

# Iron uptake regulation in *Pseudomonas aeruginosa*

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**Abstract** The *Pseudomonas* genus belongs to the  $\gamma$  division of Proteobacteria and many species produce the characteristic yellow–green siderophore pyoverdine, and often a second siderophore, of lower affinity for iron. These bacteria are known for their ability to colonize different ecological niches and for their versatile metabolism. It is therefore not surprising that they are endowed with the capacity to take up exogenous xenosiderophores via different TonB-dependent receptors. Uptake of iron is controlled by the central regulator Fur, and via extracytoplasmic sigma factors or other types of regulators (two-component systems, AraC regulators). In this review the Fur regulon (experimentally proven and/or predicted) of *P. aeruginosa* will be presented. An interesting feature revealed by this analysis of Fur-regulated genes is the overlap between the iron and the sulfur regulons as well with the quorum sensing system.

**Keywords** *Pseudomonas* · Iron · Fur · ECF sigmas · Regulators · Receptors

## The *Pseudomonas* Fur regulon

The ferric uptake regulator (Fur) is a conserved protein in different Gram-negative bacteria, which works as a repressor of iron uptake genes (siderophore biosynthesis, receptors) when bound to its co-repressor  $\text{Fe}^{2+}$  (Escobar et al. 1999). Fur is probably an essential protein in *P. aeruginosa* since no *fur* deletion mutant could be obtained in this bacterium (Vasil and Ochsner 1999). Two transcriptome studies revealed that many genes are iron-regulated in *P. aeruginosa* (Ochsner et al. 2002; Palma et al. 2003). Fur can directly control some genes involved in iron uptake, or indirectly, via extracytoplasmic sigma factors (ECF  $\sigma$ ) or via other regulators. One of these ECF  $\sigma$  is PvdS which not only controls the transcription of pyoverdine biosynthesis genes, but also of virulence genes such as the gene encoding exotoxin A or the extracellular protease PrpL (for a recent review, Visca et al. 2007). Fur also controls the transcription of two small RNAs, PrrF1 and PrrF2 (Wilderman et al. 2004; Oglesby et al. 2008). Recently, we described a new algorithm to search for Fur binding sites in *P. aeruginosa* (van Oeffelen et al. 2008). This analysis not only confirmed the data obtained in the two

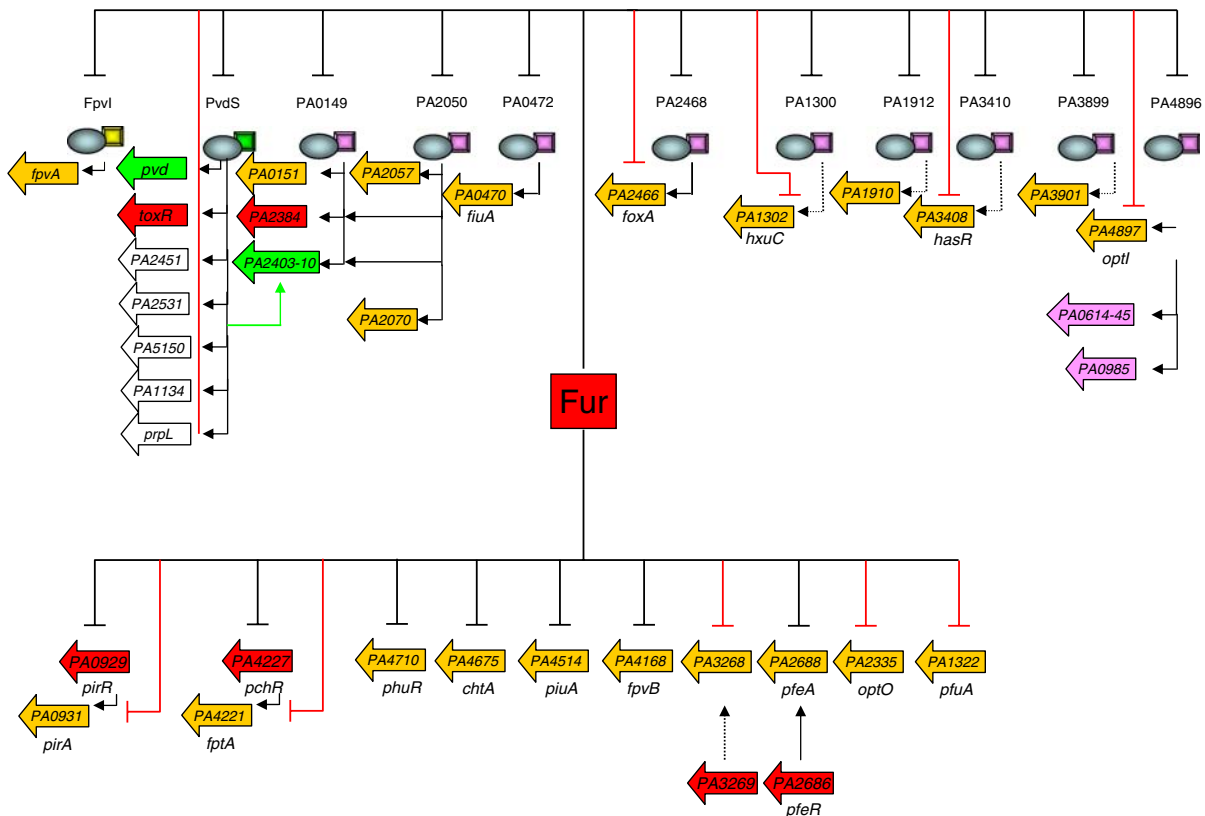
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ECF  $\sigma$  factors

Fur-regulated by van Oeffelen et al. (2008) are PA0149, PA0472 (*fiuI*), PA1300, PA1912, PA2050, PA2387 (*fpvI*), PA2426 (*pvdS*), PA2468 (*foxI*), PA3410 (*hasR*), and PA3899 (*fecA*) and are shown in Fig. 1 and Table 1. Among these genes, some were already known to be Fe-regulated, including *pvdS*, and *fpvI*, coding for ECF  $\sigma$  involved in the transcription of pyoverdine biosynthesis genes and the TonB-dependent ferripyoverdine receptor gene *fpvA*, respectively (Ravel and Cornelis 2003; Visca et al. 2007). Llamas et al. (2006) demonstrated that the FiuI ECF  $\sigma$  (encoded by PA0472) controls the expression of the FiuA receptor for ferrichrome (encoded by PA0470) and that the FoxI ECF  $\sigma$  is needed for the expression of the *foxA* gene encoding a ferrioxamine receptor. Confirming the presence of predicted Fur-binding sites upstream of these genes,



are those present in the pyoverdine locus (Ravel and Cornelis 2003). Arrows represent activation while broken lines represent repression. Black lines represent experimentally verified interactions while those in red are predicted by the van Oeffelen et al. (2008) analysis. Stippled lines are suggested, but not experimentally confirmed, interactions

PA0472, PA1300, PA2468, PA3410, and PA3899 were confirmed to be iron-repressed in the transcriptome analysis of iron-responsive genes of Ochsner et al. (2002) and/or Palma et al. (2003). The ECF  $\sigma$  encoded by PA0149, PA1912, and PA2050 did however not show up in these two microarray analysis (Ochsner et al. 2002; Palma et al. 2003). Interestingly, PA1911, encoding a trans-membrane sensor and downstream of PA1912, was found to be iron-regulated by Palma et al. (2003) and the PA1910 genes encodes a putative TonB-dependent receptor, suggesting that the PA1912 ECF  $\sigma$  controls the expression of this putative siderophore receptor. In their recent report, Llamas et al. (2008) investigated the regulons of four ECF  $\sigma$  in *P. aeruginosa*, encoded by PA0149, PA2050, PA2093, and PA4896, respectively. Among these, only PA2093 is not predicted to be Fur-regulated according to the analysis done by van Oeffelen et al. (2008) nor was it found to be iron-regulated by Ochsner et al. (2002) or Palma et al. (2003). The PA2093 encoded ECF  $\sigma$  was found to control the expression of two TonB-dependent receptor genes, PA2089, and PA2590 also not predicted to be Fur-dependent and not found to be iron-regulated (Ochsner et al. 2002; Palma et al. 2003), suggesting that these two TonB-dependent receptors are not involved in the uptake of siderophores. The PA0149 encoded ECF  $\sigma$  controls the expression of the PA0151-encoded TonB-dependent receptor, but also of the PA2403-2410 gene cluster encoding components of a transport system (Ravel and Cornelis, 2003; Llamas et al. 2008). Interestingly, the same operon is present in the pyoverdine cluster and is also regulated by PvdS (Ravel and Cornelis 2003; Visca et al. 2007) and by PA2050 (Llamas et al. 2008), which means that no less than three ECF  $\sigma$  control the expression of this operon. The PA2050 ECF  $\sigma$  also regulates the expression of two TonB-dependent receptors, encoded by PA2057 and PA2070, respectively (Llamas et al. 2008). The same authors also found that the PA4896 ECF  $\sigma$  regulon comprises the PA4897 *optI* gene encoding a TonB-dependent receptor, and a large cluster of genes (PA0614-PA0645) encoding phage and pyocin genes, and the gene for pyocin S5 (PA0985). In their *in silico* analysis, van Oeffelen et al. (2008) also found putative Fur-binding sites before four genes encoding TonB-dependent receptors (PA2466 [*foxA*], PA1302 [*hxC*], PA3408 [*hasR*], and PA4897 [*optI*]),

which are known or supposed to be ECF  $\sigma$ -dependent. This puzzling and interesting observation suggests that the ECF  $\sigma$  regulation superimposed on the Fur regulation at a later stage in the evolution.

#### Other regulators of TonB-dependent receptors

In the previous section, we saw that the expression of 11 TonB-dependent receptor genes are indirectly regulated by Fur via ECF  $\sigma$  factors while for 4 of them a “residual” Fur-binding site was found upstream of their genes. Another 10 genes encoding TonB-dependent receptors are predicted to be Fur-regulated (van Oeffelen et al. 2008). Four genes (PA0931 [*pirA*], PA4221 [*fptA*], PA3268, and PA2688 [*pfeA*]) are under the control of regulators (Fig. 1). This is the case for *fptA* encoding the ferripyochelin receptor, which is under the control of the AraC regulator PchR (Michel et al. 2005). Likewise, PA3269 encodes a putative AraC regulator and could potentially regulate PA3268 receptor gene. It is interesting to mention that both in the case of *fptA* and PA3268 a putative Fur binding site is predicted upstream of these genes (van Oeffelen et al. 2008), a situation similar to what we described in the previous section for some ECF  $\sigma$ -controlled TonB-dependent receptor genes, again suggesting that the AraC regulators superimposed on the original Fur regulation (Fig. 1 and Table 1). Two genes, *pirA* (PA0931) and *pfeA* (PA2688) each encoding a ferrienterobactin receptor (Ghysels et al. 2005), are regulated by two-component systems (Dean and Poole 1993; Dean et al. 1996), their respective response regulators being PA0929 and PA2686 (*pfeR*). Six TonB-dependent receptor genes (PA1322 [*pfuA*], PA2335 [*optO*], PA4168 [*fvpB*], PA4514 [*piuA*], PA4675 [*chtA*], and PA4710 [*phuR*]) seem to be directly regulated by Fur. Repression of *fvpB* by iron was confirmed by RT-PCR (Ghysels et al. 2004). FvpB (encoded by PA4168) is a second type I pyoverdine receptor that, together with the main receptor FpvA, takes up ferripyoverdine in *P. aeruginosa* PAO1 and in a majority of other *P. aeruginosa* strains (Ghysels et al. 2004). ChtA was found to be a receptor for the siderophores aerobactin and rhizobactin and its gene confirmed to be iron-regulated by O’ Cuív et al. (2006). All these receptor genes were previously confirmed to be iron-regulated (Ochsner et al. 2002; Palma et al. 2003),

**Table 1** List of ECF  $\sigma$  factors and TonB-dependent genes in *P. aeruginosa* and their regulators

$\sigma$	Anti- $\sigma$	Receptor	Receptor	Regulator	Receptor	
▼ PA3889	PA3890	<i>fecA</i>	S▼ <i>optO</i>	▼ PA2334	▼ <i>optH</i>	▼ <i>piuA</i>
▼ PA3410	PA3409	▼ <i>hasR</i>	▼ <i>fptA</i>	▼ <i>pchR</i>	▼ <i>pfuA</i>	
▼ PA2468	PA2467	▼ <i>optS</i>	▼ <i>pirA</i>	▼ <i>pirR</i>	▼ PA3268	
▼ PA0149	PA0150	▼ PA0151	SPA0192	▼ PA0191	▼ <i>fpvB</i>	
▼ PA1300	PA1301	▼ <i>hxcC</i>	PA2057	▼ PA2056	▼ <i>feuA</i>	
▼ <i>fpvI</i>	<i>fpvR</i>	<i>fpvA</i>	PA0781	▼ PA0780	▼ PA4156	
▼ PA1912	PA1911	PA1910	<i>fecA</i>	▼ PA3900	▼ PA4710	
▼ PA0472	PA0471	<i>fiuA</i>			▼ <i>pfeA</i>	
▼ <i>optI</i>	▼ PA4896	PA4895				
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$\sigma$	Not iron regulated					
▼ <i>pvdS</i>			PA1613	PA4837 <i>oprC</i>		
S▼ PA2050-PA2051			SPA2089	PA2289		
▼ PA2896-PA2895			PA2590	PA0434		
			PA2911	<i>btuB</i>		

The ▼ sign means that a Fur binding site has been detected upstream of the gene (van Oeffelen et al. 2008). The S in bold means that the gene is regulate by sulfate starvation (Tralau et al. 2007). See also Fig. 1 for complementary information

with the exception of PA2335 (*optO*) and PA1322 (*pfuA*). Taking into account the genes that are directly or indirectly Fur-regulated, we come to a total of 21 TonB-dependent receptors, including three involved in heme uptake (PA1302 [*hxcC*], PA3408 [*hasR*], and PA4710 [*phuR*]). It is tempting to think that the PhuR heme uptake system is the most primitive because it is regulated only by Fur while the two other systems (HxcC and HasR) are both ECF  $\sigma$ -dependent. An interesting observation made by Llamas et al. (2008) is the regulation by two ECF  $\sigma$  (PA0149 and PA2050) of the PA2384 gene encoding a Fur-like regulator, which is important for the expression of iron-uptake genes, including the pyoverdine, pyochelin and heme uptake genes (Zheng et al. 2007). Inactivation of the PA2384 regulator also results in a higher expression of the genes for the biosynthesis of the quorum sensing signal molecule PQS (Pseudomonas quinolone signal).

Other iron uptake and storage genes predicted to be Fur-regulated

Siderophore-mediated iron uptake in Gram-negative bacteria such as *P. aeruginosa* does not only involve outer membrane receptors, TonB and ExbB/ExbC, but also, by analogy with *E. coli*, other transporters in the periplasm and in the cytoplasmic membrane

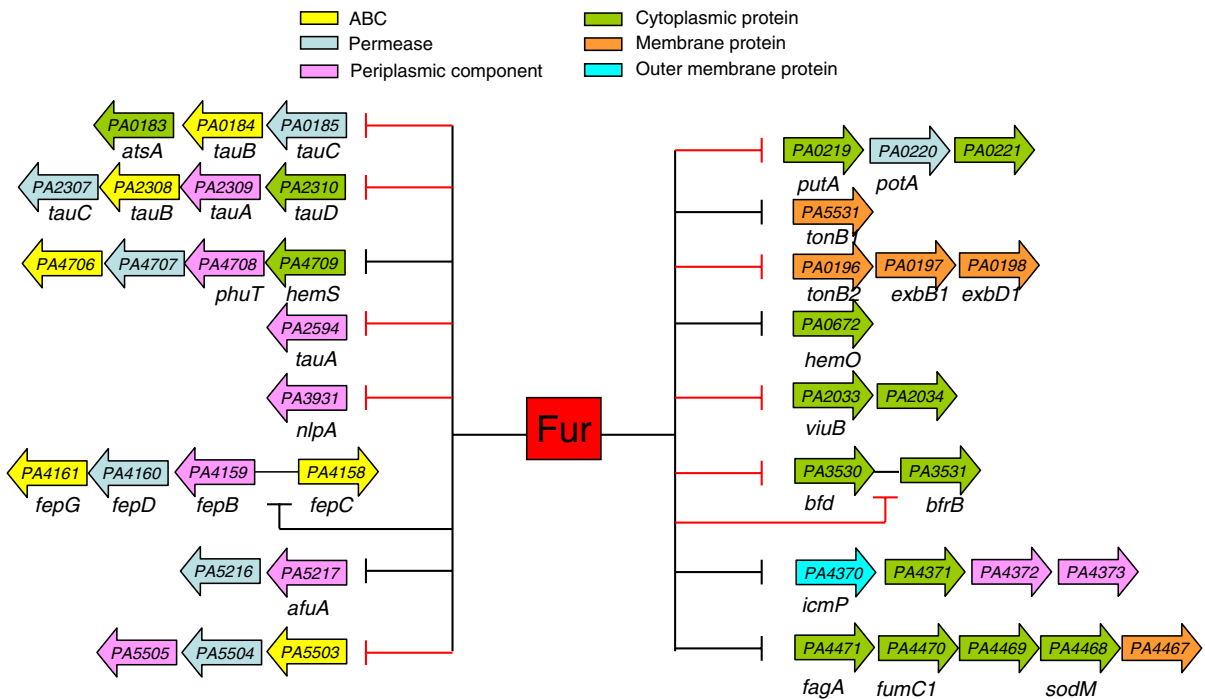
(Andrews et al. 2003). Figure 2 shows the predicted Fur-regulated genes that encodes iron uptake and storage genes. In *P. aeruginosa* there are three genes encoding TonB-like proteins (Poole et al. 1996; Zhao and Poole 2000, 2002; Huang et al. 2004). Only TonB1 (encoded by PA5531) was confirmed to be important for iron uptake. The *tonB1* gene is monocistronic while the two other *tonB* genes (PA0197 and PA0695) are in an operon structure together with *exbB* and *exbC* genes. The PA5531 gene (*tonB1*) was already known to be iron-regulated (Ochsner et al. 2002; Palma et al. 2003), but this was not described for *tonB2* although a Fur binding site is predicted upstream of the gene (van Oeffelen et al. 2008). This finding is in agreement with the fact that a *tonB2* mutation aggravates the inability of a *tonB1* mutant to take up iron (Zhao and Poole 2000). Interestingly, this PA0197-PA0199 cluster (*tonB2-exbB1-exbD1*) is also strongly up-regulated by sulfate starvation (Tralau et al. 2007). Among other known Fur-regulated genes (Ochsner et al. 2002; Palma et al. 2003), which are also predicted by van Oeffelen et al. (2008), we find PA0672 that encodes the HemO heme oxygenase, the genes coding for the Bfd and BfrB proteins (PA3530, PA3531), the PA4370-4373 cluster (*icmP*, PA4371, PA4372, PA4373) and the PA4471-4467 cluster (*fagA*, *fumC1*, PA4469, *sodM*, PA4467). A second Fur binding site is predicted in

the intergenic region between *bfd* and *bfrB* (van Oeffelen et al. 2008). The *viuB* gene (PA2033) was predicted to be iron-regulated by the two independent microarray studies of Ochsner et al. (2002) and Palma et al. (2003), and a Fur binding site is well predicted to be present upstream of the gene (van Oeffelen et al. 2008). In *Vibrio cholerae*, ViuB is a cytoplasmic protein found to be needed for the utilization of the catecholate siderophore vibriobactin, although a *viuB* mutation did not affect the production of vibriobactin (Butterton and Calderwood 1994). It is therefore likely that in *P. aeruginosa* ViuB is involved in the uptake of catecholate siderophores like in *V. cholerae*. Contrarily to the situation in *V. cholerae* where the *viuB* gene is monocistronic, there is another gene downstream of the *P. aeruginosa viuB* (PA2034) encoding a precorrin methylase. An operon for heme transport and degradation (*hemS-phuT*-PA4707-PA4706) is also predicted to be Fur-regulated, confirming the previous observations of Ochsner et al. (2002) and Palma et al. (2003). The *fepBDG* operon (PA4159-PA4161) is transcribed divergently from the *fepC* gene (PA4158) encoding an ABC transporter presumably involved in the transport of ferric enterobactin by analogy with the *E. coli fep* system (Shea and McIntosh 1991). The *fepC* gene was found to be iron-regulated in the Ochsner et al. study (2002). The two microarray analysis of Ochsner and Palma demonstrated that the PA5217 (*afuA*)-PA5216 operon is iron-regulated as well, corresponding to our prediction that it is Fur-regulated. AfuA has been described as a periplasmic iron-binding protein in the periodontal pathogen *Actinobacillus actinomycetemcomitans* (Willemsen et al. 1997). Interestingly, there is another periplasmic iron binding protein coding gene in the *P. aeruginosa* genome, *hitA* (PA4687), which was found to be iron-regulated in the analysis of Palma et al. (2003), but not predicted to be Fur-dependent (van Oeffelen et al. 2008). AfuA and HitA show 48% similarity and the genes downstream of *afuA* and *hitA* encode similar proteins as well. Probably, the regulation of *afuA* and *hitA* differs, one being directly under the control of Fur while the other gene is maybe under the control of a Fur-regulated transcriptional regulator. Our *in silico* analysis also predicted three so far non-described transport systems to be Fur-regulated: PA5503-PA5505, PA3931 (*nlpA*), and PA2594 (*tauA*). TauA is a periplasmic protein binding taurine

(2-aminoethanesulfonic acid) and was found to be up-regulated by sulfate starvation in *E. coli* (van der Ploeg et al. 1996). Two other gene clusters encoding transporters are the PA0185-PA0183 and the PA2310-PA2307 operons which were also found by Tralau et al. (2007) to be up-regulated by sulfate starvation and which correspond to transport of sulfonates. These observations show that there is an interesting overlap between the response to sulfate and iron starvation in *P. aeruginosa*.

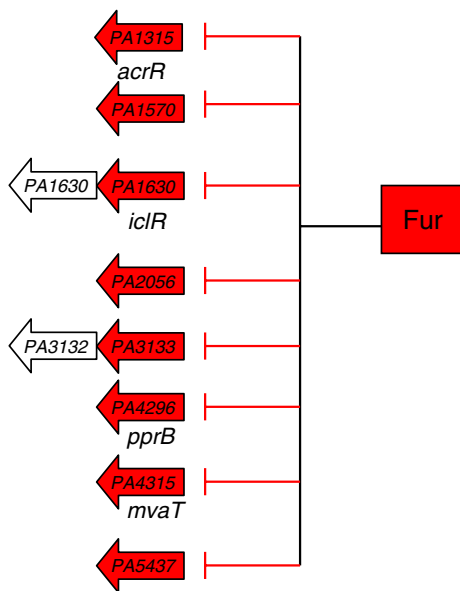
#### Other regulators not in the vicinity of TonB-dependent receptor genes

Fur binding sites are also predicted upstream of genes encoding regulators other than ECF  $\sigma$  factors (van Oeffelen et al. 2008). Figure 3 shows the regulators that are predicted to be Fur-regulated. PA1315 encodes a TetR regulator flanked by two genes (PA1313 and PA1316) encoding MFS drug efflux systems. PA1570 is a regulator belonging to the LysR family and is also flanked by a gene (PA1569) encoding a MFS transporter. PA1630 encodes an IclR type of regulator and is probably the first of two genes, the gene downstream (PA1631) encoding an acyl-CoA dehydrogenase. PA2056 is also encoding a LysR regulator, and the gene is flanked by another gene (PA2055), transcribed in the opposite orientation, and encoding a MFS protein (like in the case of PA1315 and PA1570). Another possible target for the putative PA2056 regulator is PA2057, encoding a TonB-dependent receptor, which is also regulated by the already mentioned ECF  $\sigma$  factor PA2050. PA3133 encodes an AcrR regulator (same family as PA1313) and the gene is in an operon with PA1332 encoding a putative peptidyl hydrolase. PA4296 encodes a putative response-regulator with a CheY domain. This gene is found next to the *tad* operon involved in the biogenesis of Flp type pili. MvaT, encoded by PA4315, is a global regulator which modulates quorum sensing and is involved in the regulation of *cup* gene clusters for the assembly of type IV pili (Diggle et al. 2002; Vallet et al. 2004). Interestingly the pyochelin biosynthesis genes *pchF*, *pchE*, and *pchA* were found to be down-regulated in an *mvaT* mutant (Vallet et al. 2004), suggesting a link between MvaT and iron metabolism. Finally, PA5437 encodes a putative LysR regulator.



**Fig. 2** Genes involved in iron uptake and transport and storage predicted (red and black broken lines) to be Fur-regulated. Red arrows indicate non-experimentally proven

interactions that are suggested by the analysis of van Oeffelen et al. (2008). The categories of encoded protein are color coded as indicated in the figure. See text for details



**Fig. 3** Regulator genes likely to be under Fur control according to the in silico analysis of van Oeffelen et al. (2008). See text for details

### Regulation by small RNAs

Two small RNAs, PrrF1 and PrrF2, are known to be regulated by Fur (Wilderman et al., 2004). PrrF1 and PrrF2 have a negative effect on the transcription of several genes which are induced by high iron conditions (Wilderman et al. 2004; Oglesby et al. 2008). Several of these iron-induced genes are also de-repressed in a  $\Delta prrF1,2$  mutant grown in low iron conditions (Oglesby et al. 2008). Among them is the gene encoding the Fe-superoxide dismutase (*sodB*), Fe-aconitase A (*acnA*) and succinate dehydrogenase (*sdhCDAB*) as well as other TCA cycle enzymes encoding genes (Vasil 2006). Other genes that are regulated by these two small RNAs are the *antAB* and *catAB* genes for the degradation of anthranilate (Oglesby et al. 2008). Interestingly, anthranilate is the precursor of the above mentioned Pseudomonas quinolone signal molecule (PQS), an important quorum sensing molecule (Diggle et al. 2006), establishing again a link between quorum sensing, virulence and the iron super regulon.



# Conclusion

In *P. aeruginosa* Fur is a global regulator of many genes involved in iron uptake and storage, either directly or indirectly via another regulators belonging to the families of ECF s factors, AraC or other types of regulators. Several genes are regulated by a combination of these regulators. Quite a few genes are predicted to be Fur-regulated, but were not described as iron-regulated in the two published transcriptome analysis from Ochsner et al. (2002) and Palma et al. (2003), suggesting that iron availability is not the sole factor controlling their expresión (Ghysels et al. 2004). Another interesting aspect is the cross-talk between quorum sensing (in particular the PQS system) and the iron regulon, and between the sulfate and iron starvation responses.

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