Supporting Text

Steady-state ATPase activity assay. The normal function of proliferating cell nuclear antigen (PCNA) to stimulate yeast replication factor C (RFC) ATPase activity was assayed spectrophotometrically by using a phosphoenolpyruvate kinase/lactate dehydrogenase-coupled system. The yeast RFC was purified as described (1). The ATPase activity was determined at 25°C in an assay solution containing 250 nM RFC, 250 nM PCNA or 5-[2(acetyl)aminoethyl]aminonaphthalene-1-sulfonate (AEDANS)-PCNA, 250 nM Bio62/34/36mer DNA, 1 mM ATP, 4 mM phosphoenolpyruvate, 360 μM NADH, and 4 units of phosphoenolpyruvate kinase/lactate dehydrogenase mix. The initial rates of ATP hydrolysis are reported (see Table 2).

***Saccharomyces cerevisiae* PCNA exists as a closed ring in solution.** Sedimentation velocity experiments were performed using a Beckman XL-I analytical ultracentrifuge in absorbance mode. The Q153W/C22S PCNA mutant was analyzed over the concentration up to 70 μM at a rotor speed of 48,000 rpm at 25°C. The sedimentation coefficients at each PCNA concentration were determined using the enhanced van Holde-Weischet method in the ULTRASCAN II software package (University of Texas Health Science Center, San Antonio). The replisomal sliding clamps from *Escherichia coli* (β clamp), bacteriophage T4 (gp45), yeast, and human (PCNA) have been subjected to high-resolution x-ray crystal structure studies (2-4). The solved structures revealed an intriguing conservation in the overall protein architecture despite their low sequence homology. However, recent studies revealed possible variations with respect to their solution structures. Through both hydrodynamic and FRET studies, the bacteriophage gp45 was shown to exist as an opened ring in solution (5, 6). The *S. cerevisiae* PCNA is

also a homotrimer with a molecular weight similar to T4 gp45. To address the question whether yeast PCNA exists as an open or closed ring in solution, we carried out sedimentation velocity studies to derive useful shape information. Because the wild-type yeast PCNA contains no tryptophan residues, we constructed a mutant PCNA (Q153W/C22S) that allowed us to follow the protein absorbance in the sedimentation velocity studies. The mutant PCNA retains full activity in stimulating the ATPase activity of the yeast RFC complex (data not shown). The sedimentation coefficients ($S_{20,\,W}$) of trimeric PCNA determined with differing PCNA concentration (up to 70 μM) were found to be constant (≈4.7 S; see Fig. 7), in contrast to its phage counterpart gp45 that demonstrated a strong concentration dependent variation of $S_{20,\,W}$ (5). The hydrodynamic property of PCNA suggests a symmetric closed ring structure rather than a partially opened trimeric ring as observed for T4 gp45 in solution. Our conclusion is in good agreement with an early study of human PCNA (65% sequence similarity to yeast PCNA) solution structure using a small-angle neutron-scattering technique (7).

Calculating energy transfer efficiency (E_T) and FRET-based distance (R). The energy transfer efficiency between Trp185 of PCNA and AEDANS was calculated by using Eq. **1** for fluorescent acceptor sensitization (8).

$$E_T = \left(\frac{I_{AD}}{I_A} - 1 \right) \left(\frac{\varepsilon_A}{\varepsilon_D} \right) \qquad [\mathbf{1}]$$

where E_T is the transfer efficiency of the FRET and I_{AD} and I_A are the fluorescence intensities of the AEDANS acceptor in the presence or absence of the tryptophan donor in labeled PCNA at the excitation wavelength of 290 nm. ε_A (1,800 $M^{-1} \cdot cm^{-1}$) and ε_D

(4,100 $M^{-1} \cdot cm^{-1}$) are the extinction coefficient of acceptor AEDANS and donor tryptophan at 290 nm determined in the pH 7.5 buffer, respectively.

$$R = R_0 \sqrt[6]{\frac{1}{E_T} - 1} \qquad [\mathbf{2}]$$

The distance (*R*) between the donor tryptophan and acceptor AEDANS was calculated by using Eq. **2**, where R_0 is the Förster distance and E_T is the calculated energy transfer efficiency. R_0 is determined to be 22 Å, as described below.

Determine R_0 of the Trp-AEDANS FRET pair in PCNA. R_0 is determined by using Eq. **3**, where κ^2 is the orientation factor, η is the refractive index of the medium, Q_D is the quantum yield of the donor, and $J(\lambda)$ is the overlap integral between the

$$R_0 = 0.211\left[\kappa^2 \eta^{-4} Q_D J(\lambda)\right]^{1/6} \qquad [\mathbf{3}]$$

fluorescence spectrum of the donor and the absorption spectrum of the acceptor. We assigned the value of 2/3 to κ^2 based on the fact that the measured anisotropy values for both tryptophan and AEDANS in labeled PCNA were <0.3 when exciting at 290 and 340 nm, which suggested freely rotating probes. The quantum yield of donor tryptophan in the PCNA mutant was determined to be 0.131 using L-tryptophan as reference and the following formula

$$\phi_D = \phi_R \left(\frac{F_D}{F_R} \cdot \frac{A_R}{A_D}\right) \qquad [\mathbf{4}]$$

where ϕ, **F,** and **A** are the quantum yield, fluorescence and absorbance of donor and reference compound, respectively. The overlap integral $J(\lambda)$ was calculated by using following formula

$$J(\lambda) = \int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \qquad [5]$$

where $F_D(\lambda)$ is the fluorescence intensity of the donor in the absence of acceptor as a fraction of the total integrated intensity, $\varepsilon_A(\lambda)$ is the extinction coefficient of the acceptor, and λ is the wavelength of the overlap.

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