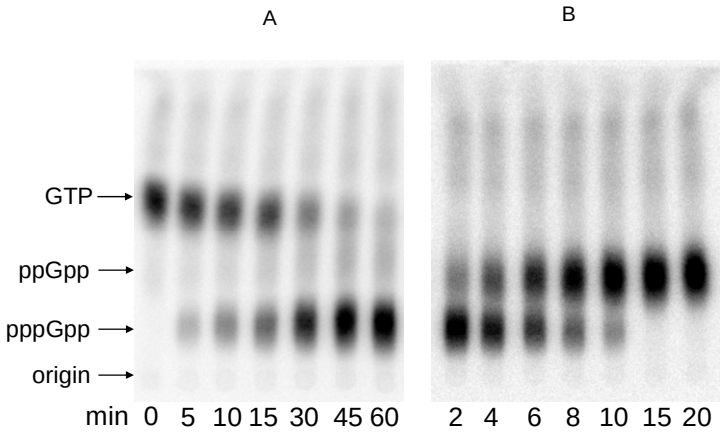
Supplementary Table 1. Data collection and refinement statistic of the *E. coli* RNA polymerase σ^{70} holoenzyme complexed with ppGpp and pppGpp

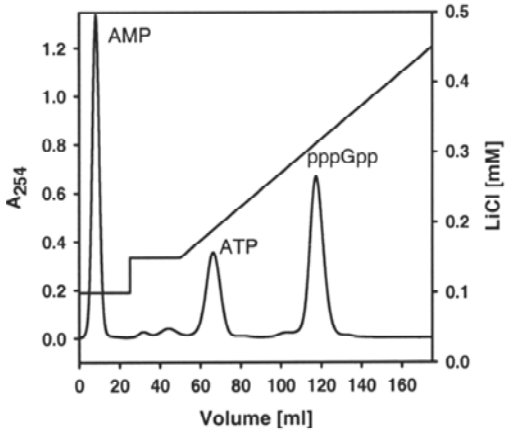
Complex PDB code	ppGpp 4JK1	pppGpp 4JK2
Data collection		
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions		
a (Å)	184.567	185.323
b (Å)	203.817	205.411
c (Å)	307.674	309.581
Resolution (Å)	30 - 3.90	30 - 4.20
Total reflections	299,711	204,492
Unique reflections	96,273	69,537
Redundancy	3.1 (1.8)*	2.9 (2.8)*
Completeness (%)	87.7 (55.8)*	80.5 (80.3)*
I / σ	6.0 (1.5)*	5.1 (1.4)*
R_{sym}	0.125 (0.531)*	0.246 (0.315)*
Refinement		
Resolution (Å)	30 - 3.90	30 - 4.20
R_{work}	0.252	0.243
R_{free}	0.320	0.320
R.m.s deviations		
Bond length (Å)	0.002	0.002
Bond angles (°)	0.49	0.50

Data sets were collected at MacCHESS-A1, Ithaca, NY
*Highest resolution shells are shown in parenthesis

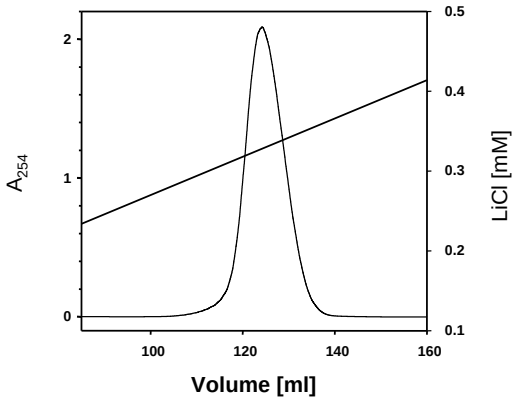
Supplementary Figures



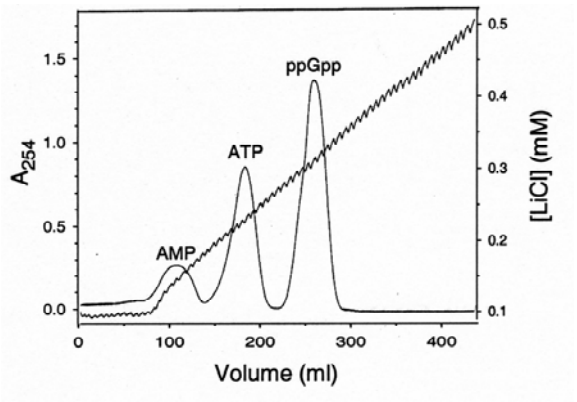
Supplementary Figure S1. Time course of pppGpp synthesis by Rel*Seq***1-385 protein and conversion to ppGpp by GppA.** (**A**) Progress of a pppGpp synthesis reaction (see Methods and Materials) using purified Rel*Seq1*-385 (specific activity 1.4 μmoles / min / mg) with ATP and ^{32}P-γ-GTP was monitored by resolving aliquots at times indicated by thin-layer chromatography. Trace amounts of ppGpp are seen to be labeled, presumably due to contamination of the unlabeled GTP substrate with GDP.(**B**) The products of the pppGpp synthesis reaction shown in Panel A were converted to ppGpp with purified GppA protein (specific activity 2.4 μmoles / min/ mg) and the subsequent reaction similarly monitored.



Supplementary Figure S2. Purification of pppGpp from ATP and GTP by ion exchange column chromatography. The proceeds of a reaction as for Figure S1 Panel A, but without isotope label, was performed so as to completely convert the GTP substrate to pppGpp, which was then purified by anion exchange column chromatography, prewashed with 0.15 M LiCl to remove AMP, then ATP and pppGpp resolved with a linear gradient of 0.15 to 0.5 M LiCl (see Materials and Methods).



Supplementary Figure S3. Verification of purity of pppGpp. The preparative peak of pppGpp recovered after purification as in Figure S2 was diluted ten-fold and again subjected an anion exchange column chromatography as in Figure S2.



Supplementary Figure S4. Purification of ppGpp synthesized from ATP and GDP by anion exchange column chromatography. A preparative reaction using purified Rel*Seq*1-385 with ATP and GDP as substrates (see Materials and Methods) was incubated so as to quantitatively convert the GDP substrate to ppGpp, and then was purified by anion exchange column chromatography and resolved with a linear 0.1 M to 0.5 M LiCl gradient as in Figure S2 but without prewashing with 0.15 M LiCl.

Supplementary Movie 1. Binding of ppGpp on the surface of double-psi β-barrel (DPBB) domain of β' subunit. This movie enables visualization of (p)ppGpp binding on the surface of the DPBB domain.