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SUPPLEMENTAL INFORMATION

SUPPLEMENTAL DATA

Table S1: Related to Figure 4. DnaA screen for divalent cations and nucleotides. Concentration of reagents, T_m and $F_{fold30\text{-}75}$.

Reagent	Conc mM	T_m (±SEM) °C	$F_{fold35\text{-}75}$ (±SEM)
ATP	5	44.7 (0.2)	0.19 (0.10)
ADP	5	40.5 (0.0)	0.01 (0.01)
AMPPNP	5	42.5 (0.0)	0.02 (0.01)
$MgCl_2$	10	51.8 (0.1)	0.10 (0.04)
$MnCl_2$	10	40.5 (0.3)	0.06 (0.03)
$MgCl_2$/ATP	10/5	55.2 (0.2)	0.03 (0.01)
$MgCl_2$/ADP	10/5	53.0 (0.0)	0.03 (0.01)

$F_{fold30\text{-}75}$ represents residual fluorescence after a melting curve protocol (30-75ºC).

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Table S2: Related to Figure 4. Effects of nucleotides and divalent cations on DnaA-GFP initial fluorescence.

Reagent	$RFU_{initial}$	Reagent	$RFU_{initial}$
ATP	4,420	$MgCl_2$	4,620
ADP	4,500	$MnCl_2$	4,100
AMP-PNP	3,830	$CaCl_2$	4,250
cAMP	3,780	$ZnSO_4$	4,000
GTP	4,630	$NiCl_2$	1,430
GDP	4,080	$CoSO_4$	140
dATP	4,275	$CuSO_4$	780
dCTP	4,700	$MgCl_2$/ATP	4,730
dGTP	3,750	$MgCl_2$/ADP	4,650
dTTP	3,820	None	2,270

Reactions were spiked with 5 mM of nucleotides or 10 mM of divalent cations. $RFU_{initial}$ correspond to fluorescence at 35°C.

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SUPPLEMENTAL FIGURE LEGEND

Figure S1: Related to Figure 2 and 3. Effect of increasing concentrations of ligands on the T_m of GK-GFP and Tus-GFP and graphical determination of K_{obs}. GK-GFP (2.5 μM in phosphate buffer pH 7.8) with increasing glycerol (1-1024 μM, grey triangles) or ATP (5-5120 μM, grey circles) concentrations. Tus-GFP (2.5 μM) with increasing concentrations of *TerB* (0.16-10 μM) in buffer D + 250 mM KCl at pH 7.5 (black circles) and pH 7.8 (black squares), and in phosphate buffer at pH 5.9 (open circles), 7.8 (black triangles) and 9.4 (black diamonds). POI-GFP were incubated at RT for 15 min with ligands. K_{obs} was obtained by linear regression and extrapolation of the linear portion of the curve and is defined as the concentration value at ΔTm = 0. CI: confidence intervals of the K_{obs}.

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

GFP cloning vectors

pET-GFP was used for cloning of RdRp-GFP, DnaG-GFP, DnaA-GFP, SSB-GFP and DnaQ-GFP harboring a N-terminal his_6-tag following a previously described strategy.[1, 2] An alternative vector pCC-Tus derived from pET20b(+) was used to produce HPR-GFP and GroES-GFP with a C-terminal his_6 tag.[2] Briefly, PCR products encoding HPR or GroES were ligated into pCC-Tus using NdeI and NheI to produce pCC-HPR-Tus and pCC-GroES-Tus. A GFP sequence derived from pET-GFP lacking a stop codon was then inserted into these vectors to yield pCC-HPR-GFP and pCC-GroES-GFP.

Table S3: Oligonucleotides for PCR amplification of genes of interest and restriction sites

Genbank # Protein	Forward Primer	Reverse Primer	Restriction sites
RdRp	CATATGCTCGATATCATGGATGTCATTGGGGAAAG	CCGCTTAAGCGCCCAAATGGCTCCCTCCG	EcoRV/AflII
SSB	AAAAAAGATATCGCCAGCAGAGGCGTAAACAAGG	AAAAAAGCTAGCGAACGGAATGTCATCATCAAAGTCCATC	EcoRV/NheI
DnaA*	AAAAAACTTAAGTCACTTTCGCTTTGGCAGCAGTGTC	AAAAAAGCTAGCCGATGACAATGTTCTGATTAAATTTG	AflII/NheI
HPR	AAAAAACATATGAAGACCGTGGGCGATAAACTCGAAG	AAAAAAGCTAGCCAGCGTCGCGCCGCCGATCG	NdeI/NheI
GroES	AAAAAACATATGAGCCTACGCCCGCTACACG	AAAAAAGCTAGCGGAATGTACGACTGCGACGATGTCC	NdeI/NheI
DnaQ	AAAACTTAAGCGCCAGATCATTCTCGATAC	AAAAGCTAGCAGCCTCGCCGACCGCCTC	AflII/NheI
DnaG	AAAAACTTAAGATTCCGCATTCGTTCCTGC	AAAAAGCTAGCCAGCCCGAGCCTCCGTTTC	AflII/NheI

*: DnaA sequence contained one mutation (P18S) compared to wildtype.

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Protein expression and purification

Except for DnaA-GFP and SSB-GFP, the POI-GFP were expressed in *E. coli* BL21(*DE3*)RIPL in Overnight Express Instant TB Medium (Novagen) at 16°C until OD reached a maximum. Cells were lysed by French press in lysis buffer (50 mM phosphate (pH 7.8), 2 mM β-mercaptoethanol, 300 mM NaCl) in the presence or absence of 10% glycerol (v/v), and the lysate was cleared by centrifugation at 40,000 g for 35 min. Except for SSB-GFP, all POI-GFP were purified with Ni-charged resin Profinity IMAC (Bio-Rad) following a previously described strategy.[1] After ammonium sulfate precipitation the POI-GFP were resuspended either in buffer A2 (50 mM sodium phosphate (pH 7.8) and 2 mM β-mercaptoethanol), buffer A10 (buffer A with 10 mM β-mercaptoethanol), buffer B (buffer A2 + 10% v/v glycerol) or Buffer C (50 mM HEPES-KOH (pH 7.6), 1 mM EDTA, 20 % sucrose, 1 mM β-mercaptoethanol). Tus-GFP was also dialyzed in buffer D (50 mM Tris (pH 7.8), 0.1 mM EDTA, 0.2 mM β-mercaptoethanol) + 250 mM KCl.

DnaA-GFP was expressed in *E. coli* KRX cells (Promega). Cells were grown in LB medium with ampicillin (100 μg/ml) at 25°C and 230 rpm until OD reached 1.8 at which point the temperature was dropped to 16 °C and rhamnose was added to a final concentration of 0.1%. Cultures were grown overnight and harvested by centrifugation at 4,000 rpm for 30 minutes. The cell pellets were resuspended at 7 ml/g of cells in ice cold buffer C + (300 mM KCl, 20 mM spermidine and 10 mM $MgCl_2$) and lysed by two French press passages at 12,000 psi. The lysate was cleared by centrifugation at 40,000 *g* for 35 min and purified

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with Ni-charged resin Profinity IMAC. The column was washed with buffer Ni (buffer C + (300 mM KCl and 10 mM imidazole)) and DnaA-GFP was eluted in buffer Ni + 200 mM imidazole. After ammonium sulfate precipitation DnaA-GFP was resuspended in buffer C.

SSB-GFP was also expressed in *E. coli* KRX cells following the same procedure as for DnaA-GFP. After centrifugation, cells were resuspended into 50 mM Tris (pH 8), 1 mM EDTA, 0.2 M NaCl, 10 % sucrose (w/v) and 15 mM spermidine trichloride. The cell lysate was precipitated with 0.15 g/ml AS and the pellet was resuspended in buffer E (20 mM Tris pH 8, 0.5 M NaCl, 1 mM EDTA, 1 mM β-mercaptoethanol, and 10 % glycerol (v/v)).

The purity of all proteins was assessed by SDS-PAGE (NEXT-GEL Amresco) and their concentrations by Bradford assay.

DNA and RNA ligands

Oligonucleotides were obtained from Sigma, diluted in either 10 mM Tris-HCl (pH 8) mM EDTA and 50 mM KCl (TE50) or 20 mM Tris (pH 8) and 150 mM NaCl (T150). Hybridization was performed by heating complementary oligonucleotides at 75-80°C for 2-5 min followed by slow cooling of complementary pairs of oligonucleotides (see following sections for sequence details).

Determination of K_{obs} of GK-GFP for ATP and glycerol.

T_m were obtained for GK-GFP samples containing glycerol (1-1024 μM) or ATP (5-5120 μM) and plotted against the log[ligand]. K_{obs} was obtained by linear regression and

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extrapolation of the linear portion of the curve and is defined as the concentration value at ΔTm = 0. At least four consecutive linear data points were required over one log to use this system for curve fitting and K_{obs} determination.

DnaA-GFP binding

The reactions containing DnaA-GFP (2.5 µM) in buffer C with different additives (see Table S1 for concentrations) were subjected to a melting curve protocol with a scan range of 30-75°C set at 0.5°C increments and a dwell time of 30 s.

The effect of DNA-binding to an ATP-DnaA box was also determined in the presence of ATP and $MgCl_2$. The ATP-DnaA box DNA (*oriC*, 100 µM) was obtained by hybridizing in buffer TE50 stoichiometric amounts of 5'-CCGGCTTTTAAGATCAACAACCTGGAAAGGATCA-3' and 5'-TGATCCTTTCCAGGTTGTTGATCTTAAAAGCCGG-3'. The *oriC* DNA was diluted in buffer C to a final concentration of 9 µM. Equal volumes of DnaA-GFP (7.5 µM in buffer C), additives (15 mM for nucleotides or/and 30 mM for divalent cations in buffer C) and *oriC* (9 µM) were mixed and incubated for 10 min at RT. For reference experiments without DNA, the fraction of TE50 in these reactions was kept identical as for reactions containing DNA.

Effect of pH and ionic strength on stability of free and Ter-bound Tus-GFP and GFP

Oligonucleotides JCU99 (5'-AATAAGTATGTTGTAACTAAAGTGGGGGCGGGG-3') and JCU100 (5'-CCCCGCCCCCACTTTAGTTACAACATACTTATT-3'), or JCU100 and JCU140 (5'-

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TATGTTGTAACTAAAGTGGGGGCGGGG-3') were hybridized in stoichiometric amounts in buffer T150 (50 μM final concentration) to obtain *TerB* and *Ter-lockB* respectively.

Equal volumes of Tus-GFP (50 μM in buffer B) and DNA (50 μM of *TerB* or *Ter-lockB*) or T150 were incubated for 15 min at RT. GFP (50 μM in buffer B) was mixed with T150 (1:1). Reactions (6 μl) were diluted in 54 μl buffer B + 250 mM NaCl at varying pH to obtain final pH values ranging from 4-12. For melting curves, the scan range was 35-80°C at 0.5°C increment with a dwell time of 30 s. The effect of pH on the T_m of *TerB* was also determined in the same conditions as for Tus-GFP (pH range: 4-12) with the exception that the reaction buffer contained SYBR green. To determine the K_{obs} of different *TerB*:Tus-GFP ratios, Tus-GFP was incubated with *TerB* as described above, however concentrations of *TerB* in T150 were altered so that the *TerB*:Tus-GFP ratio ranged from 1:16-2:1 keeping Tus-GFP constant at 2.5 μM final concentration. The T_m were determined at final pH of 5.9, 7.8 and 9.4. Both T_m and the height of the peaks were analyzed.

The effect of increasing concentrations of KCl on DNA binding of Tus-GFP was determined in buffer D (50 mM Tris (pH 7.5), 0.1 mM EDTA, 0.2 mM β-mercaptoethanol containing KCl ranging from 8.5-350 mM). Each reaction contained 2.5 μM of Tus-GFP and 3 μM of *TerB*, *Ter-lockB*, *oriC* or *oriC-lock*. The *oriC-lock* was obtained by hybridizing in buffer TE50 stoichiometric amounts of 5'-TTTAAGATCAACAACCTGGAAAGGATCA-3’ and 5’-TGATCCTTTCCAGGTTGTTGATCTTAAAAGCCGG-3’. Reactions were incubated 10 min at RT prior melting curves were started. Reference experiments of Tus-GFP without DNA were also done in identical buffer conditions.

Concentration dependence of TerB on Tus-GFP and determination of K_{obs}

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Reactions contained Tus-GFP at 2.5 μM and *TerB* at various concentrations ranging from 0.16-10 μM. Reactions were carried-out in buffer D + 250 mM KCl at pH 7.5 and 7.8 as well as in the various phosphate buffer conditions described above (pH 5.9, 7.8 and 9.4). The K_{obs} of Tus-GFP for *TerB* and buffer D + 250 mM KCl (pH 7.5 and 7.8) were obtained using the same strategy as for GK-GFP (described above).

RdRp-GFP binding

RdRp-GFP stock was in buffer A10 (145 μM). All reactions contained 20 μl of RdRp-GFP solution at 2.5 μM final concentration and were prepared by mixing three volumes of RdRp-GFP diluted in buffer A10 or buffers A10 + glycerol (5% to 40%) with one volume of additive (in water). NaCl and $(NH_4)_2SO_4$ at final concentrations of 100 mM, 300 mM or 500 mM and their combined effects were tested. The effects of $ZnSO_4$, $MgSO_4$ and EDTA at final concentrations ranging from 0.001 to 1 mM were tested in the presence of 7.5% of glycerol and 100 mM $(NH_4)_2SO_4$. The effects of ATP, GTP, GDP, cAMP and AMPPNP each at a final concentration of 1 mM were tested without glycerol.

The effect of RNA was tested in the presence of 18.75% of glycerol and ATP (1 mM) or both ATP and $MgCl_2$ (1 mM each). For this, RNAs 5'-GGGGGGGGG-3' (9-mer) and 5'-AAAAAAAAAAAAAAAAAAACCCCCCCCC-3' (28-mer) were dissolved in RNase free water at concentrations of 100 μM. Partially double stranded RNA was obtained by mixing 10 μl of 9N, 10 μl of 28N and 20 μl of T150 and heating the mixture at 70°C for two minutes followed by slow cooling. Each reaction contained RNA (2.5 μM) and RdRp-GFP (2.5 μM).

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For melting curves, the scan range was 35-75°C set at 0.5°C increments and a dwell time of 30 s.

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