## Supplemental Data

## Retention of Transcription Initiation

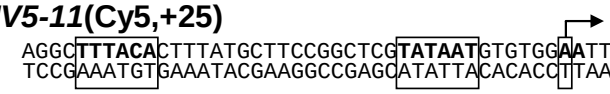
## Factor $\sigma^{70}$ in Transcription Elongation:

## Single-Molecule Analysis

**Achillefs N. Kapanidis, Emmanuel Margeat, Ted A. Laurence, Sören Doose, Sam On Ho, Jayanta Mukhopadhyay, Ekaterine Kortkhonjia, Vladimir Mekler, Richard H. Ebright, and Shimon Weiss**

**A**

***lacUV5-11*(Cy5,+25)**



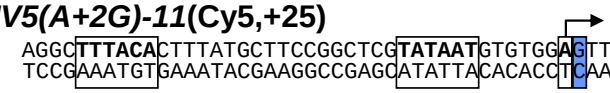

***lacUV5-14*(Cy5,+28)**


***lacUV5-50*(Cy5,+64)**


***lacUV5-11*(Cy5,-40)**


**B**

***lacUV5(A+2G)-11*(Cy5,+25)**




***lacUV5(A+2G)-14*(Cy5,+28)**


***lacUV5(A+2G)-50*(Cy5,+64)**


***lacUV5(A+2G)-11*(Cy5,-40)**

**Figure S1**. DNA fragments.

(A) DNA fragments used in the analysis of $RD_e$ initiated on *lacUV5* and halted after synthesis of 11, 14, or 50 nt of RNA (*lacUV5* derivatives having no guanine residues on the template strand from positions +1 to +11, +1 to +14, or +11 to +50)(Mukhopadhyay et al., 2001; Mukhopadhyay et al., 2003; Nickels et al., 2004). The first 3 DNA fragments are used for leading-edge FRET analysis and thus have fluorophore Cy5 at position +25, +28, and +64. The $4^{th}$ DNA fragment is used for trailing-edge FRET analysis and thus has fluorophore Cy5 at position –40. Black boxes, transcription start site (with arrow), promoter –10 element and promoter –35 element; red boxes, halt site.

(B) DNA fragments used in the analysis of $RD_e$ initiated on *lacUV5(A+2G)* (Nickels et al., 2004) and halted after synthesis of 11, 14, or 50 nt of RNA (Mukhopadhyay et al., 2001; Mukhopadhyay et al., 2003; Nickels et al., 2004). The first 3 DNA fragments are used for leading-edge FRET analysis and thus have FRET acceptor Cy5 at position +25, +28, and +64. The $4^{th}$ DNA fragment is used for trailing-edge FRET analysis and thus has FRET acceptor Cy5 at position –40.

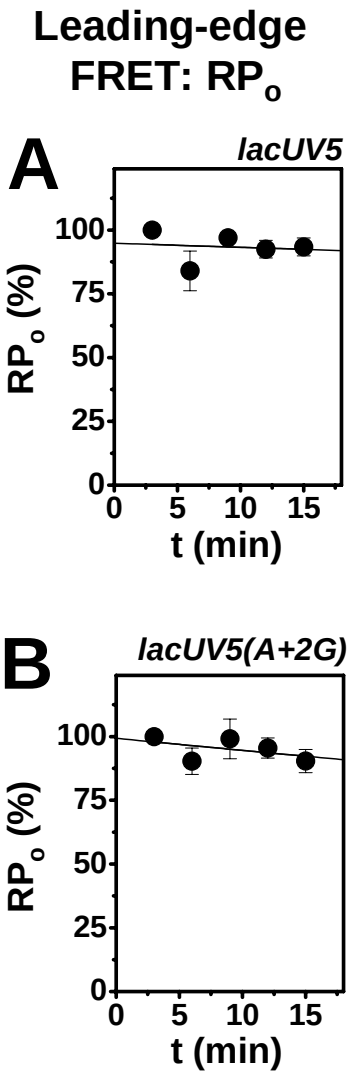



**Figure S2.** $\sigma^{70}$ retention in $RP_o$.

(A) Extent of $\sigma^{70}$ retention in $RP_o$ at *lacUV5*. The y-intercept (t = 0 min) corresponds to the beginning of data acquisition; the data are normalized to the 3-min data point. Error bars, standard error of mean (SEM) for 4 independent measurements. Solid lines, single-exponential fits. The half-life of $\sigma^{70}$ retention is >2 h.

(B) As panel (A), but for $RP_o$ at *lacUV5(A+2G)* (substituted *lacUV5* derivative lacking determinant for sequence-specific $\sigma^{70}$-DNA interaction in initial transcribed region ). The half-life of $\sigma^{70}$ retention is >2 h.

**Table S1**. Relative equilibrium binding constants ($K_{b,x}/K_{b,\ wild\text{-}type}$) for RNAP core binding of $\sigma^{70}$ derivatives used in this work (data from fluorescence-detected electrophoretic mobility shift experiments).

| **Sigma derivative** | **$K_{b,x}/K_{b,wild\text{-}type}$** |
|---|---|
| **unlabelled wild-type sigma** | [1.0] |
| **TMR366-sigma** | 0.5±0.1 |
| **TMR596-sigma** | 1.1±0.1 |