Protein SYCP2 Is an Ancient Component of the Metazoan Synaptonemal Complex

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Abstract

During the first meiotic prophase, chromosome synapsis is mediated by the synaptonemal complex (SC), an evolutionarily conserved meiosis-specific structure. In mammals, 7 SC protein components have been identified so far. Despite some controversy in the past, we have shown that SC proteins are ancient in metazoans and very likely formed an ancestral SC structure in the ancestor of metazoans. Protein components SYCP1, SYCP3, SYCE2, and TEX12 were identified in basal-branching metazoans, while other components (SYCE1 and SYCE3) are more recent elements. However, the evolutionary history of mammalian SYCP2 is not known. Here, we investigated this aspect with the aid of bioinformatic tools as well as with RNA and protein expression analysis. We conclude that SYCP2 belongs to the group of ancient SC proteins that was already present in the common ancestor of metazoans more than 500 million years ago.

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Meiosis is a type of cell division that leads to the formation of haploid cells. It is divided into 2 sequent divisions: meiosis I and meiosis II. During the first meiotic division, the chromosome set is reduced to half by the separation of the homologous chromosomes. The sister chromatids are not separated until the second division, which is very similar to mitosis, finally providing 4 haploid germ cells from 1 diploid precursor.

Three meiosis-specific features need to interplay during the first division to achieve a successful chromosome reduction: (a) the cohesion of chromatids, which is protected at the centromeres during metaphase I to prevent a premature separation of the sister chromatids; (b) the recombination of homologous chromosomes that provides interconnection (chiasmata) of the homologous chromosomes, and (c) a stable synapsis of the homologous chromosomes, which is mediated by the synaptonemal complex (SC) [Page and Hawley, 2004].

In the electron microscope, a typical SC appears as a ladder-like structure interconnecting the homologs over their entire length [Fraune et al., 2012a]. Its assembly starts during leptotema with the formation of the axial elements. As meiosis progresses, during zygonema, axial elements (now called lateral elements, LEs) recruit numerous transverse filaments (TFs), which in a zipper-like manner over-

lap and interact with TFs from the opposing LEs, hence initiating the formation of the central element (CE), which stabilizes the interactions of the TFs. SCs are fully assembled in pachynema and become disassembled during diplonema of the first meiotic prophase [Zickler and Kleckner, 1999; Page and Hawley, 2003; Fraune et al., 2012a].

The tripartite structure of the SC (LEs, TFs, CE) is highly conserved in sexually reproducing organisms [Gillies, 1975; von Wettstein et al., 1984]. Very recently, we demonstrated that mammalian SC proteins derived from an ancient structure that was present in the ancestor of metazoans [Fraune et al., 2012b, 2013]. Noteworthy, the ancient origin of SC proteins was unexpected at that time, as the mammalian SC proteins do not share any detectable sequence homologies with the characterized SC proteins of the other metazoan meiosis model organisms, i.e. *Drosophila melanogaster* and *Caenorhabditis elegans* [Fraune et al., 2012b, 2013]. An in-depth phylogenetic analysis, however, finally disclosed the conservation of most characterized mouse SC proteins from basal branching metazoans to mammals [Fraune et al., 2012b, 2013]. Our previous studies demonstrated that the major structural SC proteins SYCP1, the protein of the TFs [Meuwissen et al., 1992], and SYCP3, a component of the LEs [Lammers et al., 1994], as well as the 2 CE proteins SYCE2 and TEX12 [Costa et al., 2005; Hamer et al., 2006] are ancient in Metazoa. Orthologs of all 4 proteins were found in several animal lineages, even down to the basal species *Hydra*, and were experimentally identified as protein components of the cnidarian SC. The deduced evolutionary sequence of SC formation further suggested that the mammalian components SYCE1 and SYCE3 – both specific components of the CE [Costa et al., 2005; Schramm et al., 2011] – newly emerged in the branches leading to Bilateria (SYCE1) and vertebrates (SYCE3), respectively. It appears likely that during the divergence of the ecdysozoan species strong diversification of the existing SC proteins might have occurred, which could explain the lack of sequence homology between the mammalian SC proteins and the SC components of *D. melanogaster* and *C. elegans* [Fraune et al., 2012b, 2013].

With SYCP2, a second major protein constituent of the LEs was identified in mammals, besides SYCP3 [Offenberg et al., 1998]. SYCP2 is the largest SC protein yet described and in the mouse consists of 1,500 amino acids. It appears to be involved in LE assembly as well as linking LEs and TFs [Offenberg et al., 1998; Yang et al., 2006; Winkel et al., 2009]. In contrast to the other mammalian SC proteins, the origin of mammalian SYCP2 has not been considered in previous analyses. Here, we investigated this aspect taking

advantage of the vastly increasing number of genomic data referring to numerous different clades and species. Complementing bioinformatic results, we also performed expression analysis at the RNA and protein level.

Material and Methods

Sequence Analysis

Sequence analysis and assembly of the dataset were performed as described previously [Fraune et al., 2013]. Homologs of SYCP2 were retrieved from public databases at NCBI (<http://blast.ncbi.nlm.nih.gov>) and Ensembl (<http://www.ensembl.org/index.html>) with BLASTp and tBLASTn [Altschul et al., 1997] using the mouse SYCP2 (RefSeq: NP_796165.2) as query. Newly detected homologs were used as seeds for additional BLAST search attempts to detect more distantly related sequences as well. The identified sequences were used to build a specific HMM profile employing the HMMER 3.0 webserver (<http://hmmer.janelia.org>). The resulting profile was used to query the *non-redundant* database (hmmsearch option). The survey of public sequence databases allowed us to retrieve SYCP2 homologs. The corresponding protein sequences were aligned using MAFFT version 7 [Katoh and Standley, 2013] with the *linsi* option.

Sequence information of the *Hydra vulgaris* AEP transcriptome was obtained from the public database on the Compagen server [Hemmrich et al., 2012]. Annotated sequence alignments were designed using CHROMA version 1.0 [Goodstadt and Ponting, 2001]. The identity threshold for grouping of the residues was set to 60%. Seven groups were created, depending on different features of the amino acids: identical (*), charged (:), Ser/Thr (:), aliphatic (:), aromatic (:), polar (:), and hydrophobic (:).

Animals

H. vulgaris from the strain AEP [Martin et al., 1997] were cultured at 18°C following standard procedures [Lenhoff and Brown, 1970]. Testes formation was induced by feeding the animals daily for at least 1 week, then starving them for up to 5 days [Wittlieb et al., 2006].

cDNA Synthesis

RNA isolation was carried out as described previously [Fraune et al., 2013]. Synthesis of a *HySycp2* cDNA fragment was performed by PCR using Phusion® DNA polymerase (Thermo Scientific, St. Leon-Rot, Germany) and the following primer pair: HySycp2_part_5' ATGGTTTCAATTGATGACCG and HySycp2_shortpart_3' CATAGAATCATCTTGGACATAAGTTG (60 °C annealing temperature). The amplified fragment was cloned into the pSC-B-amp/kan PCR cloning vector (Agilent Technologies, Böblingen, Germany) and sequenced by GATC (Konstanz, Germany).

In situ Hybridization

In situ hybridization was performed on whole animals as reported in earlier studies [Grens et al., 1996; Fraune et al., 2012b]. The antisense RNA probe of 297 bp used in the current study was synthesized from the cloned cDNA of *HySycp2*.

Fig. 2. Expression analysis of the cnidarian SYCP2-homologous sequences at the mRNA (**A**, **B**) and protein (**C**) levels. The *Hydra Sycp2* mRNA is specifically expressed in the testis as can be seen by the purple staining of the *Hydra* testis base in whole-mount in situ hybridization (**A**) and the specific signal in the testis lane in RT-PCR on different tissue fractions (head, mid-piece, foot, testis)

(**B**). Actin mRNA was amplified as internal control of equal cDNA concentrations within the fractions. **C** Western blot analysis confirms testis-specific expression at the protein level. A prominent protein band of the expected molecular mass (150 kDa) is detected by the anti-HySYCP2 antibody in the testis fraction. For gel loading control, actin was detected by a mouse anti-actin antibody.

Fig. 3. Immunofluorescence staining of HySYCP2 on cryosections of *Hydra* testis. **A** The anti-HySYCP2 antibody recognizes thread-like structures specifically within the spermatocytes (SC), but not within spermatogonia (SG) or spermatids (SD). These thread-like structures correspond to SCs as shown at higher magnification (inset). **B** The same cryosection is shown after DNA labeling with Hoechst 33258. Bars = 50 μ m.

ondarily in the mammalian SC. To address this question, we investigated the function of the SYCP2 homolog found in an early diverging metazoan lineage, the cnidarian species *H. vulgaris* (GenBank Acc. No. XP_004210390).

Expression Analysis of *Hydra* SYCP2

To answer the question on the identity and possible function of the putative *Hydra* SYCP2 homolog, we per-

formed a detailed expression analysis of the identified sequence in *H. vulgaris* AEP at the mRNA and protein level. Using the detected *H. vulgaris* protein sequence as seed, we searched for *H. vulgaris* AEP transcriptome data [Hemrich and Bosch, 2008; Hemrich et al., 2012] coding for SYCP2-related sequences. We identified a related contig (HAEP_T-CDS_v02_43970) coding for a 99-amino-acid-long peptide, which aligns to the *H. vulgaris* sequence with a sequence identity of 96%. Using sequence-specific primers, we amplified a 297-bp-long cDNA sequence corresponding to part of the coding sequence of the putative *H. vulgaris* AEP SYCP2 gene (for primer sequence, see Material and Methods). This cDNA fragment was used as a template to synthesize a specific antisense RNA probe for whole-mount in situ hybridization. The analysis yielded a strong signal in the basal layer of the testes, which mainly harbors meiotic cell types (fig. 2A) [Kuznetsov et al., 2001; Fraune et al., 2014]. No additional signals were detected in the *Hydra* body. A comparison of the obtained staining pattern with those of previous in situ hybridizations against mRNAs encoding already characterized *Hydra* SC proteins demonstrated a remarkable similarity [Fraune et al., 2012b, 2013]. The testis-specific expression was confirmed by RT-PCR. For this experiment, 4 different tissue fractions (i.e. head, mid-piece, foot, and testis) were prepared [Fraune et al., 2012b]. Under these experimental conditions, a signal

Fig. 4. Immunofluorescence staining of HySYCP2 on chromosome spread preparations of *Hydra* spermatocytes. **A** Immunostaining using anti-HySYCP2 antibodies reveals localization of HySYCP2 within the SCs of *Hydra* spermatocytes. **B, C** Co-staining with antibodies to TF protein HySYCP1 in pachytene spermatocytes (**B**) and HySYCP3 of the LEs in diplotene spermatocytes (**C**) confirmed the localization of HySYCP2 within the LEs of the SC. At higher magnification, HySYCP2 can be detected along the separating LEs during SC disassembly in diplotene (**C**). Bars = 10 μm.

was exclusively detected in the testis fraction, revealing a PCR product of ~300 bp (fig. 2B). The size of the band was as expected since the same primer pair as for cDNA cloning (297 bp amplified) was used.

Next, the expression of the putative *Hydra* SYCP2 homolog was characterized at the protein level. To this end, we raised antibodies against the corresponding protein

fragment of *Hydra* (99 amino acids encoded by the amplified cDNA described above). The Western blot analysis yielded a prominent signal, which exclusively appeared in the testis lane. *Hydra* head, mid-piece and foot protein fractions were negative. The testicular protein recognized by the antibody has a calculated mass of 150 kDa (fig. 2C). The size of this protein band is in good

agreement with that of mouse SYCP2, which comprises 1,500 amino acids.

Finally, the antibodies were used to localize the protein by immunofluorescence microscopy. On cryosections from *Hydra* testes, the antibody recognized thread-like nuclear structures in spermatocytes that correspond to SCs (fig. 3, insets). The signal was specific for spermatocytes as no signal was observed within any other cell type. As in previous studies, double-label immunofluorescence microscopy on chromosome spreads at different meiotic stages was used to demonstrate SYCP2 localization (fig. 4) [Fraune et al., 2012b, 2013]. As already described in other species, HySYCP2 colocalizes with the LE protein HySYCP3 but not with the central region (CR)-protein HySYCP1. This is particularly evident during diplonema (fig. 4B, C) when LEs are still present but CR disassembly has already started. As expected for a LE-specific protein, HySYCP2 antibodies label LEs even at the sites lacking a CR (fig. 4C, insets).

Concluding Remarks

The protein investigated here is a component of SC lateral elements of the basal metazoan *Hydra*. We identified it as a homolog of mouse SYCP2, which is why we named it HySYCP2. Therefore, we conclude that protein SYCP2 belongs to the group of ancient SC components, which together with SYCP1, SYCP3, SYCE2, and TEX12 [Fraune et al., 2013] were already present in the last common ancestor of Metazoa over 500 million years ago and gave rise to modern SCs in present-day animals.

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References

- Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, et al: Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25: 3389–3402 (1997).
- Costa Y, Speed R, Öllinger R, Alsheimer M, Semple CA, et al: Two novel proteins recruited by synaptonemal complex protein 1 (SYCP1) are at the centre of meiosis. *J Cell Sci* 118:2755–2762 (2005).
- de Boer E, Lhuissier FG, Heyting C: Cytological analysis of interference in mouse meiosis. *Methods Mol Biol* 558:355–382 (2009).
- Fraune J, Schramm S, Alsheimer M, Benavente R: The mammalian synaptonemal complex: protein components, assembly and role in meiotic recombination. *Exp Cell Res* 318: 1340–1346 (2012a).
- Fraune J, Alsheimer M, Volff JN, Busch K, Fraune S, et al: *Hydra* meiosis reveals unexpected conservation of structural synaptonemal complex proteins across metazoans. *Proc Natl Acad Sci USA* 109:16588–16593 (2012b).
- Fraune J, Brochier-Armanet C, Alsheimer M, Benavente R: Phylogenies of central element proteins reveal the dynamic evolutionary history of the mammalian synaptonemal complex: ancient and recent components. *Genetics* 195:781–793 (2013).
- Fraune J, Wiesner M, Benavente R: The synaptonemal complex of the basal metazoan *Hydra*: more similarities to vertebrate than invertebrate meiosis model organisms. *J Genet Genomics* 41:107–115 (2014).
- Gillies CB: Synaptonemal complex and chromosome structure. *Annu Rev Genet* 9:91–109 (1975).
- Goodstadt L, Ponting CP: CHROMA: consensus-based colouring of multiple alignments for publication. *Bioinformatics* 17:845–846 (2001).
- Grens A, Gee L, Fisher DA, Bode HR: *CnNK-2*, an NK-2 homeobox gene, has a role in patterning the basal end of the axis in *Hydra*. *Dev Biol* 180:473–488 (1996).
- Hamer G, Gell K, Kouznetsova A, Novak I, Benavente R, Höög C: Characterization of a novel meiosis-specific protein within the central element of the synaptonemal complex. *J Cell Sci* 119:4025–4032 (2006).
- Hemmrich G, Bosch TC: Compagen, a comparative genomics platform for early branching metazoan animals, reveals early origins of genes regulating stem-cell differentiation. *Bioessays* 30:1010–1018 (2008).
- Hemmrich G, Khalturin K, Boehm AM, Puchert M, Anton-Erxleben F, et al: Molecular signatures of the three stem cell lineages in *Hydra* and the emergence of stem cell function at the base of multicellularity. *Mol Biol Evol* 29:3267–3280 (2012).
- Katoh K, Standley DM: MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 30:772–780 (2013).
- Kuznetsov S, Lyanguzova M, Bosch TC: Role of epithelial cells and programmed cell death in *Hydra* spermatogenesis. *Zoology* 104:25–31 (2001).
- Lammers JH, Offenberg HH, van Aalderen M, Vink AC, Dietrich AJ, Heyting C: The gene encoding a major component of the lateral elements of synaptonemal complexes of the rat is related to X-linked lymphocyte-regulated genes. *Mol Cell Biol* 14:1137–1146 (1994).
- Lenhoff HM, Brown RD: Mass culture of hydra: an improved method and its application to other aquatic invertebrates. *Lab Anim* 4: 139–154 (1970).
- Martin VJ, Littlefield CL, Archer WE, Bode HR: Embryogenesis in hydra. *Biol Bull* 192:345–363 (1997).
- Meuwissen RL, Offenberg HH, Dietrich AJ, Riesewijk A, van Iersel M, Heyting C: A coiled-coil related protein specific for synapsed regions of meiotic prophase chromosomes. *EMBO J* 11:5091–5100 (1992).
- Offenberg HH, Schalk JA, Meuwissen RL, van Aalderen M, Kester HA, et al: SCP2: a major protein component of the axial elements of synaptonemal complexes of the rat. *Nucleic Acids Res* 26:2572–2579 (1998).
- Page SL, Hawley RS: Chromosome choreography: the meiotic ballet. *Science* 301:785–789 (2003).

- Page SL, Hawley RS: The genetics and molecular biology of the synaptonemal complex. *Annu Rev Cell Dev Biol* 20:525–558 (2004).
- Schramm S, Fraune J, Naumann R, Hernandez-Hernandez A, Höög C, et al: A novel mouse synaptonemal complex protein is essential for loading of central element proteins, recombination, and fertility. *PLoS Genet* 7:e1002088 (2011).
- von Wettstein D, Rasmussen SW, Holm PB: The synaptonemal complex in genetic segregation. *Annu Rev Genet* 18:331–411 (1984).
- Winkel K, Alsheimer M, Öllinger R, Benavente R: Protein SYCP2 provides a link between transverse filaments and lateral elements of mammalian synaptonemal complexes. *Chromosoma* 118:259–267 (2009).
- Wittlieb J, Khalturin K, Lohmann JU, Anton-Erxleben F, Bosch TC: Transgenic *Hydra* allow in vivo tracking of individual stem cells during morphogenesis. *Proc Natl Acad Sci USA* 103:6208–6211 (2006).
- Yang F, De La Fuente R, Leu NA, Baumann C, McLaughlin KJ, Wang PJ: Mouse SYCP2 is required for synaptonemal complex assembly and chromosomal synapsis during male meiosis. *J Cell Biol* 173:497–507 (2006).
- Zickler D, Kleckner N: Meiotic chromosomes: integrating structure and function. *Annu Rev Genet* 33:603–754 (1999).