation of host cell organelles by intracellular pathogens. *Microbiol. Spectr*. 7(2). **https://doi.org/10.1128/microbiolspec.BAI-0022-2019**
Oulhen N, Schulz BJ, Carrier TJ. 2016. English translation of Heinrich Anton de Bary's 1878 speech, '*Die Erscheinung der Symbiose*' ('*De la symbiose*') [The Phenomenon of Symbiosis]. *Symbiosis* 69:131–39

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

Pagliarini DJ, Calvo SE, Chang B, Sheth SA, Vafai SB, et al. 2008. A mitochondrial protein compendium elucidates complex I disease biology. *Cell* 134(1):112–23

Pawlowska TE, Gaspar ML, Lastovetsky OA, Mondo SJ, Real-Ramirez I, et al. 2018. Biology of fungi and their bacterial endosymbionts. *Annu. Rev. Phytopathol.* 56:289–309

Perreau J, Patel DJ, Anderson H, Maeda GP, Elston KM, et al. 2021. Vertical transmission at the pathogen-symbiont interface: *Serratia symbiotica* and aphids. *mBio* 12(2). **https://doi.org/10.1128/mBio.00359-21**

Petersen LM, Tisa LS. 2013. Friend or foe? A review of the mechanisms that drive *Serratia* towards diverse lifestyles. *Can. J. Microbiol.* 59(9):627–40

Pfanner N, Meijer M. 1997. Mitochondrial biogenesis: the Tom and Tim machine. *Curr. Biol.* 7(2):R100–3

Philips JA, Rubin EJ, Perrimon N. 2005. *Drosophila* RNAi screen reveals CD36 family member required for mycobacterial infection. *Science* 309(5738):1251–53

Pieters J. 2008. *Mycobacterium tuberculosis* and the macrophage: maintaining a balance. *Cell Host Microbe* 3(6):399–407

Pons I, Renoz F, Noël C, Hance T. 2019a. Circulation of the cultivable symbiont *Serratia symbiotica* in aphids is mediated by plants. *Front. Microbiol.* 10:764

Pons I, Renoz F, Noël C, Hance T. 2019b. New insights into the nature of symbiotic associations in aphids: infection process, biological effects, and transmission mode of cultivable *Serratia symbiotica* bacteria. *Appl. Environ. Microbiol.* 85(10):e02445

Poole AM, Gribaldo S. 2014. Eukaryotic origins: how and when was the mitochondrion acquired? *Cold Spring Harb. Perspect. Biol.* 6(12):a015990

Prashar A, Terebiznik MR. 2015. *Legionella pneumophila*: homeward bound away from the phagosome. *Curr. Opin. Microbiol.* 23:86–93

Qiu H, Price DC, Weber APM, Facchinelli F, Yoon HS, Bhattacharya D. 2013. Assessing the bacterial contribution to the plastid proteome. *Trends Plant Sci.* 18(12):680–87

Randow F, MacMicking JD, James LC. 2013. Cellular self-defense: how cell-autonomous immunity protects against pathogens. *Science* 340(6133):701–6

Ray K, Marteyn B, Sansonetti PJ, Tang CM. 2009. Life on the inside: the intracellular lifestyle of cytosolic bacteria. *Nat. Rev. Microbiol.* 7(5):333–40

Renoz F, Pons I, Vanderpoorten A, Bataille G, Noël C, et al. 2019. Evidence for gut-associated *Serratia symbiotica* in wild aphids and ants provides new perspectives on the evolution of bacterial mutualism in insects. *Microb. Ecol.* 78(1):159–69

Reumann S, Inoue K, Keegstra K. 2005. Evolution of the general protein import pathway of plastids (review). *Mol. Membr. Biol.* 22(1–2):73–86

Rink J, Ghigo E, Kalaidzidis Y, Zerial M. 2005. Rab conversion as a mechanism of progression from early to late endosomes. *Cell* 122(5):735–49

Rodríguez-Ezpeleta N, Brinkmann H, Burey SC, Roure B, Burger G, et al. 2005. Monophyly of primary photosynthetic eukaryotes: green plants, red algae, and glaucophytes. *Curr. Biol.* 15(14):1325–30

Roger AJ, Muñoz-Gómez SA, Kamikawa R. 2017. The origin and diversification of mitochondria. *Curr. Biol.* 27(21):R1177–92

Rother M, Teixeira da Costa AR, Zietlow R, Meyer TF, Rudel T. 2019. Modulation of host cell metabolism by *Chlamydia trachomatis*. *Microbiol. Spectr.* 7(3). **https://doi.org/10.1128/microbiolspec.BAI-0012-2019**

Ruby T, McLaughlin L, Gopinath S, Monack D. 2012. *Salmonella*'s long-term relationship with its host. *FEMS Microbiol. Rev.* 36(3):600–615

Russell DG. 2001. *Mycobacterium tuberculosis*: here today, and here tomorrow. *Nat. Rev. Mol. Cell Biol.* 2(8):569–77

Russell DG. 2007. Phagocytosis. *eLS*. **https://doi.org/10.1002/9780470015902.a0000488.pub2**

Russell SL, Chappell L, Sullivan W. 2019. A symbiont's guide to the germline. *Curr. Top. Dev. Biol.* 135:315–51

Sachs JL, Essenberg CJ, Turcotte MM. 2011. New paradigms for the evolution of beneficial infections. *Trends Ecol. Evol.* 26(4):202–9

Sachs JL, Quides KW, Wendlandt CE. 2018. Legumes versus rhizobia: a model for ongoing conflict in symbiosis. *New Phytol.* 219(4):1199–206

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

Sapp J. 1994. *Evolution by Association: A History of Symbiosis*. Oxford, UK: Oxford Univ. Press

Sato N, Yoshitomi T, Mori-Moriyama N. 2020. Characterization and biosynthesis of lipids in *Paulinella micropora* MYN1: evidence for efficient integration of chromatophores into cellular lipid metabolism. *Plant Cell Physiol*. 61(5):869–81

Schmidt O, Pfanner N, Meisinger C. 2010. Mitochondrial protein import: from proteomics to functional mechanisms. *Nat. Rev. Mol. Cell Biol.* 11(9):655–67

Schröder D, Deppisch H, Obermayer M, Krohne G, Stackebrandt E, et al. 1996. Intracellular endosymbiotic bacteria of *Camponotus species* (carpenter ants): systematics, evolution and ultrastructural characterization. *Mol. Microbiol.* 21(3):479–89

Seoane PI, May RC. 2020. Vomocytosis: what we know so far. *Cell. Microbiol.* 22(2):e13145

Serbus LR, Sullivan W. 2007. A cellular basis for *Wolbachia* recruitment to the host germline. *PLOS Pathog*. 3(12):e190

Shigenobu S, Watanabe H, Hattori M, Sakaki Y, Ishikawa H. 2000. Genome sequence of the endocellular bacterial symbiont of aphids *Buchnera* sp. APS. *Nature* 407(6800):81–86

Siegel RW. 1960. Hereditary endosymbiosis in *Paramecium bursaria*. *Exp. Cell Res.* 19:239–52

Simonet P, Gaget K, Balmand S, Ribeiro Lopes M, Parisot N, et al. 2018. Bacteriocyte cell death in the pea aphid/*Buchnera* symbiotic system. *PNAS* 115(8):E1819–28

Simpson AGB, Eglit Y. 2016. Protist diversification. *Encycl. Evol. Biol.* 3:344–60

Singer A, Poschmann G, Mühlich C, Valadez-Cano C, Hänsch S, et al. 2017. Massive protein import into the early-evolutionary-stage photosynthetic organelle of the amoeba *Paulinella chromatophora*. *Curr. Biol.* 27(18):2763–73.e5

Sloan DB, Nakabachi A, Richards S, Qu J, Murali SC, et al. 2014. Parallel histories of horizontal gene transfer facilitated extreme reduction of endosymbiont genomes in sap-feeding insects. *Mol. Biol. Evol.* 31(4):857–71

Smith DR, Keeling PJ. 2015. Mitochondrial and plastid genome architecture: reoccurring themes, but significant differences at the extremes. *PNAS* 112(33):10177–84

Sommer F, Bäckhed F. 2013. The gut microbiota—masters of host development and physiology. *Nat. Rev.* 11:227–38

Soo RM, Hemp J, Parks DH, Fischer WW, Hugenholtz P. 2017. On the origins of oxygenic photosynthesis and aerobic respiration in Cyanobacteria. *Science* 355(6332):1436–40

Soto MJ, Sanjuán J, Olivares J. 2006. Rhizobia and plant-pathogenic bacteria: common infection weapons. *Microbiology* 152(Part 11):3167–74

Stairs CW, Dharamshi JE, Tamarit D, Eme L, Jørgensen SL, et al. 2020. Chlamydial contribution to anaerobic metabolism during eukaryotic evolution. *Sci. Adv.* 6(35):eabb7258

Starr T, Child R, Wehrly TD, Hansen B, Hwang S, et al. 2012. Selective subversion of autophagy complexes facilitates completion of the *Brucella* intracellular cycle. *Cell Host Microbe* 11(1):33–45

Steele S, Radlinski L, Taft-Benz S, Brunton J, Kawula TH. 2016. Trogocytosis-associated cell to cell spread of intracellular bacterial pathogens. *eLife* 5:e10625

Steele-Mortimer O. 2008. The *Salmonella*-containing vacuole—moving with the times. *Curr. Opin. Microbiol.* 11(1):38–45

Steenbergen JN, Shuman HA, Casadevall A. 2001. *Cryptococcus neoformans* interactions with amoebae suggest an explanation for its virulence and intracellular pathogenic strategy in macrophages. *PNAS* 98(26):15245–50

Stenmark H. 2009. Rab GTPases as coordinators of vesicle traffic. *Nat. Rev. Mol. Cell Biol.* 10(8):513–25

Stoll S, Feldhaar H, Fraunholz MJ, Gross R. 2010. Bacteriocyte dynamics during development of a holometabolous insect, the carpenter ant *Camponotus floridanus*. *BMC Microbiol.* 10:308

Swanson MS, Hammer BK. 2000. *Legionella pneumophila* pathogenesis: a fateful journey from amoebae to macrophages. *Annu. Rev. Microbiol.* 54:567–613

Szklarzewicz T, Michalik A. 2017. Transovarial transmission of symbionts in insects. In *Results and Problems in Cell Differentiation*, Vol. 63, ed. M Kloc, pp. 43–67. Berlin/Heidelberg: Springer

Tam JCH, Jacques DA. 2014. Intracellular immunity: finding the enemy within—how cells recognize and respond to intracellular pathogens. *J. Leukoc. Biol.* 96(2):233–44

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

Toju H, Tanabe AS, Notsu Y, Sota T, Fukatsu T. 2013. Diversification of endosymbiosis: replacements, co-speciation and promiscuity of bacteriocyte symbionts in weevils. *ISME J.* 7(7):1378–90

Unterman BM, Baumann P, McLean DL. 1989. Pea aphid symbiont relationships established by analysis of 16S rRNAs. *J. Bacteriol.* 171(6):2970–74

van der Wel N, Hava D, Houben D, Fluitsma D, van Zon M, et al. 2007. *M. tuberculosis* and *M. leprae* translocate from the phagolysosome to the cytosol in myeloid cells. *Cell* 129(7):1287–98

Veiga E, Guttman JA, Bonazzi M, Boucrot E, Toledo-Arana A, et al. 2007. Invasive and adherent bacterial pathogens co-opt host clathrin for infection. *Cell Host Microbe* 2(5):340–51

Vigneron A, Masson F, Vallier A, Balmand S, Rey M, et al. 2014. Insects recycle endosymbionts when the benefit is over. *Curr. Biol.* 24(19):2267–73

Walterson AM, Stavrinides J. 2015. *Pantoea*: insights into a highly versatile and diverse genus within the Enterobacteriaceae. *FEMS Microbiol. Rev.* 39(6):968–84

Watkins RA, Andrews A, Wynn C, Barisch C, King JS, Johnston SA. 2018. *Cryptococcus neoformans* escape from *Dictyostelium* amoeba by both WASH-mediated constitutive exocytosis and vomocytosis. *Front. Cell. Infect. Microbiol.* 8:108

Werren JH, Baldo L, Clark ME. 2008. *Wolbachia*: master manipulators of invertebrate biology. *Nat. Rev. Microbiol.* 6(10):741–51

Whelan J. 1999. Plant mitochondrial protein import: mechanisms and control. *Funct. Plant Biol.* 26(8):725–32

White PM, Pietri JE, Debec A, Russell S, Patel B, Sullivan W. 2017. Mechanisms of horizontal cell-to-cell transfer of *Wolbachia* spp. in *Drosophila melanogaster*. *Appl. Environ. Microbiol.* 83(7):e03425

Wilkinson TL, Fukatsu T, Ishikawa H. 2003. Transmission of symbiotic bacteria *Buchnera* to parthenogenetic embryos in the aphid *Acyrthosiphon pisum* (Hemiptera: Aphidoidea). *Arthropod Struct. Dev.* 32(2–3):241–45

Williams KP, Gillespie JJ, Sobral BWS, Nordberg EK, Snyder EE, et al. 2010. Phylogeny of gammaproteobacteria. *J. Bacteriol.* 192(9):2305–14

Wilson ACC. 2020. Regulation of an insect symbiosis. In *Advances in Insect Physiology*, Vol. 58, ed. KM Oliver, JA Russell, pp. 207–31. London: Acad. Press

Young D, Hussell T, Dougan G. 2002. Chronic bacterial infections: living with unwanted guests. *Nat. Immunol.* 3(11):1026–32

Young JP, Johnston AW. 1989. The evolution of specificity in the legume-rhizobium symbiosis. *Trends Ecol. Evol.* 4(11):341–49

Zahran HH. 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiol. Mol. Biol. Rev.* 63(4):968–89

Zaremba-Niedzwiedzka K, Caceres EF, Saw JH, Bäckström D, Juzokaite L, et al. 2017. *Asgard archaea* illuminate the origin of eukaryotic cellular complexity. *Nature* 541(7637):353–58

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

Annual Review of Cell and Developmental Biology

Volume 37, 2021

Contents

Toward a Mechanistic Understanding of Bacterial Rod Shape Formation and Regulation
Ethan C. Garner 1

Self-Organization of Cellular Units
Timothy J. Mitchison and Christine M. Field 23

Biophysical and Quantitative Principles of Centrosome Biogenesis and Structure
Sónia Gomes Pereira, Marco António Dias Louro, and Mónica Bettencourt-Dias 43

Mechanobiology of T Cell Activation: To Catch a Bond
Baoyu Liu, Elizabeth M. Kolawole, and Brian D. Evavold 65

Promoters and Antagonists of Phagocytosis: A Plastic and Tunable Response
Spencer Freeman and Sergio Grinstein 89

The Genomics and Cell Biology of Host-Beneficial Intracellular Infections
John P. McCutcheon 115

Mechanisms of Selective Autophagy
Trond Lamark and Terje Johansen 143

A New Infectious Unit: Extracellular Vesicles Carrying Virus Populations
Adeline Kerviel, Mengyang Zhang, and Nihal Altan-Bonnet 171

Spatial Organization of Chromatin: Emergence of Chromatin Structure During Development
Rajarshi P. Ghosh and Barbara J. Meyer 199

Components and Mechanisms of Nuclear Mechanotransduction
Philipp Niethammer 233

Glycocalyx Curving the Membrane: Forces Emerging from the Cell Exterior
Joe Chin-Hun Kuo and Matthew J. Paszek 257

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.

Nonmuscle Myosin II Regulation Directs Its Multiple Roles in Cell Migration and Division
Marina Garrido-Casado, Gloria Asensio-Juárez, and Miguel Vicente-Manzanares 285

Calcium Signaling Mechanisms Across Kingdoms
Sheng Luan and Chao Wang 311

Dynamic Nutrient Signaling Networks in Plants
Lei Li, Kun-hsiang Liu, and Jen Sheen 341

Cell Biology of Canonical Wnt Signaling
Lauren V. Albrecht, Nydia Tejeda-Muñoz, and Edward M. De Robertis 369

The Fertilization Enigma: How Sperm and Egg Fuse
Victoria E. Deneke and Andrea Pauli 391

Beyond Casual Resemblance: Rigorous Frameworks for Comparing Regeneration Across Species
Mansi Srivastava 415

The Visual Opsin Gene Repertoires of Teleost Fishes: Evolution, Ecology, and Function
Zuzana Musilova, Walter Salzburger, and Fabio Cortesi 441

Mechanical Patterning in Animal Morphogenesis
Yonit Maroudas-Sacks and Kinneret Keren 469

From Cell Types to an Integrated Understanding of Brain Evolution: The Case of the Cerebral Cortex
Maria Antonietta Tosches 495

Molecular Mechanisms of Sexually Dimorphic Nervous System Patterning in Flies and Worms
Stephen F. Goodwin and Oliver Hobert 519

A Tale of Three Systems: Toward a Neuroimmunoendocrine Model of Obesity
Conan J.O. O'Brien, Emma R. Haberman, and Ana I. Domingos 549

Indexes

Cumulative Index of Contributing Authors, Volumes 33–37 575

Errata

An online log of corrections to *Annual Review of Cell and Developmental Biology* articles may be found at http://www.annualreviews.org/errata/cellbio

Annu. Rev. Cell Dev. Biol. 2021.37:115-142. Downloaded from www.annualreviews.org
Access provided by 72.201.104.74 on 10/12/21. For personal use only.