Epigenetic mechanism and target therapy of UHRF1 protein complex in malignancies

Abstract: Ubiquitin-like with plant homeodomain and really interesting new gene finger domains 1 (UHRF1) functions as an epigenetic regulator recruiting PCNA, DNMT1, histone deacetylase 1, G9a, SuV39H1, herpes virus-associated ubiquitin-specific protease, and Tat-interactive protein by multiple corresponding domains of DNA and H3 to maintain DNA methylation and histone modifications. Overexpression of UHRF1 has been found as a potential biomarker in various cancers resulting in either DNA hypermethylation or global DNA hypomethylation, which participates in the occurrence, progression, and invasion of cancer. The role of UHRF1 in the reciprocal interaction between DNA methylation and histone modifications, the dynamic structural transformation of UHRF1 protein within epigenetic code replication machinery in epigenetic regulations, as well as modifications during cell cycle and chemotherapy targeting UHRF1 are evaluated in this study.

Keywords: UHRF1 protein complex, epigenetic modification, ICBP90

Introduction

Ubiquitin-like with plant homeodomain (PHD) and really interesting new gene (RING) finger domain 1 (UHRF1, also called ICBP90 or Np95) was identified as a transcription factor which could regulate the expression of topoisomerase I by binding to an inverted CCAAT box located in its promoter. The nuclear protein UHRF1 consists of multiple domains such as ubiquitin-like domain (UBL), tandem tudor domain (TTD; TTD\textsuperscript{N} and TTD\textsuperscript{C}), PHD, SET and RING-associated domain (SRA) as well as RING domain, which play an important role in the epigenetic regulation of gene expression and tumorigenesis.

Epigenetic modification refers to inherited changes in gene expression without variations in DNA structure or sequence\textsuperscript{2,4} and includes DNA methylation, histone modification, genome imprinting, X-chromosome inactivation, and microRNA regulation,\textsuperscript{4,5} where DNA methylation and histone modification are most important and their aberrant changes are always involved in cancer and neurological diseases. UHRF1 serves as a key regulator that participates in both DNA methylation and histone modifications.

UHRF1 allows crosstalk between DNA methylation and histone code as an epigenetic coordinator

DNA methylation is a fundamental epigenetic process in the regulation of gene expression. The process occurs at the carbon-5 position of cytosines mainly within CpG sites mediated by DNA methyltransferases (DNMTs). In detail, DNMT3A and DNMT3B are mainly responsible for de novo methylation during gametogenesis and early embryonic
development, whereas DNMT1 preferentially methylates hemi-methylated CpG sites for maintenance of DNA methylation by a self-inhibitory mechanism during DNA replication and cell division. Aberrant DNA methylation can lead to abnormal embryogenesis,6 neurological diseases,8 and cancers,9,10 including global DNA hypomethylation and specific CpG island hypermethylation in which the former can lead to chromosomal instability,11 activation of certain transcription factors,12 and loss of genetic imprints13 the latter often results in the inhibition of tumor suppressor genes.

Eukaryotic DNMT1 is composed of an N-terminal nuclear localization sequence, a replication foci targeting sequence (RFTS) that localizes DNMT1 to the DNA replication fork, a zinc finger CXXC domain that specifically recognizes unmethylated CpG dinucleotides,14,15 a pair of bromo adjacent homology (BAH) domains, and a C-terminal methyltransferase domain including the catalytic core and the target recognition domain (TRD). Although the occlusion of the CXXC-BAH1 linker at the catalytic site and restraint by BAH2-TRD loop preventing TRD from binding to unmethylated CpG sites facilitate DNMT1-mediated maintenance of DNA methylation,16,17 the auto-inhibitory role of RFTS domain and DNMT1-interacting proteins which affect its activity, such as UHRF1, have been revealed.18,19 UHRF1 recognizes and binds to hemi-methylated DNA (hmDNA) through its SRA domain by the thumb (444–499 residues) and NKR finger (483–496 residues) sub-domains targeting minor and major grooves, respectively,20 and recruits DNMT1 by targeting the RFTS domain which leaves the catalytic pocket of DNMT1 in the S phase of the cell cycle to replication foci21,22 and relieves the auto-inhibitory activity of DNMT1 to maintain the cytosine in newly synthesized DNA methylation with high fidelity. In addition, the UHRF1 C-terminal RING finger functions as an ubiquitin E3 ligase to establish histone H3 ubiquitination at Lys23 and Lys18 recognized by the RFTS domain of DNMT123,24 to promote its localization onto replication foci, which also has a prerequisite role in the maintenance of DNA methylation. Recently, the N-terminal UBL domain of UHRF1 was found to bind directly to DNMT1 enhancing DNMT1 enzymatic activity toward newly replicated chromatin by controlling targeted H3 ubiquitination through a hydrophobic patch.25,26 Furthermore, the UBL domain can bind the E2 Ube2D and form a stable E2/E3/chromatin complex that is equally required for the DNMT1-mediated maintenance of DNA methylation.26,27

Posttranslational modification of histone participates in DNA replication, DNA damage response, chromosome translocation, transcription activation and suppression, X chromosome inactivation, and heterochromatin replication.28 To date, the most studied histone modifications include the methylation of arginine (R) and lysine (K), acetylation of lysine, phosphorylation of serine (S) and threonine (T), and lysine ubiquitination. The histone lysine methylation is divided into monomethylation, dimethylation, and trimethylation, which greatly increases the complexity of histone modification and gene expression. Lysine acetylation of histone tails leads to transcriptional activation by neutralization of a positive charge at a lysine side chain, triggering a detachment of the side chain from the negatively charged DNA strands.

Methylation of histone H3 plays an important role in transcription, among which H3K4 methylation often causes transcriptional activation while H3K9 methylation always coordinates gene silencing. Unmethylated or methylated lysine can be modified by some proteins to regulate gene expression. Generally, UHRF1 recognizes the dimethylated or trimethylated H3K9 (H3K9me2/3) mediated by G9a29 or SuV39H through TTD4 and PHD (TTD-PHD) as well as identifies unmodified arginine 2 (R2) and unmodified lysine 4 (K4) through the PHD domain.30–36 The association with histone marks are all required for DNA methylation.34,37 Moreover, UHRF1 reportedly adopts a closed conformation in the absence of chromatin in which a polybasic region (PBR) in the C-terminus binds to the TTD and inhibits its recognition of H3K9me3, whereas the SRA domain binds to the PHD domain and inhibits recognition of unmethylated histone H3 at residue R2 (H3R2); upon binding to hmDNA by the SRA domain, UHRF1 impairs the intramolecular interactions and transfers to an open state,30,34,38 which allows TTD-PHD to recognize H3K9me3 and facilitates SRA-PBR to either recognize hmDNA or recruit DNMT1 for an accurate methylation heredity39 (Figure 1). In addition, phosphatidylinositol 5-phosphate (P15P) in the nucleus can interact with the PBR through phosphorylation of S651 to participate in the regulation of heterochromatin localization of UHRF1 and crosstalk between H3K9 methylation and DNA methylation.39,40 Phosphorylation of S298 in the linker residue abrogates the UHRF1-H3 interaction by altering the relative position of the two reader modules, indicating the linker region may act as a functional switch of UHRF1 involved in multiple regulatory pathways such as maintenance of DNA methylation, transcriptional repression, and cell cycle progression.41 In contrast, Zhao et al42 found only a 10% reduction of DNA methylation in various tissues after abolishment of the association between H3K9me2/3 and UHRF1 in mammals, indicating that H3K9 methylation binding of UHRF1 is not the only process to ensure...
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Figure 1 Schematic representation of UHRF1 with structure–function domains in the maintenance of DNA methylation.

Notes: (A) Domain architecture of UHRF1 with their corresponding epigenetic function including DNA methylation and histone modification. (B) Conformational changes of UHRF1 from close to open state mediated by hmDNA.

Abbreviations: DNMT, DNA methyltransferases; H3K9me2/3, dimethylated or trimethylated H3K9; H3R2, unmethylated histone H3 at residue R2; hmDNA, hemi-methylated DNA; HAUSP, herpes virus-associated ubiquitin-specific protease; PBR, polybasic region; PHD, plant homeodomain; PI5P, phosphatidylinositol 5-phosphate; RiNG, really interesting new gene; SRA, SeT and RiNG-associated domain; TTD, tandem tudor domain; UBL, ubiquitin-like domain.

DNA methylation. A positive correlation exists between UHRF1 and EZH2 in cancer cells, and EZH2 participates in H3K27 methylation which mediates gene silencing.

Ferry et al found nonhistone mammalian DNA ligase 1 (LIG1), which contains a conserved H3K9-like mimic can also be methylated by G9a/GLP at K126 and subsequently recruit UHRF1 to replication foci, which is similar to binding H3K9me2/3 to maintain DNA methylation.

UHRF1 can also lead to global DNA hypomethylation in cancers. Long interspersed nucleotide element-1 (LINE1) is considered a surrogate marker of global DNA methylation. Nakamura et al found overexpression of UHRF1 could drive global DNA hypomethylation in esophageal squamous cell carcinoma (ESCC). Paradoxically, Ye et al reported that knockdown of UHRF1 results in LINE1 hypomethylation in ESCC. Because the states of promoter methylation in tumor suppressor genes were not mentioned in these two studies, Hoshimoto et al hypothesized that ESCC was associated with global DNA hypomethylation and specific tumor-related gene hypermethylation.

Although DNMT1 has a 30- to 40-fold preference for hmDNA sites, it can still bind to unmethylated DNA, and exert de novo methylation activity. UHRF1 may lead to aberrant DNA methylation state in cancer cells for two reasons: 1) UHRF1 recruits DNMT1 to hm-CpG islands or CpG islands, which have not been previously methylated because DNMT1 also has the property of de novo methylation which undergoes hypermethylation, over time, finally leading to the malignancy of cancer; 2) when the UHRF1 protein levels become high, ubiquitination may occur, and DNMT1 is ubiquitylated to a proper level to maintain the malignancy state of cancer, including hypermethylation of specific tumor suppressor gene promoters as well as global DNA hypomethylation.

Self-regulation of UHRF1 macromolecular complex during the cell cycle

UHRF1, DNMT1, HAUSP, HDAC1, Tip60, Hsp90, Suv39H1, PCNA, and pRb form a macromolecular complex
termed epigenetic code replication machinery (ECREM) to undergo temporal and spatial control during the cell cycle. During DNA replication, DNMT1 is partially recruited into the replication forks by PCNA. Tip60 interacts with SRA and RING domain of UHRF1 through its enzymatic MYST domain, and overexpression of Tip60 leads to the downregulation of UHRF1. In addition, UHRF1 can repress the activity and expression of Tip60. Furthermore, overexpression of Tip60 leads to acetylation and subsequent ubiquitylation-dependent proteasomal degradation of DNMT1 mediated by UHRF1. Although increased DNMT1 abundance in multiple cancers is largely due to the reduced degradation at the protein level rather than higher mRNA level, the consequence appears diverse based on cell cycle status or cell types and DNMT1 stability might be regulated by other upstream factors such as pRB and ATM. However, the mechanism remains to be elucidated.

Conversely, HAUSP interacts with PBR of UHRF1 through UBL1 and UBL2 domains to stabilize UHRF1 via deubiquitination and protects it from autoubiquitination as well as promote the open state of UHRF1 to facilitate the binding of UHRF1 to H3K9me3. When S652 (located in PBR) is phosphorylated by M phase-specific kinase CDK1-cyclin B during mitosis, UHRF1 is separated from HAUSP and the PBR domain is exposed to Tip60 for acetylation and ultimate degradation. In addition, HAUSP can deubiquitinate Tip60.

HAUSP binds to the KG linker of DNMT1 at its acidic pocket near the C-terminal to deubiquitinate DNMT1 from proteasomal degradation. In addition, HAUSP functions as a deubiquitylating enzyme toward ubiquitylated histone H3, and is likely involved in DNMT1 recruitment to DNA replication sites and the regulation of maintenance of DNA methylation without affecting the kinetics and efficiency of DNA replication. Simultaneously, Cheng et al. found that acetylation of the KG linker lysine residues impairs DNMT1–HAUSP interaction and promotes the degradation of DNMT1. Treatment with HDAC inhibitor not only increases the level of acetylated DNMT1 but also decreases the total DNMT1 protein level. HDACs can be recruited by both DNMT1 and UHRF1 to repress gene expression, and HDAC1 can deacetylate DNMT1 to protect it from degradation. In addition, the stability of DNMT1 is maintained in part through the recruitment of Hsp90 mediated by the ubiquitin-proteasome pathway. Suv39H1 and G9a are both H3K9 histone methyltransferases found in the same macromolecular complex with UHRF1 to prevent transcriptional activation; the latter can maintain the DNA methylation state required for de novo DNA methylation and the establishment in mouse embryonic stem cells. UHRF1 is necessary for binding Suv39H1 and H3K9me3 modification, however, the underlying interaction mechanisms remain to be elucidated.

The macromolecular complex may function as follows (Figure 2): UHRF1 and DNMT1 adopt a closed conformation and an auto-inhibitory state after protein synthesis, respectively. During DNA replication, the ECREM binds to replication foci. First, the closed conformation of UHRF1 shifts to an open state due to the following reasons: 1) HAUSP binds to PBR as well as the hmDNA; 2) SRA domain binds to hmDNA; and 3) phosphorylation of S651 by P15P leads to TTD relief from PBR and forms a histone binding cassette with the PHD domain and linker. Second, UHRF1 in the open state binds to H3K9me2/3 or H3K9me3 mimic LIG1 by TTD or TTD-PHD as well as binds to H3R2 via the PHD domain. Third, the RING domain of UHRF1 ubiquinates H3K18 and/or H3K23, which recruits the RFTS domain of DNMT1. The RFTS domain of DNMT1 also binds to the SRA domain or UBL of UHRF1 to relieve the self-inhibitory activity. HDAC1 and HAUSP can prevent UHRF1 and DNMT1 from acetylation and ubiquitination for degradation mediated by Tip60 during S phase when the abundance of Tip60 is relatively low. HAUSP can also deubiquitinate H3 which recruits the RFTS domain of DNMT1 to maintain a faithful inheritance of DNA methylation. At the beginning of G2 phase, when DNA replication is completed and methylation inheritance is formed, UHRF1 and DNMT1 undergo degradation. In detail, HAUSP dislocates from the UHRF1/DNMT1 complex due to phosphorylation of the S652 position by CDK1-cyclin B followed by ubiquitination of UHRF1. Degradation of UHRF1 leads to upregulation of Tip60 and acetylation of DNMT1, which is subsequently ubiquitylated by UHRF1 and other ubiquitin-related enzymes. Finally, the abundance of UHRF1 and DNMT1 decreases to ensure the normal cell cycle.

**UHRF1 regulation and target therapy**

Due to overexpression in various types of cancers such as gastric cancer, colorectal cancer, hepatocellular carcinoma, ESCC, prostate cancer, bladder cancer, breast cancer, lung cancer, melanoma, medulloblastoma, lymphoblastic leukemia, and osteosarcoma, UHRF1 appears a potential biomarker and is promising in the target therapy of cancers. High expression of UHRF1 inhibits a variety of tumor suppressor genes (Table 1) such as p16, p53, BRCA1, p14ARF, and p15INK4a.
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Figure 2 Working model for dynamic regulation of UHRF1 macromolecular complex during cell cycle.

Notes: (A) The spatialization of UHRF1 macromolecular complex including UHRF1 at open state and other members of ECReM recruited into replication forks during S phase. (B) The separation of HAUSP from the complex owing to phosphorylation mediated by CDK1-cyclin B at the beginning of G2 phase. (C) Acetylation and ubiquitination of UHRF1 and DNMT1 by Tip60 due to release of HAUSP and HDAC1 from ECReM during G2-M phase. (D) The ubiquitination-mediated protein degradation of UHRF1 and DNMT1 in order to enter the normal cell cycle.

Abbreviations: DNMT, DNA methyltransferases; ECReM, epigenetic code replication machinery; HAUSP, herpes virus-associated ubiquitin-specific protease; HDAC1, histone deacetylase 1; PBR, polybasic region; PHD, plant homeodomain; RiNG, really interesting new gene; SRA, SeT and RiNG-associated domain; Tip60, Tat-interactive protein; TTD, tandem tudor domain; UBL, ubiquitin-like domain; UHRF1, ubiquitin-like with PHD and RING finger domains 1; PCNA, proliferating cell nuclear antigen; CDK1, cyclin-dependent kinase 1.

KISS1, RASSF1, MEG3, CDH4, Keap1, KLF17, RIP3, SHP1, RUNX3, FOXO4, CDX2, PPARG, Slit3, CDH1, GPX3, IGFBP3, RGS2, UBE2L6, and miR-145. Knowledge regarding the regulation of UHRF1 is limited; overexpression of UHRF1 may be involved in aberrantly high expression of transcription factors. Moreover, cell cycle regulators such as E2F8, E2F1, FOXM1, leptin, SP1, Hsp90, PS3/P21, and WDR79 can also protect UHRF1 from ubiquitination. Conversely, microRNAs such as miR-124, microRNA-9, miR-101, miR-378, miR-193a-3p, miR-1455/p/miR-145-3p, miR-146a/b, and let-7a-3p play an important role in the downregulation of UHRF1 (Table 2). Most of the related microRNAs have been found suppressed in multiple cancers and can regulate the expression of UHRF1 by base pairing with 3′ UTR at the mRNA level to inhibit protein synthesis.

The expression and activity of UHRF1 are also regulated by posttranslational modification. Chen et al found that casein kinase 1 delta could catalyze the phosphorylation of UHRF1 at S108 to undergo ubiquitination and degradation mediated by SCFβ-TrCP in response to DNA damage; kinase Pim1 destabilizes UHRF1 by phosphorylation at S311; methyltransferase PRMT6 can induce H3R2me2a and inhibit the association of the PHD of UHRF1 with H3R2 leading to DNA hypomethylation, which provides new insights for cancer therapy targeting UHRF1. Graf et al found the LRR domain of Pramel7 in embryos interacts with the SRA and RING domain of UHRF1 which subsequently undergoes degradation and hypomethylation. Wang et al found that globular adiponectin inhibited leptin-stimulated cell proliferation in esophageal adenocarcinoma via the inhibition of UHRF1 mediated by adiponectin receptor 2.
Table 1 Inhibition of TSGs by overexpression of UHRF1 in various types of cancers

| TSGs  | Functions                                                                 | Cancers                                                                 |
|-------|---------------------------------------------------------------------------|------------------------------------------------------------------------|
| P16^INK4 | Growth, metastasis, and apoptosis                                          | Colorectal cancer [22219067]                                            |
|       |                                                                           | Cervical cancer [23688286]                                             |
|       |                                                                           | Glioblastoma [25550546]                                               |
| BRCA1 | DNA damage repair, transcription regulation, chromatin remodeling, cell cycle checkpoint control, and apoptosis | Breast cancer [19943104]                                              |
| RUNX3 | Hypoxia and immune cell maintenance                                       | Hepatocellular carcinoma [26147747]                                    |
| KISS1 | Tumor differentiation, the depth of invasion, lymph node metastasis, and distant metastasis | Bladder cancer [25272010]                                             |
| RASSF1 | Proliferation, invasion, and apoptosis                                      | Non-small-cell lung cancer [21351083]                                  |
| MEG3  | Proliferation                                                              | Hepatocellular carcinoma [25641194]                                    |
| CDH4  | Proliferation, invasion, and metastasis                                    | Gastric cancer [26147747]                                             |
| Keap1 | Oxidative stress and reductive stress                                      | Gastric cancer [23982143]                                             |
| KLF17 | Invasion and epithelial–mesenchymal transition                            | Pancreatic cancer [26497117]                                          |
| SHP1  | Tumor differentiation or muscular infiltration depth                       | Breast cancer [28744404]                                             |
| CDX2  | Lymph node metastasis and tumor, nodes, metastasis stage                  | Gastric cancer [26147747]                                             |
| PPARG | Proliferation and migration and Wnt/β-catenin signaling pathway           | Gastric cancer [26147747]                                             |
| FOXO4 | Proliferation and metastasis                                              | Colorectal cancer [22286757]                                          |
| RIP3  | Cell survival, cell apoptosis, and cell necrosis                           | Colorectal cancer [28981102]                                          |
| Silt3 | Invasion and metastasis                                                   | Gastric cancer [23982143]                                             |
| CDH1  | Invasion and epithelial-to-mesenchymal transition                         | Prostate cancer [22330138]                                           |
| IGFBP3| Colony formation, migration, and invasion                                  | Prostate cancer [22330138]                                           |
|       |                                                                           | Prostate cancer [29507645]                                            |
| GPX3  | ROS, migration, invasion, metastasis                                       | Prostate cancer [22330138]                                           |
| UBE2L6| Apoptosis                                                                 | Cervical cancer [29157076]                                           |
| RG52  | Proliferation                                                              | Bladder cancer [25323766]                                            |
| miR-145| Proliferation, migration and invasion                                      | Non-small-cell lung cancer [25961369]                                 |

Abbreviations: UHRF1, ubiquitin-like with PHD and RING finger domains 1; TSGs, tumor suppressor genes; ROS, reactive oxygen species.

Anticancer drugs related to UHRF1 are as follows: uracil derivative NSC232003 functions as the only direct inhibitor by targeting within the SRA of UHRF1. Shikonin induces downregulation of both UHRF1 and DNMT1 in MDF-7 and hela cell lines. In addition, hinokitiol, dihydroartemisinin, epigallocatechin-3-gallate, emodin, mTOR inhibitor torin-2, luteolin, ERK1/2 pathway inhibitor PD98059, LY294002, AG490, Hsp90 inhibitor 17-AAG or 17-demethylamo-17-demethoxygeldanamycin, anisomycin, and curcumin have been reported to be used in various types of cancers.

Other functions of UHRF1 apart from epigenetic modification include its participation in the oxidative stress and DNA damage response to inhibit caspase-dependent apoptosis to promote the proliferation, invasion, and metastasis, promoting the development of embryogenesis and preimplantation of embryos, and regulating the

Table 2 Regulation of UHRF1 by microRNA in various types of cancers

| mRNAs         | Functions                                | Cancers                                                                 |
|---------------|------------------------------------------|------------------------------------------------------------------------|
| miR-124       | Proliferation, motility, angiogenesis     | Bladder cancer [26310391]                                             |
| miR-9         | Proliferation, apoptosis                  | Colorectal cancer [25940709]                                          |
| miR-101       | Migration, invasion                       | Renal cell cancer [27487138]                                          |
| miR-378       | Proliferation, apoptosis                  | Medulloblastoma [28901497]                                            |
| miR-193a-3p   | Metastasis                                | Non-small-cell lung cancer [25833338]                                 |
| miR-145-5p/miR-145-3p | Proliferation, apoptosis, migration, invasion | Bladder cancer [27628846]                                           |
| miR-146a/b    | Metastasis                                | Gastric cancer [23982143]                                            |
|               |                                          | Renal cell carcinoma [26859141]                                       |

Abbreviation: UHRF1, ubiquitin-like with PHD and RING finger domains 1.
function and development of T lymphocytes\textsuperscript{145,146} as well as the plasticity of smooth muscle cells.\textsuperscript{147}

**Prospects**

Great progress has been made in the functional and modulation mechanisms of the UHRF1 protein complex, and it will likely become a universal biomarker for cancer and specific targets for cancer therapy. Emerging evidence indicates that UHRF1 modules do not act independently of each other but establish complex modes of interaction with patterns of chromatin modifications. Due to the variant abundance of ECREM and the distinct level of components in diverse cell lines, the UHRF1 protein complex possibly undergoes temporal and spatial control during the cell cycle, although the mechanisms remain to be elucidated. Furthermore, the phosphorylation sites of amino acids among the five domains are involved in the regulation of UHRF1 activity, protein stability, DNA methylation, and histone posttranscriptional modification. As mentioned above, the mechanisms regarding degradation of UHRF1 by acetylation and ubiquitination and specific drugs targeting UHRF1 need to be further clarified. Because many drugs can downregulate UHRF1 as well as DNMT1, and lower expression of DNMT1 is more sensitive to 5-aza-CdR treatment,\textsuperscript{148} chemotherapy in cancer cells by using a DNMT1 inhibitor accompanied by UHRF1 inhibitor for the treatment of cancer is yet to be investigated. In summary, DNA methylation within CpG varies in different organs and times\textsuperscript{149} when cells are supposed to differentiate by epigenetic regulation, without DNA mutations or chromosomal translocation, indicating the important dynamics of UHRF1 in epigenetics and the various roles it plays over the life span.

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**Disclosure**

The authors report no conflicts of interest in this work.

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