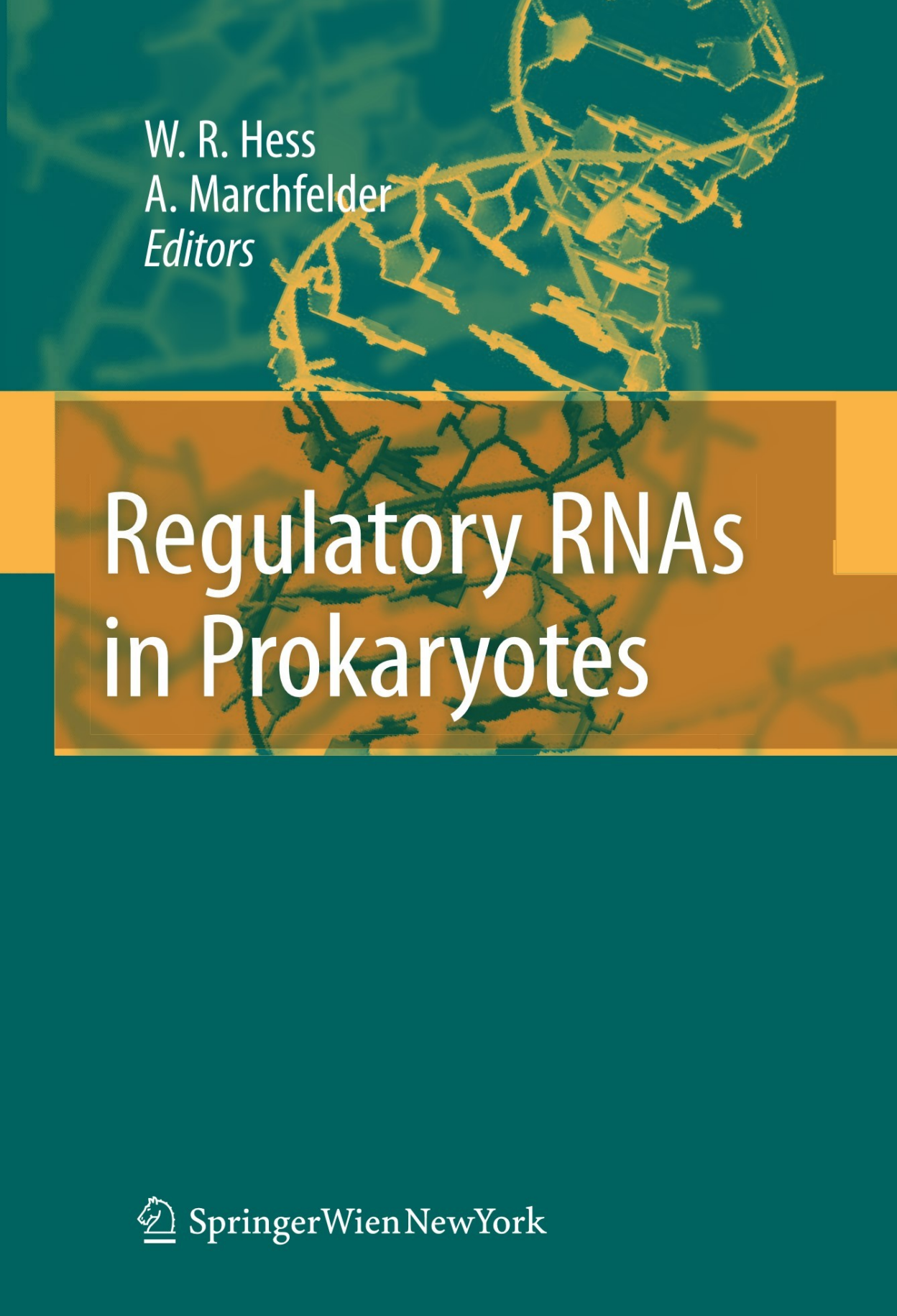
W. R. Hess
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Editors
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Editorial

Regulatory RNAs in Prokaryotes

RNA molecules play a central role in gene regulation in all three domains of life. Regulatory RNAs were originally discovered in prokaryotes as early as 1967. Fundamental mechanisms of how these molecules exert their functions were first analyzed in bacteria long before small RNAs were discovered as regulatory molecules in eukaryotes. Research on regulatory RNA in prokaryotes occurred in three major phases.

The first phase started in 1967, when Hindley (1967) identified an RNA species, later named 6S RNA, as a distinct and abundant RNA species in *E. coli*. In pioneering work four years later, its sequence and putative secondary structure were published (Brownlee, 1971). However, several decades passed before 6S RNA function in regulating RNA polymerase activity was determined (Wassarman and Storz 2000). Another enterobacterial regulatory RNA reported early on was the Spot 42 (spf) RNA (Ikemura and Dahlberg 1973). Discovering that the *spf* gene is regulated by the cAMP–CRP system (Sahagan and Dahlberg 1979) and the phenotypic consequences of its overexpression (Rice and Dahlberg 1982) suggested its functional relevance. However, a biological role was determined almost 40 years later, when its significant complementarity to the region around the start codon of the *galK* gene was noticed and its role in discoordinating gene expression of the *galETKM* galactose operon became unraveled (Møller *et al.* 2002).

About the same time the first *trans*-acting regulatory RNAs were discovered, the first regulatory *cis*-antisense RNAs were identified in bacteria. These *cis*-antisense RNAs initially appeared to be a hallmark of extrachromosomal genetic elements, bacteriophages, transposons, and plasmids, controlling their life cycle or copy number. The first of these findings was the identification of antisense transcripts for the gene *cro* in bacteriophage λ (Spiegelman *et al.* 1972). This type of transcription was confirmed for bacteriophage λ when observing that overexpression of the 77 nt OOP antisense transcript leads to its codegradation with the cII mRNA (Krinke and Wulff 1987; Krinke and Wulff 1990; Krinke *et al.* 1991). By studying the plasmid-borne RNA I, another extrachromosomally located *cis*-antisense RNA, many fundamental insights were gained early on. Among those discoveries was that RNA

I regulates maturation of the ColE1 primer for DNA replication (Stougaard *et al.* 1981; Tomizawa *et al.* 1981) and is involved in the control of plasmid incompatibility of ColE1-type plasmids (Tomizawa and Itoh 1981).

In the following two decades, a small number of additional regulatory RNAs were found fortuitously. Although important regulators were discovered, such as the chromosomally encoded small RNA MicF (Mizuno *et al.* 1983, 1984), DicF (Faubladier *et al.* 1990) and OxyS (Altuvia *et al.* 1997), the fundamental importance and broad consequences of all these findings for gene regulation were not initially appreciated. In early 2001, only 12 small RNAs (including the 6S RNA, tmRNA, RNase P RNA and 4.5 S RNA) had been identified in *E. coli* (Argamann *et al.* 2001).

A new phase started in 2001 when computational searches were introduced for more complex and systematic screening. Pioneering studies on small RNA prediction in enterobacteria employed comparative genome analysis of closely related species (Wassarman *et al.* 2001), included a search for transcriptional signals in intergenic regions (Argaman *et al.* 2001), or scored the conservation of predicted RNA secondary structure rather than of primary sequence (Rivas *et al.* 2001). However, the most significant advancement was to integrate these predictions with systematic experimental screens. As result of these seminal studies, several dozens of new *trans*-acting RNAs were identified (Argaman *et al.* 2001; Rivas *et al.* 2001; Wassarmann *et al.* 2001), yielding data for their detailed functional characterization for many years.

A third phase of prokaryotic RNA research began more recently with the advent of RNA-seq technology, triggering a wave of new studies, which have been setting new standards in this field by accelerating the identification of transcripts and transcriptional start sites. Together with progress in RNA bioinformatics and experimental structure determination, new research groups entering this exciting field of research and focusing on the biochemistry, metabolism and molecular biology of RNA, spectacular new insights into the world of prokaryotic regulatory RNAs have been obtained at an unprecedented speed and resolution. To highlight these advancements, this book focuses exclusively on prokaryotic regulatory RNAs.

Current research on regulatory RNA in prokaryotes is presented here by first providing an in depth overview of *trans*- and *cis*-acting small RNAs in various groups of bacteria and archaea and their established mechanisms of action, including the effects mediated by Hfq, an interacting protein with a pivotal role in many bacteria. These chapters are followed by reviews on regulatory mechanisms involving distinct types of RNA (e.g., 6S RNA), control of bacterial heat shock and virulence genes by RNA thermometers, and functions of *cis*-acting metabolite-sensing riboswitches. One chapter is devoted to the major recent discovery of an RNA-based prokaryotic immune system. The two last chapters provide an overview on available computational approaches to predict prokaryotic regulatory RNAs and their targets based on sequence information.

In all, this book is written by leading experts in the field and presents a timely introduction that covers all aspects of prokaryotic regulatory RNAs and their functional mechanisms.

Freiburg and Ulm in May 2011 Anita Marchfelder and Wolfgang R. Hess

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Contents

Chapter 1
Small RNAs with a Role in the Oxidative Stress Response of Bacteria

Bork Berghoff and Gabriele Klug*

1 Introduction

Most bacteria have to cope with frequent changes in their environment, which generate unfavourable conditions for growth and survival. They have evolved successful strategies as a response to these stresses. Oxidative stress is a stress factor, which is critical in most bacterial habitats and has been defined as an imbalance between pro-oxidants and anti-oxidants in the cell (Storz and Zheng, 2000). Pro-oxidants are mostly reactive oxygen species (ROS) that oxidize proteins, nucleic acids and lipids and thus lead to harmful damage to the cell (Imlay, 2003). Anti-oxidants are cellular components countering these damaging effects: i) enzymes or molecules which remove ROS like peroxidases, superoxide dismutase, thioredoxin or glutathione, ii) proteins that repair the damages like endo- and exonucleases or photolyases, and iii) sensors and regulators necessary to mount the response to oxidative stress like OxyR or SoxRS of *E. coli*. ROS are generated from the ground state (triplet state) of molecular oxygen when less than four electrons are transferred to one O_2 molecule resulting in partially reduced forms of oxygen (Imlay, 2003). Such reactions are e. g. catalyzed by respiratory enzymes and lead to the accumulation of hydrogen peroxide (H_2O_2), superoxide (O_2^-) and hydroxyl radicals ($OH^\bullet$). But ROS are also produced by exposure of cells to metals, redox-active drugs or radiation. Plants as well as animals produce ROS as a defence against pathogens. In addition to electron transfer reactions, a spin conversion of one electron of the oxygen molecule can generate the reactive singlet oxygen ($^1O_2$). This reaction occurs in the combined presence of light and a photosensitizer. In nature, porphyrins (chlorophylls or protoporphyrin) and humic acids can function as photosensitizers.

The oxidative stress response of many model bacteria has been extensively studied in the past and many regulatory proteins and protein based signalling path-

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Table 1. Small RNAs with a potential role in the oxidative stress response of bacteria.

sRNA	Bacterium[a]	Length [nt]	Stress conditions	Target mRNA	Mode of regulation	Hfq interaction	References
OxyS	E.c./S.t.	109	H_2O_2	*fhlA, rpoS*	translational repression	yes	Altuvia *et al.*, 1997
RyhB	E.c./S.t. P.a./E.ch.	90	iron limitation	*fur, bfr, ftnA, sodB, iscRSUA*[b]	mRNA destabilization	yes	Massé and Gottesman, 2002
RSs0019	R.s.	298	$^1O_2$	n.k.[c]	n.k.	no[d]	Berghoff *et al.*, 2009
RSs0682	R.s.	206, (130)[e]	$^1O_2$	n.k.	n.k.	yes[d]	Berghoff *et al.*, 2009
RSs0680a RSs1543 RSs2461	R.s.	73 83 116, (75)[e]	$^1O_2$, O_2^-, heat	n.k.	n.k.	yes[d]	Berghoff *et al.*, 2009 Nuss *et al.*, 2010
MicF	E.c./S.t.	93	O_2^-, membrane perturbation[f]	*ompF*	translational repression	yes	Blanchard *et al.*, 2007 Vogel and Papenfort, 2006
MicC	E.c./S.t.	109	O_2^-, membrane perturbation[f]	*ompC, ompD*	translational repression	yes	Blanchard *et al.*, 2007 Vogel and Papenfort, 2006 Pfeiffer *et al.*, 2009
RydB	E.c.	68	O_2^-	n.k.	n.k.	n.k.[g]	Blanchard *et al.*, 2007
CyaR	E.c./S.t.	86	O_2^-	*ompX*	translational repression	yes	Blanchard *et al.*, 2007 Papenfort *et al.*, 2008
ArcZ	E.c./S.t.	120–130, (~50)[e]	n.k.	*tpx*[h]	translational repression	yes	Papenfort *et al.*, 2009
RgsA	P.f.	~120	H_2O_2	n.k.	n.k.	n.k.	Gonzalez *et al.*, 2008
Yfr1	S.e.	65	O_2^-, salt	*sbtA*	mRNA destabilization	n.k.	Nakamura *et al.*, 2007
IsrR	S.sp.	177	iron limitation, H_2O_2	*isiA*	mRNA destabilization	n.k.	Dühring *et al.*, 2006
B11 B55 F6 ASpks	M.t.	93 61 58, 102 78, (~200)[i]	H_2O_2	n.k.	n.k.	n.k.	Arnvig and Young, 2009

Explications for Table 1 *see next page*

◄ Explications for Table 1
a E.c.: *Escherichia coli*; E.ch.: *Erwinia chrysanthemi*; M.t.: *Mycobacterium tuberculosis*; P.a.: *Pseudomonas aeruginosa*; P.f.: *Pseudomonas fluorescens*; R.s.: *Rhodobacter sphaeroides*; S.e.: *Synechococcus elongatus*; S.sp.; *Synechocystis* sp. PCC6803; S.t.: *Salmonella typhimurium*
b several other targets like *acnA*, *fumA*, and *sdhCDAB*: at least 18 transcripts, encoding 56 proteins (Massé *et al.*, 2005)
c n.k.: not known
d unpublished data (Hfq co-immunoprecipitation experiments)
e length of processed fragment is shown in brackets
f Transcription of MicF is induced and MicC is repressed by the EnvZ-OmpR system
g putative RydB homolog of *Salmonella typhimurium* interacts with Hfq (Sittka *et al.*, 2008)
h other targets: *sdaCB* and STM3216
i 200-nt fragment only detectable under stress

ways have been elucidated (e. g.: Storz and Imlay, 1999; Storz and Zheng, 2000; Mongkolsuk and Helmann, 2002; Imlay, 2008). The view emerged that the components of oxidative stress response systems overlap with components of other stress response systems, e. g. the heat shock response. It is now widely accepted that we cannot assign strictly defined regulatory systems to a single stress. Instead several components contribute to the response against different stresses and only a few components are specific to a certain stress response. In this review we will focus on responses against ROS or responses affecting genes with a clear function during oxidative stress.

Considering recent advances in the knowledge of the important regulatory roles of small RNAs (sRNAs) in bacteria, it is not surprising to find that they are also part of the oxidative stress response systems. OxyS of *E. coli* was among the first sRNAs to be discovered and analyzed in detail. It links the oxidative stress response to more global responses including other stress resistances, carbon metabolism or cell morphology. In the same organism, the sRNA RyhB plays an important role in linking the response to iron to the oxidative stress response. This review will summarize our current knowledge on the biological function of these two sRNAs and the underlying mechanisms of regulation. In the case of several other sRNAs, changed levels in response to oxidative stress have been reported or they were shown to affect the resistance to ROS (see Table 1). However, their exact function and their mechanisms of action need further elucidation. We attempt to give an overview of those sRNAs and their putative functions.

2 OxyS and the Oxidative Stress Response in Enterobacteria

When studies on the oxidative stress response in enteric bacteria were initiated, the *oxyR* gene was discovered in a screen for *Salmonella* mutants that were hyperresistant to H_2O_2 (Christman *et al.*, 1985). The OxyR protein was shown to function as a redox sensor, which is oxidized at elevated levels of H_2O_2. The oxidized protein

binds to DNA target sequences and subsequently activates a small subset of genes (Storz *et al.*, 1990). One of these genes encodes catalase that quickly removes H_2O_2 from the cytoplasm. While following OxyR mRNA levels in *E. coli* by Northern blot hybridization using a probe which in addition to the *oxyR* sequence comprised 200 bp of the upstream region, a strong signal for an sRNA, OxyS was discovered (Altuvia *et al.*, 1997). OxyS is transcribed in opposite direction to OxyR from a promoter that overlaps the promoter for OxyR and is activated by OxyR. Expression of OxyS is quickly and strongly induced upon H_2O_2 addition, while other stress factors only weakly induce OxyS (Altuvia *et al.*, 1997). Deletion of OxyS results in two-fold higher levels of intracellular H_2O_2 (Gonzalez-Flecha and Demple, 1999). Using a genetic screen, eight genes were originally found to be regulated by OxyS (Altuvia *et al.*, 1997), among them the *rpoS* gene for an alternative sigma factor and *fhlA*, a transcriptional activator of formate metabolism. While OxyR-like regulators are found in many bacteria, OxyS seems to be restricted to enteric bacteria.

The mechanism of regulation by OxyS has been best analyzed for the *fhlA* target. Altuvia *et al.* (1998) showed that OxyS represses *fhlA* translation by blocking

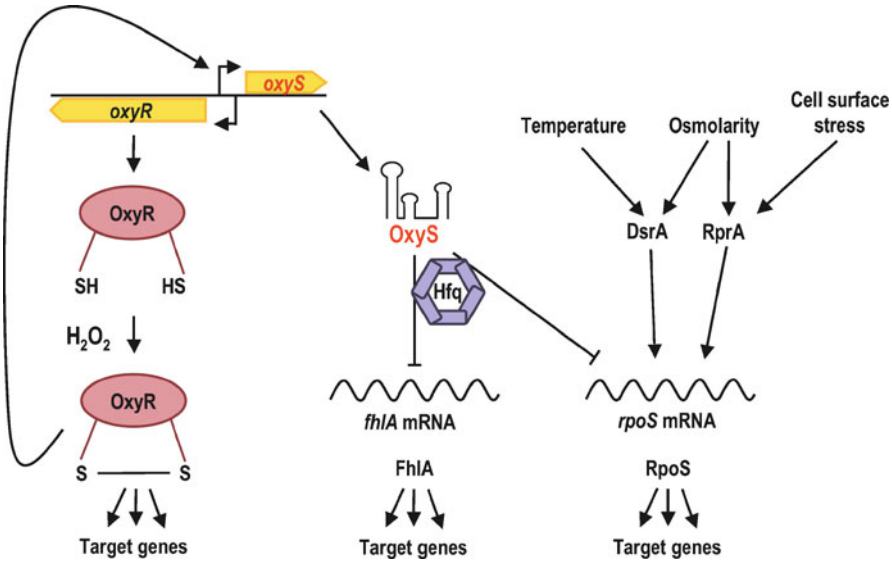

Fig. 1. OxyS is induced by H_2O_2 and acts as a negative riboregulator.
The *oxyS* gene is transcribed divergently from the *oxyR* gene, whereas the promoters are overlapping. OxyR is a transcriptional activator, which is oxidized by H_2O_2 at specific cysteine residues. Oxidized OxyR is active and induces transcription of stress-related genes. Transcription of OxyS sRNA is also induced by OxyR. Together with Hfq, OxyS negatively influences the translation of its target mRNAs, *fhlA* and *rpoS*. FhlA is a transcriptional activator and RpoS is an alternative sigma factor known to regulate gene expression during stationary phase. Translation of *rpoS* mRNA is additionally controlled by the sRNAs DsrA and RprA in a positive manner. DsrA and RprA are induced under cellular stress conditions like changes in temperature, osmolarity or cell surface stress

the ribosome-binding site. Later, the formation of a kissing complex between OxyS and *fhlA* RNAs was demonstrated (Argaman and Altuvia, 2000). Repression of *fhlA* and *rpoS* translation both depend on the RNA chaperone Hfq, since Hfq increases OxyS interaction with its target RNAs (Zhang *et al.*, 2002). Figure 1 illustrates the induction of OxyS by OxyR and its role in post-transcriptional regulation.

Several of the OxyS-regulated genes are also regulated by RpoS, an alternative sigma factor of *E. coli*. OxyS was shown to repress RpoS at post-transcriptional level, most likely by repressing translation. The A-rich single-stranded linker region between the stable OxyS hairpin loop structures is important for this repression (Zhang *et al.*, 2002). Recently it was demonstrated that growth-phase also affects stability of OxyS but altered OxyS stability does not contribute to growth-phase-dependent *rpoS* regulation (Basineni *et al.*, 2009). RpoS was considered as "stationary phase" sigma factor due to its accumulation in stationary phase (Lange and Hengge-Aronis, 1991). It is now well accepted that RpoS is not just a regulator of stationary phase but has a more general role and its target genes are involved in functions such as stress resistance (UV, osmolarity, oxidative and temperature stress), cell envelope composition, cell morphology, and carbon metabolism (Hengge-Aronis, 2002). The exact mechanism by which OxyS affects RpoS levels is less well understood than the OxyS/*fhlA* interaction. The two sRNAs, RprA and DsrA, activate *rpoS* translation in response to changes in osmolarity or temperature, respectively (Figure 1). They bind to the *rpoS* untranslated region and disrupt the formation of a hairpin that masks the ribosome-binding site (Majdalani *et al.*, 1998; 2002). In contrast to RprA and DsrA, OxyS represses *rpoS* translation, but its exact mode of action has not been explained.

The different sRNAs acting on RpoS can be present in the cell simultaneously and may compete for binding to *rpoS* mRNA. The interplay of different sRNAs thus contributes to complex regulatory networks.

3 The Link Between Iron Levels and Oxidative Stress, and the Role of RyhB

3.1 How Iron Can Cause Oxidative Stress

Iron is the most important micronutrient used by bacteria and is essential for cellular processes like respiration, photosynthesis, and nitrogen fixation. It acts as a cofactor for many enzymes and is indispensable for the biogenesis of iron-sulphur (Fe-S) clusters (Wackett *et al.*, 1989; Ayala-Castro *et al.*, 2008). However, iron acquisition and usage have to be tightly controlled in bacteria because high concentrations of free iron favour the generation of hydroxyl radicals ($OH^{\bullet}$) in a process called the Fenton reaction. In this reaction ferrous iron [Fe(II)] catalyzes the conversion of hydrogen peroxide (H_2O_2) to hydroxide ions (OH^-) and $OH^{\bullet}$. The Fe(II) is oxidized to ferric iron [Fe(III)] during this conversion.

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^- + OH^{\bullet} \qquad \text{(Fenton reaction)}$$

Accordingly, a less deleterious ROS (H_2O_2) is converted into a highly reactive ROS ($OH^{\bullet}$) by the action of free iron. In addition, H_2O_2 and superoxide (O_2^-) increase the free iron concentration by damaging Fe-S clusters and thereby accelerating the Fenton reaction (Touati, 2000; Varghese *et al.*, 2003). Since iron is a cofactor of proteins involved in defence against ROS (e. g. iron superoxide dismutase, SodB), iron limitation can also lead to elevated oxidative stress. It does not come as a surprise that the iron metabolism is in part coupled to the oxidative stress response. Here we give a review on the RyhB sRNA, which contributes considerably to iron availability and to the avoidance of oxidative stress in *E. coli*.

3.2 Mechanisms of RyhB Regulation

The 90-nt RyhB sRNA was first identified in a genome-wide screen for sRNAs using comparative genomics and microarrays in *E. coli* (Wassarman *et al.*, 2001). Cells overproducing RyhB showed only poor growth on media containing succinate as carbon source. Only one year after its identification, Massé and Gottesman (2002) demonstrated that RyhB negatively regulates a set of six iron-storage and iron-using proteins when iron is limited. Transcription of RyhB itself, is repressed by the global regulator Fur (*F*erric *u*ptake *r*egulator). Besides *ryhB*, essentially all genes involved in iron acquisition are Fur-regulated. In addition, several genes for general metabolism, pathogenicity, and defence against oxidative and acid stresses are also regulated by Fur (Escolar *et al.*, 1999). Since Fur acts as a repressor of transcription under high iron concentrations using Fe(II) as a cofactor (Bagg and Neilands, 1987), repression of *ryhB* explains the earlier observed activation of gene expression by Fur. Fur positively regulates the transcription of *sodB*, *acnA*, *fumA*, and *sdhCDAB*, all encoding iron-containing proteins. *bfr* and *ftnA*, encoding iron-storage proteins, are also activated by Fur. The existence of RyhB provides a nice explanation for this phenomenon and demonstrates that positive regulation by Fur is indirect and needs RyhB (Massé and Gottesman, 2002). RyhB-dependent repression of the *sdhCDAB* operon, encoding the Fe-S cluster containing succinate dehydrogenase, also explains the succinate defective growth of cells overproducing RyhB.

Figure 2 illustrates how the expression and action of RyhB is connected to iron metabolism and, in part, to the oxidative stress response. Under high iron conditions, Fur is active and represses transcription of siderophores and iron-siderophore transporters in order to avoid a further increase of iron concentrations. The *ryhB* gene is also repressed by Fur. As a consequence, destabilization of target mRNAs by RyhB is not possible and regular translation occurs. Translation of the *bfr* and *ftnA* mRNAs remains undisturbed and excess iron is stored by the corresponding proteins bacterioferritin and ferritin. Storage of iron will also lead to a consumption of molecular oxygen (O_2) and H_2O_2 when Fe(II) is oxidized to Fe(III) by the ferroxidase activity of these proteins (Zhao *et al.*, 2002; Bou-Abdallah *et al.*, 2002; Ceci *et al.*, 2003). Therefore, H_2O_2 is detoxified and the Fenton reaction is avoided by keeping free iron concentrations low.

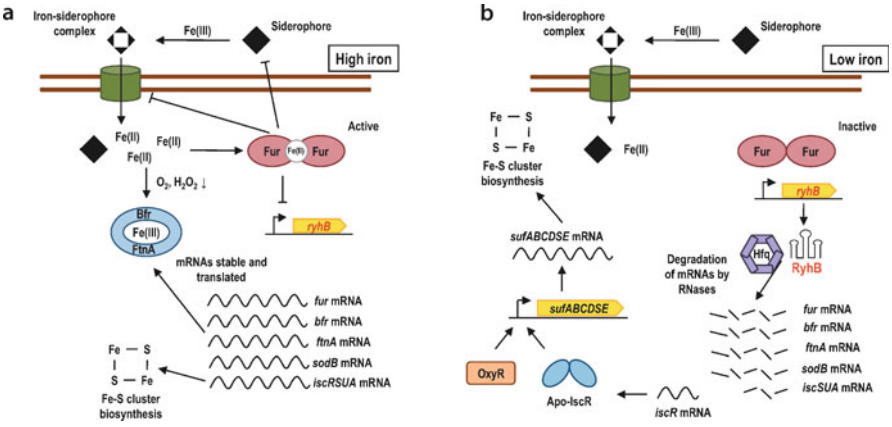

Fig. 2. RyhB links the oxidative stress response to the cellular iron concentration.
a) Under high iron conditions further iron-uptake, by iron-siderophore transport systems, is downregulated by the global iron regulator Fur. Excess iron is stored by iron-storage proteins (bacterioferritin, Bfr, and ferritin, FtnA), which is accompanied by consumption of O_2 and H_2O_2. Both processes lead to a reduced formation of $OH^{\bullet}$ during Fe(II)-mediated Fenton reaction. *sodB* mRNA is stable and translated, leading to constant SodB levels. Fe-S cluster biosynthesis is accomplished by genes of the *isc* operon.
b) Under low iron conditions Fur is no longer able to repress gene expression, which also de-represses transcription of the RyhB sRNA. With the help of Hfq, RyhB binding to targets leads to degradation of the respective mRNAs by RNases, thereby inhibiting translation. Bfr, FtnA and SodB are no longer synthesized, and selective destabilization of the *iscRSUA* mRNA results in a shift of Fe-S cluster biosynthesis. Apo-IscR and the oxidative stress activated OxyR induce the *suf* operon, which is then responsible for Fe-S cluster formation. RyhB also destabilizes *fur* mRNA, thereby avoiding high levels of inactive Fur under low iron conditions

Upon iron starvation (low iron), Fur becomes inactive and RyhB is de-repressed leading to RyhB-dependent degradation of *bfr* and *ftnA* mRNAs, thereby circumventing translation (Massé and Gottesman, 2002). RyhB does not only control iron storage but also impairs *sodB* translation, leading to dropping levels of the iron superoxide dismutase. In the well-studied RyhB/*sodB* interaction, several protein partners like Hfq and the RNases E and III play important roles. It was shown that Hfq binds RyhB (Wassarman *et al.*, 2001; Zhang *et al.*, 2003) and that RyhB transcripts are unstable when Hfq is absent (Massé *et al.*, 2003). Since RNase E is involved in cleavage of both RyhB sRNA and *sodB* mRNA (Massé *et al.*, 2003; Afonyushkin *et al.*, 2005), stabilization by Hfq is believed to be due to blocking an AU-rich region within RyhB sRNA, which is also recognized by RNase E. RyhB was also shown to be initially cleaved by RNase III when it is bound to *sodB* mRNA. Accordingly, RyhB contains an intrinsic regulatory mechanism for its own decay, which leads to dropping RyhB levels when its regulatory action is achieved.

Interestingly, RyhB also regulates *fur* expression in a negative feedback loop. Under low iron conditions Fur is inactive, a situation that supports excess *fur* transcription, since active Fur also represses its own gene. However, there is no need

for increased Fur synthesis under low iron conditions. It was shown that RyhB also destabilizes *fur* mRNA to ensure balanced synthesis of the iron-responsive repressor (Vecerek *et al.*, 2007).

RyhB also influences the assembly of Fe-S clusters, which are sensitive to high oxygen concentrations because they can be decomposed by ROS (for review see Imlay, 2006). RyhB binds the polycistronic *iscRSUA* mRNA, which encodes the regular machinery for biosynthesis of Fe-S clusters under high iron conditions. Desnoyers *et al.* (2009) showed that binding of RyhB to the second cistron of the polycistronic mRNA under low iron conditions promotes the cleavage of the *iscSUA* transcript. T he remaining 5'-fragment encodes IscR, which acts as repressor of the *isc* operon when loaded with Fe-S clusters (Holo-IscR; Schwartz *et al.*, 2001). Under low iron, IscR remains as Apo-IscR, which is believed to activate the *suf* operon, encoding an alternative machinery for the Fe-S cluster assembly (Giel *et al.*, 2006; Yeo *et al.*, 2006; Lee *et al.*, 2008). Therefore, RyhB is responsible for shifting Fe-S cluster assembly from the *isc* operon to the *suf* operon. In addition, the *suf* operon was shown to be activated by OxyR under oxidative stress conditions (Outten *et al.*, 2004), showing again the tight connection between iron regulation and oxidative stress responses.

The example of RyhB nicely demonstrates that a single sRNA can link ROS depletion and iron homeostasis by multiple targeting of mRNAs. Accordingly, several regulatory pathways are connected by a single sRNA, which enables them to work together in concert.

3.3 RyhB Homologues in Other Bacteria

RyhB and other sRNAs are best studied in *E. coli*. However, RyhB homologues are also present in other bacteria, e. g. in *Pseudomonas aeruginosa* and *Erwinia chrysanthemi*.

In *E. chrysanthemi*, a 120-nt RyhB homologue was identified that controls expression of the *ftnA* gene, encoding the iron-storage protein ferritin, in a Fur-dependent manner (Boughammoura *et al.*, 2008). Mutants of *E. chrysanthemi*, which lack *ftnA*, are more sensitive to oxidative stress. Like in *E. coli*, RyhB mediates regulation of genes responsible for iron homeostasis and oxidative stress defence.

Wilderman *et al.* (2004) identified two functional homologues of RyhB in *P. aeruginosa*, named PrrF1 and PrrF2. These sRNAs are >95 % identical to each other, appear in a tandem duplication in the chromosome and seem to have overlapping roles in the negative regulation of genes involved in diverse functions including iron storage, defence against oxidative stress, and intermediary metabolism. Like RyhB, they are transcribed under low iron conditions in a Fur-dependent manner. It was demonstrated that *sodB* and *katA* mRNAs are regulated by PrrF RNAs and are therefore involved in the detoxification of ROS. Why *P. aeruginosa* has a need for two RyhB-like RNAs is still an open question that needs to be addressed in the future.

4 Photooxidative Stress-Induced sRNAs in Photosynthetic Alpha-Proteobacteria

In bacteria, respiratory enzymes and exposure to metals, like iron, are the main sources of ROS that are generated by unspecific electron transfer. The term "oxidative stress" summarizes the generation of such ROS (H_2O_2, O_2^- and $OH^{\bullet}$). In contrast, the generation of highly toxic singlet oxygen ($^1O_2$) depends on light energy, which is absorbed by photosensitizers and then transferred to molecular oxygen (triplet oxygen; $^3O_2$). In this case the term "photooxidative stress" is used because light is pivotal for the generation of $^1O_2$. Since bacteriochlorophyll molecules and their precursors act as naturally occurring photosensitizers in the presence of light, it is obvious that photosynthetic bacteria have to cope with photooxidative stress when oxygen is present during photosynthesis. In the group of alpha-proteobacteria, there are several species that are capable of photosynthetic growth. One of the best-studied model organisms, in regard to the regulation of photosynthesis genes, is *Rhodobacter sphaeroides*, which performs anoxygenic photosynthesis in a light-dependent and oxygen-dependent manner (Gregor and Klug, 1999; Zeilstra-Ryalls and Kaplan, 2004). *R. sphaeroides* is an established model organism for studying the $^1O_2$ stress response (Anthony *et al.*, 2005; Glaeser and Klug, 2005), and recently sRNAs have been identified in a genome-wide search by pyrosequencing of cDNA (Berghoff *et al.*, 2009). Among the newly identified sRNAs, four sRNAs were found to have a putative role in the photooxidative stress response. Two of them, RSs0019 and RSs0682, are specific for $^1O_2$. RSs0019 is induced in an RpoE-dependent manner. RpoE is an alternative sigma factor, which is a major regulator in the photooxidative stress response of *R. sphaeroides* (Anthony *et al.*, 2005; Glaeser *et al.*, 2007). RSs0682 is processed after prolonged $^1O_2$ exposure and processing seems to be Hfq-dependent. It is an interesting question whether the $^1O_2$-dependent processing implies an RNA-dependent sensing mechanism for $^1O_2$, especially when taking into account the fact that no direct sensing mechanism for $^1O_2$ is known to date. Two other sRNAs, RSs0680a and RSs2461, are co-transcribed with their upstream genes and induced by photooxidative as well as oxidative stress (Berghoff *et al.*, 2009). Both sRNAs are preceded by an $RpoH_I/RpoH_{II}$-dependent promoter. The work of Nuss *et al.* (2009 and 2010) showed that the alternative sigma factor $RpoH_{II}$ is mainly responsible for the $^1O_2$, and $RpoH_I$ for the heat shock response, although overlapping regulons of the two factors exist in *R. sphaeroides*. It was verified that RSs0680a and RSs2461 really depend on both RpoH sigma factors and can also be induced by heat shock (Nuss *et al.*, 2010). In this study a third sRNA, RSs1543, was presented, which is under direct control of an $RpoH_I/RpoH_{II}$-dependent promoter. Interestingly, RSs1543 is a homologue of RSs2461 and both sRNAs genes are associated with an *ompR/lysR*-like gene, encoding transcriptional regulators. The question as to whether the two sRNAs interact with these regulators needs to be addressed in the future.

The studies on photooxidative stress-responsive sRNAs in *R. sphaeroides* demonstrated that sRNAs can be specific to a single stress, but most likely are induced by several stresses. Consequently, sRNAs enable a connective network of different stress responses, as has already been shown for OxyS and RyhB.

5 Other sRNAs Involved in Oxidative Stress Responses

Some reports present evidence for the involvement of more sRNAs in oxidative stress responses of various bacteria but exactly how they function needs further elucidation. The overview of such sRNAs as given in this chapter may not be complete and does not include all putative sRNAs, which have been found to respond to oxidative stress in global transcriptome analyses.

One important system of *E. coli* in its response to oxidative stress, in particular to superoxide stress, is SoxRS. SoxR contains a [2Fe-2S] cluster that is oxidized by superoxide and subsequently activates transcription of SoxS, an AraC family protein (Ding *et al.*, 1996). SoxS binds to its target promoters and activates genes which encode e. g. superoxide dismutase, DNA repair enzymes and enzymes of the carbon metabolism (Pomposiello and Demple, 2002). More recently, transcriptome studies have identified more protein coding genes and, in addition, sRNAs in *E. coli* that change their expression in response to superoxide. Among those sRNAs are OxyS and RyhB, which we described in previous chapters, as well as MicF, MicC, RydB, and CyaR (formerly RyeE) (Blanchard *et al.*, 2007). MicF, MicC and CyaR regulate the expression of porins (Omp: outer membrane proteins) in enterobacteria, thus linking the oxidative stress response to the outer membrane composition. MicF and MicC act by an antisense mechanism, while CyaR inhibits translation of *ompX* mRNA by sequestering the Shine-Dalgarno sequence (Papenfort *et al.*, 2008). The expression of MicF, MicC, and RydB is SoxR-dependent, whereas expression of CyaR is SoxR-independent (Blanchard *et al.*, 2007).

ArcZ is an abundant enterobacterial sRNA associated with the Hfq protein. It was shown to repress translation of several mRNAs in *Salmonella*, including the *tpx* mRNA for a periplasmic thioredoxin-like thiol peroxidase, an enzyme of the oxidative stress defence (Papenfort *et al.*, 2009). In *E. coli,* Tpx is involved in resistance to diverse oxidative stress compounds (Cha *et al.*, 1995). ArcZ binds *tpx* mRNA within the coding sequence, downstream of known translational control elements (Papenfort *et al.*, 2009). The physiological role of ArcZ in the oxidative stress response has not been analyzed to date.

In *Pseudomonas fluorescens* CHA0, transcription of the three sRNAs RsmY, RsmZ, and RsmX is controlled by the GacS/GacA two-component system (Heeb *et al.*, 2002). These sRNAs contain multiple GGA motifs and when present in high amounts titrate the RNA binding protein, RsmA, and its homologue, RsmE, which leads to increased translation of mRNAs involved in virulence and resistance to oxidative stress (Heeb *et al.*, 2005; Valverde *et al.*, 2003). Recently a novel sRNA, RgsA, was identified in *P. fluorescens* CHA0, which is also under positive control of GacA and the stress sigma factor RpoS and contains a single GGA motif. RgsA contributes to the resistance to hydrogen peroxide (Gonzalez *et al.*, 2008). It is unable to sequester RsmA and RsmE and its mode of action is unknown.

Numerous sRNAs, especially antisense RNAs, have also been identified in cyanobacteria (Georg *et al.*, 2009). The trans-encoded sRNA Yfr1 is highly conserved among cyanobacterial lineages and deletion of the *yfr1* gene results in reduced growth of *Synechococcus elongatus* PCC6301 under different stress con-

ditions, including oxidative stress, and leads to accumulation of the *sbtA* mRNA (Nakamura *et al.*, 2007). SbtA is a sodium-dependent bicarbonate transporter (Shibata *et al.*, 2002). Yfr1 is located between the *guaB* (required for synthesis of GMP) and *trxA* (encoding thioredoxin A) genes in most cyanobacteria (Nakamura *et al.*, 2007). Presently available data rather hint at an indirect effect of Yfr1 in the oxidative stress response.

In the case of the cyanobacterium *Synechocystis* sp. PCC6803, it was shown that the mRNA of *isiA* is under negative control of the antisense RNA IsrR (Dühring *et al.*, 2006). IsiA is the iron stress-induced protein A, which forms a giant ring structure around photosystem I under iron-limiting conditions (see also chapter 5 for additional details). Furthermore, IsiA dissipates excess light energy under high light and oxidative stress. Under iron-replete conditions, transcription of *isiA* is repressed by Fur and residual *isiA* mRNA is bound by its antisense regulator IsrR and degraded. When subject to iron limitation or oxidative stress (H_2O_2), *isiA* mRNA levels increase and exceed IsrR levels. As a consequence, negative control by IsrR is overcome and IsiA is synthesized under conditions where it is needed. This example demonstrates that an antisense RNA is responsible for tight control of a stress-responsive component involved in photosynthesis.

As for pathogenic bacteria, an efficient defence against oxidative stress can be crucial to escaping the host defence. This applies in particular to mycobacteria, which are able to survive and multiply in macrophages. Recently nine sRNAs were identified in *Mycobacterium tuberculosis*, four cis- and five trans-encoded (Arnvig and Young, 2009). Of those nine sRNAs, four (B11, B55, F6 and ASpks) were induced upon oxidative stress applied by hydrogen peroxide treatment. Overexpression of B11 sRNA resulted in poor growth and elongated cells of *M. smegmatis*. The question as to whether sRNAs make a major contribution to the oxidative stress response of mycobacteria needs to be elucidated in future studies.

6 Concluding Remarks

Based on our current knowledge, it emerges that sRNAs have a main function in linking different regulatory networks. This is also the case for OxyS that links the response to oxidative stress to other stress responses via RpoS, for RyhB that links regulation of iron metabolism to the oxidative stress response, and for MicF, MicC, and CyaR that are under control of the oxidative stress responsive SoxRS system and participate in regulation of the composition of the outer membrane. With an increasing number of sRNAs still being identified in bacteria and characterized in regard to their biological function, we can expect to learn much more about their role in the oxidative stress response in the future.

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