

Figure S1. Identification of the 3' end of RnaG.

A. Strategy adopted to identify the 3'-terminus of RnaG. V and N are any base and further details are provided in *Material and Methods*. **B.** Chromatogram showing the sequence of the 3' end of the RnaG provided by Bio Molecular Research (University of Padova, Italy). **C.** Northern hybridization of total RNA (lane 1, 5 μg and lane 2, 15 μg) extracted from the *E. coli* wt strain HMG11 harbouring plasmid pMYSH6601 with the 5' end 32P-labelled oligo G-290. M represents the RNA ladder. **D.** Computer prediction of the secondary structure of the RnaG 3' end using the MFOLD program (43). Positions at which the RnaG transcript terminates are indicated by arrows.

RnaG120 added (fmoles)

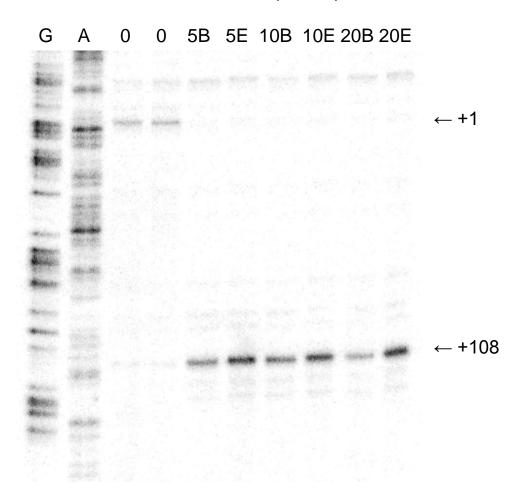


Figure 2S. The RnaG120 downregulates *icsA* transcription.

In vitro transcription was carried out on supercoiled pGT1129 as described in the text and in the legend of Figure 7 as function of the indicated amounts of RnaG120 corresponding to the antisense region (from pos +1 to pos. +120). RnaG120 was added either at the beginning (samples B) or at the end (samples E) of the transcription reaction. The *icsA* mRNA was detected by primer extension using the oligo ACC9 (from pos. +197 to pos. +219) which pairs downstream the complementary region between *icsA* transcript and RnaG. Lanes G and A represent the sequencing reactions using the same primer.

Figure S3. RNA probing of the *icsA* mRNA leader region either alone or in combination with RnaG The *icsA* mRNA (2 pmoles) was treated with DMS (0.6%) either in the absence (lanes 1 and 2) or in the presence of different amounts of RnaG: 1 pmole (lanes 3 and 4), 2 pmoles (lanes 5 and 6) and 4 pmoles (lanes 7 and 8). Modified nucleotides were detected by primer extension using the oligo G+187 and accessible sites were evaluated comparing samples incubated in the absence (-) and in the presence of DMS. Lanes C, T, A and G correspond to DNA sequencing ladders made with the same primer.

