

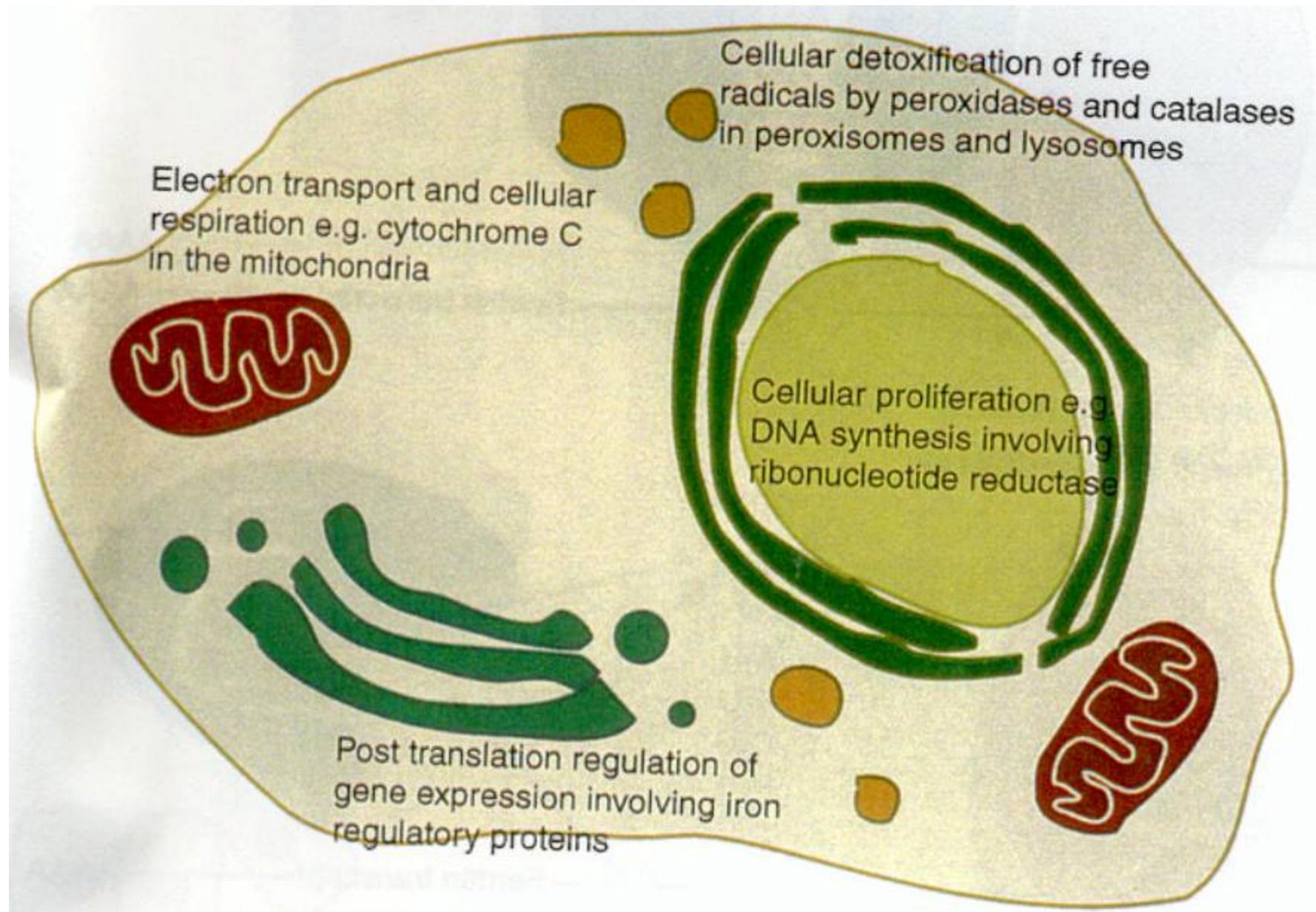
Iron: Mechanisms of Pro-oxidant Behavior, Cellular Uptake, and Organism Survival Skills

Bradley E. Britigan, M.D.

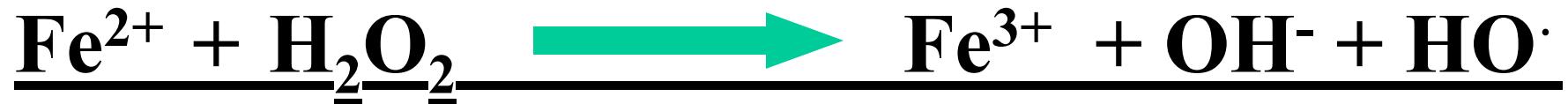
Department of Internal Medicine

**VA Medical Center- Iowa City and Roy G. and
Lucille A Carver College of Medicine**

University of Iowa



Haber-Weiss Reaction



Reaction of Iron With Lipid Hydroperoxides



Iron Can Contribute Directly or Indirectly to the Oxidation of :

- Proteins
- Lipids
- DNA
- Sugars
- Site-specific Oxidation ?

Human Iron Metabolism

- Iron exists in 2^+ (ferrous) or 3^+ (ferric) state
- Little Free Iron *in vivo*
- Chelated to Proteins or Other Molecules
 - Maintains Solubility
 - Limits Participation in Oxygen Redox Chemistry
 - Limits Availability to Microbes
- Iron-Binding Proteins Vary With Location

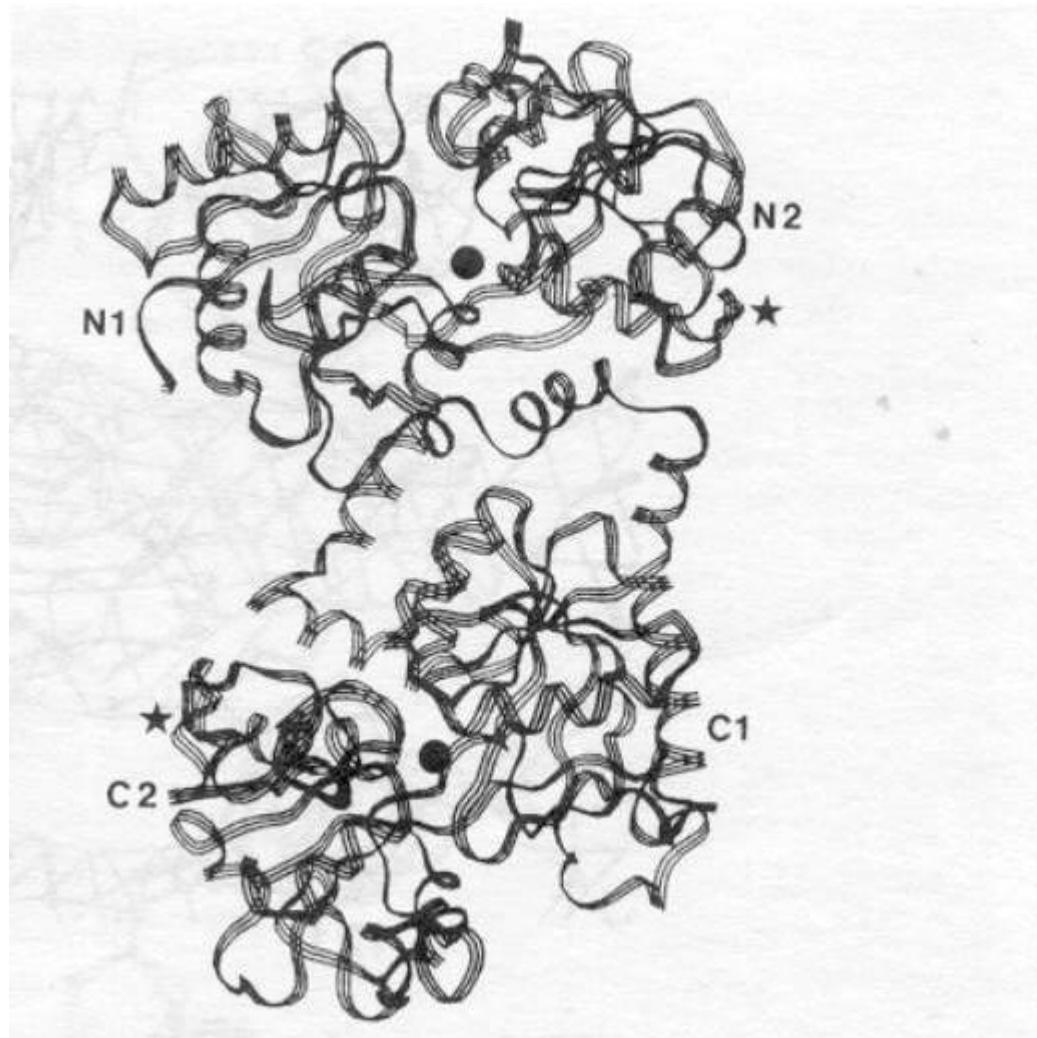
Extracellular Iron Chelates

- Transferrin
 - Serum
 - Mucosa (*e.g.* lung)
- Lactoferrin
 - Mucosa (*e.g.* lung)
 - Milk
 - Neutrophils

Transferrin and Lactoferrin

- **80 kDa glycoproteins**
- **Bind Ferric Iron With High Affinity**
- **Two Iron-Binding Sites per Molecule**
- **Enhanced by the presence of anions – *e.g.* carbonate**
- **Binding is pH sensitive**
- **Lactoferrin better iron retention at low pH**

Lactoferrin



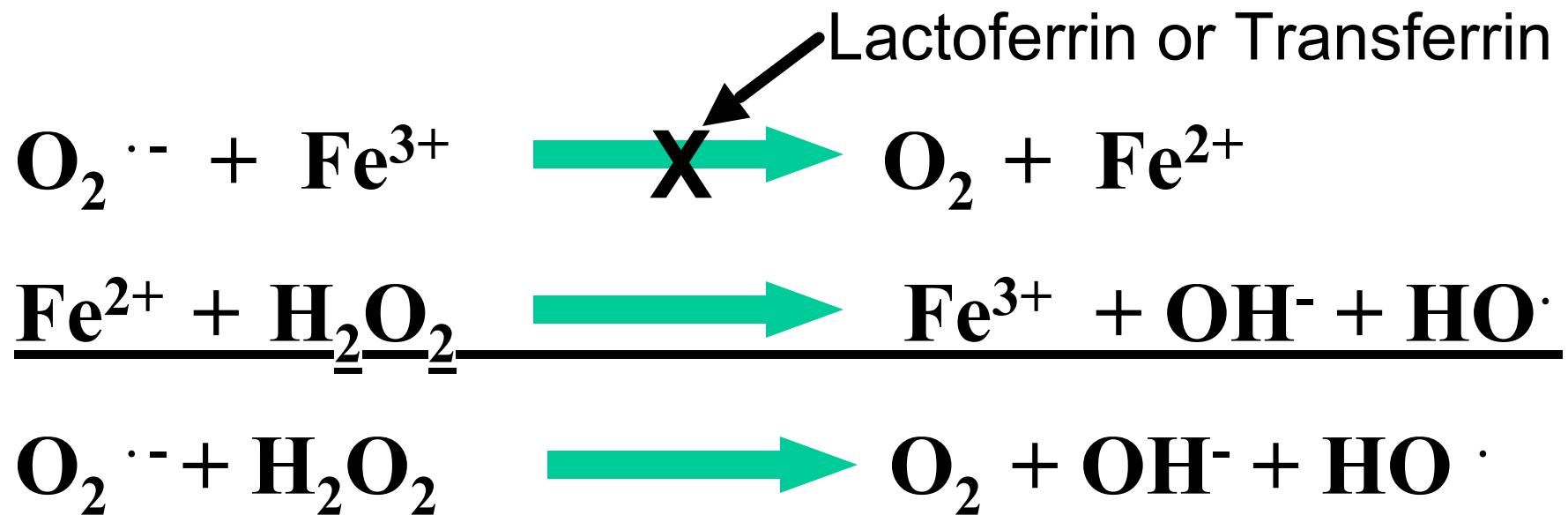
Biochemistry 31:4527-33, 1992

Iron Bound To Transferrin Or Lactoferrin Does Not Redox Cycle

Unfavorable reduction potential

	<u>E°</u>
Fe(III) Transferrin/Fe(II) Transferrin	- 400 mV
Fe(III) Ferritin, 2H ⁺ /Fe(II) Ferritin	- 190 mV
Fe(III) EDTA/FE(II/EDTA)	+ 120 mV
Fe(III) Citrate/Fe(II) Citrate	+»100 mV
Fe(III)ADP/Fe(II) ADP	+»100 mV
O ₂ /O ₂ ^{·-}	- 330 mV

Haber-Weiss Reaction



Intracellular Iron Chelates

- **Ferritin**
 - Long term storage
 - 4500 atoms Fe/molecule
 - Fe^{3+}
- **Labile Iron Pool**
 - Poorly characterized
 - Transient storage
 - Exchanges with ferritin

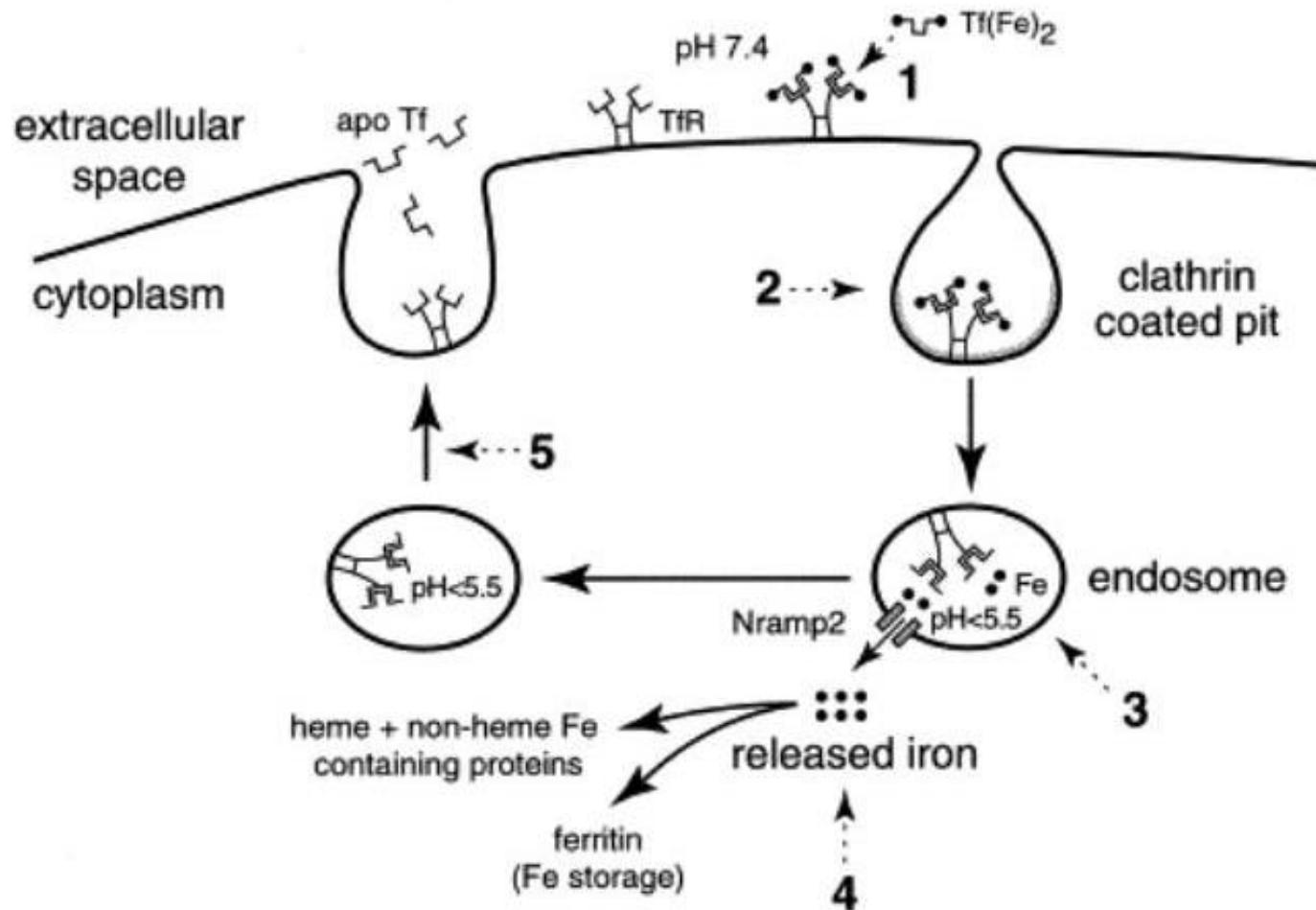
Iron Bound To Ferritin Is Also Relatively Non-Reactive

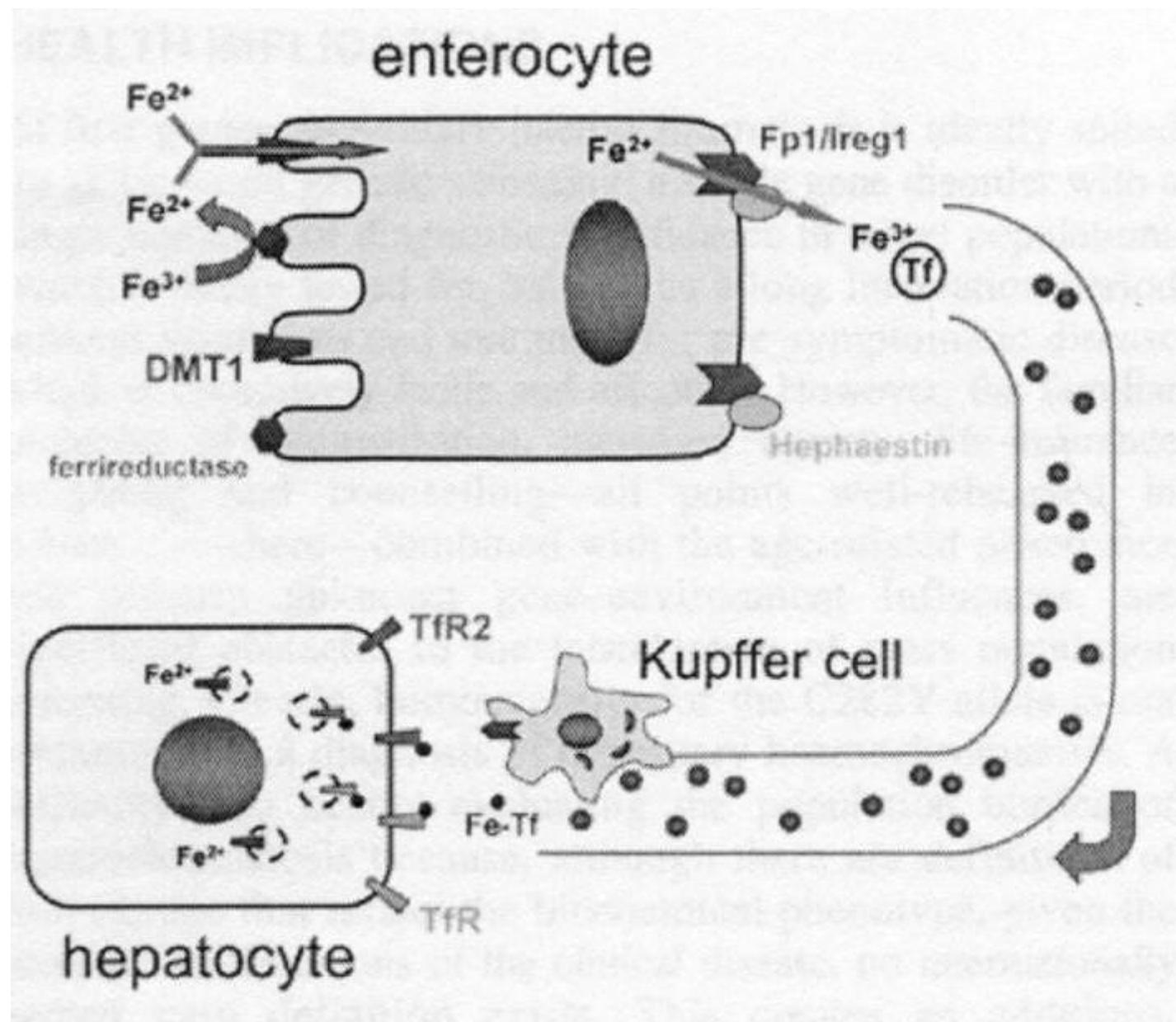
Unfavorable reduction potential

	<u>E°</u>
Fe(III) Transferrin/Fe(II) Transferrin	- 400 mV
Fe(III) Ferritin, 2H ⁺ /Fe(II) Ferritin	- 190 mV
Fe(III) EDTA/FE(II/EDTA)	+ 120 mV
Fe(III) Citrate/Fe(II) Citrate	+»100 mV
Fe(III)ADP/Fe(II) ADP	+»100 mV
O ₂ /O ₂ ·-	- 330 mV

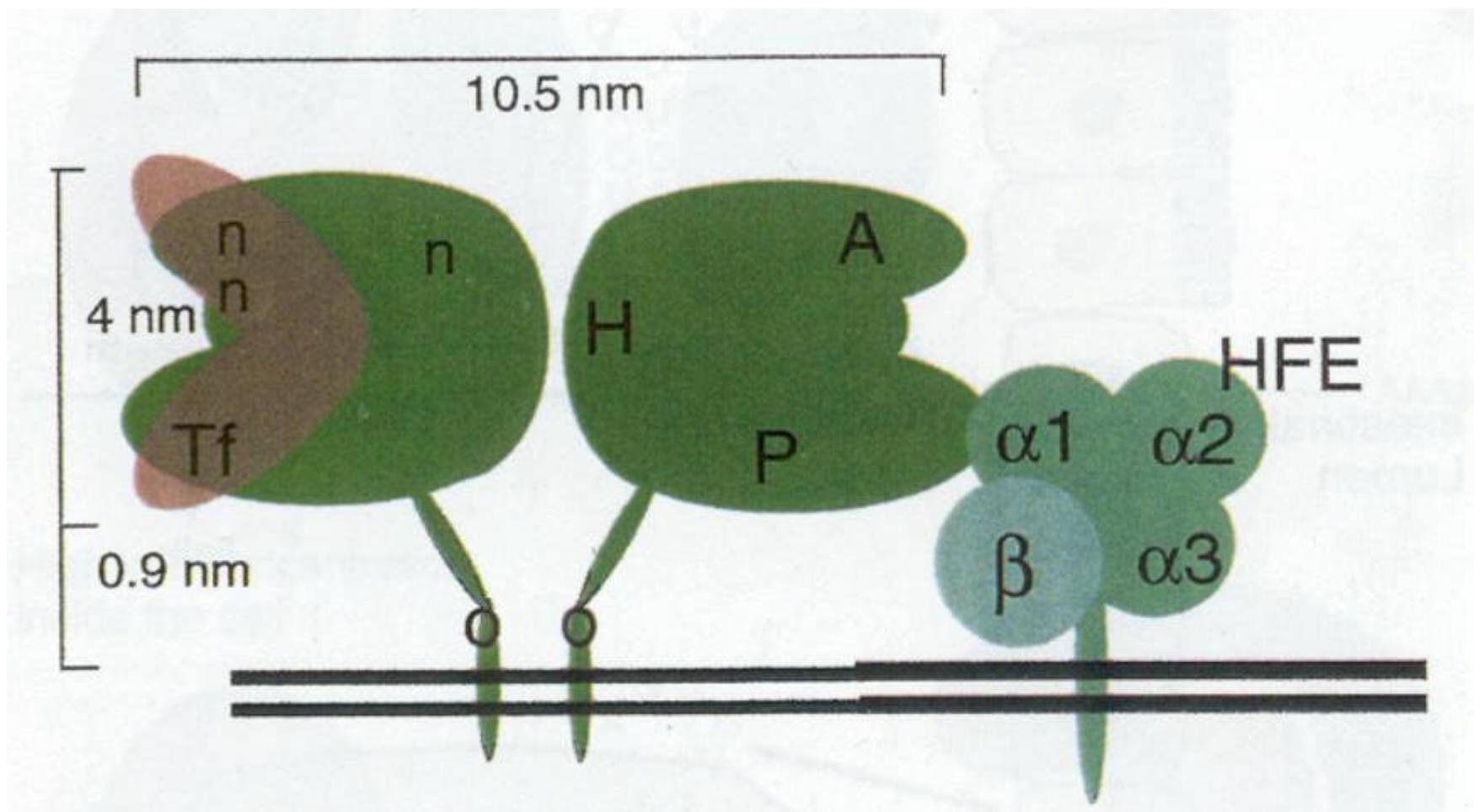
HOW IS IRON TRANSPORTED INTO CELLS?

Receptor-Mediated Iron Uptake From Transferrin



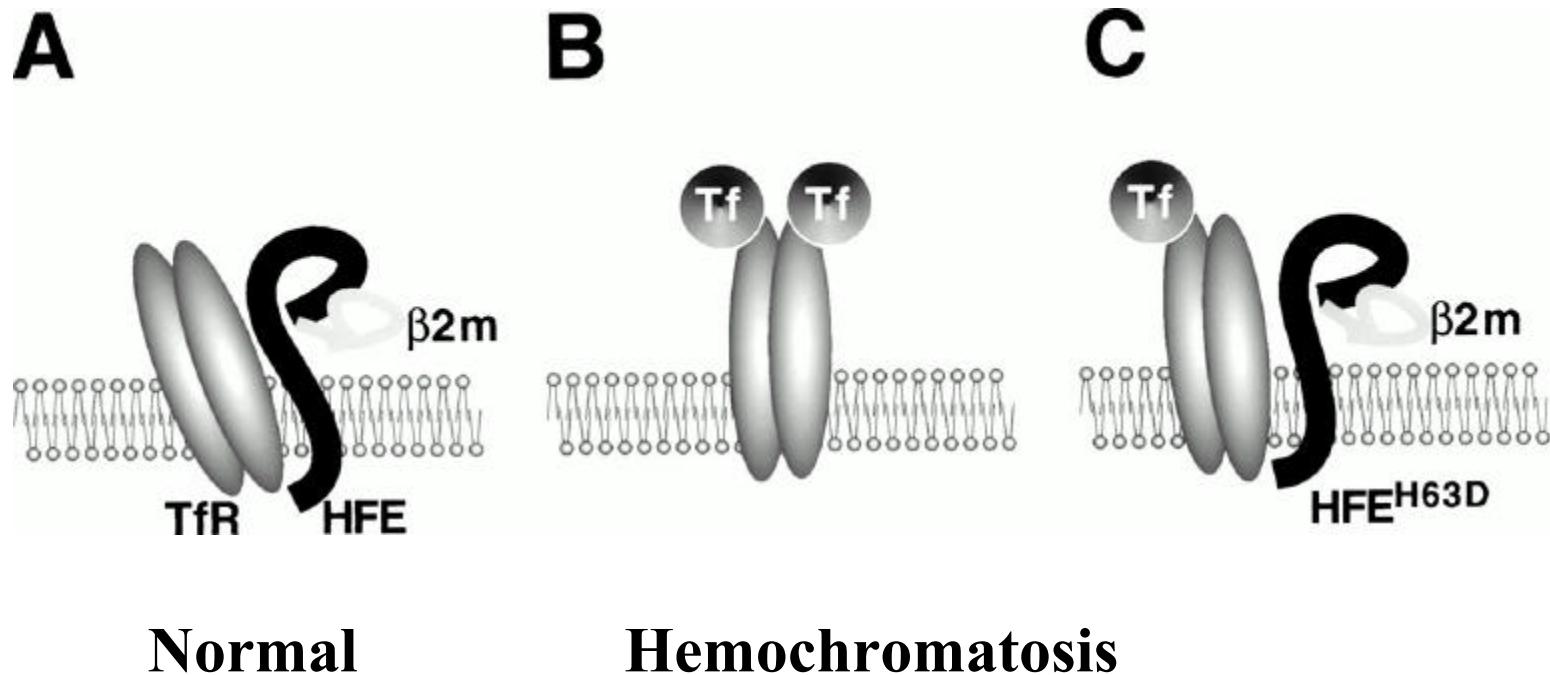


Transferrin Receptor Complex



Int Rev Cytol 211:241-278, 2001

HFE Protein Interacts With The TFR



Blood 92:1845-51, 1998

TFR2

- Newly described receptor for transferrin
- Liver and peripheral blood mononuclear cells
- Lower affinity for transferrin than TFR1
- About 60% sequence homology to TFR1
- Doesn't bind HFE
- Mutations of TFR2 are associated with hemochromatosis

Fe Uptake From Lactoferrin

- Binding to Variably Characterized Surface “Receptors”

Not TFR

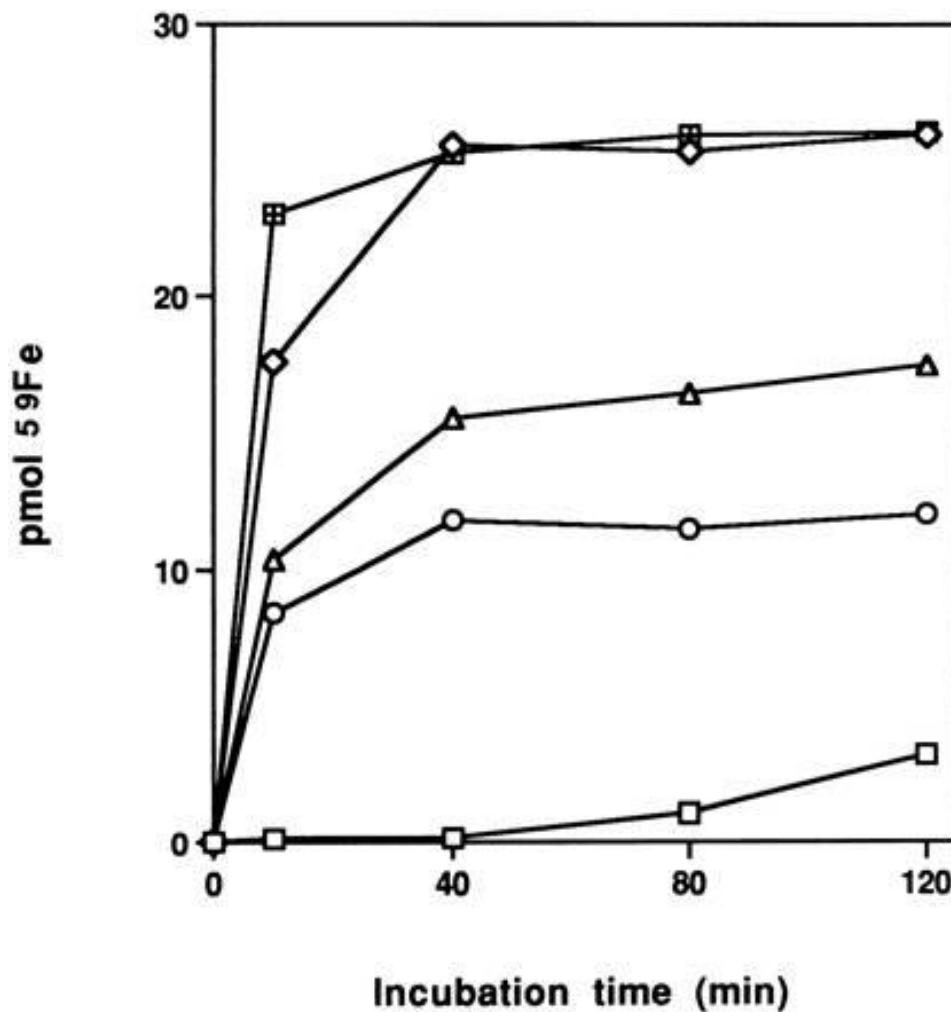
Proposed Receptors

Protein; Glycosaminoglycans; Scavenger
Receptor; Asialoglycoprotein Receptor;
Mannose Receptor

- No Agreement on Cellular Fe Acquisition from LF
- ? Fe Handled Differently than when Acquired from TF

What's Known About Fe Uptake From LMW Chelates

- Most cell types can do so
- Variable ill-defined mechanisms involved
- Inducible in myeloid cells
 - Multivalent metals
 - ATP independent
 - Not receptor-mediated endocytosis

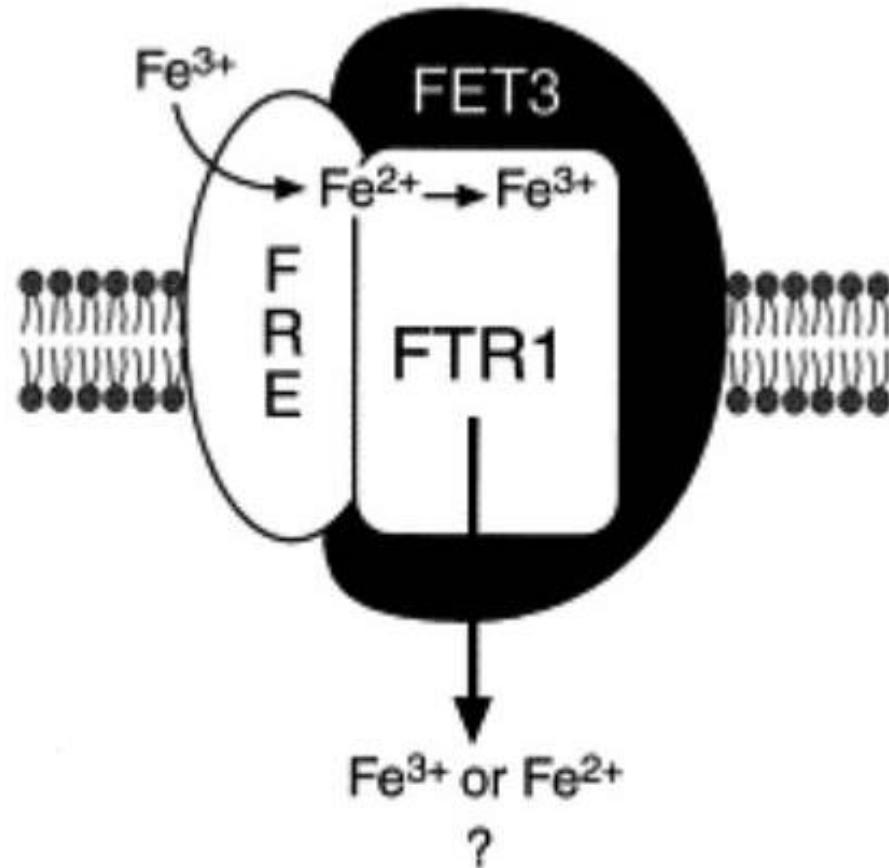


Gallium Induces Fe Uptake From LMW By HL-60 Cells

Ascorbate = NTA > ADP > citrate >> NTA (No Ga)

J Biol Chem 272:2599-2606, 1997

Pathway for High Affinity Iron Uptake in Yeast



Int J Biochem Cell Biol 33: 940-59, 2000

Iron Transporters

Yeast vs. Mammalian Cells

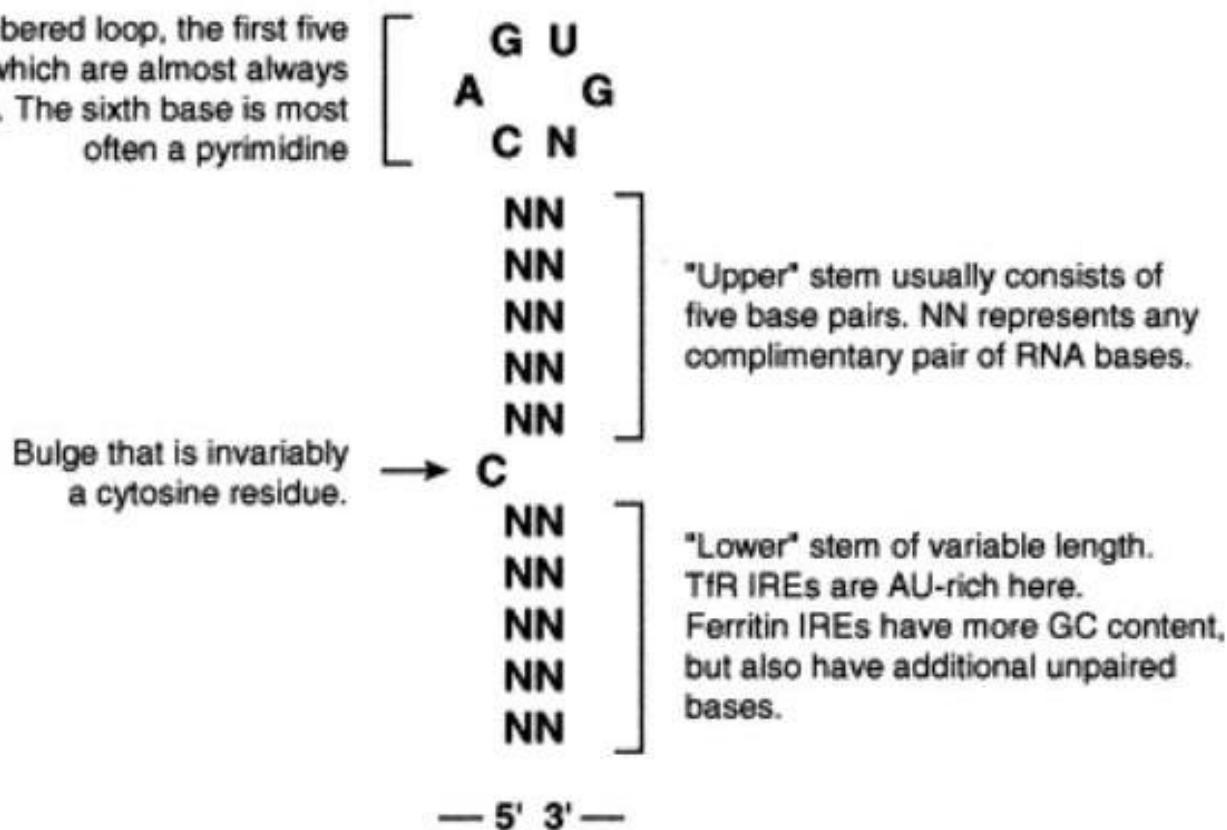
Function	Yeast cells	Mammalian cells
Ferrireduction	FRE1 and FRE2	Gp91 phos-related protein?
Divalent metal ion uptake	SMF1, other SMF family members	DMT1/Nramp2, Nramp1 and other family members?
Fe(II) uptake	FET4	Not known
FE(III) uptake	FTR1	Not known, MIP pathway?
Ferroxidation	FET3	Ceruloplasmin, hephaestin
Fe export	Not known	Ireg1/Ferroportin/MTP1

Int J Biochem Cell Biol 33:940-59, 2000

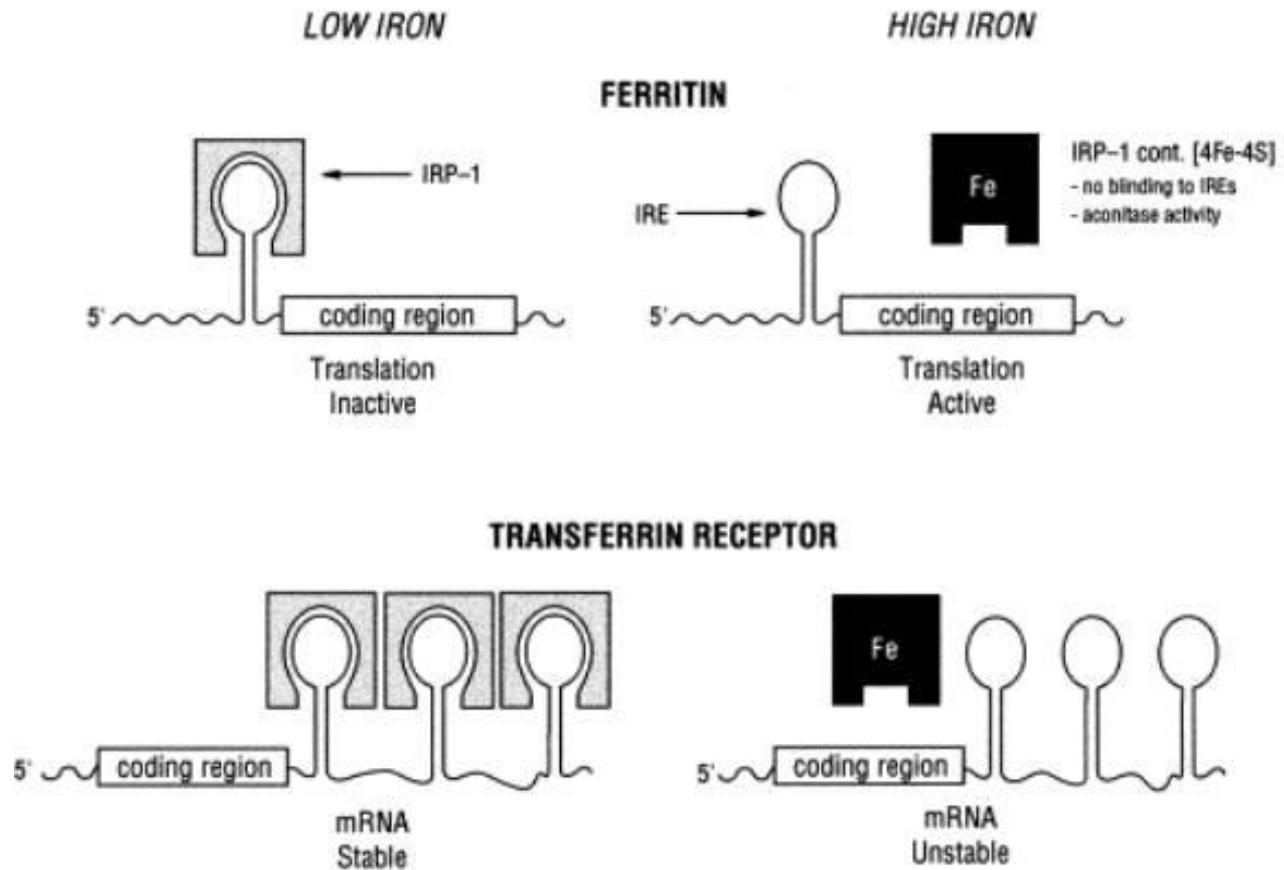
HOW IS INTRACELLULAR AND EXTRACELLULAR IRON CONTENT REGULATED?

Structure of the Consensus Iron Responsive Element

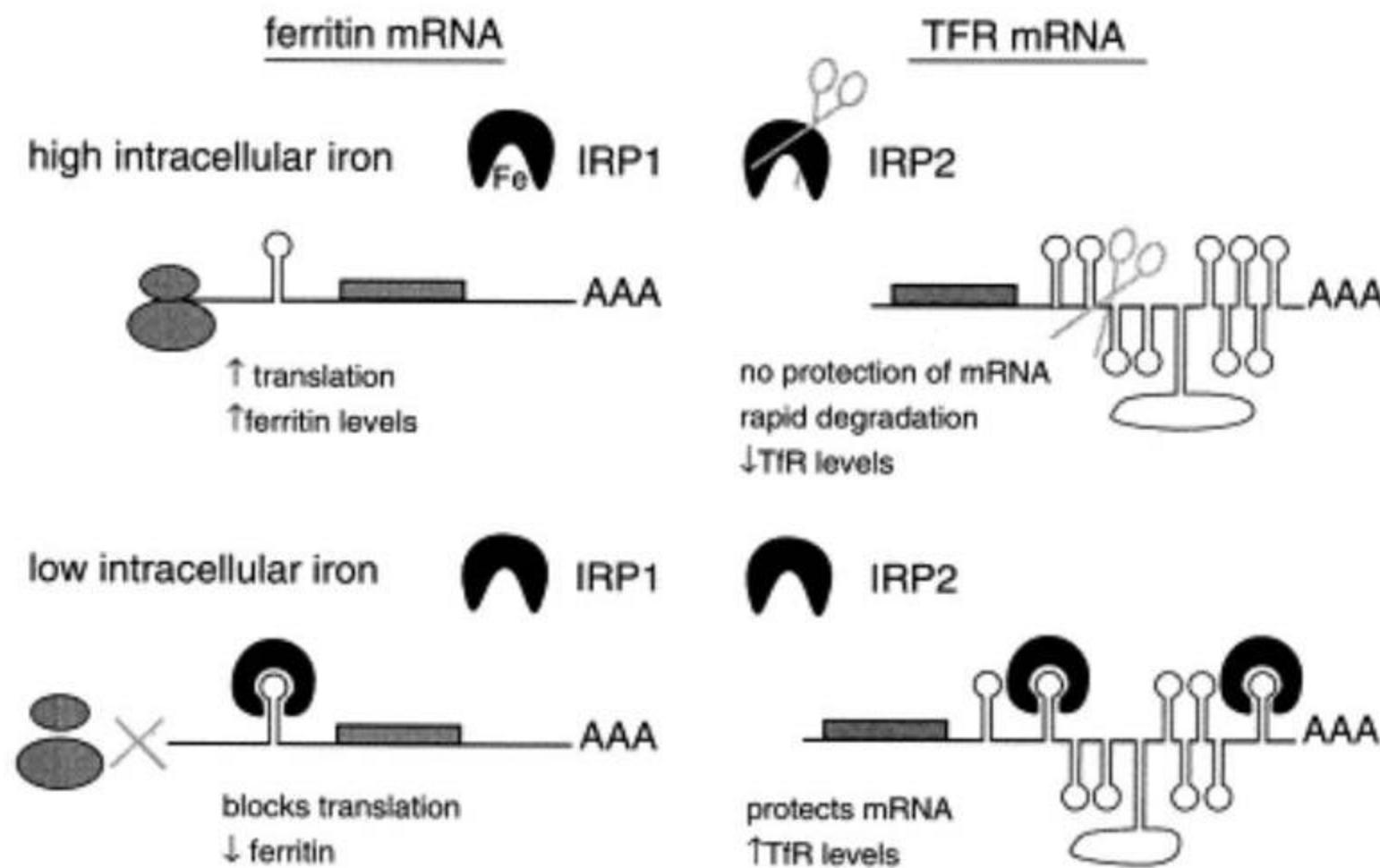
Six-membered loop, the first five bases of which are almost always CAGUG. The sixth base is most often a pyrimidine

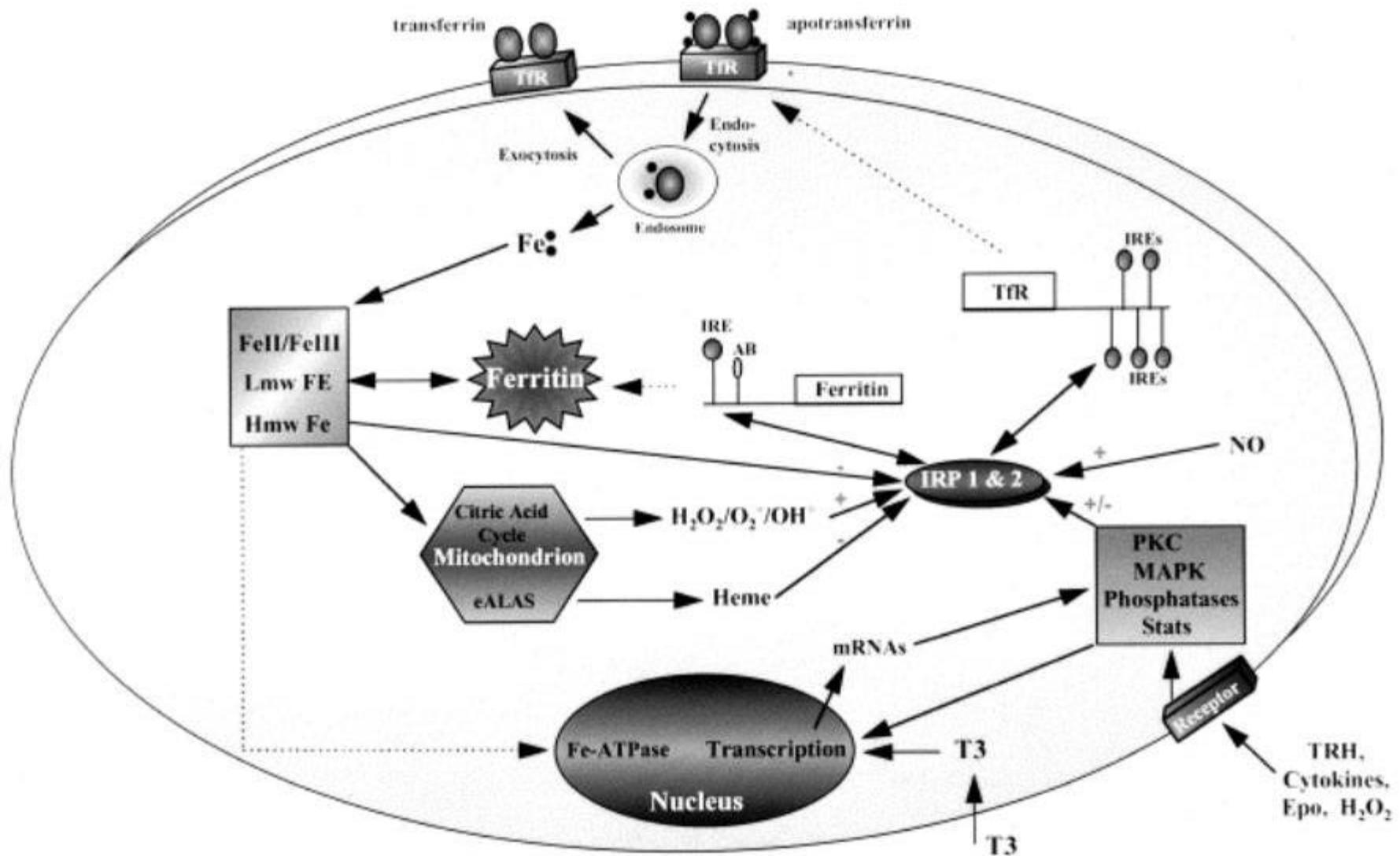


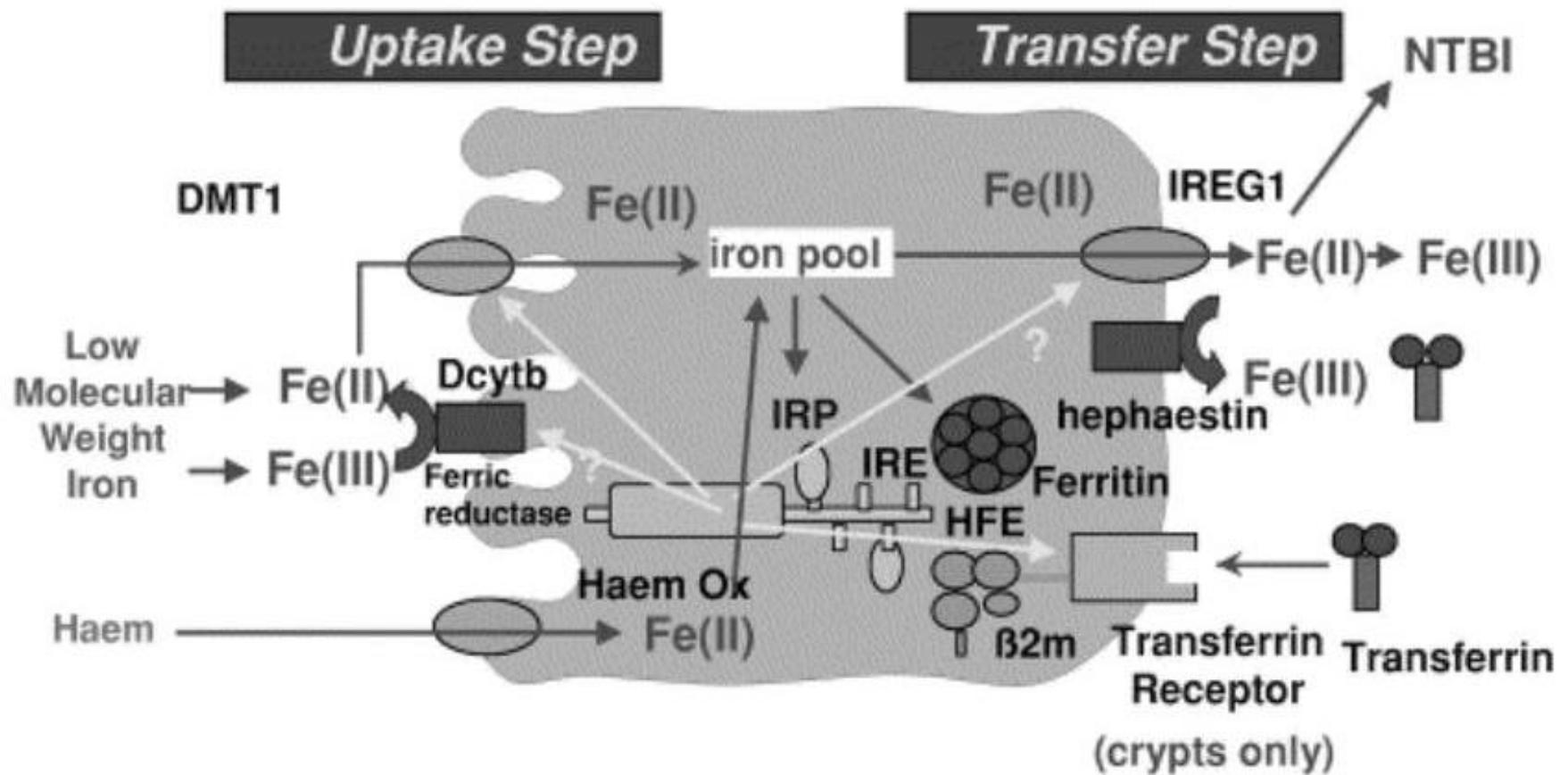
The Interaction of IRP-1 with Ferritin and Transferrin Receptor mRNA



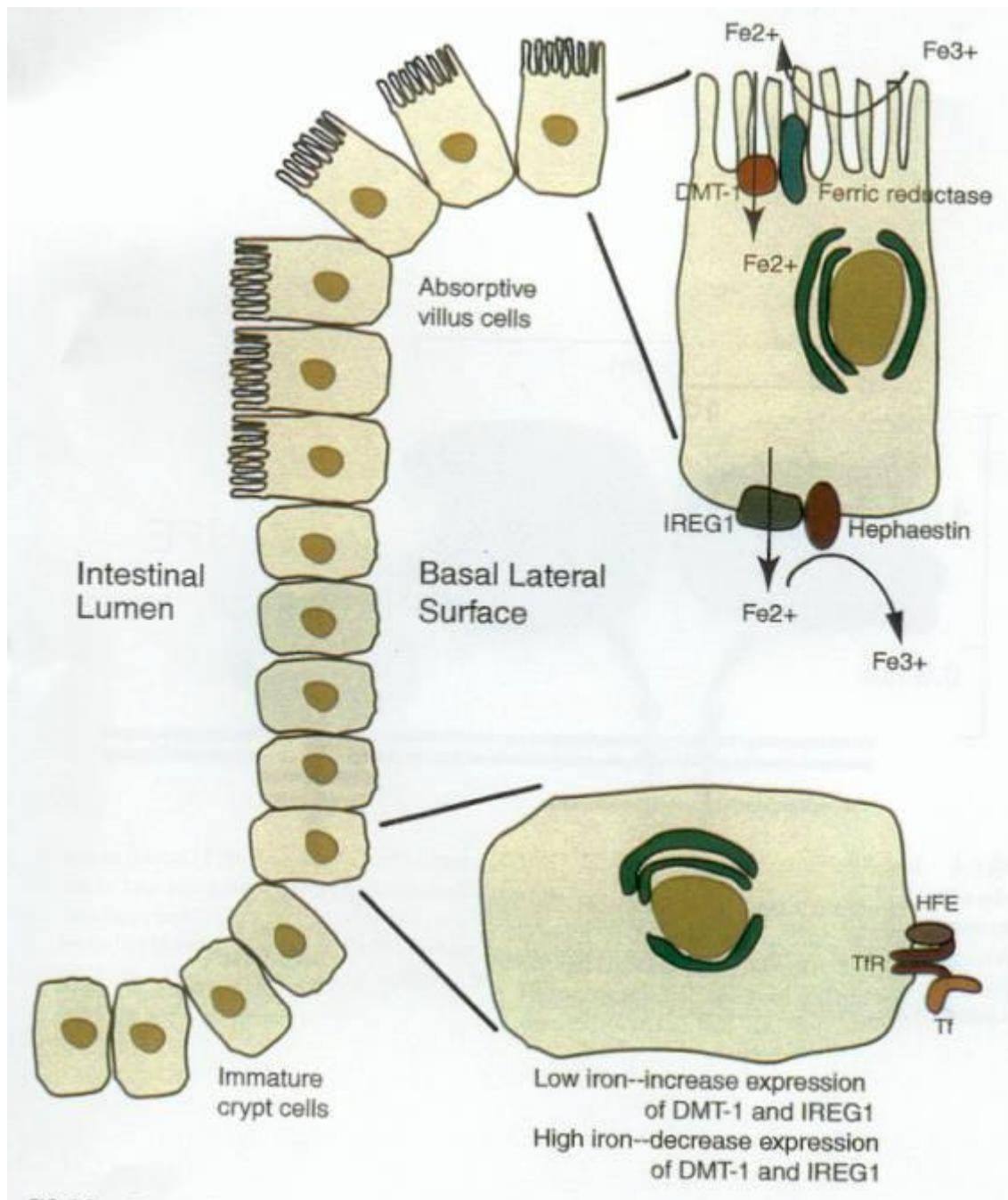
Iron Control of Translation and mRNA Stability





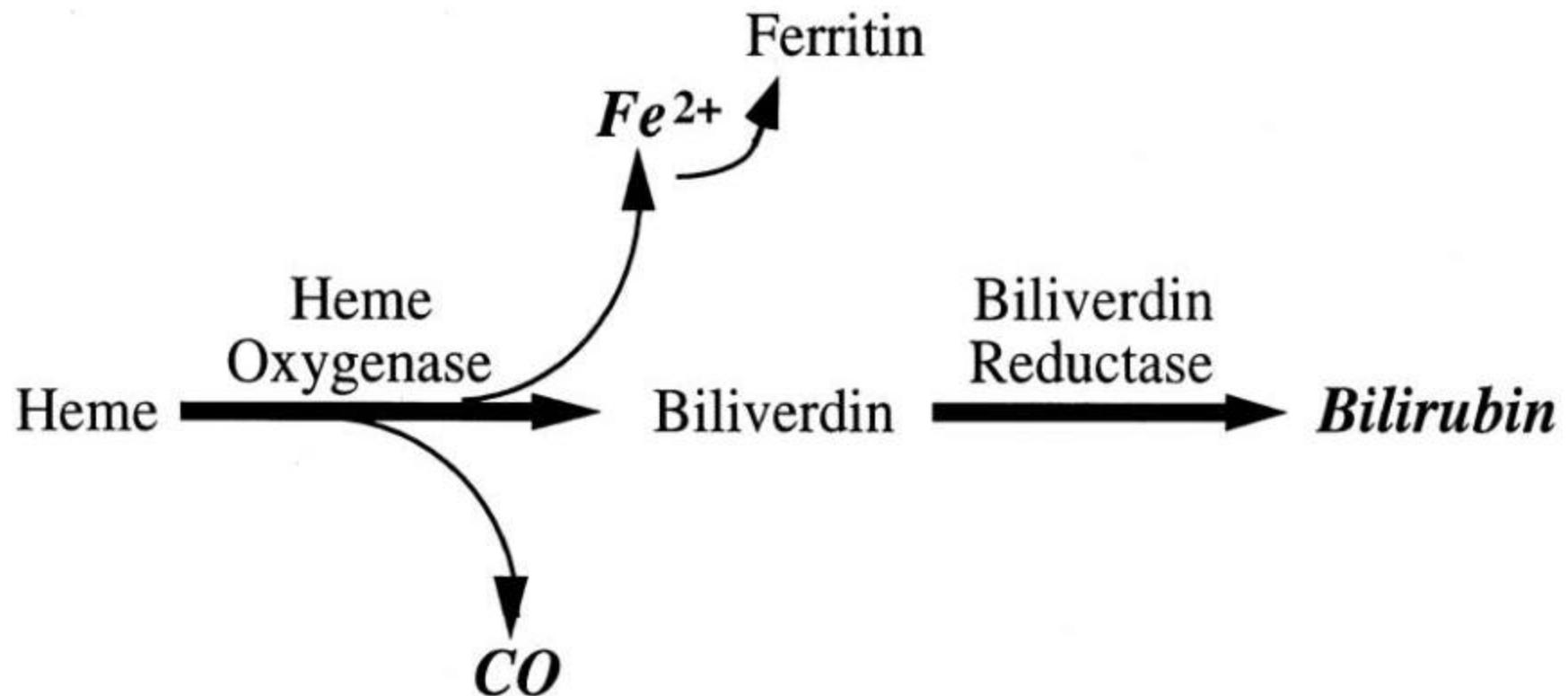


Crichton *et al.* J. Inorganic Biochem 91: 9-18, 2002

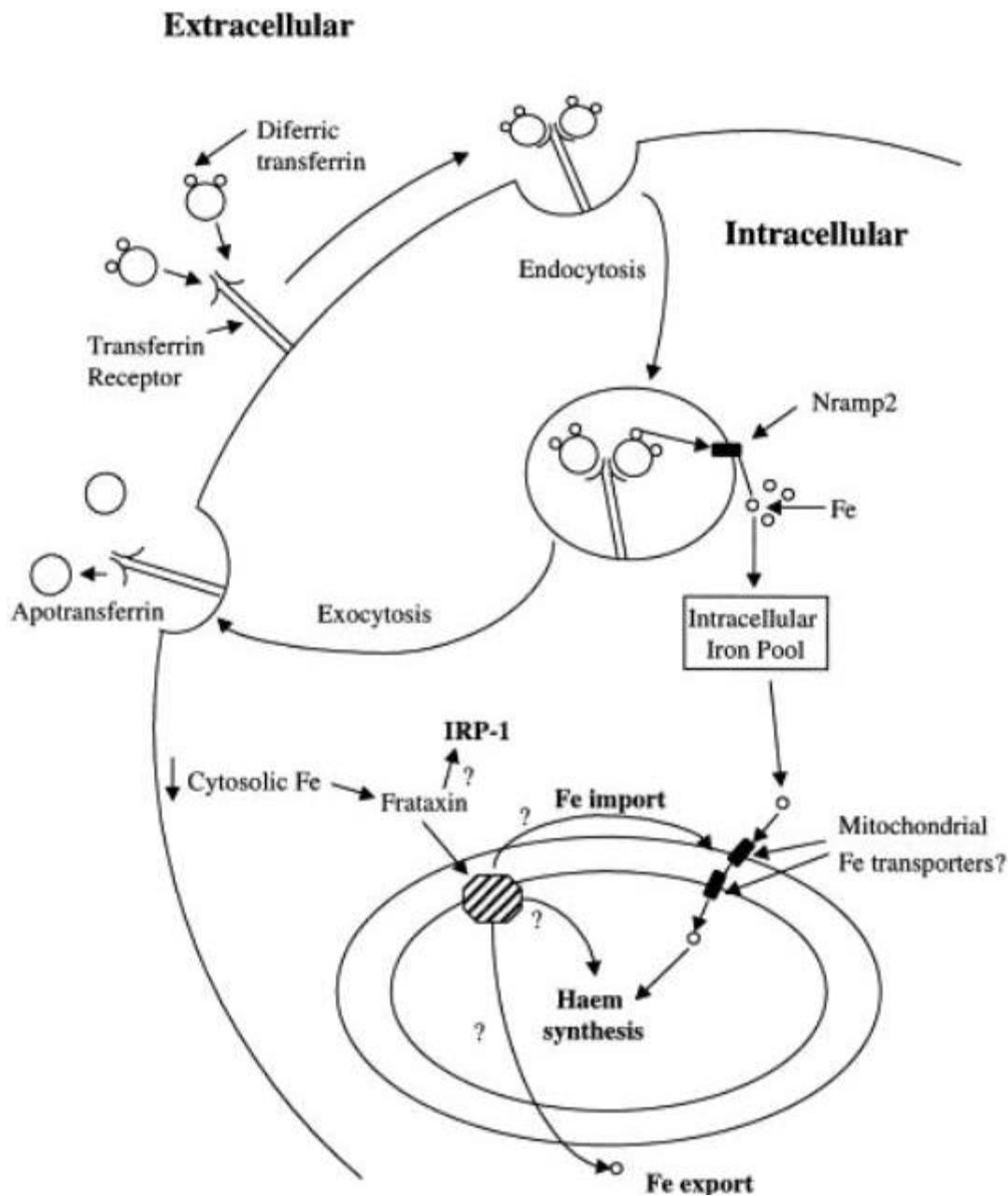


Int Rev Cytol 211: 241-278, 2001

Heme Oxygenase and Iron Metabolism



Am J Physiol 279: L1029-37, 2000



Iron Metabolism and Host Defense

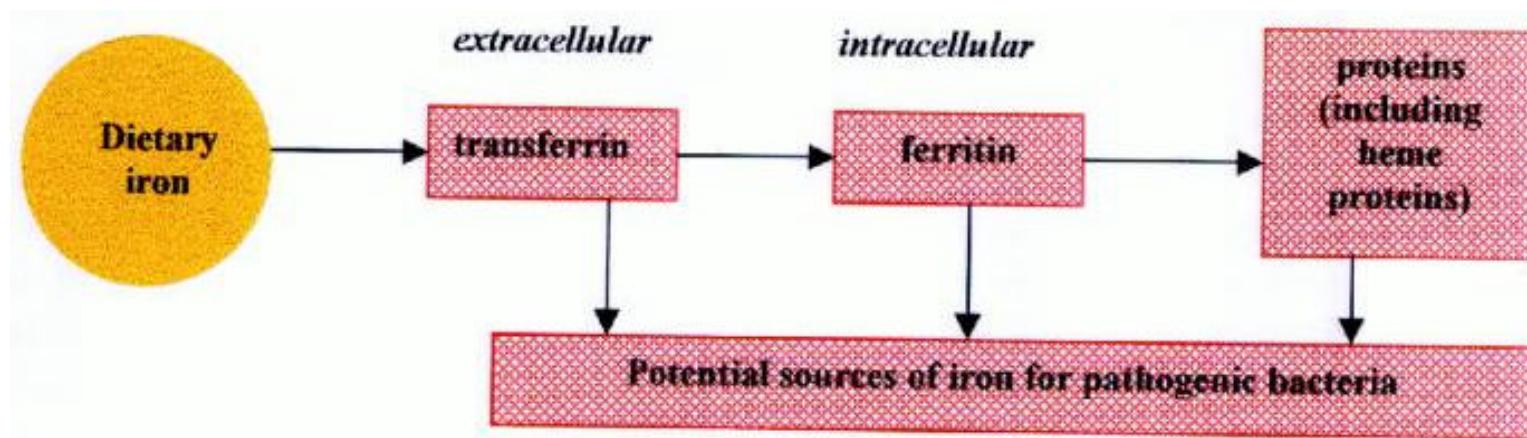
- **Nearly Every Microorganism Needs Iron for Growth and Metabolism**
 - Enzymes
 - DNA replication
 - Respiratory chain
 - Antioxidants
 - Heme centers
- **Iron Bound to Lactoferrin and Transferrin is Much Less Accessible**

Infection Shifts Iron

- Host Response to Acute or Chronic Infection
 - Shift Iron Out of Serum
 - Shift Iron Into Reticuloendothelial System Macrophages
- Good Against Extracellular Pathogens
- Perhaps Not So For Intracellular Ones

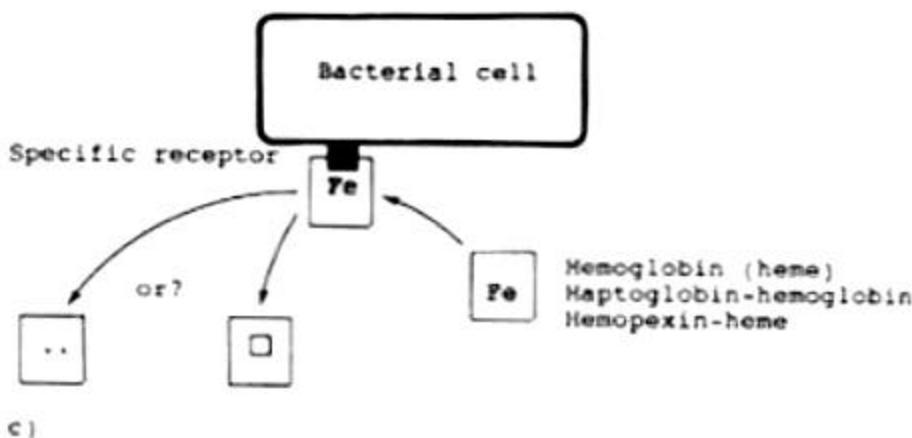
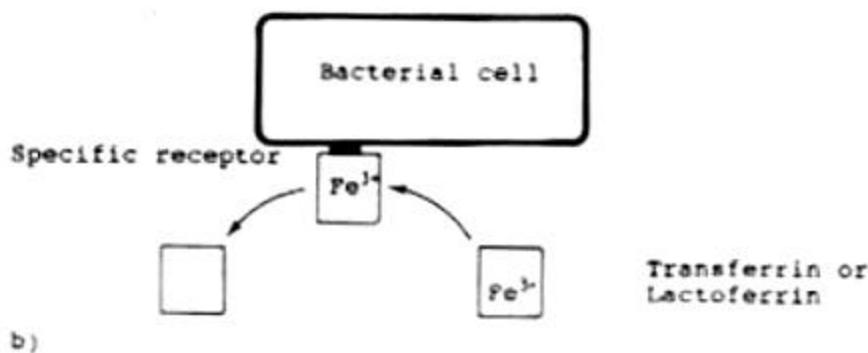
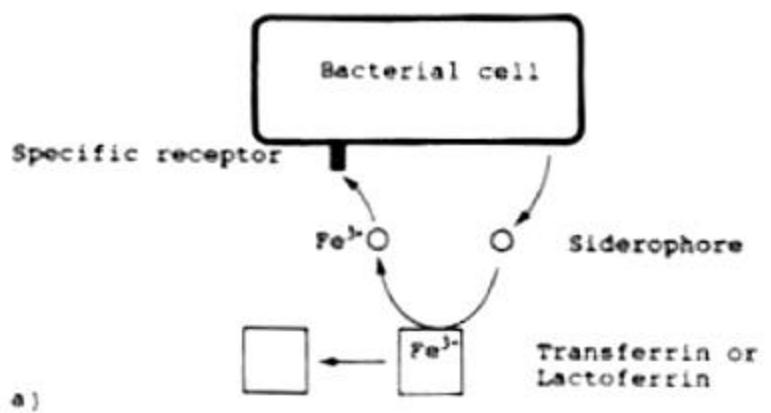
How Do Pathogens Acquire Iron From the Host?

Fe Sources Potentially Available To Pathogens



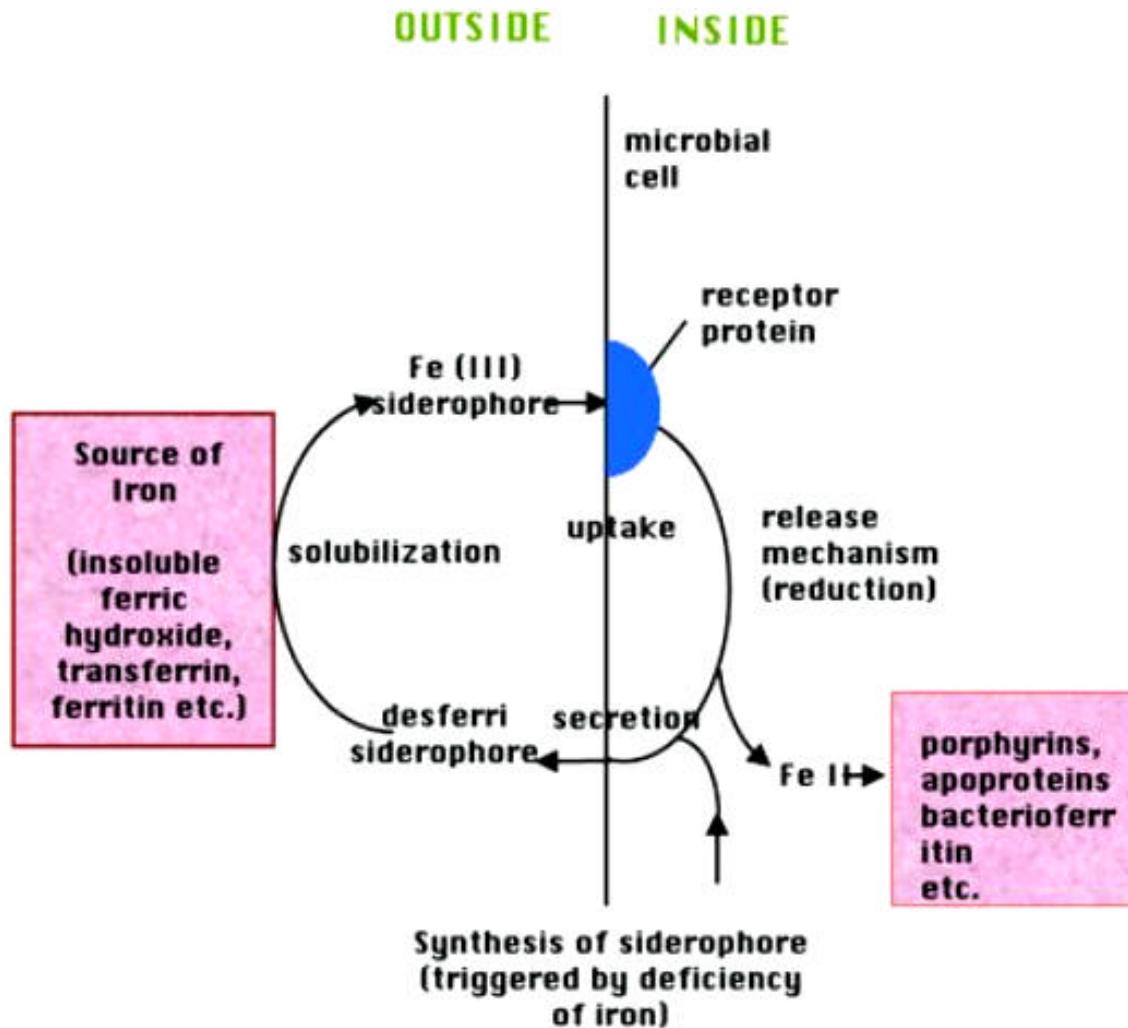
Ann Rev Microbiol. 54: 881-941, 2000

Microbial Strategies of Iron Acquisition from Extracellular Host Iron Chelates



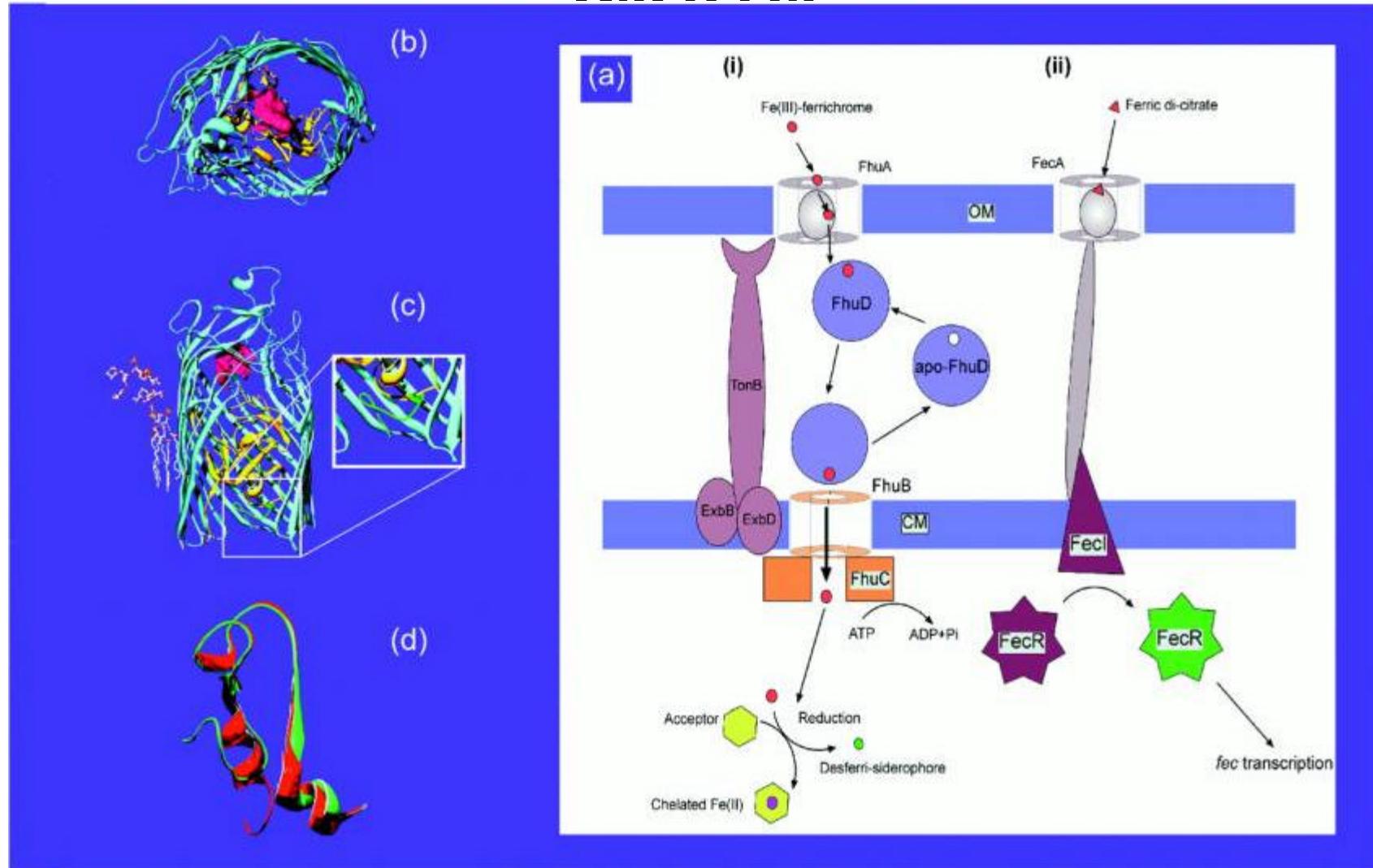
Crit Rev Micro 18: 217, 1992

Siderophore-Mediated Iron Uptake



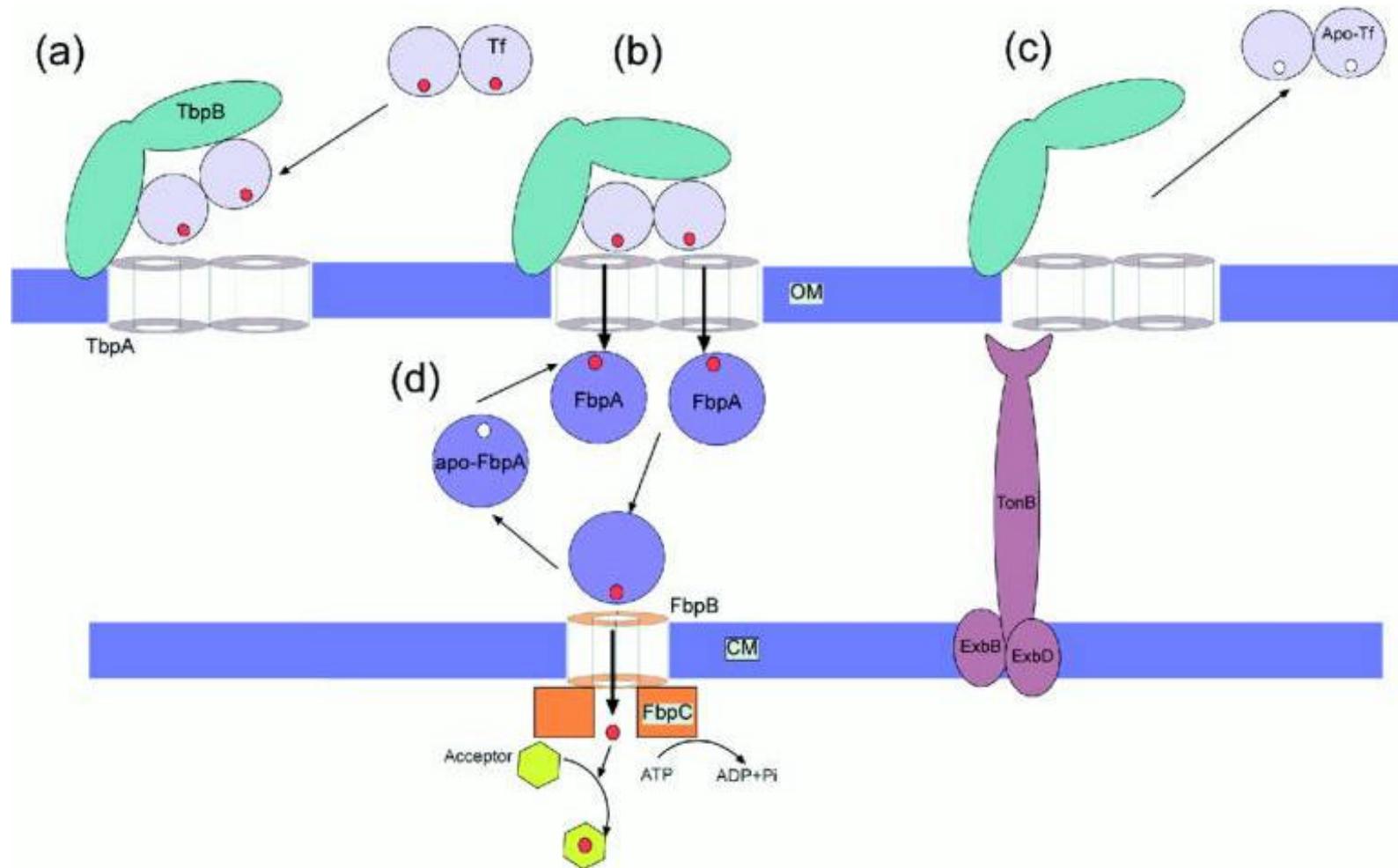
Ann Rev Microbiol. 54: 881-941, 2000

Ferri-siderophore Transport in Gram-negative Bacteria



Ann Rev Microbiol. 54: 881-941, 2000

Uptake of Transferrin Iron by Gram-negative Bacteria



Ann Rev Microbiol. 54: 881-941, 2000

Other Microbial Pathogens

- Fungi
 - Siderophores
 - Fe reduction
- Protozoan Parasites
 - Trypanosomes – TF receptor
 - Leishmania – TF or LF Receptor?
 - Trichomonas – TF or LF receptor
 - Malaria

Gene Regulation by the Fur Protein

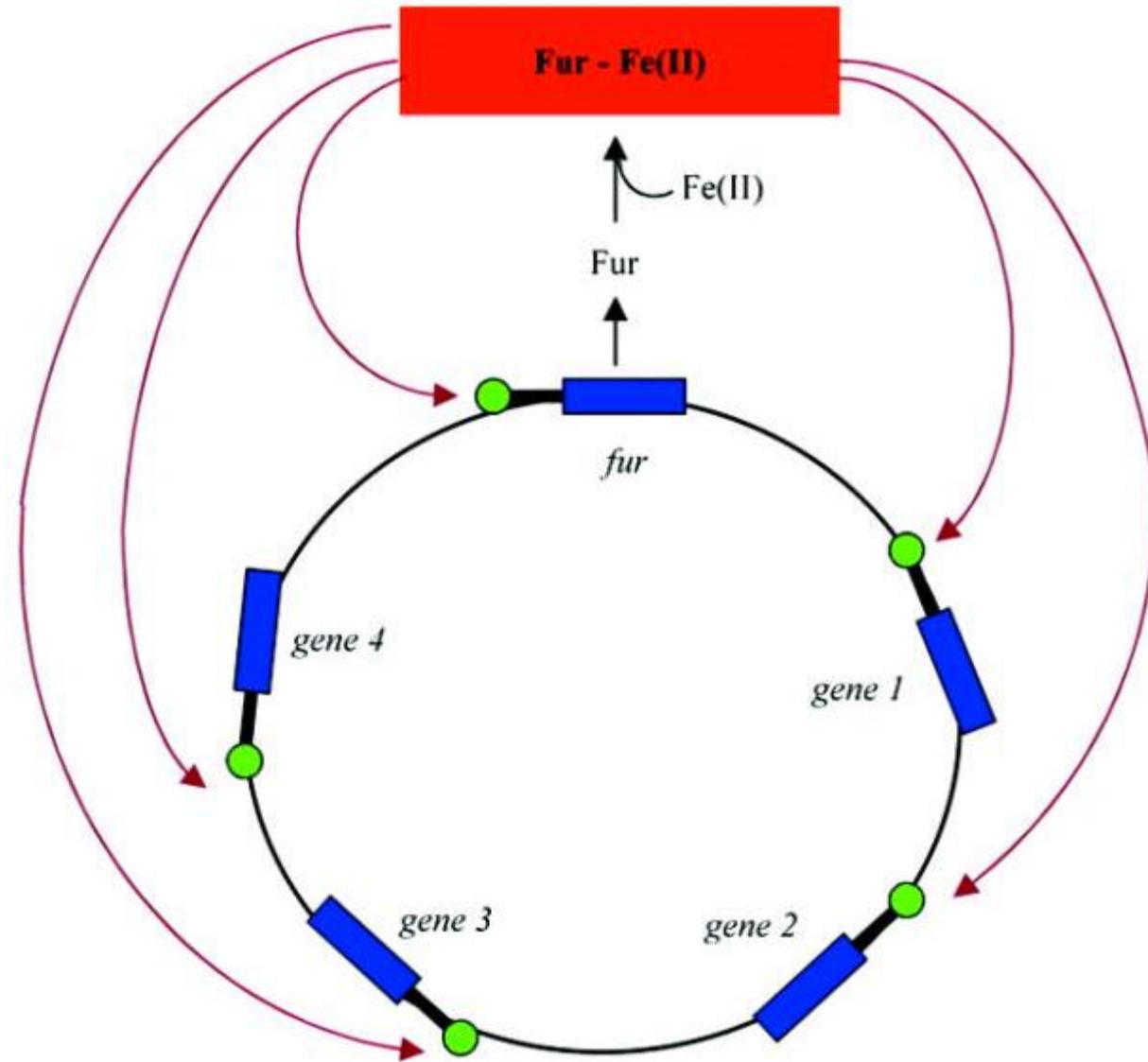


Table 3. Occurrence of the Fur protein in Gram-negative pathogenic bacteria and of related functional proteins (DtxR and IdeR) in Gram-positive bacteria

Fur

Bacillus subtilis

Bordetella spp.

Campylobacter jejuni

Escherichia coli

Haemophilus influenzae

Neisseria spp.

Pseudomonas spp.

Salmonella typhimurium

Shigella dysenteriae

Staphylococcus epidermidis

Vibrio spp.

Yersinia spp.

DtxR/IdeR

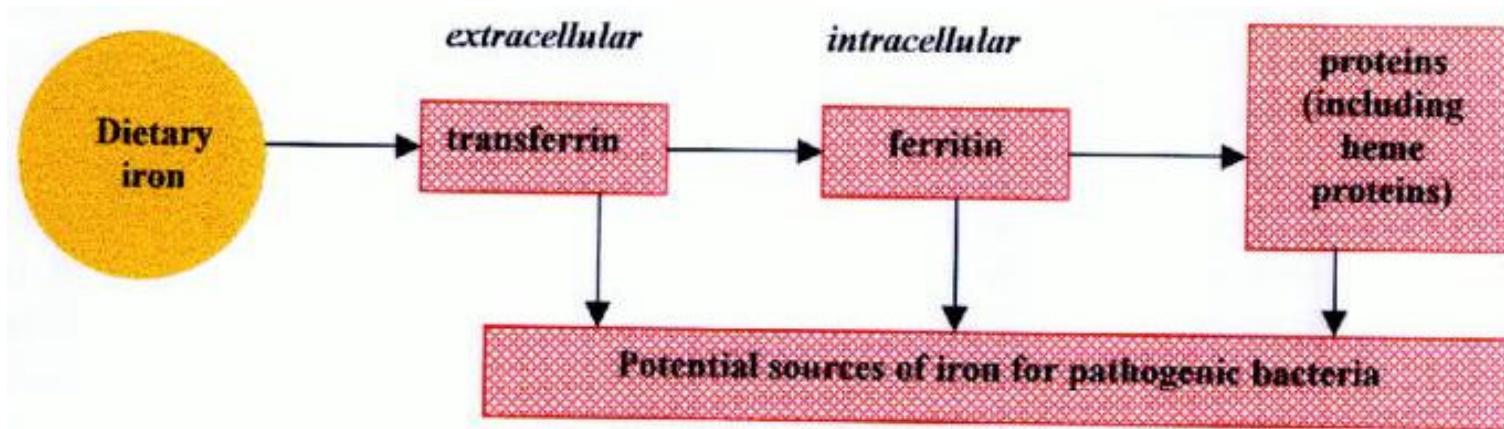
Brevibacterium lactofermentum

Corynebacterium diphtheriae

Corynebacterium glutamicum

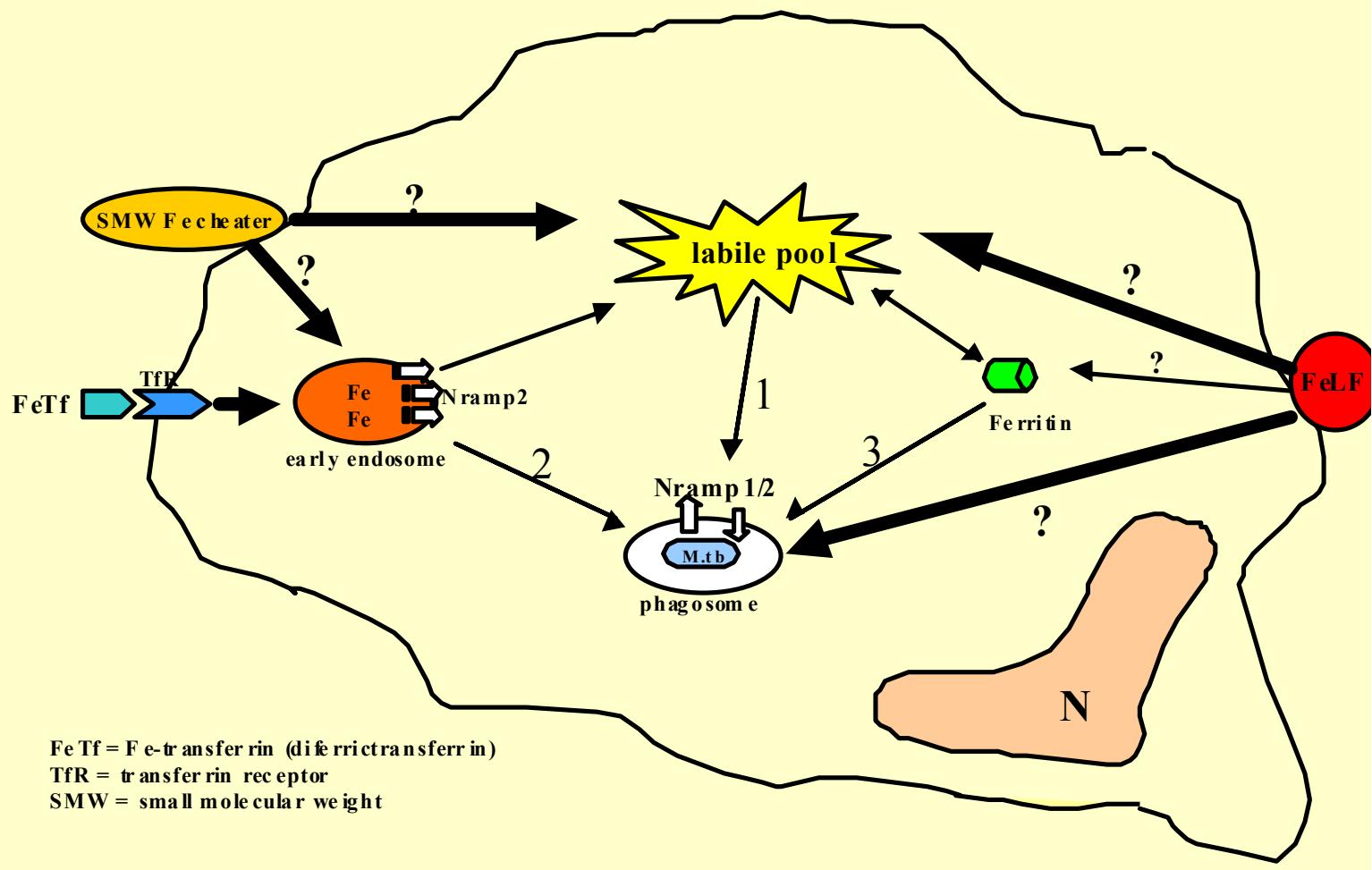
Mycobacterium spp.

Fe Sources Potentially Available To Pathogens



Ann Rev Microbiol. 54: 881-941, 2000

Iron Uptake and Trafficking in *M. tuberculosis*-infected Macrophages



Mycobacterial Iron Acquisition

Siderophores (low MW Fe chelators)

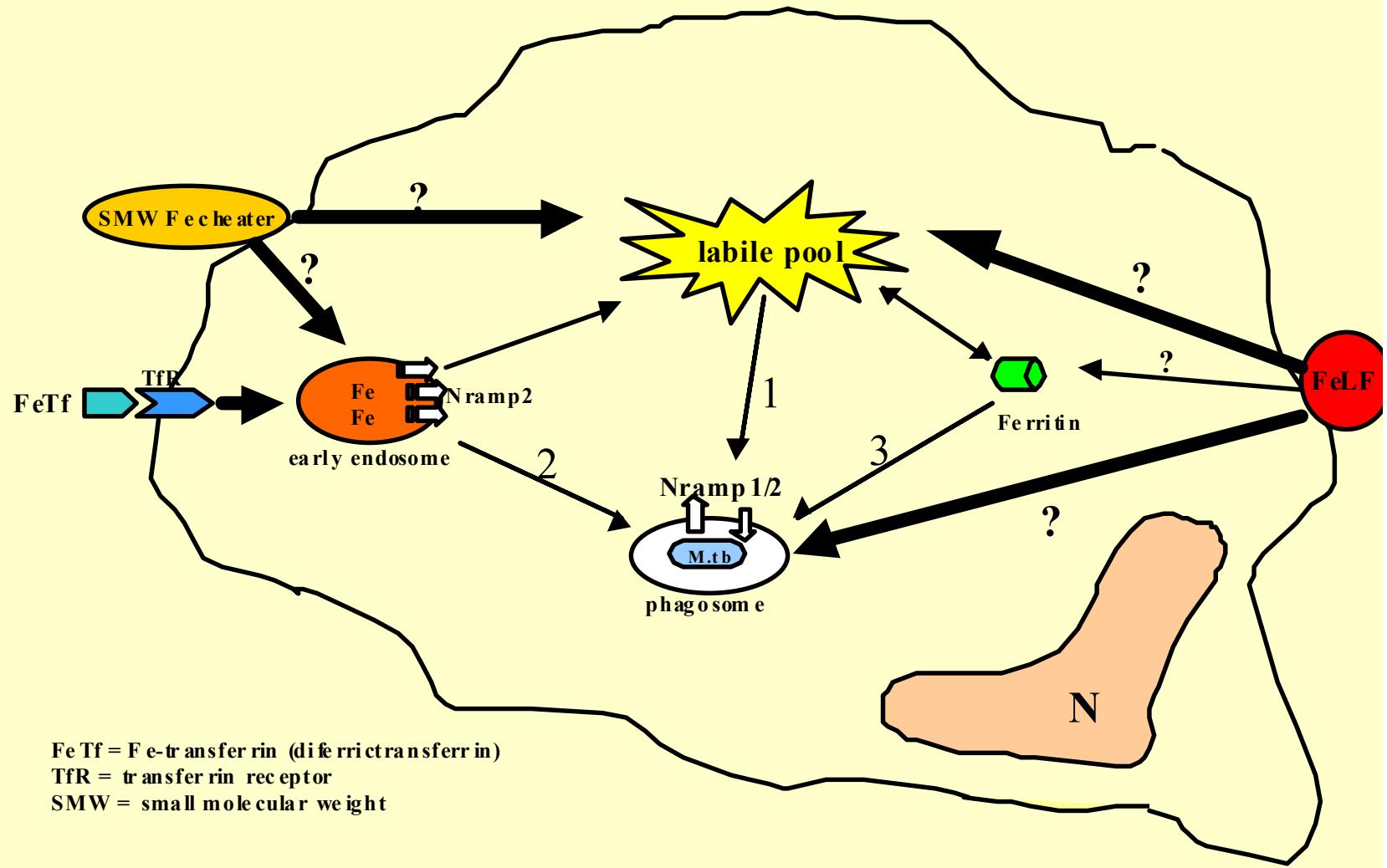
Mycobactins-hydrophobic siderophores associated with the bacterial membrane

Exochelins-water soluble, secreted siderophores

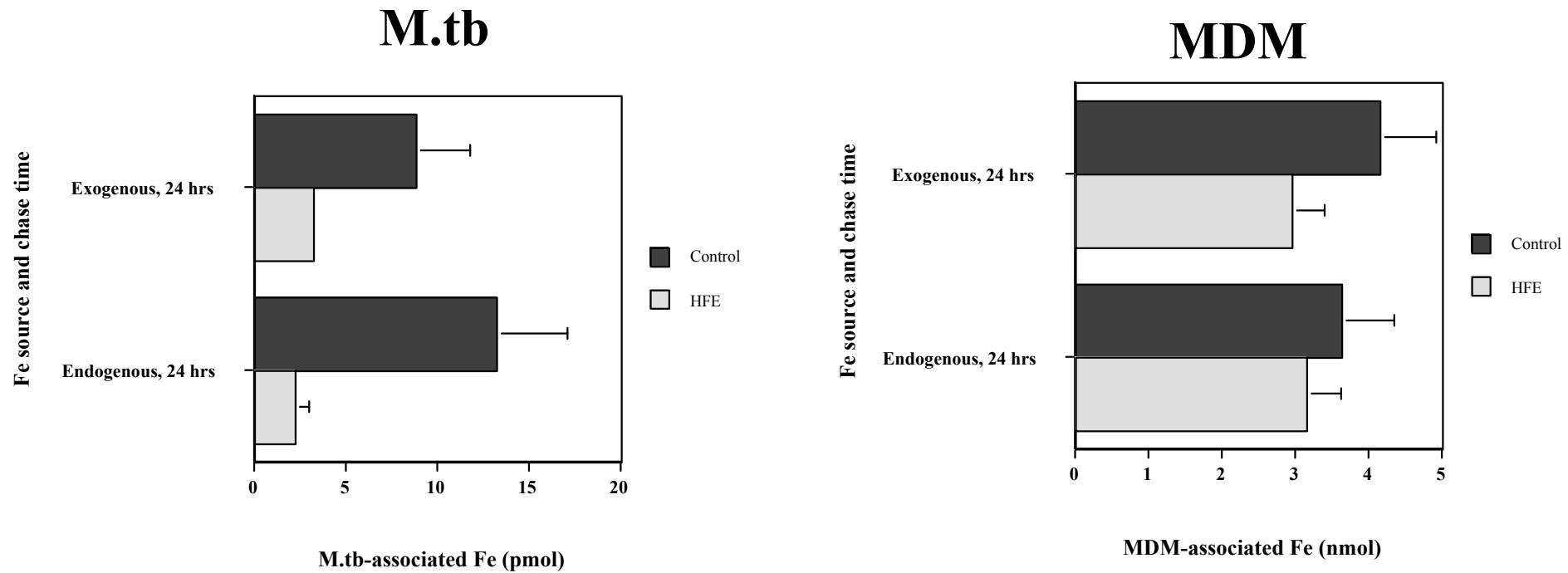
Peptidohydroxamate type (*M. smegmatis*)

Carboxymycobactin type (*M.tb*, MAC)

Iron Uptake and Trafficking in *M. tuberculosis*-infected Macrophages



M.tb Fe Uptake Decreases in MDM From Patients With Hereditary Hemochromatosis



Bacterial Iron Storage

- **Bacterioferritin**
- **Bacterial Ferritin**
- **Labile Iron Pool**
- **Mechanisms poorly defined**
- **Aconitase as a source of increased redox active iron**

THE END



Reviews and Selected Original Articles

- 1. Abraham, N. G., G. S. Drummond, J. D. Lutton, and A. Kappas. 1996. The biological significance and physiological role of heme oxygenase. *Cell.Physiol.Biochem.* 6:129-168.**
- 2. Aisen, P., C. Enns, and M. Wessling-Resnick. 2001. Chemistry and biology of eukaryotic iron metabolism. *Int.J.Biochem.Cell Biol.* 33:940-959.**
- 3. Anderson, B. F., H. M. Baker, G. E. Norris, S. V. Rumball, and E. N. Baker. 1990. Apolactoferrin structure demonstrates ligand-induced conformational changes in transferrins. *Nature* 344:784-787.**
- 4. Becker, E. and D. R. Richardson . 2001. Frataxin: its role in iron metabolism and the pathogenesis of Friedreich's ataxia. *Int.J.Biochem.Cell Biol.* 33:1-10.**
- 5. Cairo, G. and A. Pietrangelo. 2000. Iron regulatory proteins in pathobiology. *Biochem.J.* 352:241-250.**
- 6. Cavadini, P., H. A. O'Neill, O. Benada, and G. Isaya. 2002. Assembly and iron-binding properties of human frataxin, the protein deficient in Friedreich ataxia. *Hum.Mol.Genet.* 11:217-227.**
- 7. Eide, D. J. 1998. The molecular biology of metal ion transport in *Saccharomyces cerevisiae*. *Annu.Rev.Nutr.* 18:441-469.**
- 8. Eisenstein, R. S. 2000. Iron regulatory proteins and the molecular control of mammalian iron metabolism. *Annu.Rev.Nutr.* 20:627-662.**
- 9. Fleming, R. E. and W. S. Sly. 2002. Mechanisms of iron accumulation in hereditary hemochromatosis. *Annu.Rev.Physiol.* 64:663-680.**
- 10. Frazer, D. M., C. D. Vulpe, A. T. McKie, S. J. Wilkins, D. Trinder, G. J. Cleghorn, and G. J. Anderson. 2001. Cloning and gastrointestinal expression of rat hephaestin: relationship to other iron transport proteins. *Am.J.Physiol.Gastrointest.Liver Physiol.* 281:G931-G939.**

Reviews and Selected Original Articles

11. Goldenberg, H. A. 1997. Regulation of mammalian iron metabolism: current state and need for further knowledge. *Crit.Rev.Clin.Lab.Sci.* 34:529-572.
12. Griffiths, W. and T. Cox. 2000. Haemochromatosis: novel gene discovery and the molecular pathophysiology of iron metabolism. *Hum.Mol.Genet.* 9:2377-2382.
13. Hanson, E. S. and E. A. Leibold. 1999. Regulation of the iron regulatory proteins by reactive nitrogen and oxygen species. *Gene Expr.* 7:367-376.
14. Hantke, K. 2001. Iron and metal regulation in bacteria. *Current Opinion in Microbiology* 4:172-177.
15. Otto, B. R., A. M. J. J. Verweij-van Vught, and D. M. MacLaren. 1992. Transferrins and heme-compounds as iron sources for pathogenic bacteria. *Crit.Rev.Microbiol.* 18:217-233.
16. Pietrangelo, A. 2002. Physiology of iron transport and the hemochromatosis gene. *Am.J.Physiol.Gastrointest.Liver Physiol.* 282:G403-G414.
17. Ponka, P. 1999. Cellular iron metabolism. *Kidney Int.* 55:S2-S11.
18. Ponka, P., C. Beaumont, and D. R. Richardson. 1998. Function and regulation of transferrin and ferritin. *Semin.Hematol.* 35:35-54.
19. Ponka, P. and C. N. Lok. 1999. The transferrin receptor: role in health and disease. *Int.J.Biochem.Cell Biol.* 31:1111-1137.
20. Poss, K. D. and S. Tonegawa. 1997. Heme oxygenase 1 is required for mammalian iron reutilization. *Proc.Natl.Acad.Sci.USA* 94:10919-10924.
21. Radisky, D. and J. Kaplan. 1999. Regulation of transition metal transport across the yeast plasma membrane. *J.Biol.Chem.* 274:4481-4484.
22. Ratledge, C. and L. G. Dover. 2000. Iron metabolism in pathogenic bacteria. *Annu.Rev.Microbiol.* 54:881-941.

Reviews and Selected Original Articles

23. Rolfs, A., H. L. Bonkovsky, J. G. Kohlroser, K. McNeal, A. Sharma, U. V. Berger, and M. A. Hediger. 2002. Intestinal expression of genes involved in iron absorption in humans. *Am J.Physiol Gastrointest Liver Physiol* 282:G598-G607.
24. Simovich, M. J., M. E. Conrad, J. N. Umbreit, E. G. Moore, L. N. Hainsworth, and H. K. Smith. 2002. Cellular location of proteins related to iron absorption and transport. *Am.J.Hematol.* 69:164-170.
25. Snyder, A. H., M. E. McPherson, J. F. Hunt, M. Johnson, J. S. Stamler, and B. Gaston. 2002. Acute effects of aerosolized S-nitrosoglutathione in cystic fibrosis. *Am.J.Respir.Crit.Care Med.* 165:922-926.
26. Thomson, A. M., J. T. Rogers, and P. J. Leedman. 1999. Iron-regulatory proteins, iron-responsive elements and ferritin mRNA translation. *The International Journal of Biochemistry & Cell Biology* 31:1139-1152.
27. Touati, D. 2000. Iron and oxidative stress in bacteria. *Arch.Biochem.Biophys.* 373:1-6.
28. Waheed, A., J. H. Grubb, X. Y. Zhou, S. Tomatsu, R. E. Fleming, M. E. Costaldi, R. S. Britton, B. R. Bacon, and W. S. Sly. 2002. Regulation of transferrin-mediated iron uptake by HFE, the protein defective in hereditary hemochromatosis. *PNAS* 99 :3117-3122.
29. Walker, B. L., J. W. C. Tiong, and W. A. Jefferies. 2001. Iron metabolism in mammalian cells. *Int.Rev.Cytol.* 211:241-278.
30. Wandersman, C. and I. Stojiljkovic. 2000. Bacterial heme sources: the role of heme, hemoprotein receptors and hemophores. *Current Opinion in Microbiology* 3:215-220.
31. Welch, K. D., T. Z. Davis, M. E. Van Eden, and S. D. Aust. 2002. deleterious iron-mediated oxidation of biomolecules. *Free Rad.Biol.Med.* 32:577-583.