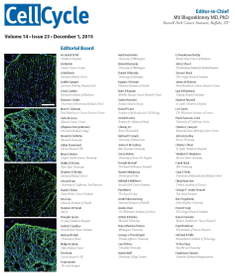

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REVIEW

How RNAi machinery enters the world of telomerase

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ABSTRACT

Human telomerase holoenzyme consists of the catalytic component TERT and the template RNA TERC. However, a network of accessory proteins plays key roles in its assembly, localization and stability. Defects in genes involved in telomerase biology affect the renewal of critical stem cell populations and cause disorders such as telomeropathies. Moreover, activation of telomerase in somatic cells allows neoplastic cells to proliferate indefinitely, thus contributing to tumorigenesis. For these reasons, identification of new players involved in telomerase regulation is crucial for the determination of novel therapeutic targets and biomarkers. In the very last years, increasing evidence describes components of the RNAi machinery as a new layer of complexity in human telomerase activity. In this review, we will discuss how AGO2 and other proteins which collaborate with AGO2 in RNAi pathway play a pivotal role in TERC stability and function.

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Introduction

Linear eukaryotic chromosomes, unlike prokaryotic circular DNA molecules, pose two main threats to genome stability: on the one hand DNA polymerases are intrinsically unable to replicate the last region of the lagging DNA strand, resulting in progressive chromosome shortening; on the other hand free DNA ends might be recognized as DNA breaks thus triggering recruitment of the DNA repair machinery [1]. In the vast majority of eukaryotes, these issues have been solved by the telomerase enzyme [2], which adds repetitive DNA sequences at chromosome ends [3].

Telomerase activity results in telomeric DNA containing repeats of the hexanucleotide sequence 5'-(TTAGGG)n-3', called telomeres, which span several kilobases at the end of each eukaryotic chromosome [4]. A specialized set of proteins is recruited on telomeric DNA sequences thus preventing the engagement of the DNA repair machinery on chromosome ends and protecting them from exonuclease attack [5–8]. Relevant exceptions are represented by diptera which have lost the genes encoding for telomerase components and rely on different classes of retrotransposons for the maintenance of telomeres [9].

Human telomerase enzyme consists of two core components, which *in vitro* are sufficient for minimally reconstituting telomerase activity: a protein component (Telomerase Reverse Transcriptase, TERT) and a non-coding RNA (Telomerase RNA Component, TERC) [10] (Figure 1(a)).

TERT has an intrinsic reverse transcriptase activity and uses the associated TERC molecule as a template to synthesize highly repetitive telomeric DNA sequences. TERT displays a high conservation across all eukaryotes with a clear organization into well-defined functional domains [11,12].

Despite the poor overall sequence conservation of TERC and the great size variability across eukaryotic cells (ranging from 147 nt to more than 2 kb), several functional domains of this non-coding RNA have been retained across evolution [13]. This is in line with the proposed pattern of evolutionary conservation of long non-coding RNAs (lncRNAs), which generally are characterized by highly conserved functional domains embedded into a less conserved RNA transcript [14]. TERC is transcribed by RNA polymerase II and polyAdenylated. However, it is worth mentioning that unlike most lncRNAs, TERC does not seem to contain any intron in any eukaryotic species with the notable exception to some fungi which rely on a non-canonical splicing event to process 3' end of TERC [15].

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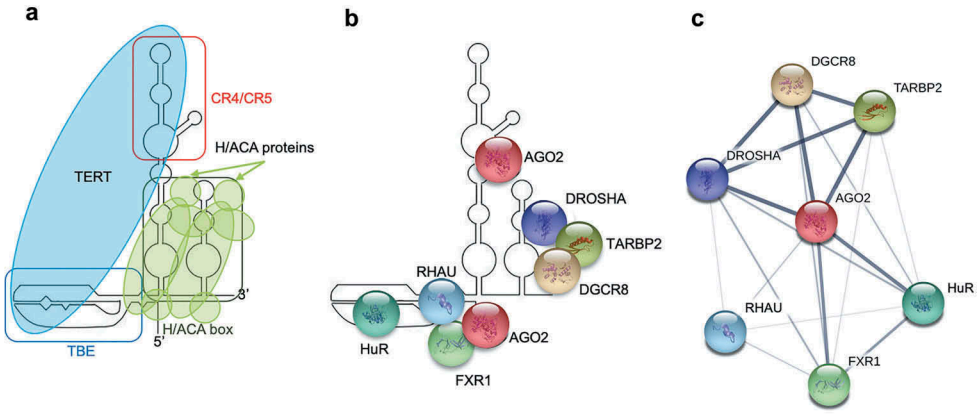

Figure 1. RNAi machinery as a new player in TERC stability and function. A. TERC (Telomerase RNA Component) contains three structural domains (TBE, CR4/CR5 and H/ACA box) which mediate interactions with TERT (Telomerase Reverse Transcriptase) and two sets of H/ACA proteins, thus forming the human telomerase holoenzyme. B. Schematic representation of the interactions between TERC and several components of the RNAi machinery . AGO2, FXR1, RHAU and HuR have been shown to associate with regions inside and surrounding the TBE at the 5′ end of TERC. AGO2 has a second binding site overlapping the CR4/C5 domain. DROSHA, TRBP2 and DGCR8 bind to the H/ACA box at the 3′end of TERC. C. Network representing functional interactions between RNAi components involved in TERC processing, assembly and function, as assessed by https://string-db.org [132].

Notably, telomere maintenance requires not only a proper assembly of TERT and TERC but also several accessory proteins needed for maturation, stability, and subcellular localization of telomerase.

Controlling telomere lengthening and telomerase activity is crucial for normal human development. Telomerase activity is undetectable in most human somatic cells but needs to be maintained in highly proliferating cell populations such as germ cells, stem cells, and expanding lymphocytes. On the other hand, telomerase production is up-regulated in many cancers, enabling neoplastic cells to proliferate indefinitely [16] and its function is impaired in telomeropathies, causing progressive telomere shortening and subsequent proliferative blocks, such as dyskeratosis congenita, aplastic anemia and idiopathic pulmonary fibrosis [17].

Interestingly, in the very last years, increasing evidence links components of the RNA interference (RNAi) pathway to telomerase activity (Figure 1 (b, c)). RNAi is an evolutionarily conserved molecular mechanism which controls expression of target genes at post-transcriptional level taking advantage of base complementarity between small non-coding RNAs (22–24 nt long, sRNAs) such as microRNA (miRNAs) [18–20], small interfering RNAs (siRNAs) [21,22] and piwi-RNAs (piRNAs) [23], and target RNAs. Most of the sRNAs are enzymatically processed by several endoribonucleases such as DROSHA and DICER and are loaded onto an RNA binding protein belonging to the Argonaute family which recruits downstream effectors [24,25]. Besides its well-known function in post-transcriptional regulation, components of human RNAi machinery have been involved in a number of other pathways, such as transcriptional regulation [26–28], RNA splicing [29–31], and DNA damage [32–35].

Here, we will discuss this emerging aspect of telomerase biology, by focusing on newly identified sRNAs originating from TERC and on primary or accessory RNAi proteins involved in TERC processing and activity.

Human telomerase RNA structure

Human TERC is organized into several domains. Starting from the 5' end of the mature RNA molecule, the following domains have been identified: the Template Boundary Element (TBE), the template region, the Pseudoknot Domain, the CR4/CR5 domain and the H/ACA box (Figure 1(a)).

Human TBE is required to pause DNA synthesis after incorporation of the GGTTAG sequence and thus allowing dissociation of the enzyme from DNA and its subsequent re-association to synthesize a further hexanucleotide [36]. The P1-b helix formed by pairing of the TBE with a downstream region of TERC itself has been proven fundamental to define the last nucleotide incorporated [37]. It is worth highlighting that appropriate pausing not only affects the

sequence of telomere repeats but is essential to preserve correct annealing of TERC to telomeric DNA for the addition of further telomeric repeats. In fact, mutations affecting the TBE also impact on telomerase processivity [37].

The template region is a short stretch of nucleotides consisting of two sequences: the alignment region pairs with telomeric DNA, while the templating region acts as a template during DNA synthesis. Human TERC relies on a 5 nt alignment region, considerably longer than the one found in rodents (2 nt) and in other vertebrates [38]. It has been shown that an alignment sequence shorter than 3 nt impairs human telomerase activity [39]. The templating region may vary slightly in different species as several different permutations of the ancestral CCCUAA template all result into $(TTAGGG)_n$ telomeric sequence. In fact, the templating region of human telomerase is CUAACC.

The pseudoknot domain is required in mammals for TERC binding to TERT, as it directly interacts with TERT protein [40]. This notion is corroborated by the finding that an engineered TERC consisting only of the pseudoknot domain is able to bind TERT yielding a functional reverse transcriptase enzyme able to extend RNA/DNA hybrid molecules [41].

The CR4/5 domain is also required for proper association of human TERC with TERT. CR4/5 and pseudoknot domains appear to interact with TERT independently of each other [42]. Direct interactions between CR4/5 domain and TERT have been characterized by UV-crosslinking. It has been suggested that binding of the CR4/5 domain contributes to the proper folding of TERT, thus explaining its requirement for telomerase activity [43].

In different phyla, the 3' terminal region of TERC RNA has extremely diverse structures. However, in most cases, despite the wide differences in sequence and conformation, this region of TERC RNA seems to play a crucial role in TERC processing [44–47]. In vertebrates, the 3' terminal region of TERC contains a structural domain common to scaRNAs (small Cajal body-specific RNAs), consisting of a pair of H/ACA box required to mediate interaction with the H/ACA proteins and TERC processing [48].

TERC processing beyond maturation: generation of small non-coding RNAs

TERC processing at 3' end is crucial for its maturation and accumulation. Indeed, TERC is initially a 3' extended product which is post-transcriptionally processed into the 451-nt mature form. These long forms are predominantly degraded by the nuclear exosome targeting complex (NEXT), and the exosome [49,50]. Moreover, TERC undergoes a cycle of oligoadenylation mediated by PAPD5 (poly(A) polymerase PAP-associated domain-containing 5), a non-canonical poly-A polymerase that is a component of the nuclear polyadenylation complex TRAMP [51,52]. Since TERC oligoadenylated isoforms are degraded by the exosome, the Poly(A)-specific ribonuclease (PARN) antagonizes degradation by removing oligo (A) tails from TERC [53–55]. Recently, it was shown that processing of TERC oligoA tails occurs in two steps with longer forms first being trimmed by the exosome-associated exonuclease RRP6 and shorter forms then being processed by PARN [56].

Involvement of the double-stranded RNA-binding protein DiGeorge critical region 8 (DGCR8) in TERC 3'end processing was recently shown [57] (Figure 1(b)). DGCR8 is known to control miRNA biogenesis. In the nuclear compartment, DGCR8 associates to the RNase III enzyme Drosha forming the Microprocessor complex which processes the primary miRNAs (pri-miRNAs) in ⊠70 nucleotide (nt) stem–loop precursor miRNAs, termed pre-miRNAs [58,59]. Pre-miRNAs are then exported in the cytoplasm and processed by another RNase type III enzyme, DICER, to give rise to miRNA duplexes [60,61]. Interestingly, DGCR8 interacts with the 3'end region of TERC and is required to recruit RRP6 onto TERC, allowing the exosome-mediated control of TERC maturation [57].

Notably, recent evidence suggests that besides the canonical processing of 3' end of TERC aimed at producing mature TERC, TERC RNA also enters an alternative pathway for the biogenesis of smaller RNA products originating from TERC 3'end sequence.

Recently, we identify a sRNA arising from positions 425–447 of TERC, lying in the right arm of the terminal hairpin of the H/ACA box (*terc*-sRNA; [62]), which belongs to the class of Transcription Termination Site-associated sRNAs (TTSa-RNAs) [63]. *Terc*-sRNA has been detected in both human cell lines and primary tissues, such as lung, colon, kidney, breast, uterus and adrenal cortex. Interestingly, *terc*-sRNA is upregulated in different tumors as compared to healthy tissues. The pattern of expression of *terc*-sRNA recapitulates what previously seen for TERC, which is ubiquitously expressed [64,65] and up-regulated in human tumor cells [66], suggesting that during tumorigenesis higher levels of TERC might promote the biogenesis of *terc*-sRNA. *Terc*-sRNA derives from a stem-loop structure, reminiscent of miRNA precursors. However, our data suggest that its processing is not dependent upon the RNase III endonuclease DICER or the sRNA binding protein ARGONAUTE2 (AGO2), which are both involved in miRNA processing and/or stability [67]. Notably, *terc*-sRNA overexpression is able to increase telomerase activity, suggesting that increasing level of *terc*-sRNA might confer a selective advantage to TERT-expressing tumor cells. The mechanism of action of *terc*-sRNA is described below.

Interestingly, in a recent paper Fish and colleagues demonstrate that a 45nt-long noncoding RNA (T3p) arises from an endonucleolytic processing of the 3 end of the TERC [68]. T3p was described as a negative regulator of miRNA function, by acting as a miRNA sponge and promoting metastasis in breast cancer. The nucleolytic biogenesis of T3p from TERC is controlled by the endoribonuclease DROSHA and the double-stranded RNA binding protein TARBP2 (RISC Loading Complex RNA Binding Subunit) (Figure 1(b)), two factors involved in miRNA processing [69]. Since T3p embeds *terc*-sRNA sequence, we can speculate that this RNA molecule might be *terc*-sRNA precursor. However, the role of T3p in telomere lengthening and telomerase activity was not investigated by the authors and further experimental evidence is required to link T3p to *terc*-sRNA biogenesis.

TERC-interacting proteins: the usual and unusual suspects

Even though for *in vitro* activity the essential components of the telomerase are TERT and TERC [10], the composition and assembly of the telomerase holoenzyme are more complex *in vivo*. Other RNA-binding proteins associate to TERC and contribute to the stability, activity and localization of telomerase holoenzyme.

In human cells, the H/ACA box at TERC 3'end fosters interactions with the same mature H/ACA proteins and chaperones as the intron-encoded small nucleolar (sno) or small Cajal body (sca) RNAs that catalyze processing and pseudouridylation of ribosomal and small nuclear RNAs [70]. H/ACA RNPs consist of four evolutionarily conserved proteins: Dyskerin [encoded by the gene *DKC1* (Dyskerin Pseudouridine Synthase 1)], NHP2 (NHP2 ribonucleoprotein), NOP10 (NP10 ribonucleoprotein), and GAR1 (GAR1 ribonucleoprotein). TERC binds co-transcriptionally with two sets of core H/ACA proteins [42,48] (Figure 1(a)). Dyskerin and NOP10 directly interact with TERC H/ACA motif, while GAR1 and NHP2 are recruited through protein–protein interactions with dyskerin and NOP10, respectively [71,72]. Association of H/ACA protein to TERC is required for TERC stability and accumulation via protection from degradation [55,56] and enhances endogenous TERC-TERT interaction [73]. Indeed, defects in genes involved in H/ACA RNP formation reduce levels of TERC and telomerase activity, thus impairing stem cell population renewal and causing a spectrum of disorders such as dyskeratosis congenita [17]. Assembly and function of H/ACA proteins were detailed elsewhere [74,75].

The 3'-apical loop in the human TERC H/ACA domain contains a Cajal body box (CAB box) which is necessary for the binding of TERC to the WD40 repeat domain-containing protein TCAB1 (telomerase Cajal body protein 1). This binding is required for telomerase trafficking to Cajal bodies, a nuclear compartment rich in RNA splicing and post-transcriptional modification machineries [70,76–78]. However, even though depletion of TCAB1 causes telomeres to shorten [79], Localization of TERC in Cajal bodies is not essential to maintain stable telomere length

homeostasis [73]. Indeed, recent evidence demonstrates that the tethering of TCAB1 to CAB box of TERC is required for the catalytic activity of telomerase by facilitating proper folding of TERC CR4/5 domain and optimal engagement with TERT [80].

Recently, we described members of the Argonaute subfamily AGO as novel TERC-binding proteins [62] (Figure 1(b)). AGO proteins are highly conserved and are found in nearly every life form, from humans to Archaea [81–84]. In humans, there are 4 AGO members (AGO1-4), among them only AGO2 has been demonstrated to have slicer activity when sRNAs and target RNAs display perfect base pairing. Also, AGO3 possesses the characteristic aspartate-aspartate-histidine catalytic triad [24,85] but requires peculiar sRNA-target complementary rules for target cleavage [86].

AGO proteins are key mediators of canonical RNAi. They mainly interact with miRNAs or siRNAs and are involved in cytoplasmic post-transcriptional gene-silencing processes [25,87]. However, recent evidence involves AGO proteins in nuclear processes such as transcriptional gene silencing [27,28], DNA damage [34,35], chromatin remodeling [88] and splicing [30,31], in association with novel classes of sRNAs [35,63,89,90].

In this scenario, we recently described an unexpected role for AGO2 in the control of human telomerase activity. Notably, AGO2 depletion in human cells decreases telomerase activity, thus resulting in shorter telomeres, lower proliferation rate *in vitro* and tumor growth *in vivo*.

We identified AGO2 as a novel TERC-binding protein. AGO2 interacts with the newly identified *terc*-sRNA, which not only originates from the 3'end of TERC but also by base complementarity targets positions 313–340 of TERC, in the CR4/CR5 domain of TERC and positions 12–31 of TERC, localized in the TBE at the 5'end of TERC [62]. We showed that disruption of TERC regions complementary to *terc*-sRNA impaired association between AGO2 and TERC. In line with our data, it was recently shown that TERC can interact with different RNA molecules by direct RNA-RNA base pairing and predicted interaction sites fall mostly within regions spanning *terc*-sRNA target sites in CR4/CR5 and in the TBE domains, suggesting that these regions are prone to form RNA duplexes [91]. Therefore, we hypothesize that *terc*-sRNA might recognize TERC by base complementarity and recruit AGO2 to TERC.

Terc-sRNA is not perfectly complementary to TERC RNA. On the contrary, *terc*-sRNA binding sites harbor some regions of complementarity with the 5' and the 3' end of the sRNA, like non-canonical miRNA target sites [92]. Therefore, we hypothesize that this newly identified sRNA might recognize TERC and recruit AGO proteins and additional factors to TERC RNA. This mechanism would mirror what happens for mammalian miRNAs, which are usually not fully complementary to their targets. In this context, miRNA targets are not cleaved, but AGO proteins recruit additional partners to mediate silencing via translational repression, mRNA deadenylation and decapping [93]. As an example, the GW182 proteins function as scaffolds to bridge the interaction between AGO proteins and downstream effectors [94]. Therefore, further investigations will disclose if AGO/*terc*-sRNA complexes require GW182 proteins to control of telomerase activity.

AGO2-TERC interaction is unlikely to control TERC stability. Indeed, depletion of AGO2 in human cells does not impact TERC expression levels but stimulates assembly of the active telomerase enzyme, by promoting the association between the telomerase core components TERT and TERC. Coherently, one of *terc*-sRNA target site spans positions 313–340 of TERC. This sequence partially overlaps the CR4/CR5 domain. In human cells when TERC residues 225–348 are depleted, TERC still accumulates but its association with TERT is greatly reduced [95]. Recently, it was reported that a mutation of TERC (G319A), mapping inside *terc*-sRNA binding site, is sufficient to cause telomere disease in a heterozygous state in humans. Interestingly, this mutation does not alter TERC overall levels but compromises telomerase function via decreasing binding of TERC to TERT in *vivo* [96]. Therefore, AGO-TERC interaction mediated by *terc*-sRNA might facilitate efficient interaction between TERC and TERT. The identification of additional cofactors will clarify the molecular mechanisms driving AGO effects on TERC-TERT association.

Recently it was shown that proper folding of the TERC CR4/5 domain is required for association with the TERT [80] and that reconstitution in *vitro* of human telomerase is highly dependent on the folding state of TERC [97,98], strongly suggesting that TERC folding is a limiting factor in vertebrate telomerase. Interaction between AGO2 and positions 313–340 of TERC might drive the folding of CR4/CR4 domain, thus recapitulating what previously seen in ciliate telomerase biogenesis. Indeed, the interaction between the Stem Terminus Element (corresponding to vertebrate CR4/CR5) telomerase RNA and a La-motif RNA binding protein in *Tetrahymena thermophila* controls structural changes in telomerase RNA, which are required to promote the association with the ciliate TERT [99].

Terc-sRNA has a second target site on TERC RNA (positions 12–31), spanning the TBE region located at the 5' of the template region. In vertebrates, TBE consists of a long-range base-paired region known as P1 helix [36,100], which physically prevents TERT from reverse transcribing flanking non-template regions. Otherwise, non-telomeric DNA is synthetized at chromosome ends, inhibiting the binding of telomeric protein and resulting in detrimental effects for telomerase function [101]. The 41 5⊠-most nucleotides of human TERC form an unusual secondary structure called guanine quadruplex (G4), which hinders the formation of P1 helix [102].

Resolution of TERC G4 structure is mediated by RHAU (RNA associated with AU-rich element alias DHX36 or G4R1), a member of the human DEAH-box family of RNA helicases [103–105]. Indeed, RHAU directly interacts with the first 43 nucleotides of TERC (Figure 1(b)) and unwinds the G4 structure thus promoting P1 helix assembly and telomere lengthening [103]. Interestingly, Booy and colleagues also demonstrate that TERC nucleotides 11–28, which are included into *terc*-sRNA binding site (TERC 12–31) are folded into an internal G quadruplex which is responsible for P1 helix inhibition. Since it was shown that G4 RNA folding can be inhibited by using antisense oligonucleotide targeting the guanines involved in the G4 folding [106,107], we can speculate that binding of *terc*-sRNA and recruitment of AGO2 to TERC 5' region might act in collaboration with RHAU and prevent the formation of G4s, thereby favoring P1 helix formation. This hypothesis is also supported by the evidence that RHAU is also implicated in direct interactions with human AGO proteins [108,109].

The 5' end of TERC physically interacts with FXR1 (Fragile X-Related protein 1) [110] (Figure 1 (b)). FXR1 is a member of the Fragile X-related family of RNA-binding proteins (FXRs), which also includes Fragile X Mental Retardation 1 (FMR1) and FXR2. FXRs can affect RNA stability, transport, or translational efficiency [111], by binding to RNA structures such as AU-rich elements (AREs) [112] and G4s [113]. FXR1 is overexpressed in different tumors [114–117], playing an essential role in cell cycle progressions and senescence bypassing. Majumder and colleagues showed that in oral cancer cells FXR1 binds and stabilized TERC thus influencing telomerase activity [110]. The G4-RNA structure at the 5'end of TERC seems to be the binding site of FXR1 since deletion of nucleotide 1–28 of TERC impairs FXR1 binding. This target site overlaps *terc*-sRNA target site on TERC positions 12–31 which can drive AGO2 recruitment on TERC. Notably, FXR1 is also associated with microRNAs and the RNAi machinery in *Drosophila* [118], in Xenopus laevis [119] and in human cells [112,120]. Indeed, members of the FXR family interacts with AGO proteins [118,120,121]. In particular, evidence demonstrates that in human cells AGO2 and FXR1 are physically associated [112], and that this interaction is mainly nuclear [122]. AGO2 and FXR1 are both recruited to the ARE sequence at the 3'end of TNFα mRNA or of a synthetic reporter RNA and are required to activate their translation [112]. Interestingly, the association of AGO2 and FXR1 to their common RNA targets is mediated by miRNAs [123,124]. Overall these data suggest that AGO2 and FXR1 recognize the same sequences on RNA targets guided by base complementarity the target RNA and a sRNA. We can speculate that this model which has been demonstrated for ARE-containing RNAs can also be applied to TERC: *terc*-sRNA targets TERC 5'end thus recruiting AGO2 and FXR1.

Recently, a role for HuR (Hu-Antigen R) has been highlighted in the maturation of TERC [125]. HuR is an RNA binding protein which is recruited onto AREs. It has been reported to stabilize mRNAs

through several different mechanisms [126]. Tang and colleagues have shown that HuR protein directly interacts with TERC RNA through two binding sites mapping at positions 38–42 and 98–103 of TERC (Figure 1(b)). Furthermore, the authors highlighted that the C residue at position 106 of TERC is a 5-methylcytosine (m5C) site whose methylation is promoted by HuR binding to TERC. Finally, by mutagenesis, the authors show that the two HuR binding sites, as well as the C at position 106 of TERC, are required for the assembly of a functional telomerase holoenzyme [125]. Interestingly, C106 lies immediately next to a GC to AG mutation (nt 107–108 of TERC) resulting in telomerase activity impairment [127]. These data suggest that, by promoting C106 methylation, HuR facilitates the binding of TERC with TERT. Indeed, the methyltransferase enzyme catalyzing C106 methylation awaits to be discovered, as well as the reader of this modification.

The complex interaction between AGO proteins and HuR has been widely investigated. HuR was first reported to counteract miRNA-mediated repression of CAT1 mRNA [128]. Nevertheless, a few years later Kim and colleagues reported that HuR synergized with RISC in the inhibition of MYC mRNA [129]. An extensive, genome-wide analysis of HuR and AGO2 binding sites on 3' UTRs of human mRNAs revealed that these two factors share several binding sites. Furthermore, experiments aiming at unraveling their functional interplay confirmed that, depending on the specific mRNA, AGO2 and HuR can either cooperatively bind or compete for binding [130].

Interestingly, one of the two HuR binding site recently reported by Tang et al. on TERC lies in close proximity to one of the two AGO2 binding sites we reported [62]. Since both loss of HuR and loss of AGO2 impair TERC/TERT association, an intriguing hypothesis would be that AGO2 and HuR might cooperatively promote TERC loading on TERT. Further investigation will be required to unravel the putative cooperation of these two factors in the telomerase holoenzyme assembly.

Concluding remarks

In order to attain appropriate telomerase activity, both TERT and TERC expression are required. Furthermore, assembly of the telomerase holoenzyme requires a plethora of enzymes and RNA-binding proteins that finely controlled processing, stability and activity of TERC. Fulfillment of these requirements provides several opportunities to fine-tune telomerase activity in the cell. Indeed, great strides have been made in recent years to increase our knowledge of how telomerase is assembled and to identify new players in telomerase regulation. Interestingly, rapidly emerging scientific findings suggest an unexpected function of components of the RNAi machinery in TERC processing and activity (Figure 1(b, c)). In this scenario, new sRNAs originating from TERC and novel TERC-binding proteins (AGO2, FXR1, RHAU and HuR) have been described. Since abnormal expression of other well-known telomerase accessory proteins (*i.e.* DCK1, NOP10, TCAB1, PARN) is linked to tumorigenesis and telomere disease, the possible deregulation of RNAi machinery in these pathological contexts deserves further investigations. These efforts will contribute not only to our understanding of natural cellular processes but also offer new possibilities for the development of novel therapeutic targets and biomarkers in a wide range of pathologies. Notably, in light of the recent advent of "RNA therapeutics" [131], the precise characterization at nucleotide resolution of interactions between TERC and several RNA binding proteins involved in telomerase biology might lay the foundation for the development of RNA-based drugs to treat telomeropathies and cancer.

Disclosure statement

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