SUPPLEMENTARY METHODS

Preparation of RNA

Short sequences of RNA derived from the antitermination region of the *M. tb* rrn operon (7mer to 13mer RNA, BoxA, BoxB and BoxC-loop, Figure 1) were purchased HPLC-purified from Curevac, Germany or gel purified from Eurogentec Ltd, Belgium. Longer RNA's (RNA63 and RNA43, Figure 1) were synthesized by *in vitro* transcription as described in (Arnvig et al., 2004). Briefly, the *M. tb* nut site sequence was cloned between a 5' hammerhead ribozyme and a 3' hepatitis δ ribozyme site in the plasmid pUC119δv, a derivative of pUC18T7Pstδv (Jovine et al., 2000) using XbaI and PstI restriction enzyme sites. The ribozyme construct was linearized with PvuII (RNA63) or HindIII (RNA43) and transcribed in a standard large-scale T7 polymerase reaction. The reaction mix was then annealed for 10 min at 65°C and 20 min at 55°C to promote ribozyme cleavage of the transcript. Product RNAs were separated from ribozyme and uncleaved sequences on a 7 M urea 10 % polyacrylamide denaturing gel, identified by UV shadowing, excised and finally eluted from the gel with PAGE elution buffer (0.5 M ammonium acetate/10 mM magnesium acetate/1mM EDTA/0.1 % SDS). The molar extinction coefficient at 260 nm was determined for each ribo-oligonucleotide or RNA by summing the contribution from the individual nucleotides, taking into account the hyperchromicity observed following digestion by snake venom phosphodiesterase (Taylor et al., 1994).

Nuclease protection assays

10 μg of *in vitro* transcribed RNA43 (see "Preparation of RNA") were 5' end-labelled in a kinase reaction using [γ-^{32}P]-ATP (Amersham Biosiences) and T4-polynucleotide kinase (Promega). The labelled RNA was Phenol/Chloroform extracted and finally purified using G50 microcolumns (Pierce). To analyse the RNA structure, roughly 1 μg of end-labelled RNA per reaction was digested using the RNases T1 (cuts specifically at single stranded G's) and CV1 (cuts specifically at double stranded regions and stacked bases). Nucleases were purchased from Ambion and the digestion reactions

carried out following the company's protocol. An RNA ladder was produced by alkaline hydrolysis of the labelled RNA. About 0.1 µg of labelled RNA were dried with 10 µg tRNA in a desiccator and then resuspended in 3 µl Na_2CO_3/$NaHCO_3$ (25 mM each, mixed 1 in 10). The samples were incubated at 95°C for 1 min and finally 3 µl of formamide loading buffer were added.

For the RNA protection assays, equimolar ratios or a 10-fold excess of NusA was added to 1 µg of end-labelled RNA and the digestion reactions were performed in the same way as for the free RNA. The products of nuclease digestion were separated on a 15 % urea PAA gel. After electrophoresis, the gel was fixed in 10 % acetic acid/ 25 % methanol/ 2 % glycerol, dried and the products visualized by autoradiography or phosporimaging.

Supplementary references

Arnvig, K.B., Pennell, S., Gopal, B. and Colston, M.J. (2004) A high-affinity interaction between NusA and the rrn nut site in Mycobacterium tuberculosis. *Proc Natl Acad Sci U S A*, **101**, 8325-8330.

Jovine, L., Hainzl, T., Oubridge, C. and Nagai, K. (2000) Crystallization and preliminary X-ray analysis of the conserved domain IV of escherichia coli 4.5S RNA. erratum. *Acta Crystallogr D Biol Crystallogr*, **56**, 1512.

Taylor, I.A., Davis, K.G., Watts, D. and Kneale, G.G. (1994) DNA-binding induces a major structural transition in a type I methyltransferase. *Embo J*, **13**, 5772-5778.

Supplementary Figure. **A**) Titration of BoxC-loop with NusADNt monitored by the change in CD at 270nm (DCD270). The points correspond to successive additions of NusADNt and the curve is the best fit to the data using a single site heterologous equilibrium model. **B**) Titration of RNA13 with NusADNt monitored by the change in CD at 270nm (DCD270). The points correspond to successive additions of NusADNt and the curve is the best fit to the data using a single site heterologous equilibrium model. Panels **C-E** are ITC titrations of NusADNt with **C**) BoxA, **D**) BoxB and **E**) RNA7.

Supplementary data