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Research Article

A Cajal body-independent pathway for telomerase trafficking in mice

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ABSTRACT

The intranuclear trafficking of human telomerase involves a dynamic interplay between multiple nuclear sites, most notably Cajal bodies and telomeres. Cajal bodies are proposed to serve as sites of telomerase maturation, storage, and assembly, as well as to function in the cell cycle-regulated delivery of telomerase to telomeres in human cells. Here, we find that telomerase RNA does not localize to Cajal bodies in mouse cells, and instead resides in separate nuclear foci throughout much of the cell cycle. However, as in humans, mouse telomerase RNA (mTR) localizes to subsets of telomeres specifically during S phase. The localization of mTR to telomeres in mouse cells does not require coilin-containing Cajal bodies, as mTR is found at telomeres at similar frequencies in cells from wild-type and coilin knockout mice. At the same time, we find that human TR localizes to Cajal bodies (as well as telomeres) in mouse cells, indicating that the distinct trafficking of mTR is attributable to an intrinsic property of the RNA (rather than a difference in the mouse cell environment such as the properties of mouse Cajal bodies). We also find that during S phase, mTR foci coalesce into short chains, with at least one of the conjoined mTR foci co-localizing with a telomere. These findings point to a novel, Cajal body-independent pathway for telomerase biogenesis and trafficking in mice.

Introduction

Telomeres are capping structures that comprise the physical ends of eukaryotic chromosomes. In vertebrates, they consist of tandemly repeated arrays of TTAGGG and a number of associated proteins [1,2]. Telomeres serve protective functions, preventing the ends of chromosomes from being recognized as double stranded DNA breaks [3] and serving as barriers to the loss of genetic information that results from the inability of DNA polymerases to fully replicate the ends of linear DNA. However, a portion of the telomere is lost with each cell division in most adult human somatic cells, and eventually telomere attrition triggers the cell to stop dividing and enter a state of proliferative senescence or undergo apoptosis. Increasing numbers of human telomere shortening diseases are being recognized and investigated [4–6].

Telomerase is the specialized reverse transcriptase that synthesizes telomeres and combats telomere erosion [7]. The telomerase enzyme is minimally comprised of two essential components: telomerase RNA (TR), which provides the template for repeat addition, and telomerase reverse transcriptase (TERT), which synthesizes the repeats. In humans, telomeres are synthesized early in development [8,9]. Telomerase activity is not detected in most adult somatic cells, but notably, the enzyme is active in over 90% of human cancers and is responsible for the prolonged proliferative capacity of these cells [10–12] (however, telomerase activity is readily detected in murine somatic cells [13–16]). Insights into the

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biogenesis and regulation of this enzyme therefore have the potential to lead to the development of novel anti-cancer therapeutics [17,18].

A recent series of studies has revealed important aspects of the trafficking of the telomerase ribonucleoprotein complex (RNP) critical to the biogenesis and function of the enzyme in human cells [19–25]. Throughout interphase, human TR is found in Cajal bodies [21,23], dynamic nuclear structures that have been implicated in the biogenesis of various cellular RNPs (reviewed in [26–28]). Localization of hTR to Cajal bodies is important for function of the telomerase enzyme [19,24]. Accumulation of hTR in Cajal bodies occurs in cancer cells where telomerase activity is present, but not telomerase-negative normal cells [22,23,25], consistent with the hypothesis that Cajal bodies serve as sites of telomerase assembly. Human TERT is detected in distinct nucleoplasmic foci, separate from Cajal bodies by immunofluorescence (IF) [23], though exogenously expressed GFP-tagged, hTERT can be detected within Cajal bodies [25] as well as nucleoli [29–31]. During S phase, both human TR and TERT move to foci found immediately adjacent to Cajal bodies, and then, peaking in mid-S phase when human telomeres are replicated and synthesized [32–34], both hTR and hTERT associate with subsets of telomeres [19,21–24]. These findings indicate that Cajal bodies are important for telomerase biogenesis and may act to deliver telomerase to the telomere.

The trafficking of telomerase components has emerged as a key process in the biogenesis and function of the enzyme, and the factors important for trafficking are being defined. The localization of human TR to both Cajal bodies and telomeres depends on TERT [22], suggesting that assembly and trafficking are tightly linked processes. The trafficking of telomerase to both Cajal bodies and telomeres depends on a newly identified telomerase component, called TCAB1 (Telomerase Cajal-body protein 1; also called WDR79) [24,35]. There is also evidence that the core telomere binding protein, TPP1 is critical for telomerase–telomere associations [36–40]. However, much remains to be learned regarding the factors and mechanisms that influence the biogenesis and trafficking of telomerase.

To gain a better understanding of the regulation of telomerase trafficking, we initiated studies in the mouse model system where genetic approaches have contributed to our understanding of basic telomere and telomerase biology, particularly with regard to cancer and aging [41–45]. Here, we report a fluorescence in situ hybridization (FISH) procedure specific for detection of mouse telomerase RNA (mTR) and the characterization of mTR localization patterns in cultured mouse cell lines using this approach. Unexpectedly, we found that mTR does not co-localize with coilin or other Cajal body markers, and instead, is found in distinct nuclear foci. However, a fraction of the mTR localizes to telomeres selectively during S phase of the cell cycle. In many cases, we observe chains of connected mTR foci during S phase, and frequently find that at least one of the foci in a chain co-localizes with a telomere. Our findings suggest an alternative mechanism for recruitment of telomerase RNA to telomeres during S phase in murine cells, which involves the convergence of non-Cajal body mTR foci at telomeres.

Materials and methods

Cell culture and transfection

MEF-26 (WT), MEF-42 (coilin−/−), MEF-14 (mTR−/−) (a gift of Carol Greider, Johns Hopkins University, Baltimore, MD), 3T3 (ATCC, Manassas, VA), n2a (a gift of Brian Condie, University of Georgia, Athens, GA), c2c12 (ATCC), and A9 cells were grown on coverslips in DMEM (Mediatech, Herndon, VA) supplemented with 10% fetal calf serum (FCS) (Mediatech) at 37 °C with 5% CO_2. For hTR overexpression, cells were transfected with pBS-U1-hTR [19] (gift of J. Lingner, EPF of Lausanne, Switzerland) using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

FISH probes

All probes were synthesized by Qiagen (Valencia, CA) as follows: mTR 117–169 (probe 1), CT*CCCGGCGAACCT*GGAGCTCCTGCGCT*GACGTTTGTTTTT*GAGGCTCGGGT*A; mTR 296–342 (probe 2), CT*CGGGGACCAGT*TCCATTCCTGT*CCTTGCGGCGCT*CGCCCGGCCT*G; mTR 224–282 (probe 3), GT*GCCCCGCGGCT*GACAGAGGCGAGCT*CTTCGCGGCGGCAGCGGAGT*CCTAAGACCCT*A; mTR 57–104 (probe 4), CT*CTGCAGGTCT*GGACTTTCCT*GGCCCGCTGGAAGT*CAGCGAGAAAT*A; U3 (probe 5), TT*CAGAGAAACTTCTCT*AGTAACACACTAT*AGAACTGATCCCT*GAAAGTATAGT*C; mU85 (probe 6), AT*TACCAAAGATCT*GTGTGTCATCT*CTCAGTGGCCAT*GACACAGCTAAGT*C; telomere (probe 7), CT*AACCCTAACCCT*AACCCTAACCCT*AACCCTAACCCT*AACCCTAACCCT*A; hTR 128–183 (probe 8), GCT*GACATTTTT*TGTTTGCTCT*AGAATGAACGGT*GGAAGGCGGCA; hTR 331–393 (probe 9), CT*CCGTTCCTCTTCCT*GCGGCCTGAAAGGCCT*GAACCTCGCCCT*CGCCCCCGAGT*G; hTR 393–449 (probe10), AT*GTGTGAGCCGAGT*CCTGGGTGCACGT*CCCACAGCTCAGGGAAT*CGCGCCGCGCT*C. T* indicates aminoallyl-modified thymidines. All mTR and hTR probes (probes 1–4 and 8–10 above) were conjugated with cy3 mono-functional reactive dye according to the manufacturers protocol (GE Healthcare, Little Chalfront, Buckinghamshire, United Kingdom). The remaining probes were labeled with Alexa Fluor 647 or Oregon green dye according to the manufacturer's protocol (Molecular Probes/Invitrogen, Carlsbad, CA).

mTR and hTR FISH

FISH was performed essentially as described [23,25]. Hybridizations were carried out overnight at 37 °C. 200 ng of each of two of the cy3-labeled mTR probes (most often probes 1 and 2) or 25 ng of each of the three cy3-labeled hTR probes (probes 8, 9 and 10) above were used per coverslip. 5–10 ng Oregon green or Alexa Fluor 647 U3 (probe 5), 100 ng Oregon green mU85 (probe 6), or 10 ng Oregon green telomere (probe 7) were also included in the hybridization when indicated. Cells were rinsed twice in 50% formamide, 2× SSC for 30 min at 37 °C. Cells were subsequently washed once in 50% formamide, 2× SSC, 0.1% NP-40 for 30 min at 37 °C. The coverslips were mounted in Prolong gold mounting media (Molecular Probes/Invitrogen), cured at room temperature for 1 h and stored at −20 °C until microscope analysis.

RNAse treatment

After fixation and permeabilization, cells were rehydrated in 1× PBS containing 1.5 mM $MgCl_2$ at room temperature for 5 min. Then the cells were incubated with RNAse A (0.2 mg/ml in 1× PBS containing 1.5 mM $MgCl_2$) at 37°C for 2 h. mTR FISH was performed after the cells were washed 3 times with 1× PBS and once with 50% formamide, 2× SSC.

Indirect immunofluorescence (IF)

Following FISH, cells were washed three times with 1× PBS. Next, cells were incubated with one of the following primary antibodies