Supporting Information

Russell and Keiler 10.1073/pnas.0904904106

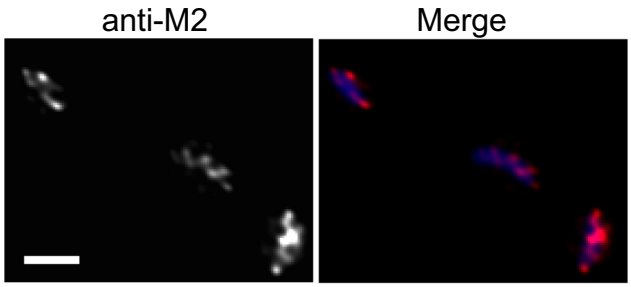

Fig. S1. SmpB-M2 is localized in a helical pattern. Localization of SmpB-M2 was observed in cells expressing SmpB-M2 using IFM with anti-FLAG M2-Cy3 antibody. Fluorescence signal from Cy3 (*Left*) and merged image of DAPI (blue) and Cy3 (magenta) signals (*Right*) are shown. Scale bar, 1 μm.

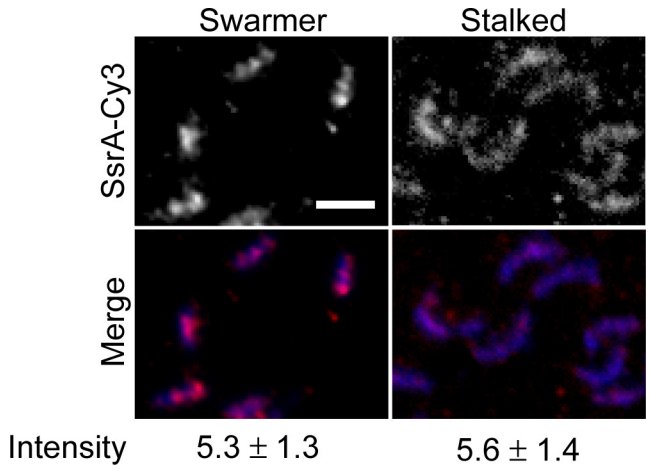

Fig. S2. tmRNA is not localized in Δ*vacB* stalked cells. Swarmer and stalked cells lacking RNase R were sampled from synchronized cultures, and tmRNA localization was determined using FISH. Fluorescence signal from SsrA-Cy3 (*Left*) and merged image of DAPI (blue) and SsrA-Cy3 (magenta) signals (*Right*) are shown. Scale bar, 1 μm. To determine the relative abundance of tmRNA in swarmer and stalked cells, the fluorescence intensity of the SsrA-Cy3 signal was quantified by measuring the average fluorescence intensity over the area of each cell. The average ($n \geq 10$) and standard deviation are shown.

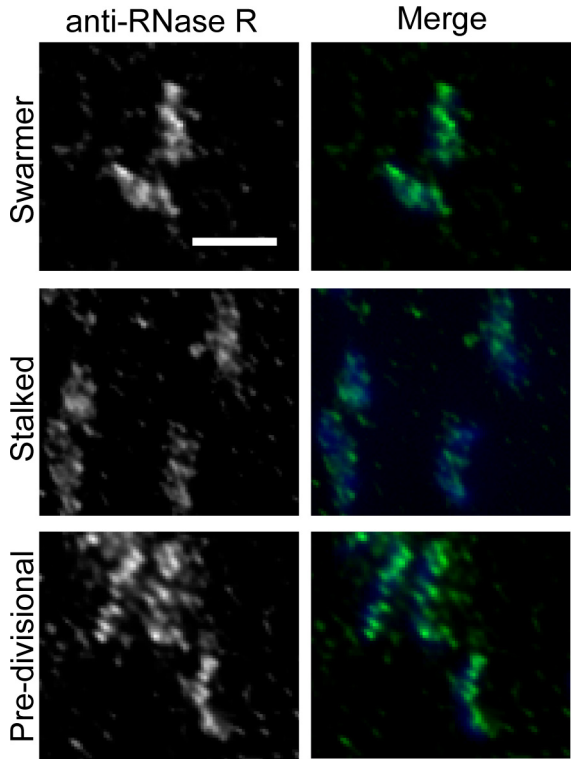

Fig. S3. RNase R localization does not change during the cell cycle. Cells were sampled from a synchronized culture of cells at 15 min (swarmer), 60 min (stalked), and 105 min (predivisional), and the localization of RNase R was determined using IFM with a RNase R antibody. Fluorescence signal from anti-RNase R (*Left*) and a merged image of DAPI (blue) and RNase R (green) signals (*Right*). Scale bar, 1 μm.

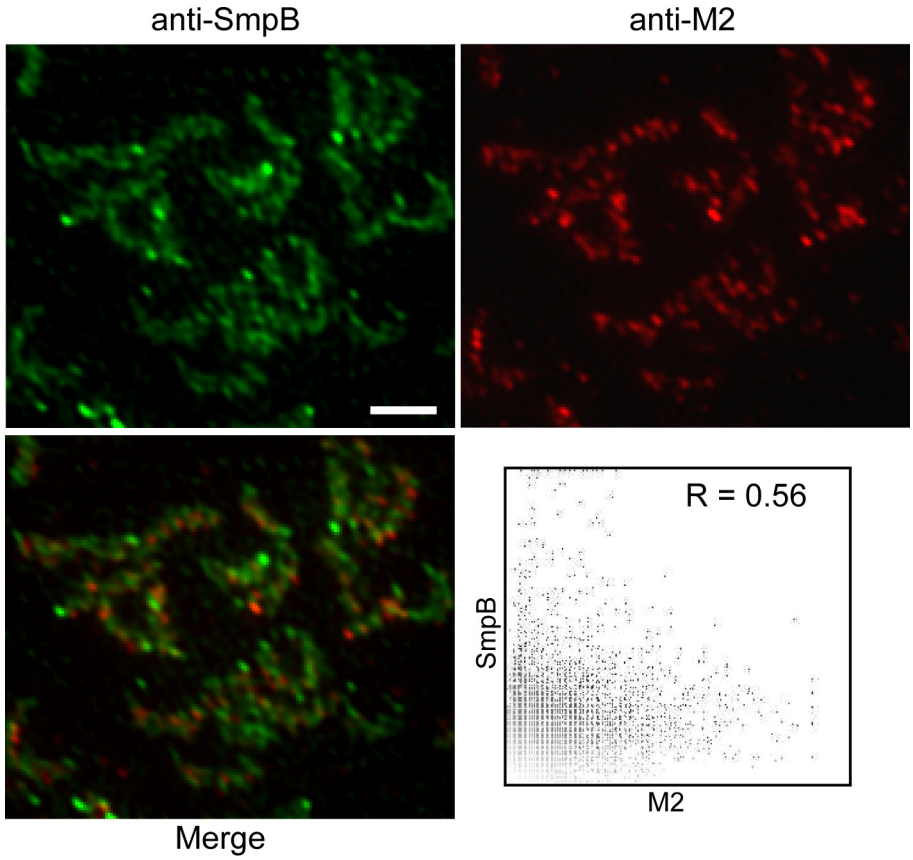

Fig. S4. RNase R and SmpB are localized in different patterns. Localization of SmpB (*Upper Left*, green) and RNase R-M2 (*Upper Right*, red) were detected in cells producing RNase R-M2 using IFM. Merged image (*Lower Left*) shows regions of overlap (yellow). For each pixel, the intensity from the SmpB channel was plotted versus the intensity from the RNase R-M2 channel (*Lower Right*), and the overlap coefficient (R) was calculated. Scale bar, 1 μm.

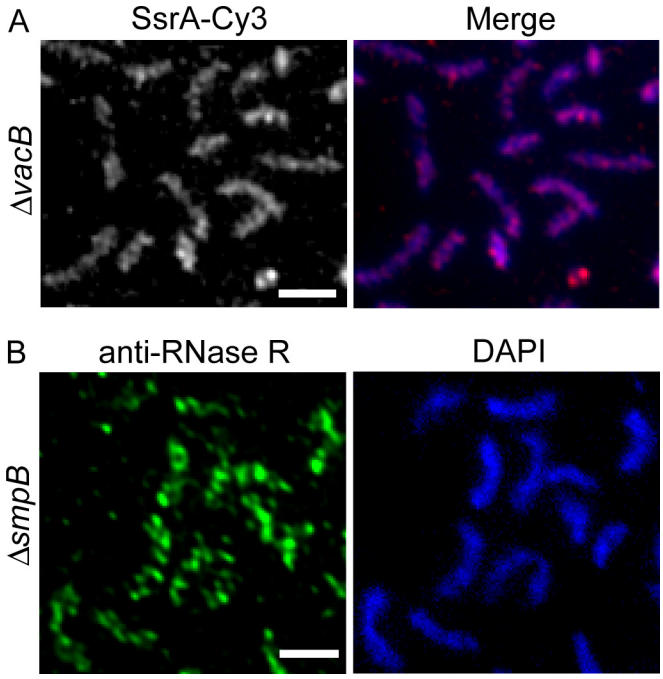

Fig. S5. RNase R localization is not dependent on tmRNA or SmpB. (*A*) tmRNA localization was determined using FISH in cells deleted for the gene encoding RNase R (Δ*vacB*). Fluorescence signal from SsrA-Cy3 (*Left*) and merged image of DAPI (blue) and SsrA-Cy3 (magenta) signal (*Right*) are shown. Scale bar, 1 μm. (*B*) RNase R localization was determined using IFM in cells lacking SmpB. Fluorescence signal from RNase R (*Left*) and the same cells stained with DAPI (*Right*) are shown. Scale bar, 1 μm.

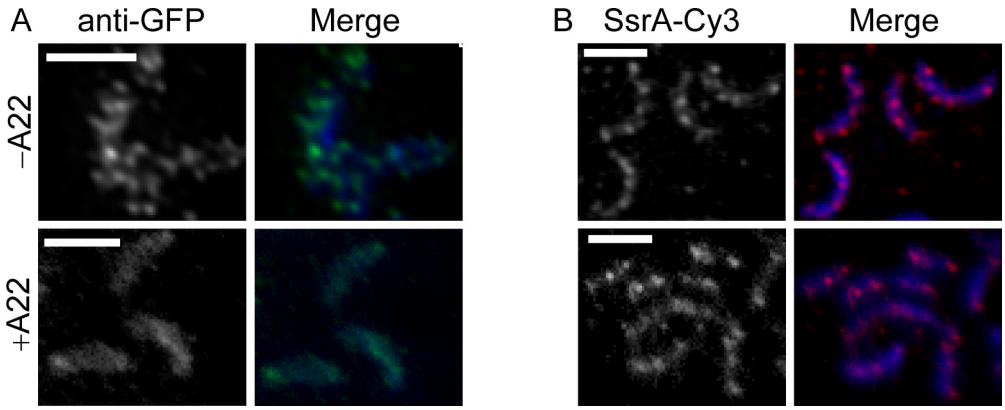

Fig. S6. tmRNA localization does not depend on the MreB helix. (*A*) Cells expressing MreB-GFP were analyzed for MreB localization in the absence (*Top*) and presence (*Bottom*) of the MreB inhibitor A22 using IFM. Fluorescence signal from MreB-GFP detected using an anti-GFP antibody (*Left*) and merged image (*Right*) of DAPI (blue) and MreB-GFP (green) are shown. (*B*) Cells analyzed for tmRNA localization using FISH in the absence (*Top*) and presence (*Bottom*) of the MreB inhibitor A22. *Left Panels* depict SsrA-Cy3 fluorescence signal; *Right Panels* are merged images of DAPI (blue) and SsrA-Cy3 (magenta). Scale bar, 1 μm.