SUPPLEMENTAL DATA

Assembly, Purification and Pre-steady-state Kinetic Analysis of an Active RNA-dependent RNA Polymerase Elongation Complex

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Figure S1

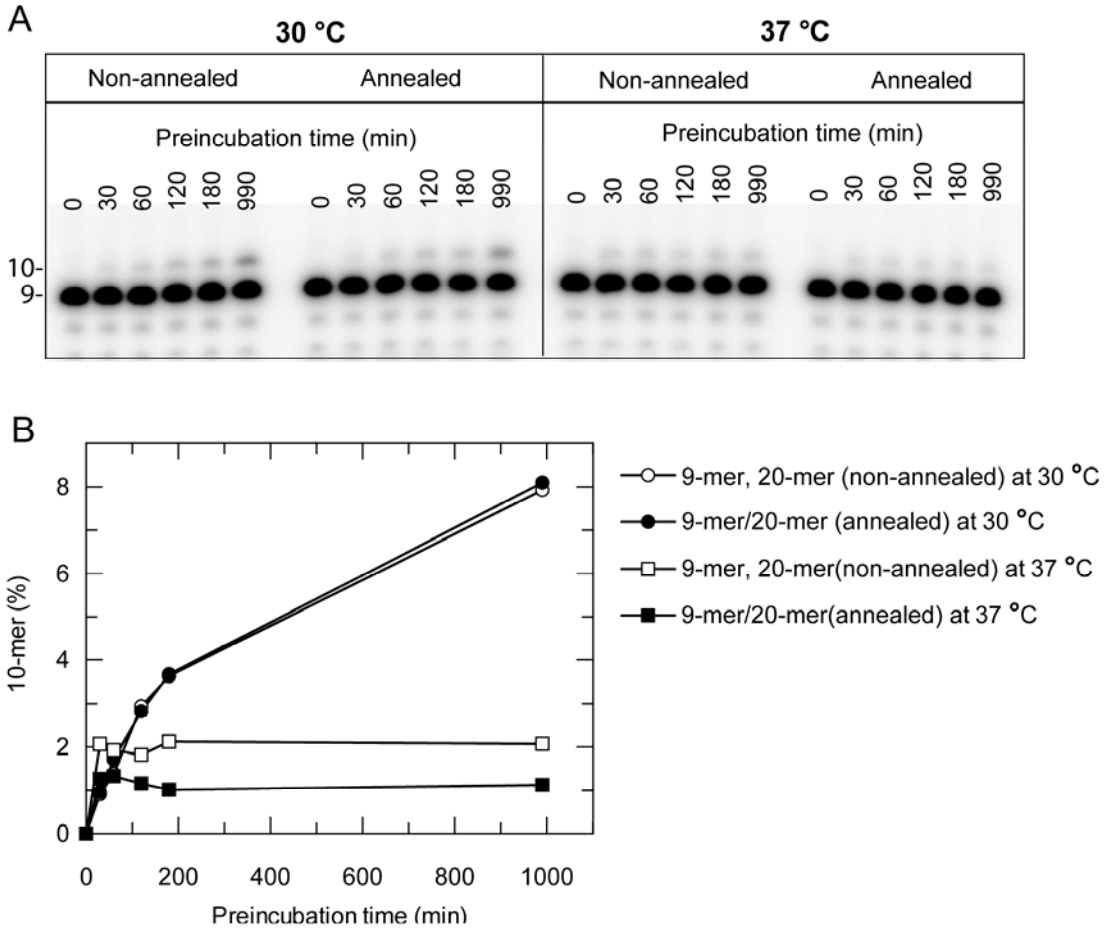

Figure S1. The formation of a functional elongation complex from the incubation of NS5B and double stranded RNA primer template (9-mer/20-mer) is very inefficient. (A) NS5B at 12 μM was mixed with 10 μM chemically synthesized 9-mer and 20-mer (annealed or non-annealed) in the reaction buffer containing 40 mM Tris-Cl, pH 7.0, 20 mM NaCl, 5 mM DTT, and 2 mM $MgCl_2$. The mixture was incubated at 30 or 37 °C for various time intervals. At each time point, an aliquot was reacted with 50 μM CTP for 20 s to measure the amount of elongation complex formed. The samples were analyzed on a sequencing gel. (B) The amount of elongation complex formed from preincubation was plotted against preincubation time. NS5B with non-annealed RNA at 30 °C (○), annealed duplex RNA at 30 °C (●), non-annealed RNA at 37 °C (□), and annealed duplex RNA at 37 °C (■).

Figure S2.

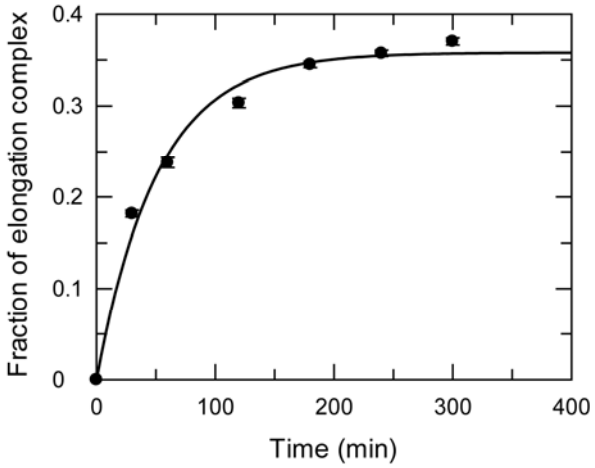

Figure S2. Time dependence of elongation complex formation. Related to Figure 1C. The fraction of elongation complex formed during the extension and pause reaction at various NS5B concentrations from Figure 1C was plotted against reaction time (up to 5 hrs). The data were fit to a single exponential, yielding a rate of 0.0195 ± 0.0023 min^{-1} (half-life of 36 ± 4 min) and an amplitude of 0.36 ± 0.01.

Figure S3.

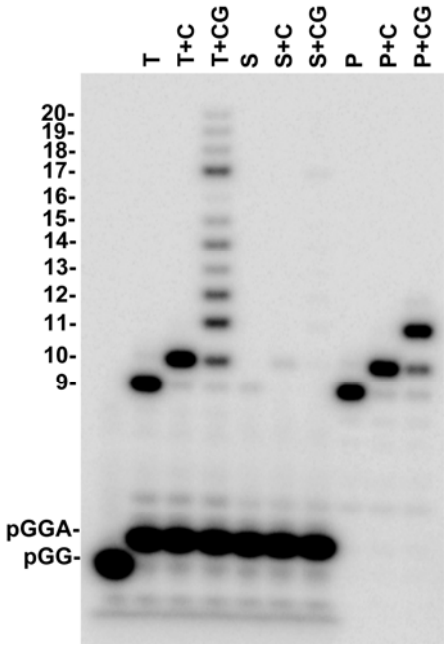

Figure S3. Assembly and purification of HCV RNA polymerase elongation complex. Related to Figure 3. An extension and pause reaction with 12 μM NS5B, 12.5 μM UTP, 25 μM ATP and 10 μM pGG/20-mer run for 2 hrs. The reaction then was centrifuged at 16,000 g for 5 min. Lane 1: pGG primer. T: the 2-hr reaction before spin. T+C: one aliquot of the 2-hr reaction was reacted with 50 μM CTP for 20 s. T+CG: one aliquot of the 2-hr reaction was reacted with 50 μM CTP and GTP for 20 s. S, S+C, S+CG: the supernatant, its reaction with 50 μM CTP for 20s, and its reaction with 50 μM CTP and GTP for 20s. P, P+C, P+CG: the resuspended pellet, its reaction with 50 μM CTP for 20 s, and its reaction with 50 μM CTP and GTP for 20 s.

Figure S4.

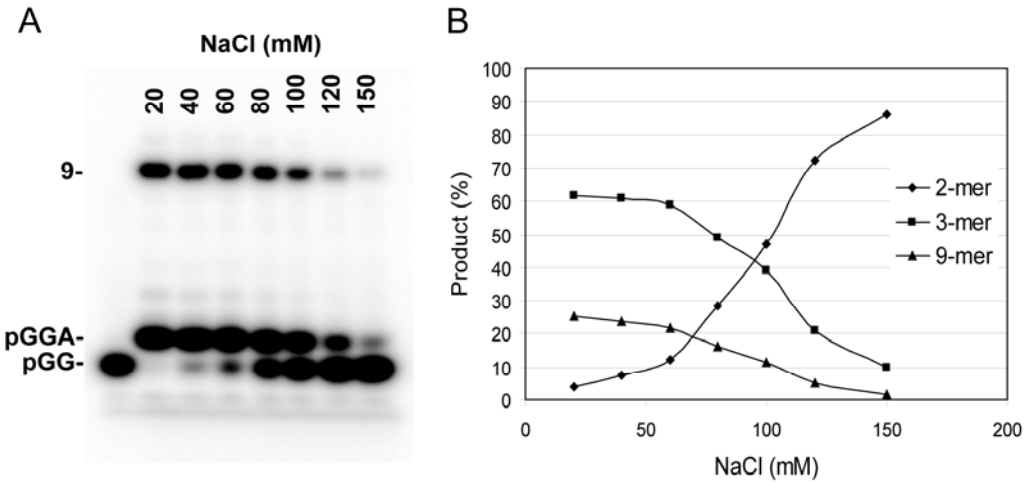

Figure S4. NaCl effect on initiation and elongation complex formation. (A) An extension and pause reaction with 12 µM NS5B, 12.5 µM UTP, 25 µM ATP and 10 µM pGG/20-mer run for 2 hrs at various NaCl concentrations. The sequencing gel image was shown. The band intensity of substrate and products was analyzed and the percentages of products and substrate were shown in (B).

Figure S5

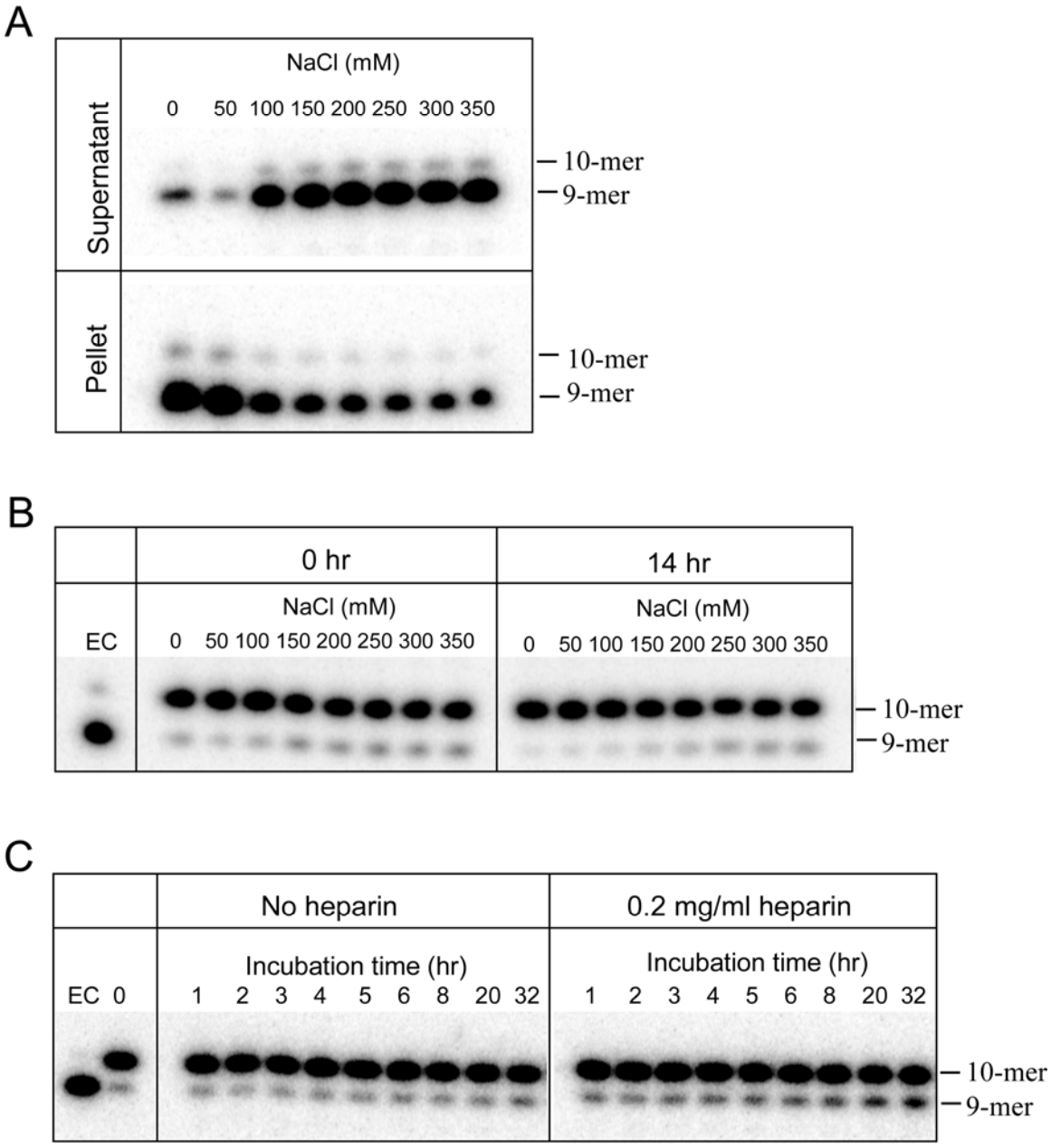

Figure S5. Solubility and stability of the elongation complex. Related to Figure 4. (A) A sequencing gel showing the solubility of the elongation complex in various NaCl concentrations. The pellet containing the elongation complex after centrifugation was resuspended in the reaction buffer with NaCl at various concentrations (0~350 mM). After 30-min incubation, the samples were spun at 16,000 g for 5 min. The elongation complex distributed in supernatant or in pellet was analyzed by sequencing gel. The percentage of the elongation complex in the supernatant (soluble EC) was plotted against NaCl concentration (Fig. 4A). (B) A sequencing gel showing the stability of the elongation complex in various NaCl concentrations. The pellet containing elongation complex was resuspended in the reaction buffer with NaCl at various concentrations and was incubated 14.3 hrs. The activity of the elongation complex pre-incubation or post-

incubation was measured by reacting an aliquot of the elongation complex with 50 μM CTP for 20 s. The percentage of the 10-mer product versus NCl concentration was shown in Fig. 4B. (C) A sequencing gel showing stability of the elongation complex in the reaction buffer with heparin. The pellet containing elongation complex was resuspended in the reaction buffer with 150 mM NaCl and 0.2 mg/ml heparin, and was incubated for various time intervals. The remaining activity of the elongation complex was measured by reacting an aliquot at each incubation time point with 50 μM CTP for 20 s. The percentage of the 10-mer product versus incubation time was shown in Fig. 4C.