Ubiquitin-Proteasome System in Spermatogenesis

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Contributor: Chao Yu, Qianting Zhang

Spermatogenesis is a prolonged and highly ordered physiological process that produces haploid male germ cells through more than 40 steps and experiences dramatic morphological and cellular transformations. The ubiquitin proteasome system (UPS) plays central roles in the precise control of protein homeostasis to ensure the effectiveness of certain protein groups at a given stage and the inactivation of them after this stage. Many UPS components have been demonstrated to regulate the progression of spermatogenesis at different levels. Especially in recent years, novel testis-specific proteasome isoforms have been identified to be essential and unique for spermatogenesis.

Keywords: proteasome ; ubiquitination ; E3 ubiquitin ligase

1. The Ubiquitination-Proteasome System

In all cell types, precise control of the effective window of a given protein relies largely on multiple machineries that regulate its abundance via tightly and precisely controlled protein synthesis and degradation. In recent years, genome-wide RNA-sequencing (RNA-seq) of purified male germ cells or isolated single cells has provided fruitful knowledge on the transcriptional regulations during mammalian spermatogenesis [1][2]. However, RNA-seq offers barely no information on protein degradation.

The ubiquitination-proteasome system (UPS) plays crucial roles in the regulation of protein degradation, and in some cases, protein activity [3][4]. Ubiquitin is a 76-amino-acid polypeptide with diversities only in 2 amino acids from yeast to human. Action of the UPS is initiated by a three-step enzyme cascade, consisted of ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase (E3). This cascade transfers ubiquitin to the substrates via formation of covalent bonds between C-terminal glycine residues (Gly76) on the ubiquitin as well as lysine residues on the substrates or ubiquitin. The recognition between E3 ubiquitin ligases and their substrates is critical. In most cases, poly-ubiquitinated substrates are then recognized by the proteasomes for proteolysis and degradation, which terminates the function of the substrates [5]. However, in some cases, the ubiquitin chain could also be removed by deubiquitinating enzymes (DUBs), which recycles both functional proteins and ubiquitin [6].

Proteasomes, comprised of a 20S core particle (CP) and one or two regulatory particles (RPs), are central executors to the UPS and protein degradation [7]. The 20S CP is a 28-subunit complex with the molecular weight of around 750 kDa. These subunits are arranged as four stacked rings, two identical outer α rings and two identical inner β rings, forming a barrel-like structure. Each ring, either α or β, contains seven evolutionally-conserved subunits, i.e., α1–α7 and β1–β7, and among which, β1, β2 and β5 subunits possess the caspase-like, trypsin-like and chymotrypsin-like proteolysis activities, respectively [8][9]. This is the most common 20S proteasome species and is termed constitutive 20S proteasome, or c20S. In immunocytes or cells under inflammatory conditions, the three catalytic β subunits are replaced by β1i, β2i and β5i subunits, forming the so-called immune-20S proteasome, or i20S, which facilitates antigen presenting [10]. Recently, several studies uncovered the physiological functions of a male germ cell-specific spermato-20S proteasome, or s20S CP, in the regulation of homologous recombination and meiotic progression [11][12][13]. In the s20S CP, the α4 subunit is replaced by its analogue, α4s [14]. Accumulative evidences have shown that the 20S CP alone has proteolysis activity to degrade unfolded or disordered proteins under stress conditions [15][16].

However, the proteolytic activity and specificity of proteasomes are significantly increased by adding RPs to the 20S proteasome [7]. The RPs are attached to the α rings of the 20S CP, acting as the gates of the proteasome to recognize certain proteins for the subsequent proteolysis and meanwhile to prevent the degradation of other proteins. 19S RP is the most common RP and is made up of 17 subunits with a molecular weight of 700 kDa. The 19S RP binds to one or two ends of the 20S CP to form the 26S proteasome, which is the most abundant proteasome isoform responsible for the degradation of majority of poly-ubiquitinated proteins [17]. Besides the 19S RP, additional RPs are found to be associated with the 20S proteasomes, such as the PA28 RPs in immunocytes and the PA200 RP in male germ cells [18][19].

Events of proteasome degradation are common and crucial to spermatogenesis [20]. First of all, male germ cells have diverse proteasome isoforms, which possess specialized activities and exert their specific roles in protein degradation at distinct stages of spermatogenesis. Secondly, massive proteomic alterations take place during spermatogenic processes. For example, H2B dissociation from the chromosomes upon ubiquitination is important for both homologous recombination and meiotic double strand break (DSB) repair [21]. The dissociation of ubiquitinated H2A and H2B also ensures successful histone-to-protamine replacement during spermatid chromatin condensation [22]. In addition to histones, the tremendous loss of mitochondria in elongating spermatids is mostly carried out by the 26S proteasome as the form of enfolded cytoplasmic lobe, which contains both mitochondria and other organelles [23][24]. Single mitochondrial degradation also occurs when its outer membrane proteins (e.g., Mitofusins) are ubiquitinated and presented to the 26S proteasome [25]. Dissociation of mitochondrial outer membrane exposes its inner contents (e.g., prohibitin) for further degradation [24]. Finally, mutations of many UPS-related genes, including genes encoding E3 ubiquitin ligases, DUBs and proteasome components, results in dysregulated spermatogenesis and male infertility in humans and mice.

2.E3 Ubiquitin Ligases in Spermatogenesis

2.1. Cullin-Ring Ligase Family

Cullin-RING Ligases (CRLs) family of multi-subunit E3 ligase complexes is the largest E3 ligase family [26]. In CRL complex, Cullin protein is the scaffold protein, recruiting substrate-targeting modules on its N-terminal for substrate-targeting specificity and RING finger proteins, usually ROC1/2, on its C-terminal for E2 combination. The diversity of CRLs depends mainly on the substrate-targeting module, i.e., the adaptor protein and the substrate receptor. The mammalian Cullin protein family comprises CUL1 to 7, as well as PARC. These CRLs are involved in multiple biological processes like cell-cycle control, DNA replication and developmental regulation [27]. Among them, CRL1, CRL2, CRL3, CRL4A and CRL4B have been identified to be spermatogenesis-related.

The CRL1 complex, or SCFβ-TrCP, is the most known E3 ubiquitin ligase and is expressed ubiquitously in all cell types. This E3 complex comprises the scaffold protein CUL1, the adaptor protein SKP1 (S phase kinase-associated protein), the substrate receptor β-TrCP and the E2-interacting protein ROC1/2. There are two paralogs of β-TrCP in mammals, β-TrCP1 (BTRC) and β-TrCP2 (FBXW11) [28]. β-TrCP1 is expressed in spermatogonia at medium level but highly expressed in spermatocytes. In contrast, β-TrCP2 shows medium expression level in spermatogonia and low expression level in meiotic cells. β-TrCP1 deficiency in mice leads to prolonged and abnormal meiosis as indicated by accumulating MI spermatocytes and multinucleated spermatids [29]. In these β-TrCP1-deleted spermatocytes, stabilization of EMI1 is identified. EMI1 is a *bona fide* substrate of β-TrCP1, regulating metaphase to anaphase progression in both mitosis and meiosis via its inhibitive effects on the anaphase promoting complex/cyclosome, or APC/C [30]. While to progress through and exit from metaphase, APC/C needs to be reactivated, depending on EMI1 degradation. However, in spermatocytes lacking β-TrCP1, APC/C complex is inhibited by the accumulated level of EMI1. On the other hand, β-TrCP2 deficiency alone doesn't cause any defects in spermatogenesis since β-TrCP1 is preserved at sufficient functional levels in all types of cells for compensation [28]. Double deletion of β-TrCP1 and β-TrCP2 leads to severe testis abnormality as indicated by absence of spermatocytes, spermatids and mature sperm. Surprisingly, these mice show dislocated spermatogonia in their seminiferous tubules, which is not observed in mice carrying either single deletion [28]. Snail1 is another substrate of β-TrCP, transcriptionally controlling the level of E-cadherin [31]. Deficiency of β-TrCP1 and β-TrCP2 results in a significantly increased level of Snail1 in these dislocated spermatogonia. As a consequence, accumulated Snail1 reduces E-cadherin and thereby disrupts the integrity of adherent junctions and the trafficking of differentiating cells from the basement of seminiferous tubules to the lumen [28].

Similar to the SCF complex, CRL4 complex is made up of the scaffold protein CUL4A/B, the adaptor protein DDB1 (DNA damage binding protein 1), the substrate receptor (DDB1- and CUL4-associated factors, DCAFs) and ROC1/2 [32]. CUL4A is predominantly expressed in primary spermatocytes at the pachytene and diplotene stages [33]. γH2AX indicates the sites of DSBs in meiotic prophase I and can be only detected on XY chromosome pairs in pachytene and diplotene spermatocytes. However, CUL4A deficiency leads to persistent γH2AX signals in mouse pachytene spermatocytes [34]. Similarly, foci of the late homologous recombination protein, MLH1, which are supposed to disappear from diplotene, remain detectable on chromosome pairs of diplotene spermatocytes [33]. As a consequence, significant decrease of post-meiotic spermatids and accumulation of malformed meiotic spermatocytes are evident in *Cul4a* knockout testes [33][34]. In contrast to CUL4A, CUL4B is expressed in spermatogonia and spermatids, predominantly regulating post-meiotic differentiation to guarantee sperm motility [33]. Although CUL4B-deficient mice is still proficient in producing spermatids, most of their spermatozoa possess low motility or become completely immotile, indicating normal meiosis but impaired post-meiotic specification [35]. Diminished mitochondrial activity with low level of ATP production and missing, superabundant or misshapen microtubule doublets on the flagella axonemes are found in spermatozoa derived from

CUL4B-deficient mice. DDB1 is the adaptor protein and deletion of which abolishes the activity of all CRL4 complexes [32][36]. Strikingly, conditional deletion of DDB1 in male germ cells with a *Ddx4-Cre* results in complete loss of male germ cells [37]. DCAF12 is one of the substrate receptors of the CRL4 complexes and directly interacts with the Moloney Leukemia Virus 10 (MOV10) [38]. DCAF12 deficiency leads to decreased sperm counts accompanied by elevated level of MOV10. MOV10 is a RNA helicase, included in RNA-induced Silencing Complex (RISC), and plays a role in RNA interference and silencing of transposons, virus, and recently duplicated genes [39]. Increased amount of MOV10 results in the abnormal lower expression of two key meiotic proteins, SYCP3 and γH2AX, suggesting that CRL4A-DCAF12 mediates the degradation of MOV10 during spermatogenesis in a UPS dependent manner to maintain proper levels of meiotic-associated proteins. However, the entire substrates of CRL4 complexes in spermatogenesis remain largely elusive.

Besides SCF and CRL4 complexes, CRL2 and CRL3 also exhibit certain roles in spermatogenesis in *C. elegans* or *Drosophila*. In *C. elegans*, CUL2 is highly expressed in germline and participates in multiple developmental processes in complex with distinct substrate receptors [40]. The Leucine Rich Repeat protein LRR-1, a substrate receptor protein of CRL2, is expressed throughout the germline [41]. In testis, CUL2 associates with LRR-1 to assembly the CRL2 complex and acts with the 26S proteasome to degrade HTP-3, which is a meiotic-related protein recruiter in the mitotic zone of the germline, preparing chromosomes for meiosis [40][42]. Normally, HTP-3 expression shows a low-to-high gradient from the most distal germ cells to the most proximal ones. However, dysfunction of the CRL2 complex leads to uniform distribution of HTP-3 along *C. elegans* gonads, resulting in premature meiotic spermatocytes [42].

Drosophila has four CUL3 isoforms and one of them is expressed exclusively in male germ cells (CUL3testis), playing an essential role during post-meiotic differentiation. CUL3testis combines with its adaptor protein Klhl10 and targets dBruce, a caspase inhibitor for degradation [43][44]. This CRL3 E3 ubiquitin ligase complex thereby ensures active function of apoptotic proteins to eliminate superfluous cytoplasm as well as unwanted organelles during post-meiotic differentiation. In mice, CUL3 emerges from post-meiotic differentiation step 9 and keeps detectable through the remaining stages of this process [44]. However, the specific roles of mammalian CUL3 during spermatogenesis remain unsolved since CUL3 deficiency in mice unexpectedly leads to early embryonic lethality by interfering cell cycle progression [45].

2.2. Other E3 Ubiquitin Ligases

Besides the CRLs family, many other E3 ubiquitin ligases also participate in the regulation of spermatogenesis. In addition to be regulated by the SCF-EMI1 ubiquitination pathway, APC/C itself is an E3 ubiquitin ligase complex and mediates poly-ubiquitination of substrates for proteasomal degradation [46]. During mitosis, APC/C associates with the spindle apparatus to promote proper separation of sister chromatids by mediating cyclin B1 and securin degradation [47]. In addition, APC/C functions to degrade proteins that are necessary for S phase and inactivation of APC/C has been proposed as the commitment point for cell-cycle entry [48]. It is likely that these functions also apply to meiosis, albeit not yet fully characterized. However, a unique role of APC/C in meiosis has been proposed as mediating piRNA-triggered PIWI destruction in late spermatids (both elongating and elongated spermatids) [49]. PIWI-interacting RNA (piRNA) is a novel germline-specific small noncoding RNA and cooperates with PIWI to maintain genome integrity by silencing transposons [50]. Spermatocytes and round spermatids show high level of piRNA/PIWI, while this level begins to decrease in late spermatids, and is ultimately eliminated in mature sperm. Knockdown of endogenous APC/C abolishes PIWI ubiquitination and degradation in late spermatids, resulting in maturation stagnation of these cells [49]. Besides, piRNA is synchronously degraded with PIWI in late spermatids, suggesting a feedforward mechanism for their elimination. That is, piRNA binds to PIWI during late spermatogenesis, causing a conformational change of PIWI, which facilitates its binding to APC/C. *Meanwhile*, PIWI degradation in turn leaves the piRNA unprotected and therefore primed for elimination [49].

SIAH1A confers cell growth arrest in somatic cells, while in male germ cells, it plays an important role to promote spermatogenesis progression beyond MI [51]. Siah1a deficiency in mice induces severe impact on spermatogenesis characterized by large amounts of spermatocytes accumulated at metaphase to telophase of meiosis I. Besides, anaphase cells are largely bi- or multi-nucleated because of incomplete chromosome segregation [52]. Since ubiquitin-dependent degradation of anaphase inhibitors such as securin is crucial for the transition from metaphase to anaphase [53], it is likely that E3 ubiquitin ligase SIAH1A also targets these proteins as substrates to facilitate anaphase entry.

HEI10 is an E3 ubiquitin ligase important for the regulation of homologous recombination during pachytene [54]. Following DSB repair mediated by RAD51, HEI10 mediates the degradation of CCNB3 to free CDK2. In HEI10 mutant mouse spermatocytes, homologous recombination initiates normally with proper DSB formation and repair. However, these spermatocytes fail to form chiasmata which leads to premature homolog separation, and ultimately undergo meiotic arrest at MI or apoptosis [55].

RNF8, or RING finger protein 8, is essentially a DNA damage response (DDR) protein that ubiquitinates histone H2A and H2AX to facilitate downstream recruitment of DDR factors to DSB-flanking chromosomes [56]. In pachytene spermatocytes, ubiquitinated H2AX (ubH2AX) is significantly enriched in the XY body, a process known as meiotic sex chromosome inactivation, or MSCI [57]. RNF8 deficiency leads to severe loss of ubH2AX in the XY body, indicating a role of RNF8 in ubiquitinating H2AX during this process. However, neither XY body formation nor the subsequent meiosis is affected upon RNF8 deficiency, indicating that RNF8 may facilitate but is not necessary for MSCI or meiosis. In contrast, RNF8 is indispensable during post-meiotic differentiation, when nucleosome removal occurs accompanied by H2A and H2B ubiquitination [56]. RNF8 deficiency in mice leads to reduced ubH2A and ubH2B in elongating spermatids as well as impaired histone-protamine transition. Sperm production in RNF8-deficient mice is significantly impaired with accumulation of defective sperm possessing malformed sperm heads [56]. Notably, although RNF8 itself has no acetyltransferase activity, it is supposed that RNF8 indirectly regulates mammalian acetyltransferase MOF through ubH2A and ubH2B. Therefore, RNF8 deficiency leads to decreased MOF level as well as H4 acetylation [58]. RNF8 also participates in epigenetic modification in post-meiotic spermatids. One study has shown that through H2A ubiquitination, RNF8 is able to achieve active epigenetic modifications such as H3K4 dimethylation (H3K4me2), which further activate escaped genes from silenced sex chromosomes in post-meiotic spermatids [59].

UBR2 also targets histone H2A for ubiquitination conjugation. UBR2 primarily localizes to the unsynapsed chromatin regions where it transfers ubiquitin from its E2 enzyme HR6B to histone H2A, mediating meiotic silencing of unsynapsed chromatin (MSUC) [60]. Furthermore, UBR2-mediated histone ubiquitination is linked to a pachytene checkpoint. UBR2 deficiency impairs multiple meiotic processes such as homologous recombination, MSUC and synapsis, which trigger meiotic arrest through the pachytene checkpoint system [61][62][63].

3. Protein Deubiquitination in Spermatogenesis

Deubiquitinases (DUBs) are cysteine proteases, which remove and recycle ubiquitin. Till now, more than 100 DUB enzymes have been identified in human [64]. DUBs participate in various biological processes such as apoptosis, cell cycle progression, and protection of proteins from degradation. DUBs are classified into six catalogues: USP (ubiquitin-specific processing proteases), UCH (ubiquitin C-terminal hydrolases), JAMM (Jab1/Pab1/MPN domain-containing metalloenzymes), OUT (Otu-domain ubiquitin aldehyde-binding proteins), MCPIPs (Monocyte chemotactic protein-induced proteases) and Ataxin-3/Josephin [65]. Some DUBs have been demonstrated to play a requisite role during spermatogenesis, which largely belong to the USP and UCH family [64].

USP7 localizes preferentially to the XY body in early pachytene spermatocytes but gradually decreases its level as meiosis progresses, which is highly concurrent with the expression manner of SCML2, a testis-specific polycomb protein [66]. Biochemically, USP7 forms a complex with SCML2 to counteract histone H2A ubiquitination in the XY chromatin during meiosis. In SCML2-deficient mice, USP7 is absent from H2A ubiquitination sites, leading to augmented H2A monoubiquitination, which in turn causes spermatogenic impairments characterized by massive pachytene spermatocyte apoptosis [66].

USP8 is expressed in both brain and testis and interacts with MSJ1, STAM2, EEA1 and VSP54 [67]. In mouse testis, USP8 shows remarkable increase during post-meiotic differentiation and forms small plaques. USP8 localizes specifically to the nuclear envelope of round spermatids, as well as the centrosome and acrosome vesicle of elongating spermatids [68]. In mouse spermatids, USP8 associates with MSJ1 and the 20S CP to move towards the developing acrosome and centrosome [67][69]. STAM2 gives rise to the endosomal-sorting complex ESCRT-0 and EEA1 is an early endosome antigen. Colocalization of USP8 with STAM2 and EEA1 is found at acrosomal vacuole and acrosome surface, respectively [70]. VSP54 is responsible for retrograde transport from early endosomes, and during post-meiotic differentiation, it follows the same migration trajectory as USP8 until complete acrosome formation is achieved [71]. Taken together, these results suggest that USP8 is a key participant of the endosome pathway during acrosome generation.

USP2 is restrictedly expressed in elongating spermatids, possessing speculative function during post-meiotic differentiation. USP2 deficiency in mice leads to defective sperm whose motility is highly vulnerable to environmental changes [72]. Besides, these sperm exhibit poor fertilization capacity due to failure in binding or penetrating to the zona pellucida. USP14 is important for post-meiotic differentiation. In *Drosophila*, USP14 deficiency impairs spermatid individualization, during which syncytial spermatids are separated into individual cells [73]. USP14 deficiency causes loss of synchronization and abnormal distribution of the actin cones. Besides, USP14-deficient mice exhibit significantly reduced sperm counts as well as severe sperm malformation such as multiple nucleus, missing of sperm head or dual sperm tails [74].

Mammalian USP9X is the functional orthologue of *drosophila* deubiquitinating enzyme fat facets (Faf), which interacts with and impedes the degradation of a DEAD-box RNA helicase Vasa, a highly-conserved marker for germ cells [75]. USP9X is predominantly expressed in spermatogonia and weakly expressed in early spermatocytes before pachytene stage. USP9X-deficiency in mouse germ cells leads to male infertility with various abnormalities along the progression of spermatogenesis: reduced number of spermatocytes, degenerated spermatids with residual body-like structures, as well as aberrantly retained mature spermatozoa in seminiferous tubules [76]. However, the maintenance and proliferation of spermatogonia were less affected in USP9X-deficient testes, indicating a critical role of USP9X since mitosis-to-meiosis transition in male germ cells.

USP26 has been considered as a potential infertility gene due to its restricted expression in mammalian testis and X-chromosome-localization (single copy in males) [77][78][79]. Several studies have reported the polymorphisms in *USP26* associated with non-obstructive azoospermia or asthenozoospermia, suggesting a causal relationship with human male infertility. However, most of the identified *USP26* polymorphisms can't disrupt its enzymatic function [80]. In addition, *USP26* is indispensable for mouse fertility in both sexes [81]. It has recently been found the effects of *USP26* mutation on male fertility dependent on the genetic background of mice [82]. *USP26* mutants in DBA/2, rather than C57BL/6 background exhibit impaired spermatogenesis with obvious deficiency and malformation of spermatozoa, resulting in infertile or sub-fertile males. These results implicate that strain-specific genetic components interact with *USP26* mutations to interfere spermatogenesis; such components may also exist in human but further investigations are needed.

UCHL3 expression exhibits differentiation-dependent pattern during spermatogenesis with minimal amount in spermatogonia but to a sequentially increasing extent in meiotic pachytene spermatocytes and post-meiotic spermatids [83]. Besides, a later study has detected intensive UCHL3 expression in sperm acrosomes and flagella, suggesting the role of UCHL3 in regulating both meiosis and post-meiotic differentiation [84]. According to the results, OA patients show significantly lower UCHL3 content and activity as compared to normozoospermia controls; UCHL3 condition is slightly better in A patients but still far from normal level [84]. Meanwhile, it is proposed that the amount and activity of UCHL3 are positively related to a series of fertility indicators including sperm counts, sperm concentration and sperm motility, highlighting the importance of this DUB during spermatogenesis.

UCHL1 shares high sequence similarity with UCHL3, however, its distribution pattern in male germ cells is totally different, indicating distinct function during spermatogenesis [85]. Predominant UCHL1 expression is found in spermatogonia as well as in Sertoli cells. The level of UCHL1 precisely determines spermatogonia fate of either self-renewal or meiotic differentiation as indicated by two markers Plzf and c-Kit, respectively [85]. In mouse testis, UCHL1 overexpression leads to drastic loss of post-meiotic germ cells and a large amount of arrested pachytene spermatocytes retards in seminiferous tubules which further undergo apoptosis [86]. On the contrary, UCHL1 deficiency significantly increases the number of spermatogonia and preleptotene spermatocytes while immensely inhibits germ cell apoptosis during the first round of spermatogenesis. Additionally, these mice have reduced sperm motility and more abnormal spermatozoa even though complete sterility is not observed [87]. Taken together, UCHL1 and UCHL3 express strongly but reciprocally during spermatogenesis, where the former mediates fate determination of spermatogonia as well as elimination of defective spermatozoa to maintain testicular homeostasis, while the latter facilitates post meiotic maturation to produce fertilization-competent spermatozoa.

Besides the DUBs in USP and UCH families, CYLD (cylindromatosis) is also found to be required for spermatogenesis by regulating early wave of germ cell apoptosis mainly in spermatogonia, a requisite process to eliminate excessive germ cells and thereby maintain the balance between germ cells and Sertoli cells [88]. CYLD directly deubiquitinates the receptor-interacting protein 1 (RIP1), to prevent the activation of IKK and NF-κB signaling and the expression of downstream anti-apoptotic genes, ultimately promote cell apoptosis [89]. Loss of CYLD leads to reduced spermatozoa, failure in radial organization of round spermatids, as well as malformation of acrosomes in elongating spermatids. On the contrary, spermatogonia and early spermatocytes are aberrantly accumulated in CYLD-deficient seminiferous tubules.

References

1. Chen, Y.; Zheng, Y.; Gao, Y.; Lin, Z.; Yang, S.; Wang, T.; Wang, Q.; Xie, N.; Hua, R.; Liu, M.; et al. Single-cell RNA-seq uncovers dynamic processes and critical regulators in mouse spermatogenesis. Cell Res. 2018, 28, 879–896.
2. Soumillon, M.; Necsulea, A.; Weier, M.; Brawand, D.; Zhang, X.; Gu, H.; Barthès, P.; Kokkinaki, M.; Nef, S.; Gnirke, A.; et al. Cellular source and mechanisms of high transcriptome complexity in the mammalian testis. Cell Rep. 2013, 3, 2179–2190.

3. Glickman, M.H.; Ciechanover, A. The ubiquitin-proteasome proteolytic pathway: Destruction for the sake of construction. Physiol. Rev. 2002, 82, 373–428.
4. Sadowski, M.; Suryadinata, R.; Tan, A.R.; Roesley, S.N.A.; Sarcevic, B. Protein monoubiquitination and polyubiquitination generate structural diversity to control distinct biological processes. IUBMB Life 2012, 64, 136–142.
5. Hochstrasser, M. Ubiquitin-dependent protein degradation. Annu. Rev. Genet. 1996, 30, 405–439.
6. Nijman, S.M.B.; Luna-Vargas, M.P.A.; Velds, A.; Brummelkamp, T.R.; Dirac, A.M.G.; Sixma, T.K.; Bernards, R. A genomic and functional inventory of deubiquitinating enzymes. Cell 2005, 123, 773–786.
7. Tanaka, K. The proteasome: Overview of structure and functions. Proc. Jpn. Acad. Ser. B Phys. Biol. Sci. 2009, 85, 12–36.
8. Huang, X.; Luan, B.; Wu, J.; Shi, Y. An atomic structure of the human 26S proteasome. Nat. Struct. Mol. Biol. 2016, 23, 778–785.
9. Groll, M.; Ditzel, L.; Löwe, J.; Stock, D.; Bochtler, M.; Bartunik, H.D.; Huber, R. Structure of 20S proteasome from yeast at 2.4 Å resolution. Nature 1997, 386, 463–471.
10. Tanaka, K.; Kasahara, M. The MHC class I ligand-generating system: Roles of immunoproteasomes and the interferon-gamma-inducible proteasome activator PA28. Immunol. Rev. 1998, 163, 161–176.
11. Zhang, Q.; Ji, S.-Y.; Busayavalasa, K.; Shao, J.; Yu, C. Meiosis I progression in spermatogenesis requires a type of testis-specific 20S core proteasome. Nat. Commun. 2019, 10, 3387.
12. Gómez-H, L.; Felipe-Medina, N.; Condezo, Y.B.; Garcia-Valiente, R.; Ramos, I.; Suja, J.A.; Barbero, J.L.; Roig, I.; Sánchez-Martín, M.; de Rooij, D.G.; et al. The PSMA8 subunit of the spermatoproteasome is essential for proper meiotic exit and mouse fertility. PLoS Genet. 2019, 15, e1008316.
13. Zhang, Z.-H.; Jiang, T.-X.; Chen, L.-B.; Zhou, W.; Liu, Y.; Gao, F.; Qiu, X.-B. Proteasome subunit alpha4s is essential for formation of spermatoproteasomes and histone degradation during meiotic DNA repair in spermatocytes. J. Biol. Chem. 2021, 296, 100130.
14. Uechi, H.; Hamazaki, J.; Murata, S. Characterization of the testis-specific proteasome subunit alpha4s in mammals. J. Biol. Chem. 2014, 289, 12365–12374.
15. Asher, G.; Reuven, N.; Shaul, Y. 20S proteasomes and protein degradation “by default”. Bioessays 2006, 28, 844–849.
16. Tenzer, S.; Stoltze, L.; Schönfisch, B.; Dengjel, J.; Müller, M.; Stevanović, S.; Rammensee, H.-G.; Schild, H. Quantitative analysis of prion-protein degradation by constitutive and immuno-20S proteasomes indicates differences correlated with disease susceptibility. J. Immunol. 2004, 172, 1083–1091.
17. Coux, O.; Zieba, B.A.; Meiners, S. The proteasome system in health and disease. Adv. Exp. Med. Biol. 2020, 1233, 55–100.
18. Preckel, T.; Fung-Leung, W.P.; Cai, Z.; Vitiello, A.; Salter-Cid, L.; Winqvist, O.; Wolfe, T.G.; Von Herrath, M.; Angulo, A.; Ghazal, P.; et al. Impaired immunoproteasome assembly and immune responses in PA28-/- mice. Science 1999, 286, 2162–2165.
19. Khor, B.; Bredemeyer, A.L.; Huang, C.-Y.; Turnbull, I.R.; Evans, R.; Maggi, L.B., Jr.; White, J.M.; Walker, L.M.; Carnes, K.; Hess, R.A.; et al. Proteasome activator PA200 is required for normal spermatogenesis. Mol. Cell. Biol. 2006, 26, 2999–3007.
20. 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.
21. Robzyk, K.; Recht, L.; Osley, M.A. Rad6-dependent ubiquitination of histone H2B in yeast. Science 2000, 287, 501–504.
22. Lewis, J.D.; Abbott, D.W.; Ausió, J. A haploid affair: Core histone transitions during spermatogenesis. Biochem. Cell Biol. 2003, 81, 131–140.
23. Hecht, N.B. The making of a spermatozoon: A molecular perspective. Dev. Genet. 1995, 16, 95–103.
24. Karbowski, M.; Youle, R.J. Regulating mitochondrial outer membrane proteins by ubiquitination and proteasomal degradation. Curr. Opin. Cell Biol. 2011, 23, 476–482.
25. Ziviani, E.; Tao, R.N.; Whitworth, A.J. Drosophila parkin requires PINK1 for mitochondrial translocation and ubiquitinates mitofusin. Proc. Natl. Acad. Sci. USA 2010, 107, 5018–5023.
26. Zheng, N.; Shabek, N. Ubiquitin ligases: Structure, function, and regulation. Annu. Rev. Biochem. 2017, 86, 129–157.
27. Sarikas, A.; Hartmann, T.; Pan, Z.-Q. The cullin protein family. Genome Biol. 2011, 12, 220.

28. Kanarek, N.; Horwitz, E.; Mayan, I.; Leshets, M.; Cojocaru, G.; Davis, M.; Tsuberi, B.Z.; Pikarsky, E.; Pagano, M.; Ben-Neriah, Y. Spermatogenesis rescue in a mouse deficient for the ubiquitin ligase SCFβ-TrCP by single substrate depletion. Genes Dev. 2010, 24, 470–477.
29. Guardavaccaro, D.; Kudo, Y.; Boulaire, J.; Barchi, M.; Busino, L.; Donzelli, M.; Margottin-Goguet, F.; Jackson, P.K.; Yamasaki, L.; Pagano, M. Control of meiotic and mitotic progression by the F box protein β-Trcp1 in vivo. Dev. Cell 2003, 4, 799–812.
30. Hsu, J.Y.; Reimann, J.D.R.; Sørensen, C.S.; Lukas, J.; Jackson, P.K. E2F-dependent accumulation of hEmi1 regulates S phase entry by inhibiting APC(Cdh1). Nat. Cell Biol. 2002, 4, 358–366.
31. Zhou, B.P.; Deng, J.; Xia, W.; Xu, J.; Li, Y.M.; Gunduz, M.; Hung, M.-C. Dual regulation of Snail by GSK-3β-mediated phosphorylation in control of epithelial-mesenchymal transition. Nat. Cell Biol. 2004, 6, 931–940.
32. Yu, C.; Zhang, Y.-L.; Pan, W.-W.; Li, X.-M.; Wang, Z.-W.; Ge, Z.-J.; Zhou, J.-J.; Cang, Y.; Tong, C.; Sun, Q.-Y.; et al. CRL4 complex regulates mammalian oocyte survival and reprogramming by activation of TET proteins. Science 2013, 342, 1518–1521.
33. Yin, Y.; Lin, C.; Kim, S.T.; Roig, I.; Chen, H.; Liu, L.; Veith, G.M.; Jin, R.U.; Keeney, S.; Jasin, M.; et al. The E3 ubiquitin ligase cullin 4A regulates meiotic progression in mouse spermatogenesis. Dev. Biol. 2011, 356, 51–62.
34. Kopanja, D.; Roy, N.; Stoyanova, T.; Hess, R.A.; Bagchi, S.; Raychaudhuri, P. Cul4A is essential for spermatogenesis and male fertility. Dev. Biol. 2011, 352, 278–287.
35. Yin, Y.; Liu, L.; Yang, C.; Lin, C.; Veith, G.M.; Wang, C.; Sutovsky, P.; Zhou, P.; Ma, L. Cell autonomous and nonautonomous function of CUL4B in mouse spermatogenesis. J. Biol. Chem. 2016, 291, 6923–6935.
36. Yu, C.; Ji, S.-Y.; Sha, Q.-Q.; Sun, Q.-Y.; Fan, H.-Y. CRL4-DCAF1 ubiquitin E3 ligase directs protein phosphatase 2A degradation to control oocyte meiotic maturation. Nat. Commun. 2015, 6, 8017.
37. Yu, J.; Lan, X.; Chen, X.; Yu, C.; Xu, Y.; Liu, Y.; Xu, L.; Fan, H.-Y.; Tong, C. Protein synthesis and degradation are essential to regulate germline stem cell homeostasis in Drosophila testes. Development 2016, 143, 2930–2945.
38. Lidak, T.; Baloghova, N.; Korinek, V.; Sedlacek, R.; Balounova, J.; Kasparek, P.; Cermak, L. CRL4-DCAF12 ubiquitin ligase controls MOV10 RNA helicase during spermatogenesis and T cell activation. Int. J. Mol. Sci. 2021, 22, 5394.
39. Fischer, S.E.J.; Ruvkun, G. Caenorhabditis elegans ADAR editing and the ERI-6/7/MOV10 RNAi pathway silence endogenous viral elements and LTR retrotransposons. Proc. Natl. Acad. Sci. USA 2020, 117, 5987–5996.
40. Burger, J.; Merlet, J.; Tavernier, N.; Richaudeau, B.; Arnold, A.; Ciosk, R.; Bowerman, B.; Pintard, L. CRL2(LRR-1) E3-ligase regulates proliferation and progression through meiosis in the Caenorhabditis elegans germline. PLoS Genet. 2013, 9, e1003375.
41. Merlet, J.; Burger, J.; Tavernier, N.; Richaudeau, B.; Gomes, J.-E.; Pintard, L. The CRL2LRR-1ubiquitin ligase regulates cell cycle progression during C. elegans development. Development 2010, 137, 3857–3866.
42. Goodyer, W.; Kaitna, S.; Couteau, F.; Ward, J.D.; Boulton, S.J.; Zetka, M. HTP-3 links DSB formation with homolog pairing and crossing over during C. elegans meiosis. Dev. Cell 2008, 14, 263–274.
43. Arama, E.; Bader, M.; Rieckhof, G.E.; Steller, H. A ubiquitin ligase complex regulates caspase activation during sperm differentiation in Drosophila. PLoS Biol. 2007, 5, e251.
44. Wang, S.H.; Zheng, H.L.; Esaki, Y.; Kelly, F.; Yan, W. Cullin3 is a KLHL10-interacting protein preferentially expressed during late spermiogenesis. Biol. Reprod. 2006, 74, 102–108.
45. Singer, J.D.; Gurian-West, M.; Clurman, B.; Roberts, J.M. Cullin-3 targets cyclin E for ubiquitination and controls S phase in mammalian cells. Genes Dev. 1999, 13, 2375–2387.
46. Peters, J.-M. The anaphase promoting complex/cyclosome: A machine designed to destroy. Nat. Rev. Mol. Cell Biol. 2006, 7, 644–656.
47. Lara-Gonzalez, P.; Moyle, M.W.; Budrewicz, J.; Mendoza-Lopez, J.; Oegema, K.; Desai, A. The G2-to-M transition is ensured by a dual mechanism that protects cyclin B from degradation by Cdc20-activated APC/C. Dev. Cell 2019, 51, 313–325.e10.
48. Cappell, S.D.; Chung, M.; Jaimovich, A.; Spencer, S.L.; Meyer, T. Irreversible APCCdh1 inactivation underlies the point of no return for cell cycle entry. Cell 2016, 166, 167–180.
49. Zhao, S.; Gou, L.-T.; Zhang, M.; Zu, L.-D.; Hua, M.-M.; Hua, Y.; Shi, H.-J.; Li, Y.; Li, J.; Li, D.; et al. piRNA-triggered MIWI ubiquitination and removal by APC/C in late spermatogenesis. Dev. Cell 2013, 24, 13–25.
50. Siomi, M.C.; Sato, K.; Pezic, D.; Aravin, A.A. PIWI-interacting small RNAs: The vanguard of genome defence. Nat. Rev. Mol. Cell Biol. 2011, 12, 246–258.

51. Liu, J.; Stevens, J.; Rote, C.A.; Yost, H.J.; Hu, Y.X.; Neufeld, K.L.; White, R.L.; Matsunami, N. Siah-1 mediates a novel beta-catenin degradation pathway linking p53 to the adenomatous polyposis coli protein. Mol. Cell 2001, 7, 927–936.

52. Dickins, R.A.; Frew, I.J.; House, C.M.; O'Bryan, M.K.; Holloway, A.J.; Haviv, I.; Traficante, N.; de Kretser, D.M.; Bowtell, D.D.L. The ubiquitin ligase component Siah1a is required for completion of meiosis I in male mice. Mol. Cell Biol. 2002, 22, 2294–2303.

53. Singleton, M.R.; Uhlmann, F. Separase-securin complex: A cunning way to control chromosome segregation. Nat. Struct. Mol. Biol. 2017, 24, 337–339.

54. Ward, J.O.; Reinholdt, L.G.; Motley, W.W.; Niswander, L.M.; Deacon, D.C.; Griffin, L.B.; Langlais, K.K.; Backus, V.L.; Schimenti, K.J.; O'Brien, M.J.; et al. Mutation in mouse hei10, an E3 ubiquitin ligase, disrupts meiotic crossing over. PLoS Genet. 2007, 3, e139.

55. Qiao, H.; Prasada Rao, H.B.D.; Yang, Y.; Fong, J.H.; Cloutier, J.M.; Deacon, D.C.; Nagel, K.E.; Swartz, R.K.; Strong, E.; Holloway, J.K.; et al. Antagonistic roles of ubiquitin ligase HEI10 and SUMO ligase RNF212 regulate meiotic recombination. Nat. Genet. 2014, 46, 194–199.

56. Lu, L.-Y.; Wu, J.; Ye, L.; Gavrilina, G.B.; Saunders, T.L.; Yu, X. RNF8-dependent histone modifications regulate nucleosome removal during spermatogenesis. Dev. Cell 2010, 18, 371–384.

57. Turner, J.M.A. Meiotic sex chromosome inactivation. Development 2007, 134, 1823–1831.

58. Sonnack, V.; Failing, K.; Bergmann, M.; Steger, K. Expression of hyperacetylated histone H4 during normal and impaired human spermatogenesis. Andrologia 2002, 34, 384–390.

59. Sin, H.S.; Barski, A.; Zhang, F.; Kartashov, A.V.; Nussenzweig, A.; Chen, J.; Andreassen, P.R.; Namekawa, S.H. RNF8 regulates active epigenetic modifications and escape gene activation from inactive sex chromosomes in post-meiotic spermatids. Genes Dev. 2012, 26, 2737–2748.

60. An, J.Y.; Kim, E.-A.; Jiang, Y.; Zakrzewska, A.; Kim, D.E.; Lee, M.J.; Mook-Jung, I.; Zhang, Y.; Kwon, Y.T. UBR2 mediates transcriptional silencing during spermatogenesis via histone ubiquitination. Proc. Natl. Acad. Sci. USA 2010, 107, 1912–1917.

61. Kwon, Y.T.; Xia, Z.; An, J.Y.; Tasaki, T.; Davydov, I.V.; Seo, J.W.; Sheng, J.; Xie, Y.; Varshavsky, A. Female lethality and apoptosis of spermatocytes in mice lacking the UBR2 ubiquitin ligase of the N-end rule pathway. Mol. Cell Biol. 2003, 23, 8255–8271.

62. Ouyang, Y.; Kwon, Y.T.; An, J.Y.; Eller, D.; Tsai, S.-C.; Diaz-Perez, S.; Troke, J.J.; Teitell, M.A.; Marahrens, Y. Loss of Ubr2, an E3 ubiquitin ligase, leads to chromosome fragility and impaired homologous recombinational repair. Mutat. Res. 2006, 596, 64–75.

63. An, J.Y.; Kim, E.; Zakrzewska, A.; Yoo, Y.D.; Jang, J.M.; Han, D.H.; Lee, M.J.; Seo, J.W.; Lee, Y.J.; Kim, T.Y.; et al. UBR2 of the N-end rule pathway is required for chromosome stability via histone ubiquitylation in spermatocytes and somatic cells. PLoS ONE 2012, 7, e37414.

64. Suresh, B.; Lee, J.; Hong, S.-H.; Kim, K.-S.; Ramakrishna, S. The role of deubiquitinating enzymes in spermatogenesis. Cell Mol. Life Sci. 2015, 72, 4711–4720.

65. Mevissen, T.E.T.; Komander, D. Mechanisms of deubiquitinase specificity and regulation. Annu. Rev. Biochem. 2017, 86, 159–192.

66. Luo, M.; Zhou, J.; Leu, N.A.; Abreu, C.M.; Wang, J.; Anguera, M.C.; de Rooij, D.G.; Jasin, M.; Wang, P.J. Polycomb protein SCML2 associates with USP7 and counteracts Histone H2A ubiquitination in the XY chromatin during male meiosis. PLoS Genet. 2015, 11, e1004954.

67. Berruti, G.; Martegani, E. The deubiquitinating enzyme mUBPy interacts with the sperm-specific molecular chaperone MSJ-1: The relation with the proteasome, acrosome, and centrosome in mouse male germ cells. Biol. Reprod. 2005, 72, 14–21.

68. Berruti, G.; Martegani, E. mUBPy and MSJ-1, a deubiquitinating enzyme and a molecular chaperone specifically expressed in testis, associate with the acrosome and centrosome in mouse germ cells. Ann. N. Y. Acad. Sci. 2002, 973, 5–7.

69. Berruti, G.; Paiardi, C. USP8/UBPy-regulated sorting and the development of sperm acrosome: The recruitment of MET. Reproduction 2015, 149, 633–644.

70. Berruti, G.; Ripolone, M.; Ceriani, M. USP8, a regulator of endosomal sorting, is involved in mouse acrosome biogenesis through interaction with the spermatid ESCRT-0 complex and microtubules. Biol. Reprod. 2010, 82, 930–939.

71. Quenneville, N.R.; Chao, T.-Y.; McCaffery, J.M.; Conibear, E. Domains within the GARP subunit Vps54 confer separate functions in complex assembly and early endosome recognition. Mol. Biol. Cell 2006, 17, 1859–1870.
72. Bedard, N.; Yang, Y.; Gregory, M.; Cyr, D.G.; Suzuki, J.; Yu, X.; Chian, R.-C.; Hermo, L.; O'Flaherty, C.; Smith, C.E.; et al. Mice lacking the USP2 deubiquitinating enzyme have severe male subfertility associated with defects in fertilization and sperm motility. Biol. Reprod. 2011, 85, 594–604.
73. Kovács, L.; Nagy, A.; Pál, M.; Deák, P. Usp14 is required for spermatogenesis and ubiquitin stress responses in Drosophila melanogaster. J. Cell Sci. 2020, 133, jcs237511.
74. Crimmins, S.; Sutovsky, M.; Chen, P.-C.; Huffman, A.; Wheeler, C.; Swing, D.A.; Roth, K.; Wilson, J.; Sutovsky, P.; Wilson, S. Transgenic rescue of ataxia mice reveals a male-specific sterility defect. Dev. Biol. 2009, 325, 33–42.
75. Lasko, P. The DEAD-box helicase Vasa: Evidence for a multiplicity of functions in RNA processes and developmental biology. Biochim. Biophys. Acta. 2013, 1829, 810–816.
76. Kishi, K.; Uchida, A.; Takase, H.M.; Suzuki, H.; Kurohmaru, M.; Tsunekawa, N.; Kanai-Azuma, M.; Wood, S.A.; Kanai, Y. Spermatogonial deubiquitinase USP9X is essential for proper spermatogenesis in mice. Reproduction 2017, 154, 135–143.
77. Wosnitzer, M.S.; Mielnik, A.; Dabaja, A.; Robinson, B.; Schlegel, P.N.; Paduch, D.A. Ubiquitin specific protease 26 (USP26) expression analysis in human testicular and extragonadal tissues indicates diverse action of USP26 in cell differentiation and tumorigenesis. PLoS ONE 2014, 9, e98638.
78. Wang, P.J.; Page, D.C.; McCarrey, J.R. Differential expression of sex-linked and autosomal germ-cell-specific genes during spermatogenesis in the mouse. Hum. Mol. Genet. 2005, 14, 2911–2918.
79. Nishimune, Y.; Tanaka, H. Infertility caused by polymorphisms or mutations in spermatogenesis-specific genes. J. Androl. 2006, 27, 326–334.
80. Liu, Y.-L.; Zheng, J.; Mi, Y.-J.; Zhao, J.; Tian, Q.-B. The impacts of nineteen mutations on the enzymatic activity of USP26. Gene 2018, 641, 292–296.
81. Felipe-Medina, N.; Gómez-H, L.; Condezo, Y.B.; Sanchez-Martín, M.; Barbero, J.L.; Ramos, I.; Llano, E.; Pendás, A.M. Ubiquitin-specific protease 26 (USP26) is not essential for mouse gametogenesis and fertility. Chromosoma 2019, 128, 237–247.
82. Sakai, K.; Ito, C.; Wakabayashi, M.; Kanzaki, S.; Ito, T.; Takada, S.; Toshimori, K.; Sekita, Y.; Kimura, T. Usp26 mutation in mice leads to defective spermatogenesis depending on genetic background. Sci. Rep. 2019, 9, 13757.
83. Kwon, J.; Wang, Y.-L.; Setsuie, R.; Sekiguchi, S.; Sakurai, M.; Sato, Y.; Lee, W.-W.; Ishii, Y.; Kyuwa, S.; Noda, M.; et al. Developmental regulation of ubiquitin C-terminal hydrolase isozyme expression during spermatogenesis in mice. Biol. Reprod. 2004, 71, 515–521.
84. Wang, M.; Yu, T.; Hu, L.; Cheng, Z.; Li, M. Ubiquitin carboxy-terminal hydrolaseL3 correlates with human sperm count, motility and fertilization. PLoS ONE 2016, 11, e0165198.
85. Luo, J.; Megee, S.; Dobrinski, I. Asymmetric distribution of UCH-L1 in spermatogonia is associated with maintenance and differentiation of spermatogonial stem cells. J. Cell Physiol. 2009, 220, 460–468.
86. Wang, Y.-L.; Liu, W.Z.; Sun, Y.-J.; Kwon, J.; Setsuie, R.; Osaka, H.; Noda, M.; Aoki, S.; Yoshikawa, Y.; Wada, K. Overexpression of ubiquitin carboxyl-terminal hydrolase L1 arrests spermatogenesis in transgenic mice. Mol. Reprod. Dev. 2006, 73, 40–49.
87. Kwon, J.; Mochida, K.; Wang, Y.-L.; Sekiguchi, S.; Sankai, T.; Aoki, S.; Ogura, A.; Yoshikawa, Y.; Wada, K. Ubiquitin C-terminal hydrolase L-1 is essential for the early apoptotic wave of germinal cells and for sperm quality control during spermatogenesis. Biol. Reprod. 2005, 73, 29–35.
88. Rodriguez, I.; Ody, C.; Araki, K.; Garcia, I.; Vassalli, P. An early and massive wave of germinal cell apoptosis is required for the development of functional spermatogenesis. EMBO J. 1997, 16, 2262–2270.
89. Wright, A.; Reiley, W.W.; Chang, M.; Jin, W.; Lee, A.J.; Zhang, M.; Sun, S.-C. Regulation of early wave of germ cell apoptosis and spermatogenesis by deubiquitinating enzyme CYLD. Dev. Cell. 2007, 13, 705–716.