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COMMENTARY

Just When We Thought We Knew Everything We Needed To Know about Zn Acquisition and Bacterial Pathogenesis

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ABSTRACT It is well established that high-affinity zinc importers play essential roles in bacterial virulence, but the studies described by Moreau et al. in this issue (G. B. Moreau, A. Qin, and B. J. Mann, J Bacteriol 200:e00587-17, 2018, https://doi.org/10.1128/JB.00587-17) demonstrate that we probably still have much to learn about how these transporters function and how the genes that encode them are regulated in different bacterial pathogens.

KEYWORDS *Francisella*, Zn acquisition

The metal zinc (Zn) is an essential micronutrient for all living organisms, and it has been estimated that approximately 5% of bacterial proteins utilize Zn as a cofactor and/or require this metal for their proper folding (1). Bacteria generally rely upon two types of Zn import systems to meet their cellular requirements for this metal. Homologs of the low-affinity, nonspecific divalent cation transporter ZupT (2) can meet the cell's need for replenishment of this essential micronutrient when Zn is readily available in the environment, and the transcription of *zupT* is often constitutive (3). In contrast, when bacterial cells are faced with Zn deprivation, they typically rely on high-affinity, Zn-specific ABC-type transporters like ZnuABC (4) or AdcABC (5). Unlike *zupT*, the expression of the genes encoding these high-affinity Zn transporters are responsive to cellular Zn levels and tightly regulated by zinc-responsive transcriptional regulators such as Zur (4) or AdcR (6). Bacteria that reside in close association with mammals face a particular challenge in acquiring sufficient levels of Zn to meet their physiologic needs because these hosts actively sequester Zn as a defense mechanism to help prevent microbial infections (7). Correspondingly, high-affinity Zn uptake systems are required for the virulence of many different bacterial pathogens (8–12).

Francisella tularensis subsp. *tularensis* strains cause the zoonotic disease tularemia (13). These bacteria infect a wide variety of wild mammals and are spread between these natural hosts and from these hosts to humans by arthropod vectors. Humans can also become infected with *F. tularensis* subsp. *tularensis* strains through direct contact with infected animals or through the inhalation of infectious aerosols. The ease with which this bacterium can be spread to humans via the latter route and the potentially lethal nature of tularemia make this bacterium a serious biohazard risk in the laboratory and a major biodefense concern. Accordingly, *F. tularensis* subsp. *tularensis* strains are considered select agents and must be handled under biosafety level 3 conditions. *Francisella novicida*, on the other hand, is a closely related bacterium that exhibits very low virulence for humans and can be handled safely under biosafety level 2 conditions but produces a rapidly fatal disease in experimentally infected mice. This combination of properties has made *F. novicida* a widely used surrogate for studying the biology and virulence of *Francisella* (13).

A paper by Moreau et al. in this issue (14) describes a comparative study of Zn acquisition by *F. tularensis* subsp. *tularensis* Schu S4 and *F. novicida* U112. A couple of

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rather surprising findings were made during the course of this study. The first unexpected finding was that although *F. novicida* U112 possesses genes predicted to encode a ZnuABC-type transporter, the authors could find no evidence that these genes are involved in Zn acquisition. Rather, they found that *F. novicida* U112 relies upon a ZupT homolog to resist Zn deprivation and that the gene encoding this transporter is regulated in a Zn-responsive manner by a Zur homolog. As alluded to previously in this commentary, this is an unusual pattern, but in fact it mimics the Zn acquisition strategy employed by *Cupriavidus metallidurans* (15, 16), a bacterium that was originally isolated from a decanting tank in a Zn factory (17) and is now widely used to study bacterial resistance to heavy metal toxicity (18). Taking this into consideration, perhaps *F. novicida* U112's use of a low-affinity, nonspecific metal transporter as its primary means of Zn acquisition is a reflection of the environment from which it was originally isolated, i.e., the Great Salt Lake in Utah (19), which has a high Zn content (20). But if *F. novicida* ZupT has the same properties that have been described in its counterparts in other bacteria (2), this importer would not be expected to provide this strain with an effective mechanism for overcoming the Zn sequestration defenses found in mammalian hosts. Maybe that is why *F. novicida* has not been isolated from mammals in the wild and is rarely associated with human infections (13). But it begs the question of why *F. novicida* U112 is so virulent in small doses in experimentally infected mice and guinea pigs (19).

An equally surprising set of results was obtained when the authors examined Zn acquisition by *F. tularensis* subsp. *tularensis* Schu S4 (14). Contrary to what was observed with *F. novicida* U112, *zupT* is a pseudogene in Schu S4 and ZnuABC is the primary Zn transporter in this strain. The authors also established a clear correlation between *znuA* expression in Schu S4 and the capacity of this strain to replicate in cultured murine macrophages (14). These experimental findings are certainly consistent with the highly virulent nature of *F. tularensis* subsp. *tularensis* because the role of ZnuABC as a virulence determinant has been well established in a variety of other bacterial pathogens (21). But what is intriguing is that *znuA* appears to be an essential gene in Schu S4. If ZnuABC is truly a high-affinity Zn transporter in *F. tularensis* subsp. *tularensis*, as it is in other bacteria, then having this system produced at all times would predispose this bacterium to Zn toxicity unless it has a highly efficient set of Zn detoxification mechanisms (e.g., Zn chaperones and storage proteins or Zn exporters), regulatory systems that tightly modulate *znu* expression or Znu activity in response to cellular Zn levels, or both. This is an important consideration because *F. tularensis* subsp. *tularensis* strains infect a wide variety of mammalian hosts and arthropod vectors (13) and Zn levels likely vary considerably between different tissues within these hosts. Notably, the authors show that Schu S4 is capable of Zn-responsive changes in gene expression, but they do not address whether or not exposure of this strain to different levels of Zn availability results in changes in *znuA* expression. So the question of whether or not Schu S4 modulates the expression of its primary Zn acquisition genes in response to the availability of this metal remains open.

Finally, considering what we have learned from the study of other bacterial Zn acquisition systems, the observation that the *Francisella* strain that employs the "low-affinity," nonspecific Zn transporter ZupT as its main Zn importer (*F. novicida* U112) is much more resistant to Zn deprivation *in vitro* than is the *Francisella* strain that uses the "high-affinity," Zn-specific transporter ZnuABC as its primary Zn importer (Schu S4) is an obvious paradox. Certainly, there are physiological differences between these strains that could explain these findings (13), but this pattern does not correlate with the different roles these two transporters play in Zn acquisition in other bacteria or with the virulence properties of these two strains in nature.

The studies described by Moreau et al. (14) tell us that we still have a lot to learn about Zn acquisition and homeostasis in bacteria and demonstrate that *Francisella* strains provide useful experimental models for expanding our general knowledge in this area. But these studies also raise a note of caution about using *F. novicida* U112 as a surrogate for studying the role of Zn homeostasis genes in *Francisella* virulence.

Considering the origin of this strain, it is probably appropriate to say that the results of such studies need to be "taken with a grain of salt."

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REFERENCES

1. Andreini C, Banci L, Bertini I, Rosato A. 2006. Zinc through the three domains of life. J Proteome Res 5:3173–3178. https://doi.org/10.1021/pr0603699.
2. Grass G, Wong MD, Rosen BP, Smith RL, Rensing C. 2002. ZupT is a Zn(II) uptake system in *Escherichia coli*. J Bacteriol 184:864–866. https://doi.org/10.1128/JB.184.3.864-866.2002.
3. Grass G, Franke S, Taudte N, Nies HD, Kucharski LM, Maguire ME, Rensing C. 2005. The metal permease ZupT from *Escherichia coli* is a transporter with a broad substrate spectrum. J Bacteriol 187:1604–1611. https://doi.org/10.1128/JB.187.5.1604-1611.2005.
4. Patzer SI, Hantke H. 1998. The ZnuABC high-affinity zinc uptake system and its regulator Zur in *Escherichia coli*. Mol Microbiol 28:1199–1210. https://doi.org/10.1046/j.1365-2958.1998.00883.x.
5. Dintilhac A, Alloing G, Granadel C, Claverys J-P. 1997. Competence and virulence of *Streptococcus pneumoniae*: Adc and PsaA mutants exhibit a requirement for Zn and Mn resulting from inactivation of putative ABC metal permeases. Mol Microbiol 25:727–739. https://doi.org/10.1046/j.1365-2958.1997.5111879.x.
6. Guerra AJ, Dann CE, III, Giedroc DP. 2011. Crystal structure of the zinc-dependent MarR family transcriptional regulator AdcR in the Zn(II)-bound state. J Am Chem Soc 133:19614–19617. https://doi.org/10.1021/ja2080532.
7. Kehl-Fie TE, Skaar EP. 2010. Nutritional immunity beyond iron: a role for manganese and zinc. Curr Opin Chem Biol 14:218–224. https://doi.org/10.1016/j.cbpa.2009.11.008.
8. Lewis DA, Klesney-Tait J, Lumbley SR, Ward CK, Latimer JL, Ison CA, Hansen EJ. 1999. Identification of the *znuA*-encoded periplasmic zinc transport protein of *Haemophilus ducreyi*. Infect Immun 67:5060–5068.
9. Campoy S, Jara M, Busquets N, Rozas AMP, Badiola I, Barbé J. 2002. Role of the high-affinity zinc uptake *znuABC* system in *Salmonella enterica* serovar Typhimurium virulence. Infect Immun 70:4721–4725. https://doi.org/10.1128/IAI.70.8.4721-4725.2002.
10. Garrido ME, Bosch M, Medina R, Llagostera M, Rozas AMP, Badiola I, Barbé J. 2003. The high-affinity zinc-uptake system *znuABC* is under control of the iron-uptake regulator (*fur*) gene in the animal pathogen *Pasteurella multocida*. FEMS Microbiol Lett 221:31–37. https://doi.org/10.1016/S0378-1097(03)00131-9.
11. Kim S, Watanabe K, Shirahata T, Watarai M. 2004. Zinc uptake system (*znuA* locus) of *Brucella abortus* is essential for intracellular survival and virulence in mice. J Vet Med Sci 66:1059–1063. https://doi.org/10.1292/jvms.66.1059.
12. Bayle B, Chimalapati S, Schoehn G, Brown J, Vernet T, Durmort C. 2011. Zinc uptake by *Streptococcus pneumoniae* depends on both AdcA and AdcAII and is essential for normal bacterial morphology and virulence. Mol Microbiol 82:904–916. https://doi.org/10.1111/j.1365-2958.2011.07862.x.
13. Kingry LC, Petersen JM. 2014. Comparative review of *Francisella tularensis* and *Francisella novicida*. Front Cell Infect Microbiol 4:e35. https://doi.org/10.3389/fcimb.2014.00035.
14. Moreau GB, Qin A, Mann BJ. 2018. Zinc acquisition mechanisms differ between environmental and virulent *Francisella* species. J Bacteriol 200:e00587-17. https://doi.org/10.1128/JB.00587-17.
15. Herzberg M, Bauer L, Nies DH. 2014. Deletion of the *zupT* gene for a zinc importer influences zinc pools in *Cupriavidus metallidurans* CH34. Metallomics 6:421–436. https://doi.org/10.1039/c3mt00267e.
16. Schmidt C, Scharzenberger C, Groβe C, Nies DH. 2014. FurC regulates expression of *zupT* for the central zinc importer ZupT of *Cupriavidus metallidurans*. J Bacteriol 196:3461–3471. https://doi.org/10.1128/JB.01713-14.
17. Mergeay M, Houba C, Gerits J. 1978. Extrachromosomal inheritance controlling resistance to cadmium, cobalt, copper and zinc ions: evidence from curing a *Pseudomonas*. Arch Int Physiol Biochim 86:440–442.
18. Herzberg M, Bauer L, Kirsten A, Nies DH. 2016. Interplay between seven secondary metal uptake systems is required for full metal resistance of *Cupriavidus metallidurans*. Metallomics 8:313–326. https://doi.org/10.1039/C5MT00295H.
19. Larson CL, Wicht W, Jellison WL. 1955. A new organism resembling *P. tularensis* isolated from water. Public Health Rep 70:253–258. https://doi.org/10.2307/4589039.
20. Domagalski JL, Eugster HP, Jones BF. 1990. Trace metal geochemistry of Walker, Mono and Great Salt Lakes, p 315–353. *In* Spencer RJ, Chou I-M (ed), Fluid-mineral interactions: a tribute to H. P. Eugster. The Geochemical Society, Washington, DC.
21. Cerasi M, Ammendola S, Battistoni A. 2013. Competition for zinc binding in the host-pathogen interaction. Front Cell Infect Microbiol 3:e108. https://doi.org/10.3389/fcimb.2013.00108.