Supplemental Methods and Figures

Conjugation of σD581C with MBP

σD581C (1000 pmol) was conjugated with the photoreactive crosslinker maleimide benzophenone (MBP; Sigma) by incubating for 30 min at 25° C with a 1000 molar excess of MBP (Sigma) in a solution (200 μl) containing 50 mM Tris-HCl (pH 8), 50 mM NaCl, 50% glycerol, 1 mM EDTA, 0.01% Triton X-100, and 0.1 mM DTT. Reactions were stopped by the addition of 1 M DTT (2.5 μl), and the protein solution was concentrated using an Amicon Ultra Centrifugal Filter (Millipore).

Crosslinking with σD581C-MBP

For photo-crosslinking using σD581C conjugated with MBP, the σ^{70} protein (2.5 or 5 pmol) was incubated as indicated, with a 5 to 6-fold excess His_6-tagged AsiA, a 2.4-fold excess MotA, and an equal amount of core and P_{uvsX} DNA for 10 min at 37° C in a solution (20 ml) containing 30 mM potassium phosphate (pH 6.5), 85 mM NaCl, 150 mM potassium glutamate, 10 mM Tris-HCl (pH 8), 40 mM Tris-acetate (pH 7.9), 0.2 mM DTT, 0.3 mM EDTA, 4 mM magnesium acetate, 0.1 mM β-mercaptoethanol, and 20% glycerol. (Before the reaction, AsiA was incubated with σ^{70} for 10 min at 37° C and then the AsiA/σ complex was incubated with core for 10 min at 37° C to assure formation of the AsiA-associated RNAP before the addition of MotA and DNA). Non-specific and unstable transcription complexes were challenged by the addition of heparin (1 ml of a 1 mg/ml solution) followed by incubation for 1 min at 37° C.

Solutions were transferred to a 96-well plate and irradiated at 366 nM using a hand held UV lamp (Minera light, Model UVGL-58) at a distance of 5 cm for 30 min at 25° C. Protein species were separated by SDS-PAGE on 10-20% Tris-tricine gels (Novex, Invitrogen) and then observed by silver staining (SilverXpress, Invitrogen Silver Staining Kit) and/or by Western blot analysis.

Western Blot Analyses

After transferring proteins to nitrocellulose membranes using the i-Blot Gel Transfer system (Invitrogen), membranes were blocked by incubating with 5% nonfat milk in 1 X PBS overnight. Blots were incubated with the primary antibody [either monoclonal anti-polyhistidine (Sigma) to detect His_6-tagged AsiA or monoclonal anti-σ, anti-β', or anti-β (Neoclone) to detect RNAP subunits] in 1% nonfat milk in 1 X PBS for 1 hr and washed 3 times with 0.1% Tween in 1 X PBS.

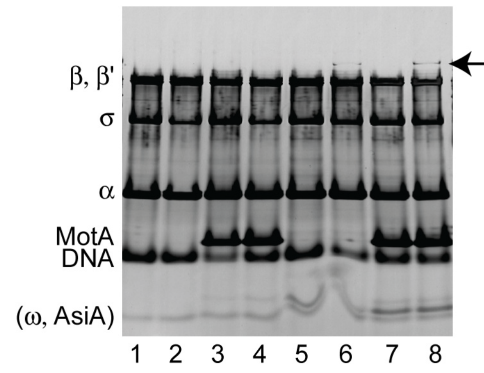
Blots were incubated for 1 hr with the secondary antibody (HRP-conjugated goat anti-mouse; Santa Cruz Biotechnology) diluted 1:10,000 in 1% nonfat milk in 1 X PBS. The blots were washed as before, and then visualized using the ECL Prime Western Blotting Detection Reagent (GE Healthcare) and detected using the BioRad ChemiDoc XRS Imaging System. In some cases, membranes were stripped using Restore Western Blot Stripping Buffer (Thermo Scientific) following the company's protocol and then reused.

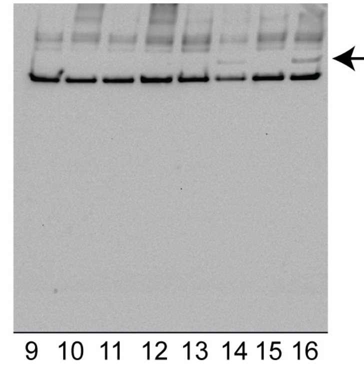
Generation of videos depicting initial and refined models of σ appropriation

Supplemental videos 1 and 2, generated first as Quicktime movies in PyMOL v1.8.0.6 Enhanced for Mac OS X, were converted to mp4 format using either FreeMovieConverter or Free Mp4 Converter. The Mp4 files were then imported into Wondershare Filmore (6.9.0) to add captions.

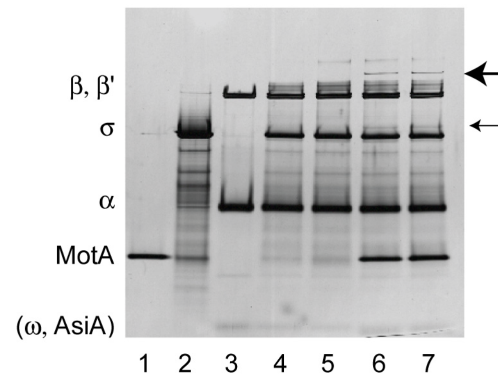
Figure S1. σ residue 581 can be crosslinked to β′ when σ is remodeled by AsiA. Solutions contained the T4 middle promoter P_{uvsX} and the indicated proteins, including either nonconjugated σD581C or σD581C conjugated with the photoreactive crosslinker maleimide benzophenone (MBP). After photocrosslinking and SDS-PAGE, proteins were visualized as indicated by either silver-staining or Western analyses using A) anti-β' antibody or B) anti-σ antibody. The positions of the RNAP core subunits (β, β', α, and ω), σ, MotA, and AsiA are indicated. Larger arrows show position of the σ-β' crosslink; smaller arrow shows the position of a species that would be consistent with a σ-AsiA crosslink. (Silver staining in panel A visualized the DNA fragment, which is indicated; DNA was not visualized in B because the exposure time was lower.)

A.





B.



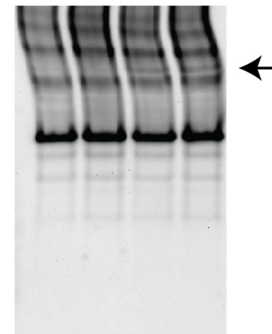
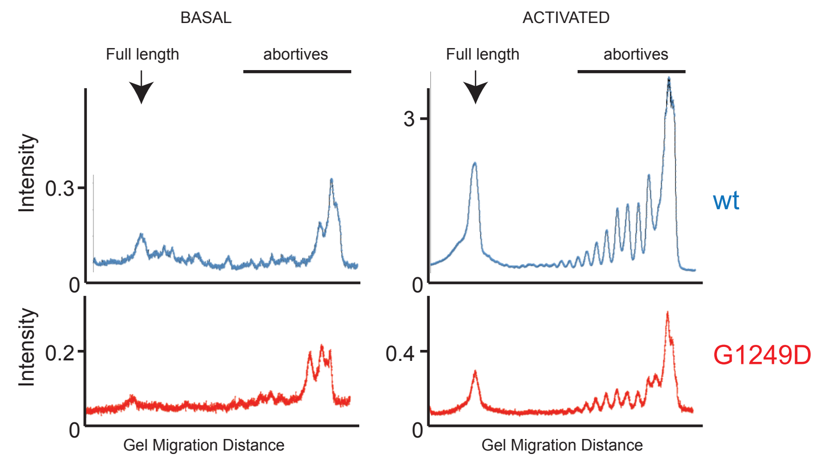


Figure S2. βG1249D does not affect promoter clearance from P_{uvsX} in the presence of MotA/AsiA. (A) Scans of a gel after promoter clearance transcription assays show amount of ^{32}P-labeled RNA products (intensity) vs. size of products (Gel Migration Distance) using either RNAP alone (Basal) or RNAP/MotA/AsiA (Activated) [wt β, blue; βG1249D, red]. DNA was incubated with proteins for 15 min at 37° C before the addition of NTPs/heparin. The positions of full length RNA (34 nt) and the abortive products (up to ~13 nt) are indicated. (B) Ratio of abortive RNAs to full length P_{uvsX} RNA with standard deviations were obtained from 4 experiments.


A
BASAL
ACTIVATED
Full length
abortives
Full length
abortives
Intensity
0.3
0
3
0
wt
Intensity
0.2
0
0.4
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G1249D
Gel Migration Distance
Gel Migration Distance

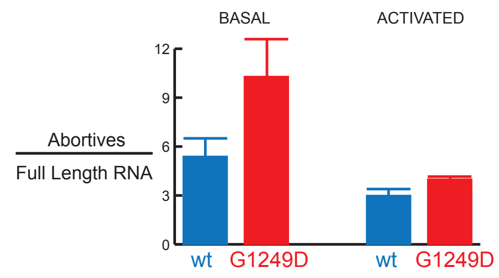
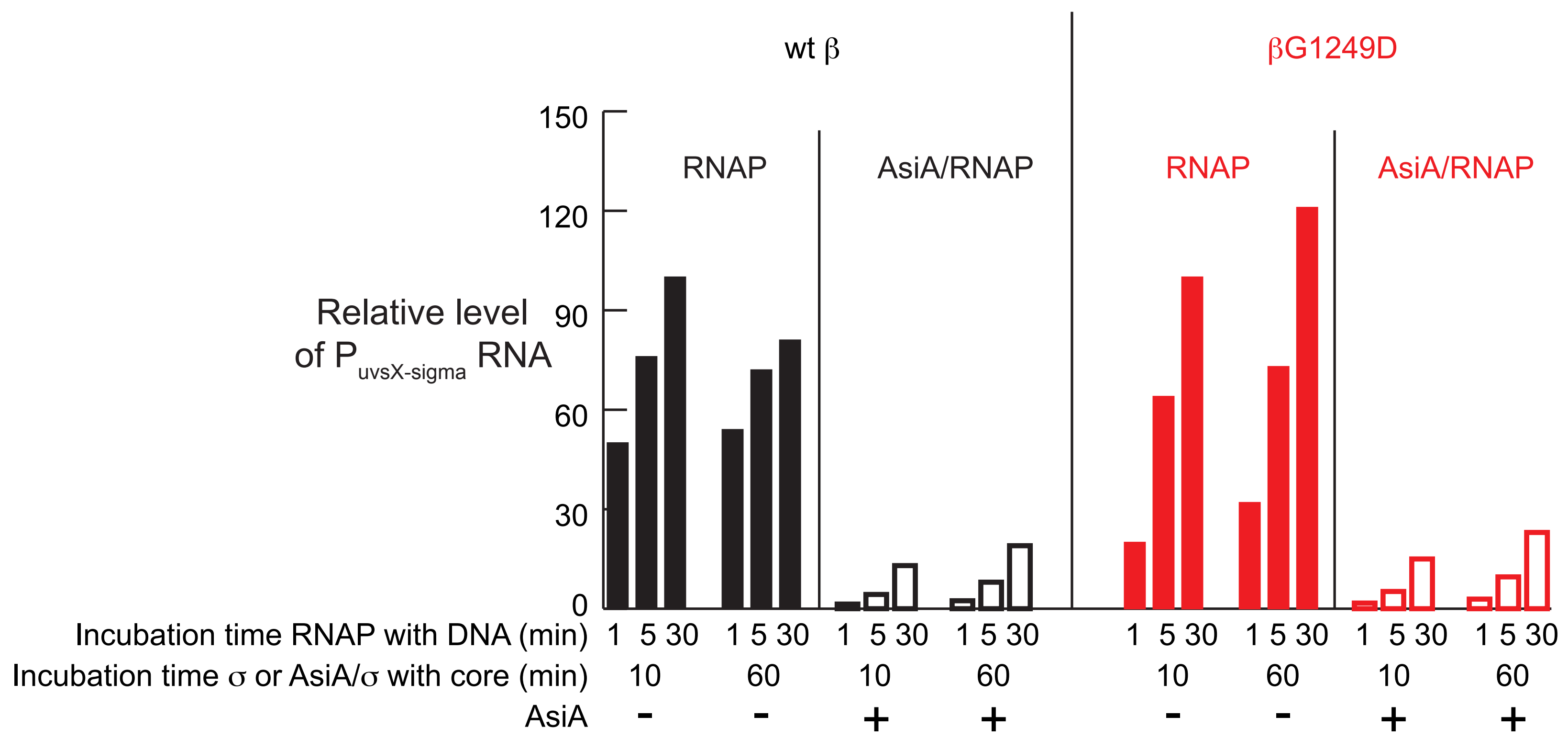

B
BASAL
ACTIVATED
Abortives
Full Length RNA
12
9
6
3
0
wt
G1249D
wt
G1249D

Figure S3. βG1249D does not affect the relative amount of transcription from a strong -10/-35 promoter by AsiA-associated RNAP. Histogram showing levels of P_{uvsX}-σ RNA for wt β (solid black plus σ alone, open black plus σ/AsiA) or βG1249D (solid red plus σ alone, open red plus σ/AsiA) relative to the amount generated after incubation of σ alone with core for 10 min and incubation of RNAP with DNA for 30 min. (σ or AsiA/σ was first incubated with core as indicated for 10 or 60 min at 37° C to generate RNAP or AsiA-associated RNAP. Polymerase was then incubated with the DNA as indicated for 1, 5, or 30 min before the addition of rNTPs and heparin to initiate a single round of transcription.)


wt β
βG1249D
RNAP
AsiA/RNAP
RNAP
AsiA/RNAP
150
120
90
60
30
0
Relative level
of $P_{uvsX\text{-}sigma}$ RNA
Incubation time RNAP with DNA (min)
1 5 30 1 5 30 1 5 30 1 5 30 1 5 30 1 5 30 1 5 30 1 5 30
Incubation time σ or AsiA/σ with core (min)
10 60 10 60 10 60 10 60
AsiA
- - + + - - + +

Supplemental video 1. Initial structure/model of σ appropriation. 1. RNA polymerase/host promoter. Subunits of core RNAP (β', cobalt; β, ice blue; ω, magenta; α, cyan) are shown as spheres; σ (yellow), nontemplate DNA strand (red), and template DNA strand (orange) are shown as cartoon. **2. sigma 581 (red sphere) close to DNA positions -36 to -40 (orange spheres); beta-flap (pale blue) holds sigma H5.** Close-up showing the position of σ residue 581 and σD581C-FeBABE template strand cut sites obtained in the presence of RNAP. **3. sigma (yellow) remodeled by AsiA (red); unremodeled sigma is in tan.** Region 4/H5 of both the σ present in *E. coli* RNAP and the AsiA-remodeled σ are presented, showing the overlap between these structures in σ residues 546-549; the ω subunit has been removed in order to see the overlap. **4. MotA (green) is added. (MotA box in the DNA is shown in blue.)** The $MotA^{CTD}$ and $MotA^{NTD}$ domains of MotA are added to the structure based on their determined positions from the FeBABE analyses; loop modeling generates the unsolved linker between the C-terminus of $MotA^{NTD}$ and the N-terminus of $MotA^{CTD}$. Remodeled σ is now in yellow. **5. sigma H5 (F610, L611 shown as yellow spheres) points toward MotA NTD hydrophobic cleft (shown as white spheres).** H5 residues F610 and L611 are pointed toward their binding partner, the basic, hydrophobic cleft of $MotA^{NTD}$ designated by residues I7, I24, A83, and Y86. **6. Initial model RNA polymerase/MotA/AsiA/DNA.** The complete initial model is shown.

Supplemental video 2. Refined structure/model of σ appropriation. 1. Refined Model RNA Polymerase/AsiA/MotA/DNA. Colors of proteins and DNA strands as in Supplemental Video 1. **2. sigma 581 (red sphere) near DNA positions -29, -30 (orange spheres).** Close-up showing the position of σ residue 581 and σD581C-FeBABE template strand cut sites obtained in the presence of RNAP, MotA and AsiA. **3. sigma 581 (red sphere) near beta' aa 80-87 (brown spheres). 4. sigma, AsiA, MotA, and DNA are shown.** Core subunits of RNAP have been removed. **5. sigma F610, L611 (yellow spheres) buried in MotA NTD cleft (white spheres).** σ residues F610 and L611 are now buried within the $MotA^{NTD}$ basic, hydrophobic cleft containing residues I7, I24, A83, and Y86 (white spheres). 6. **Refined Model RNA Polymerase/AsiA/MotA/DNA**. Zoom out to show the complete refined model.