Zinc depletion does not necessarily induce ribosome hibernation in mycobacteria

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In PNAS, Li et al. (1) recently reported analysis of *Mycobacterium smegmatis* ribosomes formed under zinc-limited conditions. A zinc chelator [*N,N,N',N'*-tetrakis (2-pyridylmethyl)-ethylenediamine] was added to growth medium and an extended purification procedure was employed to obtain zinc-depleted (C−) ribosomes for cryo-EM. The structure was solved at 3.5-Å resolution and showed five partially modeled C− ribosomal proteins in place of their zinc-binding (C+) paralogs. Additionally, a hibernation-promoting factor, MSMEG_1878 [designated by Li et al. as a mycobacterial protein Y (MPY)], associated with ribosomes at the coding region of the 30S subunit, resulting in ribosome inactivation and tolerance to aminoglycosides. While this work contributes toward understanding of ribosome hibernation in mycobacteria, we believe that differences in growth conditions used to stimulate production of C+ and C− ribosomes led the authors to erroneously conclude that MPY exclusively binds to C− ribosomes. Here, we present our data and refer to work by others to offer an alternative interpretation of the results.

We used a previously optimized protocol for gradual zinc depletion to allow analysis of actively growing *M. smegmatis* that employs C− ribosomes (2). We refer to C− as alternative (Alt) ribosomes and C+ as primary (Prim) ribosomes (2, 3). Cell-free supernatants of *M. smegmatis* were grown to late-log phase in chemically defined Sauton's medium without addition of Zn²⁺, and Alt ribosomes were purified in a single 4-h sucrose cushion step and subjected to cryo-EM. A total of 73,485 particles yielded an overall 3.4-Å-resolution reconstruction of the Alt ribosome. Upon masked classification with signal subtraction, functional states were identified with natively preserved tRNAs at 4.4- to 5.0-Å resolution, whereas no binding of MPY was detected, thus indicating the translational activity of Alt ribosomes at the time of purification (Fig. 1). In agreement with these data, a biochemical assay indicated that Prim and Alt ribosomes

have comparable overall translational activities (Fig. 2). Finally, there was no apparent difference in resistance to kanamycin and streptomycin for cultures grown in zinc-limited vs. zinc-replete medium, nor did deletion of Alt ribosomal proteins have any effect on susceptibility to these antibiotics. Therefore, our data suggest that when zinc is scarce, *M. smegmatis* employs Alt ribosomes for translation during active growth, and there is no evidence for their specific role in hibernation or aminoglycoside resistance.

We propose that the binding of MPY and hibernation of Alt (C−) ribosomes reported by Li et al. (1) might have been caused by severe zinc depletion, resulting in growth arrest. In contrast, Prim (C+) ribosomes used for comparison were present in metabolically active zinc-replete bacteria; that is, before stasis and association with MPY. Indeed, biochemical data demonstrated that MPY binds Prim ribosomes to promote hibernation and it has been aptly named ribosome-associated factor under stasis (4), which was not acknowledged by Li et al. In addition, recently published structural data showed that Prim ribosomes bind MPY in the same way as Alt ribosomes do (5). Therefore, it can be concluded that ribosome hibernation caused by MPY is not unique to zinc depletion and is likely to be associated with stress and stationary phase in general, as also previously reported for other organisms (6, 7).

The cryo-EM data of *M. smegmatis* Alt ribosomes have been deposited in the Electron Microscopy Data Bank under the accession numbers EMD-0276, EMD-0277, EMD-0278, EMD-0284, EMD-0285, and EMD-0286.

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