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Symbiosis and Pathogenesis: Evolution of the Microbe-Host Interaction

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Symbiotic and pathogenic bacteria have in common that they live in or on host organisms or host cells. To make a successful living in eukaryotic hosts, bacteria must possess the traits to recognize a given host and establish adherence. When the bacterial location is internal or intracellular, they must further have the ability to invade, to establish a niche, and finally to multiply within a host. The underlying mechanisms which allow this form of existence show similarities between symbiotic and pathogenic bacteria. The final outcome, however, may result in a wide spectrum of consequences for the host ranging from the acquisition of novel metabolic pathways to damage or death. Despite the vastly different forms of interactions, symbiotic and pathogenic bacteria have in common that they are adapted to a particular environmental niche represented by the host organism or compartment thereof. This contribution reviews the evolutionary forces which have shaped the microbial-host interactions. Particular emphasis is placed on the genetic and molecular mechanisms that drive bacterial evolution in response to the selective pressures of the host environment.

Introduction

Three types of bacteria-host interactions are generally recognized. These are symbiotic, commensal, and pathogenic interactions (Fig. 1). The term "symbiosis" was coined by de Bary (1879) for the living together of two differently named organisms. This generally implies that at least one organism benefits from the other. Pathogenic interactions are those in which one partner benefits to the detriment of the other, causing cell or tissue damage or even death of the organism. In commensal interactions one organism simply uses the body of the other as a physical environment without any evidence of benefit or detriment. The definitions of a given bacteria-host interaction should not be considered static; rather they should be viewed as an "arms race," in which changes in one partner must be matched by adaptive changes in the other to maintain equilibrium. Symbiotic bacteria-host interactions are balanced relationships towards reciprocal benefit. The fossil record and molecular evidence reveal symbioses as the most ancient forms of bacteria-host interactions dating back sometimes several 100 million years.

Evolution of the Microbe-Host Interaction

The earth is estimated to be about 4.6 billion years old (Fig. 2). Chemical evolution, which is evident particularly in the cooling of the earth's temperature and in the modification of the atmosphere, shaped the environment in which life originated more than 3.6 billion years ago. Following the diversification of micro-organisms and with the advent of photosynthesis, molecular oxygen gradually accumulated and became available to microbial metabolism, leading to a quantum leap in evolution.

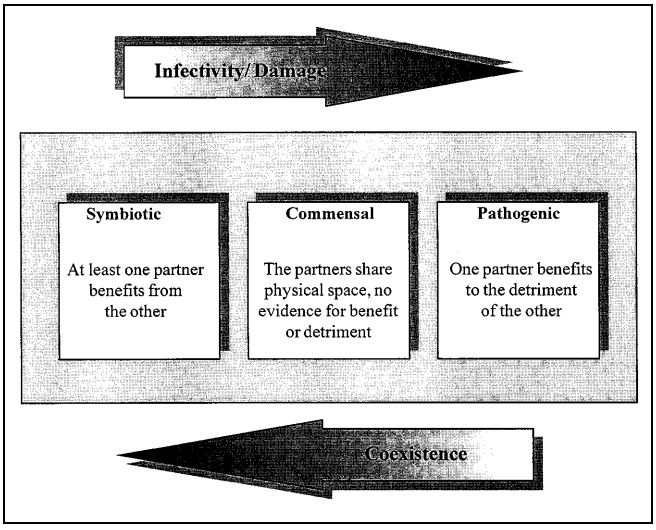

Fig. 1. Types of bacteria-host interactions

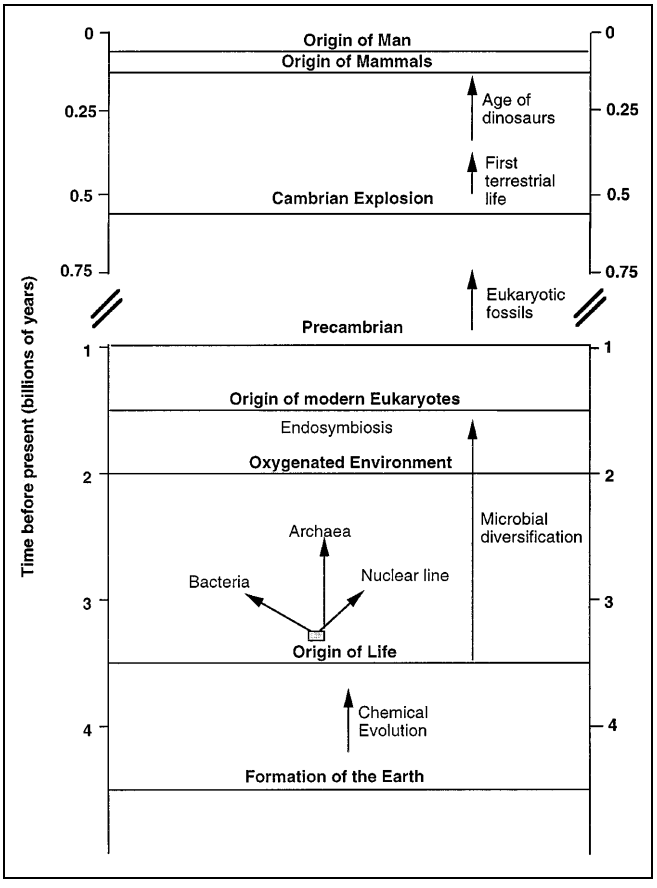

Fig. 2. Short diagram of the evolution of life

The origin of organelles of the eukaryotic cell, such as mitochondria and plastids, is generally accepted as the oldest symbiotic event, dating back more than 1.5 billion years ago (Margulis 1981). According to the serial endosymbiosis theory, these organelles were the products of a phagocytotic event of an ancestral α-proteobacterium which may have evolved into today's mitochondria and an ancestral cyanobacterium which evolved into today's chloroplasts (Margulis 1970; Taylor 1974). The acquisition of the metabolic traits such as oxygen respiration through mitochondria and photosynthesis through chloroplasts is undoubtedly the key to the evolutionary success of modern day eukaryotes.

The evolutionary event leading to the creation of the eukaryotic cell was, however, not unique. In fact, similar symbiotic events are known in all major invertebrate phyla and often had a distinct impact on evolution in terms of speciation and the population of new environmental habitats (Table 1). They are common in protozoa, where different micro-organisms occupy various niches in the host including the cytoplasm and the nuclei (Görtz and Brigger 1998; Heckmann and Görtz 1991; Jeon 1995). An indispensable, nonphotosynthetic plastid (apicoplast) that appears to have been acquired by secondary endosymbiosis of a green alga has recently been identified in protozoan parasites such as *Plasmodium* spp. and *Toxoplasma gondii* (Fichera and Roos 1997).

Porifera (sponges) are associated with often large amounts of micro-organisms (Friedrich et al. 1999; Vacelet and Donadey 1977) which are thought to contribute to the chemical defense of the sponges against predators and to prevent biofouling (Bakus et al. 1986; Paul 1992). Sponges known to contain exceptionally large amounts of bacteria belong to the genus *Aplysina* (A. *aerophoba, A. cavernicola*), and *Ceratoporella* (*C. nicholsoni)*, where the associated micro-organisms contribute up to 40% and 60% of the sponge biomass, respectively. In the phylum Cnidaria (corals, jellyfish, sea anemones) the best known symbioses are those between corals and single celled algae, *Symbiodinium* (Muscatine and Porter 1977; Rowan 1998). The algae fix CO_2 and provide nutrition to the host, allowing them build thriving reef communities in the nutrient poor, tropical waters. The evolutionary success of such symbiotic events is also evident in that tropical reefs are among the most diverse and rich environments. In the phylum Mollusca (snails, clams, octopi), symbioses are common and often evolutionary ancient (Distel 1998). Most of the symbiotic interactions are based on the transfer of nutrition from the symbionts to their hosts by translocation of low molecular weight compounds and/or by digestion of bacteria (Felbeck et al. 1983; Muscatine and Green 1973). The symbionts and hosts are often exquisitely adapted to their symbiotic existence by utilizing meta-

Table 1. A survey of bacteria-host interactions

Phylum	Eukaryotic hosts	Prokaryotic partners	Type of interaction	Proposed benefit/harm
Protozoa	*Amoeba proteus*	X-bacteria	Symbiotic	Unknown
	Paramecium	*Holospora* spp.	Pathogenic	Cell death
	Acanthamoeba	*Legionella* spp.	Pathogenic	Cell death
Porifera	*Aplysina*	Unknown	Symbiotic	Chemical defense, food source
	Ceratoporella			
Cnidaria	Corals	*Symbiodinium* spp.	Symbiotic	Provision of nutrients
Mollusca	Teredinidae	Shipworm symbiont	Symbiotic	Cellulose degradation
	Lucinidae, Solemyidae	Chemoautotrophic symbiont	Symbiotic	Provision of carbon and nitrogen
Arthropoda	Aphidae	*Buchnera* spp.	Symbiotic	Provision of essential amino acids
	Fleas, lice, ticks	*Rickettsia* spp.	Pathogenic	Insect death[a]
Vertebrates	Cows, sheeps, goats	*Bacteroides* spp.	Symbiotic	Digestion of cellulose
		Ruminococcus spp.		
	Humans	*Legionella pneumophila*	Pathogenic	Legionaire's disease
		Staphylococcus aureus	Pathogenic	Toxic shock syndrome, nosocomial infections
		Mycobacterium tuberculosis	Pathogenic	Tuberculosis
		Pathogenic *E. coli*	Pathogenic	Gastrointestinal and urinary tract infections
		Vibrio cholerae	Pathogenic	Cholera
		Bordetella pertussis	Pathogenic	Whooping caugh
		Yersinia pestis	Pathogenic	Bubonic plague
Plants	Leguminous plants	*Rhizobium* spp.	Symbiotic	Nitrogen fixation
		Bradyrhizobium spp.		
	Various plants	*Agrobacterium tumefaciens*	Pathogenic	Crown gall tumor
		Xanthomonas spp.	Pathogenic	Necrotic lesions

[a] Rocky Mountain spotted fever in humans following host spread

bolic pathways aimed at ensuring intracellular survival of the bacteria (Hentschel and Felbeck 1993, 1995).

Probably the greatest impact of bacteria upon hosts can be found in the phylum Arthropoda (Dasch et al. 1984) It has been estimated that 10% of the insects alone are associated with bacteria. Some of the associations date back 200–300 million years (Moran and Telang 1998). Through the presence of bacteria, insects can use specialized and nutritionally poor food sources. It is the combination of the new metabolic potential with the morphological complexity of the host which was fundamental in triggering various adaptive radiations. An excellent example are the insect orders Hemiptera and Homoptera, the latter ones consisting of more than 32,000 species. This enormous diversification is based on the fact that all species are uniformly associated with endosymbionts that are inherited transovarially through the eggs.

Symbioses are also common between micro-organisms and plant hosts, the most famous example being the symbiosis between N_2–fixing bacteria (*Rhizobium*) and leguminous plants (Stacey et al. 1992). Also, a wide spectrum of plant parasites are known, most noticeably the plant parasite *Agrobacterium tumefaciens* which causes crown gall tumor on infected plants (Long and Staskiawicz 1993). In many cases the interaction had evolutionary consequences for both partners. For example, new tissues or organs have evolved to house the bacteria (root nodules). Hosts have acquired a range of novel metabolic capacities (N_2 fixation). On the bacteria side, specific receptors have evolved which allow the specific recognition and attachment to the plant. Also, the intimate and long-standing host contact may be accompanied by genetic changes in the bacteria such as the amplification of specific sets of genes and/or the genome reduction. While a considerable amount of information has become available regarding the molecular mechanisms underlying *Rhizobium* symbioses, most other symbiotic systems are just beginning to be understood.

In vertebrates, the rumen microflora of cows is the best known symbiosis and permits the use of cellulose as a nutrition which is an otherwise undigestable food source (Hobson 1988). Probably due to the biased human interest in medical research, pathogenic interactions are known mostly between microbes and vertebrates, especially humans. About 200 prokaryotic pathogens are known, many of which populate the digestive tract (*Escherichia coli*, *Salmonella, Vibrio*), invade the lungs (*Legionella, Mycobacterium*) or are found in the urinary/genital tract (*E. coli, Neisseria gonorrhoeae, Chlamydia trachomatis*). Others populate the skin (*Staphylococcus*

aureus) or the oral cavity (*Streptococcus* spp.) or cross the blood-brain barrier (*Meningococcus*). Pathogenic bacteria often produce virulence or pathogenicity factors such as adhesins, capsules, and toxins which enable them to cause infections in particular host organisms. An infection is described as the successful persistence or multiplication of a pathogen on or within a host, while disease is defined as the damage inflicted upon the host. It is important to recognize that the extent of damage depends on both the potency of the micro-organism and the efficiency of the host defenses (Finlay and Falkow 1989).

Bacteria-host interactions have clearly evolved many times throughout evolution and are widespread in the animal and plant kingdom. The evolutionary success of these associations is manifested at least in two ways: firstly, in the population of diverse ecological niches and, secondly, in the species diversification of the respective host organisms. The reason why interactions of bacteria with hosts lead to such "evolutionary quantum leaps" is that bacteria are much more susceptible to genetic changes. Bacteria usually have higher generation times and are genetically more diverse than eukaryotic organisms. Also, they generate extremely large populations. It is through their close interaction with host organisms that these changes may be conferred upon their hosts, ultimately resulting in the acquisition of novel metabolic traits and leading to the formation of new tissues or to the creation of elaborate defense mechanisms, such as the development of the sophisticated vertebrate immune system. Below we discuss the genetic mechanisms which underlie bacterial evolution.

Genetic Variability Is the Motor of Evolution

Gene regulation and genetic diversity are strategies which enable bacteria to live and survive under continuously changing environmental conditions. Genetic variability is achieved by point mutations, recombinations, and horizontal gene transfer and represents the driving forces of bacterial evolution (Arber 1993; Ziebuhr et al. 1999a; Fig. 3). Genome stability, on the other hand, is achieved by the action of restriction endonucleases, DNA repair, and DNA transfer barrier. The constant interplay between these two counteracting forces leads to the evolution of new genotypes and subsequently to new phenotypes. Natural selection, according to Darwin, is the driving force selecting for the species which are more suitably adapted to their environment, and which survive better than their competitors.

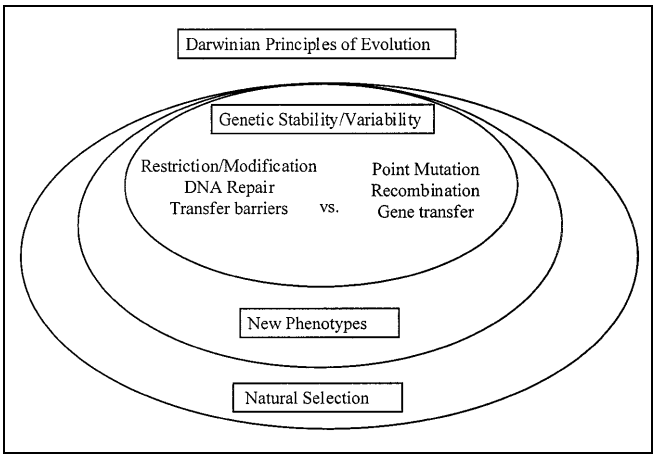

Fig. 3. Darwinian principles of evolution

Mutations

Point mutations, i.e., heritable changes in the base sequence of a genome, occur only rarely and may or may not result in a change in the associated phenotype. Therefore evolution based on point mutational events is comparably slow relative to the genetic mechanisms discussed below that are based on the acquisition, transfer, or loss of sometimes large genetic elements. However, mutations in both regulatory genes and structural genes of virulence factors may have severe consequences. Type 1 fimbriae of *E. coli* have been shown to be varied by so-called "pathoadaptive" mutations leading to variants with increased binding capacity to extracellular matrix proteins and enhanced tissue penetration (Pouttu et al. 1999; Sokurenko et al. 1998). Interestingly, particular genes of bacterial pathogens exhibit stretches of repeated DNA sequences in promoter regions or in the 5′ end of virulence genes. During replication point mutations can be generated by slipped stranded mispairing. In consequence the genes are expressed or notexpressed. In gonococci, meningococci, and *Mycoplasma* spp., phase and antigenic variation in surface-associated structures are mediated by these mechanisms (Achtman 1995; Dybvig and Völker 1996; Leathart and Gally 1998). Homologous and site-specific recombination events also contribute considerably to both antigenic variability and to the development and spread of antibiotic re-

sistance. In *N. gonorrhoeae*, for instance, the capacity for pili diversity is based on a *RecA*-dependent intragenic recombination between a silent locus and the expression locus on the same chromosome (Koomey 1987). It is likely that adaptive point mutations occur also in symbiotic systems, however, to our knowledge they have not yet been described.

Transposons and Insertion Sequences

Larger genetic rearrangements within organisms are possible through the action of extragenic elements such as transposons and insertion sequences (IS elements; Deonier 1996). These elements are able to move themselves to nearly any site on a given chromosome. Transposition is accompanied by duplication of a short sequence of the recipient site. Transposons contain genes that mediate functions in addition to transposition. In contrast, bacterial IS elements are small mobile DNA units that encode only for a transposase protein and inverted repeat sequences that exactly define the borders of the element (Mahillon and Chandler 1998). IS elements occur on the bacterial chromosome, plasmids, phages and in composite transposons and they have the capacity to cause inactivation of virulence genes. In *S. epidermidis* phase variation in biofilm formation is controlled by insertion and excision of the insertion sequence element IS 256 (Ziebuhr et al. 1999b). Sequencing of the symbiotic plasmid (replicon) of *Rhizobium* revealed the presence of numerous IS elements and other genes involved in transposition, integration, and recombination (Freiberg 1997).

Transformation, Transduction, and Conjugation

Horizontal transfer, i.e., the exchange of DNA between genetically different organisms is possible through transformation, transduction, and conjugation. Transformation describes the transfer of DNA into bacteria. This process is key to the virulence of *Neisseria* spp., *Streptococcus pneumoniae,* and *Helicobacter pylori* (Cheng et al. 1997; Hofreuter et al. 1998; Koomey 1998). Transformation occurs mainly between members of the same species and is of substantial clinical importance as it contributes to the spreading of antibiotic resistance genes. The emergence of penicillin resistance is due to recombinational exchange between the penicillin-binding protein encoding genes of *S. pneumoniae* and the corresponding DNA from related, intrinsically resistant streptococcal species (Hakenbeck and Coyette 1998). The capacity of pneumococci for uptake and integration of extracellular DNA by natural competence and transformation is regarded as the driving force in these processes.

Transduction involves the transfer of DNA through the agency of viruses and phages. These are able to integrate into various sites of the chromosome (lysogenic stage). Upon entry into the lytic stage, viruses may carry additional DNA to the newly infected host (host gene capture). Interestingly, toxin-specific genes are often found in association with bacteriophage genomes (Dobrindt and Hacker 1999). The lysogenic conversion of *Corynebacterium diphtheriae, Clostridium botulinum, Shigella dysenteriae*, and enterohemorrhagic *E. coli* into toxin positive strains generates the highly virulent pathogens (Feng et al. 1998). Also, in toxigenic *Vibrio cholerae* the CTX genetic element which carries the genes for cholera toxin is the genome of a lysogenic bacteriophage, CTXφ (Faruque et al. 1998).

The third mechanism, conjugation, relies on plasmids and usually enables a broad host range transfer. Plasmids are usually circular, extrachromosomal DNA elements which are self-transmissible or spread by mobilization. Many important pathogenic and symbiotic determinants as well as antibiotic resistance genes are encoded on plasmids (Freiberg et al. 1997; Parsot and Sansonetti 1996; Portnoy and Falkow 1981). For example, several operons relevant to the symbiotic functioning are located on the Sym-plasmid of *Rhizobium*. These include the nodulation (*nod*) genes which direct specific steps in the nodulation of the legume and the specificity genes which restrict a given *Rhizobium* strain to a particular plant host. Host specificity can be transferred across species by transferring the Sym plasmid. Moreover, the nitrogen fixation (*nif*) genes are often located on the Sym-plasmid. The bacterial endosymbiont *Buchnera aphidicola* of aphids also contains plasmids which carry operons for leucine (*leu*ABCD) and tryptophane (*trp*EG) biosynthesis. These complement the amino acid deficiency of the host which feed exclusively on plant sap (Baumann et al. 1999). Plasmids of variable sizes are also known in *Xenorhabdus* symbionts of nematodes (Steinernema; Smigielsky and Akhurst 1994) and in *Vibrio fischeri* symbionts of squids (Euprymna; Boettcher and Ruby 1994), but their function is not yet known.

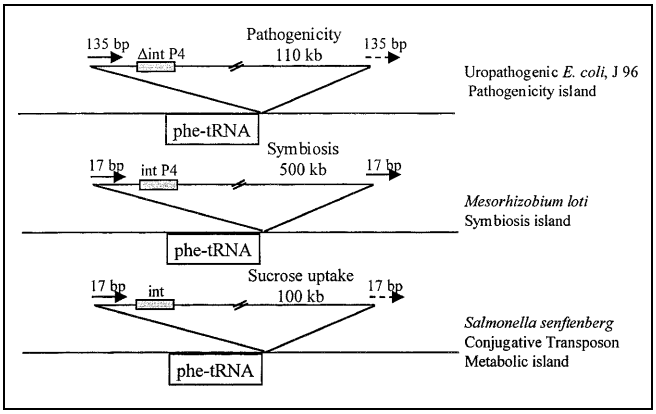

Fig. 4. Identical location of different genomic islands in *E. coli* (pathogenicity island), *Mesorhizobium loti* (symbiosis island), and *Salmonella senftenberg* (metabolic island)

Genomic Islands

Genomic islands are formally movable genetic elements which occur as distinct units on the core chromosome (Hacker and Kaper 1999). They comprise pathogenicity islands (Pais), symbiosis islands, and metabolic islands depending on which functions they encode. The general genetic structures of these islands are lined out in Fig. 4. Pais have initially been identified in various human pathogens such as *E. coli, Yersinia* spp., *H. pylori*, and *V. cholerae* (Buchrieser et al. 1998; Censini et al. 1996; Karaolis et al. 1998; Ritter et al. 1997). These genetic regions are characterized by a set of unifying features: they are often large (30–200 kb), differ in GC content from the rest of the chromosome, are flanked by specific sequences (direct repeats), are associated with tRNA loci which presumably act as targets for the integration of foreign DNA, and often possess genes or cryptic pseudogenes encoding for genetic mobility (phages, IS elements, intergrases, transposases, origins of replication; Hacker et al. 1997). Pais are notoriously unstable gene clusters and have the tendency to delete with high frequencies or may undergo duplications and amplifications. It is conceivable that the sequential deletions of Pais may also play a role in the bacterial adaptation to different environments. Current theory holds that Pais are subject to an ongoing evolutionary process aiming to stabilize clusters of functionally relevant genes on the chromosome which were formerly encoded on mobile genetic elements (Hacker and Kaper 1999; Hacker et al. 1997).

Recently a symbiosis island has been discovered in *Rhizobium* (Sullivan and Ronson 1998). This symbiosis island shares common features with Pais in that it integrates into a phe-tRNA gene and encodes an integrase of the phage P4 family within its left end. The symbiosis island comprises 10% of the entire genome and is therefore larger than any Pai known to date. Transmission of the symbiosis island has been shown in experiments in which commensal *Mesorhizobium* strains became symbiotically competent in a laboratory mating event. A genomic island has been described in *Salmonella senftenberg* (Hochhut et al. 1997). Here the genes encoding sucrose fermentation are located on a self-tranmissible element, i.e., a conjugative transposon of 100 kb, which integrates into a phe-tRNA.

Selection by Exploitation of Host Niches

The selective pressure which drives the evolution of microbes associated with host organisms is the sum of the physical, chemical, and biological parameters of the respective host microenvironment. Many bacteria are adapted to this hostile but nutritious milieu. The introduction of a new infectious agent into a virgin susceptible host population may first lead to a dramatic infection and an epidemic. Once a pathogen persists in the new population, the disease tends to become less virulent with time. This is largely due to the development of host immunity within the population (herd immunity; Brunham et al. 1993). However, the spread of the parasite is also often diminished by attenuation of the pathogen itself, and eventually a balance is established with the host. A subsequent genetic change in the parasite can again lead to a higher virulence, which then would initiate another epidemic until the host again responds, and another balance is reached.

As such, infectious pathogenic and apathogenic agents are selected to exhibit traits which allow adherence, and sometimes entrance and replication,

Table 2. Selection of bacterial pathogens by exploitation of host niches

Location	*Clostridium*	*Staphylococcus*	*Legionella*	*Listeria*	*Rickettsia*
Extracellular					
Adherence	−	+	+	+	+
Phagocytosis	−	−	+	+	+
Intracellular					
Phagosome	−	−	+	+	+
Cytoplasm	−	−	−	+	+
Nucleus	−	−	−	−	+

within the host (Table 2). One exception, in which disease occurs without colonization, is the foodborne toxinose caused by *C. botulinum* (Hatheway 1990). In most bacterial diseases, however, certain receptors on eukaryotic cell surfaces determine the tropism of a pathogen. Human fibronectin, certain carbohydrate sequences and specific collagens represent adhesion targets for S-fimbriated *E. coli* associated with meningitis (Pouttu et al. 1999; Saren et al. 1999; Schroten et al. 1998). It is noteworthy that in *E. coli*, *N. gonorrhoeae*, *V. cholerae* bacterial adhesins mainly recognize carbohydrate receptors (Jones and Freter 1976; Ölschläger et al. 1997; de Vries et al. 1998). In *S. epidermidis,* polysaccharides show adhesive properties (Ziebuhr et al. 1999b). In this context it is interesting to note that the oligosaccharide chains (glycans) of cell surface and extracellular proteins show a striking diversity. It has been proposed that the selection was mediated in part by viral and microbial pathogens (Gagneux and Varki 1999). Intracellular pathogens are often able to bind or induce host cell receptors that are involved in signal transduction. The actions of bridging molecules (*Legionella pneumophila*, *Mycobacterium tuberculosis*) or invasins (*Yersinia* spp., *S. flexneri*) induce or enhance uptake by professional and nonprofessional phagocytes (Brand and Hacker 1996; Dersch and Isberg 1999; Ölschläger and Hacker 1999; Schlesinger et al. 1990; Tran Van Nhieu et al. 1997). Once intracellular, microbes can occupy several different locations within the host cell. The least integrated are pathogens that are contained within vacuoles. However, often an intricate remodeling of the vacuole membrane occurs. *Chlamydia* spp., *L. pneumophila*, and *Mycobacterium* spp. establish a replication-competent vacuole. This includes removal of certain membrane proteins, inhibition of fusion with lysosomes, and a reduction in the vacuole acidification (Portillo and Finlay 1995). Another strategy employed by *Listeria monocytogenes* and *Shigella* spp. is the escape from the phagosome into the cytoplasm. By inducing the assembly of actin filaments in the host cell cytoplasm both pathogens spread to adjoining cells (Portnoy et al. 1992; Sansonetti 1992). A remarkable feature in terms of the exploitation of host cell compartments is the ability of *Rickettsia rickettsii* to grow within the cytoplasm and the nuclei of their host cells (Silverman and Bound 1984).

Coevolution of Pathogen Virulence and Host Resistance

The adaptation of pathogens to new niches enforces a host reaction, and in this context bacteria can be viewed as catalysts of eukaryotic evolution. If pathogens and hosts coevolve, several outcomes are possible. One possibility is that the pathogen attenuates. This attenuation of the pathogen and increasing dependence on the host may result in mutualistic or symbiotic relationships. Over evolutionary time pathogens may favor host traits that reduce the impact of the bacteria. In some cases the result is cooperation with reciprocal benefit. In another scenario the pathogen remains virulent, and as a result the host invests heavily in defense. The evolution of antimicrobial defense mechanisms of hosts and the characteristics of certain pathogens which are able to by-pass these host defenses is a good example of the escalated "arms race."

Phagocytosis by amoeboid cells represents a nonspecific defense mechanism which can be observed throughout invertebrate phylogeny. Since the appearance of free-living protozoa, bacteria have had to develop intracellular survival strategies. In this way the coevolution of the legionellae-amoebae interaction can be considered as a "living fossil" of the adaptation of bacteria to professional phagocytes (Fields 1996; Steinert et al. 1998a,b). The similarity of some protozoan species and human macrophages is striking. Upon bacterial uptake both cell systems usually employ antimicrobial mechanisms such as phagolysosome fusion and phagosome acidification. It appears that over evolutionary time the bacteria-protozoa interactions have generated a pool of genes that enabled *Legionella* to survive within human macrophages (Gao et al. 1998). In addition to the macrophage infectivity potentiator (*mip*) gene, the defect in organelle trafficking (*dot*) locus, and an iron acquisition complex have been shown to contribute to the infection of mammalian cells and protozoa (Fields 1996; Wintermeyer et al. 1995). These results provide evidence that *Legionella* infection of protozoa is related to *Legionella* infection of human cells. Interestingly, a number of other intracellular human pathogens such as *Mycobacterium avium*, *Listeria monocytogenes*, *Francisella tularensis*, and *Rickettsia* spp. exhibit intracellular survival within amoebae and macrophages (Barker and Brown 1994; Essig et al. 1997; Fritsche et al. 1999; Steinert et al. 1997, 1998a,b). Consequently it is has been suggested that amoebae are a driving force in the evolution of pathogenicity (Barker and Brown 1994).

In contrast to nonspecific defense, adaptive defense or immunity is unique to vertebrates. It is discussed that the adaptive immune system of vertebrates evolved in the gastrointestinal regions of primitive jawed fish (placoderms; Matsunaga and Rahman 1998). The extreme adaptive potential seen in higher vertebrates is based on the mechanisms of genetic recombination. The mechanisms involved include site-specific somatic mutations as well as gene conversion events for immunoglobins, T cell receptors, and the major histocompatibility complex molecules (Davis and Bjorkman 1988; Lebeque and Bearhart 1990; Tonegawa 1983). Interestingly, the genetic mechanisms employed by human hosts to respond to antigenically variant pathogens are remarkably similar to the microbial strategies (Brunham et al. 1993). Generation time is another convergent development of pathogens and certain cells of the immune system. Most vertebrate cells have relatively slow generation times. To compensate for this disadvantage in infection lymphocytes have evolved a higher rate of replication, being an order of magnitude closer to that of the pathogens (Brunham et al. 1993).

The strategies of the vertebrate immune system, including the production of antibodies and activation of macrophages and cytotoxic T cells in response to bacterial antigens, forced pathogens to evolve a wide range of countermeasures. Protective capsules, immunoglobulin proteases, altered lipopolysaccharide O antigen, toxic proteins, and mechanisms of surface variability help pathogens to evade the human immune system (Salyers and Witt 1994). An extreme adaptation to the host immune system is exemplified by obligate intracellular pathogens which can reside within human macrophages (e.g., *Chlamydia pneumoniae*; Redecke et al. 1998). The concept of exploitation of host immune response system is further reinforced by certain parasites.

Changes in Human Life-Style Select for New Pathogenic Variants

Changes in living conditions of the human population have changed niches for micro-organisms and selected new variants of pathogenic bacteria. Improving hygienic standards, vaccination programs, and chemotherapy has led to the decrease in the so-called classical pathogens in industrialized countries. However, 40 years of intense use of antibiotics in medicine and the food industry resulted in an increased antibiotic resistance. Multiple-resistant clinical strains of *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *S. aureus*, *S. pneumoniae*, and *M. tuberculosis* have appeared (Silver and Bostian 1993). Point mutations in target genes, transfer of genes encoding products that confer antibiotic resistance, and a high, man-made selection pressure for resistant strains are the evolutionary outcome of this adaptation.

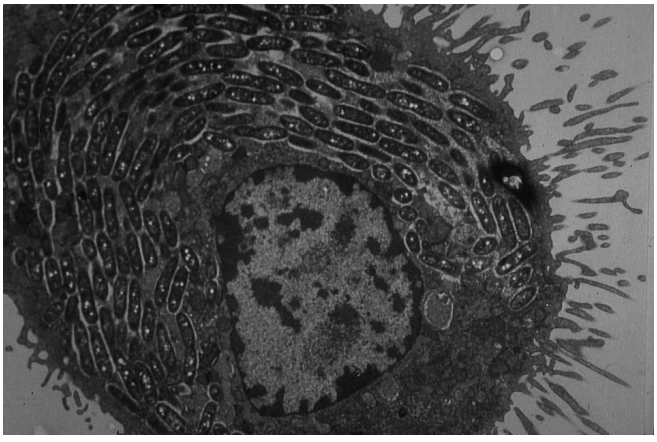

Fig. 5. Transmission electron micrograph of a human macrophage infected with *L. pneumophila*. The intracellular multiplication of the rod-shaped pathogen results in a host cell that is filled with bacteria

Legionnaires' disease is also a consequence of altering the environment for human benefit. Low numbers of *Legionella* commonly inhabit fresh water biotopes where they parasitize various protozoa species. By entering hot water systems the bacteria can multiply to high numbers (Steinert et al. 1998a,b). Infection with *Legionella* occurs by inhalation of contaminated aerosols via showers, cooling towers, and air conditioning systems (technical vectors). The strictly aerobic bacteria invade human lung macrophages where they replicate in a specialized phagosome (Brand and Hacker 1996; Gao et al. 1998). Figure 5 presents a transmission electron micrograph showing a *L. pneumophila* infection of a human macrophage. Several authors have reported that *L. pneumophila* cells are able to enter a viable but nonculturable state. This may account for the fact that *L. pneumophila* often cannot be cultured from cooling towers which are suspected to be the source of infecting bacteria. Interestingly, it has recently been shown that viability and infectivity of nonculturable *L. pneumophila* can be reactivated by intracellular replication within protozoa (Steinert et al. 1997). The intracellular replication within protozoa also complicates the eradication of *Legionella* in man-made water systems. It has been demonstrated that trophozoites and cysts of amoebae may protect *L. pneumophila* during water treatments. Especially in

protozoal cysts, legionellae survive after exposure to more than 50 mg/l of free chlorine. Therefore efficient eradication of *L. pneumophila* from man-made niches remains problematic. In addition, recolonization of plumbing systems by legionellae is readily accomplished via the water supply and other environmental sources (Steinert et al. 1998a,b). *L. pneumophila* is only one of approximately 30 species which have been described as "new pathogens" in the past two decades. As with *L. pneumophila*, many of these new pathogens gained their pathogenic potential simply by changing environmental conditions. To control the growth of these pathogens in human settings therefore requires more information on the ecophysiology of environmental bacteria.

Conclusion

Darwin's concept of evolution was not discovered on the protocyte (bacteria and archaea) level. However, the advent of microbiology and, more recently, molecular biology has made it possible to apply and expand Darwin's ideas to these two domains of the three-domain tree of life. The rapid generation time, high diversity, and extremely large populations enable bacteria to evolve with extraordinary speed in comparison with their eukaryotic hosts. The examples presented above demonstrate the success of bacteria-host interactions ranging from the oldest symbiosis, i.e., the formation of the eukaryotic cell to pathogenic infections of vertebrate hosts. While the processes leading to the evolution of either symbionts or pathogens are similar, the establishment of a symbiosis requires more time and evolutionary processing. A pathogenic interaction can be viewed as the initial stages of an arms race while in a symbiotic one the battle has been settled to mutual benefit. In all these interactions the replicative bacterial partners function as evolutionary catalysts. It is through the intimate association that bacteria drive the evolutionary changes of their hosts to new speciation and diversification.

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