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Intranuclear bacteria: inside the cellular control center of eukaryotes

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Intracellular bacteria including major pathogens live in the cytoplasm or in cytoplasmic vacuoles within their host cell. However, some can invade more unusual intracellular niches such as the eukaryotic nucleus. Phylogenetically diverse intranuclear bacteria have been discovered in various protist, arthropod, marine invertebrate, and mammalian hosts. Although targeting the same cellular compartment, they have apparently developed fundamentally-different infection strategies. The nucleus provides a rich pool of nutrients and protection against host cytoplasmic defense mechanisms; intranuclear bacteria can directly manipulate the host by interfering with nuclear processes. The impact on their host cells ranges from stable associations with a neutral or beneficial effect on host fitness to rapid host lysis. The analysis of the intranuclear lifestyle will extend our current framework for understanding host–pathogen interactions.

Bacteria in eukaryotic cells

Bacteria living within eukaryotic cells are ubiquitous. As beneficial symbionts or major pathogens they are integral to the biology, ecology, and evolution of their animal and human hosts. Intracellular bacteria generally reside directly in the host cytoplasm or in host-derived vacuoles [1]. To successfully thrive in vacuolar compartments, bacteria frequently hijack the host endocytic and secretory pathway, recruit host proteins and ribosomes to the vacuolar membrane, and ultimately structure a specialized organelle for their replication [2]. Only rarely have bacteria been observed directly within other intracellular compartments such as the endoplasmic reticulum, Golgi-related vesicles, plastids, mitochondria, or the nucleus [3–8]. Bacteria in the latter compartment are referred to as intranuclear (or endonuclear) bacteria, and currently represent the largest group of microbes targeting unusual subcellular niches.

The first evidence for intranuclear bacteria reaches back to the 19th century when enigmatic particles were observed in the nucleus of paramecia (Box 1). Today, intranuclear bacteria are known to occur in various protists, insects, marine invertebrates, and mammals. While most remain elusive, some have been described in more detail by light and electron microscopy. Information on their phylogeny and distribution, the infection process, and their impact on host cells is available for very few intranuclear bacteria. The advent of cultivation-independent methods for the identification and functional analysis of microbes in conjunction with current 'omics' technologies provides a new momentum, facilitating the characterization of intranuclear bacteria.

This review, for the first time, summarizes major findings on intranuclear bacteria obtained during the past 150 years. We first recapitulate current knowledge about the diversity of intranuclear bacteria and their respective hosts. We then provide an overview about the evidence and hypotheses regarding the infection process and invasion of the nuclear compartment. We then discuss possible advantages of exploiting this particular intracellular niche, and we end with brief evolutionary considerations.

Intranuclear bacteria in protozoa

The majority of known intranuclear bacteria are associated with protists (Figure 1). One of the most thoroughly studied and best-understood intranuclear bacteria are *Holospora* spp., which infect paramecia and are affiliated with rickettsiae (Alphaproteobacteria). In the late 19th century, three *Holospora* spp. were described and distinguished based on their characteristic morphology and their intracellular location, either in the micro- or the macronucleus [9]. The infection process of *Holospora* has been studied extensively and reviewed recently [10]. *Holospora* spp. show two distinct developmental stages: an infectious form up to 20 μm in length and a short reproductive morphotype which forms in the nucleus. Most *Holospora* spp. thrive inside the macronucleus of paramecia where up to 100% of a host population can be infected, despite a negative impact on host growth rate (reviewed in [11]). *Holospora* spp. exploiting the micronucleus are less prevalent (about 10% in the host population) because they impair ciliate sexual reproduction. The association of *Holospora* with paramecia can be stable over long time-periods, but starvation may trigger differentiation back into the infective form, which then escapes from the nuclear compartment and eventually causes host cell lysis (reviewed in [11]). Interestingly, infection with a *Holospora* sp. does not necessarily represent a burden for the *Paramecium* host but may impose a selective advantage. The presence of the symbiont enhances expression of host heat-shock proteins, resulting in increased host survival rates in the face of adverse environmental conditions such as altered salinity or temperature shifts [12–14].

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Box 1. The discovery of intranuclear bacteria

In the middle of the 19th century, rod-shaped particles were observed for the first time in the nuclei of ciliates [79]. At that time it was believed that the nucleus played an important role in 'embryo formation', and the intranuclear structures were proposed to represent spermatozoa produced in this compartment [79–81]. In the following years, similar findings were reported several times; it was noted that the particles were immobile, and the hypothesis arose that these intranuclear rods are in fact bacterial parasites [82–84]. In the early 20th century, coccoid intranuclear parasites in the flagellate *Euglena* [31] were reported that caused hypertrophy of the nucleus, discoloration of plastids, and inhibition of host cell division. Morphologically-similar bacteria were subsequently found in *Trichonympha* flagellates isolated from termites and were described as *Caryococcus* spp. [32]. Bacteria in the nuclei of multicellular eukaryotes were first noted during the analysis of rickettsiae as the causative agent of Rocky Mountain spotted fever. In addition to rod-shaped bacteria being found in the cytoplasm of tick and guinea pig cells, these bacteria were also occasionally found inside the nuclear compartment [85,86].

A second lineage of Alphaproteobacteria, represented by *Caedibacter caryophilus* and *Caedibacter macronucleorum*, is able to thrive in the nuclei of paramecia [15–17]. These bacteria were observed for the first time in the early 20th century. They are mainly transmitted vertically upon cell division of the *Paramecium* host and were originally described based on an outstanding feature, the presence of characteristic phage-like structures, so-called reticulate bodies (R-bodies; reviewed in [18]). These R-bodies mediate the 'killer trait' of *Paramecium* hosts, which serves as a defense mechanism to outcompete related symbiont-free paramecia ([19], reviewed in [18]). Two different *Caedibacter* phenotypes can be distinguished by the infective form that expresses R-bodies and the reproductive form that lacks R-bodies. Infective forms are released from symbiont-carrying hosts and can be taken up by symbiont-free paramecia through phagocytosis. Upon acidification of the phagosome, the R-body unrolls, elongates, penetrates

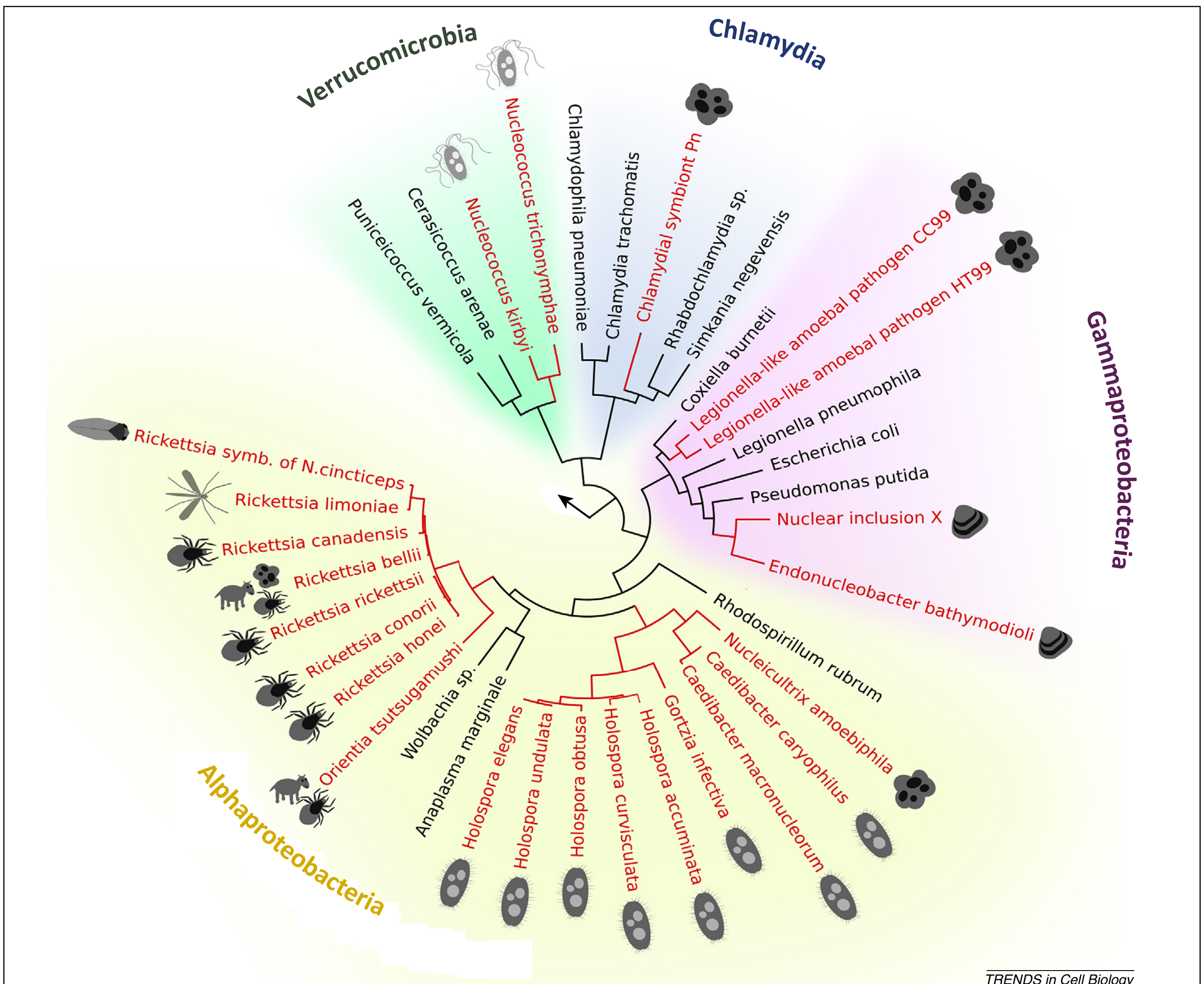

Figure 1. Phylogenetic relationships of known intranuclear bacteria. Microbes exploiting the nucleus of their hosts (shown in red) can be found in four major clades; the Alpha- and Gammaproteobacteria, the Chlamydiae, and the Verrucomicrobia. The respective host organisms are schematically indicated. A maximum-likelihood tree calculated with FastTree2 ([87] GTR+CAT approximation) based on a 16S rRNA alignment performed in Arb is shown [88].