Exploring the role of the histidine biosynthetic *hisF* gene in cellular metabolism and in the evolution of (ancestral) genes: from LUCA to the extant (micro)organisms

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ABSTRACT

Histidine biosynthesis is an ancestral pathway that was assembled before the appearance of the Last Universal Common Ancestor; afterwards, it remained unaltered in all the extant histidine-synthesizing (micro)organisms. It is a metabolic cross-road interconnecting histidine biosynthesis to nitrogen metabolism and the *de novo* synthesis of purines. This interconnection is due to the reaction catalyzed by the products of *hisH* and *hisF* genes. The latter gene is an excellent model to study which trajectories have been followed by primordial cells to build the first metabolic routes, since its evolution is the result of different molecular rearrangement events, *i.e.* gene duplication, gene fusion, gene elongation, and domain shuffling. Additionally, this review summarizes data concerning the involvement of *hisF* and its product in other different cellular processes, revealing that HisF very likely plays a role also in cell division control and involvement in virulence and nodule development in different bacteria. From the metabolic viewpoint, these results suggest that HisF plays a central role in cellular metabolism, highlighting the interconnections of different metabolic pathways.

1. Introduction

Histidine biosynthetic pathway consists in the conversion of 5-phosphoribosyl-1-pyrophosphate (PRPP) into L-histidine through 10 enzymatic reactions (Alifano et al., 1996), and it represents one of the best characterized anabolic routes, especially in the enterobacteria *Escherichia coli* and *Salmonella typhimurium* (Carlomagno et al., 1988). It is present in Bacteria, Archaea, lower eukaryotes, and plants (Kulis-Horn et al., 2014), and it was very likely assembled before the appearance of the Last Universal Common Ancestor (LUCA) (Fani et al., 1995; Fondi et al., 2009). This idea relies on extensive biochemical, genetic, and evolutionary studies that underlined the antiquity of this pathway (Fani et al., 2007 and references therein). Thus, it is an excellent model for the study and analysis of the molecular mechanisms and the forces that might have driven the origin and evolution of metabolic routes (Fondi et al., 2009). Moreover, studies about histidine biosynthesis provided an important resource to understand the mechanisms involved in transcriptional, metabolic and autogenous regulation, and to figure out the mechanisms of RNA processing and enzymology (Alifano et al., 1996; Winkler and Ramos-Montañez, 2009): for instance, the term "attenuation" was firstly coined to describe the regulation of the histidine operon (Winkler and Ramos-Montañez, 2009). Moreover, this pathway plays an important role in cellular metabolism, being interconnected to (at least) the nitrogen metabolism and the *de novo* synthesis of purines (Fani et al., 2007).

The biosynthetic pathway, the genetic organization, and the flux of intermediates show similar features in *E. coli* and *S. typhimurium*: in these microorganisms the eight biosynthetic genes are clustered in a compact operon (hisGDC[NB]HAF[IE]) (Carlomagno et al., 1988). Three of the *his* genes code for bifunctional enzymes (*i.e.hisNB*, *hisD* and *hisIE*), while another enzyme is heterodimeric, being composed of the *hisF* and *hisH* gene products (Alifano et al., 1996). The latter genes are particularly interesting from different viewpoints. This mini-review will be focused on one of them, *hisF*, and on its product, analyzing its structure, evolution, and involvement in different cellular processes in the bacterial world. Besides its involvement in histidine biosynthesis, HisF seems to take part into many other metabolic processes highlighting the complexity and the fine interconnections of histidine biosynthesis and metabolic networks.

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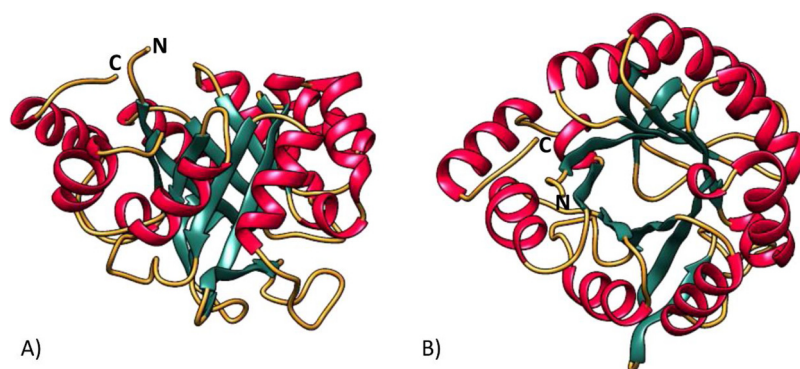[1] These authors contributed equally to this work.

Fig. 1. Three-dimensional structure of the *E. coli* HisF cyclase; A) side view; B) top view. The structure was predicted through Phyre2 (Kelley et al., 2015) software and visualized with UCSF Chimera (Pettersen et al., 2004).

2. The structure of the enzyme encoded by *hisF*

HisF belongs to the triosephosphate isomerase (TIM) barrel superfamily for its architecture, composed of eight repeats of β-strands and α-helices (Goldman et al., 2016): the parallel β-sheets are located in the internal part of the structure while the α-helices form the external surface (Fig. 1) (Gerlt, 2000). The $(\beta/\alpha)_8$-barrel architecture is a versatile structure and characterizes approximately the 10% of all enzymes (Gerlt, 2000; Goldman et al., 2016). Since this structural organization was found in several enzymes with different catalytic properties, it has been hypothesized that it could be the result of a divergent evolution event (Bork et al., 1995). TIM barrel proteins exhibit a pattern of distribution in modern metabolic pathways consistent with the patchwork model (Yčas, 1974; Jensen, 1976), according to which multiple enzymes with specific functions evolved from a smaller number of enzymes that performed multiple catalytic functions, *versus* other models of metabolic pathway evolution (Caetano-Anollés et al., 2009). Another protein of the histidine biosynthetic pathway with a $(\beta/\alpha)_8$-barrel fold is HisA (Fani et al., 1994), which carries out the reaction before that one catalyzed by HisF, through an Amadori rearrangement mechanism (Lang et al., 2000). The crystal structures of HisF and HisA proteins were determined in *Thermotoga maritima* (Thoma et al., 1999) providing the description of the structural domain. The active sites in both enzymes are located at the C-terminal face of each β/α-barrel. The central β-strands are generally short, having at most five residues, except for β1 and β5 which can contain up to eleven amino acids. The structure of the N-terminal face of each β/α-barrel is very different from the C-terminal one: the loops of the first one are very short and simple, in contrast with the other face that presents two-fold repeated motifs generating a complex structure (Lang et al., 2000).

3. The evolutionary history of *hisF*: a paradigmatic example of a cascade of gene elongations, domain shufflings, gene duplications, and gene fusions

The evolutionary history of *hisF* is quite intriguing since it represents one of the clearest compendia of the molecular mechanisms that have driven the evolution of ancestral and maybe extant genes, such as gene elongation, gene duplication, domain shuffling, and gene fusion. Moreover, it also provides important hints to understand and clarify which evolutionary trajectories have been used by primordial cells to construct the first metabolic pathways.

The analysis of the amino acid sequence of the cyclase encoded by *hisF* that was performed on a limited number of the available amino acid sequences from different microorganisms, revealed that this protein can be divided into two paralogous halves (Fani et al., 1994). In addition to this, the comparative analyses of *hisF* and *hisA* gene products in different microorganisms suggested that the two genes are paralogous; hence, they have originated from a common ancestor gene

by duplication and subsequent evolutionary divergence (Fani et al., 1994). The finding that the two genes share the same internal organization, consisting in two paralogous modules half the size of the entire gene, allowed Fani et al. (1994) to propose a two-step model for the evolution of *hisA* and *hisF*. According to this scheme, at first, a single ancestral module *hisA1* (half the length of the current *hisA*) duplicated forming *hisA2*, and the two copies fused leading to the entire *hisA*. Then the *hisF* gene originated *via* a duplication and divergence event of the ancestral *hisA* gene.

This possible evolutionary model was confirmed by the comparative analysis of the amino acid sequence of the two proteins performed on a much larger number of histidine-synthesizing prokaryotic and eukaryotic (micro)organisms (Fani et al., 2007; Fondi et al., 2009). Moreover, the comparison of the four half modules of HisF and HisA barrels, based on sequence alignment, showed that some residues are conserved and located in the same positions, as an aspartate and a valine in the first loop of the total architecture (Lang et al., 2000). The aspartate located in the center of the active site in all the four half-barrels confirmed the hypothesis of an evolutionary event that started from a $(\beta/\alpha)_4$-barrel ancestor in which this residue was involved in different catalytic reactions (Lang et al., 2000). The conserved residues and the two bound phosphate ions that bind the PRFAR substrate, support the idea of HisF evolution from a half-barrel motif (Höcker et al., 2001). This evolutionary hypothesis, based on sequence alignments, was in agreement with the crystal structures of HisF and HisA (Thoma et al., 1999). The internal symmetry of the HisF and HisA TIM barrel suggested, as previously hypothesized, that this structure arose from an in tandem duplication and divergence event, followed by a fusion, of a precursor ancestral gene encoding a $(\beta/\alpha)_4$-half barrel (Fani et al., 1994; Lang et al., 2000; Fani et al., 2007). This hypothesis is also supported by experimental data. Höcker et al. (2001) purified and characterized the two *E. coli* HisF halves (namely, HisF-N and HisF-C). They found that these HisF sub-structures were correctly folded even if separated, supporting the idea of the evolution of *hisF* from an ancestral $(\beta/\alpha)_4$-half barrel coding gene, although the catalytic activity was detected only when co-expressed. Additionally, Gerlt and Babbit (2001) demonstrated that HisF-N and HisF-C were able to form stable homodimers, but they confirmed that only the heterodimers had catalytic activity. If this scenario is correct, then, the fusion of two $(\beta/\alpha)_4$ modules after the duplication and divergence events, would have had several implications among which an enhanced stability, and thus the reduction of the system entropy. Then, in the course of evolution, the mutations occurred after the fusion of the two previously independent half-barrels would have increased and optimized the contact interface according to the 'Rosetta Stone model' of proteins interaction (Marcotte et al., 1999; Höcker et al., 2001).

Concerning the timing of the evolutionary events leading to the extant *hisF* and *hisA* genes, since they share the same structure in all histidine-synthesizing organisms, both prokaryotic and eukaryotic, it is
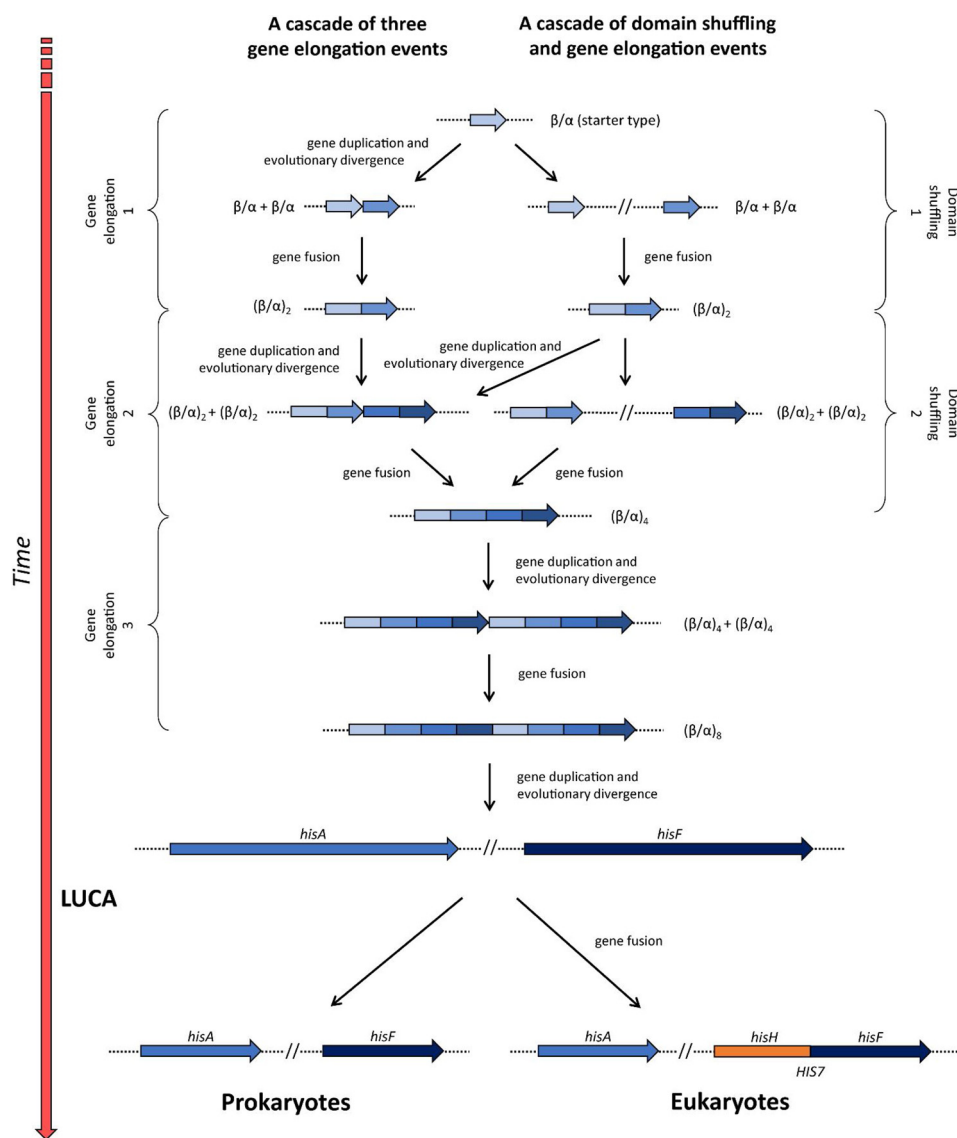
Fig. 2. Schematic representation of the model proposed for the evolution of *hisA* and *hisF* genes (adapted from Del Duca et al., 2020).

quite possible that the gene elongation event leading to the extant *hisF* occurred long before the appearance of LUCA, in agreement with the suggested antiquity of the histidine biosynthetic pathway (Shen et al., 1990; Fani and Fondi, 2009). Even though the 8 modules of the HisF and/or HisA protein do not share a degree of sequence similarity sufficiently high to suggest a common origin, we cannot *a priori* exclude the possibility that the *hisF* gene might be the result of a cascade of three gene elongation events. According to this idea, a single (β/α) module might have been able to aggregate in an unstable, inefficient and not specific homo-octamer. Then, three gene elongation events would have given rise to the complete ancestor of the extant TIM-barrels. However, since i) the degree of sequence similarity between the single (β/α) modules of the extant barrels is low, and ii) that of the two halves of HisF and HisA is higher (strongly suggesting the gene elongation event starting from a $(\beta/\alpha)_4$ module), it is also possible to hypothesize that the ancestors $(\beta/\alpha)_2$ and $(\beta/\alpha)_4$ coding genes might have been the result of domain shuffling events, in which different copies of the starter genes - (β/α) and $(\beta/\alpha)_2$ modules, respectively - scattered throughout the genome of ancestral cells might have undergone gene fusion events (Fig. 2) (Del Duca et al., 2020).

It is also worth of noticing that after the separation from LUCA, the *hisF* gene underwent another fusion event in the eukaryotic lineage, *i.e.*

its fusion to *hisH*, leading to the formation of the single bifunctional polypeptide encoded by *HIS7* (Fani et al., 1997; Brilli and Fani, 2004).

Lastly, *hisF* (and *hisA*) gene represents an excellent and paradigmatic example of how different schemes (not mutually exclusive) have been used by ancestral cells to build the first metabolic pathways. Indeed, different hypotheses on the origin and evolution of metabolic pathways have been proposed; one of them, the semi-enzymatic idea (Lazcano and Miller, 1999) will be described in the next section. In addition to this, the finding that two of the histidine biosynthetic genes are paralogous (*hisF* and *hisA*) is in agreement with the retrograde evolution hypothesis of Horowitz (1945). At the same time, it is also quite possible, according to the patchwork hypothesis proposed by Jensen in 1976, that the ancestor gene of *hisF* and *hisA* encoded a protein able to perform different, albeit chemically similar, enzymatic reactions, including the two successive reactions that are carried out by the two extant enzymes in the histidine biosynthetic pathway.

4. The roles of HisF in cellular metabolism

4.1. Histidine biosynthesis

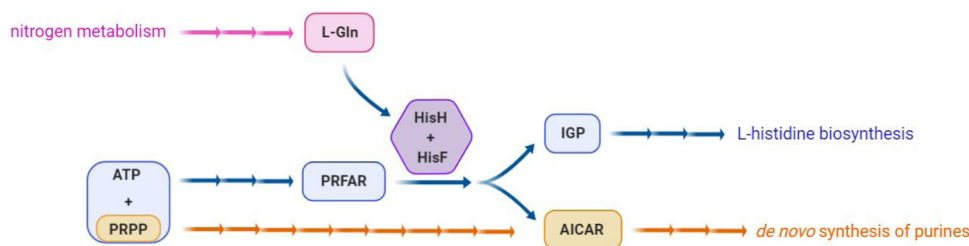The *hisF* gene codes for a cyclase that, together with HisH (a

Fig. 3. Step of histidine biosynthetic pathway (blue) connected to nitrogen metabolism (pink) and *de novo* purine biosynthesis (orange).

glutamine amidotrasferase), forms the heterodimeric holoenzyme imidazol glycerol phosphate synthase (IGPS) (Alifano et al., 1996; Fani et al., 2007). The heterodimeric complex HisF:HisH catalyzes the fifth step of the histidine biosynthetic pathway, which is the conversion of N9-[(59-phosphoribulosyl)-formimino]-5-aminoimidazole-4-carboxamide-ribonucleotide (PRFAR) into imidazole glycerol-phosphate (IGP) and 5′-(5-aminoimidazole-4-carboxamide) ribonucleotide (AICAR) (Alifano et al., 1996). This reaction is a crucial point in cellular metabolism, since the two enzymes allow the connection of three different routes (Fig. 3): histidine biosynthesis, nitrogen metabolism, and *de novo* synthesis of purines (Fani et al., 2007). Several studies highlighted the HisF:HisH *in vitro* interaction (Klem et al., 2001 and reference therein), and their *in vivo* interaction has been recently demonstrated by Chioccioli et al. (2020) through the bacterial adenylate-cyclase two hybrid system.

IGPS belongs to an enzymatic class, the glutamine amidotransferases (GATs) (Klem et al., 2001), that is well studied for the mechanisms of substrate channeling and interdomain signaling, thanks to the crosstalk of two physically separated catalytic domains: glutaminase domain at N-terminal and acceptor domain at C-terminal. The ammonia generated by hydrolysis of glutamine in the glutaminase domain (HisH) moves along an internal enzymatic channel to reach the cyclase acceptor domain (HisF) and to react with PRFAR (Chaudhuri et al., 2001). The binding of PRFAR to HisF active site is necessary to start the glutaminase reaction (Rieder et al., 1994), revealing an allosteric regulation (Rivalta et al., 2012); indeed, IGPS is classified as a V-type allosteric enzyme, since its catalytic rate is highly dependent on activation by its allosteric ligand (Lisi et al., 2017). The HisH subunit alone does not show any glutaminase activity and the presence of HisF is necessary for its stability in solution. To understand the role of the cyclase domain in the mechanism of glutamine utilization by IGPS, *E. coli hisF* mutants obtained with random PCR mutagenesis were selected; they maintained the capability of imidazole ring formation and growth in ammonium enriched medium, but formed IGPS unable of glutamine amidotransferase activity (Klem et al., 2001). The isolated glutamine-deficient mutants provided information regarding the conserved residues in the HisF acceptor domain of *E. coli* IGPS. Data obtained highlighted the importance of some HisF residues, among which the arginine 5 (R5) and the glutamate 46 (E46), which are both completely conserved in all the analyzed alignments of the wild-type proteins. In particular, E46 likely plays a role in binding the substrate glutamine and R5 in the ammonia transfer reaction (Klem et al., 2001). Rieder et al. (1994) observed that in *Klebsiella pneumoniae hisH* mutants the reaction performed by IGPS can be carried out by cyclase alone (thus, in the absence of a functional GAT) under high concentrations of NH_4^+, allowing the formation of the imidazole ring of histidine (Vazquez-Salazar et al., 2018). This phenomenon has been also studied in *T. maritima*, where it was observed that HisH is not able to perform a catalytic activity in the absence of HisF. On the other hand, the opposite scenario was observed: HisF alone showed its cyclase activity in presence of ammonia in solution (List et al., 2012). This finding provides a clear example supporting the semi-enzymatic model on the origin of metabolic pathways (Lazcano and Miller, 1999). According to the retrograde origin of metabolic routes (Horowitz, 1945), Lazcano and

Miller (1999) suggested that semi-enzymatic reactions took place as a consequence of lack of enzymes: in this condition, a catalytic step of the pathway can occur by changing the reaction conditions, through the use of other chemical compounds. Thus, the semi-enzymatic reaction that forms IGP, catalyzed by HisF alone under high ammonia concentration, represents a good example to understand how primitive biosynthetic pathways occurred by using compounds available in primordial environments (Vazquez-Salazar et al., 2018). In 2014, Reisinger et al. predicted and reconstructed the presumed ancestral architectures of HisF and HisH proteins in LUCA, called LUCA-HisF and LUCA-HisH, respectively. The predicted crystal structure of LUCA-HisF revealed several similarities with the current HisF, as the $(\beta/\alpha)_8$-barrel fold, the internal symmetry and the formation of a stable 1:1 complex with HisH. Indeed, they observed that LUCA-HisF might have bound LUCA-HisH with high affinity, suggesting that the enzymatic complex predated the LUCA era, about 3.5 billion years ago (Reisinger et al., 2014).

4.2. Cellular division

Studies performed by Casadesús and coworkers on *S. typhimurium* histidine constitutive mutants (His[c]) showed that histidine biosynthetic enzymes may be involved in the mechanism of bacterial cellular division. Indeed, in *S. typhimurium* the constitutive expression of histidine operon has pleiotropic effects, among which a strong filamentation that seems to be triggered by overexpression of *hisF* and *hisH* (Gibert and Casadesús, 1990). In fact, the overproduction of HisF and HisH is responsible for a wrinkled morphology of the colonies, as a direct consequence of extensive cell filamentation (Cano et al., 1998). The formed filaments are similar in morphology, with evenly spaced nucleoids and no septum formation, suggesting the absence of relation with chromosome partition or DNA synthesis (Cano et al., 1998). These phenotypic changes are caused directly by increased levels of HisF and HisH and not by their increased activity in histidine biosynthesis (Flores et al., 1993). Moreover, this division inhibition mechanism is *sulA*-independent, and it is not regulated by the SOS system: mutations affecting the two histidine genes cause the suppression of filamentation and wrinkledness (Gibert and Casadesús, 1990). The fact that inhibition of cell division does not involve the metabolic flux through the histidine biosynthetic pathway, suggests that *hisF* and *hisH* gene products take part to a process unrelated to their IGPS activity (Cano et al., 1998). This is further supported by the observation that certain missense mutations in *hisF* cause histidine auxotrophy but do not suppress the pleiotropic response (Mouslim et al., 1998). As reported before, one of the product of HisF and HisH enzymatic activity is AICAR, an intermediate of purine biosynthesis that is also a precursor of the potential mutagen 5-amino-4-imidazole carboxamide riboside 59-triphosphate (ZTP) nucleotide, a possible substrate for DNA and RNA polymerases. Therefore, it was hypothesized that the phenotypic effects may be derived from AICAR biosynthesis, because of the incorporation of dZTP in DNA that, as a consequence, would inhibit cell division (Flores et al., 1993). However, this scenario is not plausible because *S. typhimurium* deprived of AICAR presents the same pleiotropic effects associated to *his* operon overexpression (Flores et al., 1993). Genes that could trigger

the pleiotropic response when HisF and HisH are overproduced have been identified: this allowed the identification of novel *loci* (*e.g. sfiX, rfe*, and *metN*) on the *S. typhimurium* chromosome that resulted unrelated to histidine biosynthesis. However, data obtained suggested that the pleiotropic response of His[c] mutants involves a complex network of pathways whose ultimate physiological significance remains unknown (Flores and Casadesús, 1995; Mouslim et al., 1998). Further experiments showed that inhibitors of penicillin-binding protein 3 (PBP3) induce a filamentation pattern identical to that of His[c] strains, and that the septation defect may be due to the scarcity of PBP3 substrate (Cano et al., 1998). Filamentation of *S. typhimurium* SL1344 has been also observed in the human melanoma cell line MelJuSo. Indeed Henry et al. (2005) discovered that a *hisG* mutation was necessary to trigger SL1344 intracellular filamentation in a HisF:HisH-dependent manner. However, they could not observe inhibition of bacterial division leading to filamentation *in vitro*, suggesting that *hisG* mutation is not sufficient to block *S. typhimurium* division and that other factors of the intracellular environment are also required to inhibit SL1344 division (Henry et al., 2005).

Similar results were also obtained for *E. coli*, in which the overproduction of histidine enzymes causes pleiotropic effects very similar to those observed in *S. typhimurium*. Filamentation due to the overproduction of HisF and HisH was observed also in a *hisG* mutant, in which the substrate for these two enzymes is absent. In agreement with the studies on *S. typhimurium* (Mouslim et al., 1998), authors found out that filamentation does not require either the normal substrate or products of the overproduced histidine enzymes and must reflect another activity (Frandsen and D'Ari, 1993; Fox et al., 1993).

The nature of the hypothetical additional activity of HisF:HisH heterodimeric complex is still unknown. The simplest hypothesis is that IGPS performs a second enzymatic reaction that alters cell metabolism if it works at high levels; indeed, like other enzymes, it may have a broad specificity leading to a reaction that perturbs the cell equilibrium and becomes problematic above a critical level. However, the possibility that IGPS second role might be structural cannot be *a priori* completely ruled out (Mouslim et al., 1998).

4.3. Pathogenesis

As reported by a recent study conducted by Martínez-Guitián et al. (2019) the *hisF* gene seems to be involved in *Acinetobacter baumannii* virulence during pneumonia infection. In this work, the authors found out that, among all the histidine biosynthetic genes, only *hisF* was overexpressed in the lungs of mice affected by *A. baumannii*-associated pneumonia; moreover, the lack of the *hisF* gene in auxotroph mutant strains led to a loss of virulence, inducing a sub-lethal infection. Contrary to *hisF*, in the *in vivo* transcriptomic analysis the expression level of the *hisH* gene remained unaltered. Results suggested that HisF participates in the early and acute inflammatory responses and host defense against *A. baumannii* infection, inhibiting the initiation of the innate immunity and the inflammatory responses in lungs. Data obtained in that study suggested that conversion of PRFAR in IGP and AICAR by the dimeric complex HisF:HisH plays a key role in pneumonia caused by *A. baumannii*. Indeed, AICAR expression, which is involved in AMP-activated protein kinase (AMPK) phosphorylation, can be used by bacteria to reduce the host immune response and to favor infection. Many studies reported the AICAR inhibitory effect on inflammatory response (Giri et al., 2004; Jhun et al., 2004; Peairs et al., 2009; Yang et al., 2012; Martin et al., 2019).

To the best of our knowledge, this is the first study describing a role for HisF in the innate immune response in lungs during bacterial pathogenesis. Thus, future studies of lung infections caused by *A. baumannii* could focus on the role of *hisF* gene, which might be considered as a potential target for developing new antimicrobial therapies (Martínez-Guitián et al., 2019).

4.4. Nodulation

Histidine biosynthesis appears to be involved also in nodule development in symbiotic nitrogen fixing bacteria. Nodule formation consists in the bacterial infection of root hairs of *Leguminosae* family plants; then, bacteria grow inside a network of tubular structures and are released through endocytosis into the cytoplasm of the host cells. Intracellular bacteria, called bacteroids, show physiological and/or morphological changes, such as the activation of the nitrogenase complex making the plant able to transform atmospheric nitrogen into ammonia (Ferraioli et al., 2002).

Purine auxotrophs of various legume microsymbionts species are defective in symbiosis, showing non-infective and non-nodulating phenotypes. Nodulation was not enhanced by supplying purines or purine nucleosides (Noel et al., 1988); however, the addition of AICA (5-aminoimidazole-4-carboxamide) riboside, the unphosphorylated derivative of the purine precursor AICAR, significantly enhanced nodule development. Moreover, AICA was unable to promote infection if the purine auxotroph was also defective in converting AICA to AICAR (Newman et al., 1992). It was hypothesized that a specific factor, necessary for infection, is derived from AICAR through a pathway distinct from purine biosynthesis (Newman et al., 1994).

Other studies suggested that a perturbation of the histidine biosynthetic pathway can negatively interfere with bacterial symbiotic ability. Histidine auxotrophs can be symbiotically defective and not able to form nodules (Sadowsky et al., 1986; So et al., 1987; Malek, 1989; Yadav et al., 1998). Yadav et al. (1998) hypothesized that intermediates of histidine biosynthetic pathways may have critical roles in symbiosis, instead of the end product, *i.e.* histidine. Ferraioli et al. (2002) performed the isolation and characterization of amino acid-requiring mutant strains of *Rhizobium etli*. They obtained a histidine auxotroph mutant, with alterations in nodules development, containing a Tn5 transposon insertion in a *locus* encoding an ORF highly similar to the C-terminal portion of the *hisA* gene product of many bacteria; downstream to this ORF, a second one highly similar to the IGPS cyclase subunit (HisF) was identified.

Thus, the whole body of data reported above, *i.e.* i) the involvement of histidine biosynthesis in symbiosis ability, ii) the presence of a mutation in *hisA* (which is located upstream *hisF*, and thus a mutation in *hisA* could influence the expression of the downstream ORF, and whose product catalyzes the reaction before that of IGPS) in a histidine-requiring symbiotically-defective mutant, and iii) the importance of AICAR (one of the two products of the HisF:HisH complex) in promoting infection in purine auxotrophs, suggests a possible role of HisF, either as part of the IGPS holoenzyme or alone, in the nodulation of legume microsymbionts.

5. Conclusions

The emergence and evolution of biosynthetic pathways represented a crucial step in molecular and cellular evolution, and the ever-increasing availability of complete genome sequences allows a better understanding of the molecular mechanisms at the basis of the evolution of metabolic pathways (Fani and Fondi, 2009). Histidine biosynthesis represents an excellent model for this kind of studies, thanks to i) its antiquity, ii) the wide variety of molecular mechanisms responsible for the evolution of *his* genes, iii) its central role in cellular metabolism, and iv) the different gene organizations and structures exhibited by the prokaryotic and eukaryotic histidine synthesizing (micro)organisms (Alifano et al., 1996; Fani et al., 1998). From an evolutionary viewpoint the *hisF* gene is a paradigmatic example of the different mechanisms that have been involved in the building of complex genes (gene elongation, gene duplication, domain shuffling, and gene fusion) starting from much shorter and simple ancestral ones, the so-called starter types (Lazcano and Miller, 1994). Moreover, the findings that two *his* genes, *hisA* and *hisF* (belonging to the same
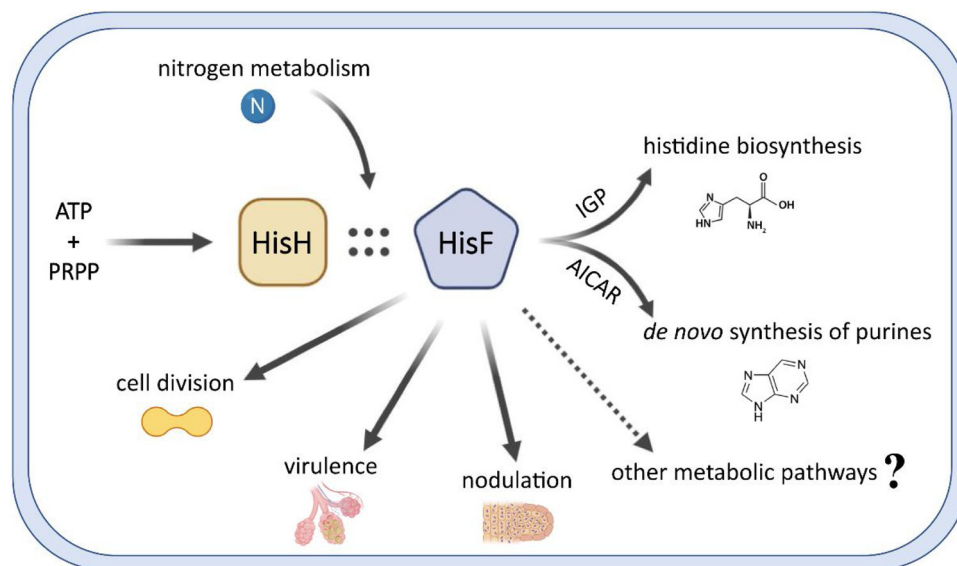
Fig. 4. Involvement of HisF in different bacterial metabolic processes.

metabolic pathway and often to the same histidine biosynthetic operon) are paralogous and that the reaction catalyzed by HisF can occur in the absence of its counterpart (HisH), demonstrate how different trajectories suggested for the origin and evolution of metabolic pathways (*i.e.* the semi-enzymatic, the patchwork, and the retrograde evolution hypothesis) can be resumed in a single gene. Moreover, HisF represents also a clear link between an evolutionary mechanism (gene elongation) and the biological significance of such event, *i.e.* the stabilization of protein subunits.

The product of *hisF* plays a crucial role in cellular metabolism connecting histidine biosynthesis with both the nitrogen metabolism and the *de novo* synthesis of purines. Indeed, its product, together with HisH, forms the heterodimeric holoenzyme IGPS, which catalyzes this cross-road metabolic reaction. Besides, HisF, either as part of the IGPS complex or alone, seems to be involved also in other bacterial metabolic processes, not directly related to the histidine biosynthetic route. Indeed, i) the overexpression of *hisF* and *hisH* appears to trigger an inhibition of cellular division in *E. coli* and *S. typhimurium*, ii) an overexpression of *hisF* gene seems to be related to *A. baumannii* virulence during pneumonia infection in mice, and iii) *hisF* may also have a role in infection and nodule development in legume microsymbionts (Fig. 4). Moreover, it cannot be *a priori* excluded the possibility that HisF might play a role also in other and still undisclosed metabolic pathways.

The whole body of data obtained on *hisF* and its product strongly suggests the possibility that HisF might be endowed with one (or more) additional function(s), besides its involvement in histidine biosynthesis. These data are in agreement with the notion of how cellular metabolism can be intricate and how apparently distinct pathways could be, otherwise, finely interconnected. It is not still clear whether HisF may perform the same (or different) additional function(s) in all the histidine synthesizing organisms. If the same additional function is performed in different organisms, it should be involved in different metabolic schemes: virulence in *A. baumannii*, nodulation in nitrogen-fixing symbiotic bacteria, cell division in *E. coli* and *S. typhimurium* and maybe other microorganisms. It is also intriguing the finding that if HisF is actually endowed with this additional function, this should be related to histidine biosynthesis, since the *hisF* gene has been disclosed only in histidine-synthesizing (micro)organisms. If this is so, histidine biosynthesis might represent a metabolic cross-road interconnecting more metabolic routes than previously thought thanks to the cyclase activity performed by HisF. Further researches performed using the omics techniques (including phenomics, transcriptomics, and

proteomics) might help in shading light on this issue.

Lastly, the finding that HisF is involved in *A. baumannii* virulence might have an interesting significance from a biotechnological and/or pharmaceutical viewpoint, since it might pave the way to the design of new synthetic drugs targeted toward this enzyme to fight clinically relevant bacterial infections.

CRediT authorship contribution statement

Sofia Chioccioli: Conceptualization, Investigation, Visualization, Writing - original draft, Writing - review & editing. **Sara Del Duca:** Conceptualization, Investigation, Visualization, Writing - original draft, Writing - review & editing. **Alberto Vassallo:** Visualization, Writing - review & editing. **Lara Mitia Castronovo:** Visualization, Writing - review & editing. **Renato Fani:** Conceptualization, Investigation, Project administration, Supervision, Visualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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