Physical Interactions Between Mcm10, DNA, and DNA Polymerase α
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SUPPLEMENTAL DATA

Fig. S1. Fit of crystallographic model to electron density. Shown is a representative section of the final refined protein model (sticks) superimposed onto a 2Fo-Fc composite omit electron density map contoured at 1σ. Several residues are labeled as landmarks.

Fig. S2. Nature of the interactions between Mcm10-ID and ssDNA. (A) Mcm10-ID sequence alignment showing secondary structure elements and DNA binding residues (black boxes) from the Mcm10-ID/ssDNA complex. Protein regions not observed in the electron density are depicted by dashed lines (coil regions) or a light blue helix (αE). (B) Isothermal titration calorimetry measurements for 25mer ssDNA titrated into Mcm10-ID at 21ºC. Upper panel, raw ITC data for sequential injections of p180 189-323; lower panel, integrated heat responses (squares) fit with a single site binding model (continuous line). The following parameters were obtained from the fit: Kd, 27 ± 0.3 µM; ΔH, -9.8 kcal/mol; TΔS, -3.6 kcal/mol. (C) Stereoview of the ssDNA binding site on the Mcm10-ID OB-fold. Annealed omit electron density contoured at 3σ is shown as blue mesh, and ssDNA-contacting residues are rendered as sticks.

Fig. S3. Crystal packing of the Mcm10-ID/ssDNA complex. (A) Crystal structure of the Mcm10-ID/ssDNA complex colored as in Figure 1, shown with the protein OB-fold (grey) and ssDNA (yellow carbons) from a symmetry-related complex. An annealed omit map for ssDNA contoured at 3σ is shown. The position of the zinc loop previously implicated in DNA binding is highlighted with an asterisk. (B) A different angle is shown to highlight the packing of the loops, precluding the ssDNA from interacting with the Zn-finger. The symmetry-related protein/DNA complex is dimly colored with yellow DNA carbons.

Fig. S4. Differences in unliganded and ssDNA bound Mcm10-ID as a result of crystal packing interactions. (A) Superposition of the Mcm10-ID/ssDNA complex, colored by motif as in Figure 1, with chain A of the unliganded crystal structure {PDB ID 3EBE`, Warren, 2008 #600} in grey. (B) Protein interactions stabilize the zinc finger helix (αE) in unliganded Mcm10-ID. The Mcm10-ID/ssDNA complex (green OB-fold, dark blue zinc finger) is superimposed onto chain A of the unliganded structure, from which the entire asymmetric unit (chains A, B, C) is shown in grey with αE helices colored light blue. Zinc finger helices from chains A and C are more ordered, and are forming contacts with a symmetry-related protomer in the crystal, whereas αE from chain B is disordered and does not make intermolecular contacts.

Fig. S5. Competition for Mcm10-ID binding by ssDNA and p180 189-323. (A) 15N-1H HSQC spectrum for Mcm10-ID alone (black), 1:1 ratio of Mcm10-ID:ssDNA (red), and a 1:1:1 ratio Mcm10-ID:ssDNA:p180 189-323 (green). (B) The reverse titration with Mcm10-ID alone (black) mixed in a 1:1 ratio of p180 189-323 (blue), and then ssDNA in a 1:1:1 ratio (gold). The region of the spectra shown in Figure 4 is boxed.

Fig. S6. DNA-induced release of Mcm10-ID from p180 189-323. (A) 15N-1H HSQC spectrum for 15N-enriched p180 189-323 (black), 1:1 molar ratio of p180 189-323:Mcm10-ID (blue), and a 1:1:1
ratio of p180^{189-323}:Mcm10-ID:ssDNA (gold). (B) The reverse titration with p180^{189-323} alone (black), 1:1 molar ratio of ssDNA (green), a 1:1:0.67 molar ratio of p180^{189-323}:ssDNA: Mcm10-ID (blue), and then a 1:1:1 molar ratio of ^{15}N-p180^{189-323}:ssDNA:Mcm10-ID (red). The region of the spectra shown in Figure 4 is boxed.

**Fig. S7.** Binding of p180^{286-310} to Mcm10-ID. (A) ^{15}N-^{1}H HSQC spectra from ^{15}N-enriched Mcm10-ID performed at Mcm10-ID:p180^{286-310} ratios of 1:0 (black), 1:0.25 (green), 1:0.5 (blue), and 1:1 (red). (B) Quantitation of chemical shift perturbations of ^{15}N-enriched Mcm10-ID upon addition of 1:1 molar ratio of p180^{286-310}. The dashed line represents 1 standard deviation above the mean. A shift of zero indicates an unassigned residue. (C) Surface representation of Mcm10-ID showing that residues exhibiting a significant shift in response to p180^{286-310} (orange) predominate on the ssDNA binding face of the protein. (D) Fluorescence anisotropy titration in which Mcm10-ID was added to FITC-labeled p180^{286-310} (black boxes). Titration of fluorescein-p180^{189-323} with Mcm10-ID (circles) and buffer only (crosses) from Figure 2 are shown for reference. The error bars represent the standard deviations from the average values from three independent measurements. The curve fits are non-linear regression of the data as described in Experimental Procedures.

**Fig. S8.** The p180^{243-256} peptide does not bind specifically to Mcm10-ID. (A) A comparison of the magnitudes of chemical shift perturbations of ^{15}N-enriched Mcm10-ID resulting from addition of unlabeled p180^{243-256} (red bars) and p180^{286-310} (grey bars). Dashed lines represent 1 standard deviation above the mean shift perturbation for all residues. (B) A surface representation of Mcm10-ID, with orange highlighting those residues that exhibit a significant shift (above the dashed line in panel A) from the p180^{243-256} titration.

**Fig. S9.** ssDNA and the N-terminal domain of p180 share the same binding site on Mcm10-ID. The protein from the Mcm10-ID/ssDNA co-crystal structure is rendered as a solvent accessible surface. Mcm10 residues exhibiting significant NMR chemical shifts perturbations in ^{15}N-^{1}H HSQC spectra are highlighted orange. Maps of p180 fragment binding were determined in the present work, and the ssDNA map is from Warren et al (2008) Structure 16, 1892-1901, and is shown here for comparison. Residues 297-302 in the L12 loop were not assigned in the NMR spectra, and thus were unable to be measured for perturbation.
Warren, et. al.
Supplementary Figure 4

A

B
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Supplementary Figure 5

A

B
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Supplementary Figure 7

A

B

C

D

Chemical shift perturbation (Δppm)

Residue number

OB-fold
zinc finger
L12
L45
αE
zinc loop

Anisotropy

xMcm10-ID (μM)
A

Chemical shift perturbation (ppm)

Residue number

B

OB-fold

L12

zinc finger

L45

zinc loop

180°
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Supplementary Figure 9

ssDNA

p180^{189-323}

p180^{243-256}

p180^{286-310}