substrates that provide strong resistance to forces applied to the cell or cytoskeleton. On these substrates, most $\alpha_5\beta_1$ integrin–fibronectin bonds were tensioned, but softer substrates in the range of 0.2 to 20 kPa provide less resistance, and the proportion of adhesive bonds in the relaxed state should increase. Fibronectin was attached to polyacrylamide gel substrates with different stiffness and $\alpha_5\beta_1$ integrin–fibronectin bonds were measured by using both spinning disc and chemical cross-linking methods (Fig. 3, A and B). Note that corrections were made for cell spreading and shape differences (see fig. S3, A and B, and SOM text, note S2). Spinning disc analysis showed that the total number of adhesive bonds that formed was independent of substrate stiffness. In contrast, the cross-linking assay showed a strong dependence of the number of tensioned bonds on substrate stiffness. Thus, as the substrate stiffness increased, the proportion of $\alpha_5\beta_1$-fibronectin adhesive bonds in the tensioned state increased. If only tensioned bonds generated downstream signals, this would provide a mechanism for cells to sense microenvironmental stiffness. Inhibitors of myosin II and actin that blocked mechanism for cells to sense microenvironmental stiffness and integrin-mediated adhesion for mechanical activation (9). Thus, the mechanical activation of src would depend on the mechanical activation of integrins, which suggests a model that contains multiple intra- and extracellular mechano-sensitive nodes to generate intracellular signaling responses. Control of intracellular signals by mechanical triggers provides a mechanism for spatial distribution of signals within cells. This is important for the cells’ capability to sense and respond to differences in microenvironmental stiffness and generates a need for mechanisms to control the cell’s “tensional” homeostasis (5).

References and Notes
12. Materials and methods are available as supporting material on Science Online.
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A Human Telomerase Holoenzyme Protein Required for Cajal Body Localization and Telomere Synthesis

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Telomerase is a ribonucleoprotein (RNP) complex that synthesizes telomeres repeats in tissue progenitor cells and cancer cells. Active human telomerase consists of at least three principal subunits, including the telomerase reverse transcriptase, the telomerase RNA (TERC), and dyskerin. Here, we identify a holoenzyme subunit, TCB1 (telomerase Cajal body protein 1), that is notably enriched in Cajal bodies, nuclear sites of RNP processing that are important for telomerase function. TCB1 associates with active telomerase enzyme, established telomerase components, and small Cajal body RNAs that are involved in modifying splicing RNAs. Depletion of TCB1 by using RNA interference prevents TERC from associating with Cajal bodies, disrupts telomerase-Cajal body association, and abrogates telomere synthesis by telomerase. Thus, TCB1 controls telomerase trafficking and is required for telomere synthesis in human cancer cells.

The telomerase reverse transcriptase (TERT) and the telomerase RNA component (TERC) comprise the minimal catalytic core of the telomerase enzyme (1), whereas dyskerin is an RNA-binding protein that recognizes the H/ACA sequence motif shared by TERC and two groups of noncoding RNAs involved in RNA modification: small Cajal body RNAs (scARNAs) and small nucleolar RNAs (snoRNAs) (2, 3). Dyskerin functions in part...
to support telomerase ribonucleoprotein (RNP) biogenesis and TERC stability (4, 5). TERT, TERC, and dyskerin are all components of active telomerase (6), and mutations in any of these genes can cause the human stem cell disorder dyskeratosis congenita (7). Other potential components of active telomerase include three evolutionarily conserved dyskerin-associated proteins, nucleolar protein 10 (NOP10), non-histone protein 2 (NHP2), and glycine/arginine-rich domain containing protein 1 (GAR1) (8–10), and ever-shorter telomeres 1A (EST1A), a homolog of the yeast telomerase protein Est1p.

We reasoned that other dyskerin-associated proteins may be telomerase components, and we therefore sought to purify dyskerin complexes.

To study dyskerin, we expressed tagged dyskerin protein at endogenous levels in the absence of competing endogenous protein (fig. S1) and isolated dyskerin complexes by using a dual-affinity chromatography strategy. Purified dyskerin complexes were analyzed by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and nano–liquid chromatography–tandem mass spectrometry (nanoLC-MS/MS) for identification of copurifying proteins (Fig. 1, A and B). Densite peptide coverage was obtained for dyskerin and for the dyskerin-associated adenosine triphosphatases (ATPases) pontin and reptin (11, 12). Each of the evolutionarily conserved dyskerin-binding proteins NHP2, NOP10, and GAR1 was detected, as were the dyskerin-associated proteins nucleolar and coiled-body phosphoprotein of 140 kD. (Nopp140) and nuclear assembly factor 1 (NAF1), a nucleoplasmic factor required for the assembly of H/ACA RNPs, including telomerase (Fig. 1B) (13). In addition, this approach identified WD repeat domain 79 (WDR79) (Fig. 1B), a protein that had not been previously implicated in dyskerin or telomerase function.

We further characterized WDR79, hereafter referred to as TCAB1 (telomerase Cajal body protein 1) (fig. S2). Endogenous TCAB1 was specifically bound to Flag-dyskerin that was immunoprecipitated from Flag-dyskerin+shRNA HeLa cells (shRNA, short hairpin RNA), as were endogenous pontin, NAF1, TERT, and TERC (Fig. 1C). Interactions between TERT and dyskerin were disrupted by ribonuclease A (RNase A) treatment of the extract, which degraded TERC. In contrast, dyskerin interactions with TCAB1, NAF1, and pontin were not RNase A–sensitive, indicating that these associations occur through protein–protein contacts (Fig. 1C). Reciprocal immunoprecipitation (IP) of Flag-TCAB1 from Flag-TCAB1+shRNA HeLa cells showed that Flag-TCAB1 not only associates with endogenous dyskerin but also with TERT and TERC, the catalytic core of telomerase (Fig. 1D). Like the TERT-dyskerin association, the binding of TERT to Flag-TCAB1 was RNase A–sensitive, suggesting that the interaction of TCAB1 with telomerase is dependent on TERC (Fig. 1D). Thus, TCAB1 interacts specifically with dyskerin, TERT, and TERC, all three known components of active telomerase.

Although TCAB1 associated with TERT, TERC, and dyskerin, it did not interact with the assembly factors NAF1, pontin, or reptin (Fig. 1D), suggesting that TCAB1 may be a component of the enzymatically active telomerase complex rather than a pre-telomerase complex. To test this hypothesis, we asked to what extent telomerase activity in cell extracts was associated with overexpressed TCAB1. Flag-tagged TCAB1, dyskerin, and NAF1 were depleted from extracts by IP (Fig. 2A, lanes 14 to 21). Flag-TCAB1 and Flag-dyskerin immunoprecipitates were associated with high telomerase activity (Fig. 2A, lane 14 to 21).
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the Cajal body accumulates in both Cajal bodies and nucleoli, the nucleoplasm (Fig. 3B). Although dyskerin
Cajal bodies, with smaller amounts distributed in
Endogenous TCAB1 was also highly enriched in
coilin and were therefore Cajal bodies (Fig. 3A).

To understand the composition of endog-
ous telomerase, IPs were performed using
antibodies to TCAB1 and NAF1, each of which
efficiently depleted its cognate protein from cell
extracts (Fig. 2B, lanes 9 to 13). High telo-
merase activity was associated with TCAB1
immunoprecipitates, and antibodies to TCAB1
quantitatively depleted telomerase activity from
cell extracts (Fig. 2B, lanes 1 to 8, and fig. S4). In contrast, antibodies to NAF1 pulled down
only a small percentage of telomerase activity.
Assessing the TCAB1 immunoprecipitates for
telomerase components revealed that TCAB1
interacts with endogenous dyskerin, TERT, and
TERC (Fig. 2B, lanes 14 to 16). IP of TCAB1
effectively depleted cell extracts of TERC by
use of Northern blot (Fig. 2B, lanes 9 to 13,
and figs. S4 and S5). We conclude that TCAB1,
like dyskerin, associates stably with a vast
majority of active telomerase and TERC and there-
fore is a component of a human telomerase
holoenzyme.

Using immunofluorescence (IF), we found
that stably overexpressed hemagglutinin (HA)–
tagged TCAB1 (HA-TCAB1) was distributed
weakly throughout the nucleoplasm but was
strongly enriched within nuclear foci resembling
Cajal bodies, sites of RNP processing shown to
strongly enrich within nuclear foci resembling
Cajal bodies, sites of RNP processing shown to

and associated telomerase components are shown in lanes 22 to 25. U1-splicing RNA was the negative
control. (A) Flag-TCAB1 or Flag-dyskerin IP quantitatively codepletes telomerase activity and TERC from extracts.

Telomeric repeat amplification protocol (TRAP) assays were performed on extracts before and after each IP (lanes 1 to 8) and on the IP (lanes 6 to 8). Depletion of endogenous NAF1 or endogenous TCAB1 and depletion of TERC are shown in lanes 9 to 13. Association of NAF1 and TCAB1 with telomerase components is shown in lanes 14 to 16. Recovery control was exogenous RNA spiked into samples after IP to control for differential RNA recovery.

localization was measured by RNA fluorescence
in situ hybridization (FISH) in TCAB1-depleted
HeLa cells. FISH revealed that TCAB1 knock-
down substantially reduced the percentage of
cells in which TERC was found in Cajal bodies
(Fig. 4, B and C) without affecting overall TERC
RNA levels (Fig. 4A). Cajal bodies have been
directly implicated in the delivery of TERC to
telomeres during S phase (19–21). Therefore,
we also examined the effect of TCAB1 knock-
down on the localization of TERC to telomeres,
using FISH for TERC and IF for telomere repeat
binding factor 2 (TRF2). Depletion of TCAB1
substantially reduced the presence of TERC at
telomeres during S phase (Fig. 4, B and C). Thus,
TCAB1 is required for TERC localization in
Cajal bodies and for delivery of TERC to
telomeres during S phase.

To determine whether TCAB1 is required for
telomere addition by telomerase, we first induced
telomere elongation through TERC overexpression in HTC75 fibrosarcoma cells. Overexpression of wild-type TERC lengthened telomeres with cell passage, but telomere lengthening was inhibited in cells overexpressing a CAB box–mutant TERC that fails to accumulate in Cajal bodies, as previously shown (19). Depletion of TCAB1 in cells overexpressing wild-type TERC substantially inhibited telomere elongation, mimicking the effect of the CAB box mutation (Fig. 4D and fig. S6). To determine whether TCAB1 is required for telomere synthesis by endogenous telomerase, we assayed telomere lengths with serial passage in HTC75 cells transduced with TCAB1 shRNAs or with the empty vector. Both shRNAs targeting TCAB1 led to progressive telomere shortening as compared with that of the empty vector control (Fig. 4E and fig. S7), indicating that TCAB1 is required for telomere synthesis in human cancer cells.

Our data identify TCAB1 as a Cajal body–enriched protein that associates with TERC and other scaRNAs and explains how TERC, and perhaps other scaRNAs, localize in Cajal bodies. TCAB1 functions as a telomerase holoenzyme component in the telomere synthesis pathway at a step after the assembly of a minimal telomerase complex containing TERT, TERC, and dyskerin. NAF1 and the ATPases pontin and reptin are required for assembly of the catalytically competent complex (14, 15). In contrast, TCAB1 stably associates with active telomerase enzyme and directs it through Cajal bodies to telomeres. In this manner, TCAB1 may act as a Cajal body–targeting or –retention factor, may facilitate additional assembly steps of the enzyme in Cajal bodies, and/or may facilitate translocation of telomerase to telomeres (Fig. 4F). The interaction of telomerase with telomeres and the activity of the telomerase holoenzyme may be enhanced by the telomere-binding proteins TPP1 and protection of telomeres 1 (POT1) (22, 23), as well as other factors that remain to be discovered.

References and Notes
Fig. 4. TCAB1 is essential for TERC localization to Cajal bodies and for telomere synthesis by telomerase. (A) HeLa cells were transduced with retroviruses expressing independent shRNA sequences targeting the indicated proteins or with empty vector control. Telomerase activity was measured by TRAP assay. (B) TERC colocalization with p80-coilin was determined by RNA FISH for TERC (green) and IF for p80-coilin (red) (top). Cells synchronized in S phase were assayed for TERC by RNA FISH (green) and for telomeres with antibody to TRF2 (red) to assess trafficking of TERC to telomeres (bottom). (C) Quantification of data in (B). (Top) Cells in which TERC colocalized with p80-coilin (+) versus cells in which TERC was not detected in Cajal bodies (–) (P < 0.0001, Fisher’s exact test). (Bottom) Cells in which TERC colocalized with telomeres (+) versus cells in which TERC was not detected at telomeres (–) (P < 0.0001, Fisher’s exact test). (D) Telomere lengths were measured using terminal restriction fragment (TRF) Southern blot in HTC75 cells overexpressing wild-type TERC (lanes 1 to 8) or mutant TERC-m1 (lanes 9 to 12). Cells overexpressing wild-type TERC were transduced with shRNA retroviruses targeting TCAB1 or with the empty vector. (E) Effect of TCAB1 depletion on endogenous telomerase was assessed by TRF Southern blot in parental HTC75 cells that were transduced with the empty vector or retroviruses expressing independent TCAB1 shRNAs. Cells were counted at each passage and population doublings are indicated. (F) Model for TCAB1 function in the telomere synthesis pathway.

References

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Supporting Online Material
www.sciencemag.org/cgi/content/full/323/5914/644/DC1
Materials and Methods
Figs. S1 to S7
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