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Cell Cycle-regulated Trafficking of Human Telomerase to Telomeres

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Telomerase synthesizes telomeres at the ends of human chromosomes during S phase. The results presented here suggest that telomerase activity may be regulated by intranuclear trafficking of the key components of the enzyme in human cells. We examined the subcellular localization of endogenous human telomerase RNA (hTR) and telomerase reverse transcriptase (hTERT) in HeLa cervical carcinoma cells. Throughout most of the cell cycle, we found that the two essential components of telomerase accumulate at intranuclear sites separate from telomeres. However, during S phase, both hTR and hTERT are specifically recruited to subsets of telomeres. The localization of telomerase to telomeres is dynamic, peaking at mid-S phase. We also found complex associations of both hTR and hTERT with nucleoli and Cajal bodies during S phase, implicating both structures in the biogenesis and trafficking of telomerase. Our results mark the first observation of human telomerase at telomeres and provide a mechanism for the cell cycle-dependent regulation of telomere synthesis in human cells.

INTRODUCTION

The ends of linear eukaryotic chromosomes are capped by nucleoprotein structures termed telomeres. In vertebrates, telomeres consist of simple DNA repeats of TTAGGG bound by several proteins (Colgin and Reddel, 2004; Smogorzewska and de Lange, 2004). Telomeres serve to maintain chromosome integrity, preventing illegitimate recombination and end-to-end joining (de Lange, 2002; Harrington, 2004; Blasco, 2005). However, because of the unidirectional nature of DNA polymerases and DNA processing events, some telomere sequence is lost with each round of DNA replication. If this loss is not compensated, the telomeres will reach a critically short length, triggering the cell to enter a state of replicative senescence or apoptosis (McEachern *et al*., 2000; de Lange, 2002).

Telomerase is the ribonucleoprotein (RNP) enzyme that synthesizes telomeres. The telomerase reverse transcriptase (hTERT) catalyzes de novo repeat addition using a short motif within the integral telomerase RNA (hTR) as a template (Greider and Blackburn, 1989). These two components are essential for the activity of the enzyme. Human telomere synthesis occurs early in development (Collins and Mitchell, 2002; Cong *et al*., 2002). The majority of adult somatic cells do not have appreciable telomerase activity and telomeres gradually shorten, limiting cell division capacity (Harley *et al*., 1990). In the majority of human cancers, however, telomerase is reactivated and provides the sustained proliferative capacity of these cells (Shay and Bacchetti, 1997). An understanding of telomerase biology thus has important implications for both cancer and aging.

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Telomeres are synthesized during S phase in human cells (Ten Hagen *et al*., 1990; Wright *et al*., 1999); however, it is unclear how telomerase is restricted to function specifically during this stage of the cell cycle. Some existing evidence is consistent with the idea that the cell cycle-dependent regulation of telomerase could occur at the level of subcellular trafficking. Redistribution of components of telomerase has been observed during S phase, when telomere synthesis occurs. Wong *et al*. (2002) showed that the subnuclear distribution of green fluorescent protein (GFP-hTERT) fusion proteins changed from predominantly nucleolar to nucleoplasmic as cells progressed through S phase. In similar studies, Yang *et al*. (2002b) also reported movement of GFP-hTERT protein during S phase, in this case into nucleoli. Regarding the other essential component of the enzyme, Jady *et al*. (2004) reported a possible influx of telomerase RNA through Cajal bodies during S phase. However, although there is some evidence that both hTR and hTERT move during S phase, movement to telomeres, and thus a direct link to regulation of function, has not been found in vertebrate cells. More persuasive evidence is available from ciliates, where telomerase RNA localizes to discrete nuclear foci throughout most of the cell cycle, and a fraction of the RNA is mobilized to the replication band, the site of DNA (and telomere) synthesis, during S phase (Fang and Cech, 1995).

In this work, we have investigated the subnuclear distribution of endogenous human TR and TERT over the course of the cell cycle and found compelling evidence that regulation of telomerase activity occurs via trafficking of hTR and hTERT in human cells. Our results indicate that hTR and hTERT move to telomeres from separate sites specifically during S phase. hTR is found in Cajal bodies, as we and others have reported previously (Jady *et al*., 2004; Zhu *et al*., 2004), throughout most of the cell cycle. Here, we provide the first clear evidence that hTERT resides in subnuclear foci that do not correspond to nucleoli, Cajal bodies, or telomeres

during most of the cell cycle. The movement of hTR and hTERT to telomeres during S phase is preceded and accompanied by other changes in localization that may relate to biogenesis and/or transport of the components. Our results suggest that hTERT moves to nucleoli and that Cajal bodies containing hTR accumulate at the periphery of nucleoli early in S phase. In addition, we find that both hTR and hTERT localize to foci adjacent to Cajal bodies during S phase, marking a potential site outside of telomeres where both endogenous telomerase components are detected. The implications of our findings with regard to telomerase biogenesis and telomere length regulation are discussed.

MATERIALS AND METHODS

Cell Culture and Synchronization

Monolayer HeLa cells were grown on coverslips in DMEM (Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal calf serum (FCS) (American Type Culture Collection, Manassas, VA) at 37°C with 5% CO_2. IMR90 primary fibroblasts were grown in minimum essential Eagle's media (American Type Culture Collection) with 10% FCS. IMR90-hTERT cells (Ouellette *et al*., 1999) were grown in a 4:1 mixture of DMEM and medium 199 (Invitrogen, Carlsbad, CA). Synchronous populations of HeLa cells were obtained by double thymidine block. Briefly, cells were treated with 2 mM thymidine (Sigma-Aldrich) for 18 h, released in normal media for 9 h, and retreated with 2 mM thymidine for another 18 h. Alternatively, HeLa cells were synchronized by treatment with 5 mM hydroxyurea for 24 h after 24 h of serum withdrawal. Cells were fixed at various time points after release and analyzed as described below.

5-Bromodeoxyuridine (BrdU) Labeling

Before fixation, cells were incubated with 100 μM BrdU (Sigma-Aldrich) for 30 min at 37°C. Cells were then rinsed once with 1× phosphate-buffered saline (PBS) (137 mM NaCl, 10 mM Na_2HPO_4, 2.7 mM KCl, and 1.4 mM KH_2PO_4, pH 7.4) and fixed with 4% formaldehyde (Electron Microscope Sciences, Fort Washington, PA), 10% acetic acid, and 1× PBS for 10 min at room temperature. After two PBS washes, cells were permeablized in 70% ethanol overnight at 4°C. Cells were denatured in 70% formamide (Sigma-Aldrich), 2× SSC for 5 min at 80°C. After three PBS washes, BrdU was detected using fluorescein isothiocyanate-conjugated anti-BrdU monoclonal antibody (mAb) (BD Biosciences, San Jose, CA; 20 μl of antibody was diluted in 70 μl of 0.05% Tween 20 in PBS [PBST]) for 2 h at room temperature. After three PBS washes, coverslips were mounted in 90% glycerol, 1 mg/ml p-phenylenediamine, 1× PBS, and 0.1 μg/ml 4′,6-diamidino-2-phenylindole (DAPI). If fluorescence in situ hybridization (FISH) was to be performed, cells were fixed again in 4% formaldehyde in 1× PBS for 10 min at room temperature and washed twice with PBS.

hTR and Telomere FISH

Probes complementary to different regions of telomerase RNA (nucleotides indicated) or telomere repeats were as follows: hTR 128–183 (probe 1), GCT*GACATTTTT*TGTTTGCTCT*AGAATGAACGGT*GGAAGGCGGCAGGCCGAGGCT*T; hTR 331–383 (probe 2), CT*CCGTTCCTCTTCCT*GCGGCCTGAAAGGCCT*GAACCTCGCCCT*CGCCCCCGAGT*G; hTR 393–449 (probe 3), AT*GTGTGAGCCGAGT*CCTGGGTGCACGT*CCCACAGCTCAGGGAAT*CGCGCCGCGCT*C; and telomere repeats (probe 4), CT*AACCCTAACCCT*AACCCTAACCCT*AACCCTAACCCT*AACCCTAACCCT*A. T* indicates aminoallyl-modified thymidines. All probes were synthesized by QIAGEN (Valencia, CA). Probes were conjugated with either Cy3 or Cy5 monofunctional reactive dye according to manufacturer's protocol (GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom). FISH was performed essentially as described previously (Zhu *et al*., 2004), http://www.singerlab.org/protocols) with the following modifications. For each coverslip, 20–30 ng of each Cy3-labeled hTR probe (1–3 above) and 5 ng of Cy5-labeled telomere probe (4) were hybridized overnight at 37°C. After hybridization, cells were washed twice with 2× SSC, 50% formamide for 1 h at 37°C. Coverslips were mounted as described above.

Coilin and TRF1 Immunofluorescence (IF)

After FISH, cells were washed twice with PBS. Cells were incubated with one or both of the following antibodies at the indicated dilution for 1 h at room temperature: mouse anti-p80 coilin (1:10,000; gift from G. Matera, Case Western Reserve University, Cleveland, OH) and rabbit anti-TRF1 (1:350; gift from Dominique Broccoli [Fox Chase Cancer Center, Philadelphia, PA] and Susan Smith [Skirball Institute, New York, NY]). Cells were washed three times with PBS and then incubated with secondary antibody (1:100 Cy2-conjugated goat anti-rabbit IgG [H+L], 1:100 AMCA-conjugated goat anti-mouse IgG [H+L], or 1:100 Cy5-conjugated goat anti-mouse IgGγ) for 1 h at room temperature. All secondary antibodies were obtained from Jackson ImmunoResearch Laboratories (West Grove, PA). After three PBS washes, slides were mounted as described above. All antibodies were diluted in PBST.

hTERT Immunofluorescence

hTERT IF was performed essentially as described in Masutomi *et al*. (2003). Cells were washed in PBS and then fixed with chilled acetone for 5 min. After two PBS washes, cells were treated with 2 M HCl at room temperature for 20 min followed by a PBS wash and neutralization with 0.1 M boric acid, pH 8.5, for 10 min. After two additional PBS washes, cells were blocked with 1% bovine serum albumin (BSA) in PBS at 4°C overnight. Cells were incubated with mouse anti-hTERT 2C4 (Abcam, Cambridge, MA; 1:2000–1:5000 in 1% BSA) for 2 h at room temperature, washed three times with PBS, and incubated with Cy2-conjugated goat anti-mouse IgM (Jackson ImmunoResearch Laboratories; 1:100 in PBST) for 2 h at room temperature. After three PBS washes, the cells were mounted as described above. When hTERT staining was combined with TRF1 or coilin, the hTERT IF protocol was followed using the above-described antibodies at the indicated dilutions except that cy3-conjugated goat anti-rabbit IgG (H+L) was used to recognize TRF1 and mouse anti-coilin was recognized with Cy5-conjugated goat anti-mouse IgGγ antibody. Both secondary antibodies were obtained from Jackson ImmunoResearch Laboratories.

Microscopy

Analysis was performed on a Zeiss Axioskop 2 Mot Plus fluorescence microscope (Carl Zeiss Microimaging, Thornwood, NY). Images were acquired at 63× or 100× magnification (Plan Apochromat objectives, numerical aperture 1.4) using a cooled charge-coupled device Retiga Exi Fast 1394 camera (Qimaging, Burnaby, British Columbia, Canada) and IPLab Spectrum software.

RESULTS

Novel Patterns of Telomerase RNA Localization during S Phase

Our laboratory developed a FISH procedure specific for the detection of endogenous hTR in human cells and was the first to describe its intranuclear localization (Zhu *et al*., 2004). Several controls established the specificity of our FISH procedure for hTR (Zhu *et al*., 2004). Using this technique, we found that hTR localized to Cajal bodies in telomerase-positive human cancer cell lines (Zhu *et al*., 2004). In the course of that study (performed with asynchronous populations of cells), we noticed that a small percentage of cells displayed different patterns of hTR localization. For example, we observed additional hTR foci that did not stain with antibodies against coilin (the marker protein of Cajal bodies).

To investigate whether the secondary hTR localization patterns observed at low frequency in asynchronous populations reflected cells in particular stages of the cell cycle, we performed hTR FISH and coilin IF on cell cycle-synchronized HeLa cervical carcinoma cells. HeLa cells were synchronized with a double thymidine block (or hydroxyurea; our unpublished data) and monitored for synchronization efficiency at the indicated time points by BrdU analysis (to distinguish S-phase cells) and DAPI staining (to distinguish mitotic cells). (Fluorescence-activated cell sorting analysis with propidium iodide-stained cells (to examine DNA content) was also performed to confirm the synchronization efficiency in some cases.) The BrdU staining patterns also allowed us to assess the S subphase (i.e., early, mid, and late) status of populations of cells as well as individual cells (O'Keefe *et al*., 1992).

As expected, throughout the majority of the cell cycle, hTR localizes exclusively to Cajal bodies (Figure 1, G_1 and G_2). However, during S phase, a dynamic change in the subcellular distribution of hTR was observed in a significant number of cells. hTR remained associated with Cajal bodies in most cells; however, several novel hTR localization patterns were observed, which peaked at distinct points in S phase. Beginning in G_1/S and peaking in early S, hTR foci were

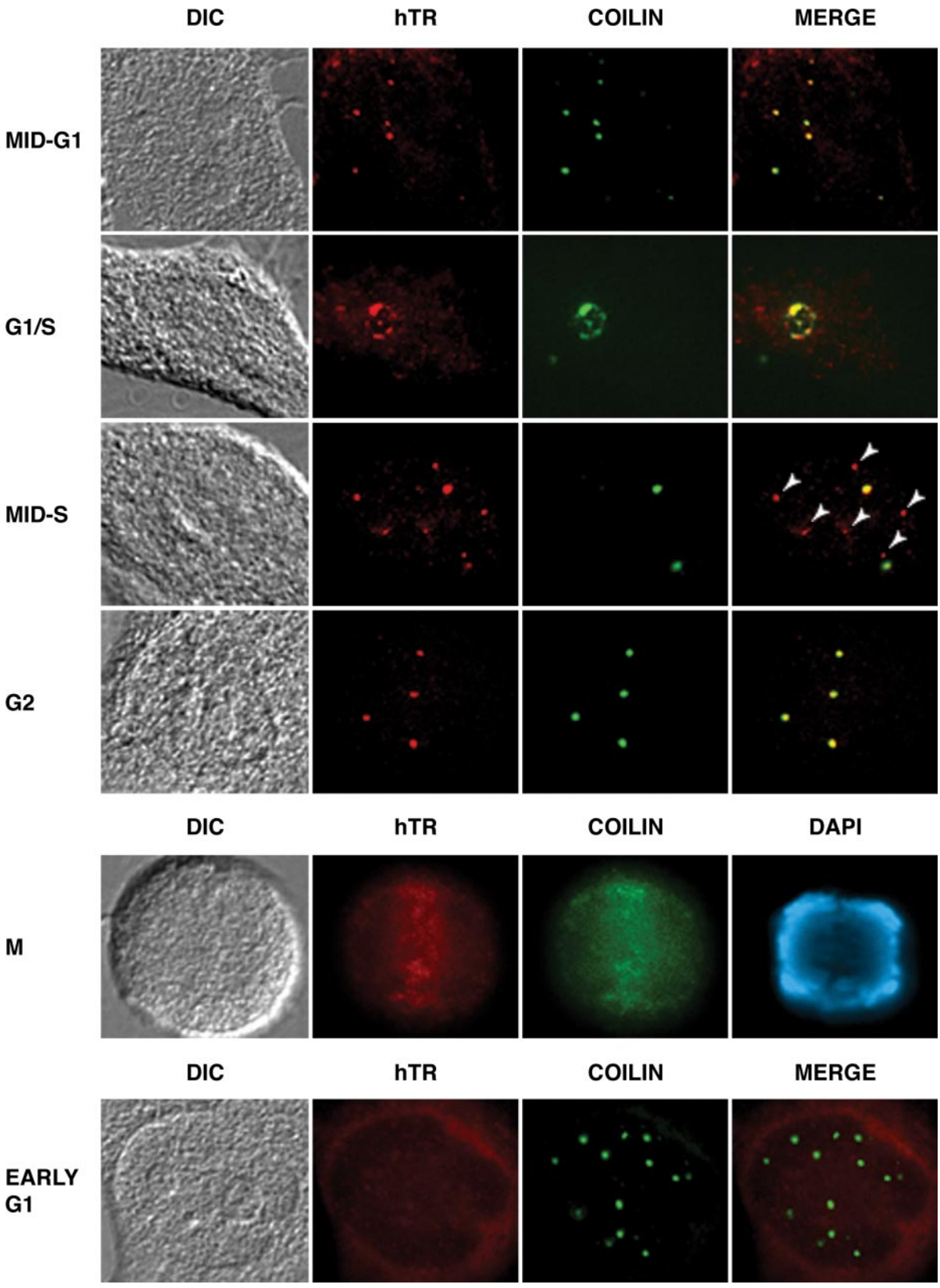

Figure 1. Localization of human telomerase RNA in HeLa cells at various stages of the cell cycle. hTR (red, detected by FISH) and coilin (green, Cajal body marker protein detected by IF) were analyzed by fluorescence microscopy in cells 0 (G_1/S), 4 (mid-S), 8 (G_2), 10 (M), 12 (early G_1), and 15 (mid-G_1) hours after release from double thymidine block. Differential interference contrast (DIC) panels show differential interference light microscopy data. DAPI panels shows DNA staining. Merge panels show superimposition of hTR and coilin fluorescence data (yellow indicates overlap of signal). Arrows in mid-S merge panel indicate hTR foci that do not colocalize with coilin.

found in ring-like patterns within the nucleus (Figure 1, row 2). In most cells, coilin colocalized with hTR in the rings, as shown (Figure 1, row 2). By mid-S phase, the distribution of hTR foci in rings had declined, and hTR occurred in small, nucleoplasmic foci that did not stain with anti-coilin antibodies (Figure 1, row 3, denoted by arrowheads). In addition, hTR was sometimes observed in foci immediately adjacent to Cajal bodies (detailed below). These patterns were specific to S phase; they were not observed in cells in G_1 or G_2 phases of the cell cycle.

During mitosis, previous research has shown that Cajal bodies dissociate, and then reform during early G_1 of the cell cycle (Andrade *et al*., 1993). We found that hTR localization parallels that of the Cajal body marker protein coilin during cell division, with only a slight temporal delay in reassociation after mitosis. Like coilin, hTR displayed a diffuse localization pattern throughout mitosis (Figure 1, row 5 shows hTR diffusely localized in interchromatin region). No association with discrete foci was observed. After mitosis, Cajal body formation precedes hTR accumulation in Cajal bodies. In early G_1, Cajal bodies had reformed in nearly all (~90%) of cells examined, but no hTR foci were found in most (~65%) cells (Figure 1, row 6). By mid-G_1 (15 h postrelease), hTR had reaccumulated in all Cajal bodies (Figure 1, row 1). Together, these results demonstrate that the cell cycle has dramatic effects on the localization of hTR and that there is a dynamic, S phase-specific rearrangement of hTR in human cancer cell nuclei.

Telomerase RNA Localizes to the Periphery of Nucleoli during Early S Phase

We found that the ring-like pattern of hTR (and coilin) foci observed during early S phase seems to correspond to localization to the periphery of nucleoli (Figure 2A). In addition to the ring pattern, we also observed hTR foci that seemed to be distributed across the surface of a nucleolus in some focal planes (Figure 2A). At early S phase, ~17% of cells (370 cells analyzed from 3 separate experiments) contained hTR foci localized around the periphery of a nucleolus. In the majority of cases, the hTR ring was observed around only one nucleolus within a given cell, suggesting a previously undescribed heterogeneity among individual nucleoli. We did not find telomeres at these rings; telomeres colocalized with hTR/coilin rings in <1% of 1197 cells examined in two experiments. The peripheral nucleolar hTR pattern was less frequent at G_1/S and mid-S than at early S phase and was not observed in cells outside S phase.

As shown in Figure 1, coilin was found with hTR in the nucleolar rings in the majority (~80%) of cells. The localization of Cajal bodies to the periphery of nucleoli is well documented (indeed, Cajal bodies were initially termed nucleolar accessory bodies; Gall, 2003; Cioce and Lamond, 2005), although an S-phase-specific association has not been described previously. We cannot exclude the possibility that the Cajal bodies and hTR are internal to nucleoli based on the studies presented here, but the distribution of the foci is suggestive of localization on the surface of the nucleolus. Elegant studies in HeLa cells have demonstrated the movement of Cajal bodies to and from nucleoli (Platani *et al*., 2000). Our results suggest that Cajal bodies containing hTR move to the periphery of nucleoli during early S phase in HeLa cells.

Telomerase RNA Localizes to Subsets of Telomeres during Mid-S Phase

To ascertain whether the coilin-negative, S-phase-specific hTR foci described above (Figure 1, row 3) were at telomeres, we coanalyzed hTR and telomeres in S-phase cells. We performed hTR FISH in conjunction with either telomere FISH (probe directed against telomere repeat sequences) or immunofluorescence with antibodies against the double-stranded telomere binding protein TRF1 (Figure 2B). Cells were examined 0 (G_1/S), 2 (early S), 4 (mid-S), 6 (late S), and 8 (S/G_2) h postrelease from a double thymidine block. At G_1/S and early S, we found that hTR colocalized with a few telomeres (1–2/cell) in ~3 and 9% of cells, respectively (562 and 370 cells analyzed from 6 and 3 separate experiments, respectively). During mid-S, there was an increase in both the number of cells that had hTR-telomere associations and in the number of associations per cell. In ~19% of mid-S-phase cells (698 cells from 5 experiments), hTR was found at telomeres—typically one to five per cell with a maximum of 11 colocalizations observed in one cell. The telomere associations declined in late S phase, to 11% of cells (336 cells from 3 experiments) and one to two per cell. hTR was also still found in or near Cajal bodies in most of these cells. hTR was not found at telomeres in G_2 phase (our unpublished data). These results indicate that human telomerase RNA moves to telomeres during mid-S phase.

hTERT Also Associates with Telomeres and Nucleoli during S Phase

Telomerase requires both hTR and hTERT for function. Previous studies found that ectopically expressed GFP-hTERT fusion protein shows striking S-phase-specific intranuclear rearrangements (Wong *et al*., 2002; Yang *et al*., 2002b), but it was not found to localize to telomeres. Here, we have investigated the localization of endogenous hTERT protein.

We performed immunofluorescence using the 2C4 mAb against hTERT (Masutomi *et al*., 2003) and observed a punctate nuclear staining pattern in HeLa cells. The staining pattern described previously for this antibody in HeLa cells was more generally nucleoplasmic (Masutomi *et al*., 2003), so we examined the specificity of the pattern observed in our experiments (Figure 3A). We found that the more restricted staining pattern that we obtained was specific to hTERT. We knocked down hTERT expression in HeLa cells by RNA interference (Masutomi *et al*., 2003) and observed a marked decrease in the fluorescence signal intensity (including complete elimination of signal in some cells), indicating that the signal in the intranuclear foci corresponded to hTERT (Figure 3A). As an additional test, we compared staining in IMR90 primary lung fibroblasts to IMR90 cells stably expressing hTERT from an exogenous construct (Ouellette *et al*., 1999). We observed some staining in the primary fibroblasts (Figure 3A), consistent with previous observations that normal cells express a low level of hTERT protein (Masutomi *et al*., 2003). The number and intensity of the foci was significantly greater in the cells expressing exogenous hTERT. Similar results were seen when hTERT was stably expressed in BJ cells (our unpublished data). Together, these results indicate that the 2C4 staining observed under these conditions is specific to hTERT and that hTERT is found in nucleoplasmic foci.

In synchronized cells, we found that hTERT (like hTR) localizes to nucleoli during the early stages of S phase (Figure 3B). Diffuse nucleolar localization of hTERT is detectable in 23% of cells at G_1/S (429 cells analyzed from 2 separate experiments) and intensifies as cells enter early S, when the localization is apparent in 35% of cells (282 cells from 2 experiments) (Figure 3B). Like hTR, hTERT was observed in a subset of nucleoli (typically 1) within a cell. Nucleolar association of hTERT declined as cells progressed through S phase (9% of 236 cells in late S phase in 2 experiments).

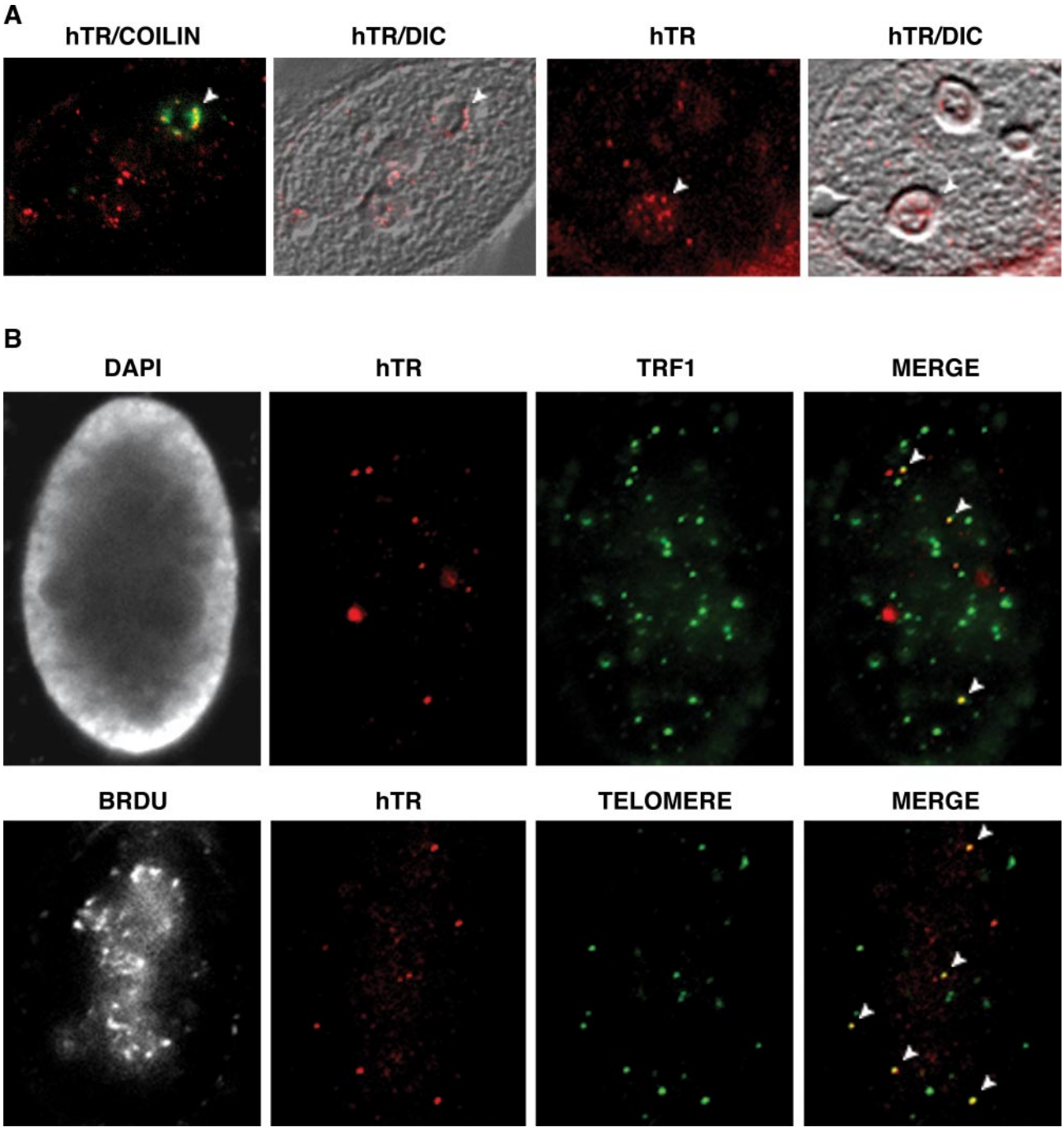

Figure 2. Human telomerase RNA is found at the periphery of nucleoli and at telomeres during S phase. (A) hTR and coilin seem to be associated with the surface of nucleoli during early S phase. The localization of hTR (red, detected by FISH) is superimposed on coilin (green, detected by IF, 1) or cellular architecture (visible by DIC light microscopy, 2 and 4) in indicated panels. The cells shown are in early S phase. Arrowheads denote nucleoli exhibiting apparent peripheral or surface hTR signals. (B) hTR associates with telomeres during mid-S phase. hTR (red, detected by FISH) and telomeres (green, detected by TRF1 IF or telomere FISH as indicated) were analyzed in mid-S-phase cells. DAPI panel shows DNA staining. BrdU panel shows a mid-S-phase pattern. Arrows in merge panels indicate foci where both hTR and telomeres are present.

Later in S phase, additional hTERT foci occurred, which corresponded to a subset of telomeres (Figure 3C). Colocalization of hTERT and telomeres (assessed by TRF1 [or TRF2; our unpublished data] antibody staining) occurred in a few (<5%) cells at G_1/S (391 cells from 2 experiments). Localization of hTERT to telomeres peaked in mid-S phase when 24% of cells (463 cells from 2 experiments) were found to contain one to five colocalizations (Figure 3C).

Unfortunately, to date we have been unsuccessful in simultaneously analyzing hTERT and hTR (because of incompatibility of the IF and FISH protocols). Independently, hTR and hTERT display remarkably similar temporal patterns of

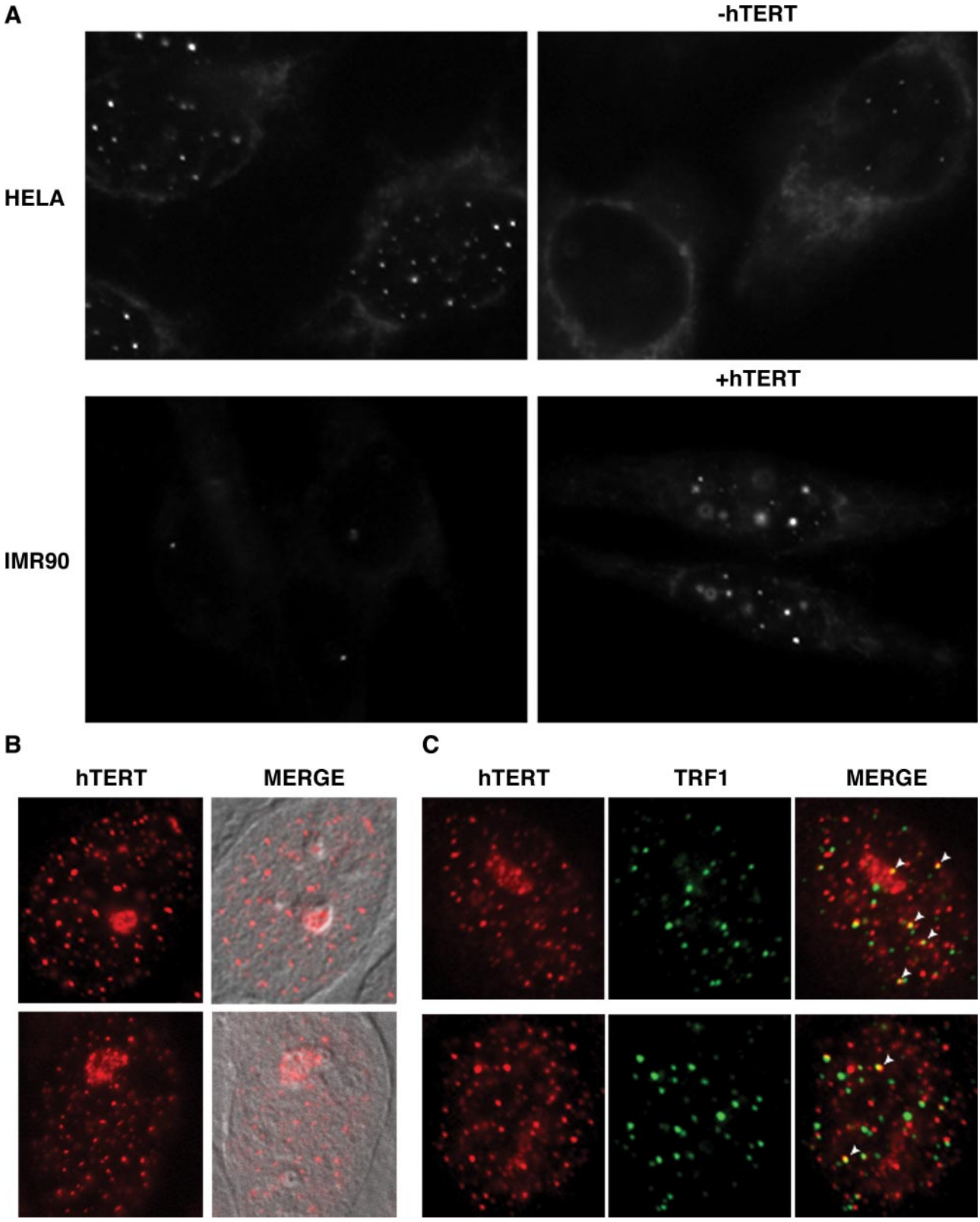

Figure 3. Cellular localization of human telomerase reverse transcriptase. (A) Intranuclear foci recognized by hTERT antibody 2C4 are specific to hTERT. The panels show 2C4 immunostaining of HeLa cells and HeLa cells within which hTERT was knocked down by RNA interference (−hTERT) and of IMR90 primary fibroblasts and an IMR90 strain that overexpresses hTERT (+hTERT). Data in each pair of panels were normalized to allow visual comparison. (B) hTERT is found in nucleoli in early S-phase cells. The localization of hTERT (red, detected by IF) is superimposed on cellular architecture visible by DIC light microscopy in merge panels. (C) hTERT is found at telomeres in mid-S-phase cells. hTERT (red) and TRF1 (green) localization is shown. Arrowheads indicate representative foci where both hTERT and TRF1 are present.

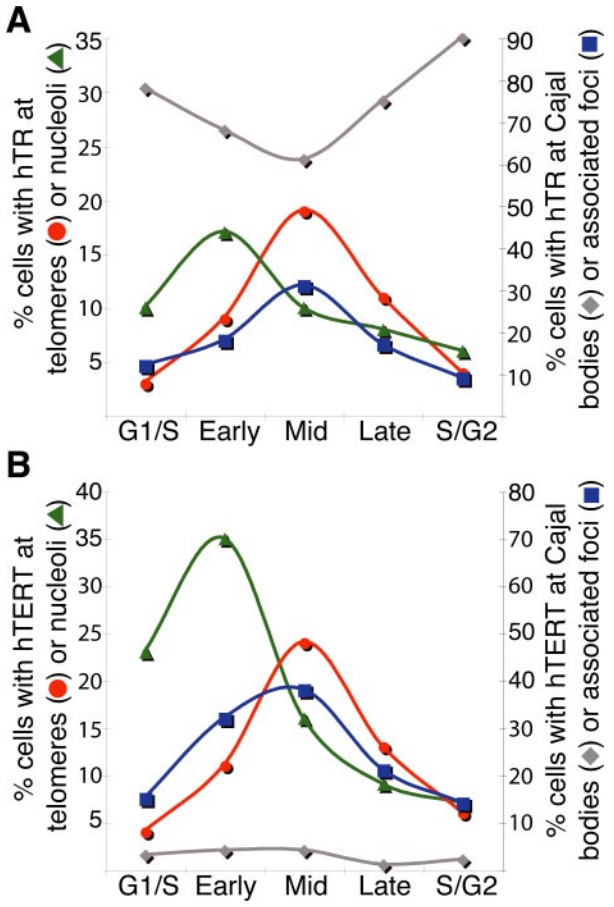

Figure 4. Temporal patterns of association of human telomerase RNA and telomerase reverse transcriptase with nuclear structures during S phase in HeLa cells. The percentage of cells in which hTR (A) and hTERT (B) was found associated with each structure in G_1/S, early S, mid-S, late S, and S/G_2 phase cells is indicated as follows: telomeres (●) and nucleoli (hTR peripheral and hTERT internal) (▲) on left axis; colocalization with Cajal bodies (◆) and Cajal body-associated foci (■) on right axis (note difference in scale of axes). Each point plotted represents an average of at least 158 (and as many as 698) total cells counted from at least two (and as many as 6) experiments, with the exception of the S/G_2 time point for hTERT, which represents data from a single experiment.

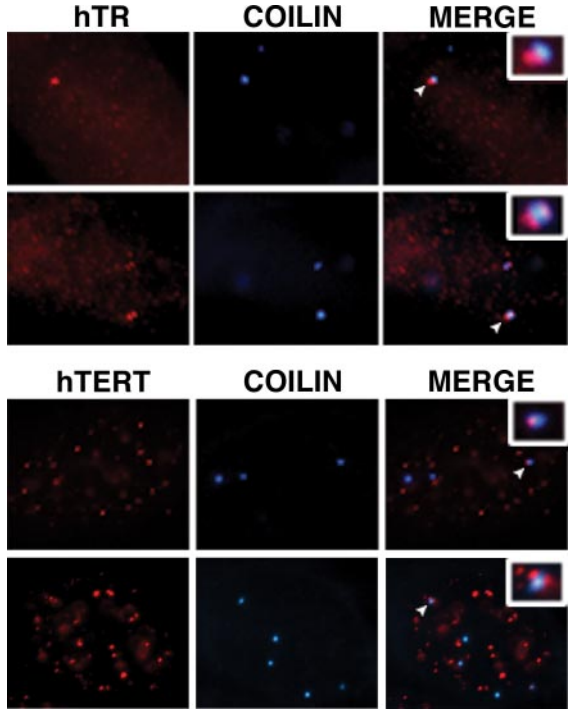

Figure 5. Human telomerase RNA and telomerase reverse transcriptase are found in foci adjacent to Cajal bodies during S phase. Coanalysis of coilin (blue) and hTR (red) or hTERT (red) in early or mid-S-phase HeLa cells is shown. Merge panels show superimposition of coilin and hTR or hTERT signals. Insets show enlargements of close associations of distinct coilin and hTR or hTERT foci (indicated by arrowheads in merge panel).

localization to telomeres and nucleoli during S phase (Figure 4). The results suggest an S-phase-specific mobilization of the components of the telomerase RNP to its functional destination, the telomere.

hTR and hTERT Are Found in Foci Adjacent to Cajal Bodies during S Phase

Our results indicate that hTR normally accumulates in Cajal bodies in HeLa cells and that hTERT does not. (Previously, we reported localization of YFP-hTERT to Cajal bodies in HeLa cells; Zhu *et al*., 2004.) However, in the current work, we did not detect significant accumulation of endogenous hTERT in Cajal bodies in either asynchronous or S-phase cells; hTERT colocalized with coilin in <5% of cells (Figure 4).) Although we did not observe endogenous hTERT in Cajal bodies, we did detect a specific accumulation of both hTERT and hTR in foci immediately adjacent to Cajal bodies during S phase. Figure 5 shows examples of the associations of hTR and hTERT with Cajal bodies observed in S-phase HeLa cells. The associations seem to represent one or two structures containing hTR or hTERT in direct contact with a Cajal body. In these cases, there is relatively little hTR (or hTERT) found in the core of the Cajal bodies. (The pattern does not represent simple pixel shifting relative to coilin data because the offsets occurred along multiple vectors within a single field and did not affect colocalizations in adjacent cells.) In some cases, particularly for hTR, it seems that the foci may be present at poles of the Cajal body (rather than in distinct adjacent structures) (e.g., Figure 5, row 2). In other cases, it is clear that the hTR or hTERT foci are distinct from the Cajal body (e.g., Figure 5, row 4). The timing and frequency with which hTR or hTERT are observed closely associated with Cajal bodies are similar to telomere associations (Figure 4). At mid-S phase, localization to foci associated with Cajal bodies was found in 31% cells for hTR (698 cells analyzed from 5 separate experiments) and in 38% cells for hTERT (280 cells from 2 experiments). At least in the case of hTERT, there is also significant association in early S phase, suggesting that the peak of localization to Cajal body-associated foci occurs just before mid-S phase (Figure 4). It is not currently known whether the hTR or hTERT foci correspond with other, previously described Cajal body-associated nuclear bodies (Liu and Dreyfuss, 1996; Yannoni and White, 1997; Schul *et al*., 1999; Zhao *et al*., 2000; Miele *et al*., 2005).

DISCUSSION

Telomere synthesis is restricted to S phase in human cells. In this study, we show that the two key components of telom-

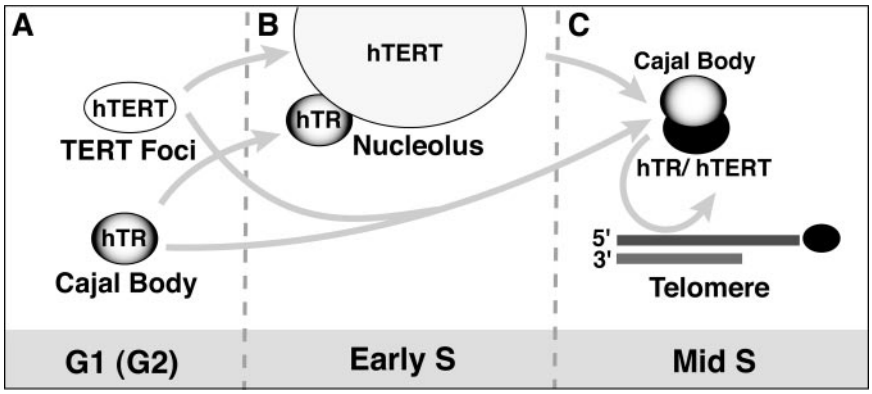

Figure 6. Model for cell cycle-regulated trafficking of telomerase subunits to telomeres during S phase. The predominant phase-specific localization of hTR and hTERT is shown for G_1 (A), early S (B), and mid-S (C). The arrows indicate possible trafficking pathways accounting for the observed localizations. See text for details.

erase, telomerase RNA and the catalytic protein subunit hTERT, are targeted to telomeres specifically in S phase. Our study marks the first time human telomerase has been visualized at telomeres and suggests that the restriction of telomere elongation to S phase of the cell cycle is achieved by subnuclear trafficking of the telomerase RNP. Furthermore, our results indicate that multiple nuclear structures play roles in the regulated transport and biogenesis of telomerase.

Telomere Synthesis

Our finding that the recruitment of telomerase to telomeres is restricted to S phase (peaking in mid-S; Figures 2B, 3C, and 4) correlates well with the known timing of telomere elongation in human cells (Ten Hagen *et al*., 1990; Wright *et al*., 1999). Strikingly, our data suggest that telomerase accumulates at only a subset of telomeres in a given cell (and in only a fraction of cells in a population) at any given time (Figures 2B, 3C, and 4). The lack of detection of telomerase at some telomeres could certainly reflect further limitations of the experimental approach. However, this finding is also consistent with the idea that telomerase may not act on every telomere during every cell cycle, as has been demonstrated in yeast where only a small fraction of telomeres (~7%) are extended within a given cycle (Teixeira *et al*., 2004). Studies in both yeast and mammalian cells indicate that telomerase preferentially elongates the shortest telomeres in a population (Ouellette *et al*., 2000; Teixeira *et al*., 2004). Alternatively, all telomeres may be extended during each cell cycle, but not simultaneously, such that telomerase is active at only a subset of telomeres at any given time point. It is known that chromosomes replicate at different rates during S phase (Woodfine *et al*., 2004; Zou *et al*., 2004), and thus the timing of telomere synthesis could vary, for example, with the timing of replication-induced changes in chromatin structure and telomere accessibility at individual chromosomes.

Telomerase Trafficking Pathway

By following the subcellular localization of endogenous hTR and hTERT molecules throughout the cell cycle, we have obtained important insight into the pathway that telomerase may follow on its way to its site of action, the telomere (Figure 6). However, it is important to note that although the successful detection of hTR and hTERT at telomeres suggests excellent sensitivity, the results of our experiments do not preclude the presence of lower concentrations of hTR or hTERT in cellular compartments other than those identified, including the nucleoplasm. In addition, as discussed above, the various localization patterns described here are not observed in every cell in a synchronized population at a given time point, which may reflect technical limitations or real differences in the timing or extent of telomere synthesis within and among individual cells.

Before S phase, during G_1 (and also following S phase, in G_2), hTR and hTERT are observed in separate intranuclear structures (Figure 6A). Telomerase RNA is present in Cajal bodies (Figure 1), consistent with previous findings (Jady *et al*., 2004; Zhu *et al*., 2004). In contrast, hTERT accumulates in distinct nucleoplasmic foci, which may represent previously unrecognized nuclear bodies or identified structures not previously known to contain hTERT (Figure 3). These findings suggest that the two key subunits of telomerase may be sequestered away from one another throughout most of the cell cycle.

Specifically during S phase, hTR and hTERT exhibit a dynamic redistribution and become targeted to common intranuclear sites. In early S phase, both hTR and hTERT can be found associated with nucleoli, although apparently not within a shared compartment (Figure 6B). hTR is present in Cajal bodies that seem to reside around the periphery of the nucleolus (Figures 1 and 2), whereas hTERT seems to be distributed throughout the interior of the nucleolus (Figure 3). Movement of Cajal bodies to and from nucleoli has been documented previously (Platani *et al*., 2000) and may account for the appearance of hTR at nucleoli in S phase. Intriguingly, we often find hTR or hTERT at a single nucleolus within a cell. Although it is well known that the nucleolus supports a number of functions beyond its conventional role in ribosome biogenesis (Pederson, 1998; Olson *et al*., 2000; Lam *et al*., 2005), our results suggest a previously undescribed division of labor among nucleoli.

Beginning in early S phase and peaking at mid-S (Figure 6C), a novel pattern of hTR and hTERT localization emerges in foci that seem to be physically associated with Cajal bodies (Figure 5). The hTERT found in Cajal body-associated foci may originate in nucleoli or come directly from the nucleoplasmic hTERT foci. The hTR foci may arise by segregation of hTR to one pole of a Cajal body, as is suggested by some of our data (e.g., Figure 5, row 2). Although we cannot demonstrate colocalization of hTR and hTERT in the Cajal body-associated foci (or at telomeres) for technical reasons, it seems possible (based on the similarity of the spatial and temporal patterns, and frequency of occurrence) that both components of telomerase are found together here (Figure 6C). Our analysis of hTERT suggests that localization to the Cajal body-associated foci precedes localization to telomeres, which peaks more distinctly in mid-S phase (Fig-

ure 4, see occurrence of Cajal body association at early S). We envision that Cajal bodies with a compartmentalized cargo of hTR and hTERT deliver telomerase to individual telomeres throughout the cell (Figure 6C).

In support of a role for Cajal bodies in the delivery of telomerase to telomeres, we have observed occasional colocalization of the Cajal body-associated hTR and hTERT foci with telomeres (our unpublished data), but the very low frequency of these associations (observed in ~2–3% of S-phase cells) suggests that the interactions with telomeres would be either transient or not preserved under our experimental conditions. In addition, live cell imaging has revealed that Cajal bodies undergo dramatic movements within the nucleus, including journeys across the diameter of the nucleus, fusion with other Cajal bodies, fragmentation into smaller bodies, and transient associations with nucleoli and specific chromosomal loci (Gall, 2000; Platani *et al*., 2000; Sleeman *et al*., 2003; Cioce and Lamond, 2005). (In contrast, the majority of telomeres seem to be anchored to the nuclear matrix with limited capacity for migration; Luderus *et al*., 1996; Molenaar *et al*., 2003.) Finally, an intriguingly similar cell cycle-regulated delivery of transcription and processing factors to histone gene loci also seems to involve Cajal bodies and closely associated foci. Emerging evidence suggests that HiNF-P, a histone gene transcription factor, and p220/NPAT, an associated protein, colocalize at or near Cajal bodies in S phase, which localize to histone gene loci resulting in activation of histone gene transcription (Frey and Matera, 1995; Ma *et al*., 2000; Zhao *et al*., 2000; Shopland *et al*., 2001; Miele *et al*., 2005). Similarly, the RNA processing factors CstF and CPSF seem to move out of Cajal bodies into adjacent structures (termed cleavage bodies) that colocalize with histone gene loci in S phase (Schul *et al*., 1999).

Although neither the detailed localization of endogenous hTR and hTERT through the cell cycle nor the visualization of hTR and hTERT at telomeres has been described previously, some aspects of the pathway described in this work are supported by previous reports. The association of hTR with Cajal bodies in human cells throughout most of the cell cycle is consistent with the previous observations of our laboratory and others (Jady *et al*., 2004; Zhu *et al*., 2004). However, Jady *et al*. (2004) reported an increase in the brightness of hTR FISH signal in Cajal bodies (relative to nucleoplasm) in cells in S phase, and it is not immediately clear how this observation relates to the pathway defined here. Consistent with our findings, a small percentage of cellular hTR has been detected in biochemical fractions containing nucleoli (Mitchell *et al*., 1999), and hTR localizes to nucleoli (as well as Cajal bodies) when injected into *Xenopus* oocytes (Lukowiak *et al*., 2001). In addition, ectopically expressed GFP-hTERT fusion proteins have been found to localize to nucleoli, and Yang *et al*. reported increased nucleolar association during S phase (Wong *et al*., 2002; Yang *et al*., 2002b). Our results place the previous observations firmly in the context of a cell cycle-regulated pathway.

Regulation of Telomerase Trafficking

Our results indicate that the transport of telomerase to telomeres is exquisitely regulated in the context of the cell cycle. On the basis of these findings, it will now be interesting to determine how movement of the RNA and protein subunits is linked to the cell cycle. One logical possibility is that some of the same kinases and phosphatases that drive other S-phase events (e.g., cdk2/cyclin A and cdc25) also modify telomerase subunits (and telomere binding proteins) to regulate molecular interactions and thereby influence telomerase trafficking (and telomere accessibility). In hTR, RNA elements termed the CAB box and H/ACA motif have been found to be important for localization of the RNA to Cajal bodies and nucleoli, respectively (Lukowiak *et al*., 2001; Jady *et al*., 2004), and the domains of hTERT that mediate nucleolar localization have been defined (Etheridge *et al*., 2002; Yang *et al*., 2002b). One would predict that these domains and proteins that interact with these domains could be modified to effectively regulate telomerase trafficking.

Telomerase Biogenesis

It is clear from our results that the trafficking of hTR and hTERT is regulated by the cell cycle. Interestingly, our findings suggest the possibility that the assembly of the telomerase enzyme may also be regulated to restrict telomere synthesis to S phase (i.e., the essential subunits may be compartmentalized away from each other as well as from their substrate). The detectable pools of hTR and hTERT are not found in common structures outside of S phase (Figures 1 and 3), suggesting that human telomerase is assembled specifically during S phase and disassembled (or destroyed in the case of hTERT; Masutomi *et al*., 2003; Kim *et al*., 2005) after each cell cycle, perhaps during M phase when the telomerase subunits do not seem to be associated with structures (Figure 1; our unpublished data).

If the biogenesis of telomerase is regulated by the cell cycle, our results suggest two likely sites for the assembly of the enzyme during S phase. hTR and hTERT are both found in foci associated with Cajal bodies and at telomeres in mid-S phase. The Cajal body-associated foci hold the potential for interaction with the SMN complex, a known RNP assembly factor that resides in Cajal bodies (Terns and Terns, 2001; Gubitz *et al*., 2004). SMN is known to interact with telomerase and specifically to interact with GAR1 (an hTR-associated protein) and hTERT, suggesting that SMN may function in the biogenesis of telomerase (Bachand *et al*., 2002; Whitehead *et al*., 2002).

Our data are also consistent with the possibility that telomerase may assemble at the telomere. There is solid evidence that yeast telomerase is assembled at telomeres. In this case, the core components of telomerase, TLC1 (telomerase RNA) and Est2p (TERT) are constitutively present at telomeres, and assembly of active telomerase is regulated by an S-phase-specific recruitment of an essential telomerase subunit, Est1, to telomeres (Taggart *et al*., 2002).

However, although telomerase activity is restricted to S phase in intact cells (Ten Hagen *et al*., 1990; Wright *et al*., 1999), catalytically active telomerase enzyme (assessed by TRAP assay) can be extracted from both human and yeast cells at any stage of the cell cycle (Holt *et al*., 1997 but see Zhu *et al*., 1996; Yang *et al*., 2002a). It is not clear whether this extracted telomerase activity reflects enzyme present in cells or assembled from individual components after cell lysis and extract preparation (as has been demonstrated to occur in the case of another RNA–protein complex; Mili and Steitz, 2004). The potential regulation of telomerase biogenesis by the cell cycle will require further investigation.

Coupling telomerase trafficking to the cell cycle may have evolved in eukaryotes as an efficient mechanism to restrict the activity of telomerase to the period when chromosomes are replicated and to limit potentially deleterious activity of telomerase at nontelomeric sites (i.e., chromosome healing at double-stranded breaks) during the remainder of the cell cycle (Sprung *et al*., 1999). Future research in the field will further delineate the intranuclear trafficking patterns of telomerase, define the molecular mechanisms of telomerase biogenesis and telomere recruitment, and determine how these processes are regulated by the cell cycle.

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REFERENCES

Andrade, L. E., Tan, E. M., and Chan, E. K. (1993). Immunocytochemical analysis of the coiled body in the cell cycle and during cell proliferation. Proc. Natl. Acad. Sci. USA *90*, 1947–1951.

Bachand, F., Boisvert, F. M., Cote, J., Richard, S., and Autexier, C. (2002). The product of the survival of motor neuron (SMN) gene is a human telomerase-associated protein. Mol. Biol. Cell *13*, 3192–3202.

Blasco, M. A. (2005). Mice with bad ends: mouse models for the study of telomeres and telomerase in cancer and aging. EMBO J. *24*, 1095–1103.

Cioce, M., and Lamond, A. I. (2005). Cajal bodies: a long history of discovery. Annu. Rev. Cell Dev. Biol. *21*, 105–131.

Colgin, L., and Reddel, R. (2004). Telomere biology: a new player in the end zone. Curr. Biol. *14*, R901–R902.

Collins, K., and Mitchell, J. R. (2002). Telomerase in the human organism. Oncogene *21*, 564–579.

Cong, Y. S., Wright, W. E., and Shay, J. W. (2002). Human telomerase and its regulation. Microbiol. Mol. Biol. Rev. *66*, 407–425.

de Lange, T. (2002). Protection of mammalian telomeres. Oncogene *21*, 532–540.

Etheridge, K. T., Banik, S. S., Armbruster, B. N., Zhu, Y., Terns, R. M., Terns, M. P., and Counter, C. M. (2002). The nucleolar localization domain of the catalytic subunit of human telomerase. J. Biol. Chem. *277*, 24764–24770.

Fang, G., and Cech, T. R. (1995). Telomerase RNA localized in the replication band and spherical subnuclear organelles in hypotrichous ciliates. J. Cell Biol. *130*, 243–253.

Frey, M. R., and Matera, A. G. (1995). Coiled bodies contain U7 small nuclear RNA and associate with specific DNA sequences in interphase human cells. Proc. Natl. Acad. Sci. USA *92*, 5915–5919.

Gall, J. G. (2000). Cajal bodies: the first 100 years. Annu. Rev. Cell Dev. Biol. *16*, 273–300.

Gall, J. G. (2003). The centennial of the Cajal body. Nat. Rev. Mol. Cell. Biol. *4*, 975–980.

Greider, C. W., and Blackburn, E. H. (1989). A telomeric sequence in the RNA of *Tetrahymena* telomerase required for telomere repeat synthesis. Nature *337*, 331–337.

Gubitz, A. K., Feng, W., and Dreyfuss, G. (2004). The SMN complex. Exp. Cell Res. *296*, 51–56.

Harley, C. B., Futcher, A. B., and Greider, C. W. (1990). Telomeres shorten during ageing of human fibroblasts. Nature *345*, 458–460.

Harrington, L. (2004). Those dam-aged telomeres! Curr. Opin. Genet. Dev. *14*, 22–28.

Holt, S. E., Aisner, D. L., Shay, J. W., and Wright, W. E. (1997). Lack of cell cycle regulation of telomerase activity in human cells. Proc. Natl. Acad. Sci. USA *94*, 10687–10692.

Jady, B. E., Bertrand, E., and Kiss, T. (2004). Human telomerase RNA and box H/ACA scaRNAs share a common Cajal body-specific localization signal. J. Cell Biol. *164*, 647–652.

Kim, J. H., Park, S. M., Kang, M. R., Oh, S. Y., Lee, T. H., Muller, M. T., and Chung, I. K. (2005). Ubiquitin ligase MKRN1 modulates telomere length homeostasis through a proteolysis of hTERT. Genes Dev. *19*, 776–781.

Lam, Y. W., Trinkle-Mulcahy, L., and Lamond, A. I. (2005). The nucleolus. J. Cell Sci. *118*, 1335–1337.

Liu, Q., and Dreyfuss, G. (1996). A novel nuclear structure containing the survival of motor neurons protein. EMBO J. *15*, 3555–3565.

Luderus, M. E., van Steensel, B., Chong, L., Sibon, O. C., Cremers, F. F., and de Lange, T. (1996). Structure, subnuclear distribution, and nuclear matrix association of the mammalian telomeric complex. J. Cell Biol. *135*, 867–881.

Lukowiak, A. A., Narayanan, A., Li, Z. H., Terns, R. M., and Terns, M. P. (2001). The snoRNA domain of vertebrate telomerase RNA functions to localize the RNA within the nucleus. RNA *7*, 1833–1844.

Ma, T., Van Tine, B. A., Wei, Y., Garrett, M. D., Nelson, D., Adams, P. D., Wang, J., Qin, J., Chow, L. T., and Harper, J. W. (2000). Cell cycle-regulated phosphorylation of p220(NPAT) by cyclin E/Cdk2 in Cajal bodies promotes histone gene transcription. Genes Dev. *14*, 2298–2313.

Masutomi, K., *et al*. (2003). Telomerase maintains telomere structure in normal human cells. Cell *114*, 241–253.

McEachern, M. J., Krauskopf, A., and Blackburn, E. H. (2000). Telomeres and their control. Annu. Rev. Genet. *34*, 331–358.

Miele, A., *et al*. (2005). HiNF-P directly links the cyclin E/CDK2/p220NPAT pathway to histone H4 gene regulation at the G1/S phase cell cycle transition. Mol. Cell. Biol. *25*, 6140–6153.

Mili, S., and Steitz, J. A. (2004). Evidence for reassociation of RNA-binding proteins after cell lysis: implications for the interpretation of immunoprecipitation analyses. RNA *10*, 1692–1694.

Mitchell, J. R., Cheng, J., and Collins, K. (1999). A box H/ACA small nucleolar RNA-like domain at the human telomerase RNA 3′ end. Mol. Cell. Biol. *19*, 567–576.

Molenaar, C., Wiesmeijer, K., Verwoerd, N. P., Khazen, S., Eils, R., Tanke, H. J., and Dirks, R. W. (2003). Visualizing telomere dynamics in living mammalian cells using PNA probes. EMBO J. *22*, 6631–6641.

O'Keefe, R. T., Henderson, S. C., and Spector, D. L. (1992). Dynamic organization of DNA replication in mammalian cell nuclei: spatially and temporally defined replication of chromosome-specific alpha-satellite DNA sequences. J. Cell Biol. *116*, 1095–1110.

Olson, M. O., Dundr, M., and Szebeni, A. (2000). The nucleolus: an old factory with unexpected capabilities. Trends Cell Biol. *10*, 189–196.

Ouellette, M. M., Aisner, D. L., Savre-Train, I., Wright, W. E., and Shay, J. W. (1999). Telomerase activity does not always imply telomere maintenance. Biochem. Biophys. Res. Commun. *254*, 795–803.

Ouellette, M. M., Liao, M., Herbert, B. S., Johnson, M., Holt, S. E., Liss, H. S., Shay, J. W., and Wright, W. E. (2000). Subsenescent telomere lengths in fibroblasts immortalized by limiting amounts of telomerase. J. Biol. Chem. *275*, 10072–10076.

Pederson, T. (1998). The plurifunctional nucleolus. Nucleic Acids Res. *26*, 3871–3876.

Platani, M., Goldberg, I., Swedlow, J. R., and Lamond, A. I. (2000). In vivo analysis of Cajal body movement, separation, and joining in live human cells. J. Cell Biol. *151*, 1561–1574.

Schul, W., van Der Kraan, I., Matera, A. G., van Driel, R., and de Jong, L. (1999). Nuclear domains enriched in RNA 3′-processing factors associate with coiled bodies and histone genes in a cell cycle-dependent manner. Mol. Biol. Cell *10*, 3815–3824.

Shay, J. W., and Bacchetti, S. (1997). A survey of telomerase activity in human cancer. Eur. J. Cancer *33*, 787–791.

Shopland, L. S., Byron, M., Stein, J. L., Lian, J. B., Stein, G. S., and Lawrence, J. B. (2001). Replication-dependent histone gene expression is related to Cajal body (CB) association but does not require sustained CB contact. Mol. Biol. Cell *12*, 565–576.

Sleeman, J. E., Trinkle-Mulcahy, L., Prescott, A. R., Ogg, S. C., and Lamond, A .I. (2003). Cajal body proteins SMN and Coilin show differential dynamic behaviour in vivo. J. Cell Sci. *116*, 2039–2050.

Smogorzewska, A., and de Lange, T. (2004). Regulation of telomerase by telomeric proteins. Annu. Rev. Biochem. *73*, 177–208.

Sprung, C. N., Reynolds, G. E., Jasin, M., and Murnane, J. P. (1999). Chromosome healing in mouse embryonic stem cells. Proc. Natl. Acad. Sci. USA *96*, 6781–6786.

Taggart, A. K., Teng, S. C., and Zakian, V. A. (2002). Est1p as a cell cycle-regulated activator of telomere-bound telomerase. Science *297*, 1023–1026.

Teixeira, M. T., Arneric, M., Sperisen, P., and Lingner, J. (2004). Telomere length homeostasis is achieved via a switch between telomerase-extendible and -nonextendible states. Cell *117*, 323–335.

Ten Hagen, K. G., Gilbert, D. M., Willard, H. F., and Cohen, S. N. (1990). Replication timing of DNA sequences associated with human centromeres and telomeres. Mol. Cell. Biol. *10*, 6348–6355.

Terns, M. P., and Terns, R. M. (2001). Macromolecular complexes: SMN–the master assembler. Curr. Biol. *11*, R862–R864.

Whitehead, S. E., Jones, K. W., Zhang, X., Cheng, X., Terns, R. M., and Terns, M. P. (2002). Determinants of the interaction of the spinal muscular atrophy

disease protein SMN with the dimethylarginine-modified box H/ACA small nucleolar ribonucleoprotein GAR1. J. Biol. Chem. *277*, 48087–48093.

Wong, J. M., Kusdra, L., and Collins, K. (2002). Subnuclear shuttling of human telomerase induced by transformation and DNA damage. Nat. Cell Biol. *4*, 731–736.

Woodfine, K., Fiegler, H., Beare, D. M., Collins, J. E., McCann, O. T., Young, B. D., Debernardi, S., Mott, R., Dunham, I., and Carter, N. P. (2004). Replication timing of the human genome. Hum. Mol. Genet. *13*, 191–202.

Wright, W. E., Tesmer, V. M., Liao, M. L., and Shay, J. W. (1999). Normal human telomeres are not late replicating. Exp. Cell Res. *251*, 492–499.

Yang, S. W., Jin, E., Chung, I. K., and Kim, W. T. (2002a). Cell cycle-dependent regulation of telomerase activity by auxin, abscisic acid and protein phosphorylation in tobacco BY-2 suspension culture cells. Plant J. *29*, 617–626.

Yang, Y., Chen, Y., Zhang, C., Huang, H., and Weissman, S. M. (2002b). Nucleolar localization of hTERT protein is associated with telomerase function. Exp. Cell Res. *277*, 201–209.

Yannoni, Y. M., and White, K. (1997). Association of the neuron-specific RNA binding domain-containing protein ELAV with the coiled body in Drosophila neurons. Chromosoma *105*, 332–341.

Zhao, J., Kennedy, B. K., Lawrence, B. D., Barbie, D. A., Matera, A. G., Fletcher, J. A., and Harlow, E. (2000). NPAT links cyclin E-Cdk2 to the regulation of replication-dependent histone gene transcription. Genes Dev. *14*, 2283–2297.

Zhu, X., Kumar, R., Mandal, M., Sharma, N., Sharma, H. W., Dhingra, U., Sokoloski, J. A., Hsiao, R., and Narayanan, R. (1996). Cell cycle-dependent modulation of telomerase activity in tumor cells. Proc. Natl. Acad. Sci. USA *93*, 6091–6095.

Zhu, Y., Tomlinson, R. L., Lukowiak, A. A., Terns, R. M., and Terns, M. P. (2004). Telomerase RNA accumulates in Cajal bodies in human cancer cells. Mol. Biol. Cell *15*, 81–90.

Zou, Y., Gryaznov, S. M., Shay, J. W., Wright, W. E., and Cornforth, M. N. (2004). Asynchronous replication timing of telomeres at opposite arms of mammalian chromosomes. Proc. Natl. Acad. Sci. USA *101*, 12928–12933.