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SYCP3-like X-linked 2 is expressed in meiotic germ cells and interacts with synaptonemal complex central element protein 2 and histone acetyltransferase TIP60

Yu-Qiang Shi [a,1], Xin-Jie Zhuang [a,b,1], Bo Xu [c,d], Juan Hua [c,d], Shang-Ying Liao [a], Qinghua Shi [c,d], Howard J. Cooke [e,*], Chunsheng Han [a,**]

[a] State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, PR China
[b] Center for Reproductive Medicine, Department of Obstetrics Gynecology, Peking University Third Hospital, Beijing 100191, PR China
[c] Hefei National Laboratory for Physical Science at Microscale, University of Science and Technology of China, Hefei 230026, PR China
[d] School of Life Science, University of Science and Technology of China, Hefei 230026, PR China
[e] Institute of Genetic and Molecular Medicine MRC Human Genetics Unit, Western General Hospital, Edinburgh EH4 2XU, Scotland, UK

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ABSTRACT

Meiosis is the process by which diploid germ cells produce haploid gametes. A key event is the formation of the synaptonemal complex. In the pachytene stage, the unpaired regions of X and Y chromosomes form a specialized structure, the XY body, within which gene expression is mostly silenced. In the present study, we showed that SYCP3-like X-linked 2 (SLX2, 1700013H16Rik), a novel member of XLR (X-linked Lymphocyte-Regulated) family, was specifically expressed in meiotic germ cells. In the spermatocyte SLX2 was distributed in the nucleus of germ cells at the preleptotene, leptotene and zygotene stages and is then restricted to the XY body at the pachytene stage. This localization change is coincident with that of phosphorylated histone H2AX (γH2AX), a well-known component of the sex body. Through yeast two-hybrid screening and coimmunoprecipitation assays, we demonstrated that SLX2 interacts with synaptonemal complex central element protein 2 (SYCE2), an important component of synaptonemal complex, and histone acetyltransferase TIP60, which has been implicated in remodeling phospho-H2AX-containing nucleosomes at sites of DNA damage. These results suggest that SLX2 might be involved in DNA recombination, synaptonemal complex formation as well as sex body maintenance during meiosis.

1. Introduction

Gametes are generated from diploid germ cells through meiosis, which is a unique cell division process encompassing a series of highly orchestrated molecular events. During meiosis, homologous chromosomes align, pair, synapse, recombine, and separate resulting in haploid cells with distinct sets of genetic materials. The accomplishment of these events depends on the complex interactions of DNAs and proteins.

One prominent feature of the initiation of meiosis is the formation of genome-wide DNA doublestrand breaks (DSBs) which are a prerequisite of homologous recombination. Most DSBs are repaired when synapsis is established and are resolved into 1–2 crossovers between each pair of homologous chromosomes. DSBs are specifically marked by the phosphorylated histone variant H2AX (γH2AX), which is distributed globally at the leptotene and zygotene stages but concentrated in the asynapsed XY body at the pachytene and diplotene stages.

The hallmark of successful synapsis is the formation of the synaptonemal complex (SC), a giant protein structure containing multiple protein subunits. Knockouts (KO) of genes encoding SC components lead to infertility or subfertility due to germ cell death or aneuploidy (Costa and Cooke, 2007). For example, KO of SYCE2 (synaptonemal complex central element 2) results in infertility in both males and females due to the failure in synapsis and the consequent germ cell lost (Bolcun-Filas et al., 2007). The phenotypes of the KO mice of SYCP3 (synaptonemal complex protein 3) are sex dimorphic. While the male germ cells die around the zygotene stage due to failed synapsis, the female ones could finish meiosis with an increased proportion of aneuploid gametes resulting in prenatal death of the abnormal embryos (Yuan et al., 2000).

SYCP3 is a member of the Xlr (X-linked lymphocyte regulated) family, whose prototype member was initially identified through mapping lymphocyte specific cDNAs to the genome (Cohen et al., 1985a). This family was linked to the X-linked immunodeficiency of the mouse, and was believed to be important in lymphocyte differentiation (Cohen et al.,

Abbreviations: SYCP3, synaptonemal complex protein 3; SLX2, SYCP3-like X-linked 2; SYCE2, synaptonemal complex central element protein 2; TIP60, histone acetyltransferase Tip60; γH2AX, phosphorylated histone H2AX; Xlr, X-linked lymphocyte regulated; Xmr, Xlr-related meiosis-regulated.

* Corresponding author.
** Corresponding author. Tel./fax: +86 10 64807105.
E-mail addresses: howard.cooke@igmm.ed.ac.uk (H.J. Cooke), hancs@ioz.ac.cn (C. Han).
[1] These authors contributed equally to this work.

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1985b). Interestingly, this family is greatly expanded in the rodents during evolution but the majority of the members were regarded as pseudogenes (Garchon et al., 1989). Calenda et al. reported the identification of the second expressed member of the family specifically present in the mouse germ cells (Calenda et al., 1994). The gene was originally named Xmr for Xlr-related meiosis-regulated. A cDNA was cloned by RT-PCR and the protein was localized to the nuclei of spermatocytes and the XY body using an anti-XLR monoclonal antibody (Calenda et al., 1994). It turned out later that the transcript of Xmr encodes a protein expressed in spermatids leaving the identity of the originally described protein unknown (Reynard et al., 2007). The gene Xmr has been renamed as Slx for SYCP3-like X-linked.

In the present study, we identified a novel expressed member of the Xlr gene family, 1700013H16Rik, by examining predicted proteins of the XLR family. The gene is located on the X chromosome and the predicted protein shares the highest homology with SYCP3, and we named it as SLX2 for SYCP3-like X-linked 2. We found that SLX2 was specifically expressed in premeiotic germ cells and that the protein was localized to the nucleus and the XY body. We also identified SYCE2 and the histone acetyltransferase TIP60 as interacting partners of SLX2. These data suggested that SLX2 could be involved in DSBs repair and SC formation.

2. Materials and methods

2.1. Animals and reagents

CD-1 mice were obtained from the Experiment Animal Center, Chinese Academy of Sciences. The animals were bred and treated in accordance with the NIH Guide for the Care and Use of Laboratory Animals in Beijing. The Institute Committee on Animal Care and Use had approved all the protocols for the animal treatment. Reagents for the cell culture and the animal treatment were obtained from Invitrogen (Carlsbad, CA), Beijing Chemical Reagents (Beijing, China) and Sigma (Sigma-Aldrich St. Louis, MO) unless specified otherwise. Matchmaker library construction and screening kit and were purchased from Clontech (BD Biosciences. San Jose, CA).

2.2. Bioinformatics mining of XLR proteins

We set out to compile a list of predicted proteins of the XLR family by using the Key Word Search form of the PANTHER classification system (http://www.pantherdb.org) (Thomas et al., 2003). When "Families" was selected and "XLR" filled in, the "XLR/SCP3/FAM9" protein family was returned, which contained 48 mouse genes. We curated this list of genes by checking their mRNA and protein expression on the linked Mouse Genome Informatics (MGI) and the Vertebrate Genome Annotation (VEGA) websites and identified 8 potential pseudogenes. As a result, we identified a list of 40 Xlr genes whose existences were supported by mRNAs and whose genomic locations were unique (Table S1). After a multiple sequence alignment and similarity comparison in the form of a tree, we finally identified 30 unique potential proteins (Fig. S1).

2.3. Recombinant protein expression and polyclone antibody production

The mouse SLX2 cDNA fragment encoding the N-terminal 120 amino acids (1–120) was subcloned into pGEX-4T1 vector (Pharmacia Biosciences). The GST fusion protein was expressed in *Escherichia coli* strain BL21 and purified using a GSTrap FF column (Amersham Pharmacia) according to the manufacturer's instructions. About 500 μg of purified protein was emulsified in complete Freund's adjuvant and injected into healthy rabbits, and 3 boosting injections with 500 μg protein emulsified in incomplete Freund's adjuvant ensued with 3-week intervals in between. Antibody was purified by affinity purification using protein G column.

2.4. RT-PCR

Total RNAs from mouse various tissues and testes at different stages of development were extracted for RT-PCR analysis. The primer pairs 5′-TTTCCCCGTGGAAGAAGGGA-3′ and 5′-AAAAGAGCGTAGACTCCTCA-3′, were used to detect the SLX2 mRNA expression. Briefly, total RNAs (5 μg) were used as templates for reverse transcription using Superscript III (Invitrogen). Detailed procedure was performed according to the manufacturer's protocol. For PCR, reaction mixture was first heated at 94 °C for 2 min. 30 cycles were then carried out with the following parameters: denaturing at 94 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 45 s. Reaction was finished with a final extension at 72 °C for 5 min.

2.5. Immunohistochemistry and immunocytochemistry

Immunohistochemistry was performed at standard manipulations. Briefly, 8 μm frozen sections of mouse testis were fixed immediately in 4% paraformaldehyde for 15 min at room temperature. After blocking, the sections were incubated with affinity-purified SLX2 antibody (diluted at 1:400 in blocking buffer) or pre-immune rabbit serum used as a negative control for 1 h at room temperature. Secondary antibody was the FITC-conjugated anti-rabbit (1:500) from Jackson Laboratories (Cambridgeshire, U.K.), followed by incubation with DAPI (Sigma-Aldrich).

The spreading chromosomes of primary spermatocytes were performed as previously described using a 'drying-down' technique (Peters et al., 1997). Briefly, mice were sacrificed, and testicular seminiferous tubules were isolated and kept in a hypotonic extraction buffer (30 mM Tris, 50 mM sucrose, 17 mM trisodium citrate dihydrate, 5 mM EDTA, 0.5 mM DTT and 0.5 mM PMSF, pH 8.2) for 30–60 min. Subsequently, the testes were torn to pieces using two fine forceps in 100 mM sucrose solution. The cell suspension was mixed with 3.7% PFA solution and spread on clean glass slides. Finally, the slides were washed twice for 2 min in 0.4% Photoflo (Kodak) and dried at room temperature. The slides were stained using anti-SYCP1 (1:200), anti-SYCP3 (1:200, Abcam), anti-SYCE2 (1:200) (Costa et al., 2005), anti-γH2AX (1:200, Upstate), and anti-SLX2 (1:400). Secondary antibody was the FITC-conjugated or TRITC- conjugated anti-rabbit (1:500) from Jackson Laboratories (Cambridgeshire, U.K.), followed by incubation with DAPI.

2.6. Yeast two-hybrid screening

Yeast two-hybrid screening was performed by using the Matchmaker library construction and screening kit (Clontech, Cat. No. K1615-1) with slight modification (Shi et al., 2009). The bait plasmid was constructed by sub-cloning mouse SLX2 cDNA fragment encoding the full length peptide into pGBKT7. To construct the testis cDNA library, mRNA (1 μg) isolated from 5 adult mice was used to synthesize the first-strand cDNA using an oligo-dT primer. Double-strand cDNA was synthesized with SMART III and CDS III anchors. The AD fusion library construction and two-hybrid screen were carried out in one step by co-transforming the yeast strain AH109 with ds cDNA, pGADT7-Rec and pGBKT7. Colonies were picked out from SD/-Ade/-His/-Leu/-Trp/X-α-Gal selection plates after culture for 5 days. The inserts of selected positive clones were sequenced and identified by searching the NCBI BLAST database.

2.7. Co-immunoprecipitation

SLX2 and SYCE2 cDNA fragments were subcloned into pFlag-cmv4 and pEGFP-N1 vector to construct Flag-SLX2 and SYCE2-GFP tagged plasmids respectively. Subsequently, the two tagged plasmid DNA were co-transfected in HEK293T cells using Lipofectamine 2000 according to the manufacturer's instruction (Invitrogen). Total protein lysates of the cells were incubated with rabbit immunoglobulin (IgG) or anti-GFP antibody for 2 h at 4 °C, followed by incubation with protein A agarose (Santa