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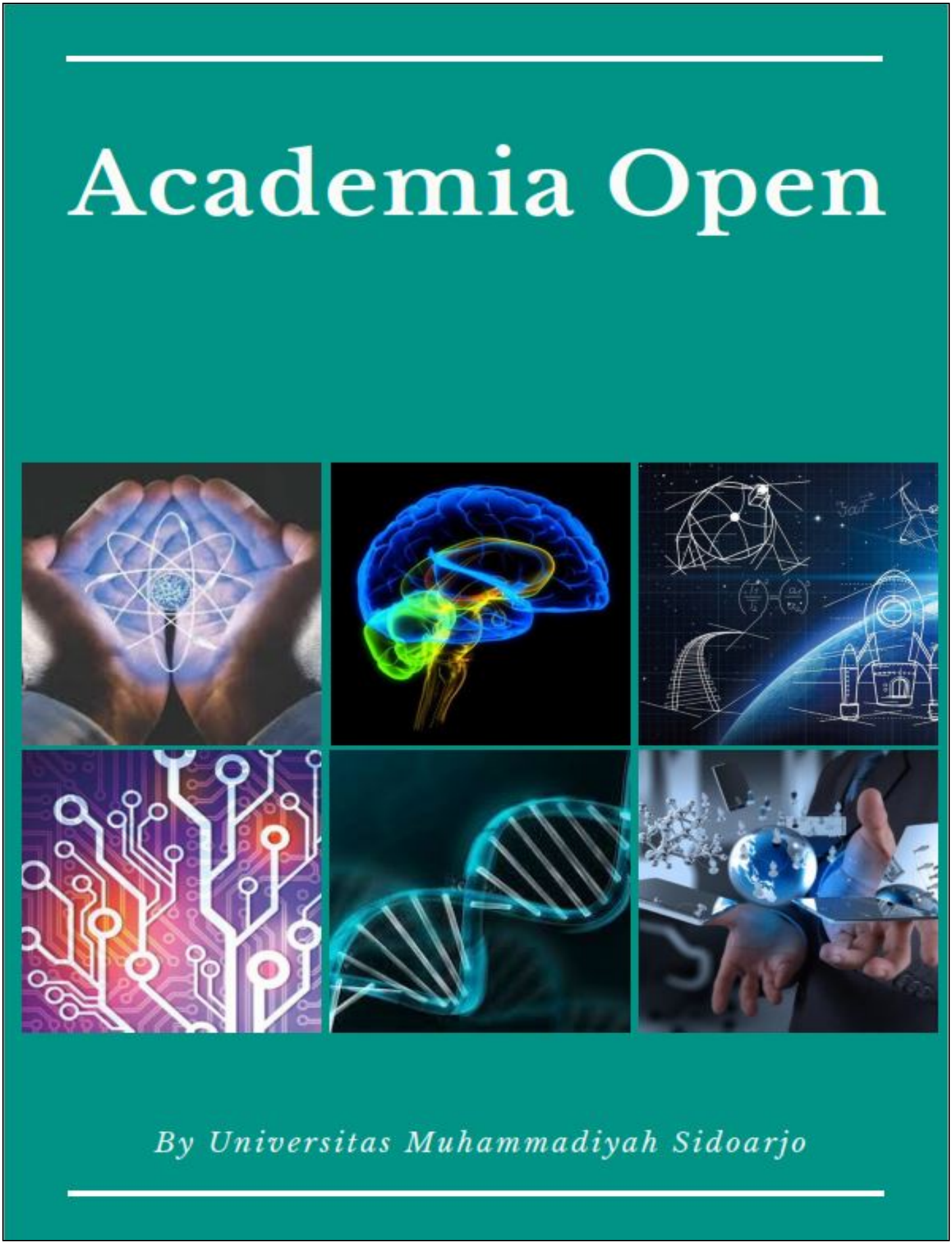

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Review Article: Role of FeoA in the Interaction with the Fe2+-Fur Complex in E. coli

Ulasan Artikel: Peran FeoA dalam Interaksi dengan Kompleks Fe2+-Bulu Kompleks dalam E. coli

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Abstract

Iron is an essential micronutrient for bacteria, functioning as a cofactor in vital processes such as electron transport, heme synthesis, and DNA replication. However, excess iron triggers the Fenton reaction, producing harmful reactive oxygen species. To manage this, *E. coli* has evolved regulatory systems to maintain iron balance despite its scarcity in the environment, with free iron concentrations as low as 10^-18 M. The iron-binding repressor protein Fur regulates genes involved in iron uptake, transfer, and storage. The FeoABC system is the primary route for Fe2+ entry, with FeoB enabling the reduction of Fe3+ to Fe2+ and transferring it to FeoA, which likely prevents wasteful iron binding to negatively charged cellular membranes. This efficient management of iron acquisition and storage not only ensures cellular survival but also provides insights into bacterial metabolic strategies for optimizing iron use while minimizing damage from reactive oxygen species.

Highlights:

1. Iron is crucial for bacterial functions like DNA replication and heme synthesis.
2. Excess iron triggers Fenton reaction, creating harmful reactive oxygen species.
3. FeoABC system regulates Fe2+ uptake and balances iron acquisition in bacteria.

Keywords: Iron homeostasis, FeoABC system, E. coli, Fur protein, reactive oxygen species

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Introduction

Iron is indispensable for several biological processes using other microorganisms. Cellular processes, including electron transfer, DNA synthesis, and respiration, are vital. Accordingly, it is not surprising that bacteria have developed a range of adaptive strategies to ensure the robustness of their cellular physiology in the face of variable ambient iron levels (Mathivanan et al.2021). At the same time, however, it is vitally important to avoid excess cellular levels of free iron, because the reactivity of this metal can result in the generation of toxic radicals. (Sadiq, 2023) Due to the pivotal importance of iron for a wide variety of roles, it is equally unsurprising that there is a very close link between iron availability and virulence in many pathogenic bacteria. It is thus the case that the modulation of iron homeostasis can confer ecological advantages to bacterial species that are free-living but can also be a powerful virulence determinant in pathogens. (Galardini et al, 2020)

E. coli FeoA is a small cytoplasmic protein whose main function is in the control of global cellular iron concentration as part of the multi-component Feo high-affinity ferrous iron uptake system. Iron homeostasis is regulated by the Fur system in E. coli. Fur is a DNA-binding molecule that represses the establishment of iron-transporting proteins by recognizing targets known as Fur boxes in their operator sites, which are typically situated in or near the relevant promoters. This section will review the known features of FeoA from E. coli with a focus on the interaction between FeoA and either the Fur regulatory complex or the metal with which FeoA must ultimately interact. Furthermore, all living systems must tightly regulate iron levels, perhaps due to the paradoxical requirement for iron in normal physiological functioning and the colossal potential for harm if iron levels spiral out of control. (Gómez Garzón, 2022; Sestok, 2022)

Result and Discussion

1.1. Overview of Iron Homeostasis in Bacteria

Iron homeostasis is essential for a variety of biological processes in bacteria. However, in contrast to the cytoplasm, which is already equipped with many iron-containing proteins and enzymes, the iron concentration in the environment is relatively low due to the limited solubility of ferric iron under aerobic conditions. (Bradley et al, 2020; Galy et al, 2024) For efficient iron utilization, living bacteria have developed complex iron homeostasis that regulates the uptake, storage, and utilization of iron. In particular, these regulatory network elements are often composed of proteins such as ferroxidases, the ferric transport protein Dps family, and/or the subfamily of ferric transporters of the ferric uptake regulator superfamily. (Cain & Smith, 2021; Kroh & Pilon, 2020) Moreover, these proteins are not unique to bacteria, and the sequences of these proteins are highly conserved, indicating that iron transport and storage mechanisms may be evolutionarily conserved across domains. Improper bacterial iron utilization often leads to reduced survival, in part because chemical isomerization of excess ferrous iron by reactive oxygen species can lead to cellular damage. Disruption of iron homeostasis can prevent bacterial differentiation and reduce virulence. (Przybyla-Toscano et al., 2021; Ray & Gaudet, 2023) Bacteria have sophisticated systems for acquiring iron from the environment, including siderophores, heme iron, and lactoferrin-specific transporters. Fur protein is the major repressor in the iron regulating system, belonging to the aquabacterial type, and is widely present in Gram-negative and Gram-positive bacteria that are involved in iron homeostasis at the post-transcriptional level, such as starvation, genetic competence, and the like. It governs a wide range of physiological processes. (Steingard & Helmann, 2023; Hou et al.2023)

1.2. The Fe2+-Fur Complex in E. coli

In natural environments, iron is typically found in insoluble form, making it an essential but scarce nutrient for most bacteria. Therefore, bacteria evolved regulatory mechanisms that control the expression of the transport systems which allow cells to recover iron from the environment (Cain & Smith, 2021). The Fe2+-Fur complex was the first regulatory system described that uses iron as a cofactor to regulate gene expression in response to iron availability. Fur is a Fe2+-dependent transcriptional repressor, which was shown in the following decades to regulate directly or indirectly dozens of genes forming a hierarchical cascade of genetic responses to iron availability. (Shimizu et al.2021)

The available evidence favours the idea that the intracellular concentration and/or cellular distribution of iron may affect the apo-activator function of Fur, leading to stronger or weaker repressing activity and a subsequent larger or smaller effect of Fur on the regulation of its direct target genes. It is likely, then, that the intracellular concentration of the entire Fur protein is regulated, but attempts to prove this point have led to conflicting results (Xia et al.2022; Sun et al., 2024). Altogether, these studies indicate that Fur has a true 'iron-uptake activities function' in enterobacteria and an additional regulon of extracytoplasmic function sigma factors. The Fur regulon overlaps to a surprisingly large extent with a series of primary and secondary regulators of its own expression and/or activity, fulfilling its regulatory functions. Therefore, considering the importance of iron availability in bacterial physiology and pathogenesis, a full comprehension of the molecular mechanisms is essential (Jaworska et al.2021; Seyoum et al., 2021).

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2. FeoA Protein: Structure and Function

The Escherichia coli FeoAB protein is one of the working lines in bacterial iron uptake mechanisms. FeoA is a small inner membrane protein consisting of 58 amino acids. It is one of the smallest proteins identified in a cell. The solution NMR structure of FeoA has shown that the protein is an ultra-stable homodimeric complex (Brown et al., 2021; Sestok, 2022). The two twisted β-sheet dimers form a β-jelly-roll topology, reminiscent of the capsid protein of a virus. The dimerization interface in the proteins studied so far is almost all hydrophobic, and a similar dimerization interface is observed in FeoA. The binary structures and actual positions of the cysteine, histidine, and aspartic acid ligands at the iron-binding centre in the active centre of the FeoA dimer are important for the functional activity of the protein. The orientation of the cysteine residues is such that it looks ideal for iron binding. (Gómez Garzón, 2022; Jaworska et al.2021)

This suggests that the metal induction ability of the protein may be related to the active centre. Understanding the binary structure and possible metal binding ability of FeoA is predicted to be an important part of iron age regulation in the expected location of Feo. The FeoA protein in E. coli contains a putative periplasmic FeoA, which facilitates the transport of essential minerals containing iron and manganese across the periplasm to the transporter. Both Fe2+-FeoB and Mn2+-FeoB have been shown to bind to FeoA, forming 1:1 stoichiometric complexes. Bacteria need a variety of iron-containing metalloproteins to adapt to changes in iron availability (Al-Aidy, 2020; Orzel et al.2023). FeoA reduces the binding affinity of the novel Fe2+-Fur and Fe2+-Fur promoters. FeoA may participate in iron homeostasis in a two-way process, on the one hand destroying the normal homeostasis of iron in E. coli bacilli and helping Bacillus adapt to an iron-limited environment where iron is competitive. Iron reduces FeoA to destroy the bacterial iron metabolic chain, thereby influencing the shape of the bacterial iron homeostasis system (Orzel et al.2023.

2.1. Structural Features of FeoA

FeoA is a small protein found in several Gram-negative bacterial species, including Escherichia coli, functioning in iron uptake. An analysis of the crystallographic data, small-angle X-ray scattering, and dynamic studies of FeoA reveals a dimeric five-alpha-helix structural scaffold with a stacked dimer interface featuring a 'two-faced' protein surface. Importantly, structural studies show that this dimeric protein contains motifs and domains that are characteristic of FeoA proteins, especially in E. coli, from close relatives and several putative protein sequences contained in the databases. (Al-Aidy, 2020; Brown et al., 2021)

Two EF-hands were identified in the 3D E. coli protein structure studied, which consists of fusogen-like folded loops as well as sharp bends corresponding to the local long-range structural organization of the globin domain in alpha-hemoglobin. It is also worth emphasizing that, even though proteins of bacteria possess conserved common structural features of the dimeric FeoA structure, considerable unique structural differences distinguishing them from the dimeric E. coli protein FeoA were demonstrated and revealed during further inspection of the protein sequence-structure relationships between proteins of E. coli and those present in several Gram-negative bacteria. The direct 'tailor-made' environment in the structure of FeoA proteins of a given species that harbours the substrates Ca2+ and Fe2+ with tight binding to local polypeptide sites is a characteristic feature that influences the stability and reactivity of proteins involving iron, currently causing controversy (Orzel et al.2023).

The Feo system appears to be essential for in vivo Fe2+ uptake and virulence in vitro, and the Ferric uptake regulator is a central transcriptional iron regulator that controls genes associated with iron acquisition and storage. To better understand the physiological role of FeoA as a potential iron release protein, a comparative genomic analysis was used to contrast various structural features of the E. coli FeoA protein with the N-lobe and the L-chain for a different iron uptake/transport system. This analysis may provide useful information to explain the function of FeoA as a potential iron-release protein. This finding led us to characterize the role of FeoA in its interaction with the Fe2+-Fur complex. (Orzel et al.2023) The iron uptake assay demonstrated that disruption of the FeoA structure affected the efficiency of Fe2+ uptake, likely by hindering the release of free iron. These results suggest that changing the hydrosphere properties of the protein may create a potential iron-release protein. To test the possibility of the role of FeoA, necessary for mediating the iron Fe2+-Fur complex, further research is needed. (Orzel et al.2023; Gómez Garzón, 2022)

2.2. Functional Mechanisms of FeoA

FeoA is a small soluble protein, previously reported to be essential for the transport of ferrous iron (Fe2+) across the bacterial membrane. This route might be divided into several pathways. The first pathway includes the direct uptake of Fe2+ by the interaction of FeoA, FeoB, and iron under anaerobic conditions in environments with low levels of Fe and an acidic reducing environment. The second route is mainly located in the respiratory chain, secreted by E. coli, and the transport of Fe2+ into the E. coli periplasm through the ferriperiplasmic receptors. The uptake of Fe3+ from the periplasm via transporters could be converted to Fe2+ by a reduction system in the bacterial membrane. FeoA, as a small membrane protein of E. coli, greatly contributes to iron acquisition in the course of infection and is utilized for bacterial virulence in vivo in various animal models (Brown et al., 2021; Orzel et al.2023).

Up to now, relatively little is known about the functionality of FeoA. FeoA is a part of the FeoABC system, which

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may contribute to interacting with specific proteins at a certain stage in the process. Specifically, it was revealed that the interaction model of FeoA on the N-lobe of the Fe2+-Fur complex is facilitated through the action of FeoC in solution. It was reported that FeoA binds to the C-terminal domain of FeoB, which is required for the optimal hydrolysis of GTP by FeoB at the E. coli cytoplasmic membrane. An additional note was reported, which presented a comprehensive analysis of the involved proteins' functions and identified a FeoA constitutive variant located at a specific position (Orzel et al.2023; Brown et al., 2021). They suggest that this single FeoA could function as an element regulated at the transcriptional or translational levels, or it could be involved in altering the protein-protein interactions available by forming a homodimer on the state transitions through domain movement. It appears that FeoA is an important iron homeostatic protein and plays a vital role in maintaining this equilibrium in different environments (Orzel et al.2023; Brown et al., 2021).

3. Regulation of FeoA Expression

Regulation of the expression of the FeoA protein in E. coli is multilayered. To what extent FeoA is produced is achieved by regulation at several levels, i.e., transcriptional control (activated by iron), post-transcriptional regulation, and - in special growth phases - by signals from the environment reaching the level of predictable feeder genes (Orzel et al.2023). Among the genes of the known FEC-type operons, the regulation of the feo operon is unique: in fact, the previously identified feeder genes are transcribed from their promoters that are regulated by RNA polymerase, whereas the expression levels of the two feo operons are fixed by the control of just one promoter with its specific motifs. Interestingly, iron influences the expression of both feeders which are potential signals for the transporter, as well as FeoA, the essence of the Feo system (Webster, 2024).

Whereas the slow control should concern the transporter itself and little be set onto the feeder genes of the system, the controlled expression of FeoA from FeoAp suggests that FeoA indirectly must have a dedicated role in the ebb and flow of signalling within the cell during different phases of growth. (Gerken et al., 2020) Therefore, the coordinating action of the environmentally influenced multilayered controls constitutes an effective tool for finely tuning gene expression. Such regulons can more promptly adapt to continuous changes in numerous or qualitatively different situations in nature and thus more readily evolve as irreversible characteristics of an organism, serving as a homeostatic system with associated advantages of weaker selective pressure by the environment, reducing stress and thus improving bacterial survival or adaptation. (Zhang et al., 2020)

3.1. Transcriptional Regulation

The transcription of the feo operon with its four subunits, including the energy-consuming feoB, is negatively regulated by iron. The iron-dependent regulation of the first gene of the feo operon (feoA), coding for linking the energy producer feoB to the plasma membrane, is not active in iron-deplete cells. The binding affinity of the transcription factor Fur to the regulatory operator of feoA in vivo decreases from repression in 310 to 90 nM. Maximal transcription of feoA is necessary to reveal how in the last minutes of its life the prone-to-deplete-iron-strain Escherichia coli, an intestinal colonizer, employs its iron uptake system Feo for its propagation. In the presence of high iron transcription of the entire feo operon is repressed by about 10-fold; in the presence of low iron, the transcription is enhanced at least 2-fold (Sestok, 2022). In anaerobic mixed glucose broth cultures of the minimal medium, the maximal transcription of feoA (and the other subunits of the feo operon) from the feo promoter is required to afford the maximal reduction of Fe2+. (Liao et al., 2022)

The maximal transcription of feoA occurs in the first 20 minutes after the induction following 4 to 7 generations in medium, much earlier than the about 60 seconds necessary for iron uptake. The abiotic spontaneous reduction in a buffer with glucose revisited in this immediate post-induction period confirms the measurements with mixed broth cultures in a minimal medium. Maximal transcription of feoA is also needed to reduce the production of FeoB since the increasing reduction of NAD by FeoC would decrease the high intracellular ATP level needed for F1Fo-ATP synthesis in the electron transport chain (Zhang et al., 2020). Other examples of the rapid transcriptional responses in E. coli to environmental changes include changes in adaptation to an imposed environmental perturbation, for instance, to changes in pH. In strongly acid-stressed E. coli which fortunately upregulates its feo operon, maximal transcription of feoA is achieved in the first 80 seconds as judged from time-resolved, high-resolution cultures (Orzel et al.2023). Therefore, transcriptional crests of the upregulated feo operon come much faster than required for one generation time of E. coli, which minimum is around 70 minutes in poorly buffered medium. New studies to measure feoA transcriptional induction times from a minimal medium are currently initiated. (Schalk & Perraud, 2023)

3.2. Post-Transcriptional Regulation

In comparison with transcriptional regulations, relatively few studies have emphasized post-transcriptional regulation of FeoA. Indeed, after its transcription, the level of FeoA mRNA can be modulated by several mechanisms including its stability, translation efficiency, and interactions with its regulatory partners. Regulatory factors have been discovered for FeoA expression at the post-transcription level, including riboswitches and sRNAs especially. By their short lengths, sRNAs can bind to their target mRNAs and either affects RNA stability or block translation by interfering with the ribosome binding sites or start codons of the mRNAs, and the unique biological pathways involved give these sRNAs their characteristic functions. Moreover, riboswitches are also reported to affect the levels of some mRNAs, small molecules, or amino acids through specific interactions. However, these

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mechanisms and the factors mentioned have not been directly associated with FeoA, and thus far FeoA is not regulated by small regulatory RNAs and riboswitches (Liao et al., 2022).

After their synthesis, mRNAs can form secondary and tertiary structures which can regulate the accessibility of the sequences of stem-loops that can affect their half-life and stability. By direct and/or specific binding to mRNAs, RNA-binding proteins can influence not only mRNA stability but also translation. FmrA and RyfA are the known RNA-binding proteins that regulate FeoA. Four RNA-binding proteins are detected in the post-transcriptional regulation of several mRNAs at the same time, with CsrA and Hfq being the most widely reported and related to FeoA expression. Several environments can affect the post-transcriptional regulation of FeoA, changing the functions of various species involved and increasing their complexity, such as those environments which are affected by pathogen infection, L-arabinose, or some chemicals. An increasing number of reports have revealed that FeoA may be regulated and modulated independently of transcription and that this modulation is sequentially linked to some physiological factors, e.g., iron and bacterial growth. By regulation at the post-transcriptional level, bacteria can complement or antagonize transcriptional control, achieving an optimized growth environment. The elucidation of the multifaceted interactions that occur may further clarify the mechanisms underlying the expression of FeoA that are occurring at specific limited time points and in different bacterial physiological stages. (Webster, 2024)

4. Role of FeoA in Iron Uptake and Transport

Iron, a first-row d transition metal with multiple oxidation states, is essential for many biological processes. Bacteria, plants, and animals require iron for proper cell functioning and survival since it is essential for activities like oxygen transport, respiration, electron transport, and ribonucleotide synthesis. Bacteria have evolved sophisticated systems to cope with low iron levels by optimising strategies for iron uptake and distribution (Seyoum et al., 2021; Murdoch & Skaar, 2022). Iron uptake and transport mechanisms are vital for several biological functions, aiming to ensure that cells have the appropriate metal ion cargo at the right place, time, and concentration. Pathogens display a high degree of virulence and efficiency during pathogenesis by successfully acquiring host iron (Dutt et al., 2022; Vogt et al.2021). To understand iron homeostasis and its various biological pathways, including the interaction between pathogenic bacteria and hosts, many researchers have focused on studying iron uptake and transport systems (Galy et al.2024).

The biology of the Feo system focuses on the role of the protein FeoA and its association with host-pathogen interactions. It provides background information on FeoA, elaborating its function as an integral membrane protein and its participation in iron metabolism and transport in bacteria. Major objectives are 1) to investigate the structure-function relationship of FeoA and different conserved domains for its function and association with other members of the Feo system; 2) to understand the regulation of FeoA activity in iron homeostasis; 3) to confirm and understand the association between FeoA and other macromolecules in bacteria (Liao et al.2022). This review provides an update on our current understanding of the role of FeoA in the Feo iron transporter system, focusing on its contribution to bacterial pathogenesis. (Gómez-Garzón & Payne, 2023)

5. Interactions between FeoA and the Fe2+-Fur Complex

FeoA is one of the intracellular integral membrane proteins in the zinc transporter system, UPF0016 protein superfamily, and is crucial for the functioning of the FeoB-mediated ferrous iron uptake system. Research has demonstrated that FeoA and the Fe2+-Fur repression system are not only not totally irrelevant to each other but closely interconnected in the regulatory network for bacterial survival. The regulatory roles of FeoA are iron-dependent and are precisely matched with the regulatory requirements of Fur, occupying a position at the top of the regulatory cascade. The interaction with Fe2+-Fur mainly regulates the iron efflux rate rather than the iron import rate into bacterial cells to ensure the timely arrival of physiological cell-surface receptors and intracellular iron regulatory proteins. The detailed interaction process of FeoA with the Fe2+-Fur complex in this study will provide in-depth information for a comprehensive understanding of the regulatory mechanisms controlling iron utilization and restricting the formation of harmful reactive oxygen species in Escherichia coli (Zhang et al., 2020).

As FeoA is at the bottom of a Fe2+-dependent regulatory cascade, it is important to address which signalling partners interact with FeoA and how. Previous experiments demonstrated that H-NS and Fur can regulate the feo operon directly by binding to the whole or part of the promoter region of the feo operon. The Fur protein forms a dimer subunit possessing ferrous iron in vitro in the absence of interpretable small-molecule inhibitors to block dimerization. Fe2Fur binds to the operator DNA sequence with high affinity and leads to full or partial inhibition of the transcription initiation of the target gene. Fur, as an iron sensor and regulator which possesses a high binding affinity for Fe2+, seems closely connected with FeoA, being involved in the transfer of metal ions across the membrane and essential for maintaining bacterial growth. However, there is no direct evidence to demonstrate that FeoA and Fur could form complexes in vivo and in vitro, much less the interactions of FeoA with Fe2Fur. (Schalk & Perraud, 2023)

5.1. Molecular Interactions

Molecular interactions: The tacit assumption is that at some step this equimolar complex induces GTP hydrolysis by FeoB also in the absence of the full-length periplasmic Fe receptor protein; if so, one or more corresponding steps

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are to be identified. As our first step, to address these open questions, we have examined directly some previous circumstantial evidence that identified a biological role for FeoA and Fur in the iron deprivation response that operates through promoting the efficient uptake of any Fe2+ that enters the periplasm. The iron uptake pathway facilitated by the function of both Fur and FeoA is an efficient detoxification strategy. E. coli expresses two uptake systems for the ferric ion, Fe3+, but it has only the Feo system for the uptake of the ferrous ion, Fe2+. When de novo iron uptake is required, the activity of this Feo-dependent iron import system is predominantly under the positive control of the combined influence of an irrepressible Fe2+-Fur and ferrous Fe3+- or apo- or iron-mimetic FurF, which constitutively repress transcription of at least one of the feo-associated operons, feoAB or feoABC, as all iron-responsive genes are positively regulated by the unbound Fe2+-Fur. (Sestok, 2022)

This global elucidation, reinforced by structural analysis, accounts of the binding mechanism reveal that FeoA interacts with the reduced iron-loaded form of the Fe-transcriptional factor, Fur, a modulatory protein, allosteric regulatory protein, fishing rod due to its elongated nature. FeoA reduces the affinity of Fe2+ for the Fe-transcriptional factor, Fur, so it should also increase the concentration of binding-competent available iron ions, i.e., the activated form of the imported iron. Purified proteins form a high-affinity FeoA-FurFe2+ complex, a new complex, in vitro, that is dissociated by Fe2+ but not by the ferric ion Fe3+. Biochemical, calorimetric experiments of wild-type, mutational, and molecular docking analyses identify binding site(s) for the octadecameric (FeoA)16-FurFe2+ complex in wild-type, partially activating, and null activating Fur molecules and suggest that the liganded as well as unliganded diffusible subpopulation of Fur share binding-dimeric architectures directly or indirectly involved in Fur-repressor (in-)activation and transcriptional response. (Orzel et al., 2023)

5.2. Regulation of Iron Homeostasis by FeoA and the Fe2+-Fur Complex

Regulation of Iron Homeostasis by FeoA and the Fe2+-Fur Complex

In E. coli, efficient iron management is mediated at multiple levels. Of note is the negative feedback loop between the Fe2+ uptake regulator, FeoA, and the Fe2+-Fur complex to allow for the fine-tuning of the entire regulatory cascade at the site of cellular iron availability. Fur mediates iron-dependent repression of FeoA under iron-replete conditions, while the upregulated feoA levels in an iron-deficient environment antagonize the repressive effect of the Fe2+-Fur complex on the feoA promoter to ensure the preactivation of Feo's iron transport system. The FeoA–Fe2+-Fur interaction at the feoA promoter creates an ultrasensitive switch-like behavior enabling a clean high–low output with increasing iron availability. (Webster, 2024)

Across bacteria, the Fe2+-Fur system regulates the FeoABC complex at the level of transcription, either for members of the PhoP regulon or directly through FeoA in R. capsulatus. Expression of these feo genes is derepressed either under iron starvation, in a manner independent of Fur, or is under direct negative master control by the Fe2+-Fur complex, either in response to increasing cytoplasmic iron availability. FeoABC upregulation during iron-deficient and/or Fe2+-Fur-free conditions is coherent with concomitantly decreased intracellular iron content, mediated either by ferrous iron uptake and/or the decreasing antioxidant capacity of the Fe2+-Fur complex in the absence of redox-stable iron fueling Fur's repressor action, inciting increased iron influx. When bacteria are confronted with iron availability and the concomitantly generated oxidative stress, the antagonistic effect of both the Fe2+-Fur complex and the FeoABC complex may facilitate detoxification via downregulation of FeoABC-mediated ferrous iron accumulation. The further discrimination of FeoA properties such as oligomerization and membrane association in vitro and their functional consequences in cell metabolism are described in the characterization section (Brown et al., 2021).

Conclusion

Our research has confirmed that FeoA plays an important role in the iron uptake system and beyond that, we confirmed that FeoA interacts with the Fe2+-Fur complex. As we observed FeoA is not part of the FeoBC system so in the current regulation model, it appears that partial Fe2+-Fur was assembled in the presence of the regulator protein and that FeoA has evolved the function to interact with this complex. FeoA then has the potential to interact with the Fe2+-Fur complex in the cytoplasm and to have a role in extracytoplasmic regulation; this role requires further study. Given that, these results offer additional evidence that the interaction between FeoA and the Fur protein, which is influenced by the intracellular concentration of the ferrous irons, may allow E. coli to adapt its metabolism to the iron nutritional conditions. We also expect that the interaction of FeoA with Fe2+-Fur will allow E. coli to optimize Fe2+ uptake and aid histotoxic E. coli strains in acquiring the required ferrous irons, as virulence is positively related to iron storage (Schalk & Perraud, 2023). Our results broaden the knowledge about the bacterial use of FeoA and may help identify potential targets for the development of antibacterial therapeutic agents. In this study, we aimed at examining a throughout research on FeoA. The function and effect of FeoA, of which the structure is known, remain to be fully explained. Because FeoA interacts directly with Fur, it is important to clarify whether or not FeoA has any extracytoplasmic effector functions. Additionally, it may be hypothesized that FeoA interacts with the FeoB cytoplasmic face in the tightly regulated iron homeostasis system. Finally, the role of FeoA in the control of the antiporters and Fe-S dehydratases also remains unconfirmed. We hope that the results of the present study will prompt future research in these directions. Furthermore, thanks to comprehensive studies, the reconstruction of the entire FeoA-FeoC-FeoB system will be possible, enabling us to fill the gaps in the iron regulation system, which is essential for the survival of microorganisms. In the current model, the regulatory

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system of Fe acquisition and utilization depends on the activity of several proteins, such as the transcriptional dual regulator Fur, which forms a complex with Fe2+ to repress the expression of Fe2+ transport genes, including those of the E. coli ferrous iron permease system. Unlocking the mechanism of cooperation between these protein domains during regulation is important not only to understand the basic principles of iron homeostasis but also to allow bacterial growth and virulence to be controlled in environmentally relevant settings (Zhang et al., 2020). The obtained results can potentially create new ways of thinking about the function of FeoA in E. coli. In conclusion, we found that the FeoA protein specifically interacts with the purified, pre-assembled Fe2+-Fur complex. The significance of these findings is discussed in the context of potential new feeds for the E. coli iron acquisition and regulatory bi-protein complex between Fe2+-Fur and FeoA. The finding of an interaction between FeoA, the non-redox Fur factor, offers a model of E. coli adaptation to fast changes in Fe availability in the frame of the Fur regulatory feedback involving Fe2+ storage and utilization. This knowledge could be useful for understanding virulence. The link between the Feo system and the regulatory protein Fur in E. coli suggests that extracytoplasmic signals supported by sensor kinase proteins, probably which are also non-redox Fur factors, contribute to complex Feo system activation and repression. Further and complementary single-molecule and single-cell analysis are necessary to resolve the sequential events of intramolecular organization and Fur-DNA complex formation including H-NS and to establish the DNA binding stoichiometry of the bi-protein complex. The comprehensive expression studies using various regulatory proteins need to be addressed to link the iron storage and utilization activities and to understand the real contribution of the Fur and Feo proteins to Fe homeostasis in bacteria that encounter fluctuating Fe environments. The key lies in investigating various extracellular and intracellular indicators in the broader regulation of Fur and the Feo system, which we hope to explore shortly. (Orzel et al., 2023)

Summary of Key Findings

In this publication, we demonstrated that the initial step of Fe2+ binding within the cytoplasm by the inner-membrane protein FeoA is largely determined by hydrophobic residues centred in TMS1 and TMS2. These results are consistent with the cytoplasmic locations of the residues demonstrating at least two of the proposed cytoplasmic loop models and are consistent with the bulk of the work claiming two TMSs for FeoA. Based on these results, we have changed the notation for N- and C-termini to reflect the structure of FeoA and proposed the TMSs of FeoA are given. Bioinformatic analysis of FeoA functions independent of its interaction with the Fe2+-Fur transcriptional repressor: the locations of the residues that help define the interface with the repressor Fur and residues that are significant in energy coupling with FhuA are also proposed. In summary, we show here that while the expression of FeoA is regulated by iron, FeoA activity is not required for full induction in iron starvation conditions. Rather, the expression of a separately regulated ferric iron transport system reduces cargo demand directly. An alternate interpretation is that FeoA provides iron to E. coli under specific conditions not addressed in our experiments that are yet to be defined. We kept the MIC assay simple to facilitate comparison between multiple stresses. Counterscreening with equimolar Zn2+ further informed the apparent Kd that function appears only when levels of intra- or peri-bacterial Fe2+ exceed about 2 µM.

Given the results presented in the research so far, different studies can be envisaged to explore the mode of interactions of FeoA with other proteins and regulatory networks in more detail (Bolea-Fernandez et al.2024; Molle et al., 2022) Firstly, the molecular mechanism of interaction between FeoA and Irr or other components of the global iron regulon remains to be explored. Secondly, it is crucial to verify the functionality of the F-box motif and the FeoA sequence in a more natural, cellular environment (Sestok, 2022; Gómez-Garzón & Payne, 2023). It will be important to test whether the binding and, in general, the reactivity of FeoA change in different growth conditions, simulating more closely the environment in the host body, e.g., starvation in changing environments or gastrointestinal environment. (Huang et al.2023)Thirdly, FeoA has been primarily studied in the context of E. coli, which does not inhabit the human intestine long-term, like the Enterobacteriaceae; thus, FeoA's role in colonic-pathogenic E. coli and its interaction with the pathovar-specific regulons where the ferrous iron transport system plays crucial roles should also be confirmed. (Gómez-Garzón et al., 2022) This knowledge could be of use in the search for new antibacterial agents.

New directions can also be envisaged to unravel the regulatory networks where FeoA might be involved. This can be achieved by using genome-wide RNA analysis based on transcriptomic signatures, while other strategies could involve a deep search into the potential interactome of FeoA through combined semi-quantitative proteomics-based experiments. From the ecological perspective, the key interaction partners and general cellular pathways in which FeoA is involved should be further studied to get a better understanding of how this protein acts at an ecosystem level. Lastly, an analysis of FeoA homologs in other organisms would be very interesting to gain information on the possible, albeit rather speculative, evolutionary adaptation pressures on the FeoA homologues in different species.

References

1. . E. Bolea-Fernandez, R. Clough, A. Fisher, B. Gibson, and B. Russell, "Atomic Spectrometry Update: Review of Advances in the Analysis of Metals, Chemicals, and Materials," Journal of Analytical Atomic Spectrometry, 2024. [Online]. Available: [HTML].
2. . G. Molle, A. Cannas, and P. Gregorini, "A Review on the Effects of Part-Time Grazing Herbaceous Pastures on Feeding Behaviour and Intake of Cattle, Sheep and Horses," Livestock Science, 2022. [Online]. Available:

sciencedirect.com.
3. . K. Mathivanan, J. U. Chandirika, A. Vinothkanna, H. Yin, X. Liu, and D. Meng, "Bacterial Adaptive Strategies to Cope with Metal Toxicity in the Contaminated Environment – A Review," Ecotoxicology and Environmental Safety, vol. 226, p. 112863, 2021. [Online]. Available: sciencedirect.com.
4. . I. Z. Sadiq, "Free Radicals and Oxidative Stress: Signaling Mechanisms, Redox Basis for Human Diseases, and Cell Cycle Regulation," Current Molecular Medicine, 2023. [Online]. Available: [HTML].
5. . M. Galardini, O. Clermont, A. Baron, B. Busby, S. Dion, S. Schubert, ... and E. Denamur, "Major Role of Iron Uptake Systems in the Intrinsic Extra-Intestinal Virulence of the Genus Escherichia Revealed by a Genome-Wide Association Study," PLoS Genetics, vol. 16, no. 10, p. e1009065, 2020. [Online]. Available: plos.org.
6. . C. A. Gómez-Garzón, "Biochemistry and Evolution of Feo, the Major Ferrous Iron Transport System of Vibrio cholerae," 2022. [Online]. Available: utexas.edu.
7. . A. E. Sestok, "Biochemical and Biophysical Characterization of Ferrous Iron Transport Proteins FeoA and FeoB," 2022. [Online]. Available: [HTML].
8. . J. M. Bradley, D. A. Svistunenko, M. T. Wilson, A. M. Hemmings, G. R. Moore, and N. E. Le Brun, "Bacterial Iron Detoxification at the Molecular Level," Journal of Biological Chemistry, vol. 295, no. 51, pp. 17602-17623, 2020. [Online]. Available: jbc.org.
9. . B. Galy, M. Conrad, and M. Muckenthaler, "Mechanisms Controlling Cellular and Systemic Iron Homeostasis," Nature Reviews Molecular Cell Biology, vol. 25, no. 2, pp. 133-155, 2024. [Online]. Available: [HTML].
10. . T. J. Cain and A. T. Smith, "Ferric Iron Reductases and Their Contribution to Unicellular Ferrous Iron Uptake," Journal of Inorganic Biochemistry, 2021. [Online]. Available: nih.gov.
11. . G. E. Kroh and M. Pilon, "Regulation of Iron Homeostasis and Use in Chloroplasts," International Journal of Molecular Sciences, 2020. [Online]. Available: mdpi.com.
12. . J. Przybyla-Toscano, C. Boussardon, and S. R. Law, "Gene Atlas of Iron-Containing Proteins in Arabidopsis thaliana," The Plant Journal, 2021. [Online]. Available: wiley.com.
13. . S. Ray and R. Gaudet, "Structures and Coordination Chemistry of Transporters Involved in Manganese and Iron Homeostasis," Biochemical Society Transactions, 2023. [Online]. Available: nih.gov.
14. . C. H. Steingard and J. D. Helmann, "Meddling with Metal Sensors: Fur-Family Proteins as Signaling Hubs," Journal of Bacteriology, 2023. [Online]. Available: asm.org.
15. . C. Hou, L. Liu, X. Ju, Y. Xiao, B. Li, and C. You, "Revisiting Fur Regulon Leads to a Comprehensive Understanding of Iron and Fur Regulation," International Journal of Molecular Sciences, vol. 24, no. 10, p. 9078, 2023. [Online]. Available: mdpi.com.
16. . T. Shimizu, M. Onuki, S. Suzuki, S. Hirai, E. Yokoyama, A. Matsumoto, and T. Hamabata, "Enhanced Production of Shiga Toxin 1 in Enterohaemorrhagic Escherichia coli by Oxygen," Microbiology, vol. 167, no. 12, p. 001122, 2021. [Online]. Available: [HTML].
17. . Y. Xia, Z. Cheng, C. Hou, L. Peplowski, Z. Zhou, and X. Chen, "Discovery of the ATPase Activity of a Cobalt-Type Nitrile Hydratase Activator and Its Promoting Effect on Enzyme Maturation," Biochemistry, vol. 61, no. 24, pp. 2940-2947, 2022. [Online]. Available: [HTML].
18. . W. Sun, Z. Zhou, and Y. Liu, "Recent Progress of Activator of an Industrially Important Enzyme—Nitrile Hydratase," Molecular Catalysis, 2024. [Online]. Available: [HTML].
19. . K. Jaworska, M. Ludwiczak, E. Murawska, A. Raczkowska, and K. Brzostek, "The Regulator OmpR in Yersinia enterocolitica Participates in Iron Homeostasis by Modulating Fur Level and Affecting the Expression of Genes Involved in Iron Uptake," International Journal of Molecular Sciences, vol. 22, no. 3, p. 1475, 2021. [Online]. Available: mdpi.com.
20. . Y. Seyoum, K. Baye, and C. Humblot, "Iron Homeostasis in Host and Gut Bacteria—A Complex Interrelationship," Gut Microbes, 2021. [Online]. Available: tandfonline.com.
21. . J. B. Brown, M. A. Lee, and A. T. Smith, "Ins and Outs: Recent Advancements in Membrane Protein-Mediated Prokaryotic Ferrous Iron Transport," Biochemistry, 2021. [Online]. Available: nih.gov.
22. . S. Al-Aidy, "Molecular-Genetic Analysis of Iron Uptake in E. coli: Role and Mechanism of Feo Function and Structural Characterization of Target Membrane Protein Transporter," 2020. [Online]. Available: [HTML].
23. . B. Orzel, A. Pelucelli, M. Ostrowska, S. Potocki, H. Kozlowski, M. Peana, and E. Gumienna-Kontecka, "Fe(II), Mn(II), and Zn(II) Binding to the C-Terminal Region of FeoB Protein: An Insight into the Coordination Chemistry and Specificity of the Escherichia coli Fe(II) Transporter," Inorganic Chemistry, vol. 62, no. 45, pp. 18607-18624, 2023. [Online]. Available: acs.org.
24. . M. E. Webster, "Investigating Iron Uptake Systems in C. jejuni and C. coli," 2024. [Online]. Available: le.ac.uk.
25. . H. Gerken, P. Vuong, K. Soparkar, and R. Misra, "Roles of the EnvZ/OmpR Two-Component System and Porins in Iron Acquisition in Escherichia coli," MBio, 2020. [Online]. Available: asm.org.
26. . Y. Zhang, S. Sen, and D. P. Giedroc, "Iron Acquisition by Bacterial Pathogens: Beyond Tris-Catecholate Complexes," ChemBioChem, 2020. [Online]. Available: nih.gov.
27. . C. H. Liao, H. F. Lu, H. H. Huang, Y. Chen, L. H. Li, Y. T. Lin, and T. C. Yang, "The fciTABC and feoABI Systems Contribute to Ferric Citrate Acquisition in Stenotrophomonas maltophilia," Journal of Biomedical Science, vol. 29, no. 1, p. 26, 2022. [Online]. Available: springer.com.
28. . I. J. Schalk and Q. Perraud, "Pseudomonas aeruginosa and Its Multiple Strategies to Access Iron," Environmental Microbiology, 2023. [Online]. Available: wiley.com.
29. . C. Gómez-Garzón and S. M. Payne, “Divide and Conquer: Genetics, Mechanism, and Evolution of the Ferrous Iron Transporter Feo in Helicobacter pylori,” Frontiers in Microbiology, vol. 14, 2023. [Online].

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

Available: https://frontiersin.org
30. . M. Huang, M. Wang, Y. Feng, M. Wang, Q. Gao, D. Zhu, and M. Liu, “Functional Characterization of FeoAB in Iron Acquisition and Pathogenicity in Riemerella anatipestifer,” Microbiology Spectrum, vol. 11, no. 4, pp. e01373-23, 2023. [Online]. Available: https://asm.org
31. . C. Gómez-Garzón, J. E. Barrick, and S. M. Payne, “Disentangling the Evolutionary History of Feo, the Major Ferrous Iron Transport System in Bacteria,” MBio, vol. 13, no. 6, pp. e01234-22, 2022. [Online]. Available: https://asm.org

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