A Thief's Toolbox: Bacterial Strategies to Acquire Iron From The Human Host

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Iron acquisition is a critical determinant in the success of bacterial infection. Bacteria are faced with a low availability of iron in the host, as it is sequestered by host proteins. In order to combat this reality, bacteria have devised multiple mechanisms to exploit iron sources by producing haemophores and siderophores themselves, or stealing them from other bacteria. A variety of different types of haemophores and siderophores are produced by both Gram-positive and Gram-negative pathogenic bacteria. The regulation of these iron uptake systems is crucial in order to maintain iron homeostasis. Knowledge of these systems can aid in the development of new therapeutic strategies such as conjugating antibiotics to the siderophores, enabling direct insertion of antibiotics into bacteria.

Introduction

Iron is an essential element for all organisms, including pathogenic bacteria. Iron can be found in two different positively charged ionic forms, Fe^{2+} and Fe^{3+} , useful in biological processes such as, oxidative phosphorylation, and DNA replication and repair. In the human host, free Fe^{3+} can react with oxygen via the Fenton and Haber-Weiss reaction, to generate reactive oxygen species that damages DNA, lipid and membrane haem proteins. To protect against the potential toxicity of Fe^{3+}, iron is sequestered by proteins such as, lactoferrin, transferrin, and ferritins. Iron may also be stored as haem, incorporated into a protoporphyrin ring in haemoproteins(Hammer and Skaar, 2011). This also reduces the availability of free iron for use by pathogenic bacteria, known as nutritional immunity. To combat this nutritional immunity, pathogenic bacteria have evolved many ways to extract

iron that is complexed to a variety of proteins. In order to acquire haem, bacteria secrete proteins known as haemophores that bind to haemoproteins to acquire haem. In order to obtain iron sequestered by lactoferrin or transferrin, bacteria can use secreted molecules known as siderophores (Caza and Kronstad, 2013). Haemophores are proteins while siderophores are not. Most haem iron uptake systems are negatively regulated by the ferric uptake regulator, Fur (Figure 1A)(Carpenter *et al*., 2009). However, this regulatory function is carried out by diphtheria toxin repressor, DtxR, in *Corynebacterium diphtheria*. *Streptococcus pyogenes* iron regulation is mediated by the metal transporter of streptococci regulator, MtsR, a DtxR divergent homolog. Analysis shows that despite their low sequence homology, MtsR, DtxR and Fur, share structural and functional similarity as transcriptional repressors of iron-responsive genes (Bates *et al*., 2005, Sheldon and Heinrichs, 2015). Some bacteria regulate iron acquisition using extracytoplasmic function (ECF) sigma factors (Figure 1B). This mechanism is employed by the has operon in *Serratia marcescens* (*S. marcescens*).

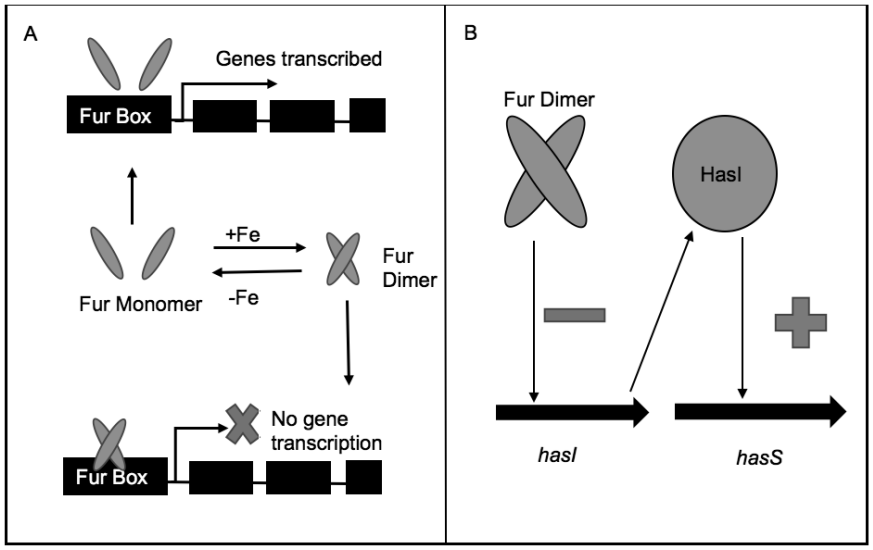

Figure 1. A) *The transcriptional repression of iron acquisition genes upon the binding of the Fur dimer to the Fur box, when iron levels are sufficient. Upon sensing of iron limiting conditions, Fur binding is diminished, resulting in the transcription of iron acquisition genes. Adapted from Nobles & Maresso (Nobles and Maresso, 2011).* B) *the regulation of iron acquisition genes by using ECF sigma factors. Fur negatively represses sigma factor gene, hasI, which results in the expression of the hasS gene which represses the hasR promoter involved in iron acquisition in S. marcescens. Adapted from Cescau et al. (Cescau et al., 2007).*

Haem-Iron acquisition strategies in bacteria

Haem accounts for 80% of the total iron available in the human host, and is found associated within haemoglobin, primarily in erythrocytes (Skaar *et al*., 2004). Many gram-positive pathogenic bacteria lyse these erythrocytes by releasing haemolysins. Upon lysis, free haem and hemoglobin are sequestered by host proteins, haptoglobin or haemopexin. Bacteria produce haemophores in order to gain access to this sequestered iron (Ma *et al*., 2015).

The NEAT-type haemophores

Haem is acquired through a relay system of proteins, known as iron-regulated surface-determinant (Isd) proteins in *Staphylococcus aureus*. The sortase B operon was found to be important for *S. aureus* pathogenesis, particularly iron acquisition. This operon, subsequently named Isd locus, encodes three of the surface-bound Isd proteins, IsdA, IsdB, and IsdC which are anchored to the cell wall by either Sortase A or Sortase B (Nobles and Maresso, 2011). This led to the proposed relay protein system, that IsdA, IsdB and IsdH are hemoprotein receptors on the cell surface that pass haem to IsdC or IsdE (Mazmanian et al., 2003). Haem is then transported through the cell wall via, ABC transport system IsdDEF or HtsBC, using energy from an ATPase, IdsF. Once inside the cytoplasm, haem oxygenases, IsdG and IsdI, break down the porphyrin ring, releasing iron for use by the bacterium (Figure 2A). The surface-bound Isd proteins, IsdB and IsdH, contain one or more near iron transporter (NEAT) motifs, which binds haem from either haemoglobin or haptoglobin. IsdH contains three NEAT domains and IsdB contains two domains, while IsdA and IsdC contain one each. IsdH (NEAT1 and NEAT2) and IsdB (NEAT1) can bind haemoglobin and haptoglobin but not haem. The haem that is stripped from the haemoglobin during the Isd protein relay, is captured by the IsdH (NEAT3) and IsdB (NEAT2) and then passed IsdA and IsdC, then passed to the ABC transporter IsdDEF (Sheldon and Heinrichs, 2015). Recent research using QM/MM and MD stimulations lead to the proposal of a reaction scheme for haem transfer between NEAT domains. The results from these experiments indicated that deprotonation of two tyrosine residues, Tyr166 and Tyr170 in IsdA , were crucial in the transfer of haem from IsdH by IsdA, to IsdC (Moriwaki *et al*., 2015).

Bacillus anthracis was found to contain homologs of the *S. aureus* Isd system, IsdC and SrtB while possessing some distinct features, such as IsdX1 and IsdX2 (Maresso *et al*., 2006, Maresso *et al*., 2008). IsdX1 and IsdX2 are secreted proteins containing NEAT domains. These were the first haemophores identified in Gram-positive bacteria. These were found to acquire haem from hemoglobin and transfer it on to IsdC. Interestingly, IsdX1 can transfer haem directly to IsdC or, pass haem to IsdX2, which then passes it to IsdC. It may seem like IsdX2 is functionally redundant, but it has been suggested that IsdX2 can act as a 'haem sponge' to control the rate of iron uptake (Honsa *et al*., 2011). The common property

of iron uptake systems in most Gram-positive bacteria, is the presence of NEAT domains. These 128 kDa NEAT domains show functional diversity, although their structural similarities are high (Honsa *et al*., 2014). Analysis of the NEAT1 (binds hemoglobin/haptoglobin) and NEAT2 (binds only haem) domains of IsdB in *S. aureus*, show 41% sequence homology. This is also the case for IsdH NEAT domains, where haem binding NEAT3 shows 38%-41% sequence homology to the first two domains (Dickson *et al*., 2014).

HasA-type haemophores

Haem acquisition system, HasA is a haemophore first identified in *Serratia marcescens* (*S. marcescens*), known as HasASM. Homologues of this system have only been identified in other Gram-negative bacteria such as, *Pseudomonas Aeruginosa* (*P. aeruginosa*), Pseudomonas fluorescens, Yersinia pestis, and Yersinia enterocolitica (Rossi *et al*., 2001, Ochsner *et al*., 2000). The HasA system allows bacteria to extract haem from haemoglobin, haemopexin and myoglobin. The crystal structure of holo-HasA has been determined, showing it to be a globular protein with two faces, one with four α-helices, and the other with seven β-strands. Between the two faces, there are two loops containing two iron axial ligands, His-32 (Loop1) and Tyr-75 (Loop 2), where haem is tightly bound (Arnoux *et al*., 1999). The Tyr-75 bond is stabilized by hydrogen bonds formed with His-83. Multiple sequence alignments of HasA from the bacteria mentioned above showed 30-50% homology, while His-32 and Tyr-75 are conserved among all species (Tong and Guo, 2009). Kinetic and biochemical studies on haem binding show that haem initially binds to Tyr-75, triggering Loop 1 to close over it, thus facilitating His-32 binding. Mutational analysis of these binding sites demonstrated that HasASM retains the ability to bind haem when one of the iron ligands is replaced by alanine, it is thought that water may help compensate the axial coordination (Caillet-Saguy *et al*., 2012). It was also suggested that His-83 may act as substitute iron ligand in the absence of Tyr-75, although this needs more examination (Kumar *et al*., 2014).

HasA then binds with high affinity to its receptor, HasR. Both holo-HasA and apo-HasA can bind to the receptor. The HasR receptor is composed of a transmembrane β-barrel, 22 antiparallel β-strands and an N-terminal plug that closes the pore of the β-barrel. Upon receptor binding, only haem is internalized while the HasA remains outside (Figure 2B) (Caillet-Saguy *et al*., 2009). Biochemical studies of this interaction revealed that haem transfer from HasA to HasR is Ton-B independent, and is actually caused by protein-protein interactions alone (Izadi-Pruneyre *et al*., 2006). It is thought that receptor binding disrupts the hydrogen bond between Tyr-75 and His-83 thus, weakening the iron binding sites affinity for haem in HasA. There are two conserved haemophore binding regions, β-strands 51-60 and 95-105. Interaction of these sites with loops 6, 8 and 9 of HasR has been indicated to cause a decrease in HasA affinity for haem, lower than HasR-haem affinity, resulting in the transfer of haem from HasA to HasR (Smith *et al*., 2015, Caillet-Saguy *et al*., 2012).

The haem that is released into the periplasm is bound by periplasmic binding haem proteins and transported across an ABC transporter into the cytoplasm, where it is degraded to biliverdin (Marvig *et al.,* 2014).

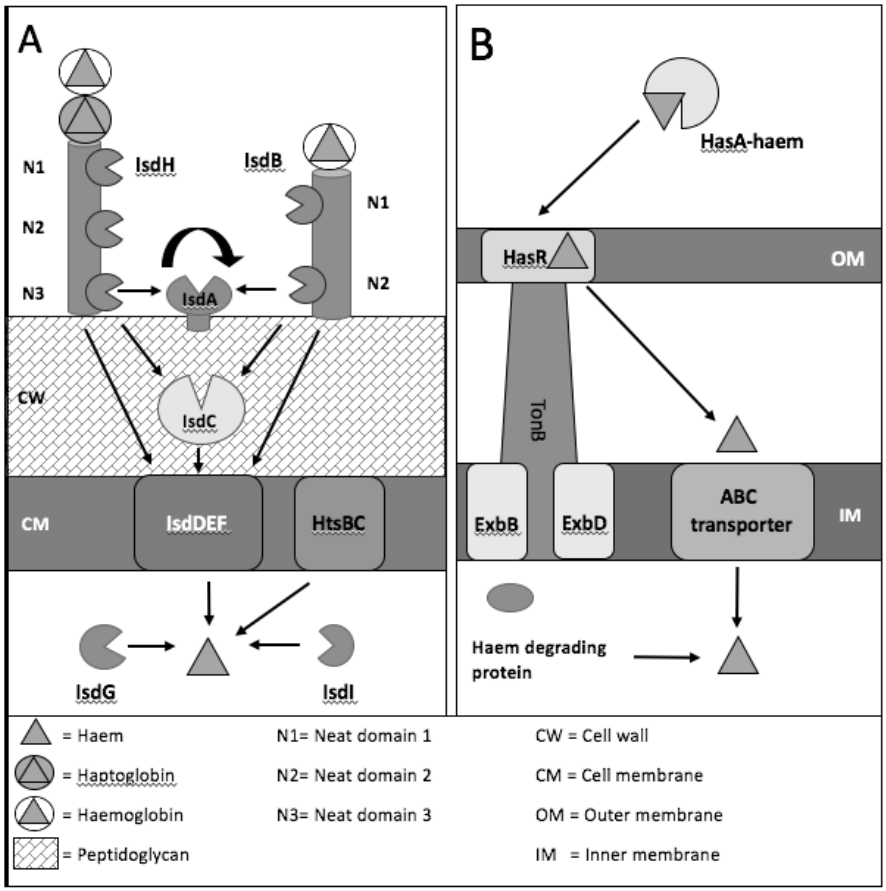

Figure 2. A) *The transport of haem along the NEAT domains (N1-N3) from IsdH, IsdB and IsdA, to IsdC or directly to IsdDEF. The black arrows represent direction of haem transport between the Isd proteins. Haem is transported across the membrane through IsdDEF or HtsBC transporters. The haem is then degraded by enzymes IsdG and IsdI. Adapted from Hammer & Skaar (Hammer and Skaar, 2011).* B) *The HasA-haem complex binding to the receptor HasR. The haem passes though the outer membrane with energy generated by the TonB analogue, HasB with proteins ExbB and ExbD. Haem transport into the cytoplasm occurs via an ABC transporter. Adapted from Contreras et al. (Contreras et al., 2014).*

Iron acquisition from transferrin, lactoferrin and ferritin

Iron complexed to lactoferrin, transferrin or ferritin can be exploited using Siderophores. Siderophores are small, secreted, high-affinity iron chelating molecules and unlike haemophores, are not proteins. Many bacterial pathogens use siderophores, but only one system from both Gram-positive and Gram-negative bacteria will be discussed in this review.

Siderophores in Gram-positive bacteria

S. aureus produces two siderophores, staphyloferrin A and staphyloferrin B, which are part of the carboxylate family of siderophores. The genes for expression of both siderophores are regulated by Fur, as previously discussed above (Hammer and Skaar, 2011).

Staphyloferrin A is encoded by the sfnABCD operon. The structure of staphyloferrin A has been determined to be a D-ornithine molecule that links two molecules of citrate through amide bonds. HtsABC is the ABC transporter involved in transporting Staphyloferrin A into the cytoplasm, as genes encoding it are found adjacent to the sfn operon (Beasley *et al*., 2009). Analysis of the crystal structure of the siderophore receptor HtsA, demonstrates that it undergoes a conformational change upon staphyloferrin A binding, trapping it. Transport across the membrane requires energy, however the snf operon does not encode an ATPase. The ferric hydroxymate uptake operon (fhuCBG) was found to encode an ATPase. FhuC was shown to be the ATPase required for both staphyloferrin A and staphyloferrin B transport, as transport was inhibited when fhuCBG operon encoding fhuC, was knocked out (Dale *et al*., 2004).

Staphyloferrin B is composed of L-2,3diaminopropionic acid, 1,2-diaminoethane and α-ketoglutaric acid. Staphyloferrin B is encoded by the sbnABCDEFGHI. The staphylococcal iron regulated transporter (sirABC) is thought to transport staphyloferrin B across the membrane in a similar manner to HtsABC with staphyloferrin A (Figure 3A). SirA, the receptor undergoes conformational change upon staphyloferrin B binding, trapping it just as HtsA does with staphyloferrin A. As with HtsABC, FhuC is the ATPase required for staphyloferrin B transport across the membrane (Madsen *et al*., 2015).

S. aureus also possesses the ability to steal siderophores produced by other bacteria, by producing xenosiderophores. These xenosiderophores are transported across the membrane using the FhuCBG system, which provides its ATPase for both staphyloferrin A and staphyloferrin B transport. FhuD1 and FhuD2 are the lipoprotein receptors and only undergo a minimal conformational change upon xenosiderophore binding, unlike HtsA and SirA. This is thought to enable broad-spectrum binding to many different types of xenosiderophores such as, erobactin, ferrichrome, ferrioxamine B and coprogen. The use of the Fhu ATPase is critical in *S. aureus* iron acquisition, making it a potential target for future therapeutic treatments.

Siderophores in Gram-negative bacteria

Similar to siderophore transport in gram-positive bacteria, ABC transporters are also required for siderophore transport in gram-negative bacteria, such as *P. aeruginosa*. *P. aeruginosa* is unable to take up iron from transferrin directly, to combat this it produces two siderophores, pyoverdine and pyochelin (Cezard *et al*., 2015). Pyoverdine has been well studied due to its fluorescent nature, however this fluorescence is diminished upon iron binding. Pyoverdine consists of a chromophore with a peptide chain bound to it. The length and composition of the peptide chain are different for each strain. *P. aeruginosa* can produce one of three structurally different types of pyoverdines. The pyoverdine-iron complex binds to the TonB-dependent membrane receptor, FpvA (Schalk, 2008). As previously discussed, this binding driven by cytoplasmic membrane potential results in a conformational change in the plug of the receptor, allowing the siderophore-iron complex passage into the periplasm. The iron is then taken by a periplasmic binding protein to the cytoplasm, via an ABC transporter. Pyoverdine is then recycled back out of the cell by an ABC efflux transporter, PvdRT-OmpQ (Yeterian *et al*., 2010). This efflux system has also been shown to be involved in the export of newly synthesized pyoverdine out of the cell, this had been disputed in the past (Imperi *et al*., 2009). Ferribactin, a pyoverdine precursor is exported into the periplasm by PvdE, where it undergoes maturation aided by PvdN, PvdO and PvdP. The newly synthesized pyoverdine is then exported out of the cell by PvdRT-OmpQ (Figure 3B).

Unlike Pyoverdine, Pyochelin does not undergo any periplasmic maturation. It is thought that Pyochelin is directly exported out of the cytoplasm through an ABC transporter composed of PchH and PchI, however little is known about the mechanisms involved. Pyochelin has a reasonably low affinity for iron and has higher affinities for both copper and zinc. However, studies indicate that pyochelin transport of copper and zinc is not as efficient as iron transport (Brandel *et al*., 2012). There is increasing evidence that both pyoverdine and pyochelin biosynthesis occur in siderosomes, in order to compartmentalize the siderophore precursors. This may be a protective mechanism for bacteria against the potentially toxic build-up of harmful molecules, such as siderophores or their precursors (Gasser *et al*., 2015). Recently, a novel iron acquisition system has been reported that is essential for the growth of *P. aeruginosa* in the airway of CF patients. *P. aeruginosa* strains defective in pyoverdine and pyochelin have been detected, suggesting an alternate iron acquisition strategy. Deletion experiments indicated that a genetic element encoding PA4834 gene was responsible for the iron acquisition of *P. aeruginosa* in airway mucosa cells. The mechanism of action of this novel iron acquisition system has yet to be determined, but suppression of this PA4834 gene may be a novel therapeutic approach against *P. aeruginosa* infection (Gi *et al*., 2015).

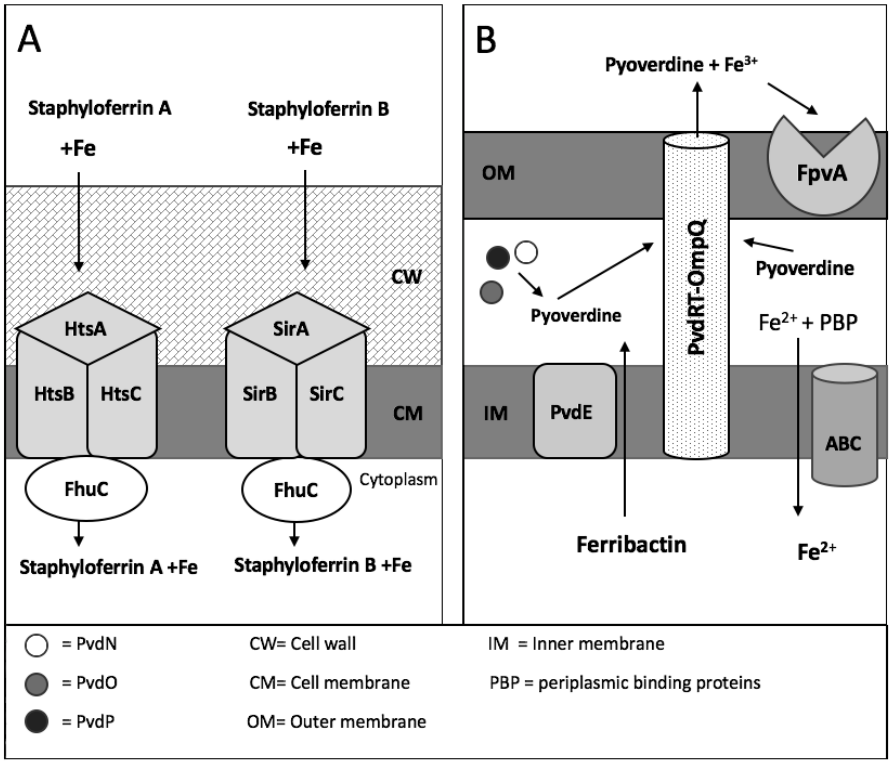

Figure 3. A) *The transport of haem complexed to siderophores, Staphyloferrin A and Staphyloferrin B produced by S. aureus. Staphyloferrin A transports iron into the cytoplasm via the HtsABC transporter, powered by ATPase, FhuC. Staphyloferrin B transports iron into the cytoplasm, via the SirABC transporter, also powered by the ATPase, FhuC. Adapted from Hammer and Skaar (2011).* B) *The generation of pyoverdine, one of the siderophores produced by P. aeruginosa. Ferribactin, a pyoverdine precursor is exported into the periplasm by PvdE, where it matures aided by proteins PvdN, PvdO and PvdP. The mature pyoverdine is then exported out of the cell by PvdRT-OmpQ. Pyoverdine extracts haem from host haemoproteins, then enters the periplasm through the receptor, FpvA. The pyoverdine molecule is recycled back out of the cell, via the PvdRT-OmpQ. The haem is transported into the cytoplasm, through an ABC transporter, aided by Periplasmic binding proteins. Adapted from Schalk (2008).*

Trojan horse strategy and medical applications

The rapid rate of antibiotic resistance is posing a great difficulty in the treatment of bacterial infections. New therapies are being developed in order to counteract this growing resistance such as, siderophore-drug complexes (SDC) known as the Trojan horse strategy. It is a way of increasing the efficacy of antibiotics by transporting it directly into the bacterium via their iron uptake systems (Page, 2012). The SDC are typically composed of three parts; siderophore, a linker and a drug. Upon uptake of the SDC, the linker region is cleaved, releasing the antibiotic, which is then functional. In the case of gram-negative bacteria, the antibiotic is transported through an ABC transporter into the cytoplasm. Naturally occurring siderophores covalently linked to an antibiotic moiety, known as sideromycins, has led to increasing interest in synthetic SDC derivatives.

The potential use of SDC against *P. aeruginosa* infections is of interest, as the bacterium is resistant to many antibiotics due to its chromosome-encoded antibiotic resistant genes. Recent studies have found that use of triscatecholate siderophores conjugated with either ampicillin, or amoxicillin, can inhibit the growth of gram-negative bacteria, such as *P. aeruginosa* (Ji *et al.*, 2012). As *P. aeruginosa* produces one of three different types of its pyoverdine siderophore, a pyoverdine-antibiotic conjugate would not be effective against all strains of *P. aeruginosa*. A pyochelin-antibiotic conjugate may be a more promising treatment, active against all strains of *P. aeruginosa* (Mislin and Schalk, 2014). Although recent research suggests that resistance to potential SDC against *P. aeruginosa* may still develop (Kim *et al.*, 2015). Research into the use of SDC against gram-positive bacteria, such as *S. aureus*, have shown some antimicrobial activity. A staphyloferrin A-fluoroquinolone conjugate has been designed to potentially treat staphylococcal skin infections (Milner *et al.*, 2013).

Conclusions

It is clear to see that iron is a critical element for bacterial survival, thus the vast amount of different iron acquisition systems that they possess in order to obtain it. While many of these systems have been determined, many are yet to be elucidated. Some systems are homologous across a wide range of bacteria, such as the presence of a TonB-ExbB-ExbD complex that provides the energy for transport of haem across the outer membrane, present in many Gram-negative bacterial iron acquisition systems. It is these common elements that provide the most hope as broad targets for future therapies against pathogenic bacteria. The Trojan horse strategy using siderophore-drug complexes, is already being used to treat certain iron related conditions, however there is increasing interest in their use against multidrug resistant bacterial infections, particularly caused by *S. aureus* and *P. aeruginosa*. Although some synthetic SDC have shown some antimicrobial efficacy, in order for these to be fully effective, the many iron acquisition pathways of bacterial pathogens need to be fully elucidated.

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References

ARNOUX, P., HASER, R., IZADI, N., LECROISEY, A., DELEPIERRE, M., WANDERSMAN, C. & CZJZEK, M. (1999). The crystal structure of HasA, a hemophore secreted by Serratia marcescens. *Nat Struct Biol*, 6, 516-20.

BATES, C. S., TOUKOKI, C., NEELY, M. N. & EICHENBAUM, Z. (2005). Characterization of MtsR, a new metal regulator in group A streptococcus, involved in iron acquisition and virulence. *Infect Immun*, 73, 5743-53.

BEASLEY, F. C., VINES, E. D., GRIGG, J. C., ZHENG, Q., LIU, S., LAJOIE, G. A., MURPHY, M. E. & HEINRICHS, D. E. (2009). Characterization of staphyloferrin A biosynthetic and transport mutants in Staphylococcus aureus. *Mol Microbiol*, 72, 947-63.

BRANDEL, J., HUMBERT, N., ELHABIRI, M., SCHALK, I. J., MISLIN, G. L. A. & ALBRECHT-GARY, A.-M. (2012). Pyochelin, a siderophore of Pseudomonas aeruginosa Physicochemical characterization of the iron(III), copper(II) and zinc(II) complexes. *Dalton Transactions*, 41, 2820-2834.

CAILLET-SAGUY, C., PICCIOLI, M., TURANO, P., IZADI-PRUNEYRE, N., DELEPIERRE, M., BERTINI, I. & LECROISEY, A. (2009). Mapping the interaction between the hemophore HasA and its outer membrane receptor HasR using CRINEPT-TROSY NMR spectroscopy. *J Am Chem Soc*, 131, 1736-44.

CAILLET-SAGUY, C., PICCIOLI, M., TURANO, P., LUKAT-RODGERS, G., WOLFF, N., RODGERS, K. R., IZADI-PRUNEYRE, N., DELEPIERRE, M. & LECROISEY, A. (2012). Role of the Iron Axial Ligands of Heme Carrier HasA in Heme Uptake and Release. *Journal of Biological Chemistry*, 287, 26932-26943.

CARPENTER, B. M., WHITMIRE, J. M. & MERRELL, D. S. (2009). This is not your mother's repressor: the complex role of fur in pathogenesis. *Infect Immun*, 77, 2590-601.

CAZA, M. & KRONSTAD, J. W. (2013). Shared and distinct mechanisms of iron acquisition by bacterial and fungal pathogens of humans. Frontiers in *Cellular and Infection Microbiology*, 3, 80.

CESCAU, S., CWERMAN, H., LETOFFE, S., DELEPELAIRE, P., WANDERSMAN, C. & BIVILLE, F. (2007). Heme acquisition by hemophores. *Biometals*, 20, 603-13.

CEZARD, C., FARVACQUES, N. & SONNET, P. (2015). Chemistry and biology of pyoverdines, Pseudomonas primary siderophores. *Curr Med Chem*, 22, 165-86.

CONTRERAS, H., CHIM, N., CREDALI, A. & GOULDING, C. W. (2014). Heme uptake in bacterial pathogens. *Current Opinion in Chemical Biology*, 19, 34-41.

DALE, S. E., SEBULSKY, M. T. & HEINRICHS, D. E. (2004). Involvement of SirABC in iron-siderophore import in Staphylococcus aureus. *J Bacteriol*, 186, 8356-62.

DICKSON, C. F., KUMAR, K. K., JACQUES, D. A., MALMIRCHEGINI, G. R., SPIRIG, T., MACKAY, J. P., CLUBB, R. T., GUSS, J. M. & GELL, D. A. (2014). Structure of the hemoglobin-IsdH complex reveals the molecular basis of iron capture by Staphylococcus aureus. *J Biol Chem*, 289, 6728-38.

GASSER, V., GUILLON, L., CUNRATH, O. & SCHALK, I. J. (2015). Cellular organization of siderophore biosynthesis in Pseudomonas aeruginosa: Evidence for siderosomes. *J Inorg Biochem*, 148, 27-34.

GI, M., LEE, K. M., KIM, S. C., YOON, J. H., YOON, S. S. & CHOI, J. Y. (2015). A novel siderophore system is essential for the growth of Pseudomonas aeruginosa in airway mucus. *Sci Rep*, 5, 14644.

HAMMER, N. D. & SKAAR, E. P. (2011). Molecular Mechanisms of Staphylococcus aureus Iron Acquisition. *Annual Review of Microbiology*, Vol 65, 65, 129-147.

HONSA, E. S., FABIAN, M., CARDENAS, A. M., OLSON, J. S. & MARESSO, A. W. (2011). The five near-iron transporter (NEAT) domain anthrax hemophore, IsdX2, scavenges heme from hemoglobin and transfers heme to the surface protein IsdC. *J Biol Chem*, 286, 33652-60.

HONSA, E. S., MARESSO, A. W. & HIGHLANDER, S. K. (2014). Molecular and evolutionary analysis of NEAr-iron Transporter (NEAT) domains. PLoS One, 9, e104794.

IMPERI, F., TIBURZI, F. & VISCA, P. (2009). Molecular basis of pyoverdine siderophore recycling in Pseudomonas aeruginosa. *Proc Natl Acad Sci U S A*, 106, 20440-5.

IZADI-PRUNEYRE, N., HUCHE, F., LUKAT-RODGERS, G. S., LECROISEY, A., GILLI, R., RODGERS, K. R., WANDERSMAN, C. & DELEPELAIRE, P. (2006). The heme transfer from the soluble HasA hemophore to its membrane-bound receptor HasR is driven by protein-protein interaction from a high to a lower affinity binding site. *J Biol Chem*, 281, 25541-50.

JI, C., MILLER, P. A. & MILLER, M. J. (2012). Iron transport-mediated drug delivery: practical syntheses and in vitro antibacterial studies of tris-catecholate siderophore-aminopenicillin conjugates reveals selectively potent antipseudomonal activity. *J Am Chem Soc*, 134, 9898-901.

KIM, A., KUTSCHKE, A., EHMANN, D. E., PATEY, S. A., CRANDON, J. L., GORSETH, E., MILLER, A. A., MCLAUGHLIN, R. E., BLINN, C. M., CHEN, A., NAYAR, A. S., DANGEL, B., TSAI, A. S., ROONEY, M. T., MURPHY-BENENATO, K. E., EAKIN, A. E. & NICOLAU, D. P. (2015). Pharmacodynamic Profiling of a Siderophore-Conjugated Monocarbam in Pseudomonas aeruginosa: Assessing the Risk for Resistance and Attenuated Efficacy. *Antimicrobial agents and chemotherapy*, 59, 7743-52.

KUMAR, R., MATSUMURA, H., LOVELL, S., YAO, H., RODRIGUEZ, J. C., BATTAILE, K. P., MOENNE-LOCCOZ, P. & RIVERA, M. (2014). Replacing the axial ligand tyrosine 75 or its hydrogen bond partner histidine 83 minimally affects hemin acquisition by the hemophore HasAp from Pseudomonas aeruginosa. *Biochemistry*, 53, 2112-25.

MA, L., TERWILLIGER, A. & MARESSO, A. W. (2015). Iron and zinc exploitation during bacterial pathogenesis. *Metallomics*, 7, 1541-1554.

MADSEN, J. L. H., JOHNSTONE, T. C. & NOLAN, E. M. (2015). Chemical Synthesis of Staphyloferrin B Affords Insight into the Molecular Structure, Iron Chelation, and Biological Activity of a Polycarboxylate Siderophore Deployed by the Human Pathogen Staphylococcus aureus. *Journal of the American Chemical Society*, 137, 9117-9127.

MARESSO, A. W., CHAPA, T. J. & SCHNEEWIND, O. (2006). Surface protein IsdC and Sortase B are required for heme-iron scavenging of *Bacillus anthracis*. *J Bacteriol*, 188, 8145-52.

MARESSO, A. W., GARUFI, G. & SCHNEEWIND, O. (2008). Bacillus anthracis secretes proteins that mediate heme acquisition from hemoglobin. PLoS Pathog, 4, e1000132.

MARVIG, R. L., DAMKIAER, S., KHADEMI, S. M., MARKUSSEN, T. M., MOLIN, S. & JELSBAK, L. (2014). Within-host evolution of Pseudomonas aeruginosa reveals adaptation toward iron acquisition from hemoglobin. *MBio*, 5, e00966-14.

MAZMANIAN, S. K., SKAAR, E. P., GASPAR, A. H., HUMAYUN, M., GORNICKI, P., JELENSKA, J., JOACHMIAK, A., MISSIAKAS, D. M. & SCHNEEWIND, O. (2003). Passage of heme-iron across the envelope of Staphylococcus aureus. *Science*, 299, 906-9.

MILNER, S. J., SEVE, A., SNELLING, A. M., THOMAS, G. H., KERR, K. G., ROUTLEDGE, A. & DUHME-KLAIR, A. K. (2013). Staphyloferrin A as siderophore-component in fluoroquinolone-based Trojan horse antibiotics. *Org Biomol Chem*, 11, 3461-8.

MISLIN, G. L. & SCHALK, I. J. (2014). Siderophore-dependent iron uptake systems as gates for antibiotic Trojan horse strategies against Pseudomonas aeruginosa. *Metallomics*, 6, 408-20.

MORIWAKI, Y., TERADA, T., TSUMOTO, K. & SHIMIZU, K. (2015). Rapid Heme Transfer Reactions between NEAr Transporter Domains of Staphylococcus aureus: A Theoretical Study Using QM/MM and MD Simulations. PLoS One, 10, e0145125.

NOBLES, C. L. & MARESSO, A. W. (2011). The theft of host heme by Gram-positive pathogenic bacteria. *Metallomics*, 3, 788-96.

OCHSNER, U. A., JOHNSON, Z. & VASIL, M. L. (2000). Genetics and regulation of two distinct haem-uptake systems, phu and has, in Pseudomonas aeruginosa. *Microbiology-Uk*, 146, 185-198.

PAGE, M. G. (2012). The role of the outer membrane of Gram-negative bacteria in antibiotic resistance: Ajax' shield or Achilles' heel? *Handb Exp Pharmacol*, 67-86.

ROSSI, M. S., FETHERSTON, J. D., LETOFFE, S., CARNIEL, E., PERRY, R. D. & GHIGO, J. M. (2001). Identification and characterization of the hemophore-dependent heme acquisition system of Yersinia pestis. *Infect Immun*, 69, 6707-17.

SCHALK, I. J. (2008). Metal trafficking via siderophores in Gram-negative bacteria: specificities and characteristics of the pyoverdine pathway. *J Inorg Biochem*, 102, 1159-69.

SHELDON, J. R. & HEINRICHS, D. E. (2015). Recent developments in understanding the iron acquisition strategies of gram positive pathogens. *Fems Microbiology Reviews*, 39, 592-630.

SKAAR, E. P., HUMAYUN, M., BAE, T., DEBORD, K. L. & SCHNEEWIND, O. (2004). Iron-source preference of Staphylococcus aureus infections. *Science*, 305, 1626-1628.

SMITH, A. D., MODI, A. R., SUN, S., DAWSON, J. H. & WILKS, A. (2015). Spectroscopic Determination of Distinct Heme Ligands in Outer-Membrane Receptors PhuR and HasR of Pseudomonas aeruginosa. *Biochemistry*, 54, 2601-12.

TONG, Y. & GUO, M. (2009). Bacterial heme-transport proteins and their heme-coordination modes. *Arch Biochem Biophys*, 481, 1-15.

YETERIAN, E., MARTIN, L. W., GUILLON, L., JOURNET, L., LAMONT, I. L. & SCHALK, I. J. (2010). Synthesis of the siderophore pyoverdine in Pseudomonas aeruginosa involves a periplasmic maturation. *Amino Acids*, 38, 1447-59.