https://doi.org/10.1186/s12958-024-01282-y

Reproductive Biology and Endocrinology

REVIEW Open Access

The role of ubiquitin-conjugating enzyme in the process of spermatogenesis

Peng Lv[1,2], Jihong Liu[1,2*] and Xiaming Liu[1,2*]

Abstract

The ubiquitination is crucial for controlling cellular homeostasis and protein modification, in which ubiquitin-conjugating enzyme (E2) acts as the central player in the ubiquitination system. Ubiquitin-conjugating enzymes, which have special domains that catalyse substrates, have sequence discrepancies and modulate various pathophysiological processes in different cells of multiple organisms. E2s take part in the mitosis of primordial germ cells, meiosis of spermatocytes and the formation of mature haploid spermatids to maintain normal male fertility. In this review, we summarize the various types of E2s and their functions during distinct stages of spermatogenesis.

Keywords Spermatogenesis, Ubiquitin-conjugating enzyme, Male infertility

Introduction

Spermatogenesis, including the mitosis of spermatogonia to self-generation, meiosis and spermiogenesis, involves multiple genes that regulate male haploid gamete formation [1, 2]. The first cells of the reproductive lineage are called primordial germ cells, which can be considered to form the first distinctive subset of embryonic cells in XY embryo, the precursors of spermatogonial stem cells. Primordial germ cells need to be transferred over a considerable distance to facilitate the development of bipotential gonad. Primordial germ cells gradually transform into prospermatogonia as cell expansions [3]. Premeiotic type A single spermatogonia ultimately generate type B spermatogonia via the mitosis of undifferentiated spermatogonia and the process of differentiation into differentiating spermatogonia after birth [4]. Meiotic phase I can br divided into the leptotene, zygotene, pachytene, diplotene and diakinesis stages, in which primary spermatocytes undergo synapsis, DNA double strand breaks, recombination and crossover. Secondary spermatocytes generate two haploid round spermatids with sister chromatid separation [5, 6]. After the maturation and release of spermatids following the 16 steps of spermiogenesis, the lumens of the seminiferous tubules and epididymis are filled with a large amount of haploid sperm [7] (Fig. 1). Unlike those in mammals, the spermatogonia of male fruit flies generate 16 primary spermatocytes, which then synchronously produce 64 round spermatids via meiosis within syncytial cysts. As the round spermatids elongate, the chromatin gradually condenses, and the nucleus transforms from round to dense and needle-like to produce giant mature sperm. The nucleus of the spermatids is located at the end of the cyst, and the tail is located at the other end [8, 9] (Fig. 2). This highly ordered process is regulated by many procedurally generated active proteins [10].

The ubiquitination-proteasome system is a major mechanism through which proteins are efficiently removed in mammals [10, 11]. Ubiquitination modifications involving ubiquitin (Ub) were first discovered to target nonfunctional or misfolded proteins for degradation [12]. Recently, studies have revealed that

*Correspondence:
Jihong Liu
jhliu@tjh.tjmu.edu.cn
Xiaming Liu
xmliu77@hust.edu.cn
[1] Department of Urology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China
[2] Institute of Andrology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China

© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

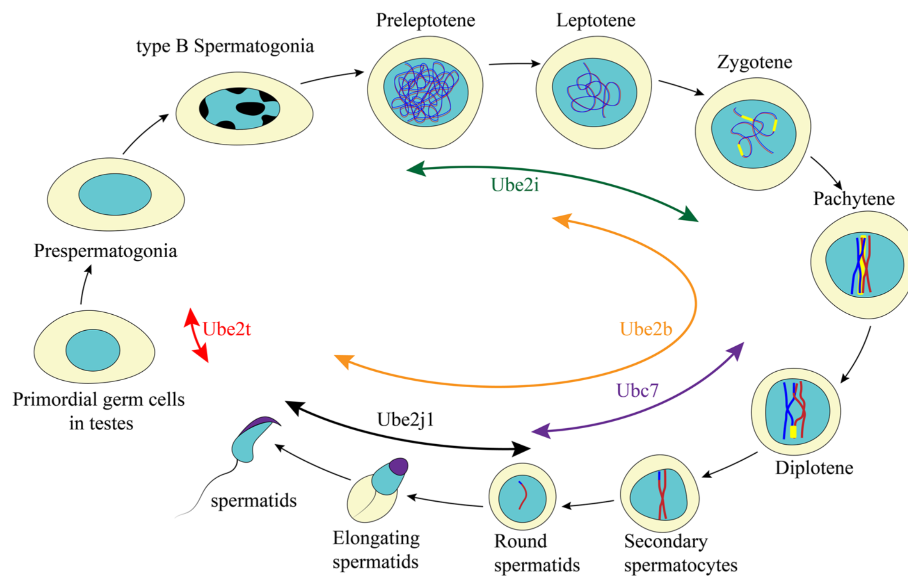

Fig. 1 The process of mouse spermatogenesis. Primordial germ cells ultimately generates type B spermatogonia through several rounds of mitosis process. The meiotic phase I can divide into leptotene, zygotene, pachytene, diplotene according on the formation procedure of synaptonemal complex. Homologous chromosomes of primary spermatocytes undergo DNA double strand breaks, synapsis, recombination and crossover (red line and bule line present homologous chromosomes, yellow columns present synaptonemal complex). Secondary spermatocyte generates two haploid round spermatids following with sister chromatids separation. After the process of spermiogenesis, mature sperm are produced to fertilize eggs. Moreover, we add the double-arrow to represent the functional stage of genes Ube2t, Ube2i, Ube2b, Ubc7, Ube2j1 in spermatogenesis

ubiquitination not only plays crucial roles in maintaining cell biological function via degradation, but also mediates the activity of multiple proteins via posttranslational modification [13–15]. Ubiquitination occurs via three-step enzyme cascades involving ubiquitin activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin ligase (E3), but this process can be reversed by deubiquitinase (DUB). E1 activates ubiquitin in a manner dependent on hydrolysing adenosine 5′-triphosphate (ATP), allowing the ubiquitin molecule to covalently conjugate with E1 to form a thioester-linked complex. The E1-Ub complex then reacts with an E2 enzyme to deliver Ub to E2 via a transthiolation reaction. Finally, an E3 ligase, which can simultaneously bind its target substrate and E2-Ub, transfers the Ub tag to target protein. This process can be arrested to release the monoubiquitinated protein for a particular purpose or can continue multiple times to covalently link a series of additional ubiquitins in a chain for polyubiquitination [16–19] (Fig. 3). Specifically, small ubiquitin-like modifier proteins are coupled to substrate proteins via a reaction of the dimeric E1 SUMO activators (Sae1/Sae2), E2 (E2 ubiquitin-conjugating enzyme I, Ube2i), and a set of E3 ligases, whereas deSUMOylation is regulated by the Sentrin or SUMO-specific proteases. The process of SUMOylation is similar to that of ubiquitination, both of which play important roles in protein posttranslational modification and are widely involved in the regulation of biological functions, including controlling germ cell development and maturation [20, 21] (Fig. 3). Many studies have reported that altered expression of ubiquitin-conjugating enzymes influences the process of spermatogenesis.

Genes in the E2 protein family have significant sequence discrepancies that result in a diversity of ubiquitin-conjugating enzymes in various species, but all of them contain a specific domain called the ubiquitin-coupled catalytic (UBC) domain, which consists of 150 to 200 amino acids [22–24]. UBC also contains a typical topological structure, as well as a motif including histidine-proline-asparagine tripeptides and catalytic cysteine residues [25]. Recently, whole-genome sequencing analysis of the sperm whale revealed 39 UBE2 genes, which may contribute to functional divergence [26]. Similarly, humans harbour forty E2 genes, including intact genes, partial genes and pseudogenes [27]. Given the pivotal role in transmission of ubiquitin and the critical role of ubiquitin-conjugating enzymes in regulation of the activity of downstream proteins, we provide a comprehensive overview on the roles of ubiquitin-conjugating enzymes in spermatogenesis (Fig. 1) and review their corresponding E3 partners in Table 1.

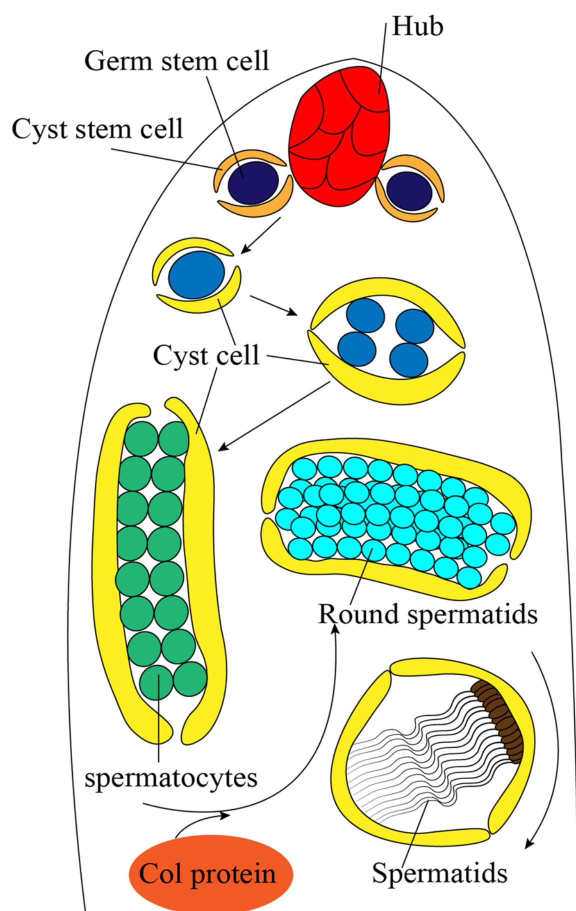

Fig. 2 The process of mouse spermatogenesis. Germ stem cell generates a spermatogonia and a new germ stem cell to maintain the stemness via mitosis. Likewise, cyst stem cell generates a cyst cell and a new cyst stem cell to maintain the stemness. Every two cyst cells encapsulate one spermatogonia. Spermatogonia produces 16 spermatocytes via mitosis. Then, spermatocytes produce 64 round spermatids via meiosis within syncytial cysts, which requiring the involvement of courtless protein. After spermiogenesis, the nucleus of the spermatids is located at the end of the cyst, and the tail is located at the other end

Ubiquitin-conjugating enzyme in primordial germ cells

Spermatogenesis is initiated by triggering spermatogonial stem cells to undergo mitotic division to generate progenitors during the migration phase, which further produces differentiated types of spermatogonia [65].

E2 ubiquitin-conjugating enzyme T (UBE2T), a crucial component of the FA pathway that serves as an indispensable way to maintain reproductive capacity, collaborates with FANCL to regulate ubiquitination [66, 67]. Ube2t deficient mice exhibit severe loss of primordial germ cells at 3 days of age and smaller ovaries or testes without differences in body weight. Knockout of Ube2t results in normal primordial germ cells migration but defective expansion with increased DNA damage, which triggers p53 pathway activation to slow the cell cycle. Moreover, Ube2t can offset transcription–replication conflicts, which are the source of replication stress by resolving R-loops, protecting replication forks and maintaining the stability of common fragile sites to maintain genomic stability in primordial germ cells. UBE2T promotes the rapid proliferation of mouse primordial germ cells to ensure the establishment of fertility [68].

Ubiquitin-conjugating enzyme in meiosis

The halved genetic reduction in a particular cell division is known as meiosis, accompanied by two successive rounds of chromosome separation after a single round of DNA replication. Homologous chromosomes separate into opposite poles at meiosis I, and then sister chromatids separate into opposite poles at meiosis II [69]. During early meiosis I, programmed DNA double strand breaks are associated with dynamic changes in chromosomal structure, and chromatin remodelling is modulated mainly by histones. Histones are involved in the resumption of meiosis, meiotic sex chromosome inactivation and asymmetric division [70–72]. Following the development of spermatocytes, the synaptonemal complex gradually forms with the assembly of lateral elements, axial elements, central elements and transverse filaments between homologous chromosomes. The synaptonemal complex maintains synapsis along the full length of each synaptonemal chromosome, sustains crossover, and promotes the exchange of DNA strands [73, 74]. Synaptonemal complex is vital for the successful progression of meiosis.

UBC7, which is located on the plasma and ER membranes along with calreticulin, participates in phagocytosis and self-polyubiquitinates on the cystine residues to yield its degradation [75–77]. The Courtless (col) gene, encoding the *Drosophila* homologue of yeast UBC7, maps to polytene chromosome band 47D. Col mutation in *Drosophila* is caused by the insertion of a P element into the 3’-UTR, which probably disrupts translational regulatory elements. Homozygous mutants led to the accumulation of courtless protein and aberrant courtship in male flies. Only 5% of the homozygous mutants finished courtship, and their matings gave no progeny. Homozygous mutants undergo normal mitotic divisions of primary spermatogonial cells to produce 16 primary spermatocytes but do not undergo meiotic divisions to generate normal haploid spermatids [78](Fig. 2). In addition, human UBE2G2, which was isolated from a human foetal-brain cDNA library, is a Ubc7 homologue [79]. Two studies including subfertile males estimated the correlation between alterations in sperm DNA methylation

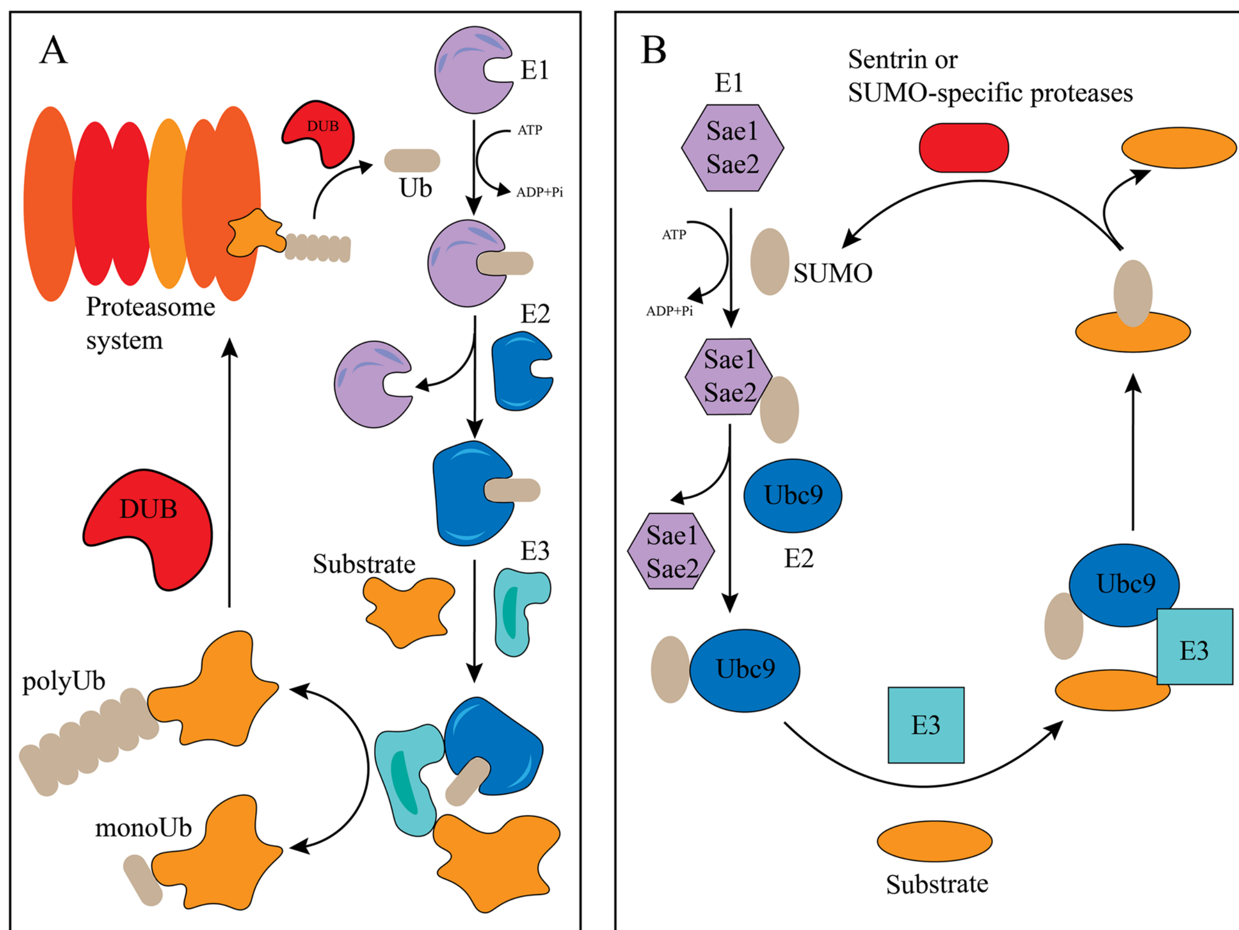

Fig. 3 The cycle of ubiquitination and SUMOylation. **A** Activation: E1 activates ubiquitin with ATP. Conjugation: E2 reacts with E1-Ub complex to deliver Ub to E2. Ligation: E3 simultaneously bind its target substrate and E2-Ub to transfer one or more Ub for substrate. Deubiquitination: ubiquitin can be removed through deubiquitinase. **B** Activation: Sae1/Sae2 is activated with ATP to bind SUMO. Conjugation: Ubc9 interacts with E1-SUMO complex to transfer SUMO to Ubc9. Ligation: E3 simultaneously reacts with its target substrate and Ubc9-SUMO to send SUMO to substrate. DeSUMOylation: SUMO can be eliminated via Sentrin or SUMO-specific proteases. E1: ubiquitin activating enzyme. E2: ubiquitin-conjugating enzyme. E3: ubiquitin ligase. DUB: deubiquitinase

levels and semen parameters via deep bisulfite sequencing. Then, they discoverd abnormal methylation levels at CpG sites in UBE2G2 gene amplicons in the oligospermic group [80, 81]. This abnormal change may indicate that UBE2G2 plays a key role during human spermatogenesis, but more rigorous and scientific evidence is lacking.

The centre of the sumoylation cascade is a single E2 SUMO-conjugating enzyme, UBE2I, which is also known as UBC9 and is required for sumoylation [82]. In the testes of Chinese mitten crabs, Ube2i expression is low during early development, reaches its highest level during rapid testes growth (Stage II), and then gradually decreases to low levels [83]. In yeast, the Ubc9-K153R/157R sumoylation deficient mutant is dispensable for growth under vegetative and stress conditions. Ubc9 sumoylation, which governs meiotic SUMO conjugates, SUMO chains, and synaptonemal complex associated genes, is a key regulatory factor in synaptonemal complex formation. Experimental results revealed that defects in the ubc9-K153/157R mutant generated on the downstream of synapsis, such as the formation of DNA double strand breaks, homology search, and the formation of synapsis initiation complexes, although the mutant was defective in producing even short stretches of synapsis [84]. Similarly, Ube2i was discovered to be expressed in pachytene spermatocytes and localized to synaptic complexes in mice [85]. Another study unveiled that in mouse spermatocytes, Ube2i was located at synaptonemal complexes, the XY body in pachytene and diplotene, and centromeres in metaphase I by immunofluorescence staining [86]. Global Ube2i knockout mice are embryonic lethal at embryonic day 7.5 because of the mitotic defects caused by abnormal chromatin condensation, chromosome segregation and nuclear reorganization [82]. Although

Table 1 The role of ubiquitin-conjugating enzyme in spermatogenesis and their corresponding E3 ligases

Gene	Alternative names[a]	Impacts on spermatogenesis	The corresponding E3 ligases
Ube2t		The defective expansion of primordial germ cells	Mule [28], Nedd4l [29], Fanci [30], Rnf1 [31], Rnf8 [32], Brca1, Fancl [33]
Ubc7	Courtless (*Drosophila*) UBE2G2 (Human)	The stalled transition of spermatocytes to round spermatids	Hrd1, Doa10 [34], Gp78 [35]
Ube2i	UBC9	Abnormal formation of synaptonemal complex	The SUMO-3 proteins, including: SPRING domain family (Pias1, Pias3 Pias4 ect.) TRIM superfamily (Trim26, Trim27, Trim33 ect.) SIM-containing SUMO E3 ligases (Ranbp2, Cbx4, Slx4, Znf451-1/2/3 ect.) The other SUMO E3 ligases (Uhrf2, Traf7, HDAC4 HDAC7 ect.) [36, 37]
Ube2b	RAD6B (Mammalian)	The apoptosis of spermatocytes Abnormal synapsis Abnormal histone modification Abnormal spermatogenesis	Ubr1, Ubr2, Bre1, Rad18 [38], Ubr4 [39], Rnf4 [40], Rnf20 [41], Rnf168 [42],, Mdm2 [43], March10a [44]
Ube2j1		Abnormal spermatogenesis	c-IAP1, Derlin-1, Rma1 [45], Rnf26 [46], Trim25 [47], Mdm2 [48], Hrd1 [49]
Ube2w		Ube2w- deficient lead to the loss and apoptosis of germ cells, but the process of spermatogenesis is normal.	Murf [50], Cbl [51], Brca1, Trim21 [52], Trim5α [53], Ubox1 [54], Rbx1 [55], Fancl [56], Rnf4, Chip [57]
Ubc4		No impact	Need4-2 [58], Cnot4 [59], Dsc1 [60], Ufd4 [61], Rsp5 [62], Tul1 [63], Pep5, Snt2, Hel1, Hel2 [64]

[a] Only in this paper

conditional Ube2i knockout in female mouse proved that the loss of Ube2i in oocytes caused the arrest of folliculogenesis and abnormal communication with ovarian somatic cells, the function of Ube2i in the spermatogenesis of mammals is still unknown [87].

Yeast RAD6, which functions as an E2 ubiquitin-conjugating enzyme, mainly regulates DNA postreplication repair and protein degradation through the N-end rule pathway [88–90]. RAD6 mediates the ubiquitination of histone 2B (H2B) at Lys 123 and then promotes the methylation of histone 3 (H3) at lysine 4 in yeast [91]. RAD6 is essential for sporulation and regulates the structure of chromatin via histone ubiquitination [92]. Unlike yeast, the mammalian RAD6 homologue is encoded by RAD6A and RAD6B (also called UBE2B). UBE2B is expressed in many cells but is especially highly expressed in spermatocytes and round spermatids [93, 94]. Although testicular tissue sections from Ube2b knockout mice exhibit abnormalities predominantly during spermiogenesis (we describe this phenomenon later in terms of *ubiquitin-conjugating enzyme in spermiogenesis*), a TUNEL assay revealed massive numbers of apoptotic spermatocytes in the seminiferous tubules of Ube2b knockout mice. The results of meiotic chromosome spread assay, which was performed with spermatocytes from Ube2b knockout mice, revealed that the average total width of the synaptonemal complex was thinner, and the average total length of the synaptonemal complex was significantly increased in late pachytene, and the loss of telomeres occurred during pachytene. Moreover, the number of chiasmata in spermatocytes from Ube2b knockout mice was increased in middle pachytene. All of these findings may be attributed to premature synaptonemal complex degradation in spermatocytes in Ube2b knockout mice [95, 96].

Homologous chromosome pairing commences at the zygotene stage and is complete at the pachytene stage, but mammalian heterologous X and Y chromosome pairing occurs only in the pseudoautosomal region. Unsynapsed X and Y chromosome arms trigger XY body formation and meiotic sex chromosome inactivation during the transition from zygotene to pachytene [97, 98]. Interestingly, histone H2AK119ub1 was enriched on the XY body with accumulation of the RAD6B enzyme [95, 99]. Additionally, H2BT119 phosphorylation, which is markedly elevated and distributed along the unpaired axial elements of sex chromosomes in pachytene and diplotene spermatocytes, extends to the XY body at gradually lower levels. However, immunofluorescence staining showed no difference in the degree of histone H2AK119 and H2BK120 ubiquitination or H2BT119 phosphorylation between the control group and the Ube2b knockout group. H2AT120 phosphorylation was found to be high in leptotene and zygotene spermatocytes but was lost in early pachytene spermatocytes, with the exception of the XY body. In Ube2b knockout spermatocytes, H2AT120 phosphorylation was strikingly increased during pachytene and diplotene, with increased signals on the XY

body. However, H2AXS139 phosphorylation did not differ between in wild-type and Ube2b knockout spermatocytes. In addition, H3K4 methylation also strongly increased in nuclei and XY body in diplotene and extended to the metaphase II phase in Ube2b knockout spermatocytes. In contrast, the levels of H3K9m2, which represents silent chromatin, were much lower on centromeric heterochromatin but normal on sex chromatin in Ube2b-deficient germ cells [100, 101].

Ubiquitin-conjugating enzyme in spermiogenesis

During spermiogenesis, round spermatids undergo giant structural alterations, including the formation of sperm acrosomes involving the Golgi apparatus, the dynamics of nuclear chromatin, the formation of the sperm flagellum, the rearrangement of mitochondria at the tail of the sperm, and the removal of redundant cytoplasm [102, 103] (Fig. 4).

The proteasomal degradation pathways strongly participate in spermiogenesis accompanied by unnecessary protein extraction form the endoplasmic reticulum to the cytosol [104]. UBE2J1 is located on the membrane of the endoplasmic reticulum and has tail-anchored protein domains [105, 106]. The SEL1L dislocation complex, which contains several factors, including UBE2J1, is known to be involved in ER dislocation [107]. A study revealed that Ube2j1 was strongly expressed in the maturation phase of elongated spermatids between steps 12 and 15, a period in which the ER undergoes massive and dynamic changes. Global Ube2j1 knockout mice were generated from heterozygous mice, and only half of all the mice survived at postnatal day 21. The survivors of the Ube2j1 knockout mice were smaller in body size and were significantly lighter in weight than the wild-type mice, but the average weight of testes was normal. Moreover, Ube2j1 knockout female mice were fertile, but male mice were sterile with severe defects in sperm morphology and mobility. The sperm of Ube2j1 knockout mice presented with excess residual cytoplasm around the acrosome, neck and midpiece of the flagellum (Fig. 4). The failure of cytoplasmic removal of Ube2j1 gene knockout in mice ultimately leads to male infertility [108]. Rnf133, a RING family E3 ubiquitin ligase, was tested for its ability to interact with Ube2j1. Knocking out Rnf133 in male mice leads to a severe subfertility with aberrant head–neck morphology. Similar abnormalities were previously shown in Ube2j1 KO sperm [109].

A previous study revealed that Ube2b knockout in mice resulted in smaller testes and male infertility. Hematoxylin-eosin staining reveled that the development of germ cells may arrest between spermatogenic stages I–IV and V–VIII [96]. Another study confirmed that Ube2b deficiency was indispensable for meiosis but resulted in failure of spermiogenesis with abnormal spermatozoa. Occasionally, a nearly complete lack of all germ cell types was detected in 10-20% of the male Ube2b knockout mice. Multiple hookless heads, including club-shaped heads, amorphous heads, and those with disintegrated nuclei or no nuclei, were observed in Ube2b knockout testes [110]. Ube2b promoted the ubiquitination of histones 2 A and 2B, and its deficiency influenced histone-to-protamine replacement during sperm head formation [103, 111] (Fig. 4). Testes-specific histone H2B, a representative marker of the elongation phase but lacking in

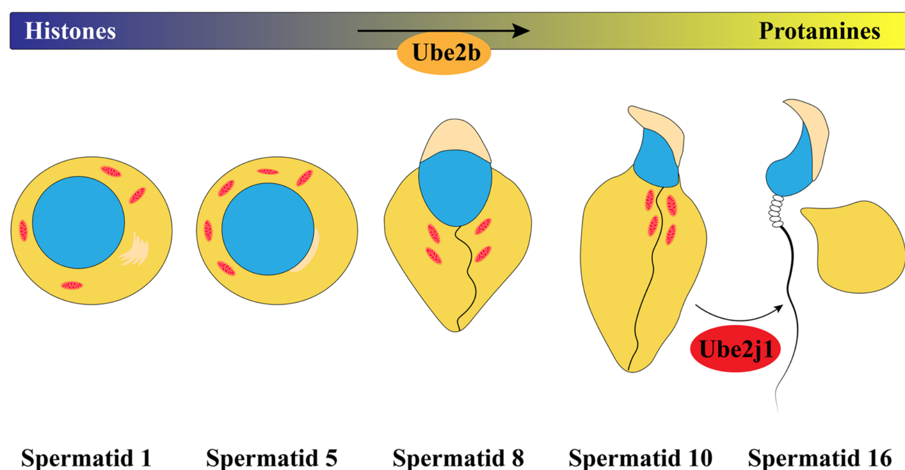

Fig. 4 The main process of spermiogenesis with the transformations of histones to protamine. Round spermatids undergo 16 steps to generate normal elongated sperm. Golgi apparatus gradually form sperm acrosome. Sperm flagellum gradually is elongated with microtubule proteins and rearrangement of mitochondria. Redundant cytoplasm was removed at last, and Ube2j1 plays an indispensable role in this process. We only show 5 stages of spermatids to present this procedure following the transformations of histones to protamine, for which Ube2b is crucial

mature spermatids, was irregularly displayed on abnormal spermatids and detected in the epididymis ducts, suggesting that round or elongating spermatids were prematurely released [111]. The genes, whose expression was repressed in normal spermatids, were increased in Ube2b-deficient cells, which may indicate that X chromosomal gene silencing was impaired in Ube2b knockout spermatids [101]. This may be related to the failure of spermiogenesis.

In humans, a study in India analysed genetic variations in the UBE2B gene in 530 infertile males (including 350 with azoospermia, 105 with oligoasthenoteratozoospermia, and 75 with oligoasthenozoospermia) and 300 control males. Sequence analysis revealed 5 single-nucleotide substitution polymorphisms in 37.5% of infertile males and 7.3% of fertile males, two (g.5197:T > G; g.9157:A > G) of which were novel and found only in infertile males [112]. Moreover, an analysis was carried out to investigate single nucleotide polymorphism differences in UBE2B in 312 fertile males and 388 infertile males in Northeast China. The UBE2B polymorphisms g.-293T > G, g.20016A > G and g.9157A > G were not associated with male infertility [113]. Another analysis of 776 idiopathic azoospermia patients and 709 fertile men revealed two novel synonymous variants in the exon region and four novel variants in the promoter region of UBE2B in the Chinese population, among which one variant (Chr5.133706925 A > G) was highly prone to idiopathic azoospermia and inhibited the transcriptional regulation activity of specificity protein 1 [114]. Single and dual CGG deletions in the 5'-UTR of the UBE2B gene increased the binding of the transcription factor specificity protein 1 to the promoter, which may be related to human male infertility [115]. Another study from the USA revealed mRNA alterations in the UBE2B gene in 326 oligozoospermic patients and 421 normal men. A total of 4.6% of the oligozoospermic patients were discovered to have nine splicing, four missense and two nonsense alterations in UBE2B mRNA, but these mutations were not found in unaffected men. The sequence of the corresponding DNA regions did not reveal causative DNA mutations, suggesting posttranscriptional defects in spermatogenesis [116]. Combined with the previous description of meiosis, we hypothesize that the UBE2B gene may play a different role at a given point in spermatogenesis.

Ubiquitin-conjugating enzyme makes no impact on the process of spermatogenesis

Various proteins are highly expressed in the progression of spermatogenesis, some of which may play indispensable roles in generating normal sperm. Some genes, such as G3bp2, Tex33, Asb15, are nonessential for mouse spermatogenesis [117–119].

UBE2W was identified as a ubiquitin-conjugating enzyme that mediates the N-terminal ubiquitination of substrates [57, 120]. The levels of Ube2w in mouse testes gradually increased with the development of newborn to adult mice. Loss of ube2w lead to susceptibility to early postnatal lethality, abnormalities in skin maturation and severe declines in male fertility accompanied by vacuolation of the seminiferous tubules. Testes of Ube2w KO mice displayed variable degeneration and atrophy of seminiferous tubules, ranging from mild vacuolation to complete vacuolation. In addition, histological staining revealed that sperm levels in the KO epididymis varied, ranging from a normal number of mature spermatozoa to a complete absence of mature spermatozoa. This suggested ube2w-deficient male mice was oligozoospermia, but the process of spermatogenesis was normal. However, ube2w deficiency did not affect the tolerance of germ cells to DNA cross-linking induced by mitomycin C or the cellular response to oxidative and endoplasmic reticulum stress [121]. Moreover, under conditions of RNF4 (a ubiquitin ligase) deficiency, inactivation of Ube2w promoted the response to DNA cross-linking damage [122]. In addition, downregulated expression of Ube2w in a mouse-derived spermatogonia cell line and spermatocyte cell line significantly increased the number of apoptotic cells via the P53/Bcl-2/caspase 6/caspase 9 signalling pathways [123]. Thus, we speculated that the decreased number of sperm in Ube2w knockout male mice due to apoptosis rather impaired spermatogenesis.

UBC4, which is induced by heat stress, is essential for the degradation of short-lived and abnormal peptides but not for long-lived proteins in *Saccharomyces cerevisiae* [124]. During spermatogenesis, massive amounts of intracellular histones are degraded in a manner dependent on UBC4 as round spermatids transform into elongated mature forms [125, 126]. A pervious study revealed that various UBC4 isoforms activate ubiquitin conjugation and participate in cellular remodelling and protein degradation during postnatal development of the rat testes [127]. However, another study revealed that Ubc4 knockout mice presented normal body weight and fertility. Compared with that of the control mice, the testicular weight of the Ubc4-deficient male mice decreased 10% after the end of the first wave of spermatogenesis, and then reached a normal weight at postnatal day 65. The number and motility of sperm isolated from the epididymides of the Ubc4 knockout mice were normal. Under heat stress, there was no difference in cell apoptosis or testes mass [128].

Conclusions and perspective

Ubiquitin-conjugating enzymes play key regulatory roles in protein degradation and affect signalling pathways, including those involved in DNA damage repair, intracellular trafficking, endocytosis, the regulation of cell membrane fluidity and inflammatory responses [129–131]. In this paper, we reviewed and illustrated the various functions of E2 enzymes involved in different stages of spermatogenesis, including expansion of primordial germ cells, spermatocyte meiosis, and sperm metamorphosis. With the exception of the E2 enzymes we summarized above, the functions of other E2 enzymes in spermatogenesis and the substrates of E2s remain unknown. In the future, with the development of biotechnology, the functions of more ubiquitin-conjugating enzymes will be better clarified during spermatogenesis. Moreover, in light of the function of E2s in the ubiquitination system and spermatogenesis, targeting E2s may be a novel strategy for the treatment of azoospermia patients via cell and gene therapy.

Abbreviations

Ub	Ubiquitin
E1	Ubiquitin activating enzyme
E2	Ubiquitin-conjugating enzyme
E3	Ubiquitin ligase
DUB	Deubiquitinase
ATP	Hydrolyzing adenosine 5´-triphosphate
UBC	Ubiquitin coupled catalytic
UBE2T	E2 ubiquitin-conjugating enzyme T
UBC7	E2 ubiquitin-conjugating enzyme 7
Col	Courtless
UBE2G2	Ubiquitin-conjugating enzyme E2 G2
UBE2B	E2 ubiquitin-conjugating enzyme B
RAD6B	RAD6 homolog B
UBE2I	E2 ubiquitin-conjugating enzyme I
UBE2W	E2 ubiquitin-conjugating enzyme W
UBC4	E2 ubiquitin-conjugating enzyme 4
UBE2J1	E2 ubiquitin-conjugating enzyme J1
H2B	Histone 2B
H3	Histone 3

Acknowledgements

The authors would like to thank all researchers included in the studies we reviewed.

Authors' contributions

Data collection, writing and editing of the manuscript: Peng Lv. Supervision and paper review: Jihong Liu and Xiaming Liu. All authors contributed to the article and approved the submitted version. All authors reviewed the manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (82072838).

Availability of data and materials

Not applicable.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 26 June 2024 Accepted: 15 August 2024
Published online: 28 August 2024

References

1. Neto FT, Bach PV, Najari BB, Li PS, Goldstein M. Spermatogenesis in humans and its affecting factors. Semin Cell Dev Biol. 2016;59:10–26.
2. Paronetto MP, Sette C. Role of RNA-binding proteins in mammalian spermatogenesis. Int J Androl. 2010;33:2–12.
3. Mäkelä JA, Koskenniemi JJ, Virtanen HE, Toppari J. Testis evelopment. Endocr Rev. 2019;40:857–905.
4. Li X, Long XY, Xie YJ, Zeng X, Chen X, Mo ZC. The roles of retinoic acid in the differentiation of spermatogonia and spermatogenic disorders. Clin Chim Acta. 2019;497:54–60.
5. Handel MA. Monitoring meiosis in gametogenesis. Theriogenology. 1998;49:423–30.
6. Ishiguro KI, Shimada R. MEIOSIN directs initiation of meiosis and subsequent meiotic prophase program during spermatogenesis. Genes Genet Syst. 2022;97:27–39.
7. Lehti MS, Sironen A. Formation and function of the manchette and flagellum during spermatogenesis. Reproduction. 2016;151:R43-54.
8. Huang Q, Chen X, Yu H, Ji L, Shi Y, Cheng X, Chen H, Yu J. Structure and molecular basis of spermatid elongation in the Drosophila testis. Open Biol. 2023;13: 230136.
9. Fabian L, Brill JA. Drosophila spermiogenesis: big things come from little packages. Spermatogenesis. 2012;2:197–212.
10. Xiong Y, Yu C, Zhang Q. Ubiquitin-proteasome system-regulated protein degradation in spermatogenesis. Cells. 2022;11:1058.
11. Li Y, Li S, Wu H. Ubiquitination-Proteasome System (UPS) and autophagy two main protein degradation machineries in response to cell stress. Cells. 2022;11:851.
12. Komander D. The emerging complexity of protein ubiquitination. Biochem Soc Trans. 2009;37:937–53.
13. Song L, Luo ZQ. Post-translational regulation of ubiquitin signaling. J Cell Biol. 2019;218:1776–86.
14. Dai X, Zhang T, Hua D. Ubiquitination and SUMOylation: protein homeostasis control over cancer. Epigenomics. 2022;14:43–58.
15. Mattiroli F, Penengo L. Histone ubiquitination: an integrative signaling platform in genome tability. Trends Genet. 2021;37:566–81.
16. Carroll EC, Marqusee S. Site-specific ubiquitination: deconstructing the degradation tag. Curr Opin Struct Biol. 2022;73: 102345.
17. Grabbe C, Husnjak K, Dikic I. The spatial and temporal organization of ubiquitin networks. Nat Rev Mol Cell Biol. 2011;12:295–307.
18. Hershko A, Ciechanover A. The ubiquitin system. Annu Rev Biochem. 1998;67:425–79.
19. Liu W, Tang X, Qi X, Fu X, Ghimire S, Ma R, Li S, Zhang N, Si H. The Ubiquitin conjugating enzyme: an important ubiquitin transfer platform in Ubiquitin-Proteasome system. Int J Mol Sci. 2020;21(8):2894. https://doi.org/10.3390/ijms21082894.
20. Vertegaal ACO. Signalling mechanisms and cellular functions of SUMO. Nat Rev Mol Cell Biol. 2022;23:715–31.
21. Yau TY, Sander W, Eidson C, Courey AJ. SUMO interacting motifs: structure and function. Cells. 2021;10(11):2825. https://doi.org/10.3390/cells10112825.
22. Dikic I, Wakatsuki S, Walters KJ. Ubiquitin-binding domains - from structures to functions. Nat Rev Mol Cell Biol. 2009;10:659–71.

23. van Wijk SJ, Timmers HT. The family of ubiquitin-conjugating enzymes (E2s): deciding between life and death of proteins. Faseb j. 2010;24:981–93.
24. Ye Y, Rape M. Building ubiquitin chains: E2 enzymes at work. Nat Rev Mol Cell Biol. 2009;10:755–64.
25. Wu PY, Hanlon M, Eddins M, Tsui C, Rogers RS, Jensen JP, Matunis MJ, Weissman AM, Wolberger C, Pickart CM. A conserved catalytic residue in the ubiquitin-conjugating enzyme family. Embo j. 2003;22:5241–50.
26. Tian R, Yang C, Geng Y, Seim I, Yang G. Genomewide analysis of sperm whale E2 ubiquitin conjugating enzyme genes. J Genet 2021, 100.
27. Stewart MD, Ritterhoff T, Klevit RE, Brzovic PS. E2 enzymes: more than just middle men. Cell Res. 2016;26:423–40.
28. Ho NPY, Leung CON, Wong TL, Lau EYT, Lei MML, Mok EHK, Leung HW, Tong M, Ng IOL, Yun JP, et al. The interplay of UBE2T and mule in regulating Wnt/β-catenin activation to promote hepatocellular carcinoma progression. Cell Death Dis. 2021;12:148.
29. Chen Y, Hong H, Wang Q, Li J, Zhang W, Chen T, Li P. NEDD4L-induced ubiquitination mediating UBE2T degradation inhibits progression of lung adenocarcinoma via PI3K-AKT signaling. Cancer Cell Int. 2021;21:631.
30. Zhang J, Wang J, Wu J, Huang J, Lin Z, Lin X. UBE2T regulates FANCI monoubiquitination to promote NSCLC progression by activating EMT. Oncol Rep. 2022;48(2):139. https://doi.org/10.3892/or.2022.8350.
31. Jiang X, Ma Y, Wang T, Zhou H, Wang K, Shi W, Qin L, Guan J, Li L, Long B, et al. Targeting UBE2T potentiates gemcitabine efficacy in pancreatic cancer by regulating pyrimidine metabolism and replication stress. Gastroenterology. 2023;164:1232–47.
32. Sun J, Zhu Z, Li W, Shen M, Cao C, Sun Q, Guo Z, Liu L, Wu D. UBE2T-regulated H2AX monoubiquitination induces hepatocellular carcinoma radioresistance by facilitating CHK1 activation. J Exp Clin Cancer Res. 2020;39:222.
33. Liu LL, Zhu JM, Yu XN, Zhu HR, Shi X, Bilegsaikhan E, Guo HY, Wu J, Shen XZ. UBE2T promotes proliferation via G2/M checkpoint in hepatocellular carcinoma. Cancer Manag Res. 2019;11:8359–70.
34. Mashahreh B, Reiss Y, Wiener R, Ravid T. Assays for dissecting the in vitro enzymatic activity of yeast Ubc7. Methods Enzymol. 2019;619:71–95.
35. Li W, Tu D, Li L, Wollert T, Ghirlando R, Brunger AT, Ye Y. Mechanistic insights into active site-associated polyubiquitination by the ubiquitin-conjugating enzyme Ube2g2. Proc Natl Acad Sci U S A. 2009;106:3722–7.
36. Wu W, Huang C. SUMOylation and DeSUMOylation: prospective therapeutic targets in cancer. Life Sci. 2023;332: 122085.
37. Shi X, Du Y, Li S, Wu H. The role of SUMO E3 Ligases in Signaling Pathway of Cancer cells. Int J Mol Sci. 2022;23(7):3639. https://doi.org/10.3390/ijms23073639.
38. Shukla PK, Sinha D, Leng AM, Bissell JE, Thatipamula S, Ganguly R, Radmall KS, Skalicky JJ, Shrieve DC, Chandrasekharan MB. Mutations of Rad6 E2 ubiquitin-conjugating enzymes at alanine-126 in helix-3 affect ubiquitination activity and decrease enzyme stability. J Biol Chem. 2022;298: 102524.
39. Hong JH, Kaustov L, Coyaud E, Srikumar T, Wan J, Arrowsmith C, Raught B. KCMF1 (potassium channel modulatory factor 1) links RAD6 to UBR4 (ubiquitin N-recognin domain-containing E3 ligase 4) and lysosome-mediated degradation. Mol Cell Proteom. 2015;14:674–85.
40. DiBello A, Datta AB, Zhang X, Wolberger C. Role of E2-RING interactions in governing RNF4-Mediated substrate ubiquitination. J Mol Biol. 2016;428:4639–50.
41. Foglizzo M, Middleton AJ, Day CL. Structure and function of the RING domains of RNF20 and RNF40, Dimeric E3 ligases that Monoubiquitylate histone H2B. J Mol Biol. 2016;428:4073–86.
42. Liu C, Wang D, Wu J, Keller J, Ma T, Yu X. RNF168 forms a functional complex with RAD6 during the DNA damage response. J Cell Sci. 2013;126:2042–51.
43. Wang C, Chang JF, Yan H, Wang DL, Liu Y, Jing Y, Zhang M, Men YL, Lu D, Yang XM, et al. A conserved RAD6-MDM2 ubiquitin ligase machinery targets histone chaperone ASF1A in tumorigenesis. Oncotarget. 2015;6:29599–613.
44. Iyengar PV, Hirota T, Hirose S, Nakamura N. Membrane-associated RING-CH 10 (MARCH10 protein) is a microtubule-associated E3 ubiquitin ligase of the spermatid flagella. J Biol Chem. 2011;286:39082–90.
45. Elangovan M, Chong HK, Park JH, Yeo EJ, Yoo YJ. The role of ubiquitin-conjugating enzyme Ube2j1 phosphorylation and its degradation by proteasome during endoplasmic stress recovery. J Cell Commun Signal. 2017;11:265–73.
46. Cremer T, Jongsma MLM, Trulsson F, Vertegaal ACO, Neefjes J, Berlin I. The ER-embedded UBE2J1/RNF26 ubiquitylation complex exerts spatiotemporal control over the endolysosomal pathway. Cell Rep. 2021;34: 108659.
47. Wang T, Jin C, Yang P, Chen Z, Ji J, Sun Q, Yang S, Feng Y, Tang J, Sun Y. UBE2J1 inhibits colorectal cancer progression by promoting ubiquitination and degradation of RPS3. Oncogene. 2023;42:651–64.
48. Zhang P, Guo H, Zhao F, Jia K, Yang F, Liu X. UBE2J1 knockdown promotes cell apoptosis in endometrial cancer via regulating PI3K/AKT and MDM2/p53 signaling. Open Med (Wars). 2023;18:20220567.
49. Burr ML, Cano F, Svobodova S, Boyle LH, Boname JM, Lehner PJ. HRD1 and UBE2J1 target misfolded MHC class I heavy chains for endoplasmic reticulum-associated degradation. Proc Natl Acad Sci U S A. 2011;108:2034–9.
50. Lord SO, Dawson PWJ, Chunthorng-Orn J, Ng J, Baehr LM, Hughes DC, Sridhar P, Knowles T, Bodine SC, Lai YC. Uncovering the mechanisms of MuRF1-induced ubiquitylation and revealing similarities with MuRF2 and MuRF3. Biochem Biophys Rep. 2024;37:101636.
51. Liyasova MS, Ma K, Voeller D, Ryan PE, Chen J, Klevit RE, Lipkowitz S. Cbl interacts with multiple E2s in vitro and in cells. PLoS ONE. 2019;14: e0216967.
52. Fletcher AJ, Mallery DL, Watkinson RE, Dickson CF, James LC. Sequential ubiquitination and deubiquitination enzymes synchronize the dual sensor and effector functions of TRIM21. Proc Natl Acad Sci U S A. 2015;112:10014–9.
53. Fletcher AJ, Christensen DE, Nelson C, Tan CP, Schaller T, Lehner PJ, Sundquist WI, Towers GJ. TRIM5α requires Ube2W to anchor Lys63-linked ubiquitin chains and restrict reverse transcription. Embo J. 2015;34:2078–95.
54. Quaile AT, Urbanus ML, Stogios PJ, Nocek B, Skarina T, Ensminger AW, Savchenko A. Molecular characterization of LubX: functional divergence of the U-Box fold by Legionella pneumophila. Structure. 2015;23:1459–69.
55. Yuan Y, Xiao WW, Xie WH, Li RZ, Gao YH. Prognostic value of ubiquitin E2 UBE2W and its correlation with tumor-infiltrating immune cells in breast cancer. BMC Cancer. 2021;21:479.
56. Zhang Y, Zhou X, Zhao L, Li C, Zhu H, Xu L, Shan L, Liao X, Guo Z, Huang P. UBE2W interacts with FANCL and regulates the monoubiquitination of Fanconi anemia protein FANCD2. Mol Cells. 2011;31:113–22.
57. Tatham MH, Plechanovová A, Jaffray EG, Salmen H, Hay RT. Ube2W conjugates ubiquitin to α-amino groups of protein N-termini. Biochem J. 2013;453:137–45.
58. Todaro DR, Augustus-Wallace AC, Klein JM, Haas AL. The mechanism of neural precursor cell expressed developmentally down-regulated 4 – 2 (Nedd4-2)/NEDD4L-catalyzed polyubiquitin chain assembly. J Biol Chem. 2017;292:19521–36.
59. Albert TK, Hanzawa H, Legtenberg YI, de Ruwe MJ, van den Heuvel FA, Collart MA, Boelens R, Timmers HT. Identification of a ubiquitin-protein ligase subunit within the CCR4-NOT transcription repressor complex. Embo J. 2002;21:355–64.
60. Raychaudhuri S, Espenshade PJ. Endoplasmic reticulum exit of Golgi-resident defective for SREBP cleavage (dsc) E3 ligase complex requires its activity. J Biol Chem. 2015;290:14430–40.
61. Bao X, Johnson JL, Rao H. Rad25 protein is targeted for degradation by the Ubc4-Ufd4 pathway. J Biol Chem. 2015;290:8606–12.
62. Stoll KE, Brzovic PS, Davis TN, Klevit RE. The essential Ubc4/Ubc5 function in yeast is HECT E3-dependent, and RING E3-dependent pathways require only monoubiquitin transfer by Ubc4. J Biol Chem. 2011;286:15165–70.
63. Sakamaki JI, Ode KL, Kurikawa Y, Ueda HR, Yamamoto H, Mizushima N. Ubiquitination of phosphatidylethanolamine in organellar membranes. Mol Cell. 2022;82:3677-e36923611.
64. Singh RK, Gonzalez M, Kabbaj MH, Gunjan A. Novel E3 ubiquitin ligases that regulate histone protein levels in the budding yeast Saccharomyces cerevisiae. PLoS ONE. 2012;7:e36295.

65. Kolasa A, Misiakiewicz K, Marchlewicz M, Wiszniewska B. The generation of spermatogonial stem cells and spermatogonia in mammals. Reprod Biol. 2012;12:5–23.
66. Alpi A, Langevin F, Mosedale G, Machida YJ, Dutta A, Patel KJ. UBE2T, the fanconi anemia core complex, and FANCD2 are recruited independently to chromatin: a basis for the regulation of FANCD2 monoubiquitination. Mol Cell Biol. 2007;27:8421–30.
67. Tsui V, Crismani W. The fanconi anemia pathway and fertility. Trends Genet. 2019;35:199–214.
68. Yu Y, Xu W, Wen C, Zhao S, Li G, Liu R, Chen ZJ, Qin Y, Ma J, Yang Y, Zhao S. UBE2T resolves transcription-replication conflicts and protects common fragile sites in primordial germ cells. Cell Mol Life Sci. 2023;80:92.
69. Zickler D, Kleckner N. Recombination, pairing, and Synapsis of Homologs during Meiosis. Cold Spring Harb Perspect Biol. 2015;7(6):a016626.
70. Ito M, Shinohara A. Chromosome architecture and homologous recombination in meiosis. Front Cell Dev Biol. 2022;10:1097446.
71. Qu W, Liu C, Xu YT, Xu YM, Luo MC. The formation and repair of DNA double-strand breaks in mammalian meiosis. Asian J Androl. 2021;23:572–9.
72. Wang L, Xu Z, Khawar MB, Liu C, Li W. The histone codes for meiosis. Reproduction. 2017;154:R65-79.
73. Llano E, Pendás AM. Synaptonemal complex in human biology and disease. Cells. 2023;12:1718.
74. Zhang FG, Zhang RR, Gao JM. The organization, regulation, and biological functions of the synaptonemal complex. Asian J Androl. 2021;23:580–9.
75. Yan L, Liu W, Zhang H, Liu C, Shang Y, Ye Y, Zhang X, Li W. Ube2g2-gp78-mediated HERP polyubiquitylation is involved in ER stress recovery. J Cell Sci. 2014;127:1417–27.
76. Kumari R, Gupta P, Tiwari S. Ubc7/Ube2g2 ortholog in Entamoeba histolytica: connection with the plasma membrane and phagocytosis. Parasitol Res. 2018;117:1599–611.
77. Jungmann J, Reins HA, Schobert C, Jentsch S. Resistance to cadmium mediated by ubiquitin-dependent proteolysis. Nature. 1993;361:369–71.
78. Orgad S, Rosenfeld G, Greenspan RJ, Segal D. Courtless, the Drosophila UBC7 homolog, is involved in male courtship behavior and spermatogenesis. Genetics. 2000;155:1267–80.
79. Watanabe TK, Kawai A, Fujiwara T, Maekawa H, Hirai Y, Nakamura Y, Takahashi E. Molecular cloning of UBE2G, encoding a human skeletal muscle-specific ubiquitin-conjugating enzyme homologous to UBC7 of C. Elegans. Cytogenet Cell Genet. 1996;74:146–8.
80. Laqqan M, Hammadeh ME. Alterations in DNA methylation patterns and gene expression in spermatozoa of subfertile males. Andrologia. 2018;50:e12934.
81. Laqqan M, Tierling S, Alkhaled Y, LoPorto C, Hammadeh ME. Alterations in sperm DNA methylation patterns of oligospermic males. Reprod Biol. 2017;17:396–400.
82. Nacerddine K, Lehembre F, Bhaumik M, Artus J, Cohen-Tannoudji M, Babinet C, Pandolfi PP, Dejean A. The SUMO pathway is essential for nuclear integrity and chromosome segregation in mice. Dev Cell. 2005;9:769–79.
83. Wang Q, Wang Y, Chen L, He L, Li W, Jiang H. Expression characteristics of the SUMOylation genes SUMO-1 and Ubc9 in the developing testis and ovary of Chinese mitten crab, Eriocheir sinensis. Gene. 2012;501:135–43.
84. Klug H, Xaver M, Chaugule VK, Koidl S, Mittler G, Klein F, Pichler A. Ubc9 sumoylation controls SUMO chain formation and meiotic synapsis in Saccharomyces cerevisiae. Mol Cell. 2013;50:625–36.
85. La Salle S, Sun F, Zhang XD, Matunis MJ, Handel MA. Developmental control of sumoylation pathway proteins in mouse male germ cells. Dev Biol. 2008;321:227–37.
86. Kovalenko OV, Plug AW, Haaf T, Gonda DK, Ashley T, Ward DC, Radding CM, Golub EI. Mammalian ubiquitin-conjugating enzyme Ubc9 interacts with Rad51 recombination protein and localizes in synaptonemal complexes. Proc Natl Acad Sci U S A. 1996;93:2958–63.
87. Rodriguez A, Briley SM, Patton BK, Tripurani SK, Rajapakshe K, Coarfa C, Rajkovic A, Andrieux A, Dejean A, Pangas SA. Loss of the E2 SUMO-conjugating enzyme Ube2i in oocytes during ovarian folliculogenesis causes infertility in mice. Development. 2019;146:146.
88. Sriram SM, Kim BY, Kwon YT. The N-end rule pathway: emerging functions and molecular principles of substrate recognition. Nat Rev Mol Cell Biol. 2011;12:735–47.
89. Varshavsky A. The N-end rule pathway and regulation by proteolysis. Protein Sci. 2011;20:1298–345.
90. Zhao M, Shirley CR, Hayashi S, Marcon L, Mohapatra B, Suganuma R, Behringer RR, Boissonneault G, Yanagimachi R, Meistrich ML. Transition nuclear proteins are required for normal chromatin condensation and functional sperm development. Genesis. 2004;38:200–13.
91. Sun ZW, Allis CD. Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. Nature. 2002;418:104–8.
92. Lawrence C. The RAD6 DNA repair pathway in Saccharomyces cerevisiae: what does it do, and how does it do it? Bioessays. 1994;16:253–8.
93. Koken MH, Hoogerbrugge JW, Jasper-Dekker I, de Wit J, Willemsen R, Roest HP, Grootegoed JA, Hoeijmakers JH. Expression of the ubiquitin-conjugating DNA repair enzymes HHR6A and B suggests a role in spermatogenesis and chromatin modification. Dev Biol. 1996;173:119–32.
94. Koken MH, Reynolds P, Jaspers-Dekker I, Prakash L, Prakash S, Bootsma D, Hoeijmakers JH. Structural and functional conservation of two human homologs of the yeast DNA repair gene RAD6. Proc Natl Acad Sci U S A. 1991;88:8865–9.
95. Baarends WM, Wassenaar E, Hoogerbrugge JW, van Cappellen G, Roest HP, Vreeburg J, Ooms M, Hoeijmakers JH, Grootegoed JA. Loss of HR6B ubiquitin-conjugating activity results in damaged synaptonemal complex structure and increased crossing-over frequency during the male meiotic prophase. Mol Cell Biol. 2003;23:1151–62.
96. Roest HP, van Klaveren J, de Wit J, van Gurp CG, Koken MH, Vermey M, van Roijen JH, Hoogerbrugge JW, Vreeburg JT, Baarends WM, et al. Inactivation of the HR6B ubiquitin-conjugating DNA repair enzyme in mice causes male sterility associated with chromatin modification. Cell. 1996;86:799–810.
97. Alavattam KG, Maezawa S, Andreassen PR, Namekawa SH. Meiotic sex chromosome inactivation and the XY body: a phase separation hypothesis. Cell Mol Life Sci. 2021;79:18.
98. Turner JM, Mahadevaiah SK, Fernandez-Capetillo O, Nussenzweig A, Xu X, Deng CX, Burgoyne PS. Silencing of unsynapsed meiotic chromosomes in the mouse. Nat Genet. 2005;37:41–7.
99. van der Laan R, Uringa EJ, Wassenaar E, Hoogerbrugge JW, Sleddens E, Odijk H, Roest HP, de Boer P, Hoeijmakers JH, Grootegoed JA, Baarends WM. Ubiquitin ligase Rad18Sc localizes to the XY body and to other chromosomal regions that are unpaired and transcriptionally silenced during male meiotic prophase. J Cell Sci. 2004;117:5023–33.
100. Baarends WM, Wassenaar E, Hoogerbrugge JW, Schoenmakers S, Sun ZW, Grootegoed JA. Increased phosphorylation and dimethylation of XY body histones in the Hr6b-knockout mouse is associated with derepression of the X chromosome. J Cell Sci. 2007;120:1841–51.
101. Mulugeta Achame E, Wassenaar E, Hoogerbrugge JW, Sleddens-Linkels E, Ooms M, Sun ZW, van Grootegoed IWF, Baarends JA. The ubiquitin-conjugating enzyme HR6B is required for maintenance of X chromosome silencing in mouse spermatocytes and spermatids. BMC Genomics. 2010;11:367.
102. Miyaso H, Ogawa Y, Itoh M. Microenvironment for spermatogenesis and sperm maturation. Histochem Cell Biol. 2022;157:273–85.
103. Govin J, Caron C, Lestrat C, Rousseaux S, Khochbin S. The role of histones in chromatin remodelling during mammalian spermiogenesis. Eur J Biochem. 2004;271:3459–69.
104. Smith MH, Ploegh HL, Weissman JS. Road to ruin: targeting proteins for degradation in the endoplasmic reticulum. Science. 2011;334:1086–90.
105. Lenk U, Yu H, Walter J, Gelman MS, Hartmann E, Kopito RR, Sommer T. A role for mammalian Ubc6 homologues in ER-associated protein degradation. J Cell Sci. 2002;115:3007–14.
106. Lester D, Farquharson C, Russell G, Houston B. Identification of a family of noncanonical ubiquitin-conjugating enzymes structurally related to yeast UBC6. Biochem Biophys Res Commun. 2000;269:474–80.
107. Mueller B, Klemm EJ, Spooner E, Claessen JH, Ploegh HL. SEL1L nucleates a protein complex required for dislocation of misfolded glycoproteins. Proc Natl Acad Sci U S A. 2008;105:12325–30.
108. Koenig PA, Nicholls PK, Schmidt FI, Hagiwara M, Maruyama T, Frydman GH, Watson N, Page DC, Ploegh HL. The E2 ubiquitin-conjugating enzyme UBE2J1 is required for spermiogenesis in mice. J Biol Chem. 2014;289:34490–502.
109. Nozawa K, Fujihara Y, Devlin DJ, Deras RE, Kent K, Larina IV, Umezu K, Yu Z, Sutton CM, Ye Q, et al. The testis-specific E3 ubiquitin ligase RNF133 is required for fecundity in mice. BMC Biol. 2022;20:161.

110. Escalier D, Bai XY, Silvius D, Xu PX, Xu X. Spermatid nuclear and sperm periaxonemal anomalies in the mouse Ube2b null mutant. Mol Reprod Dev. 2003;65:298–308.
111. Guo Y, Song Y, Guo Z, Hu M, Liu B, Duan H, Wang L, Yuan T, Wang D. Function of RAD6B and RNF8 in spermatogenesis. Cell Cycle. 2018;17:162–73.
112. Suryavathi V, Khattri A, Gopal K, Rani DS, Panneerdoss S, Gupta NJ, Chakravarty B, Deenadayal M, Singh L, Thangaraj K. Novel variants in UBE2B gene and idiopathic male infertility. J Androl. 2008;29:564–71.
113. Hu Y, Wen W, Yu JG, Qu SQ, Wang SS, Liu J, Li BS, Luo Y. Genetic association of UBE2B variants with susceptibility to male infertility in a Northeast Chinese population. Genet Mol Res. 2012;11:4226–34.
114. Mou L, Zhang Q, Diao R, Cai Z, Gui Y. A functional variant in the UBE2B gene promoter is associated with idiopathic azoospermia. Reprod Biol Endocrinol. 2015;13:79.
115. Huang I, Emery BR, Christensen GL, Griffin J, Peterson CM, Carrell DT. Novel UBE2B-associated polymorphisms in an azoospermic/oligozoospermic population. Asian J Androl. 2008;10:461–6.
116. Yatsenko AN, Georgiadis AP, Murthy LJ, Lamb DJ, Matzuk MM. UBE2B mRNA alterations are associated with severe oligozoospermia in infertile men. Mol Hum Reprod. 2013;19:388–94.
117. Yun D, Zhou L, Shi J, Li X, Wu X, Sun F. G3BP2, a stress granule assembly factor, is dispensable for spermatogenesis in mice. PeerJ. 2022;10: e13532.
118. Xia M, Xia J, Niu C, Zhong Y, Ge T, Ding Y, Zheng Y. Testis-expressed protein 33 is not essential for spermiogenesis and fertility in mice. Mol Med Rep. 2021;23:23.
119. Wu Y, Zhang R, Shen C, Xu J, Wu T, Huang X, Liu M, Li H, Xu D, Zheng B. Testis-enriched Asb15 is not required for spermatogenesis and male fertility in mice. Am J Transl Res. 2022;14:6978–90.
120. Scaglione KM, Basrur V, Ashraf NS, Konen JR, Elenitoba-Johnson KS, Todi SV, Paulson HL. The ubiquitin-conjugating enzyme (E2) Ube2w ubiquitinates the N terminus of substrates. J Biol Chem. 2013;288:18784–8.
121. Wang B, Merillat SA, Vincent M, Huber AK, Basrur V, Mangelberger D, Zeng L, Elenitoba-Johnson K, Miller RA, Irani DN, et al. Loss of the ubiquitin-conjugating enzyme UBE2W results in susceptibility to early postnatal lethality and defects in skin, immune, and male reproductive systems. J Biol Chem. 2016;291:3030–42.
122. Maure JF, Moser SC, Jaffray EG, A FA, Hay RT. Loss of ubiquitin E2 Ube2w rescues hypersensitivity of Rnf4 mutant cells to DNA damage. Sci Rep. 2016;6:26178.
123. Lei B, Xie L, Zhang S, Lv D, Shu F, Deng Y. UBE2W down-regulation promotes cell apoptosis and correlates with hypospermatogenesis. Andrologia. 2020;52:e13474.
124. Seufert W, Jentsch S. Ubiquitin-conjugating enzymes UBC4 and UBC5 mediate selective degradation of short-lived and abnormal proteins. Embo j. 1990;9:543–50.
125. Liu Z, Oughtred R, Wing SS. Characterization of E3Histone, a novel testis ubiquitin protein ligase which ubiquitinates histones. Mol Cell Biol. 2005;25:2819–31.
126. Sutovsky P. Ubiquitin-dependent proteolysis in mammalian spermatogenesis, fertilization, and sperm quality control: killing three birds with one stone. Microsc Res Tech. 2003;61:88–102.
127. Rajapurohitam V, Morales CR, El-Alfy M, Lefrançois S, Bedard N, Wing SS. Activation of a UBC4-dependent pathway of ubiquitin conjugation during postnatal development of the rat testis. Dev Biol. 1999;212:217–28.
128. Bedard N, Hingamp P, Pang Z, Karaplis A, Morales C, Trasler J, Cyr D, Gagnon C, Wing SS. Mice lacking the UBC4-testis gene have a delay in postnatal testis development but normal spermatogenesis and fertility. Mol Cell Biol. 2005;25:6346–54.
129. Bui QT, Hong JH, Kwak M, Lee JY, Lee PC. Ubiquitin-conjugating enzymes in cancer. Cells. 2021;10:70.
130. Ikeda F. Ubiquitin conjugating enzymes in the regulation of the autophagy-dependent degradation pathway. Matrix Biol. 2021;100–101:23–9.
131. Claessens LA, Vertegaal ACO. SUMO proteases: from cellular functions to disease. Trends Cell Biol. 2024;S0962–8924(24):00002–3.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.