

Letter to the Editor

## Involvement of HFq protein in the post-transcriptional regulation of *E. coli* bacterial cytoskeleton and cell division proteins

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**Abbreviations:** EM, electron microscopy; rbs, ribosome binding site; sRNA, small noncoding RNA

**Key words:** small non coding RNA, bacterial cytoskeleton, electron tomography, lanthanides, *E. coli* mini-cell, nucleoid segregation

HFq is an abundant and phylogenetically conserved bacterial protein. It was discovered as a host factor required for the activity of a bacteriophage RNA polymerase, but it was later shown that its main function was to coordinate gene expression of bacteria subjected to environmental stresses by using small noncoding RNAs (sRNA).<sup>1</sup> In *E. coli*, there are around 100 known sRNAs. These molecules often act by base pairing to mRNAs in order to regulate their translation and/or stability.<sup>2-4</sup> Nevertheless, stress-related bacterial sRNAs usually form imperfect duplexes with their targets, and they require the RNA chaperone HFq to facilitate the RNA/RNA interactions.<sup>5,6</sup> Most of the sRNAs studied were shown to act as inhibitors of translation by base pairing with the mRNA around the ribosome-binding site (rbs), although there are also some examples where the sRNA plays a role as a translational activator. In this case, the sRNA acts by exposing the mRNA rbs and promoting ribosome binding. As an example, the *E. coli* DsrA sRNA mediates the production of an RNA polymerase subunit specific to stationary-phase survival,  $\sigma^s$ , and HFq is required for its positive regulatory function.<sup>7</sup>

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In this work, we analyzed the involvement of the HFq protein in the regulation of bacterial cytoskeleton and cell division proteins. In *E. coli*, at least nine essential proteins assemble into a ring-like structure at midcell (the Z-ring) and are required for septation. These proteins include the tubulin-like FtsZ protein, and the work of Takada & et al. indicates that HFq acts as a negative regulator for FtsZ expression.<sup>8</sup> Here, we show that the role of HFq is not restricted to FtsZ post-transcriptional regulation, but it applies to other proteins composing the Z-ring or helix-like lateral-cell-division proteins, such as the actin-like MreB protein. The effects on ultrastructure related to HFq suppression also are presented by high-resolution imaging.

The effects of HFq suppression was first analyzed by quantitative western blot by using *hfq*<sup>+</sup> and *hfq*<sup>-</sup> strains.<sup>9</sup> Our results with FtsZ showed that its expression in *hfq*<sup>-</sup> strains increased by ~15% in stationary phase (result not shown), which are in accordance with the result of Takada et al. once the genetic background and growing conditions are taken into account.<sup>8</sup> This result suggested the involvement of at least one regulatory RNA. Even if the precise mechanism involved in the regulation remains indeterminate, the sRNA involved could be the 53-nucleotide DicF, that could block *ftsZ* mRNA translation.<sup>10</sup>

Besides the effect on the expression of the cell cycle *ftsZ* gene, our analysis also indicated an HFq-dependant negative regulation of MreB, which increased by ~30% in the stationary phase of *hfq*<sup>-</sup> cells (Fig. 1A). A lower (~10–15%) but reproducible negative regulation was observed for MreC in both stationary and exponential phases. In contrast, a positive regulation was observed in both phases for MinD (~20%), whereas no significant effect was observed for MinC and MinE (data not shown). Finally, we observed an interesting differential regulation for ZipA and FtsN proteins: positive regulation in exponential phase (~15–20%) and negative regulation in stationary phase (~20–30%), which suggested the involvement of at least two different regulatory RNAs. Taken together, our results suggested that sRNA riboregulators are probably involved in the regulation of at least six cell division proteins. However the requirement of HFq is not definite proof of a direct involvement of sRNAs in the regulation of these proteins, and the possibility of an indirect effect remains as is the case of BolA, where expression decreases by a factor 4 in the absence of HFq in stationary phase (result not shown). The effect on BolA is however directly dependent on  $\sigma^s$  regulation by HFq and DsrA.<sup>7,11</sup>

In order to analyze the morphological effects induced by HFq-dependant regulation of cell division proteins, we used transmission electron tomography and lanthanide-derivative staining (europium chloride, EuCl<sub>3</sub>) to visualize the effects. Note that this new approach for the direct observation of bacterial structures in 3D applies for Gram(-) bacteria, whereas it does not give any information on Gram(+) bacteria, such as *B. subtilis*, because the latter appear as fully electron-dense objects (data not shown). According to previous work,<sup>12</sup> europium accumulates inside *E. coli* cells and creates contrast within the bacteria, resulting in stain excluding regions. Interestingly, our tomograms showed that regions within the bacteria that excluded the stain (Fig. 1B) included a region corresponding to the bacterial septum (Fig. 1C(a)). As previously shown,<sup>8</sup> the *hfq*<sup>-</sup> strain forms cells of a normal size but they contain several septa that result in the formation of mini-cells. This phenotype was confirmed at high resolution by using the europium staining procedure (Fig. 1C(b)). The increased level of FtsZ in *hfq*<sup>-</sup> mutant cells was suggested to be responsible of the formation of the multi-septa.<sup>8</sup> Our results however pointed to the additional involvement of at least ZipA and FtsN.

*hfq*<sup>-</sup> mini-cells were also analyzed by this procedure. Mini-cells are usually empty of DNA apart from plasmids, which our strain lacks. Previous work about lanthanide stains suggest that they bind to nucleic acid because of their positive charge.<sup>13</sup> As seen in

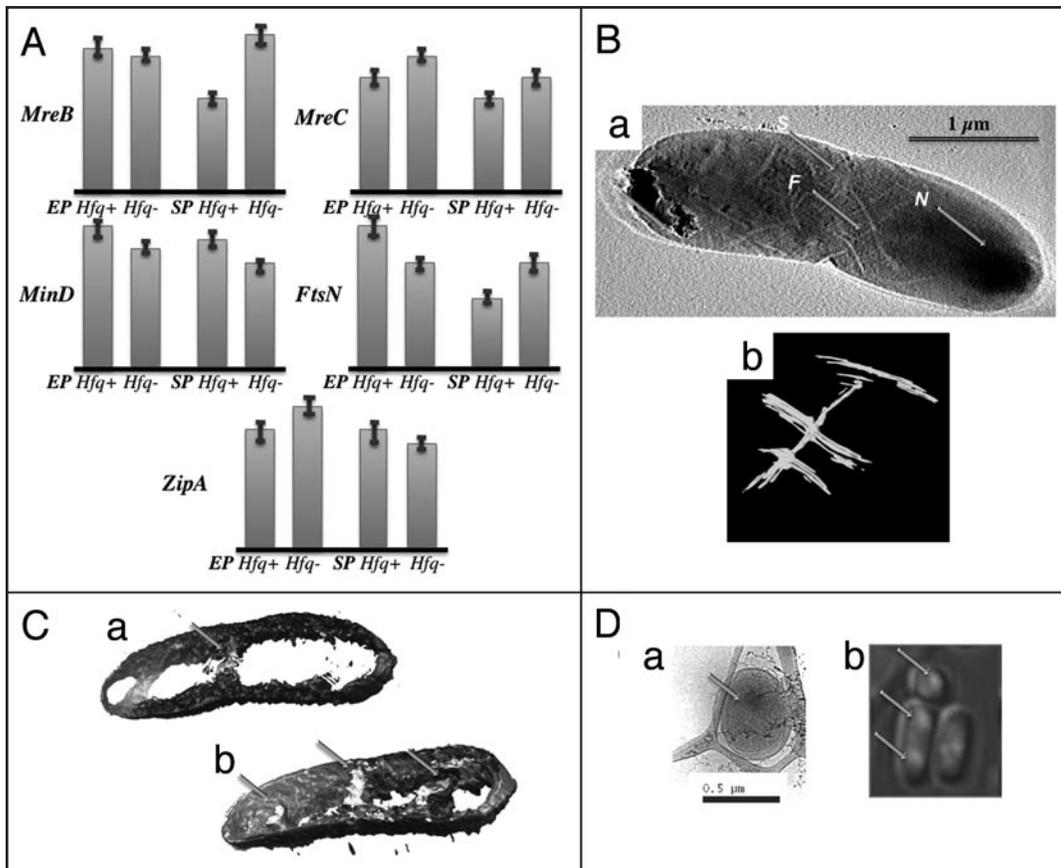


Figure 1. (A) Effect of Hfq suppression analyzed by quantitative western blot. The analysis was performed in both stationary and exponential growth phases with 6 completely independent but strongly reproducible experiments for each protein. (B) (Part a) Europium staining and electron tomography of the whole *E. coli* bacteria. For Eu staining, The cells were grown at 37°C until  $OD_{600\text{ nm}} = 1$ , collected by centrifugation at 9,000 g, re-suspended in 500  $\mu\text{l}$  of  $\text{EuCl}_3$  100  $\mu\text{M}$  in water, incubated for 10 minutes at room temperature and then washed with water. For direct observations at the electron microscope, a 10  $\mu\text{l}$  drop of  $\text{EuCl}_3$  stained sample is deposited onto a carbon coated Cu 300 mesh grid. Sample is adsorbed to the grid during 1 min and then dried with a Whatman filter paper before transfer to the electron microscope (Philips CM120 electron microscope, with a  $\text{LaB}_6$  filament, operating at 120 kV equipped of a Gatan sCCD camera). Tomographic series ( $-40^\circ$  to  $40^\circ$  with a constant tilt increment  $1^\circ$ ) have been acquired using the Gatan Digital Micrograph tomography plugin at x3000 nominal magnification. Tomography reconstruction was performed using Tomo-J software (<http://u759.curie.u-psud.fr/softwareu759.html>)<sup>18</sup> by applying ART reconstruction algorithm with 12 iterations and a relaxation coefficient of 0.1. Volume rendering has been performed using UCSF Chimera ([www.cgl.ucsf.edu/chimera/](http://www.cgl.ucsf.edu/chimera/)). Note the nucleoids (N) in black and the stain excluding regions corresponding to bacterial septum (S) and filamentous (F) ultra-structures. (Part b) Detailed 3D structure of the filaments. (C) Rendered volume dividing *E. coli* stained with  $\text{EuCl}_3$ . (Part a) wild-type *hfq*<sup>+</sup>; (Part b) *hfq*<sup>-</sup>. Dark grey corresponds to cell membranes and light grey to stain excluding regions. *hfq*<sup>-</sup> cells clearly evidence additional lateral septa. (D) (Part a) Imaging of *hfq*<sup>-</sup> minicells after cryo europium staining. In this case, 7  $\mu\text{l}$  drop of  $\text{EuCl}_3$  stained sample they were deposited onto holy-film Athene<sup>®</sup>-type grids, providing a solid rim, flat and clean, made by electroplating process (copper 300 Mesh). Sample exceed were removed with a Whatman 4 filter paper before being frozen into liquid ethane. Grids were transferred to the electron microscope using a Gatan cryotransfer accessory, and DAPI staining (Part b). For this case the cells grown at 37°C until  $OD_{600\text{ nm}} = 1$ , are collected by centrifugation at 9,000 g and fixed by method Karnovsky's Fixative modified (2.5% glutaraldehyde + 4% formaldehyde in PBS1X at pH 7.0), and 30  $\mu\text{l}$  these sample is observed with 0.5 ml of DAPI under optical microscopy.

Figure 1B(a), we confirm that europium staining of Gram(-) bacteria provides an excellent tool to visualize the bacterial nucleoid and to observe its segregation during division. But surprisingly, cryo-electron microscopy performed on mini-cells after europium staining showed electron dense regions that can be assigned to condensed DNA compatible with the presence of a "mini-nucleoid" (Fig. 1D(a)). In order to confirm this result, DAPI staining together with light microscopy was used to analyze *hfq*<sup>-</sup> mini-cells. As shown on Figure 1D(b), the presence of DNA was confirmed. This, together with the electron density observed by cryo-microscopy, definitely point out that mini-cells do contain some part of the nucleoid as a result of the abnormal division.

Finally, the electron tomography also revealed new Gram(-) ultra-structures not observed before at this resolution: Figure 1B(b) shows evidence of filaments that were variable in length but that had a uniform diameter of  $17 \pm 2$  nm ( $p = 0.95$ ). Because of their uniform diameter and their multiple orientations inside the bacteria, we ruled out the possibility that they originated from a dehydration artifact, and instead we think that they represented a true bacterial ultra-structure. Taking into account (i) the diameter of the filaments were compatible with that of MreB and MreC<sup>14,15</sup> and (ii) the filaments location were out of the Z-ring, we speculate that these filaments could be related to a "lateral" bacterial cytoskeleton.<sup>16</sup> Moreover, the recent collected images of FtsZ in *Caulobacter* by cryoEM<sup>17</sup> show that FtsZ lines the

inside of the membrane and in this way, FtsZ filaments look quite dissimilar to the filaments seen by europium staining. It thus suggests that the filaments observed here are not that of FtsZ.

## Conclusion

Two major points must be kept in mind from this work:

(1) Our results strongly suggest that sRNA riboregulators are involved in the regulation of bacterial cytoskeleton and division proteins expression at the post-transcriptional level. Because these proteins play important roles in cell division, cell shape regulation and DNA partition, this new level of regulation could be of primary importance for the bacterial cell in order to adapt to external stresses and changes in its growth conditions.

(2) We present here a versatile EM imaging method to create specific contrast within bacteria, and this allows us to directly observe the bacterial ultrastructures in 3D without requiring ultramicrotomy. This method is particularly adapted to revealing bacterial ultrastructures such as the nucleoid, the division septum and the conspicuous filaments that are likely related to the bacterial cytoskeleton. Thus it provides a useful tool for studying the morphological changes of Gram(-) bacteria at high resolution.

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## Note:

Supplementary materials may be found at:  
[www.landesbioscience.com/supplement/ZambranoCC8-15-Sup.avi](http://www.landesbioscience.com/supplement/ZambranoCC8-15-Sup.avi)

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