Supporting Information

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SI Text

SI Materials and Methods. ***Solutions and materials.*** RNA polymerase holoenzyme (RNAP) containing σ^{70} was endogenously expressed and purified from *Escherichia coli* K-12 MG1655. To label the nt strand for footprinting, DNA fragments containing the λP_R promoter were obtained from pMR288 using XbaI and SmaI cleavage; polynucleotide kinase (PNK) was used to add ^{32}P. To label the template strand, pBR81 was cut using BssHII and SmaI, and radio-labeled with ^{32}P using PNK. Fragments were isolated as described in ref. 1. Binding buffer (BB) contained 40 mM tris(hydroxymethyl)-aminomethane (Tris; pH 8.0 at 10 °C), 10 mM $MgCl_2$, 120 mM NaCl, and 100 μg/mL bovine serum albumin. RNAP storage buffer (SB) contained 10 mM Tris (pH 7.5 at 4 °C) 6.85 M glycerol, 100 mM NaCl, 100 nM Na_2EDTA, and 100 nM DTT. Enzymes were from New England Biolabs, and chemical reagents were from Sigma-Aldrich.

Population modeling. Interpolated rate constants from ref. 2 were input into Berkeley Madonna software (R.I. Macey and George F. Oster, University of California–Berkeley, www.berkeleymadonna.com) to generate a population histogram for the reaction resulting after addition of a nonperturbing competitor heparin or after a [NaCl] upshift of preformed open complexes. The population histogram was integrated with a 150-ms interval for each time point to correct for the lowering of time resolution due to the time required for the MnO_4^- reaction. The MnO_4^- reactivity for each reactive thymine position in I_2 relative to that of the RPo control was determined by comparing the observed amplitude and decay of reactivity to simulation-generated curves. To generate simulated MnO_4^- reactivity at each time point, the population of RP_o remaining was considered to retain 100% reactivity, whereas the remaining population of I_2 was considered to retain a constant fraction (0–100%) of the reactivity of the RP_o control throughout the decay.

Table S1. Simulation parameters utilized in Fig. 1

0.120 M salt	1.1 M salt
$K_1 = 2.7 \times 10^7$ M^{-1}*	$k_{-2} = 0.72$ s^{-1}†
$k_2 = 2.1 \times 10^{-3}$ s^{-1}‡	$k_3 = 0.093$ s^{-1}§
$k_{-2} = 0.72$ s^{-1}†	$k_{-3} = 10$ s^{-1}§
$k_3 = 100$ s^{-1}†	
$k_{-3} = 0.033$ s^{-1}†	

*Interpolated from the temperature dependence of values for K_1 in ref. 1.
†Ref. 2.
‡Ref. 1.
§The observed, overall salt dependence of k_d from ref. 2 was dissected into the individual salt dependence of k_3 and k_{-3} ($\frac{d \ln k_3}{d \ln[salt]} \approx -3.15$ and $\frac{d \ln k_{-3}}{d \ln[salt]} \approx 2.58$). Values of k_3 and k_{-3} in 1.1 M salt were determined by applying the individual salt dependences to the values of k_3 and k_{-3} in 0.120 M salt from ref. 2.

1 Craig ML, et al. (1998) DNA footprints of the two kinetically significant intermediates in formation of an RNA polymerase-promoter open complex: evidence that interactions with start site and downstream DNA induce sequential conformational changes in polymerase and DNA. *J Mol Biol* 283:741–756.
2 Kontur WS, Saecker RM, Capp MW, Record MT, Jr. (2008) Late steps in the formation of *E. coli* RNA polymerase-λP_R promoter open complexes: Characterization of conformational changes by rapid [perturbant] upshift experiments. *J Mol Biol* 376:1034–1047.