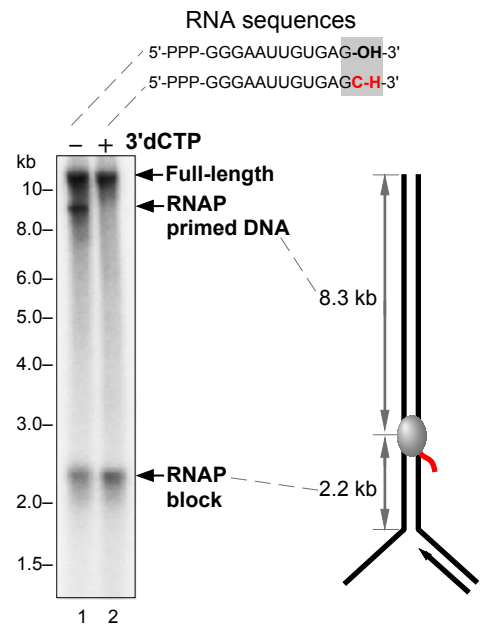


**Supplementary Figure 1**

**Leading strand products correspond to the lengths of the DNA flanking a co-directional halted RNAP.**

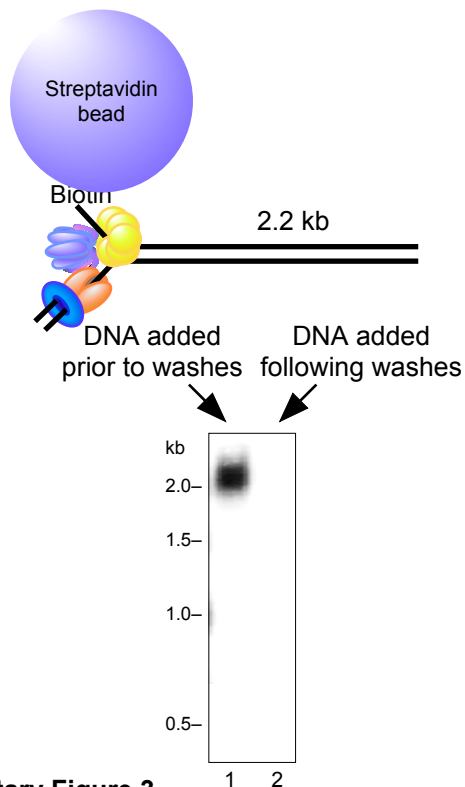
Leading strand synthesis was performed on a 10.5 kb template either with (lane 2) or without (lane 1) a RNAP halted elongation complex assembled 2.2 kb downstream from the fork. Radio-labeled DNA products were analyzed in an alkaline agarose gel.



**Supplementary Figure 2**

**Pol III uses a RNA transcript to prime leading strand synthesis on a 10.5 kb DNA template.**

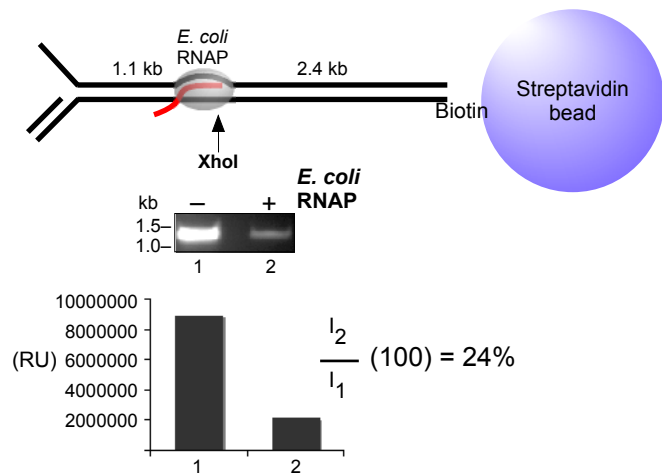
Leading strand synthesis was initiated on a 10.5 kb DNA template following assembly of a co-directional RNAP halted elongation complex 2.2 kb downstream from the fork. Extension of the RNA transcript was permitted (lane 1) or blocked (lane 2) by the addition of 3'dCTP which is incorporated by RNAP prior to replication. RNA sequences are indicated. Radio-labeled DNA products were analyzed in an alkaline agarose gel.



**Supplementary Figure 3**

**Demonstration of non-adherence of replication proteins to streptavidin beads.**

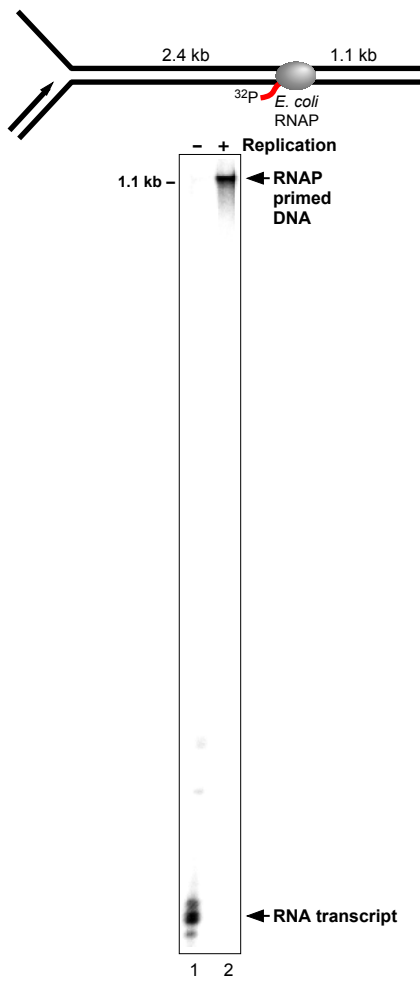
Replisome components were pre-incubated with (lane 1) or without (lane 2) a biotinylated 2.2 kb forked DNA template followed by the addition of streptavidin beads. Excess unbound Pol III\* and DnaB were removed by washing the beads. Leading strand synthesis was then initiated either with (lane 2) or without (lane 1) the biotinylated 2.2 kb forked DNA template. Radio-labeled DNA products were analyzed in an alkaline agarose gel.



**Supplementary Figure 4**

**Incomplete occupancy of *E. coli* RNAP on promoter DNA.**

A 3.5 kb biotinylated DNA either with (lane 2) or without (lane 1) a halted co-directional *E. coli* RNAP elongation complex was immobilized to streptavidin beads as in Fig. 4. The presence of the halted elongation complex was measured by *E. coli* RNAP protection of a unique XhoI restriction site immediately downstream from the promoter. Supernatant fractions were analyzed in a non-denaturing agarose gel stained with ethidium bromide. The percentage of promoters occupied by *E. coli* RNAP was determined using the following equation:  $(I_2/I_1)(100)$ , where  $I_2$  = the relative units of DNA digested in the presence of *E. coli* RNAP (lane 2), and  $I_1$  = the relative units of DNA digested in the absence of *E. coli* RNAP (lane 1). RU = relative units.



### Supplementary Figure 5

#### Pol III uses a co-directional *E. coli* RNAP transcript as a primer.

A 20 nt RNA transcript of a co-directional *E. coli* RNAP halted elongation complex assembled on a 3.5 kb linear forked DNA template was radio-labeled. The radio-labeled RNA was analyzed by 8% urea-PAGE prior to (lane 1) and following (lane 2) leading strand synthesis.