# Fitness and freezing: vector biology and human health

J. Stephen Dumler

**Commentary**

Microbes transmitted to mammals by arthropods contend with many factors that could impede survival. To survive, host fitness with infection must outweigh costs. In this issue of the *JCI*, Neelakanta et al. demonstrate that ticks infected with *Anaplasma phagocytophilum* show enhanced fitness against freezing injury owing to induced expression of tick "antifreeze glycoprotein." This allows *A. phagocytophilum* to successfully propagate and survive to cause disease in nonnatural hosts, such as humans. How an intracellular microbe with a small genome subverts host cell function for survival provides insight into the control of some cellular function programs and underscores how vector biology can have an impact on human health.

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# Fitness and freezing: vector biology and human health

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**Microbes transmitted to mammals by arthropods contend with many factors that could impede survival. To survive, host fitness with infection must outweigh costs. In this issue of the *JCI*, Neelakanta et al. demonstrate that ticks infected with *Anaplasma phagocytophilum* show enhanced fitness against freezing injury owing to induced expression of tick "antifreeze glycoprotein." This allows *A. phagocytophilum* to successfully propagate and survive to cause disease in nonnatural hosts, such as humans. How an intracellular microbe with a small genome subverts host cell function for survival provides insight into the control of some cellular function programs and underscores how vector biology can have an impact on human health.**

Emergence and reemergence of infectious diseases is often attributed to many factors, most of which are influenced by human activities, for example, climate change, environmental change, changes in human demographics and behaviors, and the rise of global trade and travel (1). Perhaps not surprisingly, pathogens evolve or acquire genetic mechanisms that enhance fitness under circumstances of human intervention, such as increasing resistance to potent antimicrobial pharmacologic agents. Emerging infectious disease is most likely to be caused by zoonotic or vector-borne agents (1). This is readily understood for zoonotic and vector-borne RNA viruses (such as tick-borne encephalitis viruses, West Nile virus, and the viruses that cause yellow fever, dengue fever, and Crimean-Congo hemorrhagic fever), which undergo frequent genetic change and thus have repeated opportunities to improve fitness (1). However, the substantial evolution of fitness needed to emerge (or reemerge) as a significant health concern in humans is surprising in the context of vector transmission for organisms with less flexible genomes, few opportunities for genetic exchange, and the need to contend with protective mechanisms in at least two distinct hosts. Yet drug-resistant malaria, African and American trypanosomiasis, Lyme disease, and rickettsial infections such as Rocky Mountain spotted fever (RMSF) and human granulocytic anaplasmosis (HGA) have emerged or reemerged to increase in prevalence over the past few decades (2). While considerable investigation is being conducted for some vector-borne emerging diseases and pathogens, bacteria of the order Rickettsiales and their resulting human diseases are understudied, in part because they are obligate intracellular pathogens, which makes biological study difficult, and in part because it is extremely difficult to establish definitive diagnosis, which makes clinical study a challenge (3). Vector biologists who study arthropod-transmitted pathogens understand the role of the vector not only in transmission, but also in disease "ecology." In this issue of the *JCI*, Neelakanta et al. demonstrate a new paradigm as to how a tick-borne pathogen can manipulate its arthropod host to foster vector survival and indirectly, pathogen transmission, mammalian reservoir maintenance, and, inevitably, human disease (4).

## Increasing rickettsial disease prevalence

While Lyme disease, which is caused by bacteria belonging to the genus *Borrelia*, is the most common vector-borne human infectious disease in the US (5), rickettsial infections are also increasingly being reported in North America, South America, and Europe, and serological studies show that rickettsial infections are common causes of febrile illness in regions where most fever is attributed to malaria or typhoid fever (5). Recognition of tick-borne rickettsial infections, such as RMSF (caused by *Rickettsia rickettsii*), African tick-bite fever (caused by *Rickettsia africae*), human monocytic ehrlichiosis (caused by *Ehrlichia chaffeensis*), and HGA (caused by *Anaplasma phagocytophilum*) has markedly expanded in recent years (3). The 4,727 confirmed and unconfirmed reports
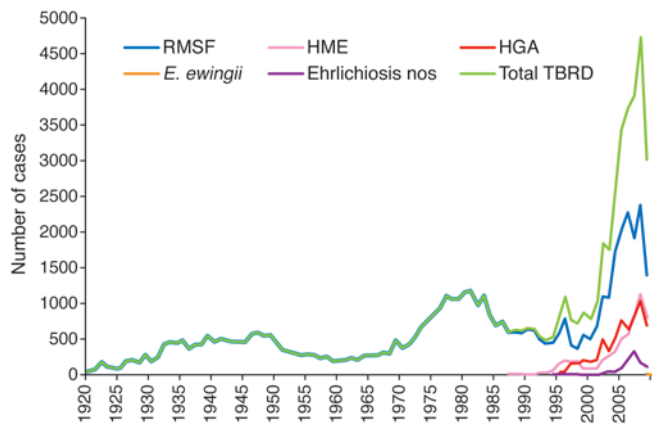
**Figure 1**
Confirmed and unconfirmed cases of tick-borne rickettsial diseases (TBRD) in the United States, 1920 to 2009. HME, human monocytic ehrlichiosis; nos, not otherwise specified. Graph generated using data obtained from Public Health Service (PHS) and Morbidity and Mortality Weekly Report (MMWR) records (www.CDC.gov/mmwr/).

of tick-borne rickettsial disease to the CDC in 2008 exceed historical highs by a factor of 4 (Figure 1 and ref. 5). Moreover, increasing evidence documents underreporting of RMSF, and likely other tick-borne rickettsial infections, by as much as 5- to 6-fold (6, 7). These observations parallel those in Brazil, where *R. rickettsii* infection, locally called *Brazilian spotted fever*, has reemerged, claiming lives in its wake (8). While at least some of the increase in the US is thought to be related to reporting issues (9), the number of additional case reports of humans infected by *Ehrlichia* spp. and *A. phagocytophilum* has more than doubled the reported incidence of tick-borne rickettsial infections between the prior peak in 1981 and 2008 (5).

### Human disease and obligate intracellular parasites
Many bacteria in the order Rickettsiales have dual life cycles, one within a mammalian host that amplifies bacterial population size and sometimes provides a safe reservoir from endogenous antimicrobial mechanisms, and one within an arthropod that is the vector for transmission (5). Moreover, their obligatory intracellular lifestyle dictates that they must contend with the substantial challenge of host cell responses to parasitism, even within primary host defense cells such as macrophages and neutrophils and in endothelium, which is continuously surveyed by host defense cells. *A. phagocytophilum* and *Borrelia burgdorferi* are both transmitted by *Ixodes* spp. ticks, and on at least 3 continents, these ticks transmit *A. phagocytophilum* to animals and on occasion to humans.

Human infection with *A. phagocytophilum* is likely subclinical in the majority of infections, but among those with illness, at least 7% require intensive care unit admission, and death can occur (10). This is of particular interest because the neutrophil "home" to *A. phagocytophilum* in human and mammalian hosts is also the primary and most abundant host defense cell. Precisely how *A. phagocytophilum* survives in this inhospitable environment is predicated on its ability to alter functions of the infected granulocyte, including diminished respiratory burst, enhanced inflammatory recruitment of new host cells, and delayed apoptosis of infected cells (10, 11). The ability of the pathogen to alter host cell transcriptional programs seems to be key for each of these phenotypic and functional alterations (12–14). Proven and potential impacts of these alterations are prolonged bacterial survival leading to expanded bacterial populations in the blood of infected mammals, thereby increasing the odds that the next tick bite will result in successful transmission via blood meal acquisition.

### Fitness and survival
The adaptation of *A. phagocytophilum* to the blood niche in a mammal is often subclinical but can result in inflammation and disease. While some aspects of the survival of *A. phagocytophilum* in mammals are now clear, how it contends with the tick is poorly understood. Around the world, the *Ixodes* spp. tick vectors of *A. phagocytophilum*, including *I. scapularis* in North America, *I. ricinus* in Europe, and *I. persulcatus* in Asia,

inhabit regions where substantial seasonal temperature swings yield freezing conditions for much of the winter. Despite the identification of *Ixodes* spp. ticks in lower temperate and subtropical latitudes, *A. phagocytophilum* tick infection and disease prevalence is nearly uniformly restricted to geographic regions that have low average temperatures during winter months (10). Infection of *Ixodes* spp. ticks by *A. phagocytophilum* was not believed to alter tick fitness under usual study circumstances that assess vector competence in field experiments or laboratories at ambient temperatures maintained well above freezing (15). It is counterintuitive that tick infection, which is mandatory for *A. phagocytophilum* survival, persists even in the absence of observed direct fitness benefits for the tick. However, in this issue of the *JCI*, Neelakanta et al. identify a mechanism by which *A. phagocytophilum* enhances tick fitness (4). The hypothesis that Neelakanta and colleagues set to test was that *A. phagocytophilum* would influence the survival of *I. scapularis* exposed to freezing temperatures since it survives the winter in tissues of the tick. Exposure of uninfected or *A. phagocytophilum*–infected ticks to temperatures well below freezing indicated that the infected ticks not only survived better but that survivors were more mobile and therefore more fit. The improved survival of infected ticks correlated with a threshold burden of *A. phagocytophilum* in the tick tissues, supporting a direct link between fitness and bacterial infection. To explain these observations, the authors identified an *Ixodes* spp. "antifreeze" glycoprotein similar to those found in other arthropods that was upregulated and promoted tick survival during freezing intervals and for which silencing reduced tick survival. Remarkably, the authors were able to demonstrate that infection with *A. phagocytophilum* further enhanced expression of the antifreeze glycoprotein and that this promoted tick fitness by enhancing ability to survive freezing intervals such as those that ticks might experience in the northern latitudes of North America, Europe, or Asia, precisely the areas where the pathogen and infection occur with greatest prevalence. This biological change was mediated by upregulated transcription of the gene encoding the antifreeze glycoprotein. Precisely how *A. phagocytophilum* accomplishes this transcriptional alteration is unclear, but the ramifications of improving tick fitness for surviving freezing conditions are
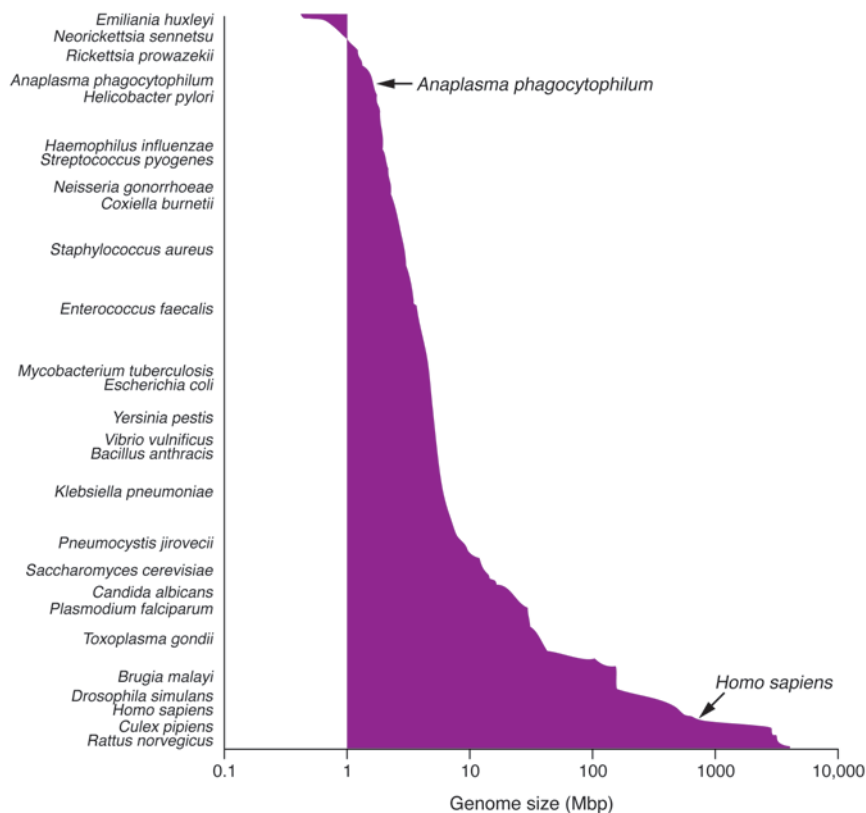
**Figure 2**
Relative sizes of sequenced eukaryotic, prokaryotic, and Achaea genomes. Graph generated using data available from the NCBI genome database (http://www.ncbi.nlm.nih.gov/sites/genome).

evident in the resulting expansion of bacterial populations and perhaps with the emergence of HGA.

### Can pathogen-induced vector fitness have an impact on human health?

The observations of Neelakanta et al. (4) underscore concepts in vector biology that are generally ignored and often misunderstood by biomedical scientists and the medical community: (a) vector ecology has its basis in biology and (b) the outcomes can weigh substantially on human infection and disease. The understanding of the former concept is best illustrated by examining endosymbionts of arthropods, such as *Buchnera aphidicola*, a symbiont of plant sap–feeding aphids that require the bacterium for survival because it supplies them with certain amino acids (16); *Wigglesworthia* spp. mutualists, which promote maternal fecundity, blood meal acquisition, and digestion in *Glossina* spp. tsetse flies (17); and *Wolbachia* spp., which infect mosquitoes and regulate iron metabolism and oxidative stress and prevent apoptosis (18).

In the cases of these endosymbionts, manipulation of the host by the microbe often is neutral to the host or mutually beneficial to both microbe and host. However, for those vector-borne microbes whose life cycles require adaptations that promote survival in several hosts, the effects can be detrimental to the vector or to human and animal health. In general, mammalian pathogens transmitted by ticks, fleas, or lice have harmful effects on their vector hosts as well, illustrated in the case of *R. rickettsii* where tick fecundity is decreased, or with *Rickettsia prowazekii* infection that substantially shortens louse lifespan and therefore reproductive capacity (19, 20). In contrast, *A. phagocytophilum* promotes tick and microbial survival, and thereby, pathogen transmission and dissemination. The ability of *A. phagocytophilum* to improve tick host fitness, as demonstrated by Neelakanta et al. (4), very likely reflects biological adaptations that further promote microbial survival and transmission in natural mammalian reservoirs. While microbe-induced transcription of the tick antifreeze glycoprotein–encoding gene is unlikely to have an impact on human health directly, the survival adaptations that result from transcriptional reprogramming likely imperil nonnatural, or "nonadapted," accidental hosts such as humans and some animals. Increasing data now demonstrate that *A. phagocytophilum* has the ability to directly alter host granulocyte antimicrobial and inflammatory transcriptional programs.

For example, it suppresses transcription of the gene encoding cytochrome b-245, beta polypeptide (CYBB), which is part of the phagocyte oxidase complex that promotes intracellular killing (13), and it induces proinflammatory gene expression that ultimately promotes recruitment of new host cells (21). Both yield microbial survival and expand microbe populations.

A critical question raised by the ability of the microbe to alter fitness with host cell infection is, how does a microbe with one of the smallest genomes control and manipulate cells with 1,000-fold excess genetic reserves (Figure 2)? The most likely scenario involves microbial targeting of host metabolic "bottlenecks" or "weak links," that is, pathways where manipulation alters entire functional programs. This could occur by modifying functional programs governed by epigenetic marks that regulate transcription from large physical regions of host chromatin (12) or by subverting host regulators of broad transcriptional programs, such as NF-κB and CCAAT displacement protein (CDP) (22, 23). Disease in humans infected with *A. phagocytophilum* is truly emerging, but the reasons for this are still under investigation. The work of Neelakanta et al. provides insight into the basic biological processes that belie manipulation of

the vector by a pathogen, providing a theoretical framework for its impact on human health and disease. These and related findings demonstrate how relevant ecology and vector biology are in protecting human and animal health, especially for emerging zoonotic and vector-borne diseases.

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# Too much PABP, too little translation

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**Posttranscriptional regulation is of critical importance during mammalian spermiogenesis. A set of mRNAs that encode proteins critical to normal sperm formation are synthesized early in the process of male germ cell differentiation and are stored in a repressed state. These mRNAs are subsequently translationally activated during the process of spermatid elongation and maturation. Of note, the translationally repressed mRNAs contain long poly(A) tails that are dramatically shortened during the translational activation process. Understanding the mechanisms that underlie this process of mRNA storage and subsequent translational activation has been a long-standing goal. The relationship of the poly(A) tail to translational control is intimately related to the functions of the cognate poly(A)-binding proteins (PABPs). In this issue of the *JCI*, Yanagiya and colleagues use a set of knockout mice to demonstrate a novel functional role for a particular modulator of PABP function, PABP-interacting protein 2a (PAIP2A), in the normal terminal differentiation of male germ cells.**

## Translational control is critical to germline formation

Modulation of mRNA translation is an effective tool to regulate gene expression in the absence of new mRNA synthesis. In part, translation is postulated to be affected by the 5′ methyl-7-guanosine (m7G) cap and 3′ poly(A) tail, two distinguishing features of a typical mature mRNA. Binding of eukaryotic initiation factor 4E (eIF4E) to the 5′ cap and poly(A)- binding protein (PABP) to the poly(A) tail precedes translational initiation. According to current models, the initiation factor eIF4G can bind simultaneously to both eIF4E and PABP, thus bridging the ends of the transcript. These interactions are proposed to circularize the mRNA, forming a postulated "closed loop" mRNA structure (1). This structure protects the mRNA termini from nuclease attack and enhances translational activity (2). Thus, poly(A) tail length and the poly(A) packaging protein PABP are considered to be critical determinants of both the stability and