Review

# The UHRF protein family in epigenetics, development, and carcinogenesis

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(Edited by Shigekazu NAGATA, M.J.A.)

**Abstract:** The UHRF protein family consists of multidomain regulatory proteins that sense modification status of DNA and/or proteins and catalyze the ubiquitylation of target proteins. Through their functional domains, they interact with other molecules and serve as a hub for regulatory networks of several important biological processes, including maintenance of DNA methylation and DNA damage repair. The UHRF family is conserved in vertebrates and plants but is missing from fungi and many nonvertebrate animals. Mammals commonly have UHRF1 and UHRF2, but, despite their high structural similarity, the two paralogues appear to have distinct functions. Furthermore, UHRF1 and UHRF2 show different expression patterns and different outcomes in gene knockout experiments. In this review, we summarize the current knowledge on the molecular function of the UHRF family in various biological pathways and discuss their roles in epigenetics, development, gametogenesis, and carcinogenesis, with a focus on the mammalian UHRF proteins.

**Keywords:** UHRF1, UHRF2, DNA methylation, DNA repair, development, cancer

## 1. Introduction

The ubiquitin-like with plant homeo domain (PHD) and really interesting new gene (RING) finger domains (UHRF) family proteins are well conserved, multidomain regulatory proteins that play important roles in many biological processes.[1),2)] Some UHRF domains, including the SET and RING-associated (SRA) and PHD domains, recognize modified or unmodified DNA or proteins, while the RING finger domain catalyzes the ubiquitylation of target proteins. Through the combined function of the different domains, UHRF proteins serve as a hub for the regulatory networks of important biological processes, including maintenance of DNA methylation, cell-cycle regulation, and DNA damage repair. Furthermore, accumulating evidence suggests that the UHRF family proteins play an important role in development, gametogenesis, and carcinogenesis.

In this review, we summarize the current knowledge on the molecular function and biological roles of UHRF proteins and discuss similarities and differences between UHRF paralogues. We focus mainly on mammalian UHRF1 and UHRF2 but also discuss evolutionary aspects in other species. The aim of this review is to provide a basis for future UHRF research.

## 2. Discovery of UHRF protein family

Mouse UHRF1 (also known as Np95) was first identified as a nuclear protein associated with cell

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Non-standard abbreviation list: 5hmC: 5-hydroxylmethyl-cytosine; 5mC: 5-methylcytocine; BER: base excision repair; DNMT: DNA methyltransferase; H3K36me3: lysine 36-trimethylated H3; H3K9me3: lysine 9-trimethylated H3; ICL: interstrand crosslinks; ICR: imprinting control regions; MMR: mismatch repair; PHD: plant homeo domain; PSG: prospermatogonium; RING: really interesting new gene; SG: spermatogonium; SRA: SET and RING-associated; TTD: tandem Tudor domain; UBL: ubiquitin-like; UHRF: ubiquitin-like with PHD and RING finger domains.

proliferation.[3),4)] Human UHRF1 (also known as ICBP90) was discovered later as a regulator of topoisomerase II, which is highly expressed in proliferating cells, including cancer cells.[5),6)] The study also showed that UHRF1 belonged to a multidomain protein family conserved in animals and plants. Unoki *et al.* found that downregulation of early growth response 2 (*EGR2*) in ovarian cancer cells may involve DNA methylation.[7),8)] They then identified UHRF1 as a protein binding to the methylated *EGR2* promoter and found that UHRF1 interacts with histone deacetylase 1 (HDAC1).[9)] This data was the first link between the UHRF proteins and epigenetics, although UHRF1 does not regulate *EGR2* directly.

Human UHRF2 (also known as NIRF) was identified as a protein that ubiquitylates PEST-containing nuclear protein (PCNP).[10),11)] PCNP can function as a tumor suppressor or an oncoprotein,[12)] but how UHRF2 regulates PCNP remains unknown. Finally, emerging data has begun to elucidate the role of UHRF2 in DNA damage repair (See section 6.2).

### 3. Fundamentals of the UHRF protein family

**3.1. Structure of the UHRF protein family.** Mammalian UHRF1 and UHRF2 are encoded by independent genes but share an identical domain organization: both proteins have ubiquitin-like (UBL), tandem Tudor domain (TTD), PHD, SRA, and RING domains (Fig. 1). The amino acid sequence identity between UHRF1 and UHRF2 proteins is 52% in humans. Their protein structure

is also well conserved among mammalian species; the amino acid sequence identity of human and mouse UHRF1 and UHRF2 is 74% and 91%, respectively.

The molecular function of each domain has been relatively well-studied for UHRF1. The TTD recognizes histone H3 di-/tri-methylated at lysine-9 (H3K9me2/3) and ligase 1 (LIG1) di-/tri-methylated at K126 (LIG1K126me2/3).[13)–16)] The PHD recognizes histone H3 unmethylated at arginine-2 (H3R2me0).[14)] The SRA domain recognizes hemi-methylated CpG sites of DNA and DNA interstrand crosslinks (ICLs).[9),17)–21)] Although a prior study reported that it recognizes not only 5-methylcytosine (5mC) but also 5-hydroxymethylcytosine (5hmC) at hemi-methylated CpG sites,[22)] this is still controversial.[23)] The SRA domain also provides a surface for the interaction with HDAC1 and DNA methyltransferase 1 (DNMT1).[9),24)] The RING domain has E3 ligase activity that mono-ubiquitylates histone H3 at K14, K18, and K23 and PCNA-associated factor 15 (PAF15) at K15 and K24.[25)–28)] Finally, the UBL domain facilitates both RING-mediated ubiquitylation and recognition of hemi-methylated DNA by the SRA domain.[29),30)] The UBL domain also provides a surface for UHRF1 interaction with DNMT1.[31)]

With respect to UHRF2, the TTD and PHD domains recognize H3K9me3 and H3R2me0, respectively, although the binding mode appears to be slightly different from binding modes of UHRF1.[32)] Recent studies also showed that the SRA domain of UHRF2 recognizes 5hmC.[33),34)] Finally, the RING domain of UHRF2 has multiple substrates for ubiquitylation including PCNP, cyclin D1, cyclin
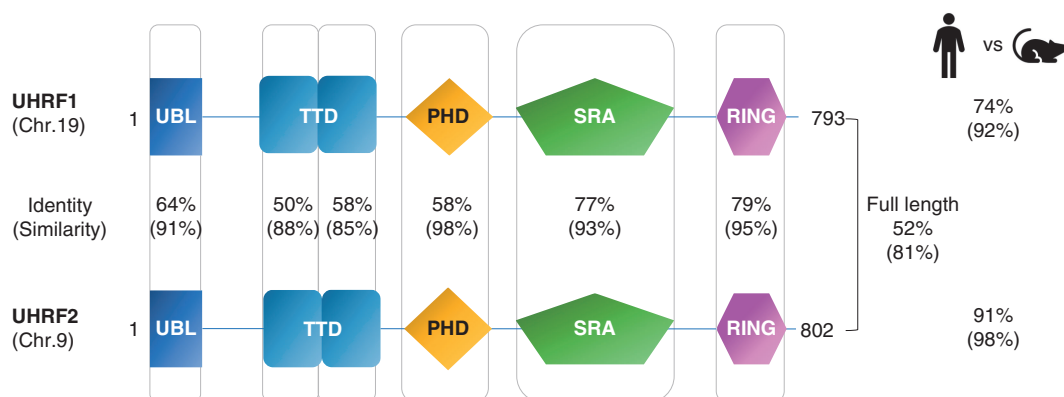


Fig. 1. Domain structure and amino acid sequence identity and similarity of human UHRF1 and UHRF2. The identity and similarity between the human and mouse homologues are indicated on the right. The chromosome number of the gene encoding each protein is shown in parentheses. UBL, ubiquitin-like; TTD, tandem Tudor domain; PHD, plant homeo domain; SRA, SET and RING-associated; RING, really interesting new gene.
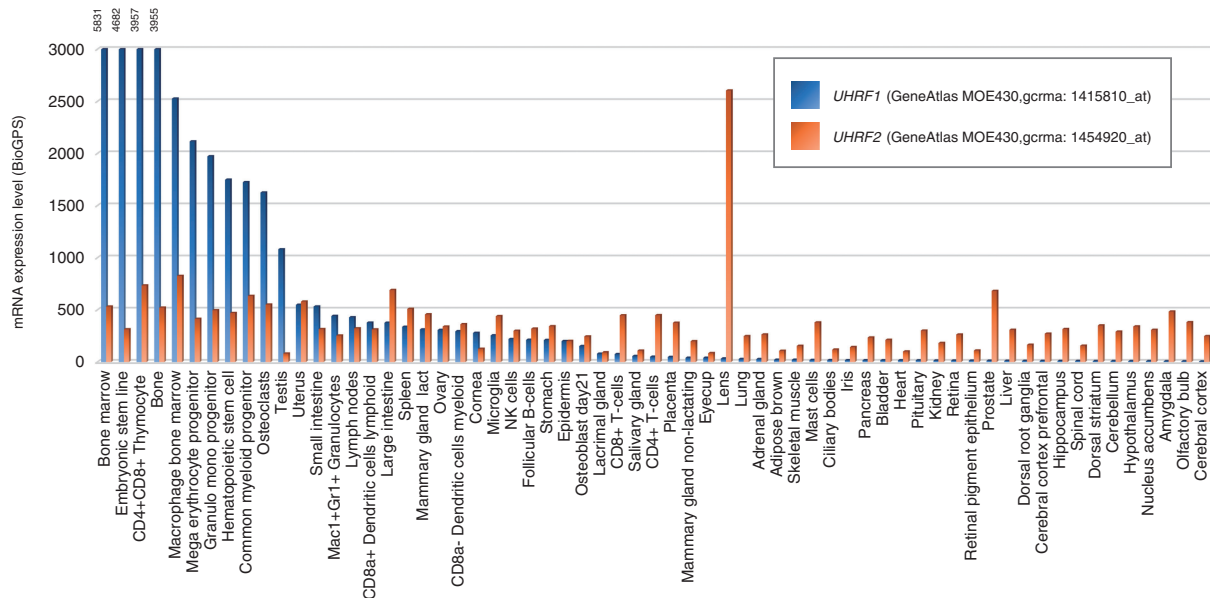
Fig. 2.   Expression of mouse *UHRF1* and *UHRF2* in various tissues and cells. Data were obtained from BioGPS expression database (http://biogps.org/).

E1, and X-ray repair cross complementing 1 (XRCC1).[11),35),36)] The function of the UBL domain of UHRF2 remains uncharacterized.

**3.2. *UHRF* gene expression in normal cells/ tissues.**    As described in section 2, UHRF1 was identified as an abundant protein in proliferating cells. According to the BioGPS expression database (http://biogps.org/),[37] mouse *UHRF1* is indeed expressed at high levels in tissues containing a high proportion of proliferating cells, such as bone marrow, testis, and small and large intestines (Fig. 2). In contrast, tissues consisting mostly of nonproliferating cells, such as cerebral cortex, cerebellum, and spinal cord, show virtually no expression. For *UHRF2*, no correlation is observed between expression level and cell proliferation (Fig. 2), suggesting that the two proteins can play distinct roles.

**3.3. Evolutionary conservation of the UHRF protein family.**    The presence or absence of the UHRF protein family in a panel of model organisms is summarized in Fig. 3. We have also indicated whether DNA methylation (5mC) exists in the species, since UHRF1 is involved in maintenance of DNA methylation in at least several organisms as will be discussed later (Fig. 3).

Both UHRF1 and UHRF2 exist in most vertebrate species except for zebrafish (*Danio rerio*), which only has UHRF1. Since many fish species including Japanese pufferfish (*Fugu rubripes*) have both proteins, zebrafish appear to be an exception. Conservation of UHRF1 among vertebrate species is consistent with the universal presence of 5mC in their genomes. We further note that, among insects, the honey bee (*Apis mellifera*), which has a considerably high level of 5mC, has UHRF1, but the fruit fly (*Drosophila melanogaster*), which has an extremely low level of 5mC, does not have UHRF1. Further, *Caenorhabditis elegans*, which does not have 5mC, is unlikely to possess a UHRF protein. UHRF2, on the other hand, is found only in vertebrates but not in other animals.

In a plant species (*Arabidopsis thaliana*), three UHRF1 homologues have been identified: VIM1 (ORTH2), VIM2 (ORTH5), and VIM3 (ORTH1).[38] All of these VARIANT IN METHYLATION (or ORTHRUS) protein family members contain a PHD, an SRA, and two RING domains and are involved in maintenance of DNA methylation.[39),40)] However, it remains unclear whether the plant has a UHRF2 homologue. Two possible candidates, VIM4 (ORTH4) and VIM6 (ORTHL), have not yet been characterized.

In fungi, *Neurospora crassa*, *Ascobolus immersus*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Phycomyces blakesleeanus* have no SRA-containing proteins, according to the Simple Modular Architecture Research Tool database
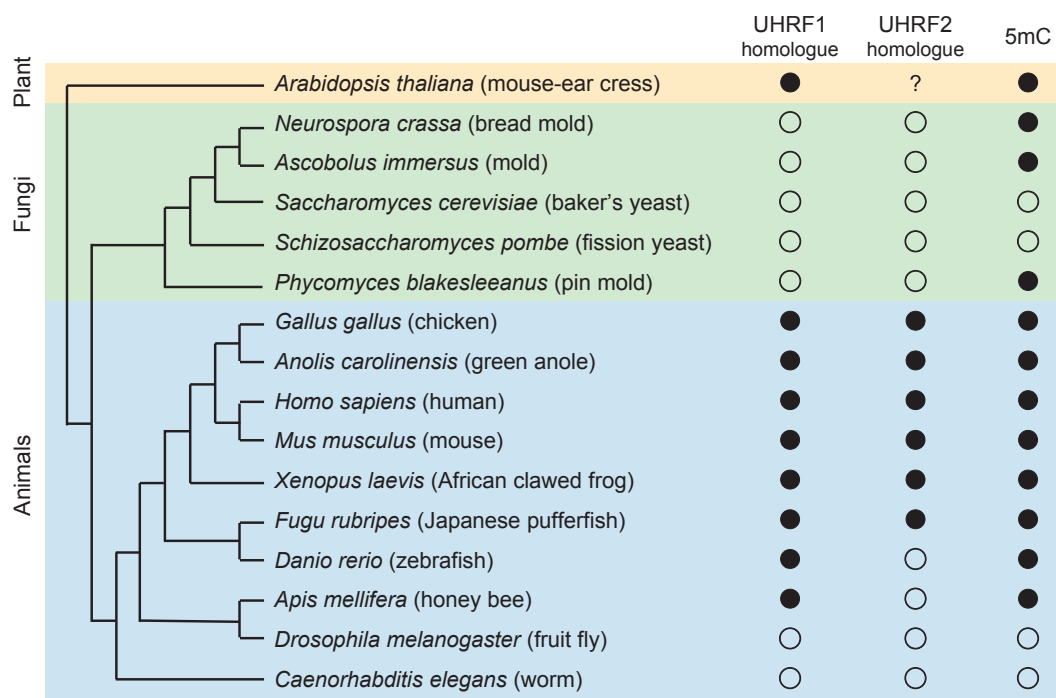
Fig. 3. Evolutionary conservation of the UHRF protein family. UHRF1 is conserved in animals and plants possessing 5mC, while UHRF2 is present only in vertebrates. No UHRF homologue has been identified in fungus species shown in this figure, but some SRA-containing proteins are present in other species.

(http://smart.embl-heidelberg.de/), despite that three of them do have 5mC. Although further investigation is needed, some other species have uncharacterized SRA-containing proteins, which could be the functional homologues of UHRF proteins.

### 4. Role of UHRF1 in maintenance of DNA methylation

In proliferating cells, CpG methylation patterns of parental genomes are propagated through DNA replication. This process, called maintenance of DNA methylation, involves the recognition of hemimethylated CpG sites and methylation of cytosines on newly synthesized strands catalyzed by the maintenance DNA methyltransferase DNMT1 in vertebrates.[41] Following the discovery of UHRF1 as a methylated DNA binding protein (See section 2), two groups examined *UHRF1* knockout (KO) mice and embryonic stem cells (ESCs) and found that UHRF1 is essential for maintenance methylation.[42),43] They showed that UHRF1 has a higher affinity for hemi-methylated DNA rather than fully methylated DNA, consistent with the role of UHRF1 in maintenance methylation. Subsequently, structural analyses revealed that the 5mC base at the hemi-

methylated CpG site is flipped out of the DNA helix and fits tightly into a pocket on the concave surface of the SRA domain.[17)–19]

It was then found that RING-mediated multiple monoubiquitylation of histone H3 at K14, K18, and K23 is critical for recruitment of DNMT1.[25] DNMT1 recognizes two of the three mono-ubiquitylated lysines through its replication foci targeting sequence (RFTS). In addition, deubiquitylation of histone by ubiquitin specific peptidase 7 (USP7) is required for maintenance methylation.[44] Nishiyama *et al.* demonstrated that RING-mediated dual monoubiquitylation also occurs for PAF15, an interacting partner of proliferating cell nuclear antigen (PCNA).[45] This dual monoubiquitylation is also recognized by the RFTS of DNMT1. Moreover, ubiquitylation of PAF15 and histone H3 appear to be associated with maintenance methylation in regions replicating early and late, respectively, during S phase.[45]

Ming *et al.* developed a technique named Hammer-seq, which enables the measurement of maintenance methylation kinetics. Using this technology, they found that maintenance occurs in both replication-coupled and replication-uncoupled manners.[46] We suggest that the replication-coupled and
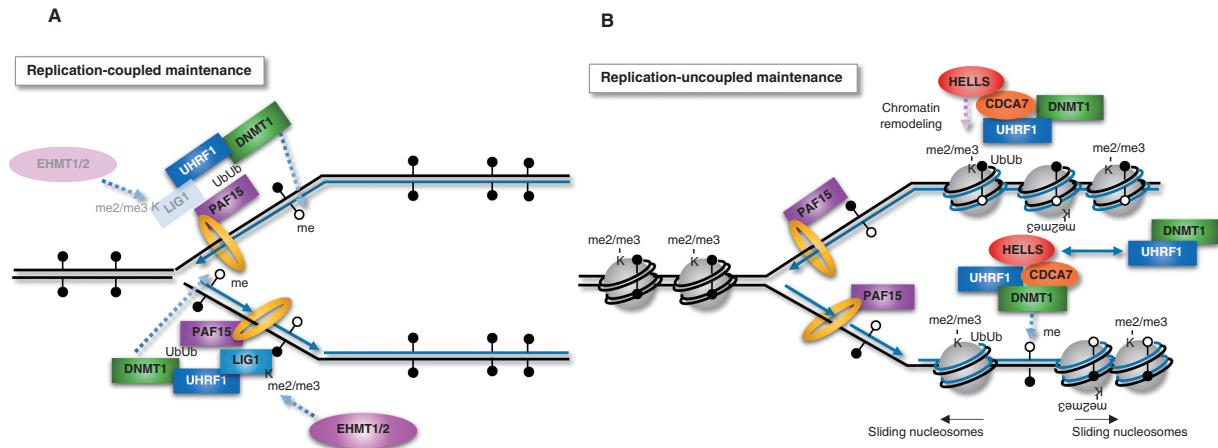
Fig. 4.    Models of replication-coupled and replication-uncoupled maintenance of DNA methylation and the role of UHRF1. (**A**) A model of replication-coupled maintenance. First, UHRF1 recognizes hemi-methylated DNA through its SRA domain and mono-ubiquitylates two lysine residues of PAF15 through the activity of its RING domain. Subsequently, DNMT1 recognizes the ubiquitylated PAF15 through its RFTS and methylates the unmethylated cytosine of the hemi-methylated DNA. Both PAF15 and DNMT1 interact with PCNA, and the interaction likely supports the replication-coupled maintenance. Further, EHMT2 and EHMT1 methylate LIG1, which is a protein required for joining Okazaki fragments during lagging strand synthesis; UHRF1 recognizes the methylated LIG1 through its TTD. This interaction also facilitates the recruitment of UHRF1 to replication sites. (**B**) A model of replication-uncoupled maintenance. First, UHRF1 recognizes hemi-methylated DNA through its SRA domain, H3K9me2/3 through its TTD, and H3R2me0 through its PHD. Subsequently, UHRF1 mono-ubiquitylates multiple lysine residues of histone H3 through the activity of its RING domain and interacts with the CDCA7/HELLS complex, which slides the nucleosomes to generate bare hemi-methylated DNA. Then, DNMT1 recognizes the ubiquitylated H3 and methylates the unmethylated cytosine of the hemi-methylated DNA. Filled circles, 5mC; open circles, unmethylated cytosines; yellow rings, PCNA. This figure is from a review article (Unoki, M. Genes Cells, 26, 349–359, 2021; Ref. 47) with slight modifications.

replication-uncoupled processes are responsible for maintenance before and after wrapping of hemi-methylated DNA around histones, respectively (Fig. 4A and 4B).[47] The replication-coupled process occurs via interactions between DNMT1, UHRF1, PCNA, methylated LIG1 (LIG1K126me2/me3), and possibly mono-ubiquitylated PAF15. Histone lysine methyltransferase 2 (EHMT2, also known as G9a) and EHMT1 (also known as GLP) are the enzymes responsible for LIG1 methylation.[15] This process restores full methylation at more than 50% of hemi-methylated CpG sites soon after fork passage occurs.[46] The primary targets of this mechanism are likely to be regions that do not require chromatin remodeling for methylation maintenance (Fig. 4A).

In contrast, the replication-uncoupled process is associated with H3K9me2/3,[46] which are abundant in heterochromatic regions and recognized by the TTD of UHRF1 (Fig. 4B). The replication-uncoupled process requires chromatin remodeling by helicase lymphoid-specific (HELLS), which forms a complex with cell division cycle associated 7 (CDCA7) (Fig. 4B),[46],48),49) consistent with previous observations that hemi-methylated DNA wrapped around histones was not a suitable substrate for

DNMT1.[50] We recently found that CDCA7 indeed facilitates recruitment of the DNMT1/UHRF1 complex on nascent DNA.[51] Importantly, *HELLS* and *CDCA7* are among the causative genes for immuno-deficiency, chromosome instability, and facial anomalies (ICF) syndrome.[52] In ICF cells, DNA methylation is significantly reduced in CpG-poor regions, such as heterochromatic regions.[53] This result further supports the significance of chromatin remodeling in the replication-uncoupled process (Fig. 4B).

Ming *et al.* reported that cell proliferation, aging, carcinogenesis, and long-term culture preferentially affect regions that adopt the replication-uncoupled mechanism.[46] Such regions include so-called partially methylated domains of tumor cells.[54]–56) They also reported that the lack of aberrant DNA methylation in *LIG1*-null ESCs[15] could be attributed to the replication-uncoupled mechanism acting as a backup.[46] Therefore, perhaps the replication-uncoupled maintenance has higher sensitivity to stress compared to the replication-coupled maintenance, as it requires an additional chromatin remodeling step and lacks a backup mechanism.
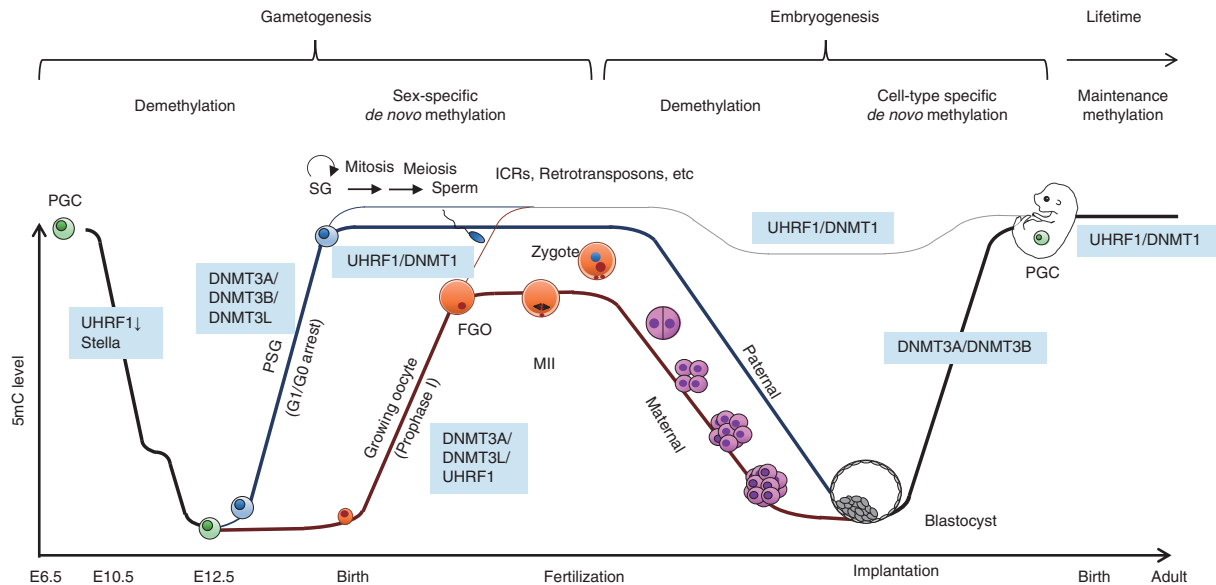
Fig. 5.  DNA methylation changes in mouse development and the roles of DNMTs and UHRF1. During gametogenesis and embryogenesis, DNA methylation is initially erased, and new methylation patterns are established. An exception is the imprinting control regions (ICRs) and retrotransposons, for which methylation is maintained throughout the wave of genome-wide demethylation in preimplantation embryos. E6.5, embryonic day 6.5; E10.5, embryonic day 10.5; E12.5, embryonic day 12.5; PSG, prospermatogonium; SG, spermatogonium; MII, metaphase II. This figure is from a book chapter (Unoki, M. Recent insights into the mechanisms of *de novo* and maintenance of DNA methylation in mammals. *In* DNA Methylation Mechanism (eds. Budak, M. and Yıldız, M.).  IntechOpen, 2019) with slight modifications.

## 5. Role of the UHRF protein family in mammalian development

**5.1. Role of UHRF1 in mammalian development.**   Levels of DNA methylation change dynamically at two developmental time windows in mouse development (Fig. 5). Sharif *et al.* showed that homozygous *UHRF1* KO embryos exhibit developmental failure and die before embryonic day 12.5 (E12.5).[42] Importantly, DNA methylation was reduced in all genomic regions examined, including retrotransposons and imprinting control regions (ICRs). The ICRs are regions that are differentially methylated between parental alleles and control allele-specific expression of nearby imprinted genes.[57] Consistent with the reduced methylation, the normally silenced retrotransposons were derepressed, and the monoallelic expression of the imprinted genes were disrupted.

In preimplantation embryos, both paternal and maternal genomes undergo global DNA demethylation (Fig. 5), mainly through a passive mechanism (lack of maintenance), partly owing to the majority of DNMT1 proteins relocalizing to the cytoplasm.[58] The ICRs and certain retrotransposons retain DNA methylation against this wave of demethylation

(Fig. 5).[58]  DNMT1, zinc finger protein 445 (ZNF445), zinc finger protein 57 (ZFP57), and tripartite motif containing 28 (TRIM28) are involved in the methylation maintenance.[58]–[60] Because UHRF1 is abundantly present in oocytes and persists throughout preimplantation development, we and others examined the role of UHRF1 by generating oocyte-specific KO mice.[61],[62] When these females were crossed with wild-type males, the derived blastocysts (called maternal KO or mat-KO blastocysts) showed significant reduction in methylation across their genomes, including the ICRs and retrotransposons. This result indicates that maternal UHRF1 is essential for maintenance methylation in preimplantation embryos.[61]

A majority of the mat-KO embryos, however, died before the blastocyst stage,[61] which was earlier than *DNMT1* mat-KO embryos,[58],[63] suggesting that maternal UHRF1 is not merely a cofactor of DNMT1. Like DNMT1,[58] UHRF1 resides in the cytoplasm of preimplantation embryos with only a small proportion detected in the nucleus.[61] It is tempting to speculate that cytoplasmic UHRF1 has a yet uncovered function.

**5.2. Role of UHRF1 in mammalian gametogenesis.**   Mammalian gametogenesis starts from

primordial germ cells (PGCs), which are derived from epiblast cells.[57] PGCs undergo genome-wide DNA demethylation through both active and passive mechanisms,[64]–[67] the latter of which is primarily attributed to the downregulation of UHRF1 in the nucleus (Fig. 5). The Stella protein (also known as DPPA3 or PGC7) may be involved in relocalizing UHRF1 to the cytoplasm.[68] Finally, sex-specific methylation patterns are established in growing oocytes and prospermatogonia (PSGs), by the action of the de novo DNA methyltransferase DNMT3A complexed with its cofactor DNMT3L.[57],[69]

Since growing oocytes are cell-cycle arrested at the diplotene stage of meiotic prophase I, they provide an ideal stage for de novo methylation, with no need for maintenance methylation.[57],[69] In growing oocytes, transcriptionally active regions marked with lysine 36-trimethylated H3 (H3K36me3) are the primary target of methylation.[70]–[74] We found that *UHRF1* KO showed a 25% reduction in global methylation, predominantly occurring in transcriptionally inactive regions with no H3K36me3.[61] This reduction is greater than that observed in *DNMT1* KO oocytes.[75] Because UHRF1 can interact with DNMT3A,[76] UHRF1 may play a role in de novo methylation in oocytes. Recently, Stella was reported to contribute to the cytoplasmic relocalization of UHRF1 and prevent excessive de novo methylation in oocytes.[77] Stella was also reported to inhibit chromatin association of UHRF1, resulting in a possible double-layer mechanism for prevention of aberrant methylation.[78]

During spermatogenesis, a wave of de novo methylation occurs in PSGs, which are arrested at the G1 phase of mitosis (Fig. 5).[57],[79] PSGs resume mitosis after birth, resulting in the formation of spermatogonia (SGs), which include both an undifferentiated and differentiated population. Using tamoxifen-inducible KO mice, UHRF1 was found to be essential for the transition from undifferentiated to differentiated SGs.[80] It was also revealed by specific disruption of *UHRF1* in differentiated SGs using stimulated by retinoic acid 8 (*STRA8*)-Cre that KO spermatocytes exhibit synaptic failure and reduction in methylation.[81] The latter may be due to defects in maintenance methylation, but the decrease in methylation was not as significant as expected. Because we and others observed increased expression of DNMT3B during the transition from undifferentiated to differentiated SGs,[79],[80] de novo methylation may fill some of the losses.

**5.3. Role of UHRF2 in mammalian development.** *UHRF2* KO mice are born normally and fertile.[82],[83] However, they exhibit partial impairment in spatial memory acquisition and retention and exhibit frequent seizures.[82],[83] Chen *et al.* reported that, while there is no significant change in 5mC, a small but statistically significant 5hmC reduction occurs in the brains of these mice.[83] *UHRF2* KO affects expression of many neuron-related genes, but their changes were modest.[82] The biological role of UHRF2 in development warrants further investigation.

## 6. Role of the UHRF protein family in DNA damage repair

**6.1. Role of UHRF1 in DNA damage repair.** While defects in some DNA damage repair pathways, such as the mismatch repair (MMR) pathway, increase the risk of developing cancer, defects in other repair pathways, such as the double strand break (DSB) repair and base excision repair (BER) pathways, increase the sensitivity of cancer cells to chemotherapeutic agents and/or radiation.[84] The involvement of UHRF1 in DNA damage repair was proposed based on observations that UHRF1 depletion increases the sensitivity of cells to various genotoxic agents and radiation.[85]–[88] Even without exogenous DNA damage inducers, UHRF1 depletion can cause cell-cycle arrest at the G2/M transition stage, along with activation of DNA damage response pathways and apoptosis.[21] Further, overexpression of *UHRF1* can decrease the radiosensitivity of cells.[89]

Breast cancer 1 (BRCA1) is an important mediator of the DSB repair pathway, which promotes homologous recombination (HR) and antagonizes p53 binding protein 1 (53BP1)-dependent nonhomologous end joining (NHEJ). UHRF1 is recruited to the sites of DSB by BRCA1 in S phase, which requires the phosphorylation of serine 661 (S661ph), located between the SRA and RING domains (Fig. 1).[90] Subsequently, the phospho-UHRF1 mediates polyubiquitylation of replication timing regulatory factor 1 (RIF1) and results in its disassociation from 53BP1 and DSBs, thereby facilitating HR initiation.[90] A more recent study found that methylation of UHRF1 by SET domain containing 7 (SET7) is required for DSB repair.[91] This methylation is induced by DNA damage, and S661ph is a prerequisite for UHRF1 interaction with SET7. After phosphorylation, the methyl-UHRF1 mediates polyubiquitylation of PCNA via its RING domain to induce the DNA damage response. Finally, UHRF1

is demethylated by lysine specific demethylase 1 (LSD1).[91]

UHRF1 may also be involved in MMR. The high efficiency of mammalian MMR surveillance is achieved through a hemi-methylated DNA-UHRF1-DNMT1 axis, in which the surveillance complex including $MutS\alpha$ is recruited by interaction with DNMT1 independent of its catalytic activity.[92] However, whether the recognition of hemi-methylated DNA by UHRF1 helps the surveillance complex to distinguish the nascent and template strands remains unclear. UHRF1 was also identified as an interacting partner of N-methylpurine DNA glycosylase (MPG), which functions in BER.[93]

Finally, recent studies reported that UHRF1 is involved in the repair of ICLs.[20),21),94] UHRF1 likely recognizes ICL lesions via its SRA domain and recruits Fanconi anemia complementation group D2 (FANCD2) and structure-specific endonucleases, such as ERCC1-XPF and MUS81-EME1. However, it remains unknown by what mechanism UHRF1 recruits these repair factors.[20),21]

**6.2. Role of UHRF2 in DNA damage repair.** There is evidence that UHRF2 is also involved in several DNA damage repair pathways. A previous study reported that UHRF2 is recruited to sites of DNA damage and promotes damage-induced H2AX phosphorylation and subsequent repair.[95] UHRF2 was also identified as a sensor protein for ICLs; UHRF2 is recruited to the sites of ICL through the SRA domain and cooperates with UHRF1 to ensure the recruitment of FANCD2.[96] In this case, a direct protein–protein interaction occurs between UHRF1 and UHRF2 and between FANCD2 and either UHRF1 or UHRF2. We note that monoubiquitylation of FANCD2 by the Fanconi anemia core complex is likely stimulated by the UHRF1/UHRF2 complex.

Like UHRF1, UHRF2 can interact with MPG, but the biological significance of this interaction remains unknown.[93] A study reported that UHRF2 is allosterically activated by 5hmC and catalyzes K33-linked polyubiquitylation of XRCC1, a member of the BER complex.[36] This nonproteolytic ubiquitylation appears to stimulate the interaction of XRCC1 with RAD23B, leading to the incorporation of thymine DNA glycosylase into the BER complex. Based on these observations in neuronal differentiation of *UHRF2* KO ESCs, the authors concluded that UHRF2 is involved in the genome-wide DNA demethylation mediated by BER.[36] Because *UHRF2* KO mice show only a neurological phenotype (See

section 5.3),[82),83] this process may occur only in limited cell types.

## 7. Dysregulation of the UHRF protein family in cancer

**7.1. Role of UHRF1 in cell-cycle regulation and carcinogenesis.** As described in section 2, UHRF1 was originally identified as a proliferation-associated nuclear protein. Unoki and others found that E2F1 regulates *UHRF1* expression through two E2F1 motifs present in intron 1.[6),9] E2F1 is a key cell-cycle regulator that targets genes promoting the G1/S transition, DNA repair, and apoptosis. Regulation of mouse *UHRF1* may be different in some respects, because adenovirus early region 1A (E1A), and not E2F1, induces *UHRF1* expression.[97] A previous study showed that the TP53/P21 pathway downregulates *UHRF1* following DNA damage, likely by modulating the interaction between E2F1 and retinoblastoma-associated protein (RB).[98]

Multiple reports have described the upregulation of UHRF1 in cancer cells (Table 1).[99)–106] Because

Table 1. Levels of UHRF family proteins in various cancer types

| Cancer type | Level of UHRF1[refs] | Level of UHRF2[refs] |
|---|---|---|
| Breast cancer | Up[99),103] | Up[117] |
| Colorectal cancer | Up[100),103),110] | Up[118)–120] |
| Intrahepatic cholangiocarcinoma | Up[104] | Up[121] |
| Osteosarcoma | Up[101] | Up[122)–124] |
| Hepatocellular carcinoma | Up[103] | Up[125] |
| Lung cancer | Up[99),103] | Down[126),127] |
| Esophageal cancer | Up[103] | Down[116),128] |
| Brain tumor | Up[103] | Down[129] |
| Gastric cancer | Up[103] | Down[126] |
| Pancreatic cancer | Up[99),103] | Down[126] |
| Cervical cancer | Up[99),103] | Down[126] |
| Head and neck cancer | Up[103] | Down[126] |
| Lymphoma | Up[4] | Down[126] |
| Bladder cancer | Up[103] | NA |
| Kidney cancer | Up[103] | NA |
| Prostate cancer | Up[99),103] | NA |
| Hepatoblastoma | Up[103] | NA |
| Astrocytoma | Up[99] | NA |
| Retinoblastoma | Up[106] | NA |
| Malignant pleural mesothelioma | Up[105] | NA |
| Thyroid cancer | Up[103] | NA |
| Melanoma | Up[102] | NA |
| Ovarian cancer | Up[103] | NA |
| Gallbladder cancer | Up[103] | NA |

NA, data not available.

higher expression is often correlated with more advanced cancer stages, multiple metastases, and/ or poor prognosis, *UHRF1* has been suggested as a good biomarker for various malignancies.[99] In addition, because *UHRF1* expression is limited to proliferating cells, inhibitors of UHRF1 may be ideal candidates for anticancer drugs that could act across a broad spectrum of cancer types, with minimal adverse effects.[99,107] Given the role of UHRF1 in various DNA repair pathways (See section 6.1), UHRF1 inhibitors may be used in adjuvant therapy for cancers that are resistant to chemotherapy and/ or radiotherapy. UHRF1 inhibitors are currently under development.[108,109]

Intriguingly, it is reported that colorectal cancer cells require the PHD and SRA domains, but not the TTD and RING domains, of UHRF1 for the maintenance of cancer specific methylation, suggesting that the ubiquitylation step could be skipped under certain circumstances.[110] More intriguingly, excess UHRF1 may also cause genome-wide DNA hypomethylation, which is a common feature of cancer cells. This may seem paradoxical given the UHRF1's role in maintenance methylation, but previous studies reported that excess UHRF1 could disrupt normal interactions between DNMT1, UHRF1, and PCNA and cause inappropriate localization and destabilization of DNMT1, the latter of which may be mediated by RING-mediated ubiquitylation.[111]–[113] Consistent with this finding, transgenic zebrafish overexpressing *UHRF1* in hepatocytes exhibit delocalization and destabilization of DNMT1, DNA hypomethylation, TP53-mediated senescence, and transformation into hepatocellular carcinoma.[114]

**7.2. Role of UHRF2 in cell-cycle regulation and carcinogenesis.** Unlike *UHRF1*, the transcriptional regulation of *UHRF2* has not been studied extensively. However, previous studies reported that overexpression of *UHRF2* induced G1 arrest.[35,115] Furthermore, UHRF2 was shown to interact with multiple cell-cycle proteins including several cyclins, TP53, and RB and ubiquitylate cyclins D1 and E1, constituting a nodal point in the cell-cycle network.[35,115] Another study reported that UHRF2 could be a negative regulator of the epithelial-mesenchymal transition.[116]

Despite these observations, the level of UHRF2 expression varies in different cancer types (Table 1). This may be consistent with the fact that there is no correlation between the proliferation state and *UHRF2* expression level in normal tissues (Fig. 2).

Because *UHRF2* KO mice do not develop any cancer within one year of the study,[82,83] the observed changes in expression in cancer are perhaps secondary.

## 8. Conclusion

The UHRF family comprises multidomain proteins that interact with other molecules and serve as a hub for several important biological processes, including maintenance of DNA methylation and DNA damage repair. The emergence of UHRF1 is perhaps linked to the presence of 5mC in animals and plants, while UHRF2 appears to have diverged in its function. The proteins are normally present in the cell nucleus and involved in nuclear processes, but they also appear to play a role in the cytoplasm of specific cells. Both proteins, particularly UHRF1, are important for embryonic development and carcinogenesis in mammals. Taken together, the UHRF family provides a paradigm of multisensing proteins that link various epigenetic modifications and important cellular processes. Some of the important questions to be addressed in future studies are: the molecular basis of the functional divergence of UHRF1 and UHRF2; identification and analysis of posttranslational modifications of the two proteins; the structural basis of their action in DNA damage repair. An understanding of this protein family has the potential to contribute to the improvement of human health.

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