References and Notes

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MICROBIOLOGY

Pathogenic Bacteria Prefer Heme

Tracey A. Rouault

Almost all cells and organisms require iron to facilitate basic cellular processes such as respiration and DNA biosynthesis. Diverse and complex iron-uptake systems have evolved throughout the biological world to provide iron for numerous proteins, particularly those involved in energy capture and oxygen transport. Indeed, in parts of the great oceans, a complete lack of iron profoundly limits bacterial growth (*1*, *2*). In most environments, however, iron uptake is limited not by its absence, but by the fact that it is insoluble and inaccessible. To facilitate iron uptake, free-living bacteria and fungi have adopted several strategies. Some secrete compounds known as siderophores that solubilize and bind to an external source of iron with high affinity; the iron-siderophore complexes are then imported into the bacteria by specific transporter proteins. Others have uptake systems that import free iron salts directly (*3*, *4*). To combat microbial infections, animals strictly limit the availability of free iron in their blood and tissues. They do this by ensuring that iron in blood and secretions is carried by the high-affinity iron-binding proteins transferrin and lactoferrin, which create a primary line of defense against infection termed the "iron blockade" (*5*). The two ferric iron-binding sites of transferrin are rarely fully saturated, and an excess of unsaturated transferrin (apo-transferrin) ensures that free iron is virtually eliminated from blood. Pathogenic microorganisms, therefore, must overcome major obstacles if they are to acquire iron and thrive in their animal hosts. On page 1626 of this issue, Skaar and colleagues (*6*) explore how the pathogenic bacterium *Staphylococcus aureus* acquires the iron that it needs for growth in two different animal hosts. By growing the bacteria in the presence of the two principal iron sources found in mammals—diferric transferrin and the iron-porphyrin heme—the investigators show that most of the iron taken up by *S. aureus* during the initial phases of infection is obtained from heme.

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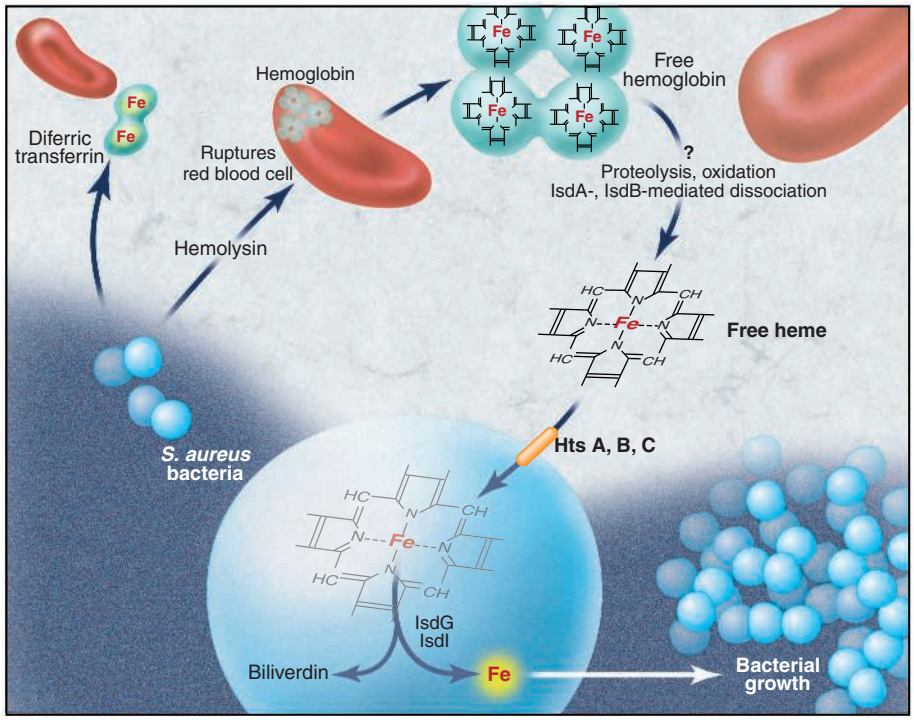
Although most bacteria are unable to grow in media in which the only iron sources are transferrin-iron and heme, some bacteria have developed strategies that enable them to obtain iron from these two sources (*7*, *8*). To determine whether *S. aureus* prefers transferrin-iron or heme as a source of iron, Skaar and colleagues labeled heme with ^{54}Fe and transferrin with ^{57}Fe, two rare and stable iron isotopes (*9*). After establishing that *S. aureus* grew well in medium supplemented with equimolar amounts of ^{54}Fe heme and ^{57}Fe transferrin, they analyzed the isotope content of cells using inductively coupled plasma–mass spectrometry (ICP-MS) at various times during growth. They discovered that *S. aureus* markedly prefers a source of iron derived from heme. Analysis of the *S. aureus* genome revealed that it encodes seven putative membrane transporter proteins that have some homology to known bacterial iron transporters. Using mutational inactivation and ICP-MS to monitor uptake of heme iron, the investigators identified a heme transport system in *S. aureus* composed of three genes



Bloodletting explained. *S. aureus* bacteria obtain most of the iron (Fe) that they need for growth in mammalian hosts from an iron-containing porphyrin ring called heme. *S. aureus* produces hemolysins that lyse red blood cells containing heme in the form of hemoglobin. It is unclear how the bacteria break down the released hemoglobin to heme, but the bacterial enzymes IsdA and IsdB may be involved. The bacteria then import heme via transporter proteins encoded by the *hts* ABC operon. The heme is then catabolized by the heme oxygenase-like enzymes, IsdG and IsdI, with the release of iron and biliverdin (a breakdown product of the porphyrin ring). The free iron released from heme is used to fuel further bacterial growth. The practice of bloodletting in the pre-antibiotic era may have been an attempt to starve pathogenic bacteria of the iron that they need for growth.

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(*hts* A, B, and C) that show homology with known heme transporter genes in other bacteria (*Yersinia enterocolytica* and *Corynebacterium diphtheriae*). Moreover, they identified a binding site for the bacterial ferric-uptake repressor protein, Fur, immediately upstream of the *Hts*A initiation codon, implying that the *hts* system is switched on in response to iron deficiency.

Although a heme-uptake system is of potential value, it would be of little use if *S. aureus* did not also possess mechanisms for liberating heme from the red blood cells where it is packaged in the form of hemoglobin (see the figure). *S. aureus* produces multiple hemolysins that breach the red cell membrane, promoting osmotic lysis of the cells (*10*). Once hemoglobin is released, it is not clear whether *S. aureus* liberates heme from hemoglobin with specific IsdB and IsdA enzymes (*11*), by secreting proteases like *Vibrio vulnificus* (*12*), or by oxidizing hemoglobin to promote its spontaneous dissociation into globin and heme (*13*). The bacteria then import the free heme, which is catabolized by the bacterial enzymes IsdG and IsdI in the same way as mammalian heme oxygenases catabolize heme, resulting in the release of iron from the heme porphyrin ring (*14*). Thus, *S. aureus* is a versatile pathogen that liberates heme from a vast erythrocyte repository, imports heme across its bacterial membrane, and degrades it to yield free iron (see the figure).

If the ability to take up iron is a potent virulence factor and the *hts* system is a major determinant of iron uptake, then mutational inactivation of the *hts* genes should attenuate *S. aureus* virulence. The authors analyze the pathogenicity of mutant and wild-type *S. aureus* in two model systems: the worm *Caenorhabditis elegans* and the mouse. Mutations in the *Hts* B and C genes markedly decreased mortality in worms infected 48 hours previously, and abscess formation markedly decreased in the livers and kidneys of mice 96 hours after intravenous injection of mutant compared to wild-type *S. aureus*. These results strongly imply that heme is the major source of nutrient iron in the critical early stages of *S. aureus* infection.

In response to bacterial infection and inflammation, humans restrict iron uptake and sequester iron within macrophages throughout the body. The peptide hormone hepcidin orchestrates these changes and causes a substantial decrease in serum iron levels (*15*). This hypoferremic response may be important for host defense by making iron even less available than usual to invading pathogens. The protective effects of hypoferremia may explain the mystery of why physicians embraced bloodletting as a therapeutic procedure for more than 2500 years. As recently as 1942, Sir William Osler's highly regarded medical textbook advocated bloodletting as a treatment for acute pneumonia: "To bleed at the onset in robust healthy individuals in whom the disease sets in with great intensity and harsh fever is good practice" (*16*). The development and widespread use of antibiotics in the mid-20th century obviated the need to employ questionable treatments such as bloodletting. However, the discovery that *S. aureus* depends on heme iron for growth in its animal hosts suggests that bloodletting in the pre-antibiotic era may have been an effective mechanism for starving bacterial pathogens of iron and slowing bacterial growth.

Bacteria continue to discover new ways to combat antibiotics and the race is on to discover new therapeutic targets to combat bacterial infection. The heme-uptake proteins of *S. aureus* may represent a new target for molecular therapy. Efficient lysis of erythrocytes increases the concentration of iron available to *S. aureus* by 100-fold compared to the normal concentration of transferrin-iron in serum. This liberated heme-iron apparently fuels the rapid growth of virulent *S. aureus* infection. Thus, pathogenic *S. aureus* applies principles of logic similar to those of accomplished bank robbers: They go for the heme, because that's where the iron is.

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MICROBIOLOGY

Noninherited Resistance to Antibiotics

Bruce R. Levin

As notorious as they may be, bacteria with inherited resistance to antibiotics are not the only reason that antibiotics fail and may not even be the major reason, at least not yet. Contributing to the humbling of these "wonder drugs" is the fact that growing populations of bacteria do not just die off when confronted with bactericidal antibiotics. Instead, their rates of mortality decline with time, and viable antibiotic-sensitive cells can be recovered even after hours of exposure to the drug (see the figure, panel A) (*1*). This phenomenon of declining sensitivity is well established for different species of bacteria and for different classes of antibiotics (*2*–*4*). Variously called "bacterial persistence" (*5*), "phenotypic tolerance" (*6*), or "adaptive resistance" (*7*), the phenomenon remains a mystery with respect to its mechanism as well as its contribution treatment failure.

One mechanism postulated to account for the declining sensitivity and survival of bacteria confronted with bactericidal antibiotics is that growing populations of genetically identical bacteria continually generate subpopulations that are less sensitive to killing by antibiotics because they either are not growing or are dividing at very low rates (*8*). On page 1622 of this issue, Balaban *et al*. provide evidence for the existence of these refractory bacterial subpopulations and explain how they may account for the persistence of antibiotic-sensitive bacteria (*9*). Meanwhile, on page 1629, Miller *et al*. present a mechanism by which this kind of noninherited resistance to antibiotics can be generated. This mechanism unexpectedly involves the SOS response, which blocks cell division during the repair of DNA damage (*10*).

Balaban and colleagues used a really cool combination of microfluidics and optical microscopy to make intimate movies of the replication of individual *Escherichia coli* bacteria, under normal conditions and when treated with the antibiotic ampicillin. In this way, the investigators were able to distinguish at least two distinct cell types in exponentially growing clones of *E. coli*: "nor-

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