

REPORT

Trim27 interacts with Slx2, is associated with meiotic processes during spermatogenesis

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ABSTRACT

Formation of the XY body is believed to prevent recombination between X and Y chromosomes during meiosis. We recently demonstrated that SYCP3-like X-linked 2 (Slx2) could be involved in synaptonemal complex formation as well as XY body maintenance during meiosis. In order to further investigate the role and composition of XY body protein complexes in meiotic processes and spermatogenesis, a yeast 2-hybrid screening was performed, and the tripartite motif protein 27 (Trim27) was found to interact with Slx2 and co-localized in the XY body. Trim27 has a tripartite motif (TRIM) consisting of a RING finger, B-box and coiled-coil domains, and is a transcriptional regulator that is expressed in various tumor cell lines. In this study, we showed that Slx2 and Trim27 were highly expressed in meiosis of mouse testis. And the Slx2/Trim27 interaction was confirmed *in vivo* by co-immunoprecipitation and mammalian 2-hybrid interaction assays. Moreover, cytoimmuno localization experiments revealed that Slx2/Trim27 was co-localized to the XY body of spermatocytes during meiosis, and immunohistochemical results revealed co-localization of Trim27 and γ -H2AX in the XY body of primary spermatocytes in the mouse testis. Trim27 may therefore be a transcriptional regulation protein connecting Slx2 and γ -H2AX, thereby promoting the formation of a more potent XY body protein complex in meiotic processes and spermatogenesis. In conclusion, Trim27 connecting Slx2 may regulate meiotic processes in multiple ways by influencing XY body formation and germ cell proliferation during spermatogenesis.

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Introduction

Spermatogenesis is a complex biological process involving both mitotic and meiotic cell division.¹ During spermatogenesis, the X and Y chromosomes are transcriptionally silenced during the meiotic prophase and form a condensed chromatin domain known as the XY body.^{2–5} Formation of the XY body is believed to prevent recombination between X and Y chromosomes and protect them from double stranded breaks (DSBs).² However, the molecular mechanisms underlying sex chromosome condensation remain known. The recent discovery of specific proteins located within the XY body has aided our understanding of its structure and function.^{6–9} The phosphorylated form of H2AX (γ -H2AX) globally distributed during the leptotene and zygotene stages, but becomes concentrated at the asynapsed XY body during the pachytene and diplotene stages.¹⁰ Moreover, XMR, a member of the X-linked lymphocyte regulated (Xlr) family, is concentrated on a chromosome segment emerging from the XY body.¹¹ We recently demonstrated that SYCP3-like X-linked 2 (Slx2) might be involved in synaptonemal complex formation as well as XY body maintenance during meiosis.^{9,12} In spermatocytes, Slx2 is distributed in the nucleus of germ cells during the preleptotene, leptotene and zygotene stages, but is restricted to the XY body at the pachytene stage.

To date, Slx2 have been found to highly express in the testis and may be involved in homologous recombination, double strand repair and sex chromosomes inactivation during the meiosis.⁹ However, the role and composition of XY body protein complexes (include Slx2) have not been clearly in testis during the meiosis.

In order to further investigate the role and composition of XY body protein complexes in spermatogenesis and identify other potential proteins that form the XY body, a yeast 2-hybrid screen was performed, and tripartite motif protein 27 (Trim27) was found to interact with Slx2. Trim27 (also known as the Ret finger protein, RFP) is a member of the tripartite motif-containing (TRIM) family and contains a TRIM/RBCC motif that consists of a RING domain, one or 2 B-box domains, and a predicted coiled-coil region.^{13,14} TRIM proteins form a superfamily with ~65 members in humans and mice,¹⁵ and they are known to play important roles in a wide range of processes including cell growth, tumor suppression, DNA damage signaling, senescence, apoptosis, stem cell differentiation, and immune responses against viral and particularly HIV infection.^{15–21} Although Trim27 is highly expressed in various tumor types and testis,²² the exact expression pattern, cellular localization and physiological function of Trim27 in testis remain unclear.

Here we showed that Trim27 were highly expressed in the mouse testis, and differentially expressed during spermatogenesis. Moreover, the Slx2 and Trim27 proteins were localized at the nucleus of primary spermatocytes, especially at the XY body during meiosis process. Furthermore, we identified Trim27 as was found to interact with Slx2 using yeast 2-hybrid screening and co-immunoprecipitation (co-IP). In conclusion, Trim27 was co-localized with Slx2 and γ -H2AX in the XY body, suggesting Trim27 connecting Slx2 could play multiple roles in the regulation of XY body formation and germ cell proliferation during spermatogenesis.

Results

Expression of Trim27 and Slx2 mRNA and protein in mouse testes

Trim27 and Slx2 expression were investigated in testes during different developmental stages. Trim27/Slx2 mRNA and protein were detected by RT-PCR (Fig. 1A) and western blotting (Fig. 1B), respectively. And Trim27 mRNA (protein) expression pattern matched Slx2 mRNA (Protein) expression in testes. Trim27 mRNA was detected in 1 dpp testis tissue after birth (Fig. 1A), and protein expression matched them RNA expression pattern. Trim27 appeared as a 58 kDa testis protein by western blotting, but a unique 68 kDa protein was also detected (Fig. 1B), suggesting Trim27 may be present in more than one form. Trim27 protein was not abundant in the 1dpp testis, but levels were higher at 14 dpp in cells undergoing meiotic prophase during the first cycle of spermatogenesis, suggesting Trim27 may be involved in male germ cell development.

Trim27 was localized to the nucleus and cytoplasm

We performed immunostaining of Trim27 on gonadal sections to examine the distribution in different germ cells (Fig. 2).

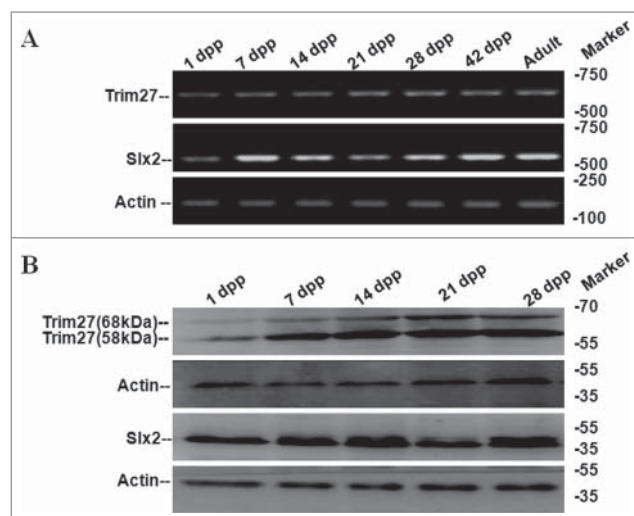


Figure 1. Expression of mouse Trim27 and Slx2 detected by RT-PCR and western blotting. (A) Trim27 and Slx2 expression were investigated in multiple developmental stages in mouse testis tissue by RT-PCR, and expression was high in various different stages. (B) Trim27 and Slx2 proteins were detected by Western blotting following immune precipitation. Two distinct bands of Trim27 were detected that correspond to 58 kDa and 68 kDa proteins.

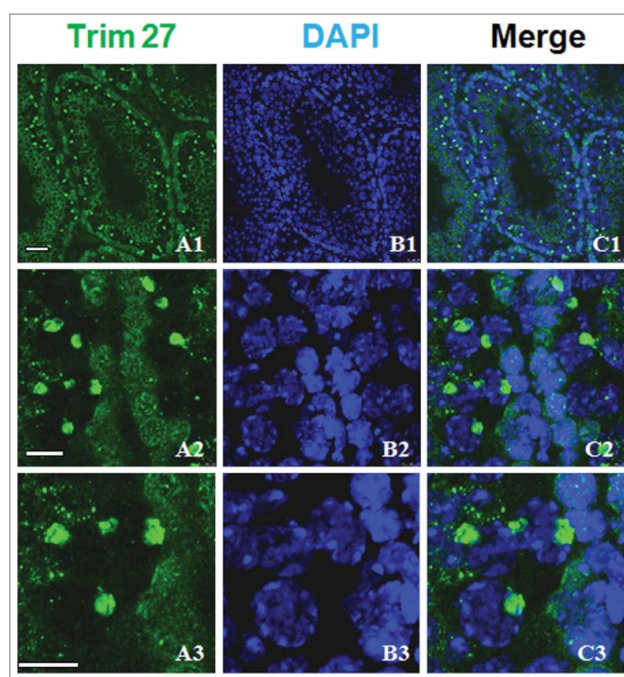


Figure 2. Trim27 expression in germ cells as detected by immunostaining of testis sections. Upper panel: Trim27 (green) and nuclei (blue) at low magnification. Lower panel: Trim27 (green) and nuclei (blue) at high magnification. Trim27 is both nuclear and cytoplasmic, and is localized in the nuclei of primary spermatocytes, but in the cytoplasm of round spermatids. Bars = 10 μ m.

Immunostaining of Trim27 in adult testis using DAPI revealed localization in the nucleus of primary spermatocytes. No specific staining was detected in spermatogenic cells when only type A or B spermatogonia were present. Trim27 was localized in the cytoplasm of round spermatids and the nuclei of Sertoli cells, and therefore appears to be a nuclear protein in spermatocytes (Fig. 2A) but a cytoplasmic protein in round spermatids, indicating a role in spermatid cell proliferation.

When meiosis reached the pachytene stage, smaller stained spots were observed in some tubules of adult mice, which were assumed to be XY bodies based on their size and morphology. Trim27 and γ -H2AX were co-localized at nuclei during the zygotene stage (Fig. 3), and since γ -H2AX is a well-known marker of the XY body, this confirmed that Trim27 was indeed associated with meiosis. We then directly examined the co-localization of Trim27 with which confirmed that Trim27 was indeed associated with meiosis. Upon further investigation of the co-localization of Trim27 and γ -H2AX in chromosome spreads of spermatocytes, co-localization at nuclei was evident at the zygotene stage, while co-localization at XY bodies was apparent in pachytene spermatocytes (Fig. 3).

Physical interaction between Trim27 and Slx2

In order to define the biochemical role of Slx2 in XY body, a yeast 2-hybrid screening was employed to identify potential binding partners, and Trim27 was identified as a candidate.

The interaction between Slx2 and Trim27 was initially observed in the yeast 2-hybrid experiment, and the interaction was further confirmed using co-IP on HEK293T cell lysates

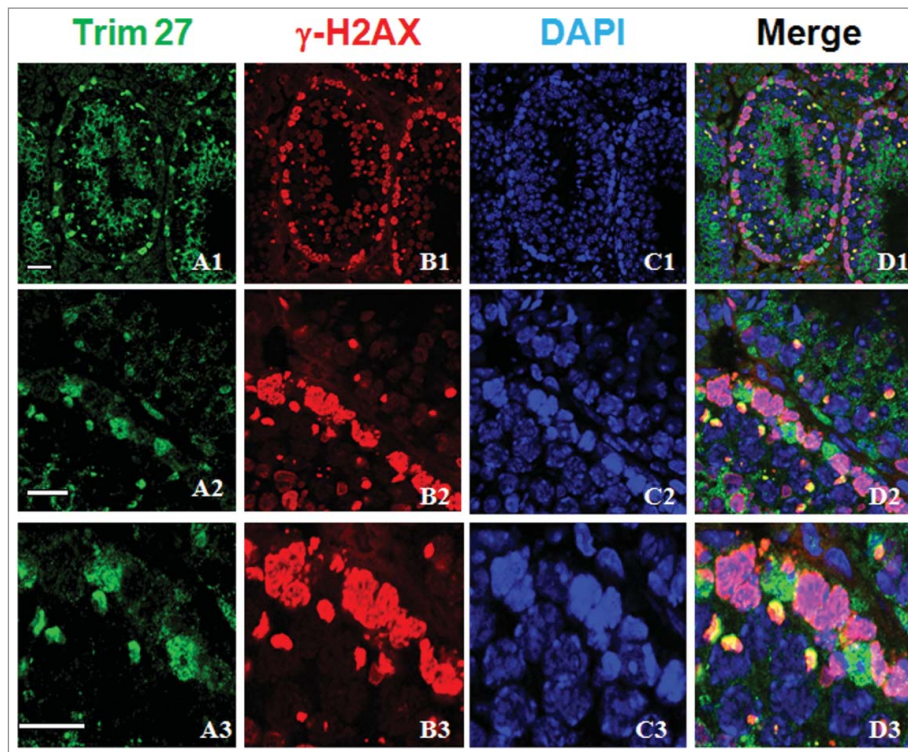


Figure 3. Co-localization of Trim27 and γ -H2AX in testis sections. Upper panel: Trim27 (green), γ -H2AX (red) and nuclei (blue) at low magnification. Lower panel: Trim27 (green), γ -H2AX (red) and nuclei (blue) at high magnification. Trim27 is co-localized with γ -H2AX in the nuclei of spermatocytes at the zygotene stage, and in the XY bodies of pachytene spermatocytes. Bars = 10 μ m.

from cells co-transfected with FLAG-Slx2 and Trim27-GFP plasmids (Fig. 4A and B). Moreover, Trim27-GFP could be pulled down by the GST-Slx2 fusion protein, and even more importantly, the *in vivo* interaction between Trim27 and Slx2

was confirmed using co-IP on the adult testicular lysates (Fig. 4C).

The Slx2-Trim27 complex was localized at XY bodies

We next examined whether Slx2 and Trim27 co-localized in meiotic spermatocytes. Immunofluorescence staining of the chromosome spreads of spermatocytes using a confocal laser scanning microscope indicated slight localization of Trim27 at bright satellite sequences (Fig. 5). Slx2 and Trim27 were found to co-localize at XY bodies in primary meiotic spermatocytes, but were separated in round spermatids, where Slx2 was restricted to XY bodies while Trim27 was diffuse in the cytoplasm of differentiating sperm.

To confirm that Trim27 was indeed localized at XY bodies of SC, chromosome spreads of cells in the leptotene to diplotene stages were immunostained for the Trim27/Scp3 and Slx2/Scp3. In preleptotene spermatocytes, Scp3 was distributed evenly across the entire nucleus, and the expression and distribution of Trim27 was similar to that of Scp3. In zygotene spermatocytes, Scp3 was beginning to be visible whereas Trim27 was diffuse but unevenly distributed. In the pachytene stage, Slx2 and Trim27 were both restricted to XY bodies while Scp3 was localized specifically at the SC. During the diplotene stage, Slx2 and Trim27 remained localized at XY bodies whereas Scp3 at the SC had begun to disassemble. In summary, Slx2 and Trim27 shared a similar distribution pattern at the zygotene and diplotene stages of meiosis, and the interaction between Slx2 and Trim27 appeared to occur in XY bodies. The results suggested Trim27 connecting

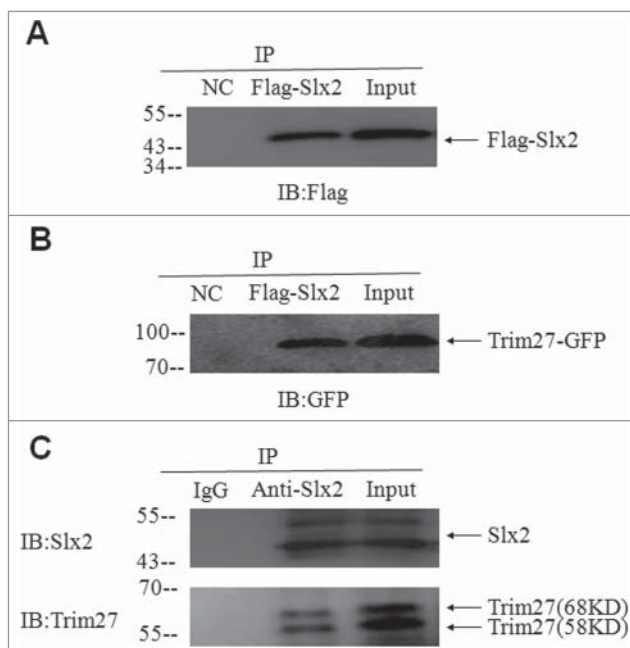


Figure 4. Interaction between Trim27 and Slx2, identified using yeast 2-hybrid analysis and confirmed using co-immunoprecipitation (CoIP). (A and B). CoIP using lysates from HEK293T cells co-transfected with Trim27-GFP and FLAG-Slx2. (C). The *in vivo* interaction between Trim27 and Slx2 was confirmed by Co-IP experiments on testicular lysates.

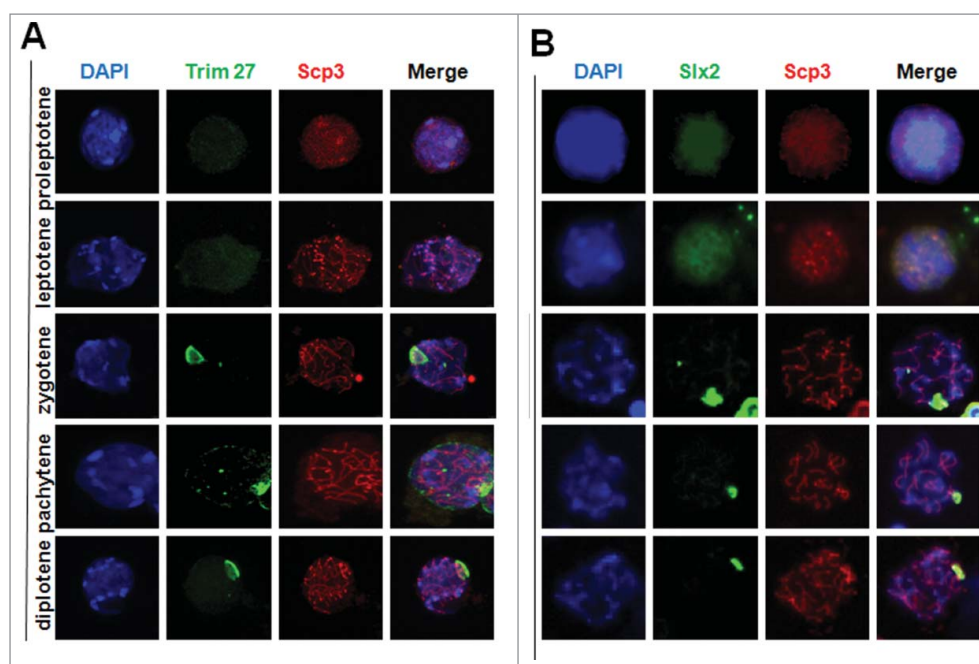


Figure 5. Immunostaining of testes chromosome spreads at the leptotene and diplotene stages for Trim27/SYCP3 and Slx2/Scp3. At the pachytene and diplotene stages, Trim27 and Slx2 are specifically restricted to XY bodies, and both proteins share a similar distribution pattern at the zygotene and diplotene stages. Interaction between Trim27 and Slx2 appears to occur in XY bodies, suggesting Trim27 is a linker protein that mediates the interaction between Slx2 and XY body proteins, and may therefore control XY body formation during meiosis. Images were captured using a laser confocal microscope. Bar = 5 μm .

Slx2 could play important roles in the XY body formation during meiosis.

To examine the interaction and localization between Trim27 and Slx2 *in vivo*, Slx2-GFP and Trim27-RFP fusion proteins were expressed in HEK293T cells (Fig. 6). Trim27-RFP was mainly localized as speckles in the nuclei, and Slx2-GFP was also displayed a nuclear localization but speckles were not

evident. Interaction between Trim27 and Slx2 may be essential for XY body formation in the nuclei of primary spermatocytes.

Discussion

Formation of the SC during the meiotic stage of spermatogenesis is a key event. During prophase of the meiotic process, X

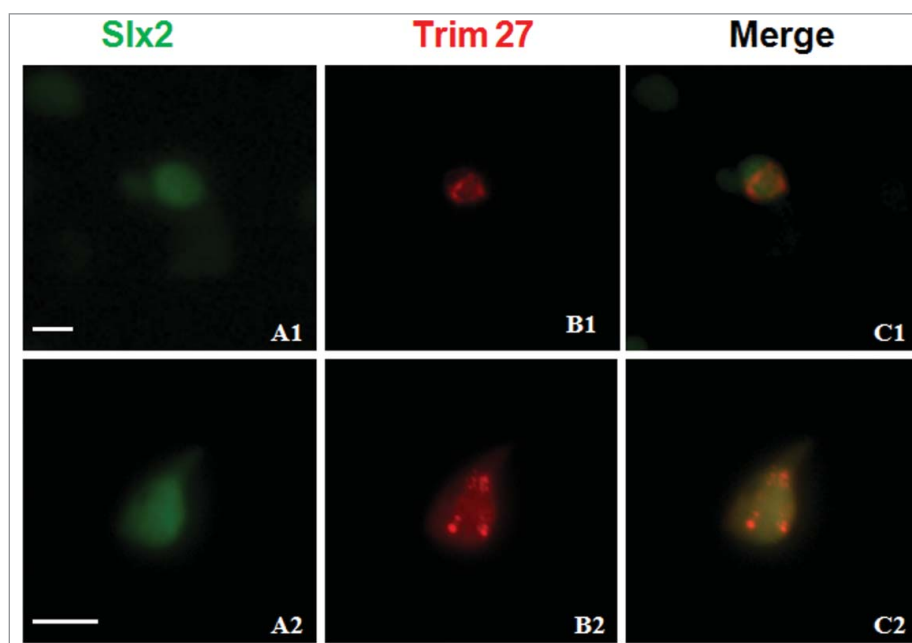


Figure 6. Subcellular localization of Trim27 and its influence on the distribution of Slx2 in HEK293T cells. Slx2-GFP is co-localized with Trim27-RFP in the nuclei of cells co-transfected with Slx2-GFP and Trim27-RFP fusion plasmids. The nuclei of cultured HEK293T cells are stained with DAPI, and images were captured using a laser confocal microscope. Upper panel: Trim27 (red), Slx2 (green) and nuclei (blue) at low magnification. Lower panel: Trim27 (red), Slx2 (green) and nuclei (blue) at high magnification. Bars = 10 μm .

and Y chromatin is remodelled to form a specialized, morphologically distinct subnuclear domain known as the XY body,⁷ the formation of which may protect the X and Y chromosomes from damaging recombination events that could occur because they are not completely homologous.^{2,7} Previously, we identified the novel XY body component protein Slx2, which is expressed in meiotic germ cells and is localized at XY bodies.^{9,12} In the present study, we found that Trim27 was highly expressed in mouse testis and localized at the same cellular substructures. The full-length Trim27 cDNA is predicted to encode a protein of 513 amino acids (Fig. 7A), and mouse Trim27 shares 98.4% homology with the human sequence. Trim27 was first identified by DNA rearrangement²³ and mapped to mouse chromosome 13. Trim27 is a member of the TRIM family of proteins, and consists of a RING domain, a B-box domain, 3 coiled-coil regions and one SPLA kinase andryanodine receptor (SPRY) domain (Fig. 7B). The RING domain plays a critical role in mediating the transfer of ubiquitin, and is a characteristic signature of many E3 ubiquitin ligases. The B-box domain includes a zinc-binding motif and is a critical determinant of the tripartite motif. The coiled coil region in TRIM/RBCC proteins is mainly involved in homo-oligomerization, promoting the formation of high molecular weight complexes, and defining discrete subcellular compartments within the cell.²⁴ E3 activity is dependent on the TRIM motif. The SRRY domain of the C-terminal region (Fig. 7B) has no known function in any of the TRIM/RBCC proteins characterized to date.²⁵ Interestingly, a major band (~68 KDa) and the expression and precise underlying mechanisms of Slx2 should be clearly research in testis. a minor one (~58 KDa) were observed on the western blots of the mouse testis (Fig. 1B). To confirm that the proteins detected by the antibody were indeed isoforms of Trim27, we

concentrated the proteins from testicular lysate by immunoprecipitation (IP) using the same polyclonal antibody as used in protein gel blotting, collected the proteins from the Coomassie Blue-stained gels, and subjected the samples to mass spectrometry analysis. The results should that both isoforms contained Trim27 peptides (data not shown). The presence of the 68kd isoform was not affected by kinase treatment or preparation of extracts in the absence of protease inhibitors suggesting that modification is not phosphorylation, ubiquitination or sumoylation.

A more detailed surface spread analysis showed that Trim27 was localized at XY bodies. Interestingly, Sumo-1 is also localized at XY bodies in pachytene spermatocytes.^{7,8} Recent results show that Trim27, Sumo-1 and PIAS all localize at a characteristic nuclear structure that is juxtaposed with the inner nuclear membrane (XY body) of primary spermatocytes in mouse testis.²⁶ Moreover, Trim27 was suggested to have an important role in the enhancement of transcriptional repression in spermatogenesis, and MBD proteins appear to be involved.²⁷ In addition, Trim27 interacts with enhancer of polycomb1 (EPC1) and functions as a transcriptional repressor in cultured cells.²⁸ The distribution of Trim27 in meiotic spermatocytes implies that it is a mediator that connects Slx2, Sumo-1 and MBD proteins to facilitate formation of the XY body during meiosis (Fig. 8). We hypothesize that Trim27 connecting Slx2 acts as an XY body component crucial for XY body formation and transcriptional regulation during meiosis.

Meiotic sex chromosome inactivation (MSCI) occurs during the meiotic phase of spermatogenesis, and involves transcriptional silencing of the X and Y chromosomes. MSCI has been shown to contribute to formation of the XY body.²⁹ It is initiated by several DNA repair proteins, and is maintained through



Figure 7. Amino acid sequence and structural domains of Trim27. (A) The full-length Trim27 cDNA is predicted to encode a protein of 513 amino acids; (B) Trim27 is a member of the TRIM family, and consists of a RING domain, a B-box domain, 3 coiled-coil regions (C1 to C3) and an SPRY domain.

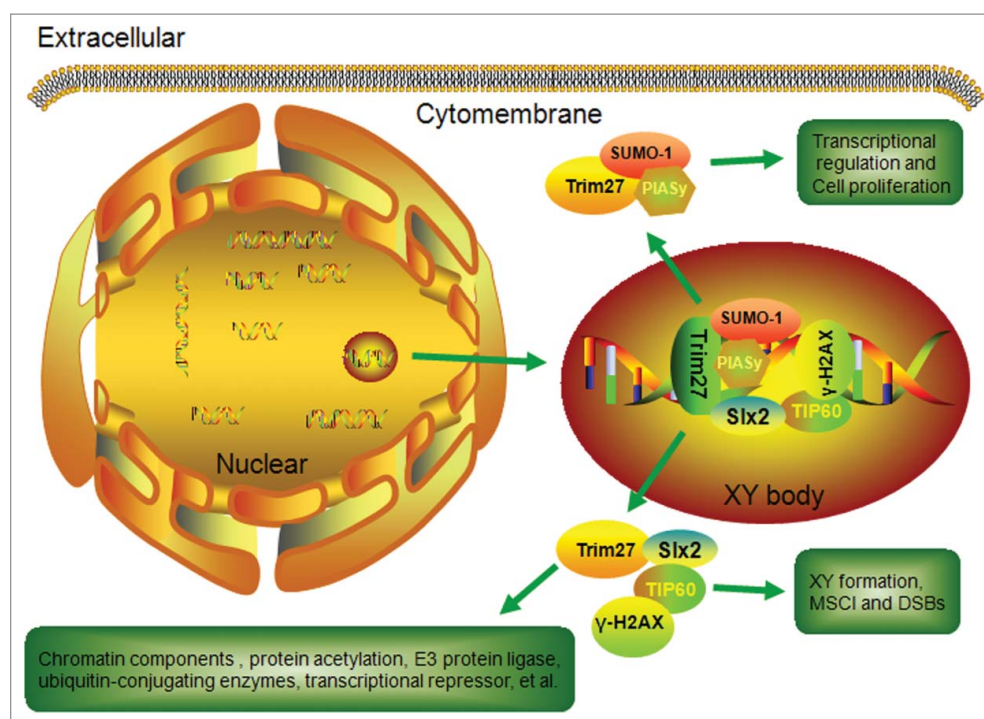


Figure 8. Summary of the functions of Trim27 in XY body formation in primary spermatocytes.

histone modifications or other modifications during the meiotic stage. At the leptotene and zygotene substages, the X and Y chromosomes are still transcriptionally active. However, they are rapidly silenced shortly after the zygotene-to-pachytene transition, which is when XY bodies are formed. Until recently, the transcriptional status of the X and Y chromosomes during spermatogenesis was poorly understood, because few published studies have focused on the activity of specific X- or Y-linked genes. H2AX performs an essential role in MSCI, and γ -H2AX is abundant in the mouse testis and is localized at XY bodies.³⁰ γ -H2AX plays an important role in the response to DNA damage such as DSBs during the leptotene stage.³¹ γ -H2AX is phosphorylated at the site of DSBs, which in turn recruits DNA repair factors that participate in DSB repair following homologous recombination.³² In the present study, we found Trim27 and γ -H2AX to be co-localized at XY bodies during the meiotic stage. The involvement of γ -H2AX in XY body formation has been associated with sex inactivation,³³ and Trim27 may mediate γ -H2AX in this process. Previously, we suggested that the interaction between Slx2 and TIP60 could be involved in DNA recombination, SC formation and XY body maintenance during meiosis.⁹ In addition to interacting with Slx2, Trim27 is known to interact with Structural Maintenance of Chromosome 3 (SMC3), a component of the multimeric cohesion complex that holds sister chromatids together and prevents their premature separation during mitosis.³⁴ It is possible that Trim27 may recruit γ -H2AX and Slx2 and/or SMC3 during DSB repair and MSCI during meiosis (Fig. 8). Given the spatial distribution of Trim27 in spermatogenesis, we hypothesize that Trim27 connecting Slx2 acts as a novel XY body component associated with MSCI during meiosis in spermatocytes.

The prototype member of the X-linked lymphocyte regulated (XLR) protein super family has been linked to X-linked

immunodeficiency in mice. XLR proteins contain a COR1 domain, and various family members including Slx1,¹ Slx2,⁹ Scp3³⁵ and Xlr5c³⁶ play an important role in meiosis during spermatogenesis. Scp3 is an important member of this family that acts as a fundamental component of the lateral elements of the synaptonemal complex during meiosis.³⁷ Along with others, Slx2 expression and interaction with Syce2 and KAT/TIP60 could contribute to meiosis and sex chromosome inactivation.⁹ And a paper showed that Slx2 KO mice are fertile and have normal meiosis progress by the restricted selection of phenotypic traits.³⁸ So the expression and precise underlying mechanisms of Slx2 were not clearly research in testis. In the present study, closer inspection showed that Trim27 was localized in the cytoplasm of round spermatids, indicating a role in germ cell proliferation. However, Trim27 was localized at XY bodies during different stages of the meiotic cycle, and interacted with Slx2, suggesting an involvement in meiotic progression (Fig. 8). In addition, other evidence indicates that Trim27 may be associated with other subcellular processes and chromatin components,³³ ubiquitin-conjugating enzymes^{39,40} the E3 sumo protein ligase PIAS²⁶ and protein acetylation,⁴¹ and may also inhibit activation of gene transcription.^{42,43} Our findings suggest that Trim27 and Slx2 may be cooperatively involved in normal spermatogenesis. It was recently reported that PIAS acts as a Sumo E3 ligases for Trim27, and the association of PIAS and Trim27 may be important for transcriptional repression in spermatogenesis.²⁶ Our results suggest that Trim27 acts as a transcriptional regulation protein that, along with Slx2, functions in the transcriptional regulation of XY body formation and germ cell proliferation during spermatogenesis.

In summary, we revealed an interaction between Trim27 and Slx2, and the localization of this complex at XY bodies during meiosis in spermatogenesis. The abundant expression of

Trim27 and Slx2 in germ cells and localization at XY bodies suggests Trim27/Slx2 interaction may perform multiple roles in the regulation of spermatogenesis including XY body formation, MSCI and germ cell proliferation.

Materials and methods

Ethics statement

This work was approved by the Institutional Animal Care and Use Committee (IACUC) of the Peking University Third Hospital (Protocol Number: 2013SZ021). All animal experiments conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals, issued by the Peking University Third Hospital in Beijing.

Animals and reagents

All animals were obtained from the Experimental Animal Center, Peking University Health Science Center. The Institute Committee on Animal Care and Use approved all protocols for animal treatment.

Reagents for cell culturing and animal treatment were obtained from Sigma (St. Louis, MO, USA), Invitrogen (Carlsbad, CA, USA) and Beijing Chemical Reagents (Beijing, China) unless specified otherwise. Protein Agarose and anti-GFP purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mouse polyclonal anti-SYCP3 antibody was obtained from Novus Biologicals (Littleton, CO, USA). Mouse monoclonal γ -H2AX and anti-Trim27 antibodies were obtained from Abcam (Cambridge, UK).

Preparation of RNA and RT-PCR

Total RNA in testes at different stages of development was extracted for RT-PCR analysis using Trizol reagent (Invitrogen, 15596018, USA) as described in our previous study.⁴⁴ Total RNA (5 μ g) was used as template for reverse transcription using Superscript III (Invitrogen) according to the manufacturer's protocol. The Matchmaker library construction and screening kit was purchased from Clontech (BD Biosciences, San Jose, CA, USA). Trim27 primer pairs (5'-ACT CTCGAGATGACCCAGGACTGTGCAGAG-3' and 5'-ACAGGTACC TCAGATTAC AGGCAACAGCACTT-3') were used to quantify Trim27 mRNA expression, and the resulting PCR products were subcloned into the pGEX-4T1 vector and sequenced to ensure there were no mutations due to PCR errors. The products of RT-PCR and PCR were analyzed by electrophoresis and stained with ethidium bromide. GAPDH was included as an internal positive control of the amplification and integrity of extracts.

Western blotting

Western blotting was performed using a standard protocol. Briefly, equal amounts of testis protein (20~50 μ g total protein/lane) were separated by 10% SDS-PAGE, transferred to nitrocellulose membranes (Amersham Biosciences AB, Uppsala, Sweden), blocked in TBST (0.5% Tween-20 in TBS)

containing 5% non-fat milk powder for 1 h, incubated with primary antibody followed by incubation with HRP-conjugated secondary antibody. Proteins were visualized using an Enhanced Chemiluminescence Kit (Pierce, Rockford, IL, USA), and protein expression levels were determined using Image-Pro plus software 5.1.

Yeast two-hybrid screening

Yeast two-hybrid screening was performed using the Matchmaker library construction and screening kit with slight modifications.¹² The bait plasmid was constructed by subcloning the full-length mouse Trim27 cDNA into pGBKT7. Fusion library construction and 2-hybrid assays were carried out in one step by co-transforming the yeast strain AH109 with dscDNA, pGADT7-Rec and pGBKT7. After selection on SD-Ade/-His/-Leu/-Trp plates, cDNA encoding the interacting polypeptides was isolated from positive colonies and sequenced. Inserts from selected positive clones were sequenced and searched against the NCBI database using BLAST.

Antibodies and immunoblotting

Immunostaining of frozen mouse testicular tissue was performed as previously described.³⁶ Frozen sections (6~8 μ m) of mouse testis were fixed immediately in 4% paraformaldehyde for 15 min at room temperature. After washing, sections were blocked with 1% BSA in PBS at room temperature for 1 h. After blocking, sections were incubated with Trim27 antibody (diluted 1:300 in blocking buffer) or pre-immune rabbit serum (negative control) for 1 h at room temperature. Incubation with the FITC-conjugated anti-rabbit antibody (1:500) (Jackson Laboratories, Cambridgeshire, UK) was then followed by incubation with DAPI (Sigma-Aldrich).

Chromosome spreading of primary spermatocytes was performed as previously described.⁹ Slides were stained using anti-Scp3 (1:200, Novus Biologicals), anti- γ H2AX (1:200, Abcam), and anti-Trim27 (1:300 Abcam). TRITC-conjugated or FITC-conjugated anti-rabbit antibodies (1:500) (Jackson Laboratories, Cambridgeshire, UK) were then applied, followed by incubation with DAPI.

Co-immunoprecipitation and confocal microscopy

Trim27 and Slx2 gene fragments were subcloned into pFlag-cmv4 and pEGFP-N1 vectors to construct the Flag-Slx2 and Trim27-GFP tagged plasmids, which were subsequently co-transfected into HEK293T cells. Total protein cell lysates were incubated with rabbit immunoglobulin (IgG) or anti-GFP antibody for 2 h at 4°C, followed by incubation with protein A agarose (Santa Cruz) overnight at 4°C. Agarose beads and captured protein complexes were washed 6 times and suspended in SDS sample buffer for immunoblotting with anti-Flag (1:2000 dilution, Sigma-Aldrich), anti-GFP (1:1000 dilution, Santa Cruz) or anti-Slx2 (1:3000 dilution).

GST pull-down assays

A full-length Slx2 cDNA fragment was subcloned into the pGEX-4T1 vector to construct the GST-Slx2 tagged plasmid. GST and GST-Slx2 were produced in *Escherichia coli* BL21 and purified using a GStap FF column (Amersham Biosciences). For the GST pull-down assay, GST and GST-Slx2 fusion proteins were immobilized on glutathione sepharose beads by incubating for 1 hour at 4°C, and beads were subsequently washed 4 times with TEN100 buffer (20 mM Tris-HCl, 1 mM EDTA, 100 mM NaCl, pH 7.4). Trim27-GFP fusion protein was over-expressed in HEK293T cells, and total protein cell lysates were extracted with lysis buffer (150 mM NaCl, 50 mM Tris-HCl pH 8.0, 0.5% Nonidet-P40) containing protease inhibitor cocktail (Roche) and incubated with beads for 2 h at 4°C. Beads were washed 6 times with lysis buffer then suspended in SDS sample buffer. Protein lysates were separated by SDS-PAGE and immunoblotted with anti-Slx2 or GFP antibody.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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