Metallomics

RSC Publishing

PAPER

Exposure of *Bacillus subtilis* to mercury induces accumulation of shorter tRNA^{Cys} species

Cite this: *Metallomics*, 2013, **5**, 398

Andrés Cruz Hernández,[a] Emmanuel Sánchez Millan,[a] Sergio de Jesús Romero Gómez,[a] José Antonio Cervantes Chávez,[a] Rocio Garcia Martínez,[b] Xóchitl Pastrana Martínez,[a] Jackeline Lizzeta Arvizu Gómez,[c] George H. Jones[d] and Juan Campos Guillén*[a]

RNA processing is an essential pathway in the regulation of genetic expression in the cell. In this work, *Bacillus subtilis* was used to understand the effects of mercury on the mechanism of tRNA metabolism. The CVAAS (cold vapor atomic absorption spectroscopy) method revealed that from the addition of $HgCl_2$ (0.75 μg ml^{-1}) during the bacterial exponential phase, *ca.* 48% of the added mercury was taken up by the cells. This led to an immediate reduction in the rate of cell division. During this response, we observed accumulation of species shorter than mature tRNA^{Cys} over a 10 h period. We did not observe this accumulation for another five tRNAs analyzed. tRNA processing is largely dependent on RNase R and PNPase in *B. subtilis*. Thus, when the exonuclease PNPase was absent, we found that the shorter tRNA^{Cys} species increased and mature tRNA^{Cys} decreased after mercury addition, but this proportion changed during the time analyzed. However, in the absence of RNase R and PNPase the accumulation of the shorter tRNA^{Cys} was more pronounced and the mature form was not recovered. In the single *rnr* mutant strain the shorter tRNA^{Cys} was not observed. All together, we provide *in vivo* evidence that PNPase and RNase R are indispensable in controlling tRNA^{Cys} quality in the presence of mercury.

Received 17th October 2012,
Accepted 26th February 2013

DOI: 10.1039/c3mt20203h

www.rsc.org/metallomics

Introduction

RNA processing plays an important role and is a major component of RNA metabolism in all organisms. In bacteria, there is a growing body of evidence to show that changes in RNA turnover play an essential role in stress adaptation, growth phase changes, pathogenesis and quality control.[1] In *Escherichia coli*, and other bacteria such as *Bacillus subtilis*, it is clear that RNA degradation is a carefully controlled biochemical pathway, involving a number of different *endo*- and *exo*-ribonucleases. Although some RNases display significant functional overlap, in *E. coli*, for example, cells lacking the 3′–5′-exonucleases polynucleotide phosphorylase (PNPase) in combination with RNase II or RNase R, which are also 3′–5′-exoribonucleases, are inviable or their growth is deficient. These findings have shown the existence of vital functions of these RNases in RNA metabolism.[2,3]

tRNAs are adapter molecules that play an essential role during translation and expression of the genetic code. tRNAs are synthesized as precursors that undergo post-transcriptional modification, such as 5′ and 3′ processing, addition of the 3′ terminal CCA sequence in some cases and nucleotide editing to give a mature tRNA and functional molecules ready for aminoacylation.[4–6] tRNA is degraded at the end of its useful life by a machinery of RNases. Any defects in this process, for example, the accumulation of shorter and defective tRNAs, could exert a deleterious effect on cells. The processing of defective RNA molecules through quality control is essential in the metabolism of all cells. It has been shown in *E. coli* that polyadenylation by poly(A) polymerase and polynucleotide phosphorylase (PNPase) activity promotes degradation of defective tRNA^{Trp} molecules. In the absence of both enzymes precursor forms of tRNA^{Trp} are accumulated.[7,8] In *B. subtilis* accumulation of a shorter tRNA^{Cys} species is observed as a band running slightly ahead of mature tRNA when CCase and RNase R are absent.[9] In both bacteria, defective stable tRNA precursors or shorter species that probably are poorly converted to their mature forms may be polyadenylated and degraded by a system of RNA quality control involving the participation of PNPase, RNase R and poly(A) polymerase. In fact, tRNA processing of the

[a] *Unidad de Microbiología Básica y Aplicada, Campus Aeropuerto, Universidad Autónoma de Querétaro, Carr. Chichimequillas s/n, km 2.5 Qro., CP 76140, México. E-mail: juan.campos@uaq.mx; Tel: +(442) 192 1200, ext. 65216*
[b] *Centro de Ciencias de la Atmósfera, UNAM, 04510, México, DF*
[c] *Departamento de Ingeniería Genética de Plantas, Cinvestav, Unidad Irapuato, México*
[d] *Department of Biology, Emory University, Atlanta, Georgia 30322, USA*

3′ end may vary from organism to organism and even between some tRNAs within the same organism.

In living systems several essential transition metals, such as copper, cobalt, iron, manganese and zinc, play important roles in the control of metabolic and signaling pathways.[10] A growing amount of evidence has shown the deterioration of biomolecules as a result of the ability of these metals, as well as metals like cadmium, chromium, vanadium, nickel and mercury, to produce reactive radicals in cells. In the environment, organisms are exposed to several chemical forms of mercury of varying toxicities. The biological effects of mercury ions are related to their affinity for residues containing thiol groups in biomolecules and are accompanied by the induction of oxidative stress.[11–13] There is evidence from several prokaryotic systems indicating an effect of mercury on RNA metabolism. For example, in *E. coli* low concentrations of $HgCl_2$ were found to induce the degradation of RNA in exponentially growing cells,[14] although the exact mechanisms involved in that induction were not determined. In the Archaeon *Sulfolobus solfataricus* the toxicity of mercury is due to TFB-1 inactivation as a consequence of transcription inhibition.[15]

The possibility that metabolism of tRNA in bacterial cells might be affected by mercury has not been investigated previously. In the present study, this possibility was investigated in *B. subtilis* during exponential growth using wild type cultures and several RNase deficient strains (*pnp*, *rnr* and *pnp rnr*). We analyzed the effects of mercuric ions on the metabolism of six tRNAs. Of these six, we observed a low level accumulation of shorter species of $tRNA^{Cys}$ in wild type *B. subtilis* but much higher level of accumulation of shorter $tRNA^{Cys}$ species in a PNPase deficient strain. These results suggest sensitivity of tRNA-metabolism activity to metals, such as mercury.

Materials and methods

Bacterial strains

The *B. subtilis* prototroph PY79[16] was used as the wild type strain and for the generation of all mutant derivatives. Mutant strains were constructed by transformation of PY79 with genomic DNA from the different strains carrying the genes of interest substituted with antibiotic resistance markers. Strains employed in this study were Δ*pnp::Cm* and Δ*rnr::spc*[2].

Growth of bacteria and isolation of RNA

To compare growth of the wild type and mutant strains with different concentrations of $HgCl_2$, single colonies were inoculated in LB medium and cultures were grown overnight at 37 °C. These were used to inoculate LB medium diluted to 50% of normal strength. Cell density was measured every hour from a starting OD_{600} of 0.02. When cell density reached 0.4–0.5, different concentrations of $HgCl_2$ (0.5, 0.75 and 1 μg ml^{-1}) were added and the growth was monitored over the course of 10 h. For RNA isolation *B. subtilis* strains were grown at 37 °C in LB medium diluted to 50% of normal strength and amended with $HgCl_2$ as above and aliquots were withdrawn every 2 h. RNA was isolated using the protocol reported previously.[17] The cell pellet was resuspended in 0.3 ml of 0.3 M sodium acetate, pH 8.8, 10 mM EDTA and 3 mg ml^{-1} of lysozyme and was incubated at 37 °C for 10 min. The next steps followed the protocol, but using all solutions at pH 8.8. Samples were treated with DNase as described by the manufacturer (Invitrogen) and RNA was re-precipitated as described.[17]

Northern blot analysis

Northern blot analysis of tRNA was done as previously described[17,18] and following the recommendations of the NorthernMax Protocol (Ambion). tRNAs were separated on 15% denaturing polyacrylamide gels. The probes used to detect the tRNAs were[5,9] $tRNA^{Cys}$, 5′-GGTTTTGCAGACCTCTGCCTTAC-3′, $tRNA^{Ala}$, 5′-CCCTGACCTCTACGCTGCCAGCGTA-3′, $tRNA^{Leu}$, 5′-CTCCACGGGTTATTCACCCACTAGG-3′, $tRNA^{Thr}$, 5′-CGCTGA CCTCTTCCTTACCATGGA-3′, $tRNA^{Trp}$, 5′-CCACACCGGAGG TTTTGGAGACCTC-3′ and $tRNA^{Val}$, 5′-CAGCGACCTCTACCC TGTCAAGGTA-3′, which were 5′-end-labeled using T4 polynucleotide kinase (Invitrogen) and [γ-^{32}P]ATP. To control for RNA loading in Northern blot analyses of $tRNA^{Cys}$ membranes were stripped and probed with an oligonucleotide derived from the sequence of the 5S rRNA gene, 5′-GGAACGGGTGTGAC CTCTTCGCCATCATCA-3′. Quantitation of radioactivity in bands on Northern blots was done using a Storm 860 PhosphorImager (Molecular Dynamics) and the digital image was analyzed using Image Quant (BioRad).

Determining mercury content from cell pellets

Cells were isolated every two hours from exponentially growing cultures (30 ml) (OD_{600} of 0.4–0.5) treated with $HgCl_2$ (0.75 μg ml^{-1}) and were pelleted by centrifugation at 10 000 × *g* and washed twice with distilled water. With each washing, the samples were treated by vortex mixing for one minute to eliminate the mercury in the growth medium. The mercury levels were determined by the cold vapor atomic absorption spectroscopy (CVAAS) method using a flame atomic absorption spectrophotometer (Perkin Elmer Mod. Analyst 100).

Statistical analysis

Data from Northern blot experiments and mercury content determinations were subjected to statistical analyses. Analysis of variance (ANOVA) was done, followed by Tukey's multiple comparison test ($\alpha = 0.05$). Tukey's tests are used in conjunction with ANOVA to compare the means of every treatment. The statistical analysis was done using the Statistical Analysis System (SAS Institute, Carry, NC, USA).

Results

Growth inhibition of *Bacillus subtilis* by mercury

Since mercury is a redox-active metal with a high affinity for reduced sulfur atoms, particularly those associated with thiol-containing biomolecules, we expected that mercury would have a significant effect on cell growth in *B. subtilis*. In our studies, the most consistent results were obtained when LB media diluted to 50% of normal strength was utilized for strain growth.

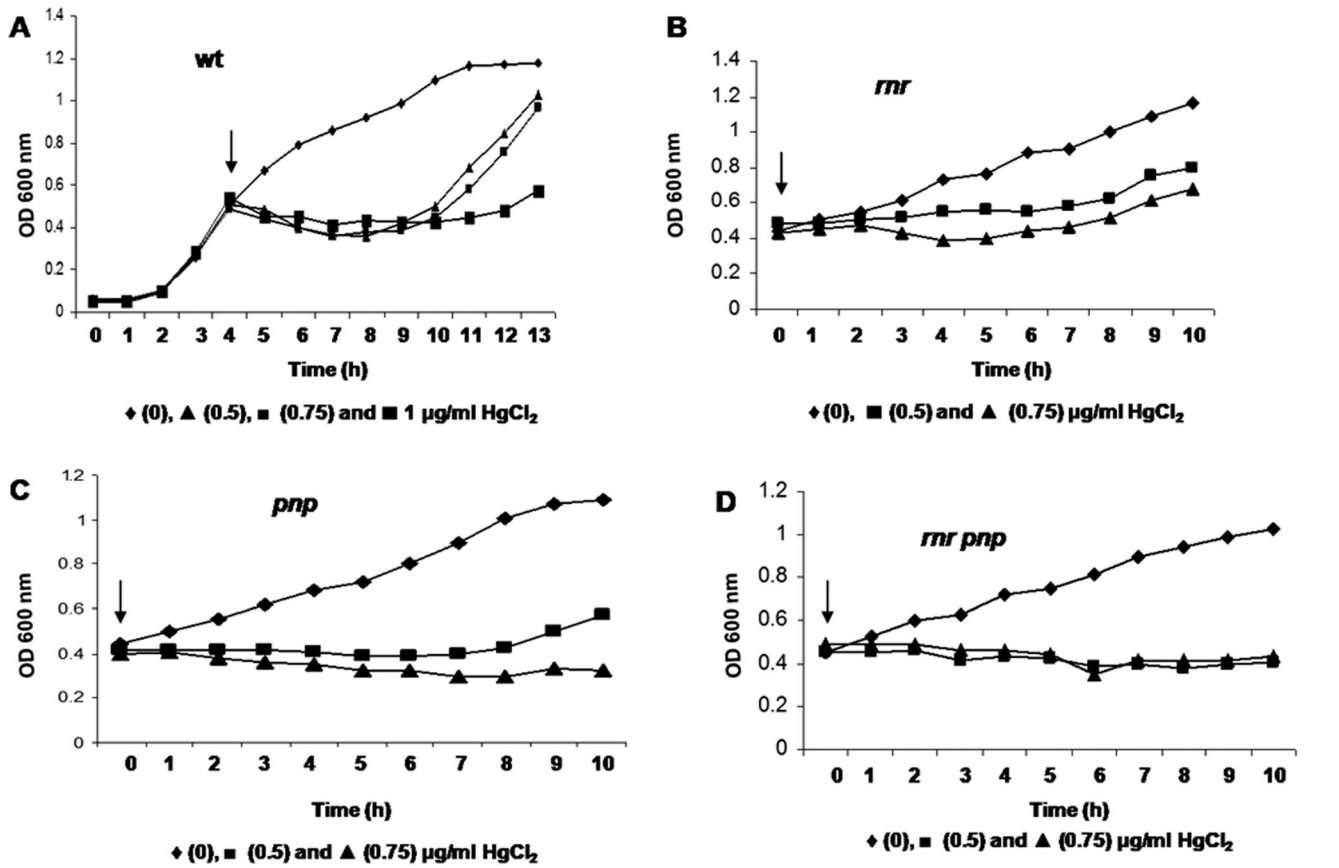

Fig. 1 Growth of *Bacillus subtilis* in the presence of $HgCl_2$. The cultures were treated at the time indicated by the arrow with various concentrations of $HgCl_2$. Exponentially grown liquid cultures of wild-type and RNase single and double mutant strains were grown to an OD_{600nm} of *ca.* 0.4–0.5 and at this optical density the cells were treated with $HgCl_2$. Cell density was monitored for several hours as described in Materials and methods section.

B. subtilis PY79 (the wild type strain) at the exponential phase (OD_{600}, 0.4–0.5) was treated with 0.5, 0.75 and 1 μg ml^{-1} of $HgCl_2$ and cell density was monitored for 10 h. Interestingly, we observed an immediate inhibition of growth for seven hours when cells were treated with 0.5 and 0.75 μg ml^{-1} of $HgCl_2$ (Fig. 1A); after this period of growth inhibition (6 h), the cells recovered and growth began again. However, when cells were treated with 1 μg ml^{-1} of $HgCl_2$ the period of growth inhibition was more pronounced and bacteria survivors were not recovered in solid medium. Concentrations above 1 μg ml^{-1} of $HgCl_2$ were lethal for *B. subtilis*, and at concentrations lower than 0.5 μg ml^{-1} of $HgCl_2$ growth was not affected.

Because of our interest in tRNA metabolism pathways, we examined the effects of mercury in several *B. subtilis* strains deficient in enzymes involved in RNA degradation. Doubling time for wild type (40 minutes), *pnp* (44 minutes), *rnr* (42 minutes) and the *pnp rnr* double mutant (58 minutes) was obtained in LB medium diluted to 50% of normal strength, where the double mutant exhibited an increase in doubling time, compared with wt (45%), while single mutants exhibited only around 6% increase in doubling time. When single RNase deficient strains were treated with 0.5 μg ml^{-1} of $HgCl_2$ during the exponential phase (Fig. 1, panels B and C), an immediate inhibition of growth was observed upon mercury addition followed by a prolonged recuperative period. However, when the *pnp* strain was treated with 0.75 μg ml^{-1} of $HgCl_2$ during the exponential phase (Fig. 1, panel C) the growth was more affected and recuperation was not observed during the time analyzed. We observed that for *rnr* mutant strain with 0.75 μg ml^{-1} of $HgCl_2$ the growth was less affected and recuperation was observed. We observed that for the double mutant strain, lacking both PNPase and RNase R, growth was affected more significantly than with either mutation alone (Fig. 1, panel D). In fact, both 0.50 and 0.75 μg ml^{-1} of $HgCl_2$ completely inhibited growth in the double mutant during the 10 h observed.

From these experiments, we conclude that the growth defects of the combined absence of exonucleases and the presence of mercury could result in an additive effect, but the fact that recovery in wild type was less pronounced than that in single or double mutants suggests that there is a physiological link between mercury and RNase activities. Further work is necessary to substantiate this observation.

Shorter $tRNA^{Cys}$ species are produced in the presence of $HgCl_2$

In order to test the hypothesis that mercury could be affecting tRNA-metabolism in metabolically active *B. subtilis* cells during growth in the presence of mercury, Northern blot analysis was performed. We were curious to know whether tRNAs with or

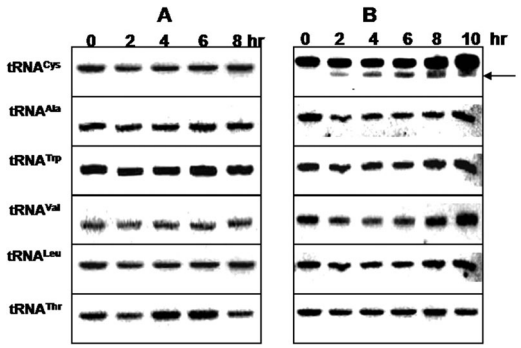

Fig. 2 Analysis of tRNA processing by Northern blotting in the wild-type strain in the absence (panel A) or presence of 0.75 μg ml^{-1} of $HgCl_2$ (panel B). Liquid cultures of wild-type and RNase single and double mutant strains were grown to an OD_{600} of *ca.* 0.4–0.5 and at this optical density the cells were treated with $HgCl_2$, as in Fig. 1. Total RNA was prepared from the cultures at the times indicated at the top of each panel. The shorter tRNA^{Cys} form is indicated with an arrow.

without encoded 3′-CCA ends would show differences in processing in the presence of mercury. We thus chose six tRNAs, those for Ala, Cys, Leu and Val which don't have the CCA end encoded in their corresponding genes, and Trp and Thr which do have the 3′-CCA encode in the tRNA genes.

Interestingly, we observed no change in the amount of six mature tRNAs during eight hours of growth in the absence of mercury (Fig. 2, panel A), but also we observed no change when cells were treated with 0.5 μg ml^{-1} of $HgCl_2$ (data not shown). However, when the wild type strain was treated with 0.75 μg ml^{-1} $HgCl_2$ during exponential growth, we observed the accumulation of shorter species for tRNA^{Cys}, beginning two hours after mercury addition, but no such effect for the other five tRNAs. Fig. 2, panel B shows a Northern blot representative of several independent experiments. Quantitative analysis of data from three independent experiments showed that of the total amount of tRNA^{Cys}, the shorter tRNA^{Cys} species represented around 5.6% at 2 h after mercury addition. The amount of this species increased with time of exposure to mercury, reaching 10% of the total at 6 h. After this time this species decreased to less than 1% of the total at 10 h, while the level of mature tRNA did not change significantly (Table 1). This experiment suggested that the accumulation of shorter tRNA^{Cys} species resulted from the effects of mercury on the system. To explore these observations further, we determined whether the absence of two enzymes involved in RNA processing, PNPase and RNase R, affected the concentration of shorter tRNA^{Cys} species following mercury treatment.

We therefore examined the tRNA^{Cys} immediately after mercury addition at 0.75 μg ml^{-1} (time 0 h, Fig. 3, panel A) but we observed no differences as compared with when mercury was absent in single or double mutant strains. Interestingly, when the PNPase was absent the shorter tRNA^{Cys} species accumulated and the mature form decreased during at least the next 6 hours analyzed, but at time 8 and 10 h, the amount of this species relative to the mature form decreased with time of exposure to mercury, suggesting a recovery of the ability to produce mature tRNA^{Cys} (Fig. 3, panel B). We expected that the level of shorter tRNA^{Cys} species would also increase

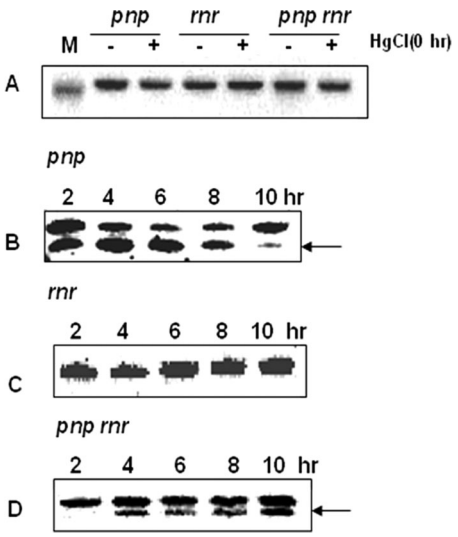

Fig. 3 Analysis of tRNA^{Cys} processing by Northern blotting in RNase single or double mutant strains treated with $HgCl_2$. Total RNA was prepared from the cultures at the times indicated at the top of each panel, as in Fig. 2. The shorter tRNA^{Cys} form is indicated with an arrow. In panel A the marker is a tRNA^{Cys} synthesized without the CCA sequence (Dharmacon RNAi Technologies, Thermo Scientific). (–) Without or (+) with $HgCl_2$.

Table 1 Quantitation of Northern blot analysis for tRNA^{Cys} in the presence of mercury (0.75 μg ml^{-1})

	wt strain		*pnp* strain		*rnr* strain		*pnp rnr* strain	
Time	Mature tRNA^{Cys}	Shorter tRNA^{Cys}	Mature tRNA^{Cys}	Shorter tRNA^{Cys}	Mature tRNA^{Cys}	Shorter tRNA^{Cys}	Mature tRNA^{Cys}	Shorter tRNA^{Cys}
0	100 ± 0^a	0 ± 0^a	100 ± 0^a	0 ± 0^a	100 ± 0^a	0 ± 0^a	100 ± 0^a	0 ± 0^a
2	94.4 ± 2^b	5.6 ± 2^b	60.7 ± 3^b	39.2 ± 3^b	100 ± 0^a	0 ± 0^a	100 ± 0^a	0 ± 0^a
4	90.4 ± 2^b	9.1 ± 2^b	35.5 ± 3^c	64.4 ± 3^c	100 ± 0^a	0 ± 0^a	90.3 ± 2^b	9.7 ± 2^b
6	90 ± 3^b	10 ± 2.5^b	30.6 ± 2^c	69.3 ± 2^c	100 ± 0^a	0 ± 0^a	68.3 ± 2^c	31.7 ± 2^c
8	91.2 ± 3^b	8.8 ± 3^b	44.4 ± 3^d	55.5 ± 3^d	100 ± 0^a	0 ± 0^a	57.6 ± 2^d	42.4 ± 2^d
10	99 ± 2^a	$<1 \pm 2^a$	85 ± 2^e	14.9 ± 2^e	100 ± 0^a	0 ± 0^a	52.4 ± 2^e	47.6 ± 2^e

Individual bands from Northern blots were analyzed with the ImageJ program for quantification. Quantitative analysis of data from three independent experiments is expressed as the average of the percentage of each form of tRNA^{Cys} present at the indicated time point following mercury addition. To control for RNA loading in Northern blot analyses of tRNA^{Cys}, membranes were stripped and probed with an oligonucleotide derived from the sequence of the 5S rRNA gene. Numbers in columns followed by same superscript letters were not significantly different between treatments according to the statistical test ($\alpha < 0.05$).

when RNase R was absent, but when the Northern blot was performed the band that represented the shorter $tRNA^{Cys}$ species was not detectable in the *rnr* strain (Fig. 3, panel C). However, when the double mutant strain was analyzed, we observed an accumulation of the shorter form of $tRNA^{Cys}$ beginning about four hours after mercury addition. Interestingly, in this genetic background the shorter form increased and the mature form decreased with time of exposure to mercury (Fig. 3, panel D).

To understand how the proportion of mature and shorter species of $tRNA^{Cys}$ changed in the presence of mercury in the single or double mutant strains, the band intensities from Northern blots were measured by densitometry. Results averaged from three different experiments are shown in Table 1. For example, when PNPase was absent, at two hours the mature form of $tRNA^{Cys}$ represented 60.7% of the total $tRNA^{Cys}$, while the shorter form represented 39.2%. These proportions changed dramatically at 4 and 6 hours, the shorter form increased to 69.3% and the mature form represented only 30.6% of total $tRNA^{Cys}$. This proportion changed again at 8 and 10 h. For example, by ten hours after mercury addition only around 15% of the shorter form was observed, while the mature form represented 85% of the total. However, when both enzymes PNPase and RNase R were absent the proportions of mature and shorter $tRNA^{Cys}$ species was completely different. For example, the shorter $tRNA^{Cys}$ species were accumulated to *ca.* 10% by 4 h after mercury addition, but the amount of this species increased with time of exposure to mercury reaching 47.6% at 10 h, while the level of mature tRNA decreased to 52.3% (Table 1), possibly due to the failure of the treated culture to recover the ability to grow after mercury treatment.

We measured the concentration of total mercury from cell pellets by CVAAS of *B. subtilis* wt, single and double RNase mutants. As shown in Fig. 4, in the wild type strain mercury accumulated in the cells so rapidly after addition to the culture that even at zero time we could not collect the cells quickly enough to prevent it. This level of accumulation represented *ca.* 48% of the total mercury used in this experiment (0.75 μg ml^{-1}) according to CVAAS analysis. As we expected, the mercury concentration decreased somewhat after four hours, as is observed in Fig. 4. At six hours, the concentration was lower than the limit of detection by CVAAS. In order to determine if the effect on the recovery of growth in single and double RNase mutants in the presence of mercury was due to the slower elimination of mercury from the mutant cells as compared with the wild type strain, the cell pellet was recovered from these conditions and analyzed by CVAAS. We observed a surprising result that mercury elimination by the mutants was similar to the wild type strain (Fig. 4), without a statistical difference ($\alpha < 0.05$). From these experiments we conclude that mercury elimination from the cells is not affected by PNPase and RNase R activities, but in the absence of these RNases there is a greater effect of mercury on growth and changes in the concentration of $tRNA^{Cys}$ species.

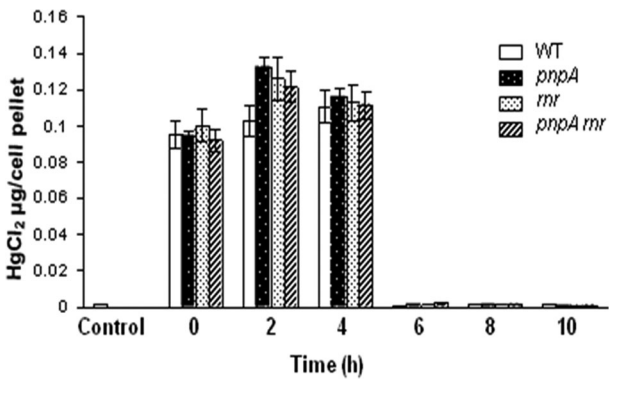

Fig. 4 Cellular concentrations of mercury in wild-type, single and double mutant strains. The cultures were treated and analyzed by CVVAS at the times indicated. Data are from 100 mg of pelleted cells. Error bars are ±standard deviation (n = 3).

Discussion

This study demonstrates for the first time that tRNA metabolism, particularly for $tRNA^{Cys}$, is affected by the heavy and toxic metal mercury in *B. subtilis*. Our results showed that the presence of mercury in the wild type strain resulted in an accumulation of a $tRNA^{Cys}$ species that is shorter than mature $tRNA^{Cys}$. This accumulation persisted for several hours after mercury addition to the culture. Studies with the *pnp* mutant revealed that the shorter $tRNA^{Cys}$ species increased to *ca.* 70% of the total amount of $tRNA^{Cys}$ while the mature form decreased to only 30% of $tRNA^{Cys}$ in six hours following mercury addition. From six hours onward, however, the level of the shorter species decreased and mature $tRNA^{Cys}$ increased in the *pnp* mutant. However when both enzymes, PNPase and RNase R, were absent the proportion of both $tRNA^{Cys}$ species did not change as occurred in the *pnp* mutant. Rather, the shorter form increased and the mature form decreased with time of exposure to mercury (Table 1). Although the levels of mature $tRNA^{Cys}$ increased significantly following mercury treatment in the absence of PNPase, some details of this process are not yet completely understood. For example, the situation in the *pnp* mutant suggests the operation of some homeostatic regulation of tRNA processing, where RNase R and other enzymes might restore the 3′ end of defective tRNAs or facilitate their degradation, as occurs in other biological systems.[3,17,18] In the absence of both PNPase and RNase R other RNases presumably cannot substitute for them. The fact that in the double mutant strain, the slow accumulation of the shorter $tRNA^{Cys}$ species was not observed until 4 h after mercury addition reveals that some mechanisms are activated in the absence of both RNases to maintain mature $tRNA^{Cys}$ levels, at least at early times following exposures to mercury. At later times, however, RNase R may enhance shorter $tRNA^{Cys}$ levels or suppress degradation of these species. Further work is necessary to understand how mercury affects this $tRNA^{Cys}$ processing.

The fact that the mercury concentrations in *pnp* and *rnr* single and double mutants were the same as those measured in the wild type, and that mercury concentrations decreased to undetectable levels during the later stages of growth after mercury addition, indicates that other mechanisms, not involving mercury excretion, may be responsible for recovery of the cells from mercury toxicity. In fact, previous studies have shown the induction of genes involved in the detoxification of heavy metals

when *B. subtilis* is grown under oxidative stress.[12,18,19] Further analyses will be required to understand the detoxification process in this system.

The fact that from six tRNAs only $tRNA^{Cys}$ was affected when mercury was present in the culture medium is interesting. *B. subtilis* encodes several isotypes of ala, val, leu and thr tRNAs and in each case, there are isotypes that lack an encoded 3′-CCA.[9] The specific oligonucleotide probes used for those four tRNAs would have detected the isotypes that do not encode the CCA end. In the case of $tRNA^{Trp}$, there is only one gene copy with encoded CCA. Nevertheless, we only observed an effect of mercury on $tRNA^{Cys}$. There is only one copy of the gene for $tRNA^{Cys}$ and that gene is located at the distal end of the *trnD* operon, without an encoded CCA. Therefore CCA addition to this tRNA species is dependent on enzymes with CCase activity. The fact that only $tRNA^{Cys}$ was affected by mercury suggests that there is a physiological link between $tRNA^{Cys}$ and cysteine concentration (S-thiolation control). Such a link presumably does not exist for the other tRNAs that were tested here.

There is a growing body of evidence for PNPase participation in mRNA decay and in the degradation of defective stable RNA. Moreover, the essential role of PNPase during oxidative stress caused by different mechanisms has focused attention on this enzyme in systems from bacteria to human cells.[20–23] Our results suggest that PNPase and RNase R are indispensable in the $tRNA^{Cys}$ quality control mechanism in *B. subtilis* under oxidative stress caused by mercury. Further elucidation of the mechanisms of tRNA repair under this condition might provide new approaches to understand tRNA metabolism in diverse situations involving oxidative stress.

Acknowledgements

This work was supported by FOMIX-CONACYT Grant no. 102052 to J.C.G.

References

1 K. L. Anderson and P. M. Dunman, *Int. J. Microbiol.*, 2009, 525491.
2 I. A. Oussenko, T. Abe, H. Ujiie, A. Muto and D. H. Bechhofer, *J. Bacteriol.*, 2005, **187**, 2758–2767.
3 B. K. Mohanty and S. R. Kushner, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 11966–11971.
4 Z. Li and M. P. Deutscher, *Cell*, 1996, **86**, 503–512.
5 O. Pellegrini, J. Nezzar, A. Marchfelder, H. Putzer and C. Condon, *EMBO J.*, 2003, **22**, 4534–4543.
6 H. Schurer, S. Schiffer, A. Marchfelder and M. Morl, *Biol. Chem.*, 2001, **382**, 1147–1156.
7 F. Carrao, A. Cruz, H. Mori and C. M. Arraiano, *Mol. Microbiol.*, 2003, **50**, 1349–1360.
8 Z. Li, S. Reimers, S. Pandit and M. P. Deutscher, *EMBO J.*, 2002, **21**, 1132–1138.
9 J. Campos, J. L. Arvizu, G. H. Jones and G. Olmedo, *Microbiology*, 2010, **156**, 2102–2111.
10 M. Valko, H. Morris and M. T. D. Cronin, *Curr. Med. Chem.*, 2005, **12**, 1161–1208.
11 N. Allocati, L. Federici, M. Masulli and C. Dillio, *FEBS J.*, 2009, **276**, 58–75.
12 F. Hochgräfe, J. Mostertz, D. Albrecht and M. Hecker, *Mol. Microbiol.*, 2005, **58**, 409–425.
13 L. L. Masip, K. Veeravalli and G. Georgiou, *Antioxid. Redox Signaling*, 2006, **8**, 753–762.
14 T. Beppu and K. Arima, *J. Bacteriol.*, 1969, **98**, 888–897.
15 V. Dixit, E. Bini, M. Drozda and P. Blum, *Antimicrob. Agents Chemother.*, 2004, **48**, 1993–1999.
16 U. Varshney, C. P. Lee and U. L. RajBhandary, *J. Biol. Chem.*, 1991, **266**, 24712–24718.
17 N. B. Reuven, Z. Zhou and M. P. Deutscher, *J. Biol. Chem.*, 1997, **272**, 33255–33259.
18 A. Neuenfeldt, A. Just, H. Betat and M. Morl, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 7953–7958.
19 F. Hochgräfe, J. Mostertz, D. Pöther, D. Becher, J. Helmann and M. Hecker, *J. Biol. Chem.*, 2007, **282**, 25981–25985.
20 H. Hayakawa, M. Kuwanoand and M. Sekiguchi, *Biochemistry*, 2001, **40**, 9977–9982.
21 H. Hayakawa and M. Sekiguchi, *Biochemistry*, 2006, **45**, 6749–6755.
22 J. Wu and Z. Li, *Biochem. Biophys. Res. Commun.*, 2008, **372**, 288–292.
23 J. Wu, Z. Jiang, M. Liu, X. Gong, S. Wu, C. M. Burns and Z. Li, *Biochemistry*, 2009, **48**, 2012–2020.