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Review

Buried Treasure: Evolutionary Perspectives on Microbial Iron Piracy

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Host–pathogen interactions provide valuable systems for the study of evolutionary genetics and natural selection. The sequestration of essential iron has emerged as a crucial innate defense system termed nutritional immunity, leading pathogens to evolve mechanisms of 'iron piracy' to scavenge this metal from host proteins. This battle for iron carries numerous consequences not only for host–pathogen evolution but also microbial community interactions. Here we highlight recent and potential future areas of investigation on the evolutionary implications of microbial iron piracy in relation to molecular arms races, host range, competition, and virulence. Applying evolutionary genetic approaches to the study of microbial iron acquisition could also provide new inroads for understanding and combating infectious disease.

An Evolving View of Host–Microbe Interactions

The outcome of an infection can have profound consequences for both host and pathogen populations. Intense selective pressures make host–pathogen interactions an attractive biological model to study evolutionary genetics over relatively short intervals of time. To date, much work has focused on rapid evolution involving canonical host immune defenses or antibiotic resistance [1,2]. However, we now know that hosts possess numerous additional means to restrict pathogens, including factors engaged in other core physiological functions. Nutrient iron sequestration provides one such alternative mode of host defense against bacteria and eukaryotic pathogens [3]. Iron is an essential micronutrient for microbes, as well as their hosts, due to its ability to readily shift between ferrous (Fe^{2+}) and ferric (Fe^{3+}) oxidative states for redox catalysis or electron transport. This ability to readily accept and donate electrons also makes iron highly volatile, necessitating a well-coordinated iron transport and storage system in metazoans to prevent the production of toxic free radicals [4]. The sequestration of free iron by host proteins simultaneously prevents acquisition by microbes, a protective effect termed **nutritional immunity** (see Glossary) [5,6]. While appreciation has grown for the role of nutrient metals in infection, these 'battles for iron' and other trace metals provide intriguing cases for investigation from an evolutionary perspective. Here we discuss emerging questions on the control of iron in microbial infection and highlight recent and potential future insights regarding the evolution of molecular arms races, host range, microbial competition, and pathogen virulence.

The Battle for Iron

A potential role for iron in immunity became apparent following an elegant series of experiments by Arthur Schade and Leona Caroline in the early 1940s [7]. While attempting to develop a vaccine against *Shigella*, the researchers observed that addition of raw egg white to their culture media severely inhibited the growth of diverse bacteria as well as fungi. The antiseptic properties of egg white have in fact been recognized since the days of Shakespeare, where it was applied to wounds during Act III of *King Lear*. While various nutrient supplements failed to reverse the

Trends

The battle between microbes and their hosts for nutrient iron is emerging as a new front of evolutionary genetic conflict.

Molecular arms races can emerge between host iron-binding proteins and microbial 'iron piracy' factors that steal this nutrient for growth. Such rapid evolution may also contribute to the host range of pathogenic microbes.

Iron acquisition plays an important role in evolutionary interactions between microbes, both in the environment and within the host. Competition for iron can prevent infection by pathogens, while genetic changes in iron acquisition systems can enhance microbial virulence.

Evolutionary conflicts for nutrient iron are revealing potential new genetic mechanisms of disease resistance as well as avenues for therapeutic development.

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antimicrobial effect of egg whites, incinerated yeast extract did, suggesting that the limiting component was elemental in nature. Of 31 individual elements tested, supplementation with iron alone was sufficient to restore microbial growth in the presence of egg white. Adding to the fortuitous nature of their discovery, the authors posited that an iron-binding component present in the egg white prevented acquisition of this nutrient by microbes, which could have important implications for immunity. Two years later the scientists reported similar activity present in human blood serum [8]. The factor responsible for this activity in both cases was later revealed to be the protein **transferrin**, which plays a central role in animal iron metabolism by binding and transporting this metal to target cells [9,10].

In the decades following Schade and Caroline's initial discoveries, Eugene Weinberg proposed that withholding iron from microbial pathogens provided an important cornerstone of host defense, which he termed nutritional immunity [11]. Weinberg's theory explained previous observations that human iron overload disorders such as hereditary hemochromatosis and thalassemia render affected individuals highly susceptible to bacterial and fungal infections. The theory of nutritional immunity was also consistent with George Cartwright's earlier observations that infection induces an acute reduction in circulating iron levels [12–14]. Subsequent microbiology and molecular genetic studies established that nutritional immunity plays a pivotal role in defense against an array of pathogens, including bacteria, fungi, and parasites [3,15]. Owing to the iron-binding properties of proteins, such as transferrin, circulating levels of free iron in the body are orders of magnitude below the requirements for optimal microbial growth.

Microbes respond to iron starvation by actively scavenging this nutrient from host proteins to meet their metabolic requirements (Figure 1) [16]. One of the most common microbial iron acquisition strategies involves the secretion of **siderophores**, small molecule chelators, which possess an affinity for iron unmatched even by proteins such as transferrin [17,18]. Microbes then recover iron–siderophore complexes via cell surface receptors. Obviating the need for siderophores, several microbes also express receptors that directly recognize and extract iron from host proteins including transferrin and lactoferrin [19–23]. Additional mechanisms involve the acquisition of heme, the iron-containing porphyrin cofactor, from abundant host proteins such as hemoglobin [24–26]. Ferric reductases are an important class of iron acquisition systems in fungal pathogens, which convert transferrin or lactoferrin-bound ferric iron into a soluble ferrous form [27]. The identification of iron acquisition genes as pathogen **virulence factors** further underscores the role of iron in infection, as well as the potential for evolutionary conflicts to arise in the struggle for this precious nutrient.

New Perspectives on Ancient Evolutionary Arms Races

Novel mutations that alter host–pathogen interactions can provide a substantial fitness advantage and spread in a population through **positive selection**. Recurrent bouts of positive selection at such interfaces can give rise to so-called 'molecular arms races', in which the host and pathogen must continually adapt to maintain comparative fitness [1]. Genes subject to such evolutionary conflicts are often characterized by an increased rate of nonsynonymous to synonymous substitutions (termed dN/dS or ω), reflecting recurrent selection for novel amino acid substitutions that alter protein interaction surfaces. Instances of such molecular arms races also exemplify Leigh Van Valen's **Red Queen Hypothesis**, which proposed that antagonistic coevolution leads to a perpetual cycle of adaptation in which neither opponent gains a permanent advantage [28]. Several core components of the vertebrate immune system have subsequently been shown to engage in such conflicts, some of which are able to dictate the outcome of an infection [29–35].

Our recent work highlighted the battle for iron as a new interface for Red Queen evolutionary conflicts [36]. As described earlier, transferrin was among the first vertebrate proteins to be

Glossary

Antagonistic pleiotropy: also termed an evolutionary 'trade-off', in which a single gene controls multiple traits with opposing beneficial and deleterious effects.
Black Queen Hypothesis: describes the process by which gene loss can progress via natural selection, particularly in cases where individuals reduce investment in costly metabolic functions provided by other members of a microbial community.
Microbiota: the collection of microorganisms inhabiting a particular environment, such as the human body.
Nutritional immunity: a host immune defense mechanism by which essential nutrients, such as iron, are withheld in order to limit microbial growth and prevent infection.
Positive selection: the process by which new, beneficial genetic variation accrues in a population.
Red Queen Hypothesis: posits that antagonistic coevolution (e.g., between predators and prey or pathogens and hosts) produces a state in which constant adaptation is required to maintain comparative evolutionary fitness.
Siderophore: a diverse class of small molecule iron chelators, which are secreted by microbes and then internalized via surface receptors to mediate iron acquisition.
Transferrin: a serum glycoprotein in animals containing two iron-binding 'lobe' domains that deliver ferric iron to host cells via receptor-mediated endocytosis, as well as withholding iron from microbes.
Virulence factor: a gene or molecule that contributes to microbial infection, while not necessarily required for viability in nonpathogenic settings.
Zoonosis: an infectious disease naturally transmitted from animals to humans.