Structural analysis of the human SYCE2–TEX12 complex provides molecular insights into synaptonemal complex assembly

Owen R. Davies, Joseph D. Maman and Luca Pellegrini

Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Old Addenbrookes Site, Cambridge CB2 1GA, UK

1. Summary

The successful completion of meiosis is essential for all sexually reproducing organisms. The synaptonemal complex (SC) is a large proteinaceous structure that holds together homologous chromosomes during meiosis, providing the structural framework for meiotic recombination and crossover formation. Errors in SC formation are associated with infertility, recurrent miscarriage and aneuploidy. The current lack of molecular information about the dynamic process of SC assembly severely restricts our understanding of its function in meiosis. Here, we provide the first biochemical and structural analysis of an SC protein component and propose a structural basis for its function in SC assembly. We show that human SC proteins SYCE2 and TEX12 form a highly stable, constitutive complex, and define the regions responsible for their homotypic and heterotypic interactions. Biophysical analysis reveals that the SYCE2–TEX12 complex is an equimolar hetero-octamer, formed from the association of an SYCE2 tetramer and two TEX12 dimers. Electron microscopy shows that biochemically reconstituted SYCE2–TEX12 complexes assemble spontaneously into filamentous structures that resemble the known physical features of the SC central element (CE). Our findings can be combined with existing biological data in a model of chromosome synapsis driven by growth of SYCE2–TEX12 higher-order structures within the CE of the SC.

2. Introduction

Human fertility and genetic diversity depend on the successful execution of the genetic programme of meiosis. At the physical and functional centre of meiosis is the synaptonemal complex (SC), an enigmatic proteinaceous superstructure that holds together homologous chromosome pairs, providing the structural framework within which meiotic recombination and crossover formation occur [1–5]. The SC is essential for the successful completion of meiotic cell division: its disruption in mice leads to complete meiotic failure and resultant infertility [6–10], and its defective function in humans is associated with infertility and recurrent pregnancy loss (affecting 15% and 5% of couples, respectively), in addition to non-lethal aneuploidies such as Down's syndrome [1,6,11,12].

Initially discovered in crayfish spermatocytes [13], the SC has since been observed in a wide range of sexually reproducing organisms, from humans to yeast [14,15]. In all cases, it adopts a remarkably conserved tripartite ribbon-like structure that holds homologous chromosomes together along their entire length. This tripartite structure consists of lateral elements (LEs) running along each chromosome axis, a central element (CE) along the
midline and an array of juxtaposed transverse filaments (TFs) that bridge between LEs by interdigitating—much like the teeth of the ‘zipper’—within the CE [2,16–18] (figure 1a). In addition to the overall structure, the dimensions of the SC are also well conserved: the central region (comprising TFs and CEs) typically spans 100 nm, whereas LEs and CEs have widths of approximately 50 and 20–40 nm, respectively [14,15].

Figure 1. Physical features and protein constituents of the synaptonemal complex. (a) Schematic of a synapsed homologous chromosome pair, with electron micrograph of the mouse synaptonemal complex in which central element (CE), lateral element (LE) and transverse filaments (TF) are labelled. The inset electron micrograph image is reproduced from Kouznetsova et al. [10] under the Creative Commons Attribution Licence. (b) Schematic of the mammalian synaptonemal complex; SYCP1 molecules are orientated according to current models with N-terminal regions in the CE, C-terminal regions in the LE and central regions forming the TF. The LE contains SYCP2 and SYCP3, whereas the CE contains SYCE1, SYCE2, SYCE3 and TEX12. (c) Yeast two-hybrid (Y2H) analysis of human SC protein interactions. Y187[pGBKT7-bait] strains were mated with Y2HGold[pGADT7-target] strains, plated on SD/-Ade/-His/-Leu/-Trp/Aba/X-a-Gal plates and then transferred to filters for visualization. Positive reactions depend on activation of the four independent reporter genes: ADE1, HIS3, AUR-1C and MEL1. These data are representative of three repeats.
Assembly and disassembly of the SC are timely events within meiotic prophase I. SC assembly follows the induction of 200–400 double-strand breaks (DSBs) per cell that, through homology searching, establish local alignments between homologous chromosomes [3,6,17,18]. Short tracts of LEs begin to form along chromosome arms and are brought into 400 nm apposition at local alignments. Synapsis of homologous chromosomes nucleates at these sites by bringing LEs into 100 nm apposition and is extended by growth of the CE and TF array along the chromosome axis. SC assembly thus converts local alignments into fully synapsed homologous chromosome pairs. Its three-dimensional architecture further provides the necessary structural framework for completion of meiotic recombination, resulting in DSB resolution and crossover formation [11]. Once accomplished, the SC is disassembled, leaving crossovers as the sole physical links between homologous chromosomes during metaphase I [1–3].

Over the past two decades, seven essential protein constituents of the mammalian SC have been identified [19–25]; all contain predicted α-helical structure, and some contain putative coiled-coils. On the basis of immunofluorescence and immunogold electron microscopy studies, a rudimentary protein map of the SC has been formulated (figure 1b). TFs are formed by SYCP1, an elongated protein containing a large central region of predicted coiled-coil with flanking N- and C-terminal domains [21,26]. The N-terminal domain is located within the CE, wherein it is closely associated with CE proteins SYCE1, SYCE2, SYCE3 and TEX12 [23–27], whereas the C-terminal domain localizes to the LE, wherein it contacts LE proteins SYCP2 and SYCP3 [16,22,26–28]. Deficiency of each known SC protein abrogates synapsis, DSB resolution and crossover formation, resulting in complete male/female infertility for SYCP1 and CE proteins, and a sexual dimorphism of male infertility and female subfertility for LE proteins [6–9,25,29,30].

An apparent dichotomy has emerged between CE proteins. SYCE1 and SYCE3 co-localize in a continuous pattern identical to that of SYCP1, and their disruption leads to complete failure of tripartite structure formation [23–25]. By contrast, SYCE2 and TEX12 co-localize in a distinct punctate pattern (although this may reflect antibody properties rather than the underlying protein distribution) and their disruption leads to synaptic failure, albeit with the presence of short stretches of close associations that contain CE-like structure [7,9,23]. Furthermore, SYCE2 and TEX12 co-immunoprecipitate from mouse testis lysate [23]. These findings have led to the suggestion that SYCE1 and SYCE3 function in the initiation of synapsis, whereas SYCE2 and TEX12 function in its extension [4,7,9,25].

Since its discovery over 60 years ago and the recognition of its critical role in meiosis, the accumulating wealth of biological evidence has led to tentative models of SC assembly and disassembly [31–35], and to suggestions of functional roles in mediating recombination, crossover formation and late interference [8,18,36,37]. However, the absence of any detailed biochemical and structural information about the SC and the physical organization of its constituent proteins hampers rational attempts to test current models of SC function, and consequently our understanding of its role in meiosis remains rudimentary. In order to provide a molecular basis of SC function, we have embarked upon the biochemical and structural characterization of purified, recombinant SC proteins.

Here, we describe the reconstitution and biophysical characterization of a stable, constitutive complex between human CE proteins SYCE2 and TEX12. The first biochemical and structural analysis of an essential SC protein component provides molecular insight into assembly of the human SC.

3. Material and methods

3.1. Yeast two-hybrid

Sequences corresponding to human SYCP1 (1–811), SYCP2 (1399–1530 and 1358–1530), SYCP3 (1–236), SYCE1 (1–315, 1–144 and 141–269), SYCE2 (1–218 and 57–165) and TEX12 (1–123 and 49–123) were cloned into pGBKTK7 and pGADT7 vectors (Clontech). Yeast two-hybrid (Y2H) analysis was performed using the Matchmaker Gold Y2H system (Clontech), with protocols based on the manufacturer’s instructions. pGBKTK7 and pGADT7 vectors were transformed into yeast strains Y187 and Y2H Gold, respectively, according to a standard PEG/ssDNA/LiAc procedure. Y187[pGBKTK7-bait] strains were mated with Y2H Gold[pGADT7-target] strains by mixing single colonies of each in 0.5 ml 2xYPDA and incubating at 30°C, 50 r.p.m. for 24 h. Cultures were then diluted 1 in 10 using 0.5xYPDA; 100 μl was plated onto SD/-Leu/-Trp to select for mated colonies, and a further 100 μl was plated onto SD/-Ade/-His/-Leu/-Trp containing aureobasidin A (AbA) and X-α-Gal to select for mated colonies with activation of ADE1, HIS3, AUR1-1C and MEL1 reporter genes. Plates were incubated at 30°C for 5 days. Colonies were lifted onto filters (Whatman No. 5, 70 mm) that were dried, scanned and displayed aligned against a black background.

3.2. Recombinant protein expression

For co-expression, sequences corresponding to human SYCE2 (1–218, 57–165, 57–88 and 88–165) with N-terminal MBP-tag and TEX12 (1–123, 24–123, 45–123, 49–123 and 87–123) or SYCE2 (1–218) with N-terminal His-tag (both linkers containing tobacco etch virus (TEV) protease cleavage sequences) were cloned into the two open reading frames of pRSFDuet-1 (Novagen). For separate expression, sequences corresponding to SYCP1 (1–144 and 141–269), SYCE2 (1–218 and 57–165) and TEX12 (1–123, 24–123, 45–123, 49–123 and 87–123) with N-terminal MBP- or His-tags were cloned into pMAT11 and pHAT4 vectors, respectively [38]. All constructs were expressed in Rosetta 2 (DE3) cells (Novagen), in 2xYT media, induced with 0.5 mM IPTG for 16 h at 25°C. In the text, usage of the protein names, SYCE2 and TEX12, relates to the full-length sequences, unless stated otherwise, in which case construct boundaries are provided in subscript.

3.3. Purification of SYCE2–TEX12 protein complexes

MBP–SYCE227–165 was co-expressed with His–TEX12 or His–TEX12124–125 (described earlier). Fusion protein complexes were co-purified from clarified lysate by sequential affinity chromatography using Ni–NTA resin (Qiagen) and amylose resin (NEB); cleaved protein complexes were eluted from the latter column through incubation with TEV protease (Invitrogen). Further purification was achieved through anion-exchange chromatography using a Resource Q 6 ml column (GE Healthcare). Protein complexes were eluted from the Resource Q column.
in 20 mM Tris pH 8.0, 145 mM KCl, 2 mM DTT, at concentrations of 2–5 mg ml⁻¹. All biophysical assays were performed using freshly prepared material. Protein samples were analysed by SDS–PAGE using the NuPAGE Novex Bis-Tris system (Invitrogen), with Coomassie staining performed using SimplyBlue SafeStain (Invitrogen). Densitometry was performed using ImageJ [39]. Protein concentrations were determined by UV spectrophotometry (Varian Cary 50 spectrophotometer), with extinction coefficients and molecular weights calculated by ProtParam (http://web.expasy.org/protparam/). Edman degradation analysis of SYCE257–165–TEX12 solution samples was performed by the Protein and Nucleic Acid Facility (Department of Biochemistry, University of Cambridge).

3.4. Circular dichroism spectroscopy

Circular dichroism (CD) data were collected using an Aviv 410 spectropolarimeter (Biophysics facility, Department of Biochemistry, University of Cambridge). Protein complexes SYCE257–165–TEX12 and SYCE257–165–TEX1249–123 were analysed at 0.20 and 0.22 mg ml⁻¹, respectively, in 10 mM NaH₂PO₄ pH 7.5, 150 mM NaF, using a 1 mm path-length quartz cuvette, with 1 nm slit width and 1 s averaging time. CD spectra were recorded at 4°C (between 260 and 185 nm) with 0.5 nm increments; for each sample, raw data from three measurements were averaged, corrected for buffer signal, smoothed and then converted into mean residue ellipticity (Θ). Deconvolution was performed using the CDSSTR algorithm [40] on the DichroWeb server (http://dichroweb.cryst.bbk.ac.uk) [41]. CD temperature melt data were recorded at 222 nm, for 1°C increments between 5°C and 95°C, with 1°C per minute ramping rate, 0.5°C deadband, 30 s incubation time, 1 nm slit width and 1 s averaging time. Raw data were converted to mean residue ellipticity ([Θ]222) using standard equations.

3.5. Analytical ultracentrifugation

Sedimentation velocity experiments were performed using a Beckman XL-A analytical ultracentrifuge (Biophysics facility, Department of Biochemistry, University of Cambridge). Protein complexes SYCE257–165–TEX12 and SYCE257–165–TEX1249–123 were analysed at 57 and 289 µM, respectively, in 20 mM Tris pH 8.0, 145 mM KCl, 2 mM DTT. Sedimentation velocity experiments were performed at 30 000 r.p.m, 20°C, with absorbance data at 285 nm recorded across cell radii at 0.003 cm intervals, at 3.2 min time intervals, over a total period of 320 min. Protein and buffer parameters were calculated using SEDNTERP, and data were analysed through direct boundary modelling to a continuous c(S) distribution of Lamm equation solutions using SEDFIT [42].

3.6. Size-exclusion chromatography – multi-angle light scattering

Absolute molar masses of proteins were determined through size-exclusion chromatography multi-angle light scattering (SEC–MALS). Protein samples (100 µl; 1–5 mg ml⁻¹) were loaded onto a Superdex 200 10/300 GL SEC column (GE Healthcare) in 20 mM Tris pH 8.0, 150 mM KCl, 2 mM DTT, at 0.5 ml min⁻¹ using an AKTA Purifier (GE Healthcare). The column output was fed into a DAWN HELEOS II MALS detector (Wyatt Technology), in which light scattered from a polarized laser source of 664 nm is detected by eight fixed angle detectors, followed by an Optlab T-Rex differential refractometer (Wyatt Technology), which measures absolute and differential refractive index using a 664 nm LED light source at 25°C. Data were collected and analysed using ASTRA 6 software (Wyatt Technology). Molecular masses were calculated across eluted protein peaks through extrapolation from Zimm plots using a dn/dc value of 0.1850 ml g⁻¹; quoted molecular weights and estimated errors relate to the overall mass calculation across a single peak.

3.7. Amylose affinity pulldown assay

MBP-fusion SYCE2 constructs were co-expressed with His-tagged TEX12 or SYCE2 constructs (described earlier). For each condition, 1 l cultures were grown, and cells were resuspended in 25 ml of 20 mM Tris pH 8.0, 500 mM KCl, 2 mM DTT, lysed by sonication, clarified by high-speed centrifugation and incubated with 4 ml of amylase resin (NEB) for 1 h at 4°C. After thorough washing, bound complexes were eluted in 10 ml of 20 mM Tris pH 8.0, 150 mM KCl, 30 mM d-maltose, 2 mM DTT. Total protein concentrations were equalized to 3 mg ml⁻¹ through dilution or concentration (Millipore Amicon Ultra-4) as appropriate, and analysed by SDS–PAGE (described earlier). This purification method was also used in the preparation of individually expressed MBP–SYCE2 and MBP–TEX12 fusion proteins for analysis by SEC–MALS.

3.8. Electron microscopy

Electron microscopy analysis was performed using an FEI Philips CM100 transmission electron microscope (Multi Imaging Unit, University of Cambridge). Protein samples at 100 µM were applied to transmission electron microscopy carbon-coated grids, and negative staining was performed using 0.1 per cent (v/v) uranyl acetate.

3.9. Protein sequences and analysis

Protein sequences were extracted from UniProtKB; multiple sequence alignments were performed using MUSCLE (EBI) and were displayed using JALVIEW v. 2.0 (www.jalview.org) [43]. Secondary structure predictions were performed using JNET (http://www.compbio.dundee.ac.uk/www-jpred/), PsiPRED v. 3.0 (http://bioinf.cs.ucl.ac.uk/psipred/), PORTER (http://distill.ucd.ie/porter/) and SOPMA (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html).

4. Results

4.1. Identification of a constitutive SYCE2–TEX12 complex

Given the apparent intricacy of the molecular architecture of the SC, we reasoned that SC proteins might exist in constitutive multi-component complexes. We thus set out to identify interactions between human SC proteins that would facilitate their biochemical and structural analysis. This was achieved by a yeast Y2H grid screen of human SC components using
the Matchmaker Gold Y2H system, in which positive inter-
actions are determined by the activation of the four
independent reporter genes ADE1, HIS3, AUR1-C and
MEL1. This revealed self-association of SYCP1, SYCP2,
SYCP3, SYCE1 and TEX12, consistent with previous reports
[21,23,24,30,32], and a robust interaction between CE proteins
SYCE2 and TEX12 that was detected in both directions
(figure 1c). We did not identify other heterotypic SC protein
interactions. This may be due to their non-binary nature,
the high stringency nature of this screen (designed to identify
only strong interactions), or steric interference of the Y2H
fusion proteins. Complex formation between SYCE2 and
TEX12 is entirely consistent with previous reports of their
co-localization, co-immunoprecipitation and the phenotypic
similarity of their individual knockouts [7,9,23]. Accordingly,
we decided to focus our efforts on the putative
SYCE2–TEX12 interaction.

Sequence analysis of SYCE2 reveals that this 218 amino
acid protein consists of a central evolutionarily conserved
domain of three predicted α-helices, the first of which
forms a putative coiled-coil (at confidence level greater than
90%), flanked by divergent N- and C-terminal extensions
(see figure 2a; electronic supplementary material, figure S1).
TEX12 is a highly conserved 123 amino acid protein,
containing three predicted α-helices in its central
and C-terminal regions, with a divergent N-terminus (see
figure 2a; electronic supplementary material, figure S2).
Expression and purification of individual SYCE2 and
TEX12 only allowed for recovery of small amounts of
material after removal of affinity tags, which was unsuitable
for biophysical analysis. By contrast, SYCE2 and TEX12 co-
expression conferred a large increase in the solubility and
stability of both protein components. In the case of the full-
length protein complex, removal of affinity tags revealed
considerable degradation of SYCE2. As the N- and C-terminal
extensions of SYCE2 are divergent or absent in other species
(see electronic supplementary material, figure S1), and are
dispensable for interaction with TEX12 (figure 1c), we co-
expressed TEX12 with the central conserved region of SYCE2,
spanning residues 57–165. This eliminated degra-
expressed TEX12 with the central conserved region of
(see electronic supplementary material, figure S1), and are
extensions of SYCE2 are divergent or absent in other species
length protein complex, removal of affinity tags revealed con-
stitutive. Furthermore, SDS–PAGE band densitometry
in which the SYCE2–TEX12 complex is disrupted. We thus
could not identify a non-denaturing biochemical condition
in which the SYCE2–TEX12 complex is unstructured,
validating our subsequent use of SYCE257–165–TEX1249–123
in structural analysis, and demonstrate high helical content
within the central region of SYCE2 and the central and C-terminal
regions of TEX12.

We assessed the thermal stability of the SYCE2–TEX12
complex by measuring the α-helical signature ellipticity at
222 nm over the temperature range 5–95°C (figure 3b). SYCE257–165–TEX12 showed a reversible linear decline in
ellipticity (i.e. typical of α-helical fraying [44]) up to 65°C,
with irreversible cooperative unfolding beyond this point.
Similar data were obtained for SYCE257–165–TEX1249–123,
albeit with irreversible conformation change and subsequent
unfolding occurring at the slightly lower temperature of
55°C. The considerable resistance to thermal denaturation
confirmed the high conformational stability of the
SYCE2–TEX12 complex.

4.2. High helical content and thermal stability of
the SYCE2–TEX12 complex

As the first stage of structural characterization, we assessed
secondary structure composition of SYCE2–TEX12 by CD
spectroscopy (figure 3a). Far UV spectra of SYCE257–165–
TEX12 showed the presence of 65 per cent α-helical content
(153 helical residues), remarkably close to its predicted α-heli-
cal content of 64 per cent (150 helical residues). CD analysis of
the SYCE257–165–TEX1249–123 complex showed an increase in
relative α-helical content to 82 per cent (157 helical residues)
with concomitant reduction in unordered signal. These data
confirm that the N-terminal region of TEX12 is unstructured,
validating our subsequent use of SYCE257–165–TEX1249–123
in structural analysis, and demonstrate high helical content
within the central region of SYCE2 and the central and C-terminal
regions of TEX12.

4.3. The SYCE2–TEX12 complex is a hetero-octamer

We next set out to determine the oligomeric status of the
SYCE2–TEX12 complex. Analytical ultracentrifugation
(AUC) sedimentation velocity data for SYCE257–165–TEX12
were fitted to a continuous c(s) distribution, resulting in a
single skewed peak of sedimentation coefficient 4.53 S,
with fitted frictional ratio 1.92 and estimated molecular
weight 118 kDa (figure 4a). As we have previously deter-
ned the complex to be equimolar, the AUC analysis is
most consistent with a hetero-octameric assembly formed
by four chains each of SYCE2 and TEX12, corresponding to
a theoretical molecular weight of 109 kDa. The skewed
peak and slight disparity between estimated and theoretical
molecular weights are likely due to the unstructured
nature of SYCE257–165–TEX12, with concomitant reduction in unordered signal. These data
confirm that the complex is equimolar, the AUC analysis is
most consistent with a hetero-octameric assembly formed
by four chains each of SYCE2 and TEX12, corresponding to
a theoretical molecular weight of 109 kDa. The skewed
peak and slight disparity between estimated and theoretical
molecular weights are likely due to the unstructured
N-terminal region of SYCE2–TEX12. AUC analysis of SYCE257–165–
TEX1249–123 showed a single symmetrical peak of 4.38 S,
with fitted frictional ratio 1.65 and estimated molecular
weight 89.9 kDa (figure 4b), closely matching an equimol-
ular hetero-octamer size of 89.0 kDa. Reduction in frictional
ratio confirms the flexible unstructured nature of the TEX12
N-terminus, and a frictional ratio of 1.65 for SYCE257–165–
TEX1249–123 indicates significant asymmetry within this
central core, suggesting that the complex adopts an extended
rather than a globular conformation.

To confirm the size of the SYCE2–TEX12 complex, we
employed SEC–MALS, in which native molecular weights are
determined absolutely, overcoming the ambiguity of fric-
tional ratio fitting in AUC. SYCE257–165–TEX12 eluted in a
majority peak of molecular weight 110 kDa (figure 4c), with
some high molecular weight aggregation, whereas
SYCE257–165–TEX1249–123 eluted in a single peak of
Figure 2. Identification of a constitutive equimolar complex between central element proteins SYCE2 and TEX12. (a) Schematic of human SYCE2 and TEX12 protein sequences. The central region of SYCE2 (residues 60–165) shows evolutionary conservation; \( \alpha \)-helical structure is predicted for residues 66–83 (\( \alpha \)1), 87–140 (\( \alpha \)2) and 143–160 (\( \alpha \)3), and the coiled-coil (CC) formation is predicted for residues 60–87. The central and C-terminal region of TEX12 (residues 24–123) show evolutionary conservation; \( \alpha \)-helical structure is predicted for residues 52–56 (\( \alpha \)1), 62–79 (\( \alpha \)2) and 86–121 (\( \alpha \)3). For full sequence alignments, secondary structure and coiled-coil predictions, see electronic supplementary material, figures S1 and S2. (b) Coomassie-stained SDS–PAGE showing co-expression in bacteria and co-purification of the SYCE2\text{57–165}–TEX12 complex by Ni–NTA affinity chromatography, amylose affinity chromatography, TEV cleavage and anion-exchange chromatography. (c) Coomassie-stained SDS–PAGE showing size-exclusion chromatography analysis of SYCE2\text{57–165}–TEX12 in comparison with His-TEX12; elution positions of gel filtration standards are shown. (d) Densitometry analysis of purified SYCE2\text{57–165}–TEX12; for analysis, the sample was diluted until peaks for constituent proteins became clearly defined, as shown. (e) Coomassie-stained SDS–PAGE of SYCE2\text{57–165}–TEX12\text{49–123}; this complex was purified in an identical manner to SYCE2\text{57–165}–TEX12. (f) Densitometry analysis of SYCE2\text{57–165}–TEX12\text{49–123}; integrated intensities of SYCE2\text{57–165} and TEX12\text{49–123} peaks account for 59.5% and 40.5% of the total signal, closely matching their theoretical equimolar mass percentages of 59.1% and 40.9%, respectively.
molecular weight 89 kDa (figure 4). Thus, the molecular weights determined by SEC–MALS match closely the theoretical sizes of 109 and 89 kDa for equimolar hetero-octameric assemblies of SYCE2 57–165–TEX12 and SYCE2 57–165–TEX1249–123, respectively.

4.4. SYCE2 is a constitutive tetramer that multimerizes via its central α2–3 region

The realization that interaction of SYCE2 and TEX12 leads to an octameric assembly raises the question of their oligomeric status in the absence of the protein partner. As production of isolated recombinant SYCE2 and TEX12 proved difficult, we resorted to the use of MBP-fusion tags in order to improve solubility and stability. SEC–MALS analysis of MBP–SYCE2 fusion protein (see figure 5a; electronic supplementary material, figure S3a) revealed a single peak of molecular weight 274 kDa, consistent with an MBP–SYCE2 tetramer of theoretical molecular weight 278 kDa. These findings were confirmed by a SEC–MALS analysis of His-tagged SYCE2 (see figure 5b; electronic supplementary material, figure S3b) that, despite significant instability and aggregation of the sample, indicated a molecular weight of 126 kDa, against a theoretical tetramer size of 115 kDa. We thus conclude that SYCE2 exists as a tetramer in solution.

We note that SYCE2 self-association was not detected by Y2H (figure 1c); this may be due to the lack of dynamic exchange between the two populations of SYCE2 complexes upon yeast mating, or steric interference of Y2H fusion proteins.

To explore the molecular determinants of SYCE2 tetramerization, we dissected the SYCE2 sequence on the basis of the three predicted α-helices within its central evolutionarily conserved domain. As an MBP-fusion protein, the α1 region of SYCE2 (amino acids 57–88) proved highly stable, and was determined by SEC–MALS to have a molecular weight of 48.7 kDa (figure 5b; electronic supplementary material, figure S3c,d), precisely matching its theoretical monomer size. We could not obtain SEC–MALS data for MBP-fusion proteins corresponding to the α1–3 (amino acids 57–165) and α2–3 (amino acids 88–65) regions of SYCE2, presumably owing to their instability in the absence of TEX12 (data not shown). To overcome this, we assessed the ability of MBP–SYCE2 fusion constructs to self-associate with His–SYCE2 by amylose pull-down following
co-expression in bacteria (figure 5d). Pull-down experiments revealed His–SYCE2 binding to full-length, α1–3 and α2–3 regions of SYCE2, but not to its α1 region. These data confirm self-association of full-length and central α1–3 region of SYCE2, and further demonstrate that while the N-terminal α1 region is monomeric, the central α2–3 region is sufficient for oligomerization (figure 5e,f).

4.5. TEX12 is a constitutive dimer that self-associates via its central α1–2 region

We next assessed the oligomer status of isolated TEX12. As for SYCE2, it proved necessary to express and purify TEX12 as an MBP-fusion protein (see electronic supplementary material, figure S3e) in order to obtain recombinant protein suitable for biophysical analysis. SEC–MALS revealed a single peak of molecular weight 110 kDa (figure 6a), consistent with an MBP–TEX12 homodimer (its theoretical homodimer size is 118 kDa). The molecular determinants of dimerization were explored by a SEC–MALS analysis of a series of MBP-fusion proteins in which the N-terminus of TEX12 was progressively truncated (see figure 6b–d; electronic supplementary material, figure S3e). Dimerization was retained for MBP-fusion constructs TEX1224–123 and TEX1249–123 that contain the three predicted helices of the evolutionarily conserved domain, α1–3 (observed molecular weights of 107 and 102 kDa, respectively, and theoretical dimer sizes of 113 and 108 kDa). Thus, self-association of TEX12 is maintained in the SYCE257–165–TEX1249–123 complex. However, deletion of the α1–2 region abrogated dimerization, as the MBP–TEX1257–123 construct containing only the C-terminal α3 region had a molecular weight of 49.9 kDa (theoretical monomer size of 49.5 kDa).
conclude that TEX12 dimerizes via its central $\alpha$2–$\alpha$3 region (figure 6e,f).

4.6. A molecular model for the SYCE2–TEX12 hetero-octamer

As a further step in the analysis of the SYCE2–TEX12 complex, we investigated the molecular determinants of the SYCE2–TEX12 interaction by amylose pull-down of bacterial extracts containing over-expressed MBP–SYCE2 and His–TEX12 constructs. First, we assessed TEX12 binding by SYCE2 (figure 7a). His–TEX12 binding was detected for full-length, $\alpha$1–3 and $\alpha$1 regions of SYCE2, but not for its $\alpha$2–3 region. Thus, the N-terminal $\alpha$1 region of SYCE2 spanning residues 57–88 is necessary and sufficient for interaction with TEX12. Interestingly, despite being monomeric in solution, SYCE2$_{57–88}$ contains a predicted coiled-coil, suggesting that the SYCE2–TEX12 interaction may take the form of a heterotypic coiled-coil. These data suggest a modular structure for SYCE2,
with mutually independent functions pertaining to the N-terminal α1 region that binds TEX12, and the central α2–3 region that is responsible for tetramerization (figure 7c).

We next assessed SYCE2-binding by TEX12. Interactions with MBP–SYCE2 and MBP–SYCE257–165 were detected for all N-terminal truncations of TEX12 down to and including its α3 region alone (figure 7b,c), indicating that the C-terminal α3 region of TEX12 spanning amino acids 87–123 is necessary and sufficient for interaction with SYCE2. Thus, TEX12 structure contains mutually independent functional modules as observed for SYCE2, with a central α1–2 region that mediates dimerization, and a C-terminal α3 region responsible for SYCE2 binding (figure 7c). Interactions with MBP–SYCE257–88 were further detected for all N-terminal truncations of TEX12 down to and including its α3 region alone (figure 7d), but not for the N-terminal or α1–2 regions of TEX12 (see electronic supplementary material, figure S4), confirming a direct interaction between the N-terminal α1 region of SYCE2 and the C-terminal α3 region of TEX12. Interestingly, the stabilizing effect conferred by TEX12 onto MBP–SYCE2 (as assessed by proteolytic degradation of the fusion protein) is substantially diminished for the α3 region of TEX12 (figure 7b,c), suggesting that stabilization is dependent on TEX12 dimerization.

On the basis of these findings, we propose a molecular model for SYCE2–TEX12 hetero-octamer formation in which an SYCE2 tetramer binds two TEX12 dimers through 1:1 interactions between N-terminal α1 regions of SYCE2 and C-terminal α3 regions of TEX12 (figure 7f,g).
4.7. Higher-order structure formation of SYCE2–TEX12

The observation that SYCE2 and TEX12 associate constitutively in a hetero-octameric assembly raises the question of the biological role of the SYCE2–TEX12 interaction in SC function. As SYCE2 and TEX12 co-localize to the same molecular network that extends throughout the CE, we decided to investigate whether the SYCE2–TEX12 complex could self-associate in large supramolecular structures of comparable size to the known physical dimensions of the SC. Electron microscopy analysis of SYCE257–165–TEX12 and SYCE257–165–TEX1249–123 complexes revealed their
concentration-dependent assembly into extended, filamentous structures that are approximately 40 nm wide and range in length from 300 nm to 1 μm (figure 8a,b). The dimensions of the filaments resemble those of the CE within the SC [14,15], raising the possibility that the SYCE2–TEX12 filaments might represent structural components of the CE. To relate this to our solution studies of SYCE2–TEX12, while the majority species observed were hetero-octamers, a minority of higher-order species were observed, the proportion and size of which were reversibly increased by protein concentration (data not shown). It is sensible to envisage that assembly of SYCE2–TEX12 complexes into higher-order filamentous structures within the CE might be a dynamic process driven by low-affinity interactions between SYCE2–TEX12 complexes, in contrast to the high-affinity, constitutive interactions that hold together the SYCE2–TEX12 hetero-octamer (figure 8c).

5. Discussion

Since its discovery in 1956, the tripartite structure of the SC has become recognized as a physical hallmark of meiosis. However, despite its essential role in meiotic cell division, the molecular structure, mechanism of assembly and function of the SC remain largely unknown. One of the principal challenges of studying the SC at the molecular level is the difficulty in producing recombinant versions of the SC proteins,
which has precluded so far their biochemical and structural analysis. Here, we have overcome this problem by defining a stable and constitutive complex between CE proteins SYCE2 and TEX12, as an equimolar hetero-octamer, resulting from the constitutive interaction of one SYCE2 tetramer with two TEX12 dimers. The assembly of SYCE2–TEX12 hetero-octamers into higher-order structures suggests a possible architectural role of the complex in CE structure.

The constitutive nature of the SYCE2–TEX12 interaction is consistent with their co-localization pattern and co-immunoprecipitation, as well as the similar phenotype of synaptic failure induced by their individual disruption [7–9,23–25]. It is likely that the SYCE2–TEX12 hetero-octamers form immediately upon expression in meiotic cells and that they constitute the dynamic form that is transported to chromosomes for SC assembly. The realization that SYCE2 and TEX12 associate spontaneously into hetero-oligomers raises the question of whether other SC protein components exist in constitutive complexes. Clear candidates are SYCE1 and SYCE3, which, similar to SYCE2 and TEX12, have a shared functional role and localization pattern within the CE [8,24,25].

A molecular model of the SYCE2–TEX12 hetero-octamer was constructed from biophysical and pull-down analyses of protein truncations (figure 7f,g). SYCE2 and TEX12 share a modular structure in which both proteins contain distinct self-association and heterotypic interaction sites. SYCE2 undergoes tetramerization through its central α2–3 region, whereas TEX12 dimerizes through its central α1–2 region. Heterotypic association is mediated by the N-terminal α1 region of SYCE2 and the C-terminal α3 region of TEX12, possibly through coiled-coil formation. Thus, assembly of the SYCE2–TEX12 hetero-octamer results from four 1:1 interactions between an SYCE2 tetramer and two TEX12 dimers. The strong reciprocal affinity of SYCE2 and TEX12, and high stability of the resulting complex, indicates a large degree of reciprocal stabilization of the two protein partners.

The regular filamentous appearance of the higher-order structures formed by SYCE2–TEX12 complexes that extend to micrometre scale suggest that they might represent 'bona fide' architectural components of the CE. Given the high asymmetry of the SYCE2–TEX12 hetero-octamer, we postulate that the long dimension of the complex constitutes the width of the higher-order structures and that formation of extended filaments occurs by lateral associations of hetero-octamers (figure 8c). As the large majority of the SYCE2–TEX12 complex exists in solution as individual hetero-octamers, lateral associations are probably low-affinity and dependent on high protein concentrations of the complex. These weak associations between SYCE2–TEX12 hetero-octamers may exert considerable cooperativity, creating a stable higher-order structure. Within the cell, the formation of such structures might be induced by high local concentration of the complex at the developing SC and may be further stabilized by specific interactions with other SC proteins.

To assess the potential role of SYCE2–TEX12 higher-order structures within the CE, we refer to previous electron microscopy three-dimensional reconstruction studies of the SC central region. In insects, the CE has well-defined, ladder-like structures, provided by pairs of stacked pillars orientated perpendicular to the axis, which are connected vertically, transversely and longitudinally by fibrous bridges [45–47]. The mammalian CE is, by contrast, far more amorphous; nevertheless, putative transverse and longitudinal components have been reported [45,46]. The filamentous nature of the SYCE2–TEX12 higher-order structures is most consistent with a role as longitudinal CE components that extend synopsis in recurrent discrete steps along the chromosome axis. This is in agreement with the observed failure of extension, but retention of synaptic initiation, upon disruption of SYCE2 or TEX12 in meiotic cells [7,9], and provides molecular explanation for their distinct punctate staining pattern along the length of the SC [23,24].

Our findings can be combined with existing biological data in a model for SC assembly. At sites of initiation, growth of SYCE2–TEX12 filaments may extend the CE, in synchrony with concomitant extension of the SYCP1 TF array. Full synopsis of homologous chromosomes may be achieved through repeated episodes of initiation and extension of SYCE2–TEX12 filaments, resulting in concurrent, reciprocal stabilization of the CE and the flanking arrays of TFs. While it remains unknown how SYCE2–TEX12 complexes associate with TFs, possibilities include direct interactions with SYCP1 or indirect interactions mediated by synaptic initiation proteins such as SYCE1 and SYCE3 [23–25]. To extend the familiar analogy of the SC as a ‘zipper’, if SYCP1 molecules are the teeth, SYCE2–TEX12 seemingly acts as the slider, pulling the teeth together from initiation sites and extending synopsis along the entire chromosome axis.

As a complete catalogue of protein factors important for SC assembly and functions emerges from biological studies, it will become increasingly possible to attempt the partial or complete biochemical reconstitution of the process of SC assembly that takes place during meiosis. An important outcome of this work is the demonstration that biochemical and biophysical studies of SC proteins are both feasible and necessary in order to understand the molecular basis of SC function.

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