

## Noncoding RNAs: Different roles in tumorigenesis

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A major portion of the mammalian genome is transcribed to produce large numbers of noncoding RNAs (ncRNAs). During the past decade, the discovery of small RNAs, including the microRNAs (miRNA) and small interfering RNAs (siRNA), has led to important advances in biology. The breadth of the ncRNA field of study has substantially expanded and many recent results have revealed a range of functions that can be attributed to the miRNAs and other ncRNAs. For example, *H19* RNA, *HOTAIR* RNA, transcribed ultraconserved regions (T-UCRs), natural antisense RNA, transfer RNA and mitochondrial noncoding RNA have been suggested to play important roles in cancers and other diseases as well as in diverse cellular processes. In this review, we focus on the current status of several classes of ncRNAs associated with cancer with the emphasis on those that are not microRNAs.

**noncoding RNA, cancer, microRNA**

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Mounting evidence has revealed that a major portion of the mammalian genome is transcribed to produce large numbers of noncoding RNAs (ncRNAs), that is, RNAs that either do not have an open reading frame or RNAs that have a short poorly conserved open reading frame and do not code for a protein [1,2]. During the past decade, the discovery of small RNAs including the microRNAs (miRNAs) and small interfering RNAs (siRNAs) has led to major advances in biology. After miRNAs were first connected to cancer pathogenesis [3], accumulating data have pointed to a central regulatory role for miRNAs in the initiation and progression of most of the cancers that have been analyzed so far. Recently, the breadth of the ncRNA field of study has substantially expanded. A series of studies have revealed the essential roles of many ncRNAs in diverse cellular processes including cancer; these ncRNAs include *H19* RNA, *HOTAIR* RNA, transcribed ultraconserved regions (T-UCRs), natural antisense RNA, transfer RNA and mitochondrial noncoding RNA (Figure 1). In this review, we focus on the current status of the research into the several classes of ncRNAs

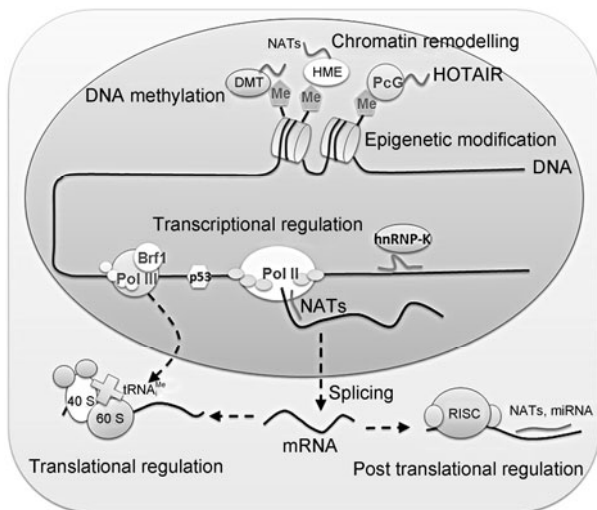
associated with cancer.

### 1 MicroRNAs and cancer

MicroRNAs (miRNAs), a category of small ncRNA molecules with lengths of 18–25 nucleotides, were discovered recently. MiRNAs act as post-transcriptional regulators of genes by sequence-specific binding to the 3' untranslated regions (3' UTRs) of target mRNAs. Release 17 (April 2011) of the miRBase database (<http://microrna.sanger.ac.uk/>) catalogues 1424 human miRNAs. Over 60% of human protein-coding genes have been suggested to be under selective pressure to maintain their pairing to miRNAs [4]. Depending on the degree of sequence complementarity between the miRNA and 3' UTR, the pairing might result in inhibition of translation and/or degradation of the target mRNA [5,6].

Several miRNAs that previously were described as suppressors have been found to be either deleted or mutated in various human malignancies. Calin et al. [3] first reported that *miR-15a* and *miR-16-1*, located in a cluster on chromosome 13q14, a frequently deleted region in B cell chronic

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**Figure 1** Summary of ncRNAs involved in tumorigenesis and cancer biology. The roles of each of the ncRNA are described in the corresponding sections of the review.

lymphocytic leukemia (CLL), could function as tumor suppressor genes. This finding prompted the mapping of all miRNA genes to chromosomal locations. The mapping revealed that a significant fraction of miRNAs mapped to cancer-associated genomic regions in human [7] and mouse [8], suggesting a connection between the deregulation of miRNAs and tumorigenesis accompanied by chromosomal aberrations such as deletions and amplifications.

The loss of members of the let-7 family of miRNAs resulted in the constitutive overexpression of the *ras* oncogene [9]. It was found that *miR-15a* and *miR-16-1* target the *BCL2* oncogene that inhibits apoptosis [3]. It is clear that the loss of the miRNAs could contribute to malignant transformation through the dysfunction of cellular oncogenes.

However, in various human tumors, the overexpression of miRNAs has been observed and the evidence indicated that some of these miRNAs could function as oncogenes. The two miRNAs that were first found to be overexpressed in cancers were the miR-155 and miR-17-92 clusters [10,11]. Interestingly, in solid cancers of epithelial origin and in leukemias and lymphomas, *miR-155* was highly expressed and acted as an oncogene. However, in endocrine tumors it was significantly down-regulated and possibly had suppressive functions [12]. These studies suggest that the roles of miRNAs in cancers are tissue and tumor specific.

Various biological processes associated with cancer are regulated by microRNAs; they include transcription, cell-cycle regulation, apoptosis, angiogenesis, invasion and metastasis [13]. Numerous miRNA-target gene pairs have been reported to be involved with tumorigenesis (for a review, see [14]).

Because miRNA expression profiles reflect tumor origin, stage and other pathological variables, the profiles could be used as diagnostic or prognostic tools. Furthermore, in tumors in which miRNAs are either lost or overexpressed,

corresponding miRNAs or anti-miRNAs [15] might be considered as drugs that could induce apoptosis and/or cell cycle arrest in cancer cells depending on their deregulation role for growth and survival. Here we have briefly reviewed the relationship between miRNAs and cancer; however, there are many reports and reviews that discuss this subject and readers are referred to the related references for more detailed information [16,17].

## 2 Roles of other noncoding RNAs in cancer

While recent studies strongly support the role of microRNAs in the pathogenesis of the majority of cancers studied so far, recent investigations into the functions of other ncRNAs have indicated that they also play essential roles in the pathogenesis of cancers. These results add a new layer of complexity to the molecular architecture of human cancers. Here we briefly review several types of ncRNAs that drive cancer development and progression through different mechanisms in human cancers.

### 2.1 Large intergenic noncoding RNAs (lincRNAs)

There is growing recognition that mammalian cells produce many thousands of large intergenic transcripts [18]. To date, about a dozen functionally well-characterized large intergenic noncoding RNAs (lincRNAs) with transcript sizes ranging from 2.3 to 17.2 kilonucleotide (knt) have been reported in mammals [19,20]. These lincRNAs have distinctive biological roles in diverse molecular mechanisms that include imprinting (e.g. *H19* RNA), trans-acting gene regulation (e.g. *HOTAIR* RNA), mediating global gene repression in the p53 response (e.g. *lincRNA-p21*), functioning in X-chromosome inactivation (e.g. *Xist*) [20] and regulating nuclear import (e.g. *Nron*) [21]. Recent studies suggest that some of the lincRNAs, for example *H19* and *HOTAIR*, are involved in cancer progression and metastasis.

(i) *H19* RNA. *H19* is a maternally imprinted gene located on chromosome 11p15.5. It transcribes into a spliced 2.5-knt polyadenylated long ncRNA (*H19* RNA). Postnatally, its expression is shut off in most tissues; it is reactivated during adult tissue regeneration and tumorigenesis [22,23]. The imprinted cluster loci have been implicated in a variety of disorders and cancer predispositions for both pediatric and adult tumors. This observation implied that *H19* was a potential tumor suppressor. Studies by Hao and Crenshaw [24] demonstrated that the induced expression of a transfected copy of *H19* RNA suppressed cellular proliferation and tumorigenesis in certain tumor cell lines. This further placed the *H19* as a candidate gene with some kind of role in tumorigenesis. Later studies found that *H19* expression was reduced or extinguished in a proportion of Wilms' tumors and embryonal rhabdomyosarcoma [25,26]. These data show that *H19* can act as a tumor suppressor gene.

Numerous reports have linked *H19* expression and carcinogenesis, providing evidence for *H19* as an oncogene [23,27] and recent studies have shown that altered *H19* expression is associated with different stages of tumorigenesis, such as cell de-differentiation [28], blood vessel development [29] and tumor metastasis [30].

Even though many related studies have identified *H19* RNA that are aberrantly expressed in cancers, how *H19* fulfills its tumor suppressing or oncogenic tasks remains poorly understood. Berteaux and Lottin [31] found that *H19* overexpression had a significant impact on breast cancer cell proliferation; *H19* expression accelerated S-phase entry and cell cycle progression by controlling the direct and specific binding of transcription factor E2F1 to its promoter. A miRNA (*miR-675*) has recently been profiled in exon 1 of the human *H19* genes, suggesting that *H19* RNA may have two different functions: one as the spliced full-length transcript and the other as the *miR-675* precursor [32]. This duality could explain the conflicting functions (either as a growth suppressor or as a growth enhancer) that have been ascribed to *H19* in the control of tumor growth.

*IGF-2* is an oncogene which was identified as an oppositely imprinted (paternally imprinted) gene to *H19* in the same imprinting cluster [33]. *H19* and *IGF-2* have similar expression patterns in the same tissues at the same developmental stages during embryogenesis [34]. Losses of imprinting of the *IGF2* gene and inactivation of the *H19* gene have been implicated in the pathogenesis of embryonal tumors [35]. This pattern of gene expression suggests that these two genes may be co-regulated. In addition, *H19* RNA was reported to be involved in the repression of the *IGF-2* oncogene by affecting either its transcription [36] or its translation [37].

Different research groups have reported several different elements that regulate *H19* expression in tumors. Boutros et al. [23] identified c-Myc that directly binds to the *H19* promoter and substantially up-regulates the transcription of the maternal *H19* allele by recruiting histone acetyltransferase (HAT). Their results have further indicated that up-regulation of *H19* by Myc contributes significantly to the tumorigenic phenotype of breast and lung cancer cells. In breast cancer, *H19* was reported to be a target gene for hepatocyte growth factor (HGF) [38]. These results suggest the possibility of using the *H19* promoter in gene therapy to drive the expression of cytotoxic genes in tumorigenic cells [39].

(ii) *HOTAIR*. Rinn and Kertesz [40] have characterized a 2158 nucleotide lincRNA, termed *HOTAIR* (for *HOX* antisense intergenic RNA), and made the surprising discovery that this ncRNA acts *in trans* to regulate a *HOX* gene cluster on a completely different chromosome, not the *HOX* gene cluster that encodes the ncRNA itself. Mammalian homeobox (*Hox*) genes (*HOXA–D*), which establish the body plan during early development, are clustered at four chromosomal loci. *HOTAIR*, which is transcribed from the *HOX C* locus, can target Polycomb repressive complex 2 (PRC2)

and other chromatin regulators to the *HOX D* locus and potentially many other genomic loci [40–42]. Rinn and Kertesz [40] have profiled expression patterns of the transcriptional landscape of these loci and identified ncRNAs corresponding to chromatin regions with different epigenetic modifications, suggesting the mechanism might be epigenetic; however, this still remains an open question. Nevertheless, this is the first example of an RNA expressed on one chromosome influencing transcription on another chromosome. These findings reveal a new mechanism whereby ncRNAs can determine the silencing of distant chromosome regions that might have important implications for both disease and development.

Recently, Gupta and Shah [41] reported that *HOTAIR* is systematically dysregulated in breast carcinoma and, notably, that *HOTAIR* regulates metastatic progression. They found that *HOTAIR* recruits the PRC2 complex to specific target genes genome-wide, leading to alteration of histone H3 lysine 27 methylation, epigenetic silencing of metastasis suppressor genes (such as *JAM2*, *PCDH10* and *PCDHB5*), and inducing positive regulator genes of metastasis (such as *ABL2*, *SNAIL*, and *laminins*). *HOTAIR*-altered gene expression was reversed after PRC2 depletion.

The interdependence between *HOTAIR* and PRC2 may indicate potential therapeutic applications. Tumors that are sensitive to inhibitors of PRC2 could be identified by monitoring the increased level of *HOTAIR* [43]. Conversely, suppression of endogenous *HOTAIR* or inhibition of the *HOTAIR*-PRC2 interaction may provide potential therapeutic targets to tumors that overexpress Polycomb proteins.

(iii) *lincRNA-p21*. In investigations into the potential biological roles of lincRNAs, several lincRNAs were identified to be regulated by *p53*. Huarte et al. [44] have demonstrated that numerous lincRNAs are important components in the *p53*-dependent transcriptional pathway. Of these, *lincRNA-p21* functions as a direct *p53* transcriptional target in response to DNA damage; it is required for *p53*-dependent apoptotic responses to DNA damage. *lincRNA-p21* acts by modulating nuclear ribonucleoprotein K (hnRNP-K) localization through its interaction with hnRNP-K.

Various hypothetical mechanisms by which *lincRNA-p21* could contribute to repression at specific loci have been suggested. They include: (1) *lincRNA-p21* might direct a protein complex to specific loci by Crick-Watson base pairing; (2) *lincRNA-p21* might act by forming DNA-DNA-RNA triple-helical structures; or (3) *lincRNA-p21* might alter the binding specificity of DNA-binding proteins to influence their target preference. The precise mechanism needs further confirmation.

Because *p53* is a well-known tumor suppressor gene, it is clear that *lincRNA-p21* and several other lincRNAs function in an important pathway for cancer. It is tempting to speculate that other lincRNAs may also play important roles in numerous other tumor suppressor or oncogenic pathways, suggesting that the lincRNAs represent an unknown para-

digm in cellular transformation and metastasis. It is important to determine whether lincRNA genes can serve as tumor suppressor genes or oncogenes in future studies.

## 2.2 Transcribed ultraconserved regions (T-UCRs)

Evolutionarily conserved sequences within or adjoining orthologous genes often serve as critical *cis*-regulatory regions. Recent studies have identified long, noncoding genomic regions that are perfectly conserved in human, mouse and rat genomes; these regions are termed ultra-conserved regions (UCRs). UCRs represent a group of sequences 200–779 bp in length that were first discovered by Bejerano in 2004 [45,46]. UCRs are frequently located at fragile sites and genomic regions involved in cancers. A large fraction of the genomic UCRs encode a particular set of ncRNAs called transcribed ultraconserved regions (T-UCRs) that exhibit altered expression in human cancers [47].

Calin et al. [47] demonstrated that a new class of UCRs was significantly altered at both the DNA and RNA levels in adult chronic lymphocytic leukemias (CLL), colorectal and hepatocellular carcinomas. In colon cancer, *uc.73A*, one of the most statistically up-regulated transcribed-UCRs (T-UCRs), was found to induce cell proliferation by reducing apoptosis, suggesting its function as an oncogene.

Scaruffi et al. [48] suggested that deregulation of the microRNA/T-UCR network may play an important role in the pathogenesis of neuroblastoma and tumor-associated T-UCRs in CLL were found to be regulated by certain miRNAs [47]. These results led to the hypothesis that T-UCRs along with miRNAs may define signatures associated with the diagnosis, prognosis and treatment of tumors. The roles of T-UCRs in cancer cells remain to be defined.

## 2.3 Natural antisense RNA

Natural antisense transcripts (NATs), also called antisense RNAs, are a group of RNAs that contain sequences that are complementary to other endogenous transcripts. Both sense and antisense RNAs can either encode proteins or be noncoding transcripts; however, the most prominent product of antisense transcription in the mammalian genome is a noncoding antisense RNA partner of a protein-coding transcript [49]. To date, numerous NATs with repressive functions have been described in different organisms. They can be transcribed *in cis* from opposing DNA strands at the same genomic loci (*cis*-NATs), or *in trans* from separate loci (*trans*-NATs). Most of the studies in the past few years have focused on *cis*-NATs.

Earlier studies identified a certain numbers of sense-antisense transcripts exhibiting interactions that were associated with cancer. For example, *Bcl-2* antisense RNA *IgH* deregulates *Bcl-2* gene expression in human follicular lymphoma cell lines [50] and the NAT of EPR-1 (effector cell protease receptor-1) down-regulates survivin expression, thus, func-

tioning as an inhibitor of apoptosis in a human colon cancer cell line [51]. Although many NATs have been identified recently, our understanding of how antisense transcripts regulate gene expression in human cells remains mostly incomplete. Recent functional validation investigations have indicated that antisense transcripts are not a uniform group of regulatory RNAs but instead belong to multiple categories that have some common features. Pioneering studies in several eukaryotic systems have identified a number of proposed mechanisms for the antisense-mediated regulation of sense mRNA gene expression [52]. The proposed mechanisms have been categorized into four main groups: mechanisms related to transcription, RNA-DNA interactions (such as genomic imprinting, alteration of DNA methylation and chromatin modification), nuclear RNA duplex formation, and cytoplasmic RNA duplex formation (such as the formation of endogenous siRNAs and masking miRNA-binding sites). For a review of these mechanisms, see [53].

The growing list of validated sense-antisense transcript pairs includes many important developmental genes as well as genes known to be involved in various human disorders, including cancer. It is well known that suppression of transcription is usually brought about by DNA and chromatin modification at the promoter region of the sense strand. Previous studies have reported a few examples of antisense transcripts that are implicated in cancer via their role in altering DNA and chromatin modification. *p15* is a well-documented tumor suppressor gene that is frequently genetically deleted or epigenetically silenced in a wide variety of tumors including leukemia, melanomas, gliomas and lung cancers. Recently, Yu et al. [54] identified a *p15* antisense RNA (*p15AS* RNA), which can induce epigenetic silencing of *p15*. This study demonstrated that the antisense RNA could silence its sense gene at the transcriptional level by affecting DNA methylation and histone modifications. This result was confirmed by Morris [55] who found that *p21AS* RNA directed the trimethylation of histone H3 at lysine 27 (H3K27me3) in the *p21* sense promoter region, resulting in the suppression of *p21* gene expression. In addition, *p53* as a tumor suppressor gene plays an important role in the prevention of human cancer and its mutations result in human tumors. Recent studies have revealed that *Wrap53*, a natural antisense transcript of *p53*, plays an important role in the regulation of *p53* mRNA and in the induction of the p53 protein by targeting the 5' UTR of *p53* mRNA. Unlike the *p15* and *p21* antisense RNAs, *Wrap53* antisense and *p53* are co-expressed in cells [56].

NATs associated with genomic imprinting were identified in several reports. A region on chromosome 6 that contains three maternally imprinted protein-coding genes (*Igf2r*, *Slc22a2* and *Slc22a3*) has been shown to be controlled by a paternally expressed noncoding antisense *Igf2r* RNA (*Air*) [57]. *Air* is a 108-kb unspliced and repeat-rich transcript which only overlaps in an antisense orientation with *Igf2r* and not with *Slc22a2* or *Slc22a3* [58]. However, the expres-

sion of *Air* can silence all three protein-coding genes in the imprinted cluster [59].

Significantly, the development of tools to identify antisense transcripts that are associated with cancer loci should provide a better insight into the functions of different antisense transcripts and the molecular etiology associated with many important human cancers. Moreover, the ability of NATs to regulate the expression of sense transcripts could provide numerous new potential drug targets in the emerging field of functional antisense transcripts.

## 2.4 Transfer RNA

The increased expression of RNA polymerase (pol) III transcription factors and of pol III products (such as tRNA and 5S rRNA) has often been observed in transformed and tumor cells in, for example, ovarian and cervical carcinomas and breast cancer [60–62]. However, whether pol III is a causative factor in cancer is unclear because recurrent mutations in pol III subunits or the associated transcription factors have not been found in tumors.

tRNAs are one large class of pol III noncoding transcripts. Earlier research showed that the tRNA methyltransferase Misu was overexpressed in breast and colon cancers, and that Misu RNAi (RNA interference) inhibited growth of carcinomas [63], supporting the conclusion that tRNA metabolism can have a strong influence on oncogenic transformation.

Recently, White et al. [64] reported that elevated tRNA production can have a causal role in oncogenic transformation. They demonstrated that the phenotypic effects of pol III transcription factor Brf1 induction was mimicked by elevated levels of a pol III product tRNA<sub>i</sub><sup>Met</sup>, the initiator tRNA<sup>Met</sup>. Moreover, a modest overexpression of tRNA<sub>i</sub><sup>Met</sup> was found to significantly stimulate translation, suggesting the activation of tRNA expression that was often observed in transformed cells might have functional consequences for the development of cancer [64]. White et al. created stably transfected lines of immortalized mouse embryonic fibroblasts carrying cDNA encoding tRNA<sup>Met</sup> that expressed elevated levels of cyclin D1 and c-Myc proteins without corresponding changes in the levels of their mRNA. Mei et al. [65] microinjected tRNA into cells and found that this led to significant inhibition of cytochrome *c*-induced apoptosis. Therefore, the tRNA levels in cells may be relevant for the translation of the important genes required for the tumorigenic process and may play a central role in cancer development and progression. The precise mechanism and possible therapeutic applications of tRNA in cancer need to be explored further.

## 2.5 Noncoding mitochondrial RNAs

The mammalian mitochondrial genome is a double-stranded circular DNA about 16500 nucleotides long [66]. It encodes

the 12S and 16S ribosomal RNAs, 22 tRNAs and 13 polypeptides [67]. Besides these coding regions, the mitochondrial DNA (mtDNA) contains an approximately 1-kb noncoding region called the D-loop region that contains the origin of H-strand replication and the promoters for transcription of the H-strand and L-strand templates [68].

Several models that link mitochondrial dysfunction to cancer have been proposed. However, only a few of these models discuss the role of noncoding mitochondrial RNA (ncmtRNA) in cancer, and the precise mechanism of their involvement is yet to be defined.

mtDNA mutations have been linked to a broad spectrum of malignancies. Mutations have been described in both rRNAs, in all 22 tRNAs, in all 13 of the mtDNA-encoded subunits of the respiratory chain complexes and in the mitochondrial D-loop regions [69]. Interestingly, about half of the disease-related mutations (237 in total 480) are located within the ncmtRNA genes (see <http://www.mitomap.org/MITOMAP>) and, given that these sequences comprise only 10% of the mitochondrial genome, this result indicates a possible role for the ncmtRNAs in various diseases including cancer [70].

Mei et al. [65] have reported that when mitochondrial tRNAs along with cytosolic tRNAs bind to cytochrome *c* they impair the interaction of cytochrome *c* with Apaf-1, a caspase activator, and prevent the formation of the apoptosome. This finding raised the possibility that mitochondrial tRNAs may play a role in determining cellular responsiveness to apoptotic stimuli.

In mouse cells, Villegas et al. [71,72] identified the presence of a mitochondrial RNA (mtRNA) that contained an inverted repeat (IR) of 121 nucleotides that covalently linked to the 5' end of the mitochondrial 16S RNA. Later the same workers demonstrated that human cells contain a transcript of 2374 nt with similar structural features to the mouse mtRNA. This transcript contained a stem-loop structure with an 820-bp double-stranded region and a 40-nt loop, and that it was expressed in human proliferating cells but not in resting cells, suggesting that this ncmtRNA may play a role in cell proliferation [73]. Recently, Burzio et al. [74] reported that in addition to this transcript, normal human proliferating cells in culture expressed two ncmtRNA transcripts, sense ncmtRNA (SncmtRNA) and antisense ncmtRNA (ASncmtRNA). The expression of SncmtRNA and the down-regulation of the ASncmtRNAs was observed in 15 different tumor cell lines and in tumor cells present in 273 cancer biopsies corresponding to 17 different cancer types. The correlation of SncmtRNA and the replicative state of the cells suggests that SncmtRNA may play a role in regulating cell cycles. However, how this molecule might participate in the regulation of cell cycles remains unknown. Our understanding of ASncmtRNAs is even lower; the down-regulation of ASncmtRNAs in tumor cells suggests its possible role as a unique mitochondria-encoded tumor suppressor [74].

## 2.6 Other ncRNAs

Many other ncRNAs including Alu RNA [75], Y RNA [76], and small nucleolar RNA [77], show abnormal expression patterns in cancerous tissues. Their roles and possible mechanisms are not reviewed here; however, some details about them are described in the references.

## 3 Conclusion

An understanding of ncRNA is crucial to the early detection and better diagnosis of cancer, for a more accurate description of the tumor type, and for a more effective therapy. Potentially, an attractive model of cancer treatments is either to activate tumor-suppressor genes or to silence oncogenes by programming specific noncoding RNAs.

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