Figures and figure supplements

A molecular model for the role of SYCP3 in meiotic chromosome organisation

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**Figure 1.** Homologous chromosome synapsis by the synaptonemal complex. (A) The synaptonemal complex (SC) is a molecular ‘zipper’ that holds together homologous chromosomes during meiotic prophase I, enabling recombination and crossover formation. The SC has a tripartite ultrastructural appearance in which transverse filaments bridge between a midline central element and lateral elements that coat the chromosome axes. The inset electron micrograph is reproduced from Kouznetsova et al. (2011) under the Creative Commons Attribution License. (B) Model for assembly of the mammalian SC from its key components. SYCP1 forms the transverse filaments, with its N- and C-terminal regions located in the central and lateral elements respectively. The central element also contains SYCE1, SYCE2, SYCE3 and TEX12, whilst the lateral element contains SYCP2 and SYCP3. DOI: 10.7554/eLife.02963.003
Figure 2. Structure of human SYCP3. (A and B) SEC-MALS analysis of SYCP3<sub>core</sub> and SYCP3<sub>FL</sub>, in which light scattering (LS) and differential refractive index (dRI) are plotted in conjunction with fitted molecular weights (M<sub>W</sub>). (A) SYCP3<sub>core</sub> has a fitted molecular weight of 78.6 kDa (±0.259%), with polydispersity 1.000 (±0.365%); its theoretical tetramer size is 79.5 kDa. (B) SYCP3<sub>FL</sub> has a fitted molecular weight of 110 kDa (±0.064%), with polydispersity 1.000 (±0.091%); its theoretical tetramer size is 111 kDa. (C) The crystal structure of SYCP3<sub>core</sub> is shown with a 90° rotation around its longitudinal axis; chains A-D are depicted in purple, salmon, teal and blue. The tetramer provides a length of 20 nm between the extremes of its C-terminal coiled-coils (measured at 196.1 and 199.9 Å between Glu220 Ca atoms of chains A and B, and chains C and D, respectively). The structure is made up of a central coiled-coil and two flanking arms. Each arm contains a four-helix bundle (with proximal aromatic-rich and distal Trp111 regions) and a C-terminal coiled-coil at the distal end. The four-helix bundle regions become continuous with the central coiled-coil through transition points that are distinct for each arm. DOI: 10.7554/eLife.02963.004
Figure 2—figure supplement 1  The helical core of human SYCP3 is defined by amino acids 66-230.
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Figure 2—figure supplement 2. Stereo images of sample electron density and the overall SYCP3 structure. DOI: 10.7554/eLife.02963.006
Figure 2—figure supplement 3. Iodide sites used for SAD phasing. DOI: 10.7554/eLife.02963.007

Figure 2—figure supplement 4. Comparison of SYCP3 tetramers present within native and iodide derivative crystals. DOI: 10.7554/eLife.02963.008
Figure 3. Structural details of the SYCP3 tetramer arm and central coiled-coil regions. (A–C) The SYCP3 tetramer arm is formed by a bipartite four-helix bundle of a proximal aromatic-rich core and distal W11 region, which becomes continuous with a C-terminal coiled-coil. (A) The aromatic (F/Y)-rich core is assembled through hydrophobic associations of Y125_{A/C}, F129_{A/C}, F133_{A/C}, Y179_{A/D}, and F182_{A/D}, with hydrogen bonds between Y125_{A/C} and Q181_{B/D}, and between Y179_{B/D} and S126_{A/C}. (B) In the distal region, tryptophan residues W111 adopt distinct conformations in chains A and C, undergoing hydrogen
bonding with Q201<sub>B</sub> (W111<sub>A</sub>) and D194<sub>D</sub> (W111<sub>C</sub>). (C) At the distal end of the SYCP3 tetramer, a parallel coiled-coil is formed between the C-terminal ends of chains B and D, involving residues L211<sub>A</sub>, L215<sub>B</sub>, and T219<sub>B</sub>. Flanking chains A and C diverge and are oriented through interactions of L92<sub>A</sub>, L100<sub>A</sub>, and M216<sub>B</sub>. (D–F) The central region of SYCP3 is asymmetrical, containing of a parallel coiled-coil flanked by transition points that are distinct between the two tetramer arms. (D) The central parallel coiled-coil is formed between chains B and D, with equivalent chains A and C held apart by steric exclusion. Packing is driven by aromatic interactions between F154 residues, and a network of hydrogen bonds between Q157<sub>B/D</sub> and Q158<sub>B/D</sub> (Q157<sub>A/C</sub> and Q158<sub>A/C</sub> adopt alternative solvent-exposed conformations). (E) At transition point 1, chains B and D are pulled together for coiled-coil formation through hydrogen bonding between W136<sub>A/C</sub> and Q168<sub>B/D</sub>. The interaction is further stabilised by salt bridges between R171<sub>B/D</sub> and D139<sub>C/A</sub>. (F) At transition point 2, coiled-coil formation between chains A and C is prevented through an alternative hydrogen bonding pattern in which W111<sub>B/D</sub> interacts with Q168<sub>C/A</sub>. Salt bridges between R171<sub>A/C</sub> and D139<sub>D/B</sub> are unchanged. (G) The SYCP3 native (top) and iodide derivative (bottom) structures coloured according to their backbone atomic crystallographic B-factors from red (high) to blue (low). Residues of the central region have B-factors of up to four times higher than those of the four-helix bundle regions.

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Figure 3—figure supplement 1  Non-crystallographic symmetry (NCS) between chains of the SYCP3 tetramer.

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Figure 3—figure supplement 2  NCS differences between chains of the SYCP3 tetramer.
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Figure 4. SYCP3 interacts directly with double stranded DNA. (A) Multiple sequence alignment of the N-terminal (left) and C-terminal (right) regions of SYCP3. The N-terminal region contains two basic patches, BP1 (amino acids 52–57) and BP2 (amino acids 88–91), that flank conserved patch Nt6 (amino acids 69–74). The C-terminus contains conserved patch Ct6 (amino acids 231–236). Arrows indicate N82 and Q221, the N- and C-terminal most amino acids present in all chains of the SYCP3 structure. (B–G) Electrophoretic mobility shift assays in which 187 base pair double stranded DNA (dsDNA) substrates at 18.7 μM (per base pair) were incubated with (B) SYCP3ΔCt6, (C) SYCP3core, (D) SYCP3ΔCt6ΔBP1 (mutation of BP1 to alanines), (E) SYCP3ΔCt6ΔBP2 (mutation of BP2 to alanines), (F) SYCP3ΔCt6ΔBP1+2 (mutation of BP1 and BP2 to alanines) and (G) GST-SYCP3ΔCt6 at concentrations shown. (H) Fluorescence anisotropy analysis in which SYCP3ΔCt6 WT, ΔBP1, ΔBP2 and ΔBP1+2 were incubated with 60 base pair FAM-dsDNA (25 nM per molecule)
at concentrations shown. Data points represent the mean and standard deviation (n = 3). The $K_d$ for SYCP3$\Delta_{Ct6}$ WT tetramers was determined as 0.20 μM by fitting to a standard curve. (I) Schematic diagram showing how SYCP3 may interact with two DNA molecules. DNA-binding is mediated by BP1 and BP2, which lie at the extreme N-terminus of the SYCP3$_{core}$ structure. The two ends of the SYCP3 tetramer may bind DNA, leading to two DNA molecules being held apart by 20 nm, with torsional rotation permitted around the longitudinal axis.

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**Figure 4.** Continued

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**Figure 4—figure supplement 1.** SEC-MALS analysis of SYCP3$\Delta_{Ct6}$ and basic patch mutants.

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Figure 5. SYCP3 undergoes self-assembly into regular filamentous structures. (A) Transmission electron micrograph of SYCP3FL, loaded onto an EM grid at 1 mg/ml (32 μM) in buffer containing 250 mM KCl, with negative staining performed using uranyl acetate. (B) SYCP3 fibres vary in length and width but show a constant pattern of light and dark striations, with a periodicity of 23 nm (mean = 23.3 nm, standard deviation = 0.95 nm). These striations may be explained by SYCP3 tetramers lying along the longitudinal axis, with the 20 nm rigid rod providing the bulk of the 23 nm spacing. (C and D) Transmission electron micrographs of 1 mg/ml (C) SYCP3ΔCt6 and (D) SYCP3ΔNt6. (E) Transmission electron micrograph of 1 mg/ml SYCP3FL incubated with 350 base pair double-stranded DNA (dsDNA) at 190 μM (per base pair). (F) The N- and C-terminal regions of SYCP3 are implicated in self-assembly. They are predicted to interact in an interlaced fashion within the remaining 3 nm space, creating arrays of self-association sites within discrete layers that define three-dimensional lattice assembly of SYCP3 fibres.

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**Figure 5—figure supplement 1.** SEC-MALS analysis of SYCP3ΔNt6.

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**Figure 6.** Model for organisation of the chromosome axis by SYCP3 assembly. Each SYCP3 tetramer contains two DNA-binding regions, separated by a distance of 20 nm owing to the central rigid rod-like structure. Upon binding to the chromosome axis, SYCP3 tetramers may pinch off portions of the axis such that short stretches of chromosomal DNA are looped back on themselves with a separation of 20 nm. The loading of further SYCP3 tetramers may bridge between the initial pinched-off portions, creating a continuous structure that extends along the chromosome axis. Thus, the final assembly consists of a three-dimensional lattice of SYCP3 tetramers that organise the chromosome axis in a concertina-like manner such that the length of the axis is shortened and the chromatin loops (that will flank the SC) are lengthened. For clarity, other meiotic factors that are known to perform important functions in the organisation of the chromosome axis, such as SYCP2, cohesin and condensin, are not depicted.

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