SUPPORTING MATERIAL

POLRMT transcription control experiment for ^{32}P incorporation during elongation

For promoter binding of transcription factors, 100 nM mtTFA and 24 nM mtTFB2 were incubated at 32 ºC for 5 min with 4 nM LSP or HSP templates in POLRMT transcription buffer (10 mM HEPES, pH 7.9, 10 mM $MgCl_2$, 20 mM NaCl, 0.1 μg/μL bovine serum albumin, 1 mM dithiothreitol). For "cassette labeling" reactions for LSP template binding contained 100 μM ATP, 20 μM GTP, and 10 μM UTP and 0.1 μM [α-^{32}P] UTP (3000 Ci/mmol) and those for HSP template binding contained 100 μM ATP, 20 μM GTP, 10 μM CTP and 0.1 μM [α-^{32}P] CTP (3000 Ci/mmol). For "hot elongation" reactions, [α-^{32}P] NTP was omitted from the initiation mix. To test for heparin inhibition of initiation, heparin was included in the initiation mix for one "cassette labeling" reaction (Lane 1). Addition of 20 nM POLRMT initiated transcription at each promoter and continued incubation at 32 ºC allowed POLRMT extension of transcripts to the end of the "labeling cassette" in each template. By excluding the next ribonucleotide required for transcript extension (CTP for the LSP templates and UTP for the HSP1 templates), POLRMT complexes were stalled until ribonucleoside triphosphate (NTP) addition. After 5 min for LSP template reactions and 10 min for HSP template reactions, heparin was added at 50 ng/μL to inactivate uninitiated POLRMT, then elongation by the stalled POLRMT complexes was induced by increasing all four NTP concentrations to 400 μM. For "hot elongation" reactions, this mix contained [α-^{32}P] NTP to provide a 1/4000 ratio of [α-^{32}P] UTP to cold UTP or [α-^{32}P] CTP to cold CTP during the elongation incubation. After elongation times up to 30 min at 32 ºC, reactions were stopped with 0.3% SDS/225 mM EDTA, proteins digested with 0.4 mg/mL proteinase K, and nucleic acids precipitated with ethanol by centrifugation. Samples were dried under vacuum, resuspended in 80% formamide dye, and resolved by denaturing 5% polyacrylamide gel electrophoresis in TBE (89 mM Tris-borate, 1 mM EDTA, pH 8.0). The gels were dried, and then transcripts were detected by phosphorimaging analysis using a Storm 840 system with Image Quant software (GE Healthcare Bio-sciences Corp, Piscataway, NJ).

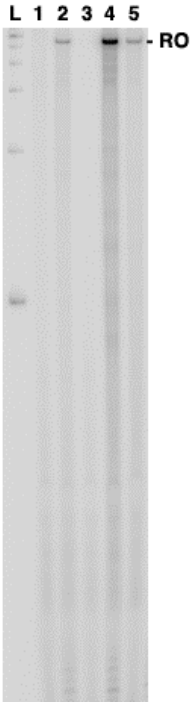

Figure S1. No POLRMT transcript labeling occurs during elongation under single-round transcription conditions. The gel depicts transcripts from the HSP Xts G:C template. Lanes: (**L**) 100 bp ladder, (**1**) "cassette labeling" reaction with heparin added prior to POLRMT initiation yielded no transcripts confirming that the heparin concentration used for single round transcription blocked re-initiation by POLRMT, (**2**) "cassette labeling" reaction produced full-length transcript (*RO*) from single-round transcription, (**3**) "hot elongation" reaction under single round conditions produced no labeled transcripts, (**4**) "cassette labeling" reaction without heparin produced full-length transcripts from multiple rounds of initiation, (**5**) "hot elongation" reaction without heparin showed that incorporation can occur at 1/4000 ratio of [α-^{32}P] NTP to NTP during multiple rounds of initiation.

Results and Conclusion

Heparin effectively blocks re-initiation of POLRMT (*Lane 1*) and no incorporation of [α-^{32}P] NTP occurs at the 1/4000 ratio of [α-^{32}P] NTP to NTP present during the single-round elongation conditions (*Lane 3*). Therefore, no correction is required for the calculation of % of transcripts arrested at the adducts in our transcription experiments, as all transcript band intensity results from [α-^{32}P] NTP incorporation at the labeling cassette.