Recognition of the ring-opened state of proliferating cell nuclear antigen by replication factor C promotes eukaryotic clamp-loading

Supplementary material

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Methods

Model construction

Models of the yeast PCNA clamp alone and in complex with the pentameric RFC clamp loader were constructed based on the available crystal structures (PDB accession code: 1PQL and 1SXJ, respectively). In the subunit A of RFC a residues whose side chains were unresolved in the crystal structure were modeled from the sequence using the program Modeller 8. In the simulations the non-hydrolysable ATP analog (ATP$_\gamma$S) was replaced by ATP at the interfaces between the RFC subunits A through D. One ADP molecule occupied the interface between subunits E and D. The models were completed by adding hydrogen atoms and solvated with 25194 TIP3P water molecules for PCNA and 71374 water molecules for the PCNA/RFC model using the XLeaP utility of AMBER 9. Charge neutralization was accomplished by the addition of Na$^+$ and Cl$^-$ ions. Excess salt concentration (~0.1 M) was introduced in order to mimic physiological conditions.

Simulation Protocol

The systems underwent 2000 steps of initial energy minimization to remove unfavorable contacts followed by 50,000 steps of constant volume molecular dynamics. The temperature was gradually increased from 0 K to 300 K during this period. Harmonic restraints (with force constant of 4 kcal×mol$^{-1}$×Å$^{-2}$) were imposed on the protein C$_\alpha$ atoms. These restraints were scaled down in four stages and eventually removed in the course of an extensive 2 ns equilibration in the canonical ensemble.
The production runs were performed in the isothermal-isobaric ensemble with the program NAMD 2.6 and the AMBER Parm99SB force field. The long-range electrostatic interactions were treated using the smooth particle mesh Ewald (SPME) algorithm. For the non-bonded short-range interactions a cutoff of 10 Å with a switching function between 8.5 Å and 10 Å was employed. The r-RESPA multiple time step method was employed with a 2 fs time step for bonded interactions, 2 fs for short-range non-bonded interactions, and 4 fs for long-range electrostatic interactions. The bonds between hydrogen and heavy atoms were constrained with the SHAKE algorithm.

Steered MD

To enforce separation of the two interfacial beta strands of PCNA we relied on steered molecular dynamics (SMD): a method that involves the use of a harmonic constraint moving at a constant velocity to “steer away” the centers of mass of groups of atoms and, thus, promote enhanced sampling (see also Figure S1). Specifically, we selected the Cα atoms of six residues from the first beta strand and applied external forces to steer the center of mass of these atoms away from the corresponding Cα atoms on the second beta strand (Figure 1d). SMD was used to force open the PCNA subunit interface in the in-plane direction for two cases: (i) an isolated sliding clamp and (ii) a clamp in complex with the clamp loader. The force constant k for the pulling runs was 6 kcal mol⁻¹Å⁻² and the steering velocity v was 3.0 Å/ns. At no point during the SMD run or during subsequent equilibration runs did we apply external forces in the orthogonal direction.
Since the SMD simulations for the yeast RFC/PCNA complex were carried out in a highly non-equilibrium pulling regime, the runs were followed up by extensive molecular dynamics equilibration. To prevent the system from snapping back into a closed conformation, the center of mass distance between the two beta strands was restrained to vary between 25 and 40 Å during the first 45 ns of MD simulation. The system was allowed to move freely within the above range of distances but prevented from exceeding them by potential barriers placed at the limiting values. For the subsequent 32 ns all restraints were removed and the system was allowed to evolve freely toward an equilibrium conformation. While the SMD technique could, in principle, be used for PMF reconstruction $^{10,11}$, it proved less advantageous for our systems. The reason for this became clear in retrospect, upon examination of the resulting force versus extension profiles, and is also illustrated in Figure S1. To circumvent this difficulty, we opted for a technique that more closely resembles equilibrium sampling and does not suffer from large non-equilibrium effects, namely, the adaptive biasing force (ABF) method.

**Adaptive biasing force calculations**

The adaptive biasing force (ABF) method $^{12}$ as implemented in the NAMD2.6 package $^{5,4}$ by Chipot and Henin $^{13}$ was used to determine the effective free energy profiles along two predefined intuitive coordinates $\xi_1$ and $\xi_2$, representing approximate directions for clamp opening in the absence and presence of the clamp loader. The $\xi_1$ coordinate corresponds to the center of mass distance between six C$\alpha$ atoms from the two interfacial beta strands, projected onto the in-plane opening direction. Likewise $\xi_2$ was defined as the inter-stand distance (same six C$\alpha$ atoms) projected onto the out-of-
plane opening direction connecting the centers of mass of the interfacial beta strands in the ring-open PCNA/RFC model. This choice poses a difficulty at small values of $\xi_2$ as it requires out-of-plane shearing of the subunit interface during PCNA opening. Thus, to avoid atomic clashes at the interface, for the first 4 Å of the PMF the in-plane direction was followed.

The ABF method couples ideas from thermodynamic integration and average force formalisms with unconstrained molecular dynamics and the introduction of an adaptive biasing potential. The average force experienced by the simulated system at any point along the reaction coordinate is estimated from the averaging of instantaneous forces experienced by the system at that position. Cancellation of the average force through the gradual introduction of an adaptive bias allows the system to overcome barriers and achieve enhanced sampling along the $\xi$ coordinate. Reconstruction of the PMF is accomplished through straightforward integration of the average force. The reaction coordinate $\xi$ was subdivided into discrete windows with a confining wall potential placed at the boundaries ($k_{\text{wall}} = 100$ kcal/mol). Within each window the average force was accumulated in 0.1 Å sized bins and continuously updated as the simulation progressed. Smoothing within 0.2 Å was applied by averaging the content of adjacent bins. Application of the adaptive bias was initiated only after the accumulation of 1000 samples in the individual bins. Subsequently, the biasing force was introduced progressively in the form of a linear ramp. Production runs in each window were continued for 6 ns to 24 ns, resulting in a total simulation time of more than 200 ns for the free energy runs.

**Electrostatic Potential**
Poisson-Boltzmann electrostatics calculations were carried out using the APBS package. The charge and radius parameters for APBS were assigned by using PDB2PQR server. AMBER force field charges were used. The detailed set of APBS parameters included: (i) protein dielectric of 2.0; (ii) solvent dielectric of 78.54; (iii) solvent radius 1.4 Å; (iv) temperature of 298.15 K; (v) ionic salt concentration 0.1 M; (vi) grid dimensions: 194 x 194 x 194 and (vii) grid spacing ~ 0.5Å.

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Supplementary Figures
Figure S1 The harmonic restraint used in SMD can be described as a moving bead attached to the system by a Hookean spring. When the underlying free energy profile features a narrow and deep minimum, large extensions of the harmonic spring become possible during a pulling run. As a result, when the external force becomes sufficient to overcome the free energy barrier, the spring snaps, leading to a large non-equilibrium displacement of the steered atoms. The highly non-equilibrium nature of the motion makes PMF reconstruction from the SMD data extremely challenging.
Figure S2 Fast reclosing of an open PCNA clamp. a) Initial ring-open configuration (taken window 8 of the ABF simulation of an isolated sliding clamp); b) Releasing the constraints imposed by ABF and running free molecular dynamics for 4 ns leads to a ring-closed state. The sliding clamp is displayed in cartoon representation with elements of secondary structure show as follows: (i) helix in red; (ii) beta sheet green; (iii) turn and coil in cyan. A van-der-Waals surface for the sliding clamp is displayed in gray. The surface was generated using all protein atoms except hydrogen using a probe radius of 1.4 Å.

Movie 1 Animation that qualitatively illustrates the reclosing process. The movie was generated from the trajectory of the above simulation run. (mpeg file 2.9 Mbytes in size).
**Figure S3** Domain displacement in subunits S2 and S3 of the open structure relative to the closed conformation of PCNA in three different orientations: a) lateral view from the side of the open subunit interface; b) lateral view with S3 positioned toward the front; c) posterior view of PCNA. Subunits S1, S2 and S3 in the open structure are shown in red, green and blue, respectively. The closed PCNA structure is shown in gray.

**Table S1** Backbone RMSD values for the open PCNA model relative to the closed PCNA structure. In addition to the overall RMSD for PCNA, values are listed also for the individual subunits S1-S3. In each case a fit to the corresponding subunit in the closed PCNA structure was performed prior to calculating the RMSD. Thus, the values denoted as S1, S2, S3 reflect internal rearrangement within a particular subunit. These values increase from S1 to S2 and S3 but remain relatively small. To highlight the displacement of subunits S2 and S3 relative the subunit S1, we fit separately onto to S1 before carrying out the RMSD calculation. The next two values in the table reflect a moderately large relative displacement for S2 (~10 Å) and a very large displacement of S3 (~34 Å). Figure S2 provides a visual illustration of this result.
Figure S4 Distance and cross-correlation maps for the open PCNA/RFC complex (RFC subunits D and E only). (RFC residue numbering by chain A-E: A1-446; B447-762; C763-1084; D1085-1412; E1413-1729 and PCNA residue numbering by chain A-C: A1730-1987; B1988-2245; C2246-2508) The covariance matrix elements \( c_{ij} \) can be expressed as:

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where \( t_{\text{ave}} \) is the averaging time, \( \Delta t \) is the time step, the positions atoms \( i \) and \( j \) at time \( t \) are \( r_i(t) \) and \( r_j(t) \), respectively, and the angular brackets denote time averaging. The cross-correlation matrix is the normalized covariance matrix \( (c_{ij} = \frac{c_{ij}}{c_{ii}^{1/2} c_{jj}^{1/2}}) \). Both maps (distance and covariance) readily display the two residue clusters (Fig. 2) with PCNA.
Figure S5 Directionality of the lowest frequency mode from normal mode analysis of a coarse-grain elastic network model (ENM) of the RFC/PCNA complex in the ring-open state. Each residue was represented by one node in the ENM and the distance cutoff parameter was set to 10 Å. To visualize the motion of the complex six structures along the direction of the first normal mode were overlaid in ribbon representation and colored from blue to red. Notably, the largest amplitude region corresponds to the open clamp and the direction of the mode is toward closing (opening) the clamp interface.