Monitoring the Retention of Human PCNA at Primer/Template junctions by Proteins that Bind Single-stranded DNA

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Supporting Information

Supporting Information Experimental Procedures (Materials and Methods)

ATPase Activity of RFC - The ATPase activity of RFC (hRFCp140ΔN555) in the presence of a forked P/T DNA substrate and a PCNA was assayed spectrophotometrically via an NADH oxidation enzyme-coupled assay exactly as described previously[1]. The initial rates of ATP hydrolysis were determined in the presence of either wild-type (native) PCNA, mutant PCNA, or Cy5-labeled PCNA and all values were normalized to wild-type (native) PCNA. The ATP hydrolysis activities relative to wild-type (native) PCNA are reported in **Figure S1** (Relative ATPase activity).

Supporting Information Figure Legend

Figure S1 Cy5-labeling efficiency does not affect the interaction of PCNA with RFC. RFC utilizes ATP binding and hydrolysis to load PCNA onto DNA. Accordingly, PCNA stimulates ATP hydrolysis by RFC in the presence of DNA. The initial rates of ATP hydrolysis by RFC were determined in the presence of DNA and either wild-type (native) PCNA, mutant PCNA, or Cy5-labeled PCNA. Values for the mutant and Cy5-labeled PCNA preparations were normalized to wild-type (native) PCNA (represented by dashed line at 1.0) and plotted versus the Cy5 labeling efficiency of PCNA monomers (X-axis) or the average number of Cy5-PCNA monomers per PCNA homotrimer (X2-axis). Each value represents the average ($\pm$ standard deviation) of at least three independent measurements. Compared to the procedure described in the main text (35.5 - 38.8 % labeling efficiency, ~1.0 Cy5-PCNA monomer/Homotrimer), the labeling efficiency can be increased by either decreasing the concentration of TCEP to 200 μM (54.39 $\pm$ 4.947%) or using two Cy5 maleimide mono-reactive dye packs (85.74 $\pm$ 3.251%). However, the Cy5-labeled PCNA preparations (38.32 – 85.74% labeling efficiency) as well as the mutant PCNA (0.00% labeling efficiency) stimulated the ATPase activity of RFC to approximately the same level ($\geq$ 90%) as wild-type (native) PCNA. This indicates that the mutations and the Cy5-labels do not affect the interaction of PCNA with RFC or the ability of RFC to load PCNA onto DNA.

Table S1 RPA and SSB stabilize Cy5-PCNA in the same FRET state at a P/T junction. The duplex region within the Cy3P/BioT70 DNA substrate (**Figure 2**) is 29 bp and the 5' ssDNA overhang is 70 nt long, sufficient to accommodate a single SSB molecule. The FRET values within the plateau regions (FRET max) observed for the Cy3P/BioT70 DNA substrate (from **Figure 4**) in the presence of SSB are indicated in row 2. In a previous report[2], identical titrations were carried out with RPA on a Cy3P/BioT DNA substrate in which the duplex region was identical to the duplex region within the CyP/BioT70 DNA substrate but the length of the 5' ssDNA overhang was only 33 nt long, sufficient to accommodate a single RPA molecule. The FRET values within the plateau regions observed for this substrate in the presence of RPA are indicated in row 1 and are identical to those observed for the Cy3P/BioT70 DNA substrate with SSB. This suggests that Cy5-PCNA encircling a P/T junction is in the same FRET state when the adjacent ssDNA is bound by either RPA or SSB.

Supporting Information Figures

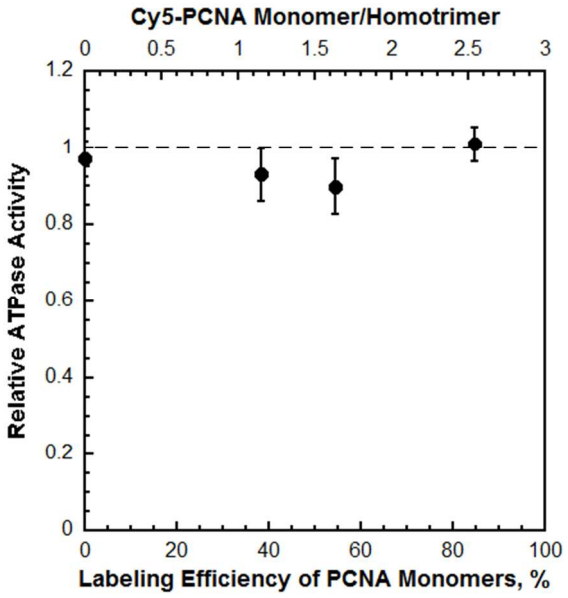

Figure S1

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Length of 5' ssDNA Overhang, nt	**RPA**	**SSB**	**RFC[3]**
33[1]	0.548 ± 0.00857		0.565 ± 0.0252
70[2]		0.549 ± 0.0123	0.557 ± 0.0263

[1] Values are from reference 2 below.

[2] Values are from **Figure 4** in the main text.

[3] RFC titrations were carried for each Cy3P/BioT DNA substrate under conditions where the 5' ssDNA overhang was saturated with the respective ssDNA-binding protein.

Table S1

Supporting Information References

[1] Hedglin, M., Perumal, S. K., Hu, Z., and Benkovic, S. (2013) eLife 2, e00278.

[2] Hedglin, M., and Benkovic, S. J. (2017) Biochemistry 56, 1824 - 1835.