RESEARCH ADVANCE

Figures and figure supplements

DNA passes through cohesin's hinge as well as its Smc3–kleisin interface

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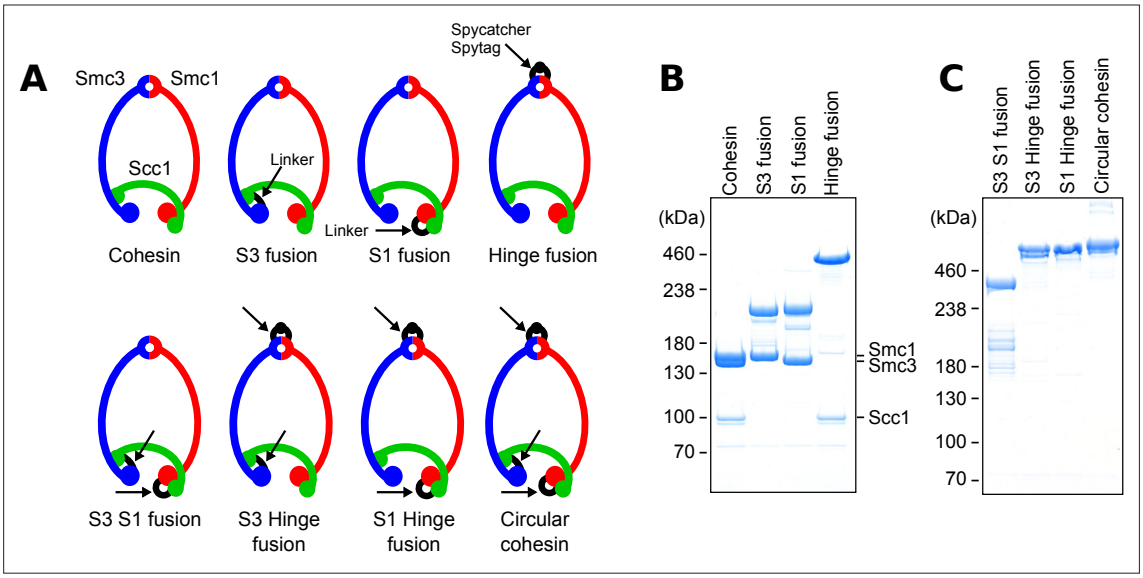

Figure 1. Covalent closure of cohesin's interfaces. (**A**) The cohesin ring and fusion variants. Covalently sealed interfaces are linked in black and are indicated with arrows. (**B**) Coomassie stain of purified cohesin with either the Smc3/Scc1 (S3 fusion), Scc1/Smc1 (S1 fusion), or hinge interfaces (Hinge fusion) covalently sealed. (**C**) Coomassie stain of purified cohesin with multiple interfaces covalently sealed.

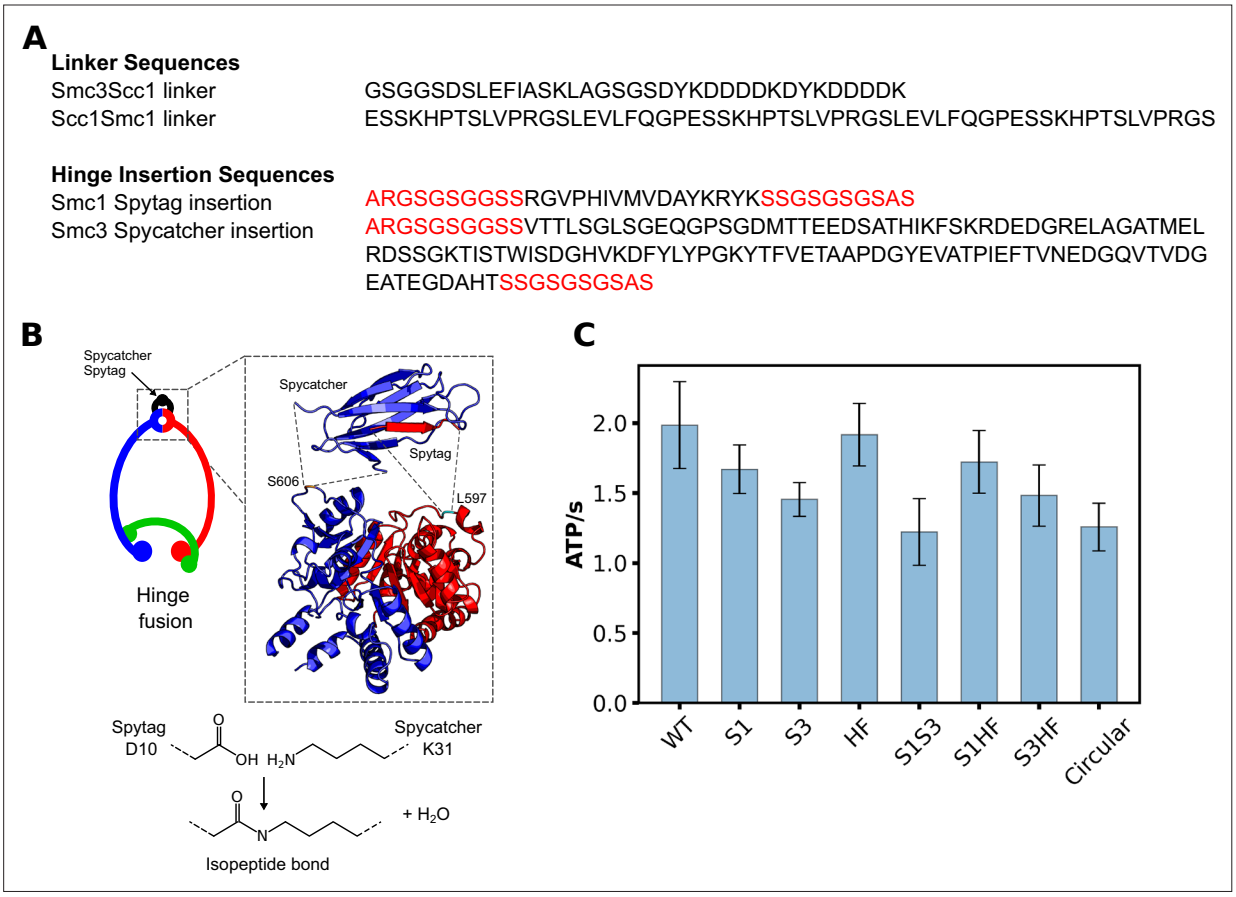

Figure 1—figure supplement 1. Covalent closure of cohesin's interfaces. (**A**) Top: the polypeptide sequences used to fuse Scc1 to Smc1 and Smc3. Bottom: the polypeptide sequences of the spytag and spycatcher inserted into Smc1 (after residue L597) and Smc3 (after residue S606), respectively. Linker sequences in red and spytag/spycatcher sequences in black. (**B**) Composite model (4MLI and 2WD5) of the spytag and spycatcher inserted into the Smc1 and Smc3 hinge domains, respectively. An isopeptide bond forms between D10 of the spytag and K31 of the spycatcher. (**C**) ATPase activity for cohesin and fusion proteins performed in the presence of Scc2, Scc3, and DNA. $n = 4$.

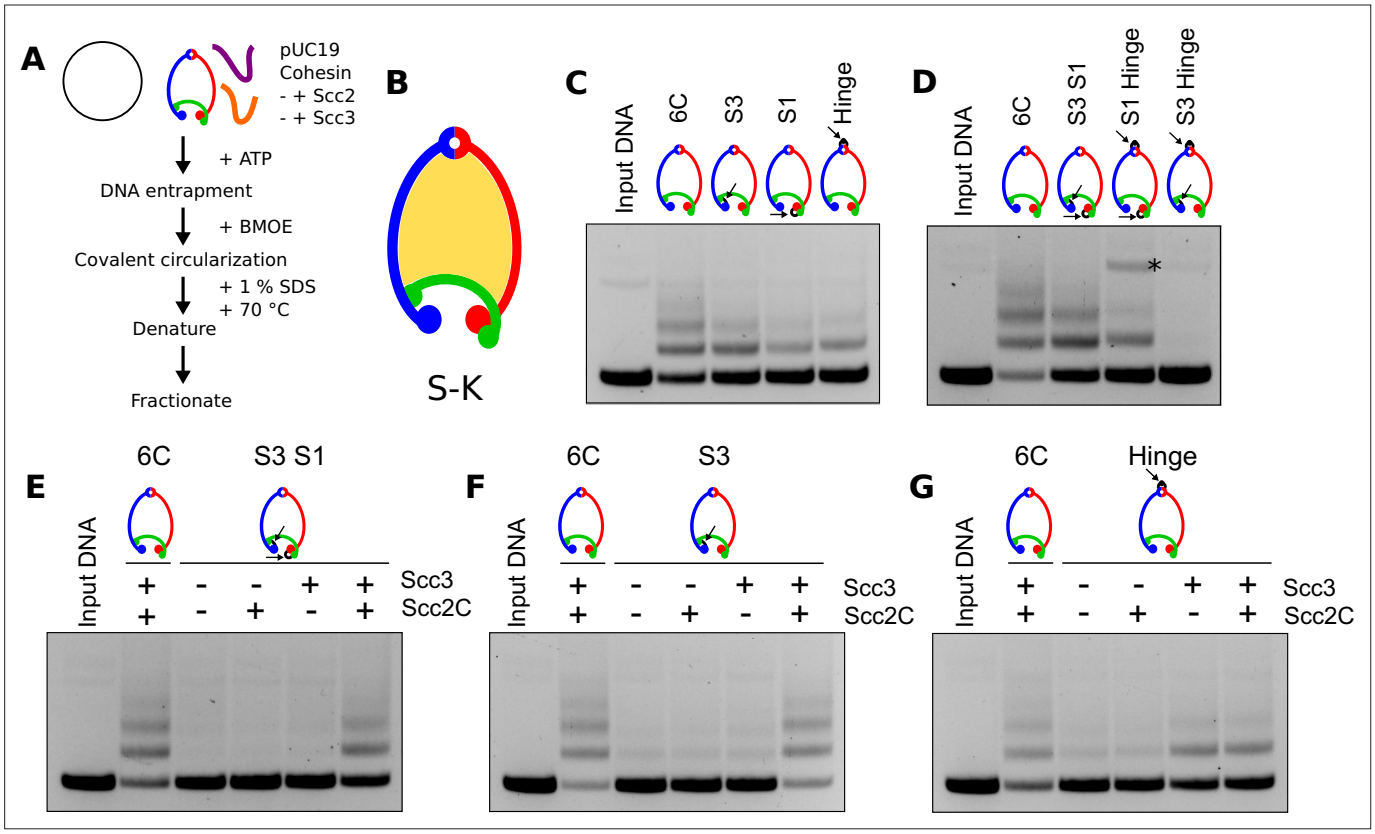

Figure 2. DNA passes through cohesin's hinge and Smc3/Scc1 interfaces. (**A**) Schematic of the in vitro DNA entrapment assay. (**B**) SMC–kleisin (S–K) ring. (**C**) DNA entrapment comparing WT cohesin (6C) with the Smc3–Scc1 (S3), Scc1–Smc1 (S1), or hinge (Hinge) fusion proteins in the presence of Scc2 and Scc3 after 40 min. (**D**) DNA entrapment comparing WT cohesin (6C) with the Smc3–Scc1–Smc1 (S3 S1), Scc1–Smc1–Hinge (S1 Hinge), or Smc3–Scc1–Hinge (S3 Hinge) fusions in the presence of Scc2 and Scc3 after 40 min. * = nicked DNA. (**E**) DNA entrapment comparing WT cohesin (6C) in the presence of Scc2 and Scc3 with the Smc3–Scc1–Smc1 (S3 S1) fusion in the presence or absence of Scc2 and Scc3 after 40 min. (**F**) DNA entrapment comparing WT cohesin (6C) in the presence of Scc2 and Scc3 with the Smc3–Scc1 (S3) fusion in the presence or absence of Scc2 and Scc3 after 40 min. (**G**) DNA entrapment comparing WT cohesin (6C) in the presence of Scc2 and Scc3 with the Hinge fusion construct in the presence or absence of Scc2 and Scc3 after 40 min.

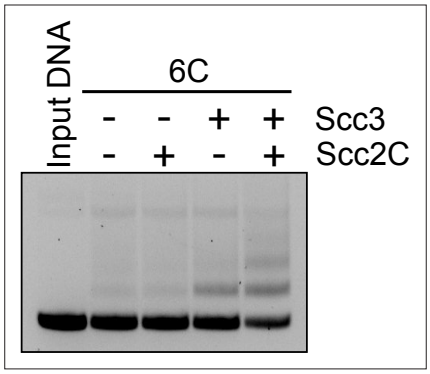

Figure 2—figure supplement 1. DNA entrapment by SMC–kleisin (S–K) rings. DNA entrapment for WT cohesin (6C) in the presence or absence of Scc2 and Scc3 after 40 min.

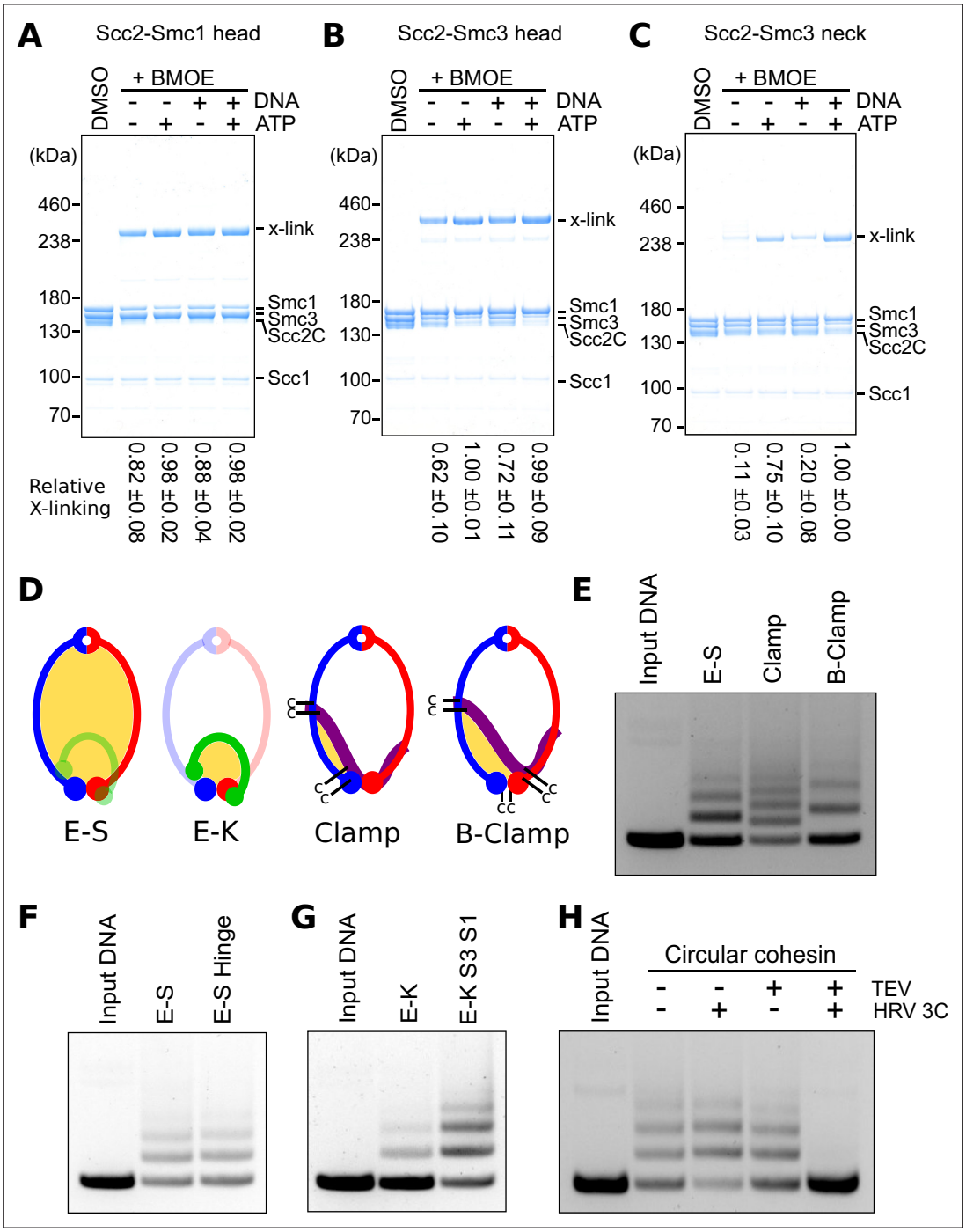

Figure 3. DNA passes through cohesin's ATPase domains. (**A**) Crosslinking Scc2 to the Smc1 head in the presence or absence of ATP and DNA. *n* = 3. (**B**) Crosslinking Scc2 to the Smc3 head in the presence or absence of ATP and DNA. *n* = 3. (**C**) Crosslinking Scc2 to the Smc3 neck in the presence or absence of ATP and DNA. *n* = 3. (**D**) Models of cohesin showing the E-S, E-K, Clamp, or below the clamp (B-Clamp) compartments, highlighted in yellow. For the Clamp and B-Clamp compartments Scc2 is in purple. (**E**) Entrapment of DNA in either the E-S, Clamp, or B-Clamp compartments in the presence of Scc2 after 2 min. (**F**) Entrapment of DNA in the E-S compartment by cohesin with either an open (E-S) or covalently closed (E-S Hinge) hinge in the presence of Scc2 after 2 min. (**G**) Entrapment of DNA in the E-K compartment by cohesin with either both kleisin interfaces open (E-K) or both covalently closed (E-K S3 S1 fusion) in the presence of Scc2 after 2 min. (**H**) Entrapment of DNA by covalently circular cohesin in the presence of Scc2 after 2 min. After crosslinking, BMOE was quenched by addition of dithiothreitol (DTT) and then the samples were treated with tobacco etch virus (TEV) and/or human rhinovirus (HRV) 3C proteases and incubated at 24°C for 30 min.

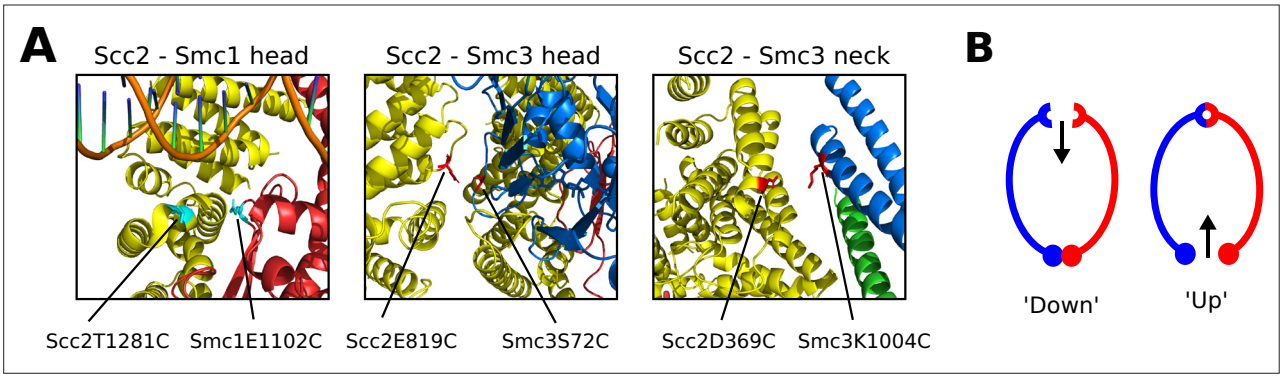

Figure 3—figure supplement 1. DNA passes through cohesin's ATPase domains. (**A**) Left panel: Scc2–Smc1 head crosslinking pair. Middle panel: Scc2–Smc3 head crosslinking pair. Right panel: Scc2–Smc3 neck crosslinking pair. (**B**) DNA could enter the E-S compartment by going 'down' through the hinge or 'up' through the heads.

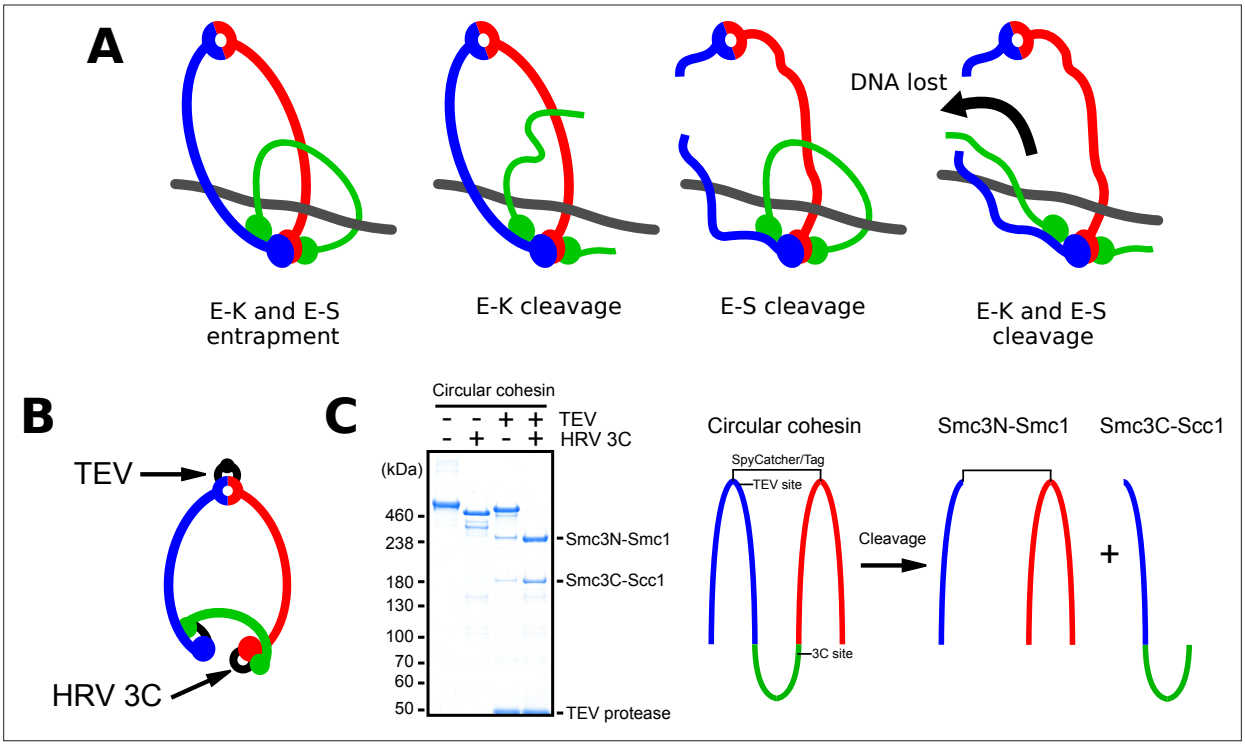

Figure 3—figure supplement 2. Entrapment in the E-S and E-K compartments occurs simultaneously. (**A**) Models showing that cleavage of both the E-S and E-K compartments is required to release DNA that has passed 'up' through the heads. (**B**) A TEV protease site is located in the linker between the spycatcher and the C-terminal half of Smc3. A HRV 3C protease site is located in the linker between Scc1 and Smc1. (**C**) Left panel: Coomassie stain of covalently circular cohesin incubated with either TEV and/or HRV 3C proteases for 1 hr at 30°C. Right panel: schematic showing the polypeptide topology of covalently circular cohesin and the position of the protease cleavage sites. Incubation with both TEV and HRV 3C proteases will cleave the construct into a C-terminal-Smc3–Scc1 fragment and an N-terminal-Smc3–Smc1 fragment.

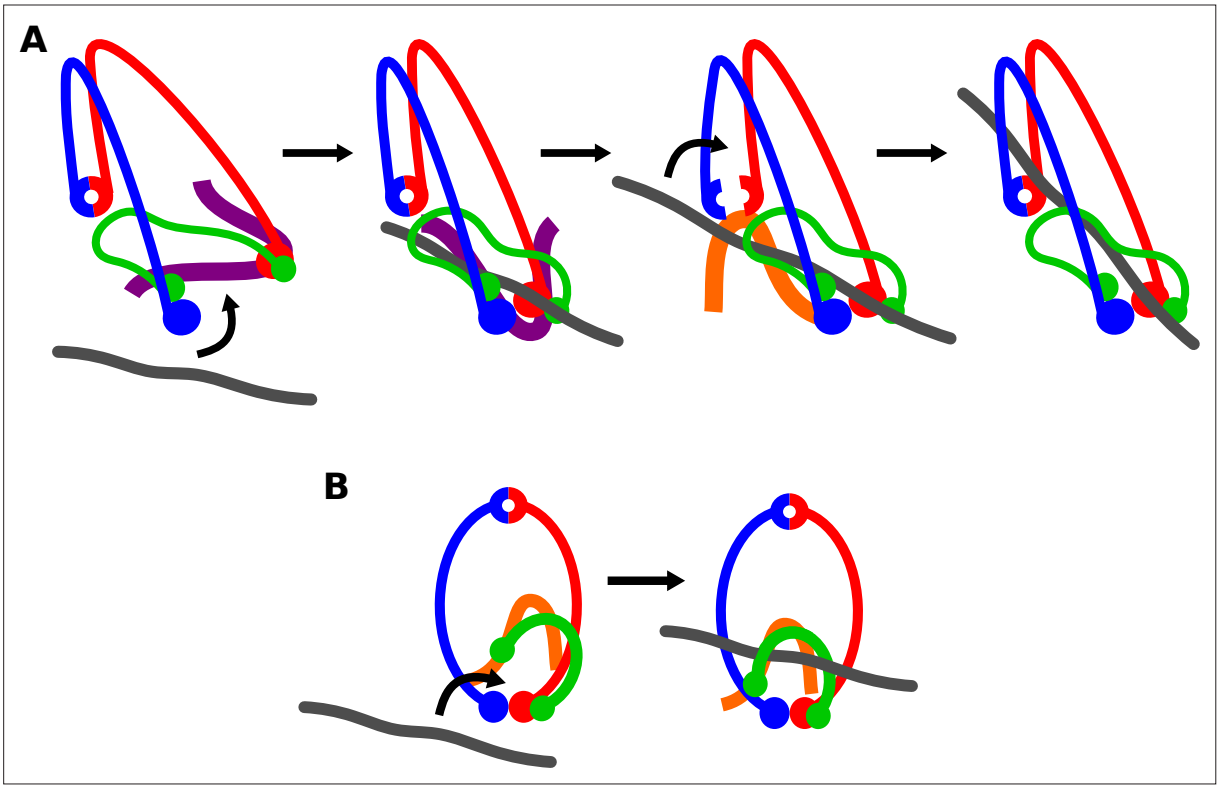

Figure 4. Models for DNA entry. (**A**) Model for DNA passage through the hinge. DNA passes 'up' through the ATPase heads and binds to Scc2 (purple). The SMC heads engage in the presence of ATP, and DNA is then clamped between Scc2 and the Smc3 neck. Scc3 (orange) is involved in opening and passing a downstream section of DNA through the hinge. (**B**) Model for DNA passage through the Smc3–Scc1 interface. ATP binding leads to the opening of the Smc3–Scc1 interface. DNA then binds to Scc3 and the interfaces closes.