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Small RNAs, Ribosomes, and the Adaptation of *Bacillus subtilis* to Metal Ion Limitation

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We have identified metalloregulatory proteins that coordinate metal ion homeostasis in *Bacillus subtilis* including Fur (Fe), MntR (Mn), and Zur (Zn) (1). Each of these proteins mediates the metal-dependent repression of high affinity uptake systems specific for their cognate metal ions. However, adaptation to metal ion stress also involves a complex remodeling of the cell proteome to release and recycle metal ions from non-essential to essential uses.

The Fur regulon has been characterized using genomic, genetic, and bioinformatic approaches (2). Fur regulates at least 23 operons as an Fe-dependent transcriptional repressor and indirectly regulates many more. These operons include genes for siderophore (corynebactin) biosythesis and transport, transport of hydroxamate siderophores, Fe-citrate uptake, and elemental Fe uptake (1). Fur also regulates a complex metabolic remodeling of the cell to enable a more efficient use of limiting Fe. For example, Fur represses the expression of two flavodoxin proteins that can functionally replace the FeS-containing ferrodoxin when Fe is limiting.

Genomic analyses have indicated that *fur* mutants have greatly decreased levels of several different FeS-containing enzymes. Recent work has identified three Fur target genes as small, non-coding RNAs (*mrgC, ydbN*, and *fsrA*) that together mediate a metabolic remodeling of the cell to more efficiently use Fe. For example, *fsrA* is complementary to the leader region of the succinate dehydrogenase operon and mediates translational repression when Fe is limiting. Since SDH is an abundant FeS containing protein, repression enables a rerouting of Fe to other essential functions. In a *fur* mutant, which constitutively expresses *fsrA*, cells are unable to grow on succinate as carbon source. Fur also positively regulates several other non-essential FeS containing enzymes in response to Fe availability.

Zur is a Fur paralog that senses Zn instead of Fe and represses two operons that mediate Zn(II) uptake (3). Previous studies have demonstrated that Zur also represses YtiA, a paralog of the Zn(II)-containing ribosomal protein (r-protein), L31 (4). Zur also regulates expression of YhzA (a paralog of S14) and RpmGC (a paralog of the RpmGA and RpmGB L33 proteins). The regulation of these r-

proteins by Zn(II) was originally predicted based on bioinformatic analyses of Zur boxes and their locations in the genome (5). Together, these observations lead to a model in which Zn starvation derepresses alternative, non-Zn-binding r-proteins that may displace small, surface-exposed Zn-containing r-proteins. Degradation of the released r-proteins may release the "stored" Zn for essential cellular uses. Interestingly, *rpmGC* is a pseudogene in *B. subtilis* 168, although it is intact in *B. licheniformis*. Both L31 and L33 are non-essential r-proteins required for optimal growth and the slow growth phenotype of the L31 mutant can be suppressed by a *zur* mutation. Physiological studies to test the hypothesis that Zn stored in r-proteins can be recycled for other cellular functions are in progress.

These two examples emphasize the complex nature of cellular adaptations to metal ion limitation. While derepression of high affinity uptake systems for essential metal ions is certainly a primary response, this can only alleviate the growth restriction if metal ions are available in the environment. Metabolic remodeling of the cell to release and recycle metals from non-essential sites provides a complementary strategy to enable limited growth under conditions of severe metal starvation.

References

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