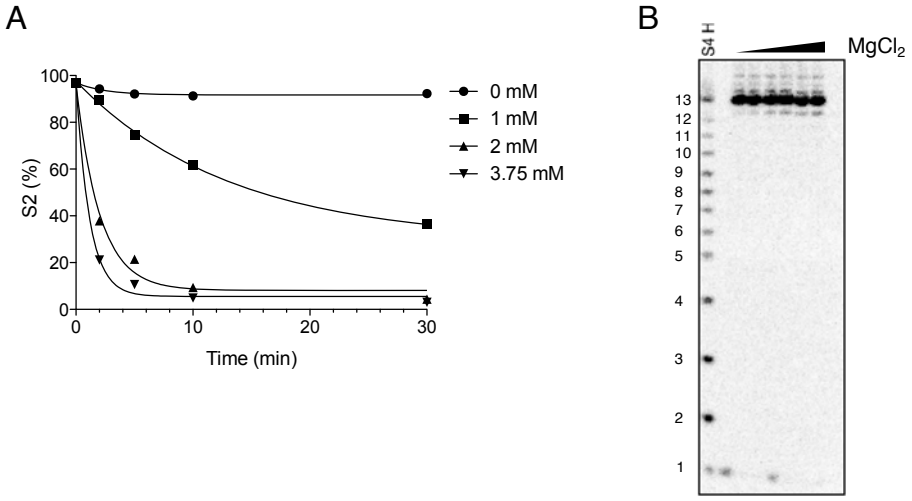

Fig. S1: Magnesium concentration dramatically modulates release rate but not pRNA size. **A.** Release kinetics of 25 nM 6S RNA bound to 200 nM Holoenzyme released with 225 μM each NTP in the presence of the indicated amounts of $MgCl_2$. Percentage bound to Holoenzyme plotted against time. The curves are fitted to eqn. 4. **B.** pRNA sizes in the S4 released state as a function of magnesium concentration, Lanes 2 to 8: 0, 1, 1.5, 2, 2.5, 3, and 3.5 mM $MgCl_2$. Lane 1: alkaline hydrolysis ladder.

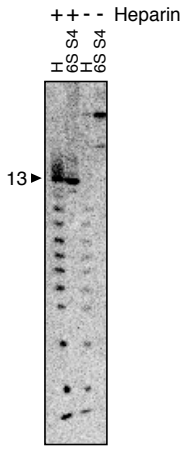

Fig. S2: Addition of Heparin simulates *in vivo* pRNA size observed during 6S RNA release. 6S RNA was bound to the holoenzyme in the presence or absence of heparin as indicated and the release was induced by the addition of 147 μM each NTPs and 4 mM $MgCl_2$ with a trace amount of γ–[^{32}P]-ATP to ensure pRNA labeling. The products were loaded onto a 23% Denaturating PAGE gel. H: alkaline hydrolysis ladder for the lane to the immediate right.

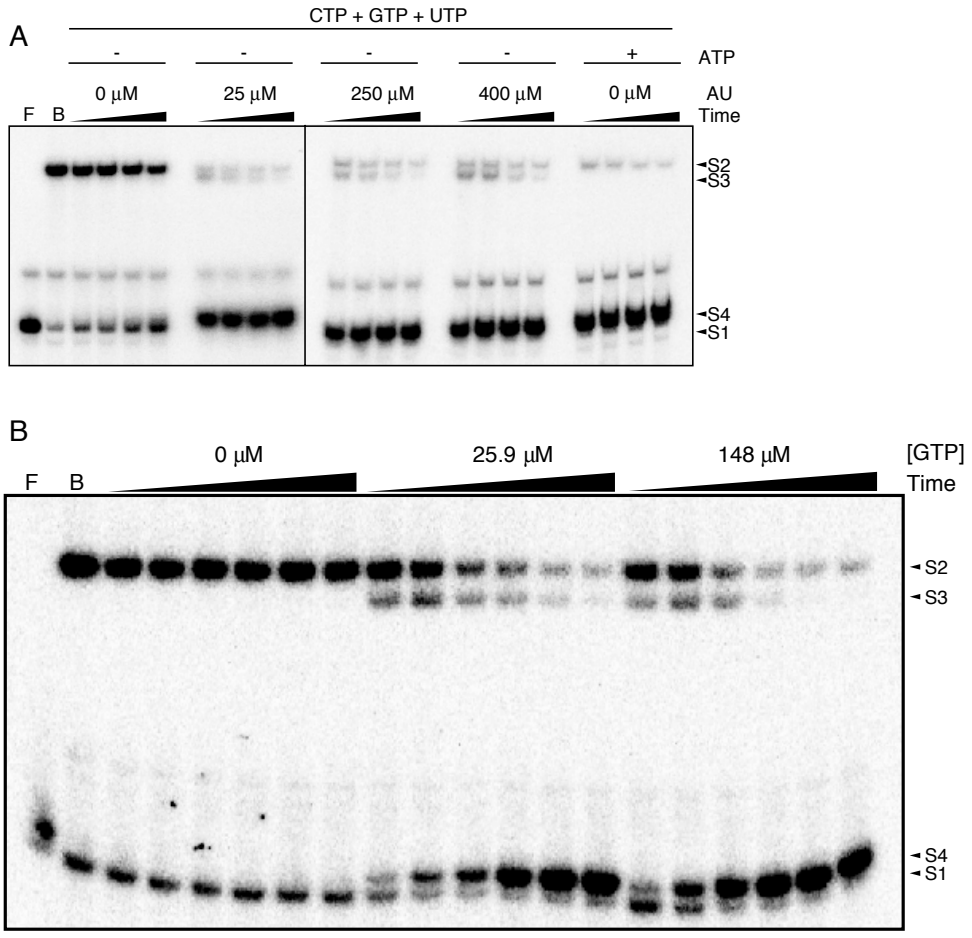

Fig. S3: Dynamically populating the release intermediate 6S RNA S3 state by titrating AU dinucleotide and GTP concentrations. **A.** 25 nM of body labeled 6S RNA was bound to an excess (200 nM) holoenzyme and was released with the indicated amounts of AU. CTP, GTP and UTP were held at 145 μM and $MgCl_2$ was at 3.75 mM. F: free 6S RNA, B: bound $E\sigma^{70}$ complex. Time points were 2, 5, 10, and 30 min and loaded in a 5% Native PAGE gel with 5% Glycerol. **B.** Bound body labeled 6S RNA was released with 25 μM AU, 147 μM each CTP, UTP. GTP concentrations were as indicated. Time points were taken at 2, 5, 10 and 30 min and processed as in panel A.

A **B**

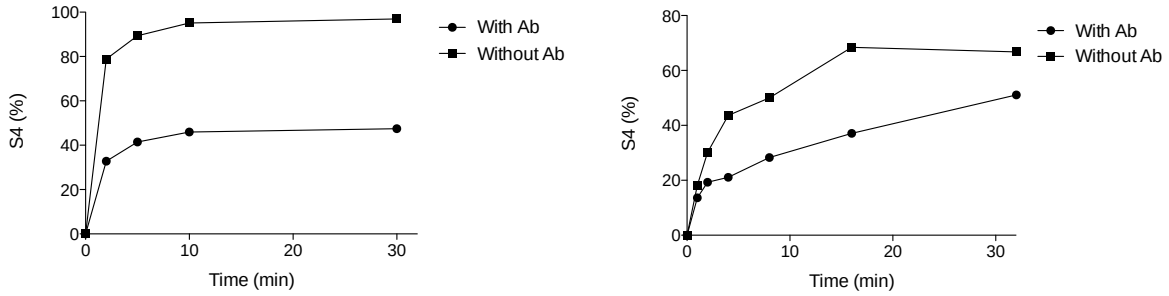

Fig S4: Rate of release of 6S RNA decreases with the addition of Anti σ^{70} Antibody. **A.** Release kinetics of 25 nM 6S RNA bound to 200 nM Holoenzyme incubated with or without mouse anti σ^{70} antibody and released with 147 μM of each NTP and 3.75 mM $MgCl_2$. Data for release with σ^{70} antibody corresponds to Fig 4A while the gel for 6S RNA release under the same conditions without σ^{70} antibody is not shown. **B.** Release kinetics of 25 nM 6S RNA bound to 200 nM Holoenzyme incubated with or without mouse anti σ^{70} antibody as indicated and released with 147 μM of each CTP, GTP, UTP, 1.5 mM $MgCl_2$ and 25 μM AU. Data corresponds to Fig 4B.

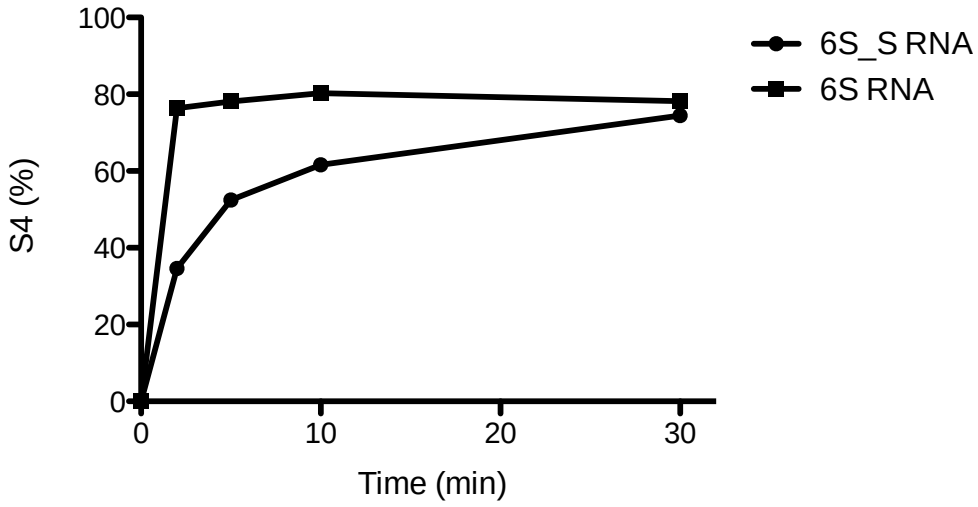

Fig S5: Rate of 6S_S release is slower than that of 6S RNA. Release kinetics of 25 nM RNA bound to 200 nM Holoenzyme incubated and released with 147 μM of each NTP and 3.75 mM $MgCl_2$. Data Correspond to Fig 5C.

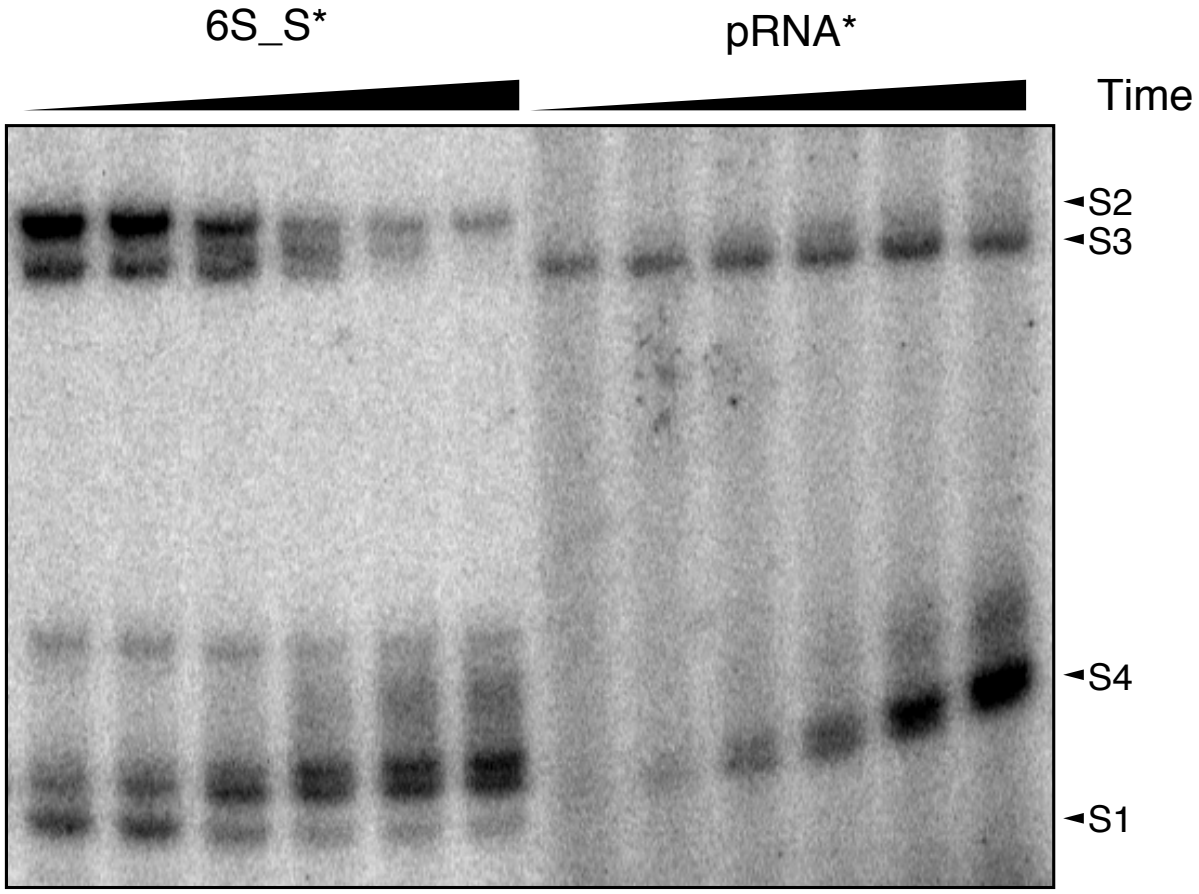

Fig. S6: The 6S_S RNA construct does not produce a pRNA in the S2 state. Left time course shows bodylabeled 6S_S RNA released using each NTP at 147 μM, and 1.5 mM $MgCl_2$. Right time course [^{32}P]-γ-ATP at 25 μM, CTP, GTP and UTP at 147 μM. Times are: 1, 2, 4, 8, 16, 32 min for each.

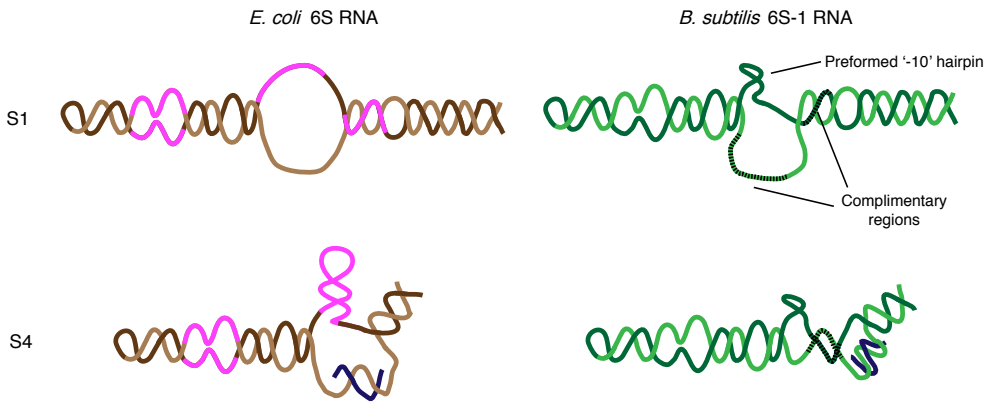

Fig. S7: Secondary structure changes for the *E. coli* (left) verses *B. subtilis* (right) between the S1 (initial unbound) and S4 (final released state).

Equations derived to fit the S2 and S3 states:

The differential equations for 6S release are:

$$\frac{dS2}{dt} = -k_{23}S2 \quad \text{..... 1}$$

$$\frac{dS3}{dt} = k_{23}S2 - k_{34}S3 \quad \text{..... 2}$$

$$\frac{dS4}{dt} = -k_{34}S3 \quad \text{..... 3}$$

The Solutions for 6S release are

$$S2 = S2_i\, e^{-k_{23}t} \quad \text{..... 4}$$

$$S3 = \frac{k_{23}}{(k_{34}-k_{23})} S2_i\, (e^{-k_{23}t} - e^{-k_{34}t}) \quad \text{..... 5}$$

$$S4 = -\frac{k_{23}k_{34}}{(k_{34}-k_{23})} S2_i\, (\frac{e^{-k_{34}t}}{k_{34}} - \frac{e^{-k_{23}t}}{k_{23}} - \frac{1}{k_{34}} + \frac{1}{k_{23}}) \quad \text{..... 6}$$