SUPPLEMENTARY INFORMATION

SUPPLEMENTARY DATA

Displacement of σ region 1.1 by a downstream fork junction

Fluorescein was incorporated into σ^{70} region 1.1 at position 38 as described in (Knight *et al*, 2005). Fluorescence of RNAP holoenzyme assembled from wild type core RNAP and the labeled at position 38 σ^{70} subunit decreased upon RNAP binding to Rifampicin (Rif) due to FRET between fluorescein and Rif (Mekler and Ebright, 2005). Figure S1 shows the values of FRET efficiency measured in RNAP and RNAP complexes with -60/+30 T5N25 promoter DNA fragment and with [-12/+18][+3/+18] downstream fork junction (shown in Fig. 1A).

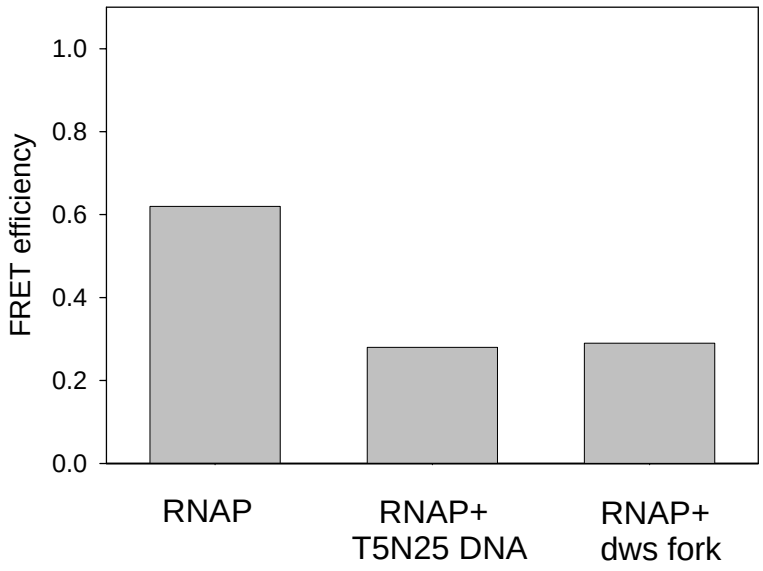

Figure S1 Fluorescence resonance energy transfer assay reveals displacement of σ region 1.1 by a downstream fork junction. RNAP (1 nM) was incubated with 100 nM Rif for 20 m followed by incubation with either 2 nM of T5N5 DNA or 2nM of the fork junction probe for 1 h. FRET efficiency was measured as in (Knight *et al*, 2005).

Determination of affinity of -8T substituted downstream fork junction probes using a competition binding beacon assay

The signal generated by -8T substituted downstream fork junctions is low (Fig. 1B), which complicates calculation of K_d from dependence of the fluorescent signal amplitude on DNA probe concentration (i.e. by using titration assay). Affinity of RNAP beacon to these probes was evaluated by a competition binding assay using -12/+2 oligo (5'-TATAATAGATTCAT) as a competitor.

Figure S2 shows changes in florescence intensity after addition of 500 nM -12/+2 oligo to free RNAP beacon and to samples in which RNAP beacon was preincubated with 2 nM of each of the studied -8T substituted fork junctions for 2 hours. Upon the addition of -12/+2 to free RNAP beacon, the signal increased and reached saturation intensity for a few seconds. Upon similar oligo addition to the samples containing fork junctions, a rapid increase in the signal intensity was followed by further slow enhancement of the signal. The initial fast intensity increase in the samples containing fork junctions apparently corresponds to oligo interaction with free RNAP beacon molecules, while the slow kinetics reflects reaching of equilibrium between the oligo and bound to RNAP fork junctions. Consequently, amplitude of the rapid increase is proportional to concentration of RNAP beacons remained unbound to fork junctions before the oligo addition. Since characteristic time of the slow signal increase is considerably longer than the time required for signal amplitude measurement (~ 30 s), amplitude of the rapid signal increase could be readily found from the data shown in Fig. S3. Therefore, occupancy (X) of RNAP beacon by a fork junction probe was determined as $X = 1-I/I_0$, where I_0 is amplitude of the oligo signal measured with free beacon and I is

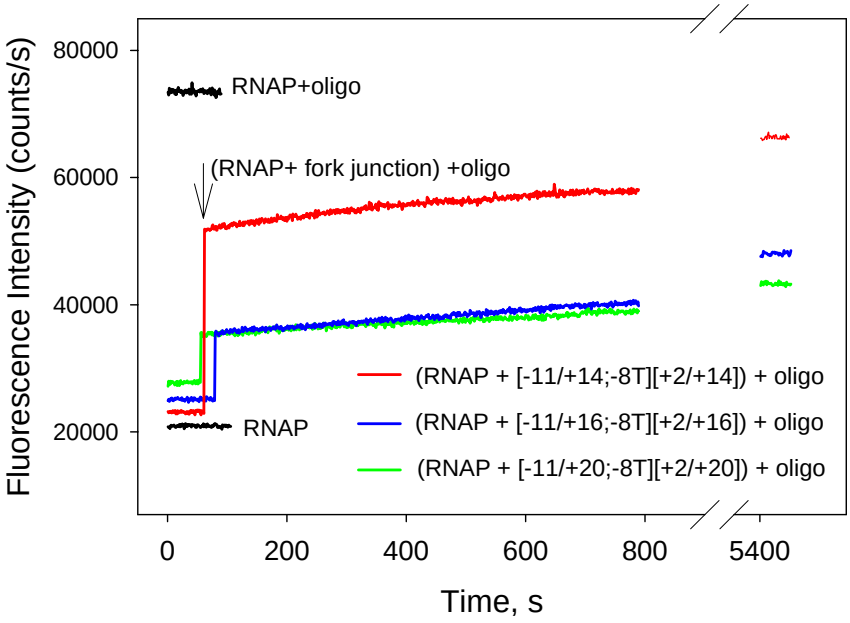

Figure S2 Protein beacon assay for RNAP occupancy by downstream fork junctions. Time-dependent change of the fluorescence signal upon addition of 500 nM -12/+2 oligo to samples containing 1 nM RNAP beacon preincubated with 2nM of -8T substituted downstream fork junctions for 2 hours. Fluorescence signal measured with free RNAP beacon and signal measured upon addition of the oligo to free RNAP beacon are indicated as "RNAP" and "RNAP + oligo", respectively.

amplitude of the rapid increase measured in sample containing beacon preequilibrated with a fork junction. The Kd values were calculated from these data by using a chemical equilibrium equation [S1].

$(1-X)(C-[RNAP]X) = K_d X,$ [S1]

where C is fork junction concentration. The RNAP occupancies and K_d values calculated from data shown in Fig. S3 are shown in Fig. 4B and Table 1.

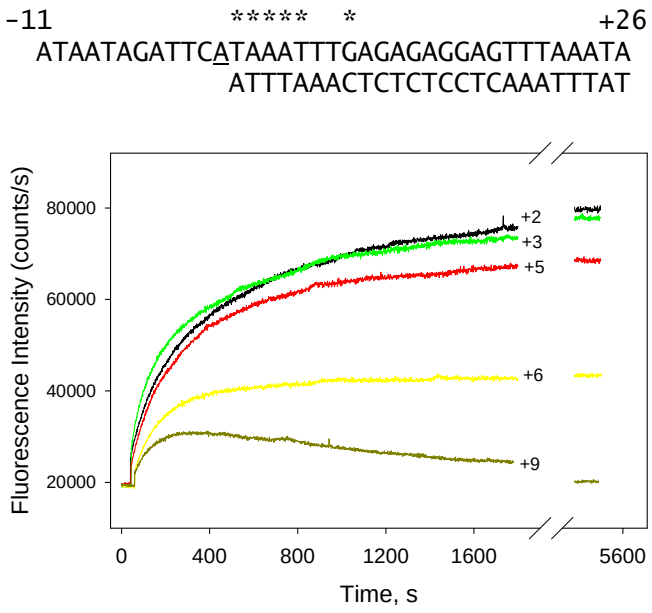

Figure S3 The structure of assayed probes with junction points at +2, +3, +5, +6 and +9 and time-dependent change of the fluorescence signal upon probe addition to RNAP beacon. The concentrations of RNAP beacon and fork junctions were 1 and 2 nM, respectively.

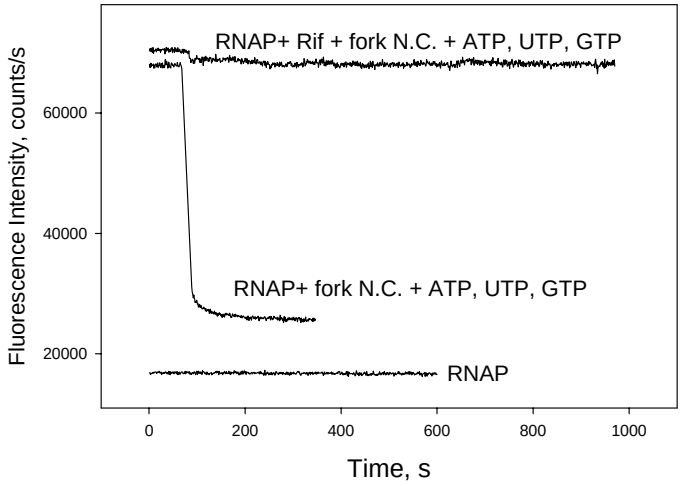

Figure S4 Beacon assay for transcription activity of partially non-complementary downstream fork junction. Time-dependent change of the fluorescence signal upon addition of a mixture of ATP, GTP and UTP (to a final concentration of 0.5 mM each) and 10 nM of unlabeled wild type σ^{70} to complexes of the N.C. fork junction with (211Cys-TMR) σ^{70} holoenzyme preformed with and without 1 μM rifampicin. The structure of the partially non-complementary fork junction (fork N.C.) is shown in Fig. 6A.

-40 +20
TTTATTTGCTTTCAGGAAAATTTTTCTGTATAATAGATTCATAAATTTGAGAGAGGAGTT
AAATAAACGAAAGTCCTTTTAAAAAGACATATTATCTAAGTATTTAAACTCTCTCCTCAA

Figure S5 The sequence of the parent double-stranded [-40/+20] probe that is derived from the N25 promoter of the phage T5.

REFERENCES

Knight JL, Mekler V, Mukhopadhyay J, Ebright RH, Levy RM (2005) Distance-restrained docking of rifampicin and rifamycin SV to RNA polymerase using

systematic FRET measurements: developing benchmarks of model quality and reliability. *Biophys J*. **88:** 925-938

Mekler V, Ebright RH (2005) Structural transitions in transcription initiation: Identification of the promoter-DNA segment required to displace DNA-molecular-mimic sigma R1.1 from the RNA-polymerase active-center cleft. *Biophys J* **88:** 568A