
Figures and figure supplements

The primary σ factor in *Escherichia coli* can access the transcription elongation complex from solution in vivo

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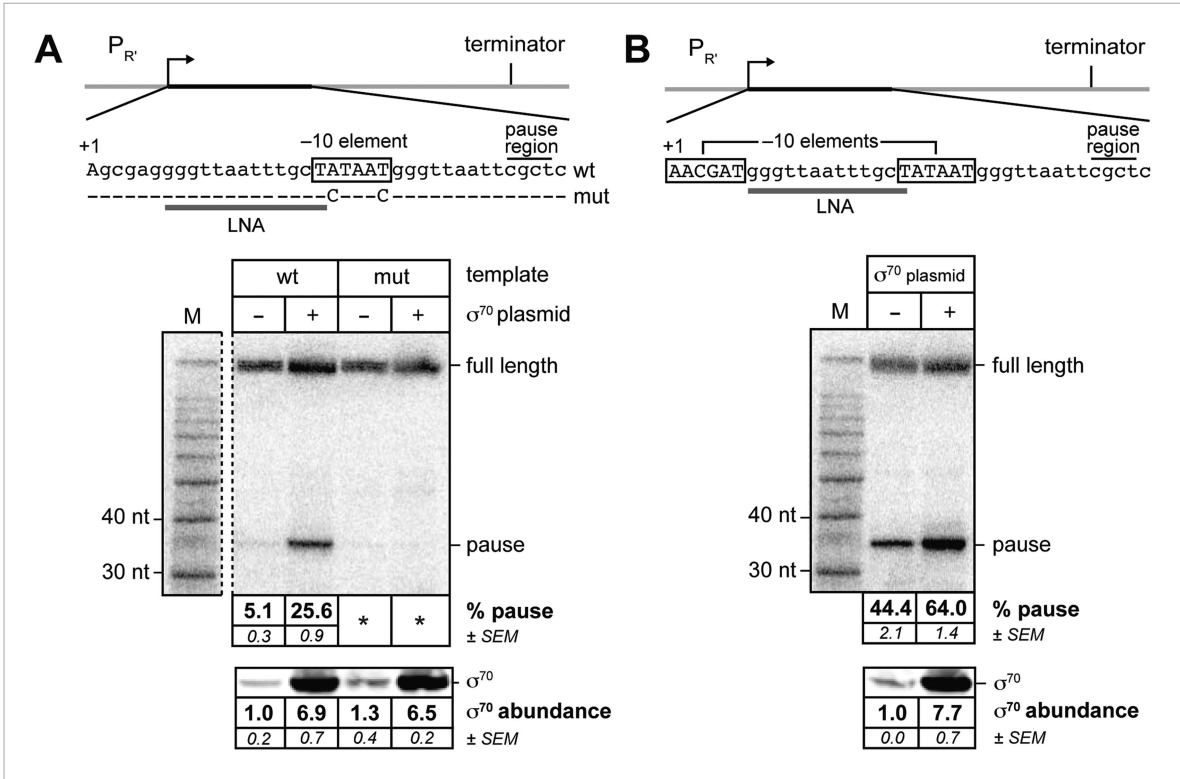


Figure 1. σ^{70} trans loading on a σ^{70} -dependent transcription unit in vivo (MG1655). **(A)** Top: schematic of DNA template carrying $\lambda P_{R'}$, transcribed-region consensus extended -10 element (wild-type or mutant) and terminator (see 'Materials and methods' for the $\lambda P_{R'}$ promoter sequence). Transcribed-region sequences that are complementary to the LNA probe are underlined (grey bar) and the positions corresponding to pause sites are indicated. middle Analysis of RNA transcripts in vivo by LNA probe-hybridization. RNA was isolated from MG1655 cells harvested at an OD_{600} of 0.8–1.0 (see 'Materials and methods'). Pausing is quantified by dividing the signal in the ~35-nt pause RNA band by the sum of this signal and the signal in the terminated (full-length) band; this ratio is expressed as a percentage (relative abundance). Mean and SEM of six independent measurements are shown. Asterisks (*) designate values that were too low (<approximately threefold above background) for accurate quantification. M, 10-nt RNA ladder. bottom Analysis of σ^{70} levels by Western blot. Amount of soluble σ^{70} is normalized to the amount in cells carrying the experimental template (wt) and a vector that does not direct σ^{70} over-production. Mean and SEM of three independent measurements are shown. **(B)** Top: schematic of DNA template carrying $\lambda P_{R'}$, initial-transcribed-region σ^{70} -dependent pause element, transcribed-region consensus -10 element and terminator. middle Analysis of RNA transcripts in vivo by locked-nucleic-acid (LNA) probe-hybridization, as in panel A. bottom Analysis of σ^{70} levels by Western blot. DOI: 10.7554/eLife.10514.003

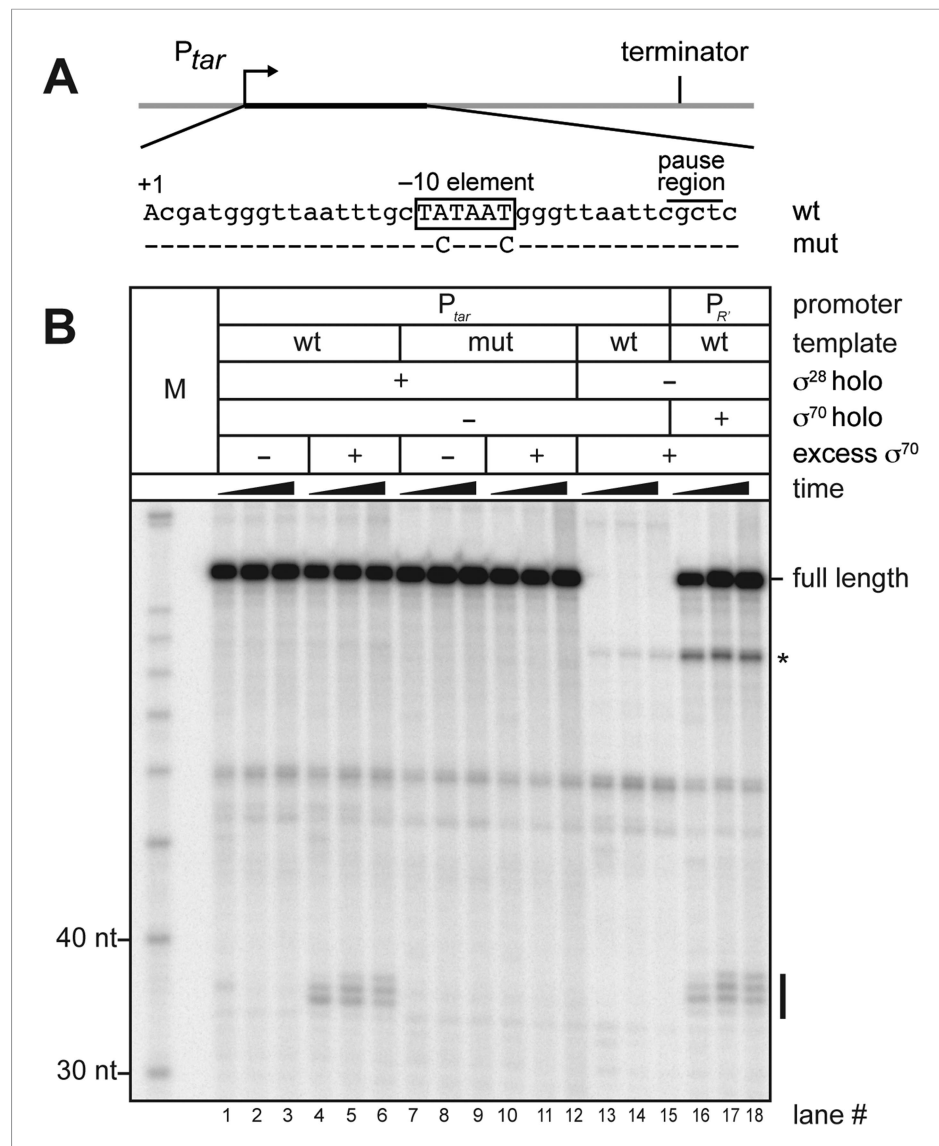
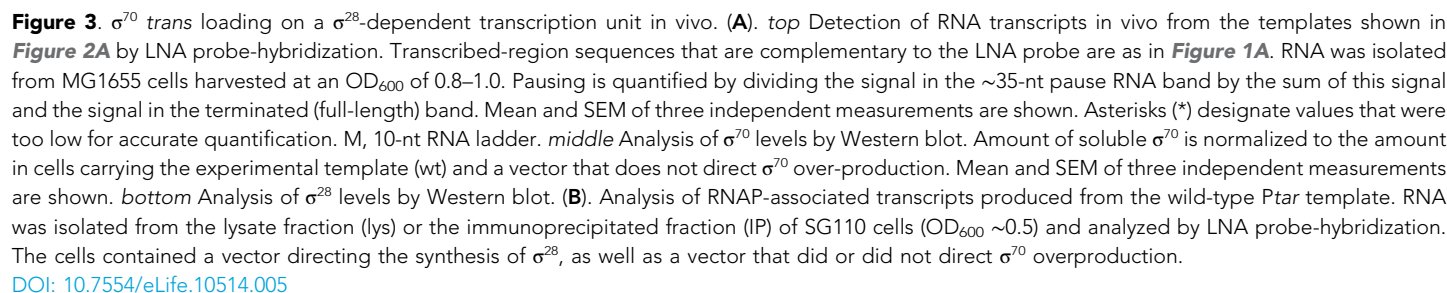


Figure 2. σ^{70} trans loading on a σ^{28} -dependent transcription unit in vitro. **(A).** Schematic of DNA template carrying P_{tar} , transcribed-region consensus -10 element (wild-type or mutant) and terminator. Template positions corresponding to pause sites are indicated. Note that the pause sites and terminated transcripts emanating from the P_{tar} promoter are located one base closer to the transcription start site (+1) than on the $\lambda P_{R'}$ template (**Figure 1A**). (See 'Materials and methods' for the P_{tar} promoter sequence.) **(B).** Analysis of RNA transcripts in vitro. Single-round in vitro transcription reactions were performed with reconstituted RNA polymerase (RNAP) holoenzyme containing σ^{28} (lanes 1–12), RNAP core enzyme (lanes 13–15) or reconstituted RNAP holoenzyme containing σ^{70} (lanes 16–18) and three different templates: P_{tar} with a wild-type (wt) transcribed-region -10 element (lanes 1–6 & 13–15) or a mutated (mut) transcribed-region -10 element (lanes 7–12) and $\lambda P_{R'}$ with a wild-type transcribed-region -10 element (lanes 16–18). The reactions were performed as a time course with samples taken at 1, 6 and 18 min after transcription was initiated; these reactions were performed in the absence of transcript cleavage factors GreA and GreB, resulting in a characteristic pattern of long-lived pause species (Deighan et al., 2011). Where indicated, excess σ^{70} (1 μ M) was added with the 'start mix' after open complex formation. RNAs associated with paused transcription elongation complexes (TECs) (pause) and terminated transcripts (full length) are labeled. The asterisk (*) indicates a shorter terminated transcript that is the result of transcription initiating under the control of the transcribed-region -10 element when the σ^{70} -containing holoenzyme is present in the reaction.

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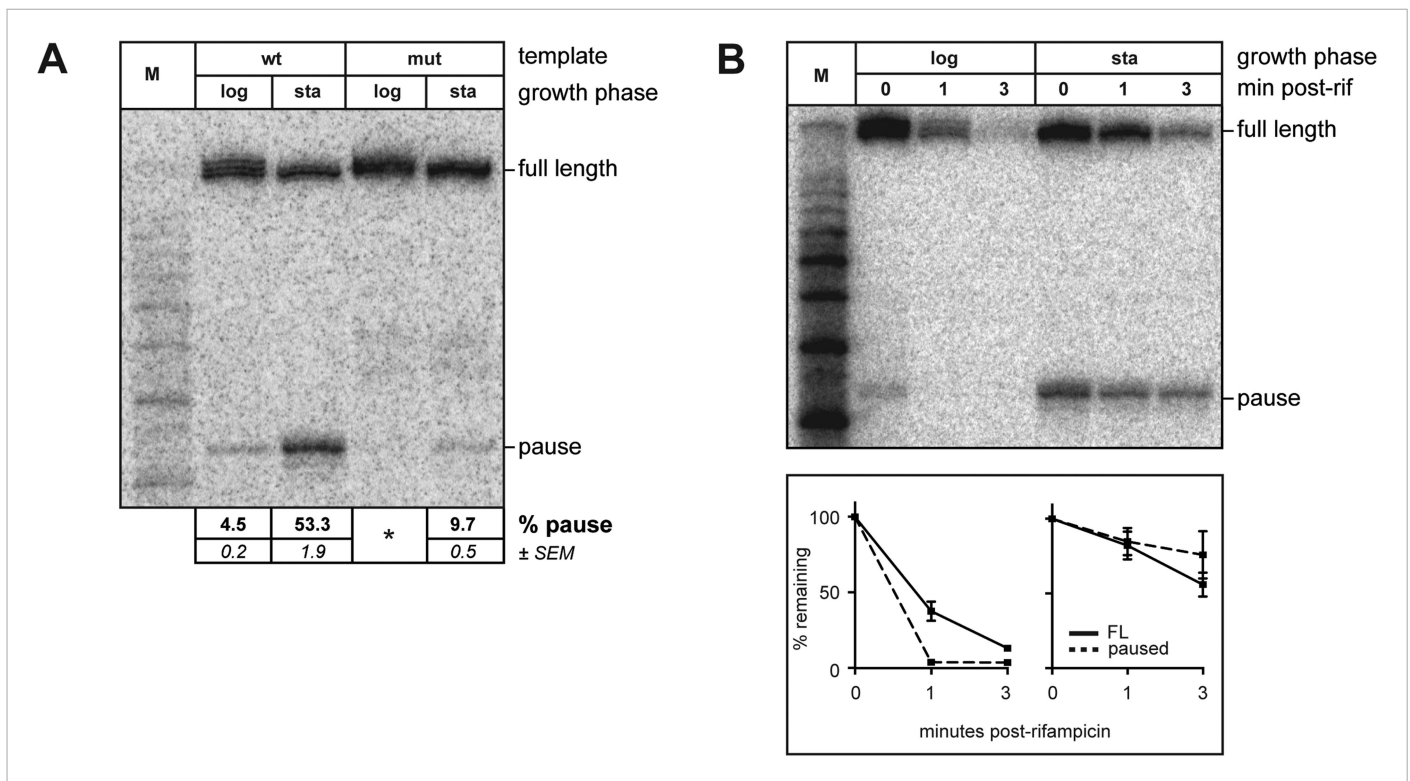


Figure 4. Growth phase dependent σ^{70} trans loading on a σ^{28} -dependent transcription unit in vivo. **(A).** Detection of RNA transcripts in vivo from the templates shown in **Figure 2A** by LNA probe-hybridization. Transcribed-region sequences that are complementary to the LNA probe are as in **Figure 1A**. RNA was isolated from SG110 cells harvested at an OD_{600} of ~ 0.5 (log) or ~ 2.5 (sta). Pausing is quantified by dividing the signal in the ~ 35 -nt pause RNA band by the sum of this signal and the signal in the terminated (full-length) band. Mean and SEM of six independent measurements are shown. Asterisks (*) designate values that were too low for accurate quantification. M, 10-nt RNA ladder. **(B).** *top* Detection of RNA transcripts derived from the wt template in vivo after treatment with rifampicin. *bottom* Percent of transcript remaining relative to T = 0 at indicated time points after addition of rifampicin. Mean and SEM of ten (log, 1 m), eight (sta, 1 m), or six (log and sta, 3 m) independent measurements are shown.

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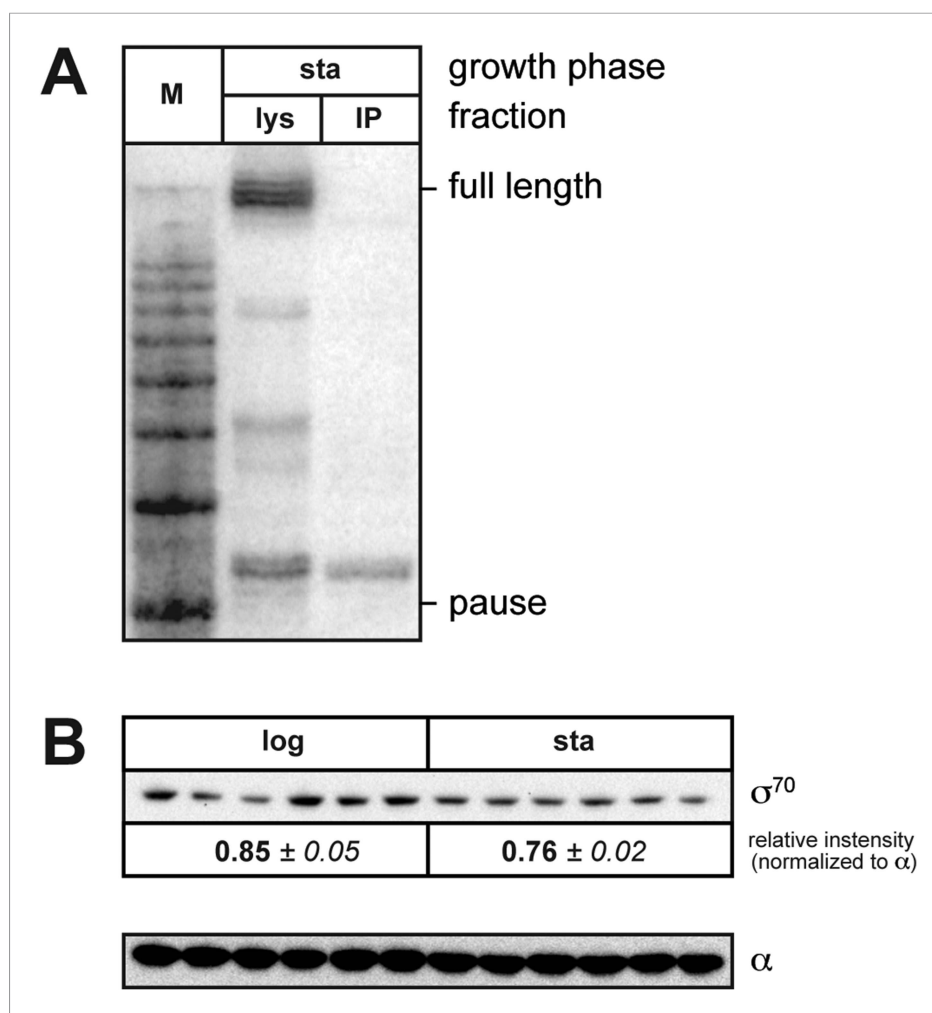


Figure 4—figure supplement 1. (A). Analysis of RNAP-associated transcripts produced from the wild-type *P_{tar}* template. RNA was isolated from the lysate fraction (lys) or the immunoprecipitated fraction (IP) of SG110 cells ($OD_{600} \sim 2.5$) and analyzed by LNA probe-hybridization. The cells contained a vector directing the synthesis of σ^{28} . (B). Analysis of σ^{70} levels by Western blot. Relative quantification of σ^{70} (top) is normalized to the abundance of *rpoA* (α) in each sample (bottom). Mean and SEM of six independent measurements are shown.
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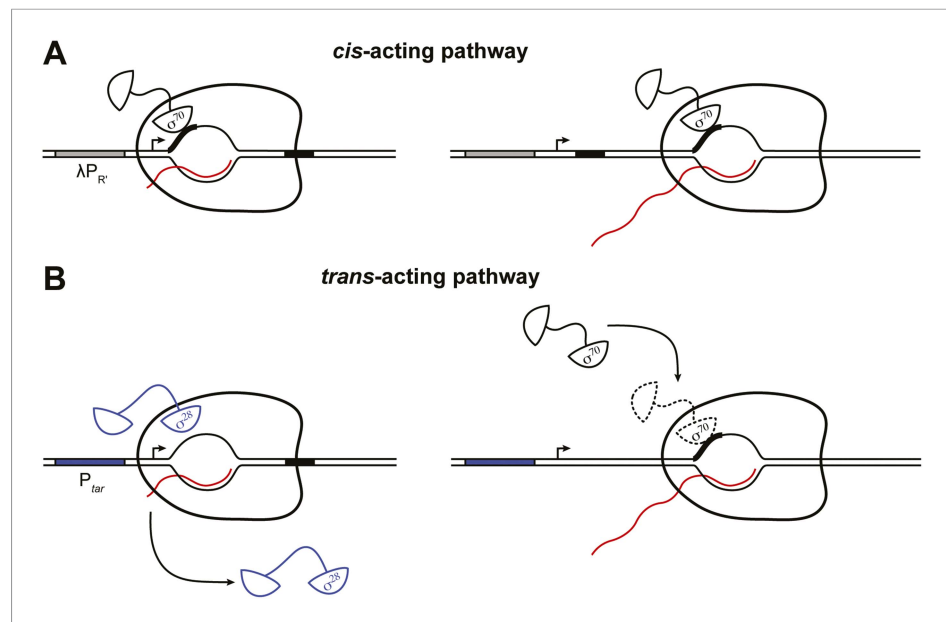


Figure 5. Dual pathways for σ^{70} to associate with the TEC in vivo. **(A).** Cis-acting pathway (Deighan et al., 2011). The retention in the TEC of the σ^{70} that was used during initiation enables pausing at transcribed-region -10 -like elements on transcription units that are expressed under the control of σ^{70} -dependent promoters. Presence of an initial-transcribed-region σ^{70} -dependent -10 -like element increases the σ^{70} content of downstream TECs and increases the efficiency of pausing at a second σ^{70} -dependent pause element further downstream. Promoter, grey rectangle; σ^{70} -dependent pause elements, black rectangles; RNA, wavy red line. **(B).** Trans-acting pathway. Binding of σ^{70} to TECs that have lost the σ factor used during initiation (here, σ^{28}) increases the efficiency of pausing at a transcribed-region σ^{70} -dependent pause element. Promoter, blue rectangle; σ^{70} -dependent pause element, black rectangle; RNA, wavy red line.

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