How Microorganisms Transport Iron

In the midst of plenty, microorganisms are often in danger of iron-starvation; the mechanism by which they transport iron has now been elucidated

Iron is a component of many important enzyme systems in virtually every type of living cell. Given the immense abundance of this element in the earth's surface—it is exceeded only by aluminum, silicon, and oxygen—one might expect that cells would face few problems in fulfilling their requirements. Wrong. Ever since photosynthetic organisms started pumping oxygen into the atmosphere 2 billion years ago, available iron has been converted to its ferric form, which is virtually insoluble. Living cells have therefore had to go to extraordinary lengths to obtain this vital metal. The means by which some of them do it has finally been worked out in considerable detail.

By exploiting the process that the gut bacterium *Escherichia coli* sometimes uses in becoming a virulent pathogen in other parts of the body, Albrecht Bindereif and Joe Neilands of the University of California, Berkeley, have been able to dissect the genetic system that underlies the elaboration of this iron-transport machinery. They are also able to speculate on how the system is regulated at the molecular level.

Very simply, microorganisms use the same general approach for getting iron into the cell at anything more than a residual rate. This high-affinity system has two parts: the production of an iron-binding molecule, which is excreted from the cell; and the elaboration of a membrane receptor molecule, which binds the iron complex and transports the metal to the cell's interior. What the Berkeley biochemists have shown is that the membrane receptor and the enzymes that synthesize the iron-binding molecule are coded for by five genes in one operon, which is turned off when iron levels in the cell have been satisfied. The off switch appears to be a complex formed between iron and a polypeptide (a repressor protein), which is coded for at a separate location from the operon.

The fact that no other metal requires a transport mechanism as elaborate as this one emphasizes the very great problems to be overcome in getting iron into the cell in the face of very great need. Iron can get into cells without the aid of this system, but the rate of this low-affinity system is minuscule and the mechanism obscure.

The interchange between the ferrous and ferric states of iron generates a large redox potential, which is one reason why it is so effective a part of certain enzyme systems. Several components of the electron transport system of the respiratory chain exploit this property. And so too does ribonucleotide reductase, without which cells cannot manufacture deoxyribose, which is part of the backbone of DNA.

Exactly why iron was chosen for these functions during primordial life is a matter of conjecture. Its redox potential and ready availability (in the ferrous form) clearly made it a strong candidate, but so too was manganese. In any case, iron prevailed, its choice perhaps being partly a matter of chance, but certainly one that would bring problems. Life, for the most part, was hooked on iron. "When the atmosphere became strongly oxidizing and the iron was transformed into the insoluble ferric form, microorganisms were faced with a crisis on a grand scale," says Neilands. "They had to find some way of making ferric iron soluble—and the answer was siderophores."

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Siderophore is Greek for "iron bearer," and the term is applied to molecules that can bind the metal at very high affinities. By now several hundred different siderophores have been identified from microorganisms. These molecules fall into two main groups: those based on phenol catechols, such as enterobactin, which is made by *E. coli*; and those based on hydroxamic acid, such as ferrichrome. Generally, any particular microorganism will produce just one siderophore.

The general feature of all siderophores, however, is that they are capable of forming a "molecular cage," at the center of which ferric iron is held as part of a six-coordinate, octahedral complex. The six coordinates are typically set up by three sets of bidentate ligands, in which oxygen atoms interact with the metal's molecular orbitals. Oxygen is described as a "hard base," because of its small size and high potential negative charge, and ferric iron is a "hard acid," for the same reason, except the charge is positive. Interactions between hard acids and hard bases tend to be very strong, and this is one reason why siderophores have such enormous affinities for ferric iron. Another reason is that the niche into which the ion slots has been "tailored" to suit its size.

At neutral *p*H ferric hydroxides, for instance, have vanishingly small solubilities, in the order of 10^{-16} gram per liter. Siderophores, with their great affinities for ferric iron, can mop this up and even pluck ferric ions from minerals such as hematite. Competitors for that central niche are either much more weakly bound, such as aluminum, or extremely rare in nature, such as gallium.

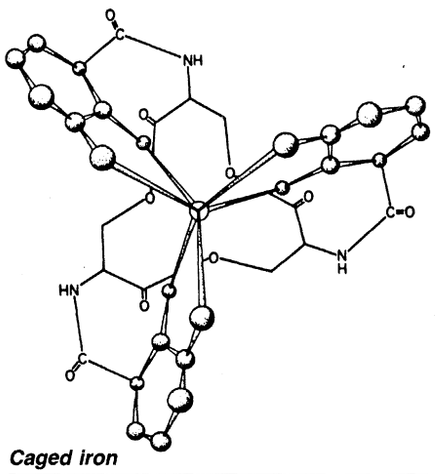
"Nature did pretty well in 'designing' these siderophores," says Neilands, but in solving one problem it made itself another." Most siderophores fall somewhere between 500 and 100^0 in molecular weight, which is not very big. Unfortunately, it is big enough to hinder any ready passage through membrane pores in many microorganisms. "The solution was to make a membrane receptor specific to the siderophore complex."

No one has yet managed to crystallize a siderophore receptor, and so the details of their disposition in the membrane and their interaction with the appropriate iron-containing complex remain obscure. The molecular weight, however, is usually between 70,000 and 80,000. (For the *E. coli* system that Bindereif and Neilands investigated it was 74,000 daltons.) "What is known about the receptor," says Neilands, "is that, once again, in solving this second problem in

getting iron into the cell, nature has brought upon itself another, unrelated, problem." It turns out that certain "lethal agents," bacteriophages, and "killer proteins" sneak into bacteria by binding specifically to these siderophore receptors. The fact that bacteria have not abandoned the receptor system in the face of this abuse indicates how dependent they are on this high-affinity transport mechanism, observes Neilands.

The general scheme of iron transport is as follows. Once the iron-siderophore complex binds with the membrane receptor, one of two things happens. Either the iron is popped through the membrane, which would probably involve reduction to the ferrous form, leaving the siderophore on the exterior. Or the whole complex passes through, whereupon the iron is released, again through reduction or through total or partial degradation of the siderophore. Intact siderophores might pass back through the membrane to begin the cycle over.

Bindereif and Neilands were able to analyze the molecular biology of one of these transport systems—from virulent *E. coli*—through a piece of good fortune. A couple of years ago Peter Williams, of the University of Leicester, England, was investigating what factor coded for by a large plasmid—pColV—in clinical isolates of the bacteria endowed its virulence. The plasmid was known to code for a lethal protein, colicin V, which was thought to give its producer a potential edge over other microorganisms that were susceptible to it.



Caged iron

This siderophore, enterobactin, traps iron at the center of a six coordinate, octahedral complex. The complex has a high affinity for ferric iron, which can nevertheless be rapidly exchanged.

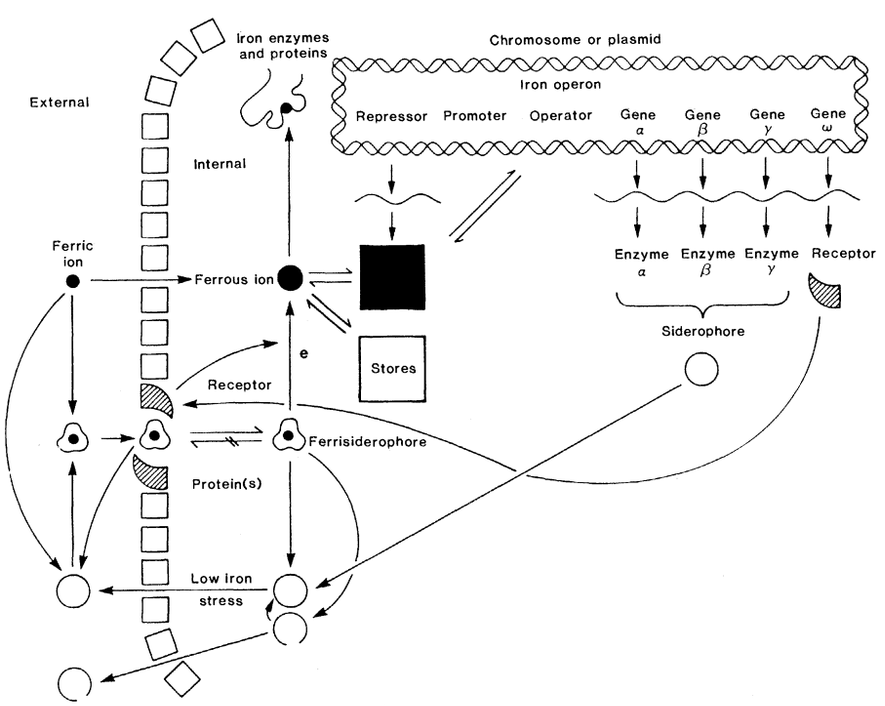
To everyone's surprise, however, the crucial factor turned out to be the capacity to synthesize and use a siderophore, which Neilands showed to be aerobactin, the system normally found in *Aerobacter aerogenes*. This siderophore clearly allows *E. coli* to obtain iron in territories where its indigenous system, enterobactin, could not operate. Since that time Bindereif and Skye McDougall, also a graduate student at Berkeley, have found that the aerobactin region on the plasmid is bounded by sequences reminiscent of a transposable element.

Given the good fortune of finding an entire siderophore producing and utilizing region on an accessible plasmid, Bindereif was able to snip it out and manipulate it in a much more manageable form, specifically on a smaller plasmid. He was therefore able to analyse the arrangement of the genes involved and the regulatory region that controls them. And by putting the plasmid in *E. coli* minicells he could characterize the polypeptides produced by the string of genes: there were five, which come off the operon in the order of 63−, 33−, 32−, 53−, and 74,000 daltons. The last and largest of the five, the 74,000, is the membrane receptor, while the rest, apart from the 53,000, are involved in siderophore synthesis. There is some evidence from a Dutch research group that this 53,000 protein is involved in uptake of the iron-siderophore complex.

The study of siderophores and their function in microorganisms might seem like a recondite pursuit, but the ubiquity of the need to transport iron against natural odds puts it in a more prominent perspective. Although plants and animals appear to use different iron-transport systems, what is being learned with the simpler organisms is helping to pioneer approaches to investigating those higher up the scale. In any case, the molecular biology of transport regulation promises to be similar between microorganisms and people.

At a more practical level, making a better siderophore has been the aim of pharmaceutical companies for some years, for any one of several reasons. For a start, by specifically blocking a bacterial pathogen's iron-transport mechanism one might achieve a new antibiotic, although, says Neilands, "antibiosis by iron starvation is a tough way to go." Another interest is simply that of mopping up excess iron in patients who have too much, either through faulty uptake or because of repeated blood transfusions, such as is necessary in thalassemia. And there is growing interest too among rheumatoid arthritis researchers in the potential therapeutic benefits of preventing free-radical formation, specifically hydroxyl radicals, which is thought to be catalyzed by iron.

After three decades of steady application to the problem, Neilands notes wryly that "the biochemistry of iron transport is suddenly becoming an extremely popular subject."—**Roger Lewin**



Schematic model of low- and high-affinity iron transport

Low iron stress coordinately turns on the siderophore synthesizing system and the required membrane acceptor. Once formed, the iron-siderophore complex binds with the membrane receptor, bringing iron into the cell. When iron requirement has been satisfied excess iron combines with a repressor protein and switches off the system.