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# Role and Mechanism of the Ubiquitin-Proteasome Pathway in Germ Cell Meiosis (Postprint)

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## Abstract

The ubiquitin-proteasome pathway (UPP) represents the principal protein degradation pathway in eukaryotic cells and plays pivotal roles in diverse biological processes, including the regulation of protein-protein interactions, protein activity, signal transduction, and cell cycle progression. Research has demonstrated that UPP also exerts critical regulatory functions in meiotic homologous recombination, sex chromosome inactivation, meiotic resumption, and first polar body extrusion during gametogenesis in humans and animals. This review synthesizes the signal transduction and regulatory mechanisms of UPP in meiotic processes and gametogenesis of animal germ cells, aiming to provide a reference framework for future investigations.

## Full Text

### Roles of the Ubiquitin-Proteasome Pathway in Meiotic Division of Germ Cells and Its Mechanism

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**Abstract:** The ubiquitin-proteasome pathway (UPP) represents the primary protein degradation pathway in eukaryotic cells and plays a crucial regulatory role in various biological processes including protein-protein interactions, protein activity modulation, signal transduction, and cell cycle progression. Accumulating evidence demonstrates that UPP is also essential for regulating key events during gametogenesis in humans and animals, such as meiotic homologous recombination, sex chromosome inactivation, meiotic resumption, and first polar

body extrusion. This review summarizes the signal transduction and regulatory mechanisms of UPP during meiotic division and gametogenesis in animals, providing a reference for future research in this field.

**Keywords:** ubiquitin-proteasome pathway; ubiquitination; germ cells; meiotic division

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## 1 Overview of the UPP

The ubiquitin-proteasome pathway (UPP) mediates protein degradation in eukaryotes through a three-enzyme cascade that covalently attaches ubiquitin to target proteins. Beyond protein degradation, ubiquitination critically regulates protein stability [1], protein trafficking [2], cell differentiation [3], cell cycle progression [4], and physiological processes including lipid metabolism [5], muscle development [6], and neuronal morphogenesis [7]. Dysregulation of UPP leads to pathological conditions such as muscle atrophy [8], inflammatory responses [9], and testicular tumors [10]. Studies have confirmed that ubiquitin-related components and deubiquitinating enzymes are ubiquitously expressed during animal gametogenesis and play vital roles in meiotic processes including homologous recombination [11], meiotic sex chromosome inactivation [12], oocyte meiotic resumption [13], first polar body extrusion [14], and sperm-egg fusion [15]. Disruption of UPP or mutations in its components impair these biological processes and cause germ cell developmental defects. For example, during pachytene chromosome synapsis and recombination in primary spermatocytes, histone polyubiquitination occurs, and artificial knockout of histone ubiquitination leads to meiotic arrest and eventual infertility [16]. In round spermatids, histone ubiquitination facilitates protamine replacement [17]. Cullin4 (CUL4) deficiency in oocytes delays meiosis I resumption [18], while knockdown of ubiquitin-conjugating enzyme E2C (UBE2C) blocks first polar body extrusion and causes chromosome segregation defects [19]. Investigating the biological functions and mechanisms of UPP in animal gametogenesis not only advances our understanding of reproductive processes but also holds significant implications for treating human infertility and improving reproductive performance in livestock. This review summarizes the signal transduction and regulatory mechanisms of the ubiquitin-proteasome pathway during meiotic division and gametogenesis in animals.

### 2.1 Oocyte Meiosis I Resumption

After mammalian oogonia differentiate into primary oocytes during embryonic development, the cell cycle arrests at prophase of meiosis I (M I) until puberty, when oocytes resume meiosis to develop into fertilization-competent eggs. The anaphase-promoting complex/cyclosome (APC/C) plays a critical role in M I resumption. APC/C is a Cullin-RING ligase (CRL) that exerts E3 ligase activity by forming APC/C^Cdh1 or APC/C^Cdc20 complexes through association

with adaptor proteins CDC20 homolog 1 (Cdh1) and cell division cycle protein 20 (Cdc20), which bind substrates and present them to APC/C [23]. Cyclin B and securin are major APC/C substrates whose premature accumulation must be prevented during the meiotic arrest period. At puberty, resumption of meiosis involves Cyclin B accumulation and activation of the Cyclin B-Cdk1 complex (maturation promoting factor, MPF) through kinase/phosphatase regulation, leading to nuclear envelope breakdown and exit from M I prophase. This process is intimately associated with the fine-tuning of APC/C^Cdh1 activity [13,24]. Studies show that APC/C^Cdh1 is the predominant form in mammalian oocytes during M I prophase and prometaphase, and its activity is essential for slowing Cyclin B accumulation and maintaining the balance between securin and Cyclin B [25], thereby playing a crucial role in maintaining meiotic arrest [26]. Additionally, the E2 enzymes UBE2C, UBE2S, and UBE2D regulate APC/C activity during mouse oocyte meiosis, promoting chromosome segregation and spindle formation. Deletion of any of these enzymes reduces M I cytokinesis by 50%, while their overexpression doubles the cytokinesis rate, with high UBE2C activity driving M I resumption [27].

The Cullin family member CUL4 also plays important physiological roles during M I. Cullin proteins lack a complete RING domain but can form CRL E3 complexes by binding RING family proteins like RBX1/2 (ROC1/2) at their C-terminus to interact with E2 enzymes, while their N-terminus recruits different substrate recognition subunits via adaptor proteins to target specific substrates [28]. CUL4 forms the DDB1-CUL4-RBX1 E3 complex by binding RBX1 at its C-terminus and the adaptor protein DDB1 (damaged DNA binding protein 1) at its N-terminus [29]. DDB1 recruits the WD40 repeat-containing substrate recognition subunit DCAF1 (DDB1-CUL4 associated factor 1)/VPRBP to bind and degrade specific substrates. Studies show that DCAF1 recognizes the PP2A-A subunit of protein phosphatase 2A for polyubiquitination and proteasomal degradation. As a cell cycle regulator, PP2A-A functions during oocyte meiosis [18] and is required for maintaining normal arrest at the germinal vesicle (GV) stage of M I prophase. PP2A-A protein levels begin to decline at germinal vesicle breakdown (GVBD). Oocyte-specific deletion of DDB1 or DCAF1 in mice causes PP2A-A accumulation and delays meiosis I resumption, while CRL4 deficiency also inhibits homologous chromosome segregation [18].

### 2.2 First Polar Body Extrusion

Extrusion of the first polar body marks the completion of meiosis I in oocytes and is closely associated with APC/C^Cdc20 activity. Studies show that APC/C^Cdh1 is activated first during M I prophase and prometaphase, while Cdk1-mediated activation of APC/C^Cdc20 becomes the predominant active form during M I anaphase. APC/C^Cdc20 targets securin and Cyclin B1 for degradation, thereby regulating chromosome condensation/segregation and first polar body extrusion [14,30]. Cdk1 protease inhibitors cause Cdk1 inactivation and first polar body extrusion in oocytes, while inhibitor removal

prevents entry into meiosis II (M II). The spindle assembly checkpoint (SAC) surveillance mechanism ensures proper kinetochore-microtubule attachment and also regulates APC/C^Cdc20 activity. Before metaphase, SAC remains active and may inhibit APC/C^Cdc20 by binding Cdc20 with the SAC component mitotic arrest deficient 2 (Mad2), thereby blocking APC/C-Cdc20 interaction [31]. When all chromosomes achieve correct attachment, SAC is silenced and APC/C^Cdc20 activity peaks. APC/C^Cdc20 then degrades Cyclin B1 and securin, allowing separase to cleave the cohesin rings connecting sister chromatids and drive chromosome segregation, which is critical for subsequent first polar body extrusion. Additionally, the SCF -TrCP-EMI1-APC/C pathway plays an important role in mouse oocyte M I progression and first polar body extrusion. Similar to the DDB1-CUL4-RBX1 ubiquitin ligase complex, SCF (Skp1-Cullin-F-box) is also a CRL E3 ligase, but uses Cullin1 as its scaffold protein. Cullin1 binds RBX1 at its C-terminus and Skp1 at its N-terminus, which bridges to F-box proteins for substrate recognition.

The SCF ligase substrate early mitotic inhibitor-1 (Emi1) is an APC/C inhibitor that suppresses APC/C activity. The SCF component RBX1 migrates around and along with spindles and condensed chromosomes during oocyte maturation. Knockdown of Rbx1 with siRNA reduces first polar body extrusion rates and causes M I metaphase arrest in most oocytes, while also leading to Emi1 accumulation and significantly increased securin and Cyclin B1 expression [32].

Ubiquitin linkage types and proteasome activity also critically influence first polar body extrusion. Studies have identified K11-linked ubiquitin chains as essential signals for this process [19,29]. Microinjection of ubiquitin mutants into mouse GV-stage oocytes to block chain elongation revealed that injection of K11-mutated ubiquitin (with arginine substitution) significantly impaired first polar body extrusion and severely disrupted chromosome segregation [19]. Compared to wild-type ubiquitin-injected controls, microinjection of K11-mutated polyubiquitin chains caused chromosome segregation failure and markedly reduced first polar body extrusion [29]. Proteasome catalytic activity is also essential for MPF inactivation and first polar body extrusion. After meiotic resumption, proteasomes translocate to the spindle, and treatment of rat oocytes with the proteasome inhibitor MG132 causes Cyclin B accumulation, enhanced MPF activity, and M I metaphase arrest, blocking first polar body extrusion [33]. Furthermore, knockdown of any of the E2 enzymes UBE2C, UBE2S, or UBE2D impairs first polar body extrusion and disrupts spindle formation and chromosome segregation [19]. However, the mechanisms of APC/C action in mammalian male germ cell meiosis require further investigation.

In summary, the role of UPP in oogenesis is illustrated in Figure 1 [Figure 1: see original paper]. "↑" indicates increased expression, while "↓" indicates decreased expression.

**Figure 1** The role of UPP during oogenesis [14,18,23-27,30-31]

### 3.1 Meiotic Sex Chromosome Inactivation

During meiosis in mammalian male germ cells, the X and Y chromosomes synapse and condense at the pseudoautosomal region during M I prophase to form the XY body, which exhibits transcriptional silencing known as meiotic sex chromosome inactivation (MSCI) [34]. This process is closely related to meiotic progression and essential for spermatogenesis by preventing expression of spermatogenesis-inhibiting genes [35]. The XY body is enriched in ubiquitinated histone H2A (uH2A), which peaks during the pachytene stage and serves as a marker for sex chromosome transcriptional silencing [36]. Studies in Drosophila and mammals have linked uH2A to gene repression [37], suggesting its importance for MSCI. The E3 ligase UBR2 interacts with the E2 enzyme HR6B to mediate H2A ubiquitination; UBR2-deficient mice show loss of H2A ubiquitination and MSCI in M I spermatocytes, which may activate the pachytene checkpoint and cause M I arrest [38].

Another hallmark of MSCI is reduced dimethylation of histone H3 at lysine 4 (H3K4) [39], mediated by HR6B interaction with the E3 ligase RAD18. Loss of HR6B or RAD18 function increases H3K4 dimethylation on X and Y chromosomes and causes derepression of silenced genes [40]. RAD18 binds the recombinase RAD51C to mediate homologous recombination repair after DNA damage. Rad18-silenced mice exhibit failed X-Y chromosome synapsis during M I pachytene, increased H3K4 dimethylation, corresponding derepression of X-linked genes, along with reduced fertility, lower body weight, and decreased testicular volume [41]. The E3 ligase RING finger protein 8 (RNF8) mediates phosphorylation of H2AX (a H2A variant) at serine 139 ( -H2AX) through the ATM (ataxia telangiectasia mutated kinase) pathway [42]. Rnf8-deficient mice show normal -H2AX accumulation and MSCI initiation in sex chromosomes, but XY bodies fail to undergo ubiquitination [13]. Additionally, the ubiquitin ligase Ret finger protein (RFP) acts as a transcriptional repressor that interacts with nuclear matrix-binding proteins and double-stranded DNA, playing an essential role in asynchronous synapsis of sex chromosomes during M I in spermatocytes [43].

### 3.2 Meiotic Progression

UBA6, an E1 component of the UPP, functions as a meiosis initiation factor. UBA6 mRNA and protein expression levels are highest in testicular tissue compared to other tissues in both humans and mice. UBA6 is expressed in neonatal germ cells, with peak expression in the cytoplasm of spermatogonia and preleptotene spermatocytes at postnatal day 10 (PND10), potentially functioning to initiate M I [44], while at PND20 it is expressed in the nuclei of leptotene and zygotene spermatocytes. The E3 ligase SCF -TrCP also participates in male germ cell meiosis in addition to its role in oocyte M I and first polar body extrusion. -transducin repeats-containing proteins ( -TrCP) are F-box proteins in the Skp1-Cullin-F-box ubiquitin ligase complex. -TrCP-deficient mice show increased M I metaphase spermatocyte production but reduced spermatid gener-

ation, with elevated levels of SCF -TrCP substrates including Emi1 and Cyclin A, which may represent the molecular mechanism underlying the meiotic defects [45]. Additionally, the polyubiquitin gene Ubi-p63E, which maintains free ubiquitin monomer balance in the testis, is essential for normal chromosome condensation and the G2-to-M transition during M I in Drosophila spermatocytes, with Ubi-p63E mutation causing M I arrest [46].

The E3 ligase CUL4 also plays important physiological roles in male germ cell meiotic progression. RNA interference inactivation of CUL4 in Caenorhabditis elegans causes failure of DNA replication initiation protein CDT-1 degradation. Gene targeting of Cul4 in mice leads to accumulation of CDT-1, phosphorylated p53, and mismatch repair protein MLH1, along with increased cell death during M II pachytene and diplotene stages [29]. Despite normal synaptonemal complexes and DNA double-strand break (DSB) repair, mouse M II diplotene spermatocytes show delayed dissolution of recombination nodules containing MLH1, which may represent the molecular mechanism causing M II interruption. In the Chinese mitten crab (Eriocheir sinensis), CUL4, proliferating cell nuclear antigen (PCNA), and cell cycle proteins p21, p27, and p53 are highly transcribed in primary spermatocytes but decrease during spermiogenesis (except p27). p53-mediated spontaneous germ cell apoptosis may serve as a quality control mechanism to eliminate defective germ cells, while the CRL4 complex may regulate male germ cell M II progression by maintaining the balance of p53, p21, and p27 in primary spermatocytes [47].

In summary, the role of UPP in spermatogenesis is illustrated in Figure 2 [Figure 2: see original paper].

**Figure 2** The role of UPP during spermatogenesis [11,16,29,38-41,44-45,47-48]

### 4 UPP and Meiotic Recombination in Germ Cells

DNA double-strand breaks (DSBs) and their repair are critical events during prophase I of meiosis. Homologous recombination (HR) represents one DSB repair pathway, and meiotic recombination is a key step ensuring efficient genetic exchange and genome integrity in germ cells during prophase I. Studies in yeast show that the E2 enzyme RAD6 interacts with the E3 ligase BRE1 to regulate monoubiquitination of histone H2B at K123 [48]. RAD6 mutation causes defects in spore formation and DNA repair [49], while BRE1 inactivation or H2B residue mutation reduces DSB formation during meiosis, and H2B K123 mutation also causes M I arrest, indicating that RAD6/BRE1-mediated H2B monoubiquitination is required for DSB formation [50].

In mammals, the E2 enzymes HR6A and HR6B (homologous to yeast RAD6) mediate DNA damage repair [51]. Hr6b knockout mice are infertile, showing increased apoptosis of primary spermatocytes during the first wave of spermatogenesis, longer synaptonemal complexes in M I pachytene, loss of synaptonemal complex proteins in subtelomeric regions, and increased MLH1 content, suggesting that HR6B may suppress meiotic recombination [52-53]. Studies indicate

that HR6A/HR6B interact with the E3 ligase UBR2 [54], which is important for maintaining genome integrity and homologous recombination repair (HRR) of DSBs [55]. Ubr2-deficient mice lack intact synaptonemal complexes in testicular cells, causing apoptosis and infertility. Additionally, single nucleotide polymorphism analysis of the Ubr2 gene suggests its association with non-obstructive azoospermia in men [56].

Following DNA DSB formation, -H2AX is marked at damage sites, and its phosphorylated serine 139 recruits MDC1 (mediator of DNA damage checkpoint 1) to activate the RNF8-RNF168-mediated ubiquitination pathway [57]. The E3 ligases RNF8 and RNF168 interact with the E2 enzyme UBC13 to catalyze K63-linked polyubiquitination of histones H2A and H2AX [58], promoting recruitment and binding of DNA damage response proteins 53BP1 (P53 binding protein) and BRCA1 (breast cancer susceptibility gene 1) [59]. The N-terminal RING domain of BRCA1 forms a heterodimer with BRCA1-associated RING domain protein (BARD1) that possesses E3 activity and interacts with the E2 enzyme UBCH5C to catalyze K6-linked polyubiquitin chain synthesis for DSB repair [60]. However, the RING finger protein RNF169 negatively regulates homologous recombination repair by competing with 53BP1 and BRCA1 for binding to uH2A [61]. The SUMO-dependent ubiquitin ligase RNF4 can ubiquitinate SUMOylated MDC1 and BRCA1, and interacts with the homologous recombination repair protein RAD51 to participate in DSB repair [11]. RNF4-deficient mice show increased spermatocyte apoptosis and spermatogenesis defects, while Rnf4hypo/hypo mouse embryonic fibroblasts exhibit permanent DNA damage after ionizing radiation [11].

CUL4 also plays important roles in meiotic recombination. The C-terminus of CUL4 binds the RING domain protein ROC1/RNF75 while its N-terminus binds DDB1 to form the DDB1-CUL4-ROC1 E3 complex, where DDB1 recruits specific target proteins and ROC1 binds E2 enzymes to mediate substrate ubiquitination [62]. Mammals possess two Cul4 genes: Cul4A (autosomal) and Cul4B (X-linked) [16]. Studies show that mice with truncated Cul4A expression are infertile due to failed ROC1 binding, while Cul4A / mice with exons 4-8 deletion (no truncated protein detected) show permanent DSBs in pachytene spermatocytes, homologous recombination defects, significant testicular cell apoptosis, lack of M I prophase primary spermatocytes, and severe spermatogenic failure [16].

### 5 Role of Deubiquitinating Enzymes in Meiosis

Deubiquitinating enzymes (DUBs) reverse protein ubiquitination by hydrolyzing polyubiquitin chains from substrate proteins, thereby maintaining protein metabolic balance. Studies demonstrate that DUBs play critical roles in animal germ cell meiosis. For example, the ubiquitin C-terminal hydrolase family (UCHs) among DUBs is crucial for mammalian oocyte maturation by regulating oocyte development and spindle formation. Uchl-1 and Uchl-3 mRNAs are highly expressed in mouse and rhesus monkey oocytes at both the GV and M

II stages, with Uchl-1 associated with the oocyte cortex and Uchl-3 related to meiotic spindle formation. Microinjection of UCHs enzyme inhibitor ubiquitin-aldehyde (UBAL) into GV-stage oocytes prevents M I metaphase entry and causes abnormalities in spindle formation and first polar body extrusion, while Uchl-3 antibody injection affects oocyte maturation and leads to abnormal spindle morphology and meiotic defects [63]. Additionally, UCHs-deficient oocytes show reduced fertilization rates, and mutant embryos fail to form blastocysts [64]. UCHs also play important physiological roles in spermatogenesis [65]; for instance, Uchl-1 functions in mitotic proliferation of spermatogonial stem cells, while Uchl-3 acts during meiosis in spermatocytes and sperm maturation in the epididymis [65].

Studies show that Uchl-1 is important for regulating apoptosis during spermatogenesis. Overexpression of Uchl-1 in mouse testes increases apoptotic spermatocytes and causes pachytene arrest [66], while Uchl-1 knockout increases premeiotic germ cell numbers and levels of apoptotic proteins TRP53, Bax, and caspase-3 at PND7-14 [67].

### 6 Conclusion

The UPP is indispensable during meiotic division of germ cells and represents the genetic foundation for ensuring genome integrity and normal development of male and female gametes. Deficiency or mutation of UPP components causes defects in meiotic recombination, MSCI, and cell cycle progression. Moreover, UPP plays important regulatory roles in sperm acrosome formation, tail development, sperm-egg binding, paternal mitochondrial degradation in zygotes, and maternal protein degradation. Studies demonstrate that numerous UPP components function at different developmental stages of germ cells, with the same component exerting different regulatory effects in female versus male germ cells, and the same E2 enzyme producing different physiological effects when combined with different E3 ligases. This complex and precise regulatory mechanism of UPP ensures normal meiotic division and generation of fertilization-competent gametes. Currently, research on UPP effects on germ cell meiosis has focused primarily on mice and humans, with relatively few studies in pigs. Therefore, investigating the biochemical properties, subcellular localization, related substrates, and specific functional mechanisms of UPP during meiotic division in porcine germ cells will further advance our understanding of its reproductive functions and holds great significance for improving reproductive performance.

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