

IRON METABOLISM IN BACTERIA

MAIN TOPICS

- **Role of iron in bacterial metabolism**
- **Iron-uptake mechanisms in bacteria**
- **Regulation of bacterial iron uptake systems**
- **Role of iron homeostasis in bacterial virulence**
- **Bacterial iron metabolism as drug target**

Role of iron in bacterial metabolism

Iron can exist in two oxidation states, the ferric form (Fe^{3+}) and the ferrous form (Fe^{2+})

Iron is a very versatile biocatalyst, due to its extremely wide redox potential

Iron is a cofactor of many cellular proteins, which are involved in:

- electron transport
- ROS detoxification
- amino acid and nucleoside synthesis
- DNA synthesis
- photosynthesis

Iron is essential for almost all living organisms

[with few exceptions (Mn instead of Fe)]

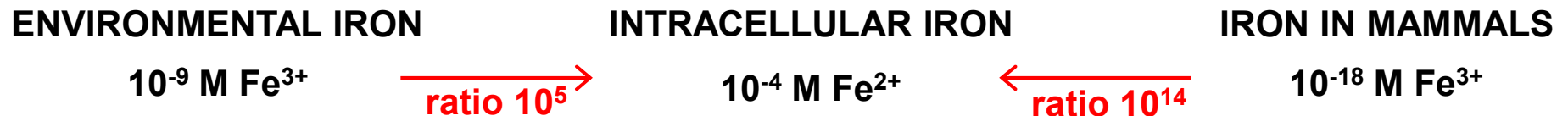
Iron-uptake mechanisms in bacteria

Iron bioavailability

In the environment iron is mostly present in the ferric form (Fe^{3+}), which is very poorly soluble in aerobic neutral environments
[$\text{Fe}^{3+} + 3\text{OH}^- \rightarrow \text{Fe}(\text{OH})_3$]

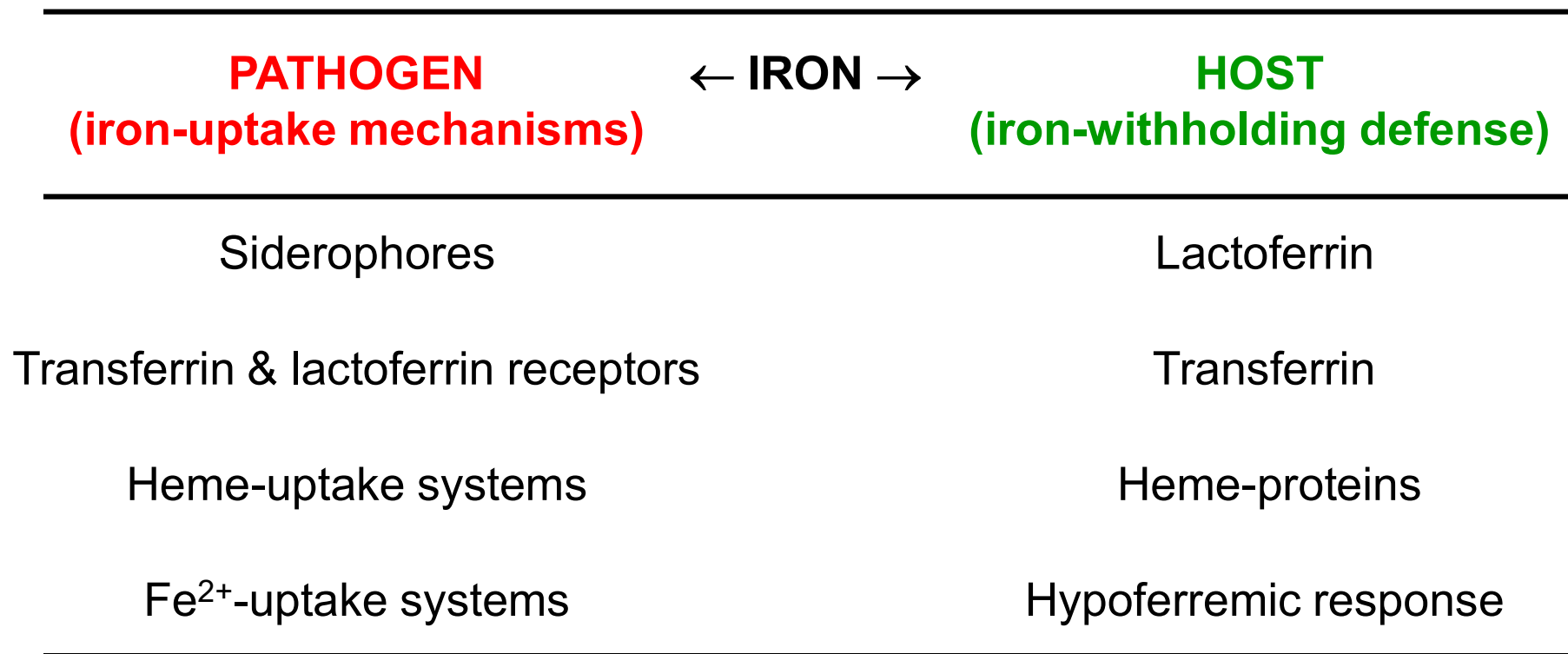
In the host iron is almost completely sequestered by iron-binding proteins

Iron is not a freely-available nutrient

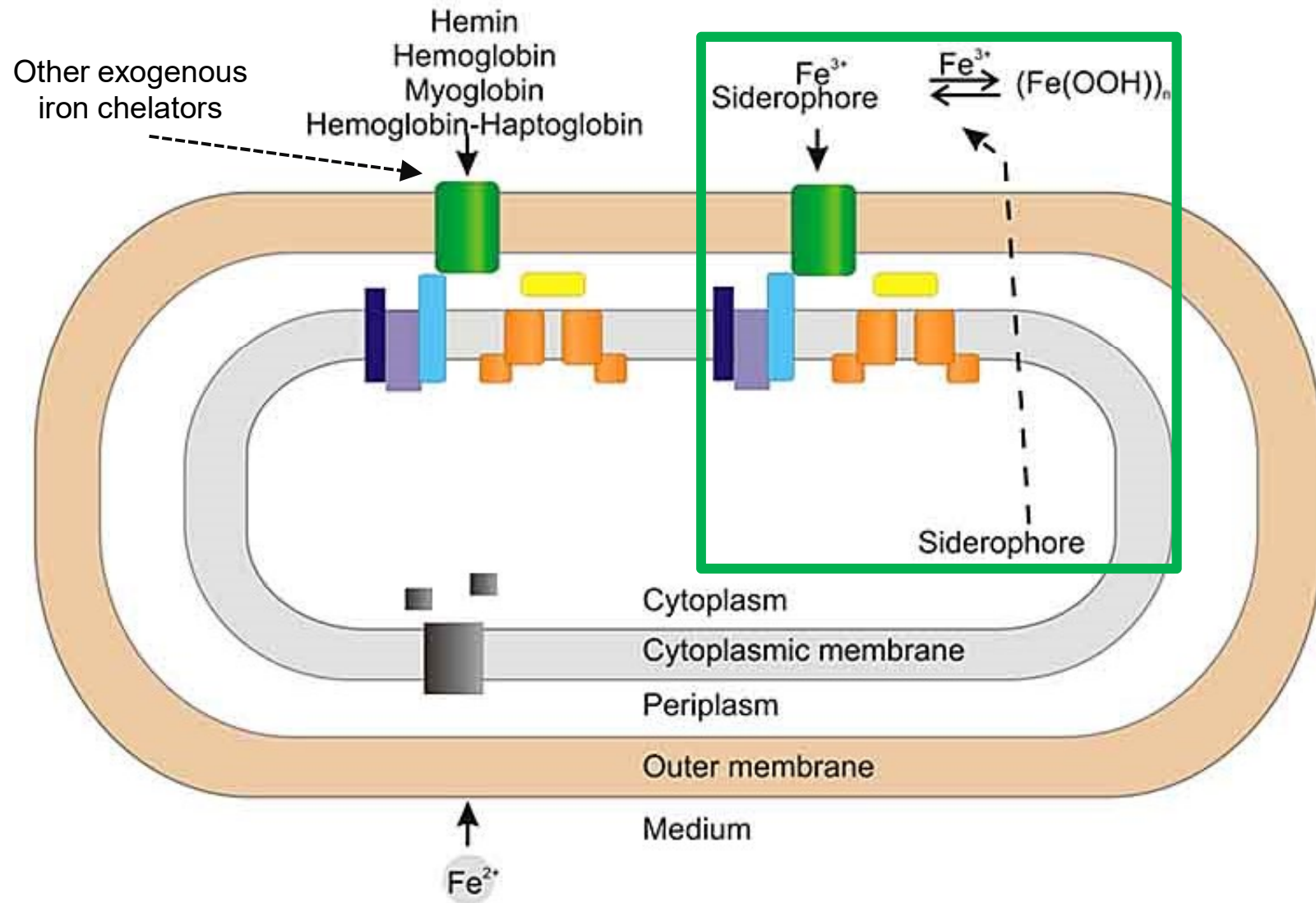


BACTERIA NEED ACTIVE UPTAKE OF IRON

Iron-uptake mechanisms in bacteria

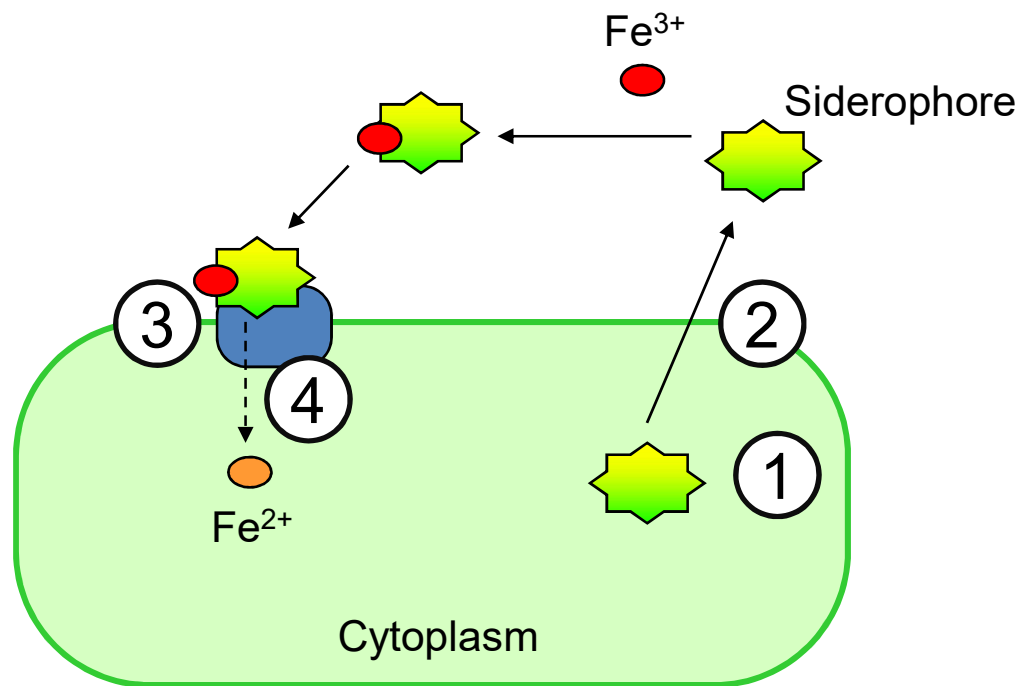


Iron-uptake mechanisms in bacteria



Iron-uptake mechanisms in bacteria

General features of the siderophore-mediated iron uptake



1. Synthesis
2. Export
3. Uptake
4. Iron release

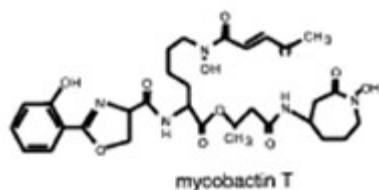
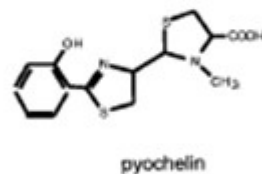
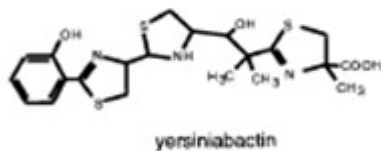
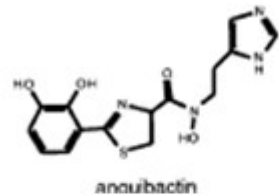
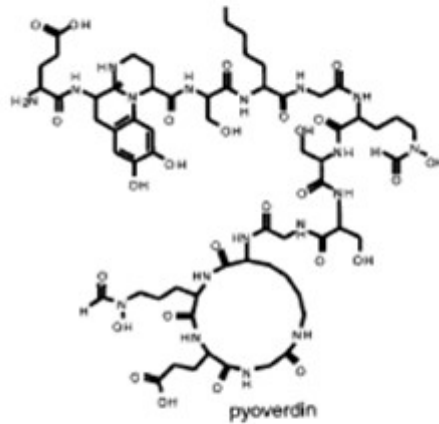
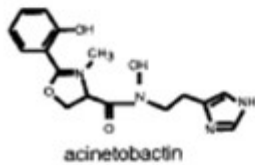
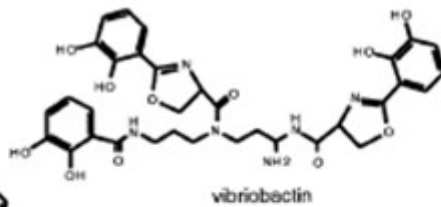
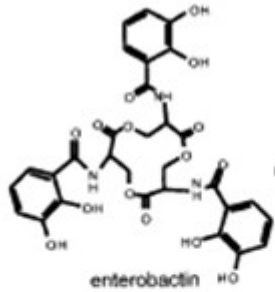
Iron-uptake mechanisms in bacteria

Siderophores

Highly variable structures

Common features:

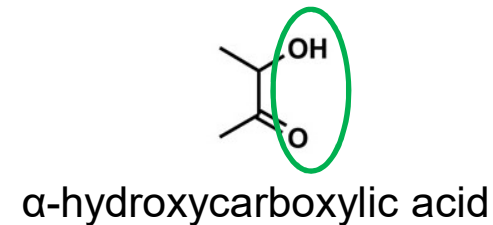
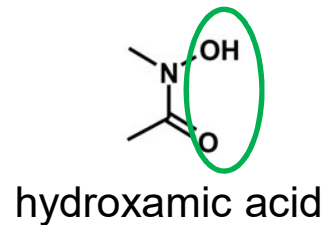
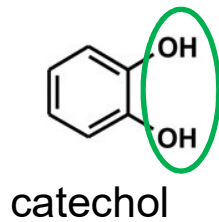
- small peptidic molecules (including non-proteinogenic, modified and D-amino acids)
- functional groups with high-affinity for ferric ions (Fe^{3+})
- synthesized by short, dedicated metabolic pathways



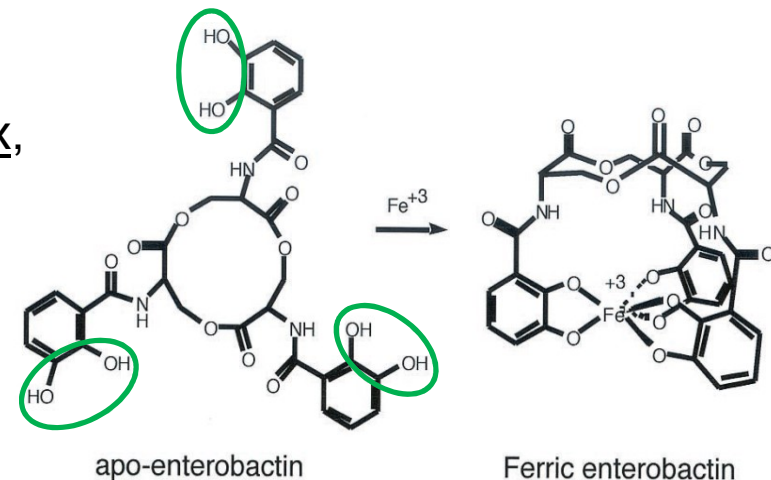
Iron-uptake mechanisms in bacteria

Iron binding by siderophores

The functional groups for Fe^{3+} coordination are limited: siderophores usually contain the following metal-chelating functional (bidentate) groups:



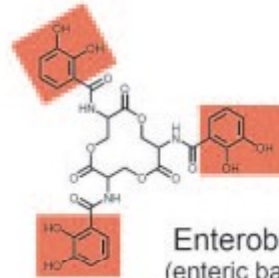
Fe^{3+} prefers a hexa-coordinate chelation complex, which requires three iron-chelating groups



Iron-uptake mechanisms in bacteria

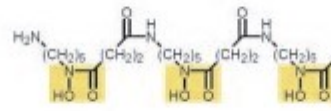
Siderophores are classified according to their functional groups

Catecholate Type



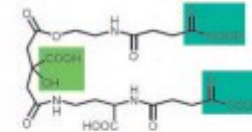
Enterobactin
(enteric bacteria,
Streptomyces spp.)

Hydroxamate Type



Desferrioxamine B
(*Streptomyces pilosus*)

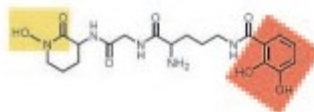
Carboxylate Type



Achromobactin
(*Erwinia chrysanthemi*)

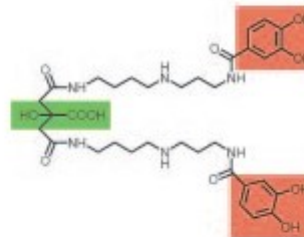
Mixed Types

Catecholate-Hydroxamate



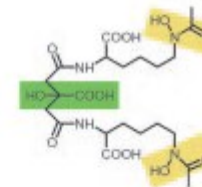
Heterobactin B
(*Rhodococcus erythropolis*)

Citrate-Catecholate



Petrobactin
(*Bacillus anthracis*,
Bacillus cereus,
Marinobacter hydrocarbonoclasticus)

Citrate-Hydroxamate



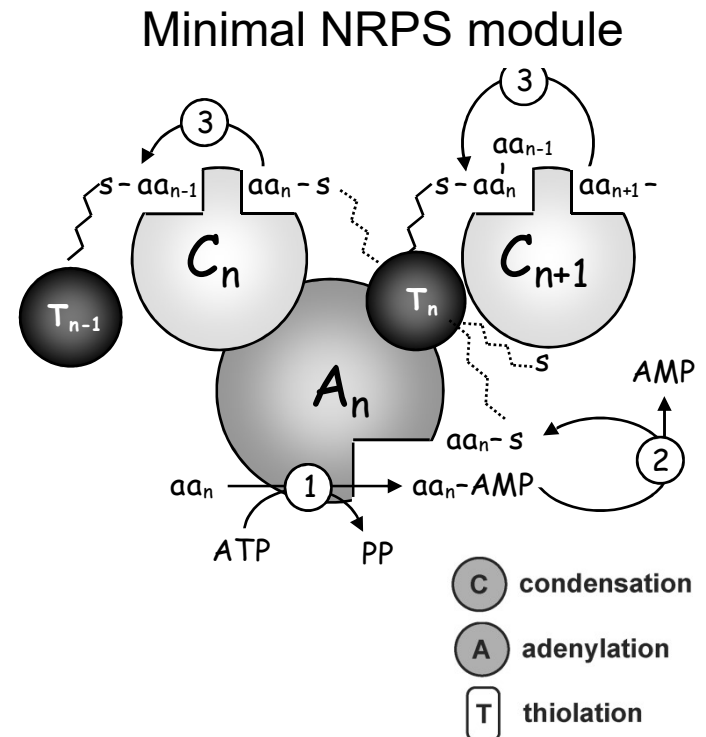
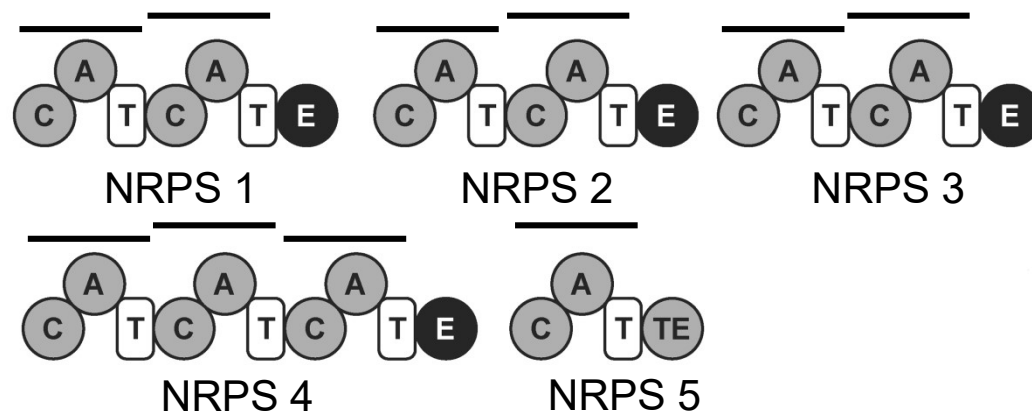
Aerobactin
(*Enterobacter* spp.,
Escherichia coli,
Shigella flexneri)

Iron-uptake mechanisms in bacteria

Siderophore biosynthesis

Siderophore biosynthesis generally occurs through “non ribosomal peptide synthesis”

..which is catalyzed by large multi-modular enzymes (non ribosomal peptide synthetases or NRPSs)



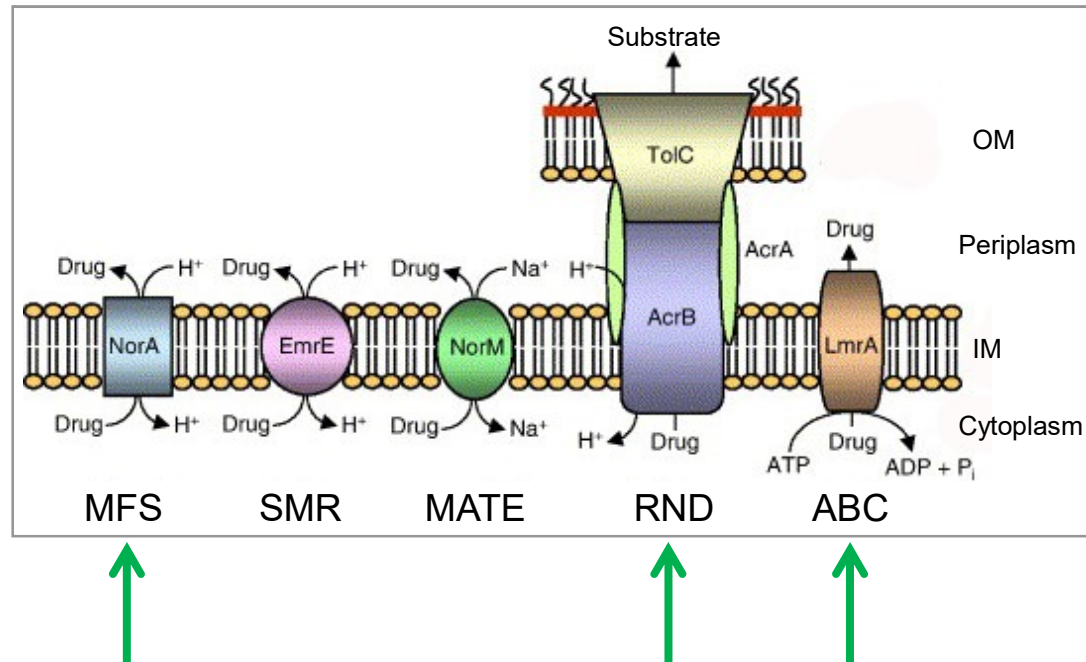
Accessory domains or accessory enzymes are involved in amino acid modification

Iron-uptake mechanisms in bacteria

Siderophore export

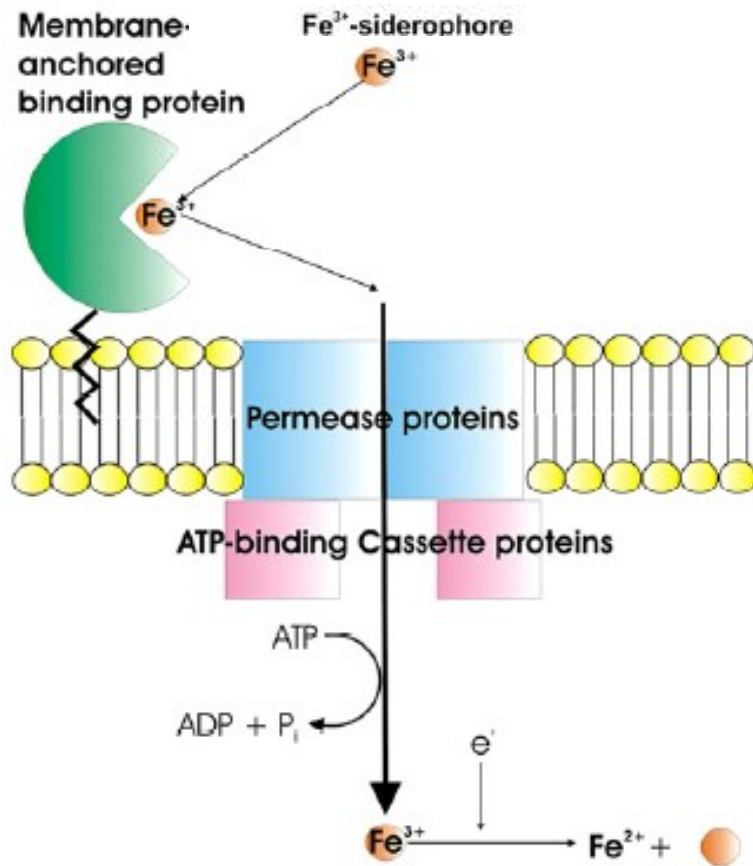
Siderophore secretion occurs through specific efflux systems (belonging to the same classes of those involved in antibiotic extrusion)

Main types of bacterial drug efflux systems



Iron-uptake mechanisms in bacteria

Siderophore-mediated iron uptake in Gram⁺ bacteria

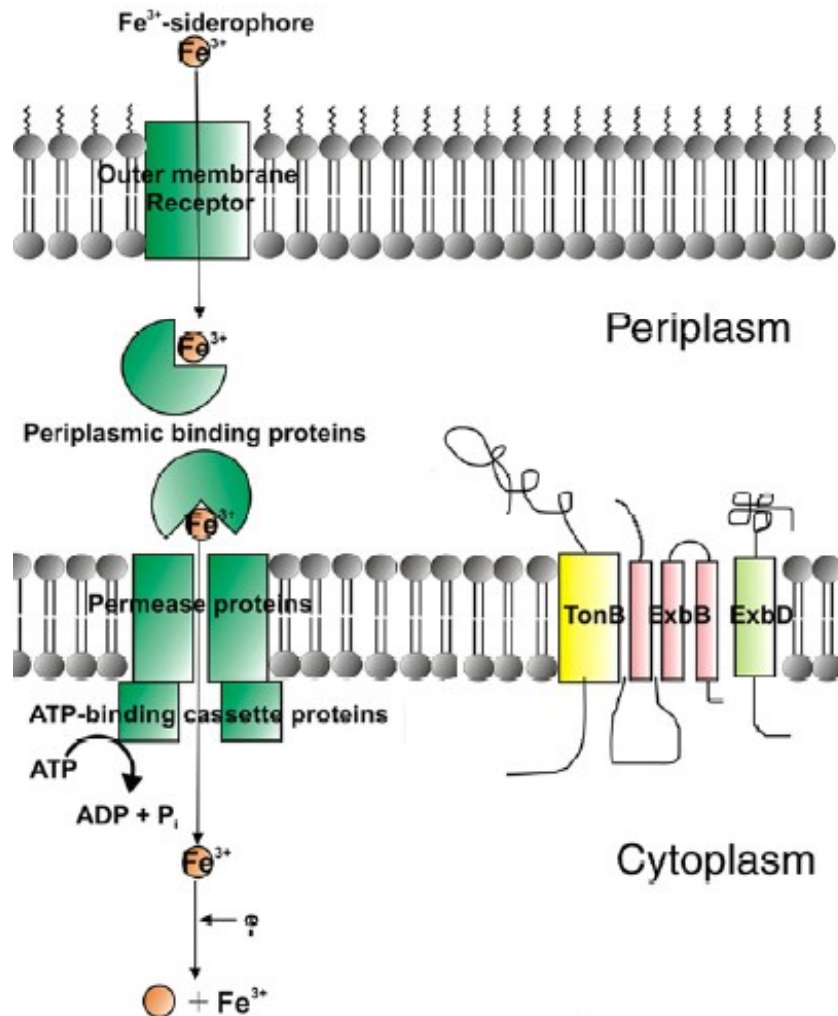


Two proteins are involved:

- a membrane-anchored binding protein (the receptor)
- a membrane-associated ABC transporter (energy from ATP hydrolysis)

Iron-uptake mechanisms in bacteria

Siderophore-mediated iron uptake in Gram- bacteria

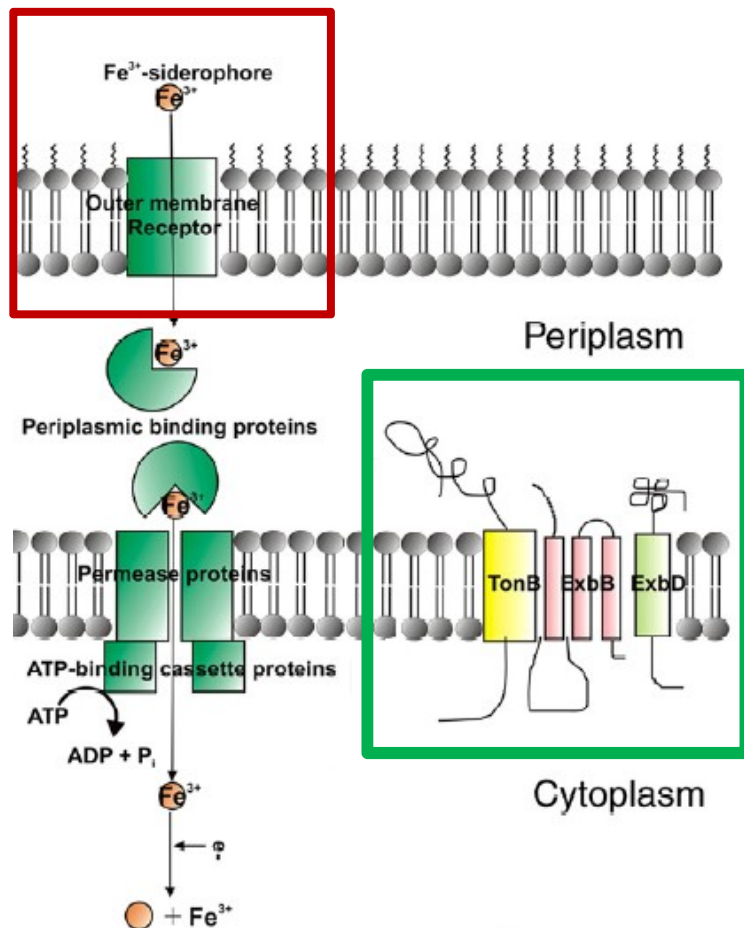


Many proteins are involved:

- an outer membrane receptor
- a periplasmic binding protein
- an inner membrane ABC transporter (ATP hydrolysis)
- the **TonB system**

Iron-uptake mechanisms in bacteria

The TonB system of Gram- bacteria

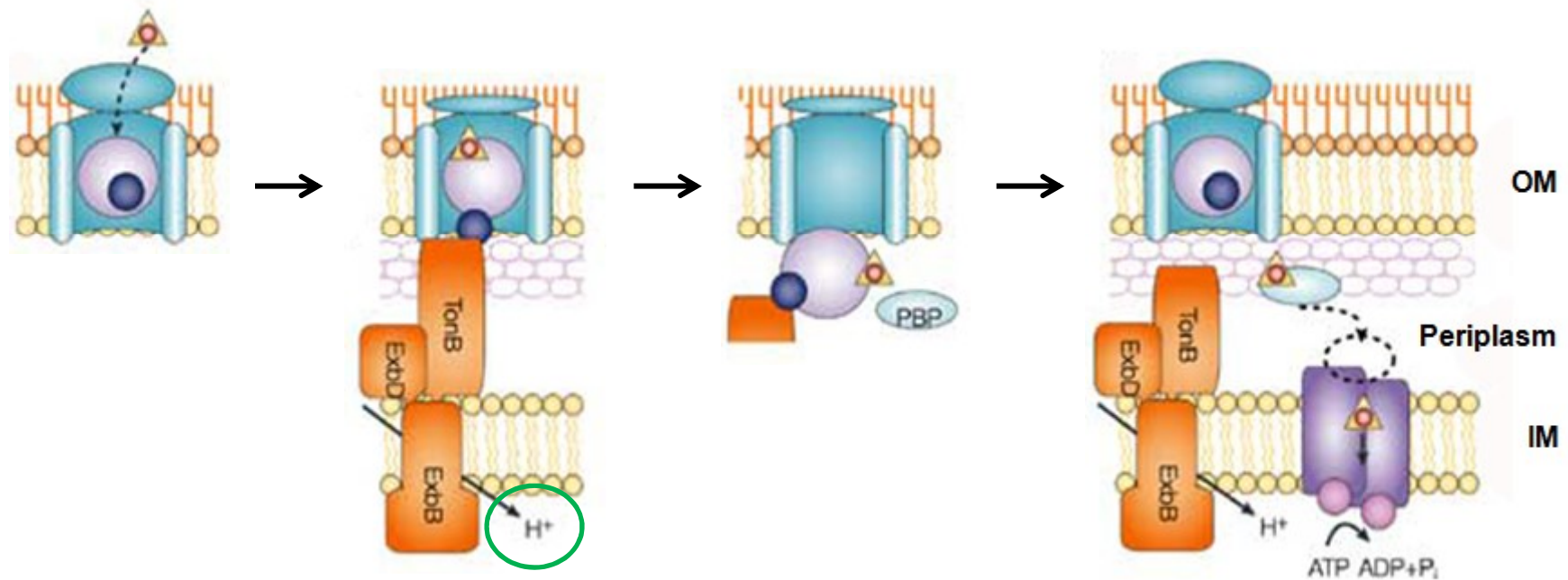


- Uptake by OM receptors requires energy
- The OM is not energized
- No readily available energy sources in the periplasm

The TonB system transduces the energy of the proton-motive force from the IM to the specific OM receptor

Iron-uptake mechanisms in bacteria

The TonB system of Gram- bacteria

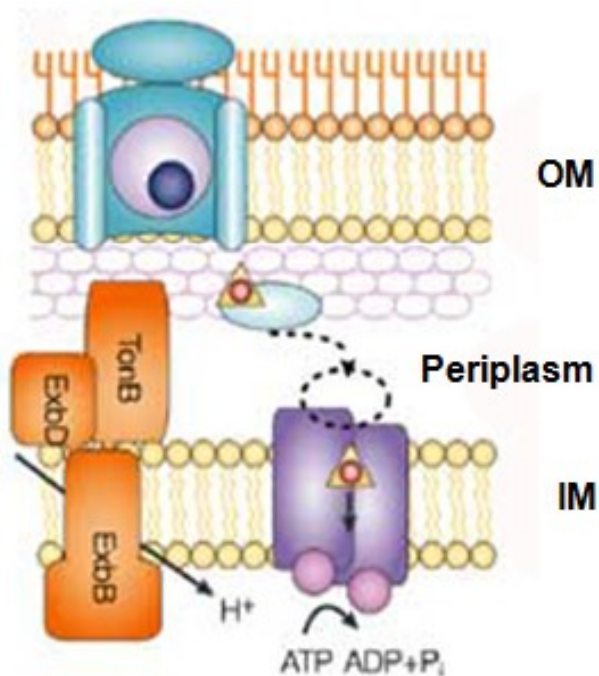


Energy from the IM proton gradient

The TonB system is also involved in the transport of vitamin B12, nickel, different carbohydrates, etc.

Iron-uptake mechanisms in bacteria

Iron release from siderophores



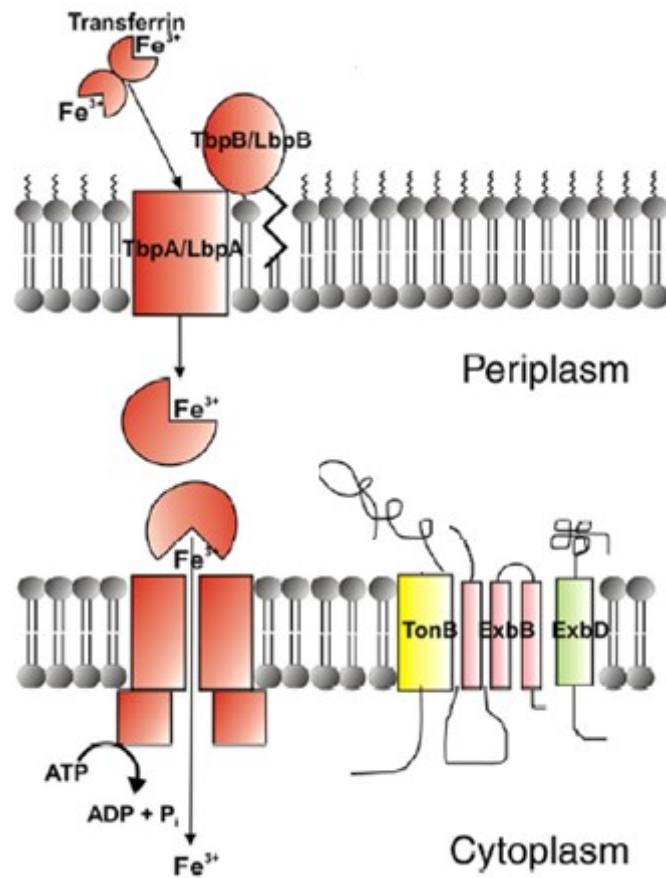
Two mechanisms:

Siderophore degradation !

1. Fe-siderophore hydrolysis (specific enzymes)
2. Reduction of siderophore-bound Fe³⁺ to Fe²⁺ (Fe-siderophore reductases)

Siderophore recycling !

Iron-uptake mechanisms in bacteria



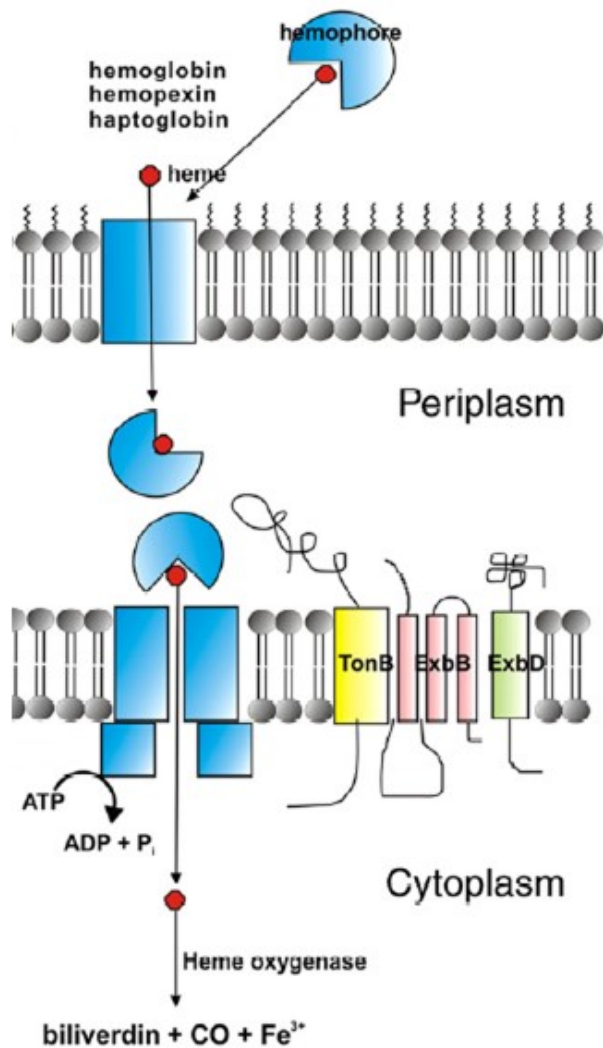
The same molecular mechanisms are involved in iron uptake mediated by:

- transferrin
- lactoferrin
- heme (and heme-binding proteins)
- other iron chelators (exogenous siderophores)

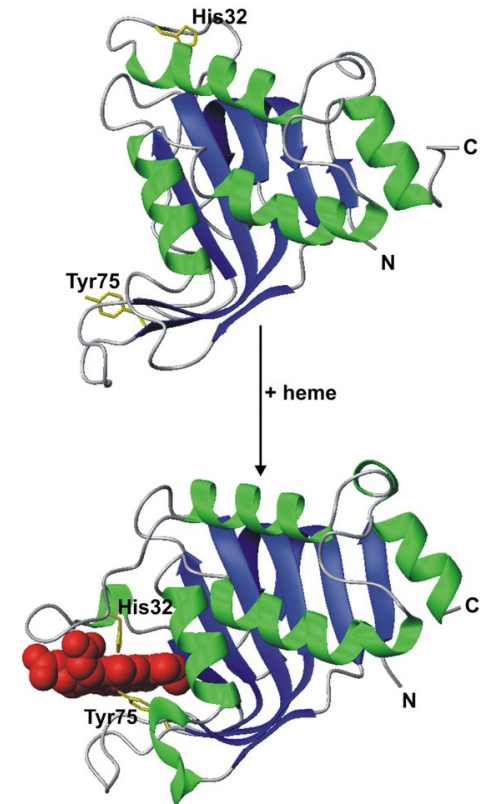
Based on specific outer (Gram-) or inner (Gram+) membrane receptors

Iron-uptake mechanisms in bacteria

A second heme uptake system: the hemophore HasA



HasA is a secreted protein with a structure resembling a “fish biting the heme”



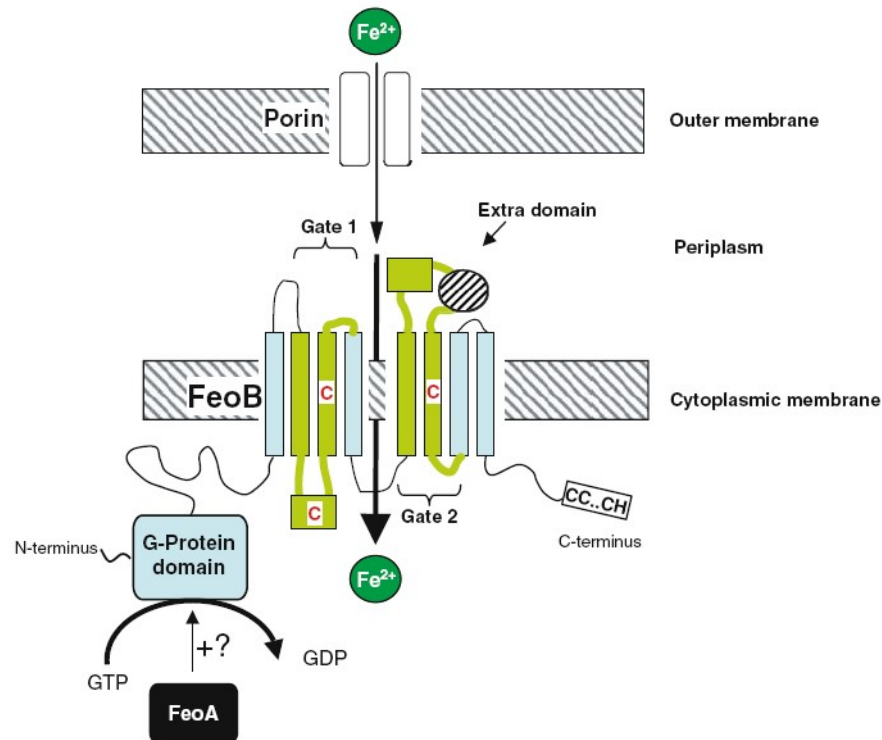
Present in some Gram- bacteria, such as *Pseudomonas* spp., *Serratia marcescens* and *Yersinia* spp.

Iron-uptake mechanisms in bacteria

Ferrous iron uptake: the Feo system

Unlike the ferric form, ferrous iron is relatively soluble (0.1 M for Fe^{2+} versus 10^{-18} M for Fe^{3+} at pH 7).

However, Fe^{2+} oxidizes spontaneously to Fe^{3+} unless it is under reducing conditions (anaerobic conditions or low pH).



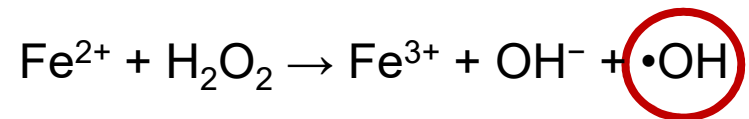
The Feo gene cluster (*feoA*, *feoB*, *feoC*) is present in about 50% of completely sequenced bacterial genomes

Two types of “Feo system”:
one that is Fe^{2+} specific and
another (Meo) that is Mn^{2+} specific

Regulation of iron uptake systems

The dual role of iron: essential but toxic

Intracellular Fe^{2+} catalyses the formation of hydroxyl radicals (a highly ROS) in the presence of hydrogen peroxide (through the Fenton reaction)



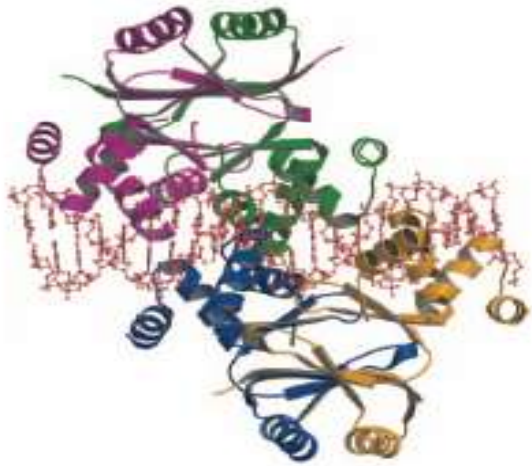
Hydroxyl radicals (as other ROS) can damage almost all macromolecules, including proteins, lipids and nucleic acids

BACTERIA NEED MECHANISMS TO SENSE AND CONTROL INTRACELLULAR LEVELS OF IRON

Regulation of iron uptake systems

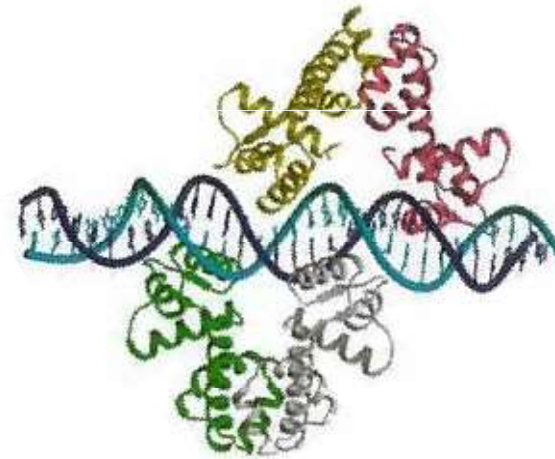
Fur and DtxR as master regulators of iron metabolism

Fur (ferric uptake regulator)



Gram-negative bacteria

DtxR (Diphtheria toxin repressor)



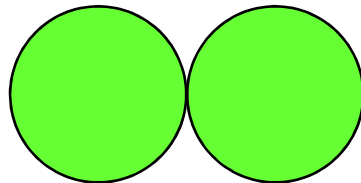
Gram-positive bacteria

Fur and DtxR are transcriptional repressors

Regulation of iron uptake systems

Fur (or DtxR) dependent gene repression

Inactive Fur repressor



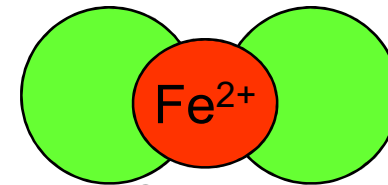
TTGACAGATAATGATAATCATTATCTATAAT

-35

-10

Low iron: transcription of Fur-regulated genes

Active Fur repressor



TTGACAGATAATGATAATCATTATCTATAAT

-35

-10

High iron: repression of Fur-regulated genes

→ ←
GATAATGATAATCATTATC

The Fur binding site (Fur box) is present in all iron-repressed genes directly controlled by Fur

Regulation of iron uptake systems

Bacteria also need to express specific iron-using proteins in response to high iron levels

Gene name	Gene number	Function or activity
<i>E. coli</i> ^a		
<i>ftnA</i>	b1905	Fe storage
<i>cyoA-E</i>	b0428-0432	Cytochrome <i>bo</i> oxidase
<i>cydAB-ybgE</i>	b0733-0735	Cytochrome <i>bd</i> oxidase 1
<i>appCB</i>	b0978-0979	Cytochrome <i>bd</i> oxidase 2
<i>narK</i>	b1223	Nitrite exporter I
<i>narGHJI</i>	b1224-1227	Respiratory nitrate reductase 1
<i>paaY</i>	b1400	Phenylacetate degradation?
<i>narZY</i>	b1468-1467	Respiratory nitrate reductase 2
<i>nap-ccmA</i>	b2208-2201	Periplasmic nitrate reductase
<i>nuoA-N</i>	b2276-2288	Complex I
<i>tdcG-A</i>	b3112-3118	Anaerobic Thr dehydratase
<i>garPLRK</i>	b3127-3124	Glycerate kinase 2
<i>nirBDC</i>	b3365-3367	NAD(P)H nitrite reductase
<i>frdABCD</i>	b4154-4151	Fumarate reductase
<i>nikABCDE</i>	b3476-3481	Ni transport
<i>sodB</i>	b1656	Fe superoxide dismutase
<i>pyrL</i>	b4246	Asp transcarbamoylase
<i>P. aeruginosa</i> ^b		
<i>antABC</i>	PA2512-2514	Anthranilate dioxygenase
<i>HUU</i>	PA2511	Transcriptional regulator?
<i>HUU</i>	PA2682	Dienelactone hydrolase
<i>fdnH</i>	PA4811	Formate dehydrogenase
<i>HUU</i>	PA4880	Bacterioferritin?
<i>KatA</i>	PA4236	Catalase
<i>napA</i>	PA1174	Periplasmic nitrate reductase
<i>sodB</i>	PA4366	Superoxide dismutase
<i>sdhCDAB</i>	PA1581-1584	Succinate dehydrogenase
<i>bfrB</i>	PA3531	Bacterioferritin B

Genes induced under iron-replete conditions are involved in:

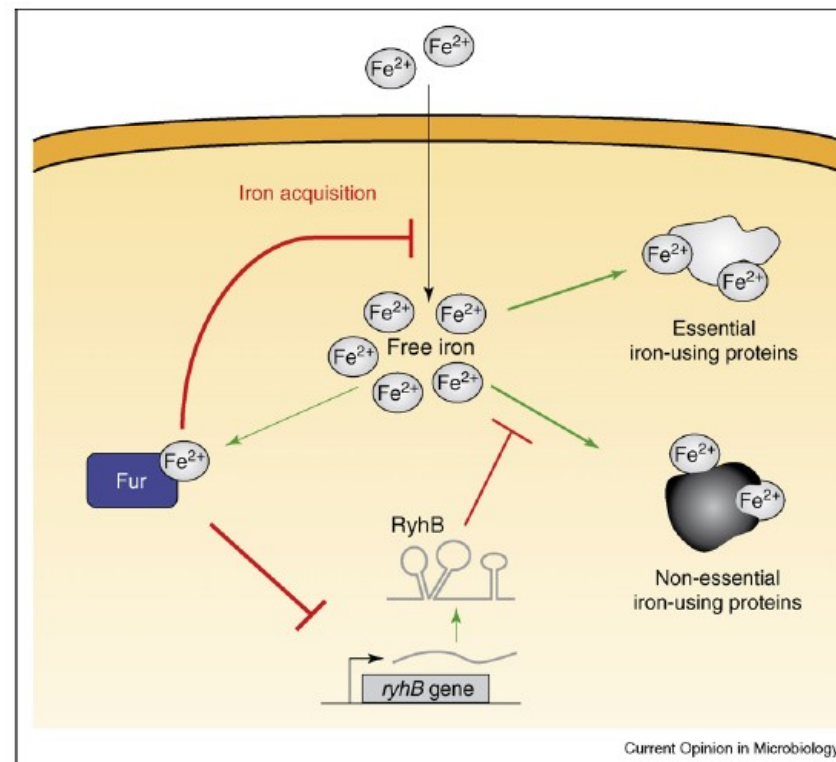
- iron storage
- defense against oxidative stress
- basic intermediary metabolism
- other cellular processes

HOW ?

Regulation of iron uptake systems

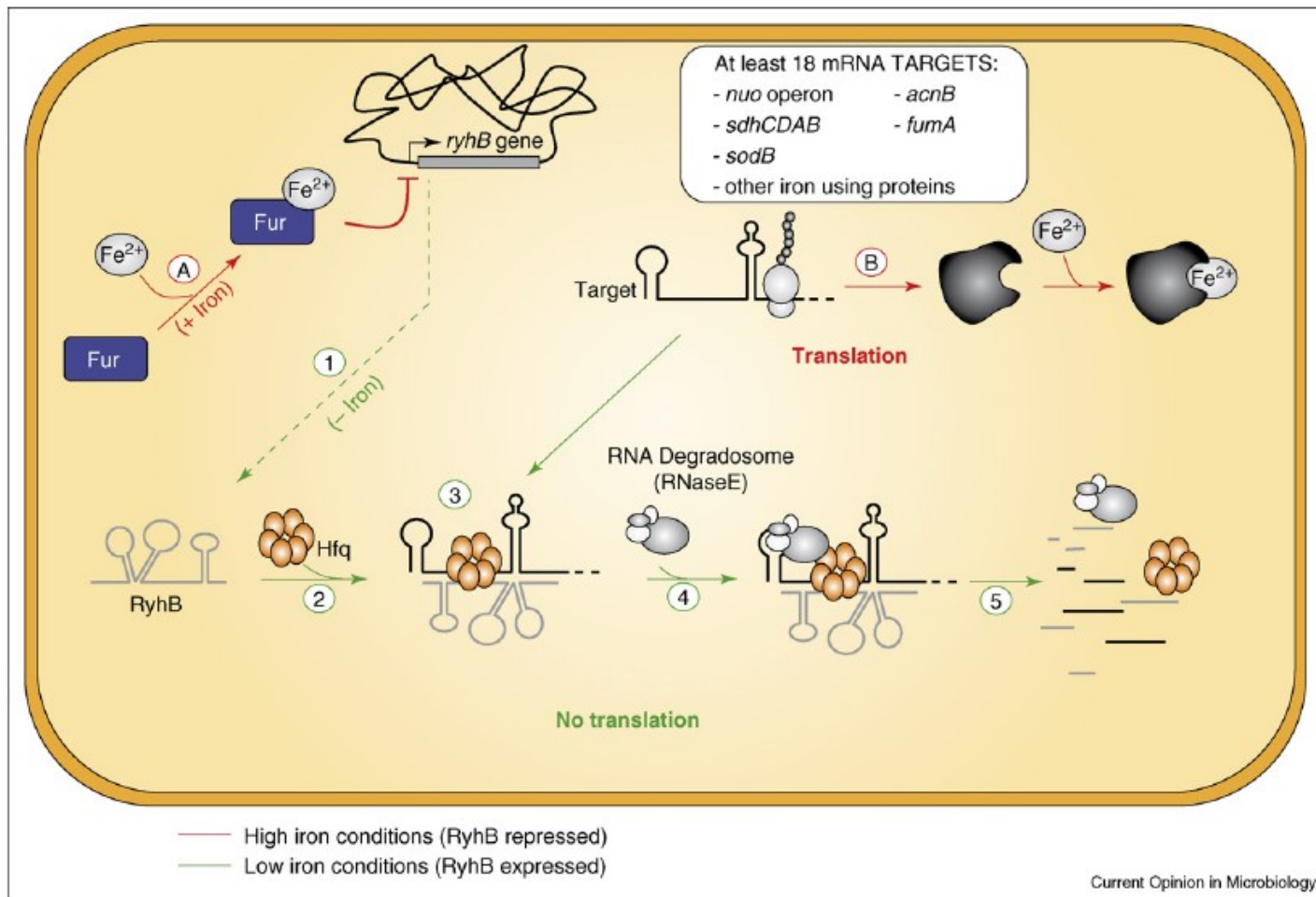
.. by coupling two negative regulatory systems

The case of the small RNA RyhB in *E. coli*



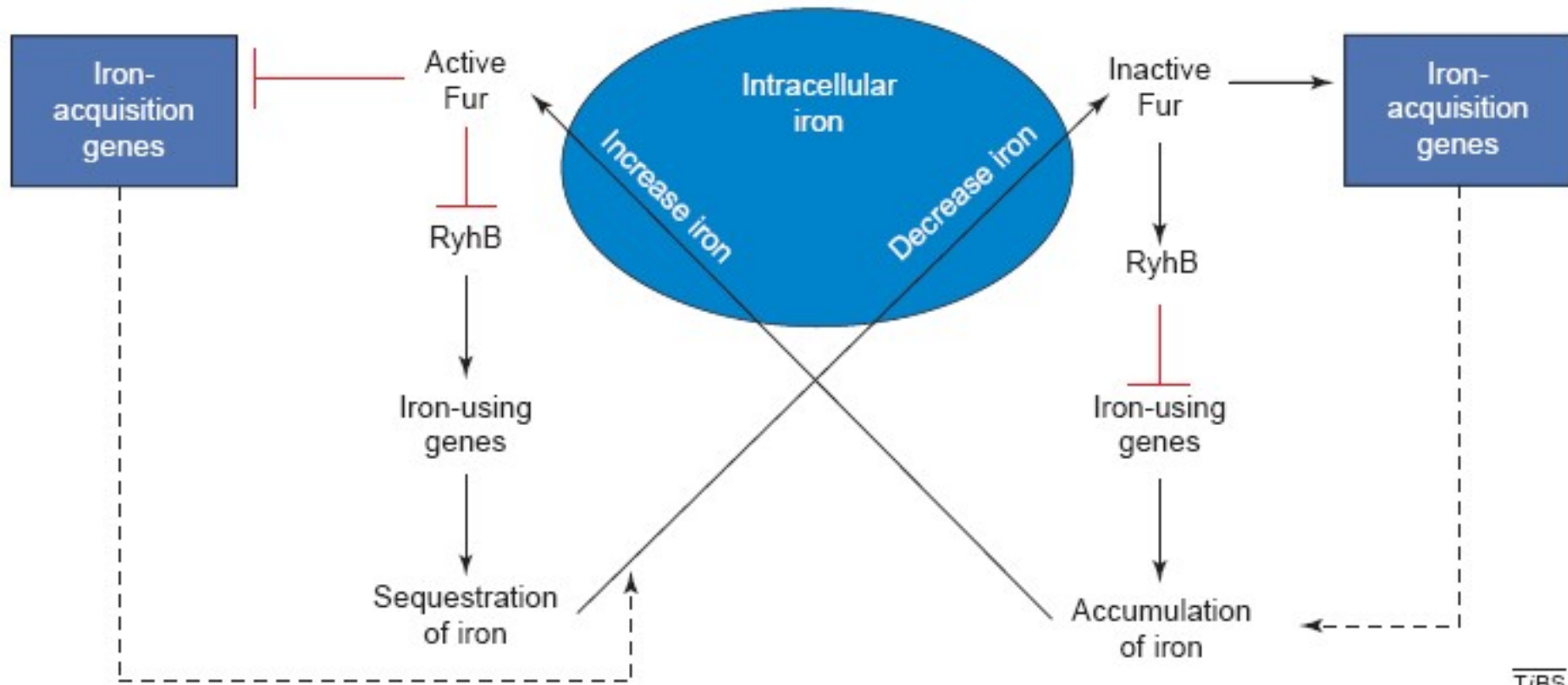
Regulation of iron uptake systems

Mechanism of action of the small RNA RyhB



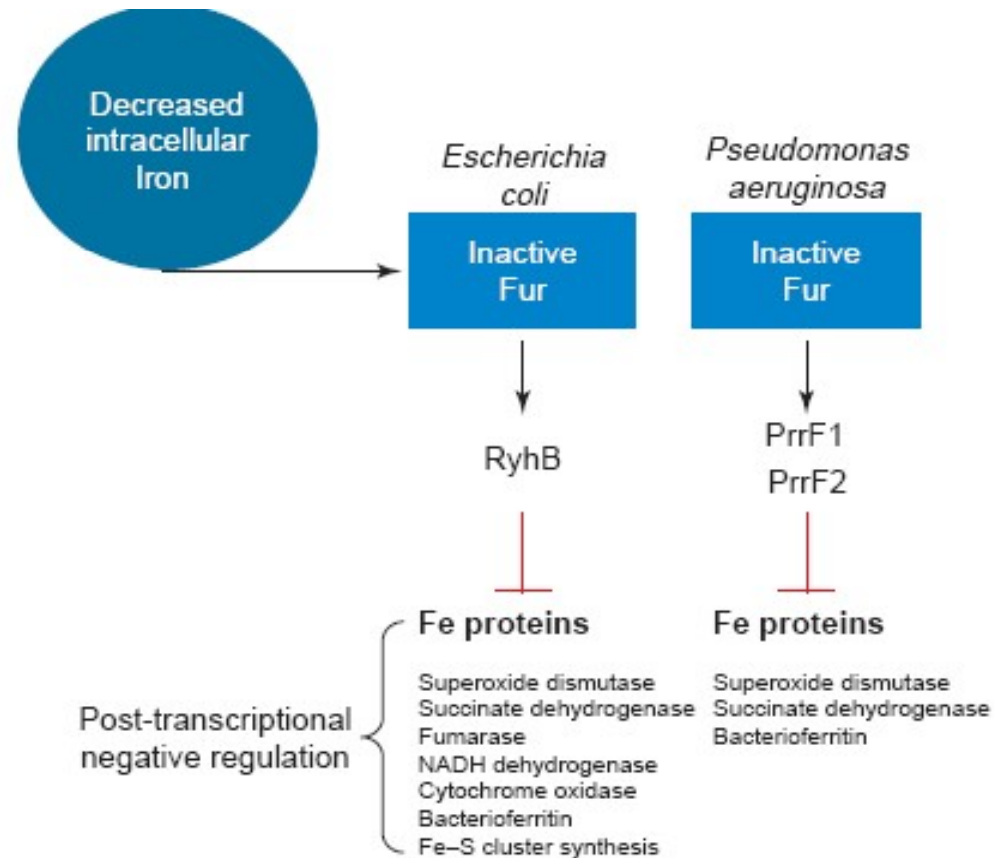
Regulation of iron uptake systems

A fine-tuned balance between Fur and RhyB activities allows bacteria to maintain iron homeostasis



Regulation of iron uptake systems

RhyB is functionally conserved in bacteria



Regulation of iron uptake systems

..a second step of complexity

Bacteria can produce multiple siderophores..

..but synthesize a given siderophore only when it is effective in delivering iron

Bacteria can also prey on heterologous siderophores and utilize exogenous iron chelators..

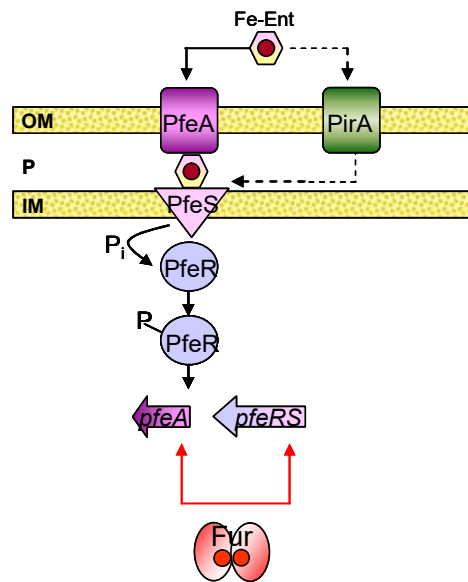
..but express the specific receptors only in the presence of the cognate ligand

**NEED FOR POSITIVE REGULATION
AND SIGNALING**

Regulation of iron uptake systems

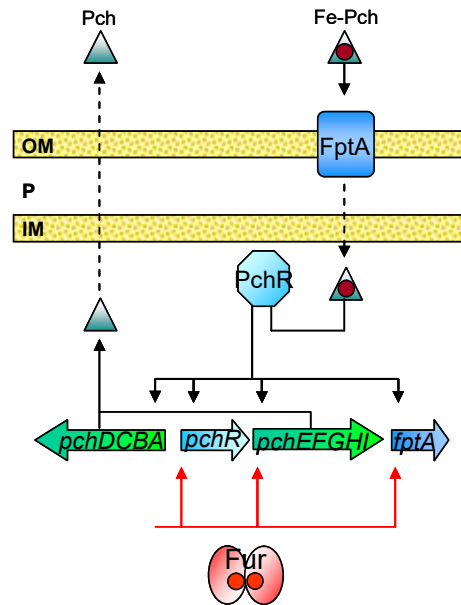
Siderophore signaling: different strategies, same result

Enterobactin (*E. coli*)



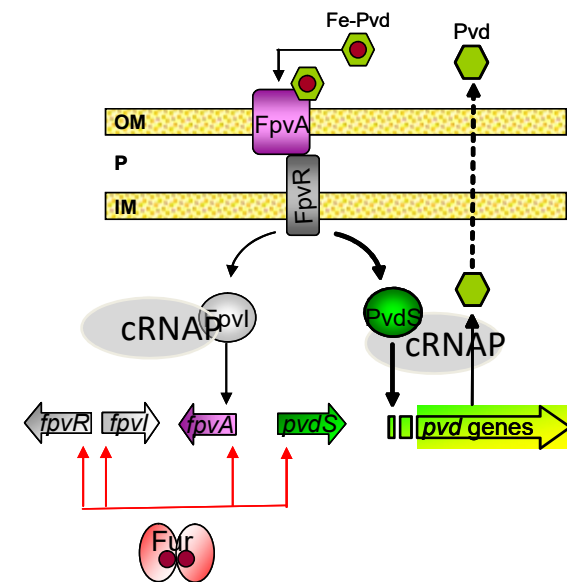
Two-component system
(*periplasmic signaling*)

Pyochelin (*P. aeruginosa*)



AraC-like regulator
(*cytoplasmic signaling*)

Pyoverdine (*P. aeruginosa*)

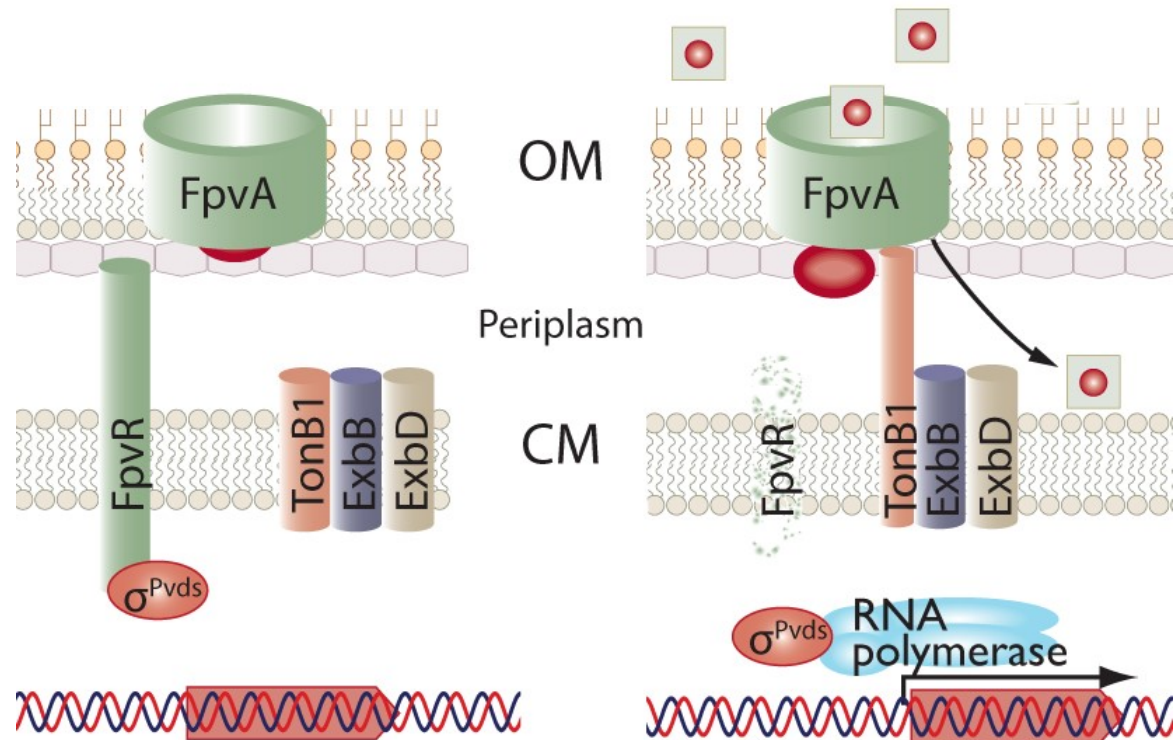
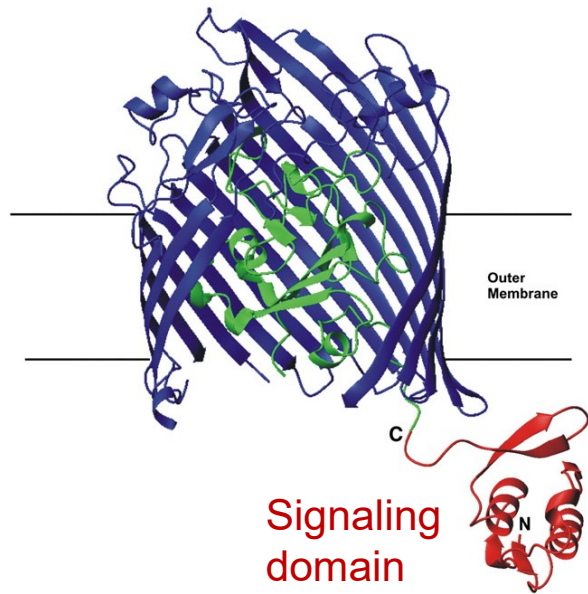


Sigma-antisigma factors
(*surface signaling*)

Each siderophore is only produced when it is effective in delivering iron

Regulation of iron uptake systems

Surface signaling receptors have a specific N-terminal signaling domain



Role of iron homeostasis in virulence

Many virulence factors are specifically expressed under low iron conditions

Shiga and Shiga-like toxins (*Shigella*, *E. coli*)

Fur box

Diphtheria toxin (*Corynebacterium diphtheriae*)

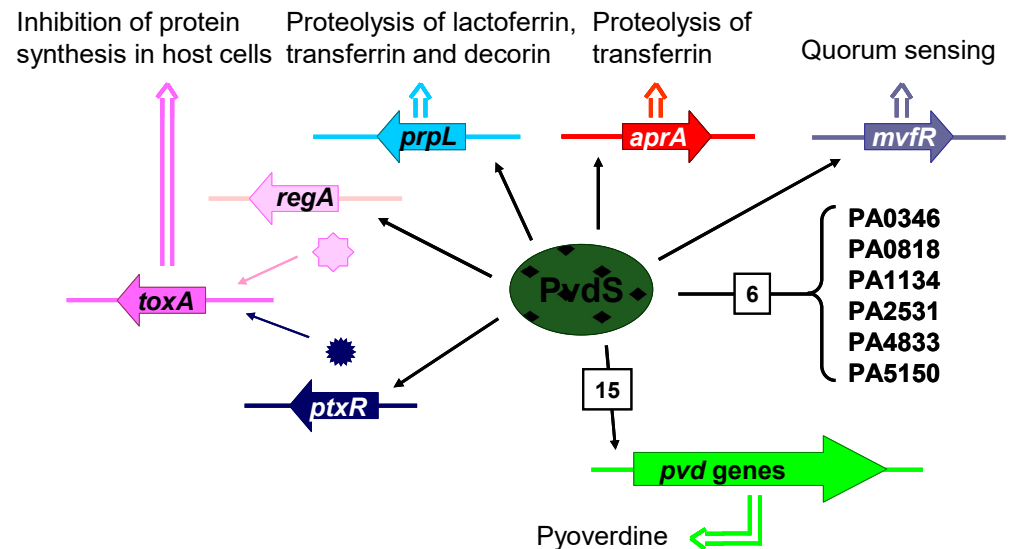
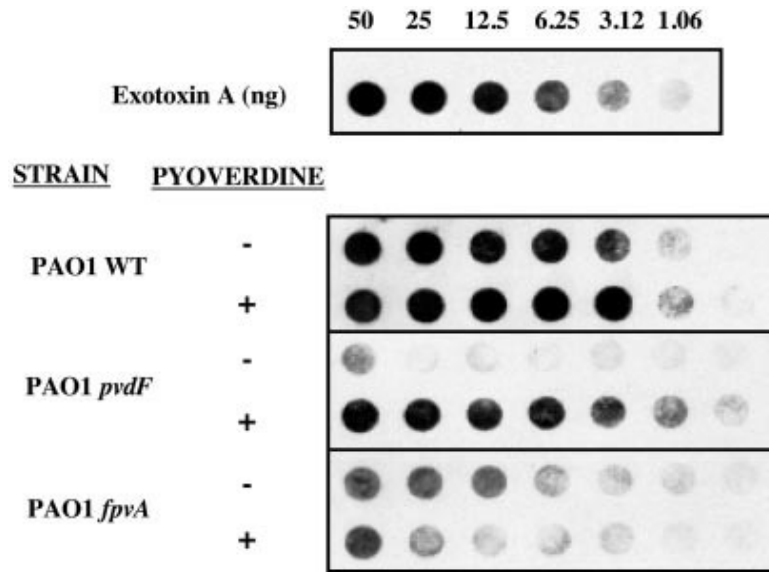
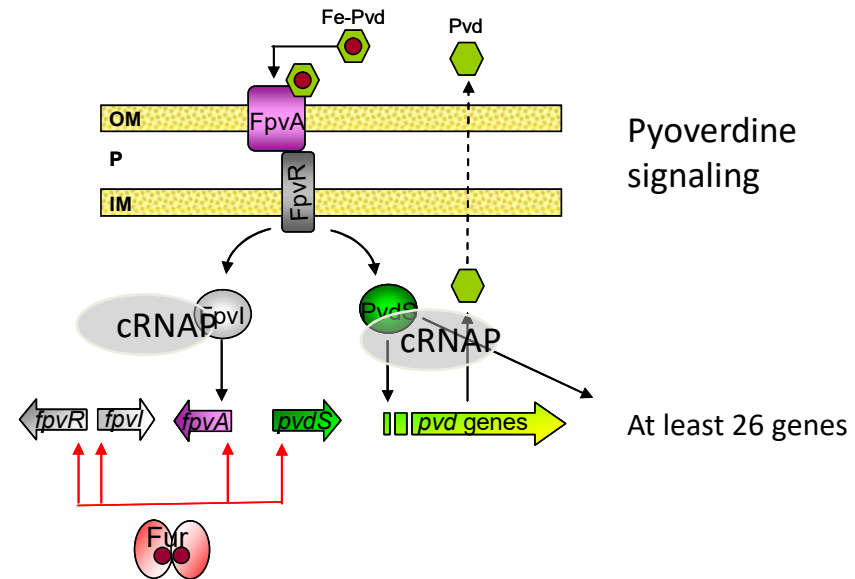
DtxR binding site

Exotoxin A (*Pseudomonas aeruginosa*)

No Fur box

Role of iron homeostasis in virulence

Iron-dependent regulation of virulence factors in *P. aeruginosa*: the role of pyoverdine and PvdS



Iron as “biofulcrum” in bacterial infectious diseases

PATHOGEN (iron-uptake mechanisms, adaptation)	← IRON →	HOST (iron-withholding defense)
Siderophores		Lactoferrin
Transferrin & lactoferrin receptors		Transferrin
Heme-uptake systems		Heme-proteins
Virulence-related factors		Hypoferremic response

Iron is a master regulatory signal of bacterial pathogenicity

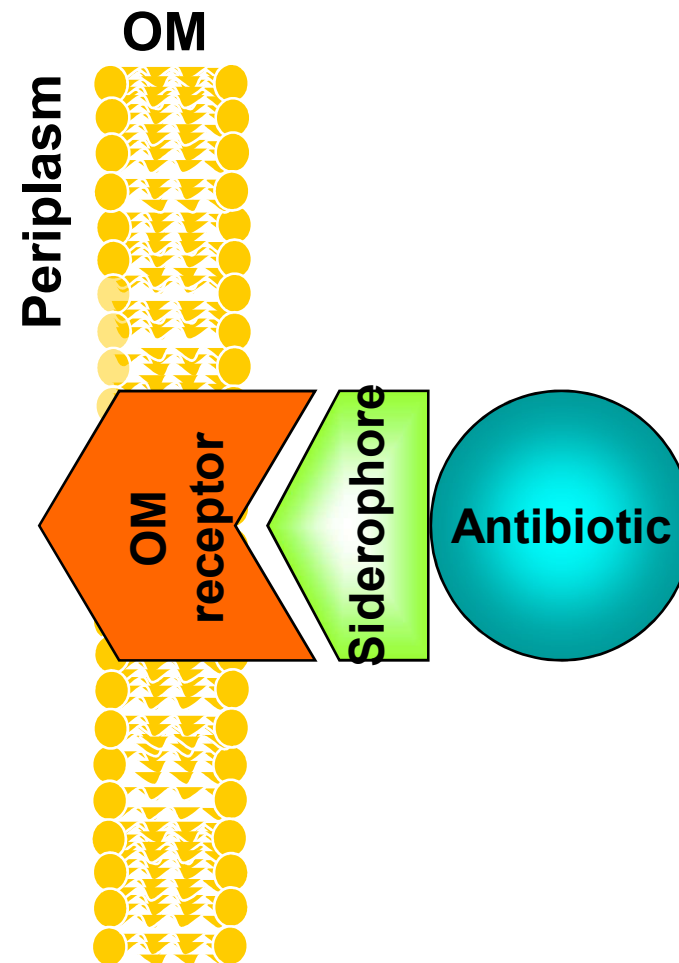
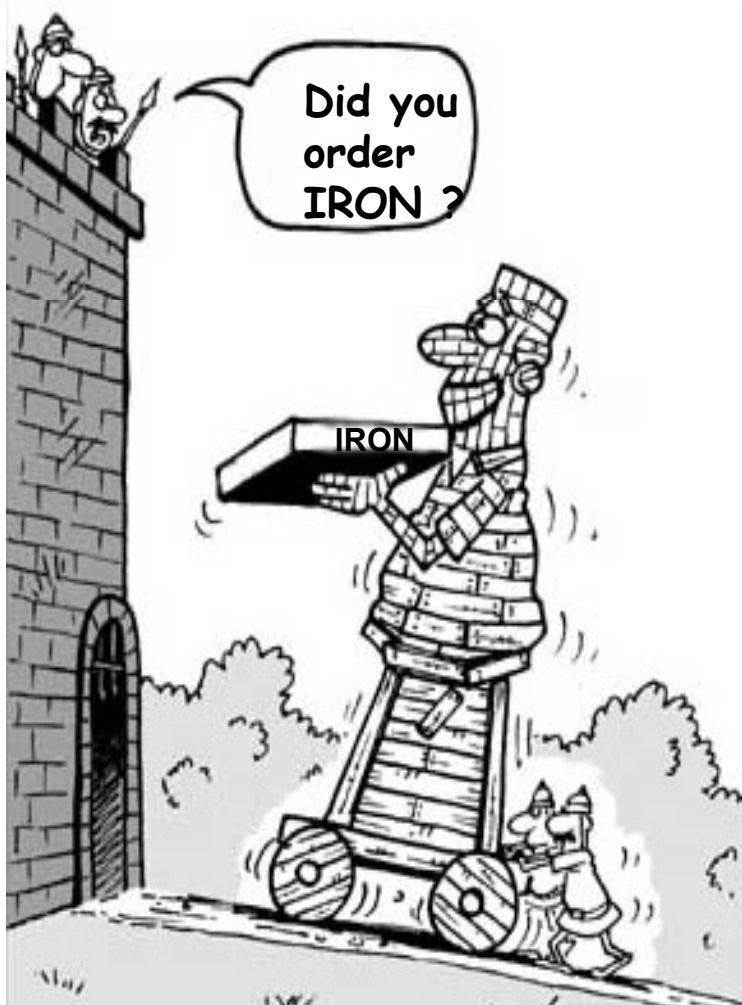
Stimulus = low iron concentration *in vivo*



Response = co-ordinate regulation of virulence and iron-uptake genes

Bacterial iron metabolism as a drug target

Development of siderophore-antibiotic conjugates
(the Trojan horse strategy)



Bacterial iron metabolism as a drug target

Research article

The transition metal gallium disrupts
Pseudomonas aeruginosa iron metabolism
and has antimicrobial and antibiofilm activity

Yukihiro Kaneko,¹ Matthew Thoendel,² Oyebode Olakanmi,³
Bradley E. Britigan,^{3,4} and Pradeep K. Singh¹

Use of the transition metal gallium (Ga)
to disrupt bacterial iron metabolism

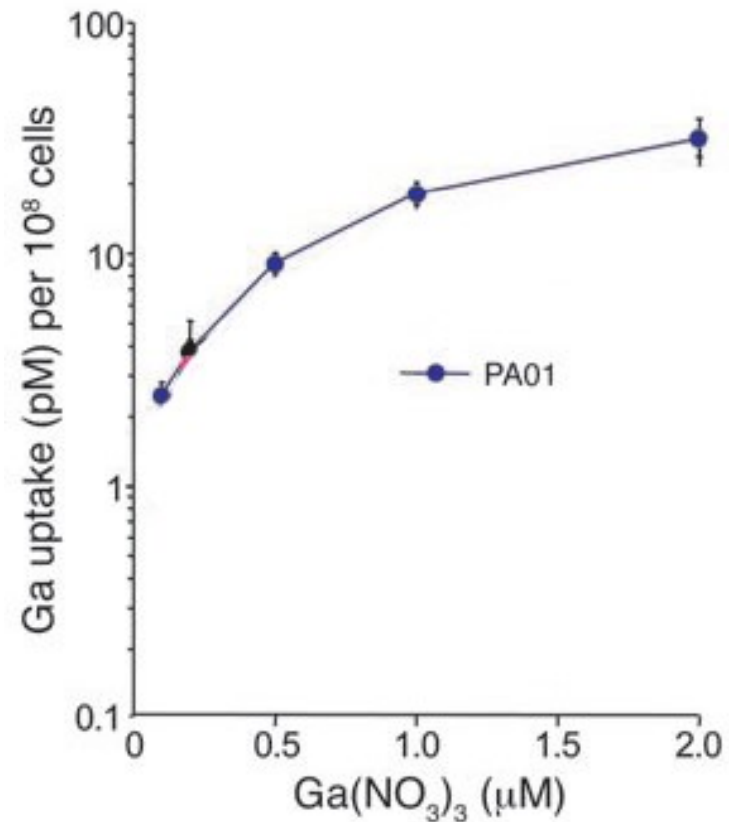
RATIONALE:

- Ga has an ionic radius nearly identical to that of Fe
- many biological systems do not distinguish Ga^{3+} from Fe^{3+}
- unlike Fe^{3+} , **Ga^{3+} cannot be reduced**

Ga could inhibit Fe-dependent processes, by inhibiting Fe-dependent redox reaction critical for many biological functions

Bacterial iron metabolism as a drug target

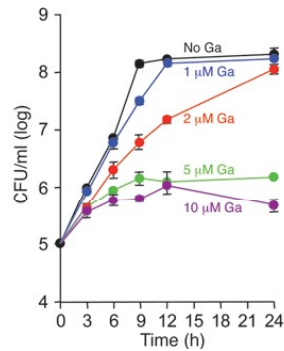
Pseudomonas aeruginosa cells take up Ga in a concentration-dependent manner



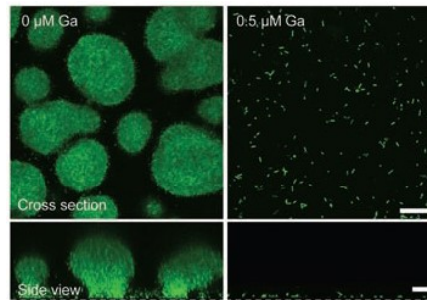
Bacterial iron metabolism as a drug target

In *Pseudomonas aeruginosa* Ga inhibits:

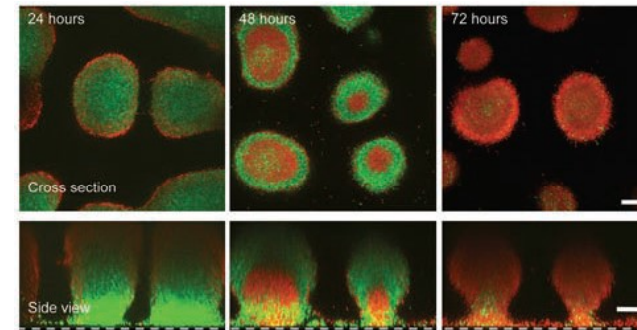
Growth



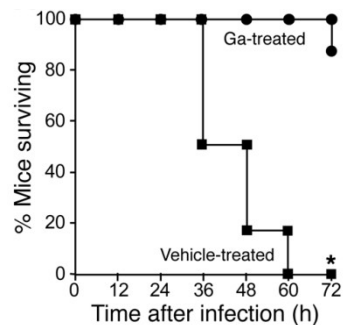
Biofilm formation



Biofilm persistence



Pathogenicity



Ga has been proved to inhibit many other bacterial pathogens,
and it is currently in phase I clinical trials for the treatment of *P. aeruginosa* pulmonary infection in cystic fibrosis patients

DRUG DEVELOPMENT

Gallium disrupts bacterial iron metabolism and has therapeutic effects in mice and humans with lung infections

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