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Telomerase in the human organism

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The intent of this review is to describe what is known and unknown about telomerase in somatic cells of the human organism. First, we consider the telomerase enzyme. Human telomerase ribonucleoproteins undergo at least three stages of cellular biogenesis: accumulation, catalytic activation and recruitment to the telomere. Next, we describe the patterns of telomerase regulation in the human soma. Telomerase activation in some cell types appears to offset proliferation-dependent telomere shortening, delaying but not defeating the inherent mitotic clock. Finally, we elaborate the connection between telomerase misregulation and human disease, in the contexts of inappropriate telomerase activation and telomerase deficiency. We discuss how our current perspectives on telomerase function could be applied to improving human health.
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Telomere maintenance is essential for the continued viability of proliferating cells. Incomplete genome replication by DNA-dependent DNA polymerases and end processing by nucleases result in the attrition of an average of 50–100 base-pairs of terminal (T_2AG_3•C_3TA_2) telomeric repeat sequence with each population doubling of cultured human somatic cells (reviewed by Harley, 1995; Huffman *et al*., 2000). This erosion eventually generates a 'critically short' length of telomeric repeat on one or a few telomeres. It is unclear what constitutes a dangerously short telomere at the molecular level; the number of double-stranded repeats and the length of the single-stranded 3′ overhang may both play a role. Whatever this signal may be, the presence of critically short telomere(s) induces one of three fates: proliferative senescence, apoptosis or continued proliferation accompanied by genomic instability (reviewed by Stewart and Weinberg, 2000).

The replication-dependent erosion of telomeric DNA can be counteracted by telomerase-mediated addition of single-stranded T_2AG_3 repeats. The extension of chromosome 3′ ends by telomerase is presumably coupled to second-strand synthesis by a lagging-strand DNA replication complex in human cells, as it is in yeast and ciliate systems (Diede and Gottschling, 1999; Fan and Price, 1997). The sequence-specificity of repeat synthesis derives from a template provided within the RNA component of a telomerase ribonucleoprotein (RNP) complex (Greider and Blackburn, 1989; Yu *et al*., 1990). Numerous proteins associate with telomerase RNA, including one telomerase-specific protein, telomerase reverse transcriptase (TERT), that is common to active telomerase RNPs in all species (Lingner *et al*., 1997; reviewed by Nakamura and Cech, 1998). In human somatic cells, telomerase function is regulated by processes that establish the levels of telomerase accumulation, catalytic activation and telomere recruitment. This review addresses the molecular mechanisms of these processes and their implications for telomere synthesis, sustainable cell proliferation and human health.

The human telomerase enzyme

Endogenously assembled human telomerase RNPs fractionate as large complexes with approximately megadalton molecular mass (Schnapp *et al*., 1998). Apparent mass varies with the purification strategy, suggesting that there are telomerase RNPs of different compositions. This complexity may be partly extractor purification-dependent, but may also reflect a physiological heterogeneity that is inherent in the pathways of telomerase RNP assembly, activation or regulation. We lack a complete molecular description of telomerase from any organism. Here, we will use 'telomerase RNP' when referring to all complexes containing telomerase RNA; we will use 'telomerase holoenzyme' when referring to the subset of telomerase RNPs that are active at least by *in vitro* assay. In human cells, at least three steps in the creation of a biologically functional telomerase holoenzyme can be distinguished by their requirements for regions of telomerase RNA and associated proteins. These three steps, RNA accumulation, catalytic activation and telomere recruitment (Figure 1), are discussed individually in greater detail below.

RNA accumulation

The stable accumulation of most cellular non-protein coding (nc) RNA requires active processing of a

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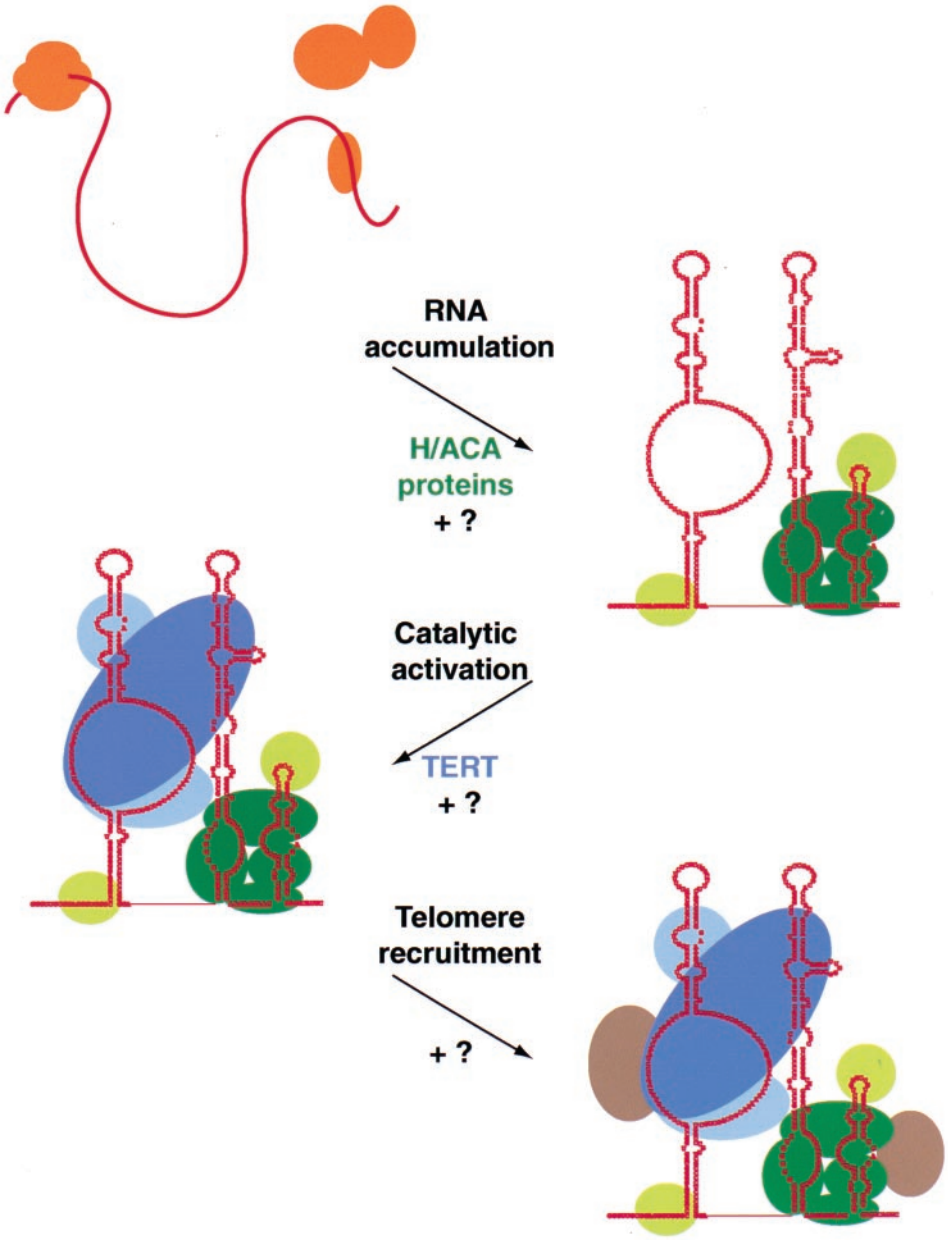

Figure 1 Biogenesis of biologically active human telomerase enzyme. Step 1: telomerase RNA synthesis and maturation require the participation of numerous cellular factors, which together will establish the level of mature RNA accumulation. Some factors (orange) will associate only transiently with telomerase RNA (red). H/ACA proteins (darker green) and other hTR binding proteins (lighter green) become bound to telomerase RNA, providing RNA stability. Step 2: catalytic activation requires the association of telomerase RNP with TERT (dark blue) and possibly other proteins as well (lighter blue). TERT binds directly to telomerase RNA in at least two separable regions. Step 3: active holoenzyme must be recruited to telomere substrates. Proteins that interact with active telomerase (brown) may regulate its recruitment to telomeres by interacting with telomeric chromatin directly or by affecting telomerase subnuclear localization

primary transcript, with events such as precursor cleavage, base and sugar modifications and directed assembly of proteins that protect the RNA from nuclease digestion. Telomerase RNAs in organisms of different phylogenetic groups are strikingly divergent, not only in RNA sequence and structure but also in requirements for transcription, 3′ end formation and cellular stability. The 451-nucleotide human telomerase RNA (hTR) is a product of RNA polymerase II, as are the telomerase RNAs of other vertebrates and yeasts (Chapon *et al*., 1997; Chen *et al*., 2000; Hinkley *et al*., 1998). Ciliate telomerase RNAs are instead transcripts of RNA polymerase III (Yu *et al*., 1990). If hTR sequence is artificially transcribed by RNA polymerase III, it is processed differently and only a truncated RNA accumulates (Mitchell *et al*., 1999a). This observation is consistent with increasing evidence for coordination of many different RNA processing events,

including capping, editing, splicing and cleavage, by factors associated with the transcribing polymerase itself (reviewed by Hirose and Manley, 2000).

RNA polymerase II-mediated transcription of telomerase RNA continues beyond the mature RNA 3′ end, creating a precursor that must be cleaved to generate the final product. Proper 3′ end formation is a critical step in the formation of all telomerase RNPs but is accomplished by different organisms in different ways. In budding yeast, the telomerase RNA precursor is initially polyadenylated and then processed after association with Sm proteins, a pathway common to many small nuclear (sn) RNAs (Chapon *et al*., 1997; Seto *et al*., 1999). In humans, neither the important features of the telomerase RNA precursor downstream of the mature 3′ end nor the processing machinery required to generate the mature RNA 3′ end are known. We have found no evidence for a polyadenylated precursor of endogenous hTR. Instead, transcription termination without polyadenylation may occur.

The requirements for hTR 3′ end formation and stability appear to be linked. The cellular accumulation of mature hTR requires an H/ACA RNA motif composed of primary and secondary structure elements in an overall hairpin-Hinge-hairpin-ACA configuration (Mitchell *et al*., 1999a; Mitchell and Collins, 2000). This motif was previously thought to be exclusive to the H/ACA family of small nucleolar (sno) RNAs, which as RNPs are responsible for the post-transcriptional conversion of uridine to pseudouridine in rRNA, snRNA and potentially other RNA targets as well (reviewed by Kiss, 2001, and Smith and Steitz, 1997). Each hairpin of a snoRNA H/ACA motif contains a bilaterally unpaired pocket within its paired stem. Sequences in the pocket hybridize with an RNA target to direct the sequence-specificity of base conversion. The H/ACA motif is phylogenetically conserved among vertebrate telomerase RNAs (Chen *et al*., 2000) but not those of yeasts or ciliates, each of which appear to employ a different strategy for accomplishing telomerase RNA accumulation *in vivo* (Aigner *et al*., 2000; Seto *et al*., 1999). Most H/ACA motif RNAs demonstrate a steady-state localization to the nucleolus, but at least some are concentrated in other subnuclear bodies (reviewed by Kiss, 2001). In human cells, telomerase distribution within the nucleus is partially nucleolar (Mitchell *et al*., 1999a) in a manner that may be regulated by transformation and the cell cycle (J Wong and K Collins, unpublished data).

The H/ACA motif associates with four proteins common to all known H/ACA snoRNPs. These proteins were first identified in budding yeast as Gar1p, Cbf5p, Nhp2p and Nop10p (Girard *et al*., 1992; Balakin *et al*., 1996; Ganot *et al*., 1997; Lafontaine *et al*., 1998; Watkins *et al*., 1998; Henras *et al*., 1998). Loss of expression of any one of these proteins results in growth arrest with aberrant rRNA, and loss of any one except Gar1p prevents the accumulation of H/ACA snoRNAs. Cbf5p contains the active site for pseudouridine base conversion, initially inferred from the substantial homology of the central region of this protein with other pseudouridine synthases (Koonin, 1996). Interestingly, although null alleles are not viable, substitutions that cripple the Cbf5p active site result in only slow and temperature-sensitive growth (Zebarjadian *et al*., 1999). This suggests that the binding of H/ACA RNPs to RNA targets may be more important than the subsequent base modification. It would appear that the essential Cbf5p function is promote H/ACA RNA stability, which in turn could promote an ordered pathway of target RNA folding and RNP assembly. No requirement for H/ACA proteins in yeast telomerase RNA accumulation is observed, consistent with its lack of an H/ACA motif (Dez *et al*., 2001).

In human cells, the yeast H/ACA protein equivalents dyskerin (the Cbf5p homologue), hNHP2, hNOP10 and hGAR1 associate with H/ACA snoRNAs and with hTR as well (Mitchell *et al*., 1999b; Dragon *et al*., 2000; Pogacic *et al*., 2000; JR Mitchell and K Collins, unpublished data). All sequence variants of hTR that retain the ability to accumulate *in vivo* also retain the ability to interact with dyskerin (and by extension, cooperatively bound hNHP2 and hNOP10); conversely, substitutions of H/ACA motif elements that are predicted to eliminate binding of the H/ACA proteins likewise eliminate hTR accumulation (Mitchell and Collins, 2000). These findings reveal that the hTR H/ACA motif recruits the same proteins stably bound to H/ACA snoRNAs and, like a snoRNA, requires these proteins for stability *in vivo*.

At least one region of vertebrate telomerase RNA distinct from the H/ACA motif itself, at the distal end of the 3′ H/ACA motif hairpin, is also required for RNA stability *in vivo* (Mitchell and Collins, 2000; Martin-Rivera and Blasco, 2001). This suggests that the H/ACA proteins are necessary but not sufficient for hTR accumulation. It seems likely that numerous other maturation events also remain to be incorporated into models of telomerase RNP biogenesis. For example, we have detected base modifications in functionally critical regions of hTR that may promote proper RNA folding or step-wise RNP assembly (JR Mitchell and K Collins, unpublished data). Differences in the efficiency of any event required for hTR accumulation (step 1 in Figure 1) could contribute to establishing the observed cell type-specific differences in levels of telomerase RNP (described below).

Catalytic activation

All human somatic cells and almost all human cell lines transcribe and accumulate hTR, generating telomerase RNP. However, the distribution of catalytically active telomerase holoenzyme is much more restricted. Inactivity is most frequently correlated with the absence of TERT mRNA (e.g., Meyerson *et al*., 1997). Accordingly, forced expression of recombinant TERT is sufficient to direct enzyme activation (Weinrich *et al*., 1997). *In vitro*, expression of TERT and telomerase RNA from human, mouse or the ciliate

Tetrahymena thermophila in rabbit reticulocyte lysate (RRL) reconstitutes many features of telomerase holoenzyme activity (Weinrich *et al*., 1997; Beattie *et al*., 1998; Greenberg *et al*., 1998; Collins and Gandhi, 1998). For these recombinant telomerases, RRL appears able to bypass or recreate some of the cellular requirements for RNP assembly and activation (Holt *et al*., 1999; Licht and Collins, 1999). Even under the best of conditions, however, *in vitro* reconstitution remains inefficient. Recombinant expression of human TERT and hTR in budding yeast or insect cells can also reconstitute enzyme activity (Bachand and Autexier, 1999; Masutomi *et al*., 2000; Wenz *et al*., 2001). Cellular requirements for hTR accumulation and telomerase RNP assembly are not circumvented by this heterologous expression however, because at least in yeast, recombinant human telomerase production requires the host cell H/ACA proteins (Dez *et al*., 2001).

In human somatic cells, it is logical to propose that TERT associates with hTR that has been pre-assembled into stable telomerase RNP (step 2 in Figure 1). TERT association with telomerase RNP appears to be mediated predominantly by direct binding to two independent regions of telomerase RNA, both of which are required for activity *in vivo*: the template region (including nucleotides 44–186) and a putative double hairpin element in the 5′ stem of the H/ACA domain (a region within nucleotides 243–326). *In vivo*, hTR variants internally deleted for either or both TERT-binding motifs assemble to form stable but inactive RNPs; H/ACA proteins are present but TERT association is reduced (Mitchell and Collins, 2000). *In vitro*, a low level of activity can be reconstituted with TERT and the template region alone (Beattie *et al*., 1998) but is dramatically stimulated by the presence of the hairpin element. These two hTR regions can be combined in trans to stimulate telomerase activity (Tesmer *et al*., 1999) with each RNA region demonstrating independent interaction with TERT (Mitchell and Collins, 2000). Neither TERT nor the TERT-interacting regions of hTR are required for RNA accumulation *in vivo*, but at least under some conditions, constitutive expression of TERT can increase the steady-state level of endogenous or recombinant hTR and increase endogenous hTR half-life (Yi *et al*., 1999; J Wong and K Collins, unpublished data).

The absence of TERT mRNA guarantees an absence of catalytic activity, but TERT mRNA expression alone is not necessarily sufficient for activation. Alternative splicing of the endogenous human TERT pre-mRNA creates transcripts predicted to encode isoforms of TERT without catalytic function (Kilian *et al*., 1997). At least one such TERT protein can have a dominant negative effect on telomerase activity and telomere length maintenance (Colgin *et al*., 2000; Yi *et al*., 2000). Because this truncated form of TERT lacks a complete set of active site motifs but still contains an intact RNA binding domain (Lai *et al*., 2001), the simplest model is that it competes with full-length TERT for binding to a limiting pool of telomerase RNP. In addition, post-translational TERT modifications including phosphorylation also appear to influence catalytic activation (e.g. Liu *et al*., 2001). Given our incomplete knowledge of the composition of different telomerase RNPs or telomerase holoenzymes, there may well be additional forms of regulation not yet envisioned.

Telomere recruitment

Telomere maintenance requires the telomerase holoenzyme to find and extend a chromosome 3′ end. It is not known whether telomerase interacts with all telomeres in any given cell cycle or where in the nucleus this interaction occurs. Also unclear is the extent of telomerase-telomere interaction that is accomplished by direct binding of telomerase to single-stranded DNA, rather than by protein–protein interactions. For most of the cell cycle, telomeres are packaged into the chromatin structure responsible for end protection (reviewed by McEachern *et al*., 2000). In vertebrates, the terminal telomeric repeat 3′ overhang pairs back within the duplex repeat region of the same chromosome end to form a t-loop (Griffith *et al*., 1999). Among other roles, the t-loop seems likely to inhibit telomere elongation by telomerase. There may be an interval within S phase when t-loops are disassembled by the DNA replication machinery which provides the best opportunity for telomerase access to a chromosome 3′ end. Even then, the relative scarcity of telomeres and telomerase suggests that an active recruitment mechanism is likely to exist.

Candidates for proteins that could fulfill the active recruitment role include the hnRNP proteins A1 and C. Recombinant hnRNP A1 can bind simultaneously to hTR and a telomeric DNA substrate (Fiset and Chabot, 2001) and over-expression of hnRNP A1 in an hnRNP A1-deficient mouse cell line results in telomere elongation (LaBranche *et al*., 1998). Recombinant hTR can also interact with hnRNP C (Ford *et al*., 2000). Antibodies against hnRNP C immunopurify endogenous telomerase but not telomerase assembled from C-terminally epitope-tagged TERT. C-terminally tagged TERTs produce telomerase holoenzyme that is active on primers *in vitro* but not on telomeres *in vivo* (Counter *et al*., 1998; Ouellette *et al*., 1999). Amino acid substitutions within TERT can have the same effect, implying that the requirements for catalytic activation are a subset of those for physiological telomere length maintenance (Armbruster *et al*., 2001). These findings support an active recruitment mechanism and implicate an interaction involving TERT itself (step 3 in Figure 1). It is perhaps surprising that recombinant TERT over-expression does not inhibit telomere length maintenance, if TERT alone would be sufficient to act as a competitive inhibitor of holoenzyme–telomere interaction. Clearly, these and many other interesting possibilities for the mechanism(s) of telomere–telomerase association remain to be explored in greater detail.

More questions remaining

Antibodies raised against molecular chaperones or several different RNA binding proteins can immunoprecipitate telomerase activity from cell extracts (Nakayama *et al*., 1997; Harrington *et al*., 1997; Holt *et al*., 1999; Le *et al*., 2000). Deciphering the significance of these proteins for telomerase function is made more complex by the species-specificity of enzyme composition. In addition, it seems highly likely that some telomerase RNP proteins will be specific for a subset of the total telomerase RNP pool. For example, there could be protein that protects the template region of telomerase RNA in cells without TERT. A major challenge for the future will be to more completely characterize the composition of human telomerase RNPs and their interactions with other cellular factors. Transient interactions may be as critical for the ultimate production of functional telomerase as the proteins that remain integral parts of holoenzyme architecture.

Telomerase regulation in human somatic cells

Most human somatic cells do not produce active telomerase and do not maintain stable telomere length with proliferation. Most or all do have telomerase RNP, which raises the possibility of a second telomerase function independent of DNA synthesis. However, the long-term viability of yeast and mice engineered to lack telomerase RNA reveals that no form of telomerase RNP is required for genome maintenance in every cell cycle. Instead, the significance of telomerase function in any given cell is dependent on both telomere length and the number of future cycles of proliferation. It becomes more difficult to interpret the importance of telomerase regulation in settings where this is means something other than just the constitutive absence or overabundance of activity. Even if telomerase activation is not required at a particular point in time to prevent the onset of short telomere-induced phenotypes, its activity is tallied as part of the cumulative history of telomere length maintenance in the cell lineage. Below, we describe what is known about telomerase regulation in human somatic cells and its significance.

Tissue specificity and developmental regulation

Studies of telomerase function in the human organism are limited. Studies of telomerase function in mice are more facile but are also distinguished by differences as well as similarities of telomerase and telomere regulation (reviewed by Artandi and DePinho, 2000; Wright and Shay, 2000). In humans, telomerase activity detectable at the blastocyst stage and in most embryonic tissues before 20 weeks of gestation is subsequently lost (Wright *et al*., 1996). Fetal tissues show temporally distinct patterns of regulation, with activity remaining longer in liver, lung, spleen and testes than in heart, brain and kidney (Ulaner and Giudice, 1997). These assays examine the aggregate of numerous cell types present in whole tissue samples, including migrating or circulating cells present at the time of the assay (for example, fetal spleen and liver are sites of hematopoiesis). However, some correlation can be made between telomerase inactivation, differentiation and increased rate of telomere loss with time (Ulaner and Giudice, 1997; Ulaner *et al*., 2001). In heart tissue, loss of activity occurs concomitant with loss of TERT mRNA expression; loss of activity in kidney instead occurs concomitant with a change in the pattern of TERT pre-mRNA splicing (Ulaner *et al*., 1998). In the adult, most somatic cells lack detectable telomerase activity or TERT mRNA. Notable exceptions include lymphocytes in bone marrow and peripheral blood (Hiyama *et al*., 1995; Counter *et al*., 1995; Broccoli *et al*., 1995); a subset of proliferating epithelial cells in at least the skin, hair follicle, gastrointestinal tract and endometrium (Yasumoto *et al*., 1996; Taylor *et al*., 1996; Härle-Bachor and Boukamp, 1996; Hiyama *et al*., 1996; Ramírez *et al*., 1997; Tanaka *et al*., 1998; Yokoyama *et al*., 1998; Bachor *et al*., 1999); and a subset of cells in the testis (Wright *et al*., 1996; Yashima *et al*., 1998).

These various telomerase-positive human somatic cell types produce different relative amounts of catalytic activity, as judged either by the activity of cell extracts assayed *in vitro* or by the extent of telomere length maintenance *in vivo*. There are several interesting implications of this heterogeneity. First, cellular telomerase activation does not necessarily act to maintain a constant telomere length. In some cases, telomeres erode with cell proliferation despite telomerase activation (e.g., Bodnar *et al*., 1996; Stöppler *et al*., 1997); in other cases, telomeres make dramatic gains in net length despite cell proliferation (e.g. Weng *et al*., 1997). Second, strong telomerase activation in human somatic cells is transient, not within a given cell cycle but over the course of multiple cell divisions. A stem or progenitor cell with weak telomerase activity can generate strongly telomerase-positive lineage-committed descendants, which will subsequently lose telomerase activity with additional differentiation. Clear examples of this have been demonstrated in blood (Hiyama *et al*., 1995; Chiu *et al*., 1996; Weng *et al*., 1997; Hu *et al*., 1997) and skin (Bickenbach *et al*., 1998). This transient telomerase activation in normal human soma contrasts sharply with the constitutive activation of telomerase in most cancers.

The findings described above suggest an overall rationale for human somatic cell telomerase regulation. Telomerase repression to protect against the generation of overly long telomeres or to suppress tumor development is balanced against telomerase activation to extend tissue renewal capacity. Telomerase is active early in development, to offset telomere loss in the rapid cycles of proliferation necessary for tissue growth and differentiation. Enough telomere length is retained to endow the descendant, predominantly telomerase-negative somatic cells with a lifetime's worth of

proliferative capacity. High proliferative demand within the first year(s) of life may continue to drain the telomere reserve of at least some tissues at a higher rate than observed in adult life, as judged by the more rapid loss of telomere length in lymphocytes and granulocytes during this time (Frenck *et al*., 1998; Rufer *et al*., 1999) and the greater telomerase activation evident in extracts of peripheral blood mononuclear cells at an early age (Hiyama *et al*., 1995). This peak of proliferative demand in prenatal development and childhood may account for the early onset of HHS (Hoyeraal–Hreidarsson syndrome) and some cases of DKC (dyskeratosis congenita; see below). In cell lineages that must continue to renew throughout adult life, the potential for telomerase activation is retained. The somatic cells that do up-regulate telomerase to offset proliferation-dependent telomere shortening do so only transiently, in concert with rapid cell division cycles.

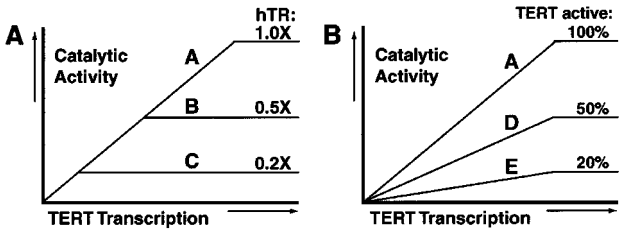

Figure 2 Telomerase regulation by hTR accumulation, TERT gene expression and the presence of inactive TERT protein. The level of catalytic activation (Y-axes) is established by multiple regulatory influences. TERT mRNA transcription (X-axes) can limit telomerase activation in human somatic tissues if the low level of expression is insufficient to saturate the available RNP. (**a**) The level of hTR accumulation limits maximal activation (compare lines A, B, C). (**b**) The presence of inactive TERT still capable of binding telomerase RNA reduces catalytic activity at any level of TERT transcription, from minimal to maximal (compare lines A, D, E)

Regulatory mechanisms

Several levels of regulation combine to determine the amount of active telomerase in any given cell. Specific differences among human somatic cells are detailed in the following paragraphs, after a consideration of the general principles illustrated in Figure 2. Differences in levels of hTR or TERT have unique effects on telomerase activation. Cells with less steady-state hTR will have less maximal telomerase activation (Figure 2a). At low levels of TERT transcription (left side of the X-axis), when TERT protein is insufficient to saturate binding sites on available telomerase RNP, then differences in hTR accumulation will not impact total catalytic activity (compare lines A, B and C). In contrast, at maximal activation, a reduced level of hTR can have a substantial impact on telomerase activation (right side of the X-axis). The level of TERT gene expression also influences catalytic activation, up to the maximum set by the availability of hTR (Figure 2a,b, X-axes). The presence of inactive TERT will reduce telomerase activation by competition for a limiting amount of telomerase RNP (Figure 2b). In contrast with differences in hTR accumulation, differences in the percentage of TERT that is inactive will impact total catalytic activity at all levels of activation (compare lines A, D and E). We consider below what is known in human somatic cells about the regulation of hTR and TERT and also regulation at the telomere (extending beyond the complexity shown in Figure 2).

Even from the limited number of studies described to date, it is evident that hTR accumulates to different levels in different tissues at different stages of development. *In situ* hybridization experiments with human embryonic tissues revealed the highest levels of hTR in undifferentiated neuroepithelium, with reduced but readily detectable levels in other epithelial tissues (Yashima *et al*., 1998). In adult tissues, hTR accumulation is greatest in primary spermatocytes of testis, moderate in germinal centers and weak but detectable in regenerative epithelia; notably, hTR levels in the central nervous system are below the level of detection (Yashima *et al*., 1998). In total RNA prepared from adult tissues and assayed by Northern blot hybridization, levels of hTR normalized to 5S rRNA are greater in breast and liver tissues than in brain, muscle or lung (Avilion *et al*., 1996). Among cultured cells, fibroblasts have less telomerase RNA per molecule of control RNA than lymphoblasts, both of which have less telomerase RNA than immortalized cancer cell lines (Mitchell *et al*., 1999b). T cell activation results in a rapid, 10–20-fold increase in steady-state hTR (Bodnar *et al*., 1996). In all cases, the factor(s) responsible for directing these differences in hTR accumulation are unknown. In other contexts, regulation of hTR transcription is known to increase steady-state hTR level in cancer cells (Yi *et al*., 1999) or to reduce steady-state hTR level in ALT cells (Hoare *et al*., 2001). The level of hTR accumulation could also be affected by changes in the efficiency of precursor processing or the stability of assembled RNP(s). Because hTR can have an extraordinarily long cellular half-life (Yi *et al*., 1999), regulated changes in RNA accumulation would require substantial chronological and proliferative time to have full impact.

The patterns of TERT transcriptional regulation are best fit by a model combining factor-induced activation and chromatin-mediated repression. Factor-induced activation appears to be restricted to a small number of somatic cell types. Although stem cells were originally considered the most likely somatic cells to utilize telomerase-dependent telomere maintenance, stem cells in blood and skin demonstrate only weak telomerase activation. Their activity is at least an order of magnitude less than that found in an equal number of cancer cells *in vitro* and is insufficient for telomere length maintenance *in vivo* (Vaziri *et al*., 1994). Instead, it is some mitotically hyperactive descendants of these stem cells that exhibit a level of telomerase activation sufficient to offset, balance or even reverse (Weng *et al*., 1997) proliferation-dependent telomere shortening.

Induction of telomerase activity in germinal center B cells undergoing affinity maturation, or in T cells and B cells activated in culture, is accompanied by an increase in TERT mRNA (Hu and Insel, 1999; Liu *et al*., 1999). In these cell types, it seems likely that the same signals stimulate factor-induced TERT transcriptional activation and cell proliferation.

A far greater number of cell types appear to repress TERT gene expression. Numerous studies have documented that fibroblasts have undetectable telomerase activity and TERT mRNA under all conditions of proliferation. Even in the mitotic descendants of activated lymphocytes, telomerase activity ultimately decreases and TERT mRNA expression is reduced (Bodnar *et al*., 1996; Liu *et al*., 1999). Likewise, the telomerase activity and TERT mRNA expression in proliferating basal layer skin keratinocytes is lost upon additional differentiation (Härle-Bachor and Boukamp, 1996; Kolquist *et al*., 1998; Hiyama *et al*., 2001). The lack of even basal TERT gene expression in fibroblasts and most terminally differentiated cells suggests that some form of chromatin-mediated repression is in effect. This could underlie the lower frequency of spontaneous immortalization in fibroblasts than in epithelial cells (Shay *et al*., 1993; Romanov *et al*., 2001). Viewed in this context, the weak telomerase activity of stem cells in skin and blood may reflect basal or spuriously activated transcription. Low-level telomerase activity may be the physiological indicator of progenitor cells that avert TERT gene repression, thereby conferring their descendants with the potential to undergo factor-dependent transcriptional activation.

In addition to differences in the levels of hTR, TERT and potentially other holoenzyme components, telomerase function is likely to be regulated by differences at the telomere. Cell-type specific differences in telomerase regulation by telomere structure could have a major impact on telomere maintenance. Molecular details of this level of regulation have not been as well characterized in mammalian cells as in other model systems (reviewed by Evans and Lundblad, 2000). Some human cells die if forced to express high levels of telomerase activity (MacKenzie *et al*., 2000), perhaps due to a toxicity of overly long telomeres. However, other cell types do not appear to be sensitive to excessive levels of telomerase holoenzyme activity, suggesting a more effective telomerase inhibition at the telomere or a more permissive requirement for telomere structure. High levels of telomerase activation may be inherently advantageous: substantial telomere elongation could be accomplished in the fewest number of cell cycles. But high levels of telomerase could also lead to overly long telomeres, potentially deleterious for growth and viability. The transience of telomerase activation in normal human somatic cells may solve this dilemma: strong telomerase activity rapidly elongates telomeres during a restricted interval of cell divisions, but telomerase inactivation occurs before telomeres reach a length long enough to induce cell death.

Future studies

There are several critical directions for future studies of telomerase regulation in human somatic cells. Conclusions about the extent of telomerase regulation and its physiological significance need to be supported by a more careful analysis of telomerase-dependent telomere maintenance in normal tissues. What changes in telomere length occur in different types of human tissues with age and insult? How do these changes correlate with the amount and specific activity of telomerase RNP? It will also be important to address whether there is evidence for a cell type specificity to the telomerase-independent processes that impact telomere function. These could include differences in the rate of telomere shortening in the absence of telomerase (Huffman *et al*., 2000) or the telomere structure(s) that provoke a cellular 'short telomere' response.

Telomerase and human disease

Arguably, more is known about human telomerase misregulation than about normal physiological regulatory mechanisms. This may derive in part from a lack of incentive for studying endogenous telomerase regulation in vertebrate model systems such as mice, which can prosper for several generations without any telomerase enzyme (Blasco *et al*., 1997). However, recent studies demonstrate that adequate telomerase function in somatic cells is critical for human health and viability within a single life span (Mitchell *et al*., 1999b; Vulliamy *et al*., 2001b). Mutations in genes encoding two telomerase holoenzyme components reduce the maximal level of telomerase activation and also dramatically compromise the proliferative renewal of hematopoietic and epithelial tissues. These findings reveal a much greater requirement for telomerase in the processes of human growth and development than previously suspected. In this section, we will describe the two extremes of telomerase misregulation, overabundance and deficiency, and their consequences on human health.

Telomerase excess

The vast majority of human cancers and cancer cell lines possess active telomerase and maintain stable telomeres with proliferation (Kim *et al*., 1994; reviewed by Shay and Bacchetti, 1997). Their requirement for telomerase activity may be greater and more immediate than that of normal somatic cells, due to chromosome aneuploidy and rapid cell division cycles. Recent studies have begun to define the *in vivo* requirements for assembly and activity of hTR and human TERT (Mitchell *et al*., 1999a; Mitchell and Collins, 2000) and their murine equivalents (Martin-Rivera and Blasco, 2001). This information could be used to design high throughput screens for small molecule inhibitors of RNP biogenesis and function that should have a

different specificity than small molecule inhibitors sought using activity assay protocols. In a clinical setting, telomerase inhibition may work best in conjunction with surgery rather than as a primary means to reduce tumor load, due to a possible lag phase between inhibition and the loss of telomere integrity. Some immortal human cell lines lack telomerase activity and instead maintain atypically long and heterogeneous length telomeres by a telomerase-independent alternative lengthening of telomeres (ALT) mechanism (reviewed by Colgin and Reddel, 1999). The murine ALT lines that arise readily in the absence of telomerase maintain atypically short rather than long telomeres (Hande *et al*., 1999), suggesting some species-specificity in ALT pathway requirements.

Telomerase insufficiency

The impact of telomerase regulation on human health is likely to be much greater than represented by inappropriate activation of telomerase in tumor cells alone. Telomeres shorten in normal human somatic tissues with age, suggesting that telomerase activation does not completely rescue proliferation-dependent telomere attrition even in telomerase-competent cell lineages (reviewed by Harley, 1995). This telomerase 'insufficiency' could have a major impact on the capacity for cellular renewal. In extreme form, this train of thought could be extended to suggest that telomere shortening is responsible for human aging: eventually enough somatic cells will senesce, apoptose or otherwise lose viability that the organism can no longer function. In more modest form, we suggest that the incomplete offset of telomere shortening in human somatic cells imposes limits on cellular renewal. As a tumor suppression mechanism, limits on cellular renewal would be beneficial. However, when tissue failure is a greater risk than tumorigenesis, limits on renewal might compromise rather than promote human health. It is not possible to test the role of telomerase in the human soma using forward genetics, to establish whether proliferation-dependent telomere loss can limit human somatic cell renewal in a single life span and whether telomerase activation extends renewal capacity. Nevertheless, one disease with at least two different patterns of inheritance provides the answers: telomerase activation does offset a telomere attrition that otherwise limits the renewal of highly proliferative somatic tissues.

Dyskeratosis congenita

Dyskeratosis congenita (DKC) is initially evident as a triad of cutaneous symptoms: reticulate skin pigmentation, nail dystrophy and mucosal leukoplakia (patchiness). These features typically precede a progression to pancytopenia and ultimately bone marrow failure, the primary cause of mortality. The pattern of inheritance, age of onset and severity of the symptoms varies widely (reviewed by Dokal, 2000). The vast majority of cases are X-linked (MIM 305000), although a small number of autosomal dominant (AD; MIM 127550) and putative autosomal recessive (AR; MIM 224230) forms of the disease have also been described (OMIM, 2001). X-linked DKC patients are young males typically diagnosed in their first decade of life, with a median age of 16 years at death (Knight *et al*., 1998). Other common but not universal disease symptoms include damaged teeth, premature hair loss and graying, gastrointestinal hemorrhage, pulmonary disease, developmental delay, short stature, testicular atrophy and a predisposition to malignancy (reviewed by Dokal, 2000; OMIM, 2001). AD and putative AR cases have a similar range of symptoms; the AD cases are generally later onset and less severe (reviewed by Drachtman and Alter, 1992; Dokal, 1996).

Positional cloning identified the gene affected in numerous patients with X-linked DKC as the human homologue of yeast *CBF5*, termed dyskerin (Heiss *et al*., 1998). At least 20 different DKC-associated alleles of dyskerin have now been identified (Dokal, 2000; Heiss *et al*., 2001). None of the predominantly single-amino-acid dyskerin substitutions are predicted to affect residues important in the catalysis of pseudouridine formation, as judged from sequence alignments with other pseudouridine synthases in the TruB family (Koonin, 1996). A small number of cases may reflect changes in the level of dyskerin expression, rather than changes in amino acid sequence (Knight *et al*., 2001). At least some of the DKC isoforms of dyskerin are expressed at wild-type levels with wild-type electrophoretic mobility (Mitchell *et al*., 1999b) and wild-type nucleolar localization (Heiss *et al*., 1999). Despite initial suggestions that DKC would reflect a 'ribosomal rebellion' (Luzzatto and Karadimitris, 1998), no defect in rRNA processing or pseudouridine modification has been detected in DKC cells. In addition, cells expressing altered dyskerins have wild-type levels of all tested H/ACA snoRNAs, snRNAs and rRNAs (Mitchell *et al*., 1999b). However, our limited knowledge of functional ncRNAs in the human genome makes it impossible to test for wild-type accumulation of all H/ACA RNAs.

Our identification of an H/ACA motif in hTR and its requirement for hTR stability (Mitchell *et al*., 1999a) raised the possibility that DKC reflects the phenotypes of a telomerase deficiency. In support of this hypothesis, we demonstrated that cells from young male patients in X-linked DKC families have an hTR accumulation defect (Mitchell *et al*., 1999b). An approximately fivefold decrease in the steady-state level of hTR is evident in patients relative to maternal carriers in two families with different dyskerin alleles. The carrier cells express only wild-type dyskerin from their single active X chromosome, providing normal controls. As expected from the reduced hTR accumulation, DKC cells forced to transiently over-express recombinant TERT produce about fivefold less telomerase activity than cells expressing wild-type dyskerin.

Fibroblasts and lymphoblasts from these patients, and peripheral blood mononuclear cells from numer-

ous other DKC patients, all have dramatically and critically shortened telomeres, as judged by comparison to age-matched controls or maternal carriers (Mitchell *et al*., 1999b; Vulliamy *et al*., 2001a). This molecular evidence for abnormally short telomeres is also supported at the cellular level by the premature proliferative senescence of cells from DKC patients in culture and by the age- and disease-dependent accumulation of chromosomes with aberrant fusions and rearrangements (Dokal *et al*., 1992). The premature telomere loss observed in DKC patients is particularly striking in the absence of any indication of a DNA repair deficiency (Coulthard *et al*., 1998). Thus, there should not be an increase in proliferative demand linked to a higher rate of cell turnover. This distinguishes DKC from phenotypically overlapping diseases induced by mutations in genes important for DNA damage repair.

In a large DKC family with AD disease inheritance, a region of chromosome 3 containing the hTR gene was implicated in disease transmission (Vulliamy *et al*., 2001b). The hTR gene itself contains a relatively small (<1 kb) deletion in one allele present in affected individuals, which leaves the 5′ template region intact but removes the 3′ end including much of the H/ACA motif. No aberrant forms of hTR are detectable in patients from this family, consistent with the essential role of the H/ACA motif in promoting hTR accumulation. These results suggest that the AD transmission of DKC can be caused by hTR haploinsufficiency. In support of this hypothesis, sequencing of the hTR gene locus in DKC patients from two other families with AD disease transmission (Vulliamy *et al*., 2001b) revealed one substitution in a region already known to be important for RNA accumulation (the top of the H/ACA 3′ stem; see above) and a dinucleotide substitution within a region dispensable for RNA accumulation but essential for catalytic activity. The latter RNA accumulates substantially, raising the possibility that in this case the defect is in the association of TERT with telomerase RNP.

The similarity of phenotypes resulting from mutations in dyskerin and hTR genes provides strong evidence in favor of the conclusion that DKC is exclusively a telomerase deficiency. It is striking that X-linked DKC has an earlier age of onset than AD DKC. The fivefold reduction in hTR observed in X-linked DKC limits maximal catalytic activation to 20% of normal (Figure 2a, line C). To have biological impact, somatic cells would have to require more than 20% of normal holoenzyme activity. The twofold reduction in hTR predicted in AD DKC would limit maximal catalytic activation to 50% of normal (Figure 2a, line B). The biological impact of such a modest hTR deficiency suggests that somatic cells in fact employ more than 50% of the holoenzyme maximum in the effort of telomere maintenance. The phenotypic and molecular differences between X-linked and AD disease suggest that the greater the telomerase deficiency, the greater the disease severity (i.e., 20% of maximal activation provides for fewer years of life than 50% of maximal activation). All forms of DKC are accompanied by premature telomere shortening (Mitchell *et al*., 1999b; Vulliamy *et al*., 2001a,b). DKC patients in total may reflect the range in phenotypic severity arising from different extents of telomerase deficiency (different gene mutations) combined with different requirements for telomerase activation (different genetic and environmental backgrounds).

How does telomerase deficiency induce the spectrum of phenotypes associated with DKC? Reduced hTR level will reduce telomerase activation under any condition in which the level of TERT expression saturates the RNP pool. The combination of a greater rate of telomere loss in early development and a cell-type specific exaggerated rate of telomere loss in the adult (Figure 3) can account for the observed symptoms of DKC. In fetal development and early childhood, a large number of tissues will experience a high proliferative demand only partially offset by telomerase activation. This offset would be less in DKC patients (intermediate activity, purple lines). In the adult, somatic cells that lack telomerase activity experience the same rate of telomere shortening with time comparing normal and DKC individuals, because DKC does not impose extra proliferative demand via an increased rate of cell turnover (blue and red lines). However, because telomeres begin at shorter lengths, DKC individuals will still have shorter telomeres than normal individuals of the same age even in lineages that lack potential for telomerase activation (e.g., fibroblasts). The rates of telomere loss with chronological age will be faster in rapidly dividing telomerase-negative cells (red lines) than in more slowly dividing ones (blue lines). Some somatic cell lineages induce transient but strong telomerase activation that is sufficient to slow or even reverse proliferation-dependent telomere shortening (e.g., lymphocytes). Telomere elongation will be less in DKC than normal individuals (strong activity, green lines), in fact proportionally less as the percentage of telomerase RNP activated in the normal cell increases above the reduced amount of telomerase RNP in the DKC cell. Highly proliferative, strongly telomerase-positive cell lineages would suffer the greatest disparity in telomere maintenance in DKC patients relative to normal individuals. Critically short telomeres will arise prematurely in DKC cells and limit additional proliferation.

The variable phenotypic severity of DKC even within individual families is entirely consistent with the stochastic nature of telomere erosion (Levy *et al*., 1992). Epithelial cells respond to short telomeres by entering a sustained growth arrest (Romanov *et al*., 2001). This could promote excess skin pigmentation in several ways, for example as the result of an altered immune response, a decrease in keratinocyte turnover (if cells gain pigment at the same rate over more time), an increase in melanin synthesis associated with melanocyte senescence (Haddad *et al*., 1999) or aberrant melanin uptake. Exhaustion of epithelial stem cells would cause the nail dystrophy and other

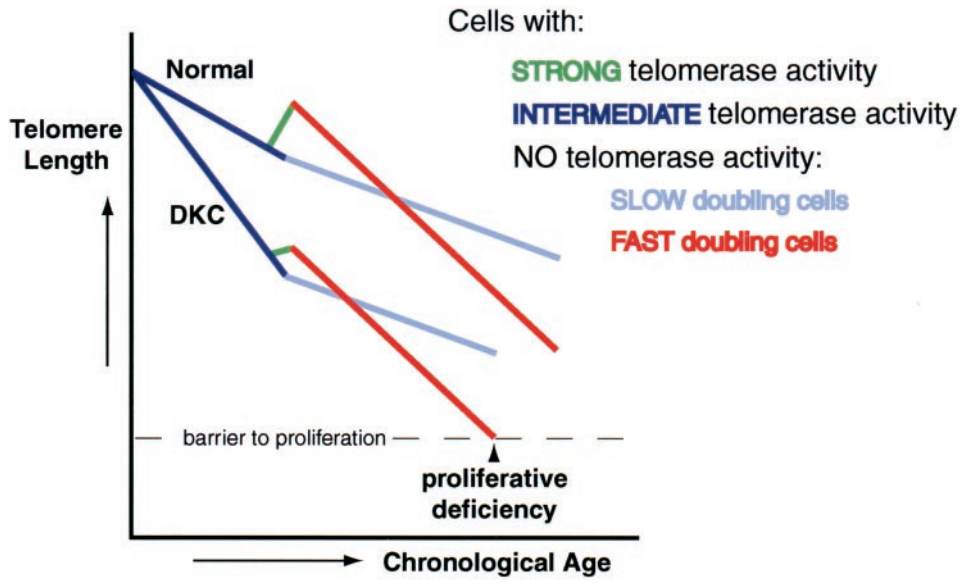

Figure 3 Telomere length with age in normal individuals and DKC patients. Details of this model are described in the text. Not depicted is the large degree of tissue heterogeneity expected in the rates of telomere loss

cutaneous phenotypes. Pancytopenia and bone marrow failure could follow from apoptosis triggered by short telomeres in hematopoietic cells.

Another feature of DKC in patients who live long enough for its detection is an increased risk of cancer (reviewed by Dokal, 2000). Prematurely short epithelial cell telomeres in DKC patients would enhance genomic instability (Romanov *et al*., 2001) as do short telomeres in epithelial cells in some genetic backgrounds of mice (reviewed by Chang *et al*., 2001). Although hematopoietic cells in DKC patients also have critically short telomeres, they may more readily succumb to apoptosis, reducing the collective incidence of lymphoma, myelodysplasia and leukemia to less than that of carcinoma in DKC patients as well as in the human population in general. If telomerase deficiency facilitates cancer progression in DKC patients, how is telomere maintenance accomplished in the cancer once established? Human epithelial cells may be capable of telomerase-mediated telomere maintenance with less than maximal telomerase activation, or tumor progression could select for an increase in telomerase RNP accumulation that counteracts the DKC deficiency. We find that cultures of DKC fibroblasts can be readily immortalized by constitutive over-expression of full-length TERT, suggesting that DKC cancers do not necessarily require an ALT pathway for telomere maintenance (JR Mitchell, J Wong and K Collins, unpublished data).

Dyskerin gene mutations have recently been associated with the rare childhood Hoyeraal–Hreidarsson syndrome, which affects only males (HHS; MIM 300240). HHS is characterized by prenatal growth retardation, microcephaly (a small brain), cerebellar hypoplasia, severe immunodeficiency and aplastic anemia (pancytopenia). Because of the early mortality, typically within the first few years of life, the cutaneous features of DKC may not develop with the same penetrance in HHS. Three missense mutations in the dyskerin gene have been identified in patients with HHS (Knight *et al*., 1999; Yaghmai *et al*., 2000). One results in a conservative S121G substitution within the region of dyskerin homologous to TruB family pseudouridine synthases, a substitution not detected in DKC patients (Knight *et al*., 1999). In contrast, a 4-year-old boy with classic symptoms of both HHS and DKC was found to express A353V dyskerin (Yaghmai *et al*., 2000), which is the most common, independently arising X-linked DKC allele, accounting for more than one quarter of total cases. It seems possible that the genetic background in which a particular dyskerin allele is inherited or expressed could play a large role in determining the spectrum of disease symptoms. The severe, prenatal neurological deficiencies of HHS may reflect an essential developmental role of telomerase in the generation of largely post-mitotic adult tissues, which may have no enduring requirement for telomerase activation.

Molecular requirements for dyskerin function

How could different dyskerin amino acid substitutions affect the steady-state level of hTR and do so specifically, without impacting snoRNA accumulation? Before we can answer this question, an understanding of the normal role of dyskerin in the biogenesis of telomerase and other H/ACA RNPs is essential. Regrettably, cellular biogenesis is not well characterized for any RNP let alone for telomerase. Several plausible roles for dyskerin in the production and function of telomerase are described below, based on studies of other RNPs in several model systems. Much insight remains to be gained in

understanding the maturation, transport and turnover of cellular RNPs.

If full-length TERT is transiently over-expressed in X-linked DKC patient fibroblasts, about five times less telomerase activity is detected in cell extracts when compared to extracts of similarly treated fibroblasts from maternal carriers in the same family (Mitchell *et al*., 1999b). This difference in activity parallels that in steady-state hTR, suggesting that the specific activity of telomerase holoenzyme is not altered. Consistent with this conclusion, if epitope-tagged wild-type and DKC dyskerins are immunopurified from telomerase-positive cells, similar amounts of telomerase RNA and telomerase activity are associated per dyskerin protein. Furthermore, if DKC is a defect in hTR accumulation without a defect in holoenzyme specific activity, no difference in telomerase activity should be apparent in DKC patient and normal cells under conditions of minimal TERT expression. In other words, at TERT expression levels sufficient to associate with only a small percentage of telomerase RNP, differences in RNA accumulation should not limit telomerase activation (Figure 2a, left side). This is true in unstimulated lymphoblasts and lymphocytes, which have a very low level of telomerase activation (JR Mitchell and K Collins, unpublished data; Vulliamy *et al*., 2001a).

Several hypotheses for the role(s) of dyskerin compromised by DKC gene mutations can be proposed, any of which would account for a reduction in hTR accumulation (Figure 4). First, a role for dyskerin in synthesis or processing of the hTR precursor could be affected (Figure 4a). While hTR is generated from an independent transcription unit, other vertebrate H/ACA snoRNAs are processed from spliced introns. Therefore, hTR requirements for transcription termination and 3′ end formation will be unique, possibly involving recognition of downstream RNA sequence by RNA polymerase II-associated factors (Fatica *et al*., 2000; Steinmetz *et al*., 2001), while snoRNA maturation will instead be aided by the splicing machinery (Hirose and Steitz, 2001). In yeast, where some snoRNAs are expressed as independent transcription units, we note that a genome-wide two-hybrid screen detected the interaction of Cbf5p with an RNA polymerase mediator subunit, Srb4p (Ito *et al*., 2001).

Second, RNP assembly could be affected at a step subsequent to precursor synthesis (Figure 4b). The independently transcribed yeast U3 snoRNP, from the C/D motif family, undergoes several sequential steps of precursor processing. The final assembly of C/D motif binding proteins with the partially 3′ processed U3 precursor requires the yeast La homologue. This protein associates with the partially processed U3 precursor 3′ end, promotes assembly of the C/D motif binding proteins, then dissociates to allow the additional RNA 3′ end cleavage necessary to generate a mature RNA (Kufel *et al*., 2000). By analogy, DKC forms of dyskerin could be deficient in a chaperoned process of loading onto RNA, as a consequence of defective binding to the relevant protein chaperones or to telomerase RNA itself. Either of these defects could be specific to hTR: the former due to differences in chaperone requirements for RNP assembly from different precursors, the latter due to RNA sequence differences within and beyond the conserved H/ACA motifs. It may be relevant to this model that over-expression of La in telomerase-positive human cells results in telomere shortening, and that several different La antibodies can immunoprecipitate active telomerase (Ford *et al*., 2001). As a final consideration, DKC dyskerin substitutions could compromise the processing of all H/ACA RNAs, with feedback mechanisms restoring only snoRNA accumulation to wild-type levels.

Third, DKC forms of dyskerin could have a telomerase-specific impact on assembled RNP stability (Figure 4c). If H/ACA RNP conformation is slightly altered by DKC dyskerins, this change could affect the affinity of hTR association with additional proteins that need not be recruited to H/ACA snoRNAs. Or, perhaps the architecture of an H/ACA RNP is normal in DKC cells but less favorably adopts a telomerase-specific conformation. Fourth, DKC dyskerins could have an altered interaction with the cellular factors that establish RNP subcellular localization (Figure 4d). These requirements could be different for telomerase relative to snoRNPs and could alter the regulation of RNP turnover. In this regard, it is of potential interest that the rat dyskerin homologue NAP-57 has been purified in association with the intranuclear trafficking protein Nopp140 (Meier and Blobel, 1994). These hypotheses for roles of dyskerin in establishing the steady-state level of hTR can tested in future studies, for example by measuring the half-life of hTR in somatic cells from X-linked DKC and normal individuals.

One possible approach for exploration of dyskerin function would be to introduce various dyskerin gene mutations into mice. However, there are several caveats involved in gaining meaningful information about human cell requirements from this method. First, if some DKC dyskerins alter telomerase RNA interaction or RNP conformation, they may not have the same impact in mice due to substantial divergence in telomerase RNA sequence. Second, there are differences between mouse and man in telomerase and telomere regulation. At least some cell types in these two species are likely to have different limitations on telomerase RNA accumulation, telomerase RNP catalytic activation, the telomerase–telomere interaction (Figure 1) or telomerase-independent telomere regulation. Indeed, no impact of mouse telomerase RNA (mTR) haploinsufficiency has been described (Niida *et al*., 1998) although hTR haploinsufficiency is lethal (Vulliamy *et al*., 2001b). The haploinsufficiency of TERT in cultured mouse ES cells (Liu *et al*., 2000) suggests that at least in this cell type, TERT gene transcription rather than mTR accumulation or the percentage of inactive TERT establishes the level of telomerase activation (see Figure 2). Third, there is a

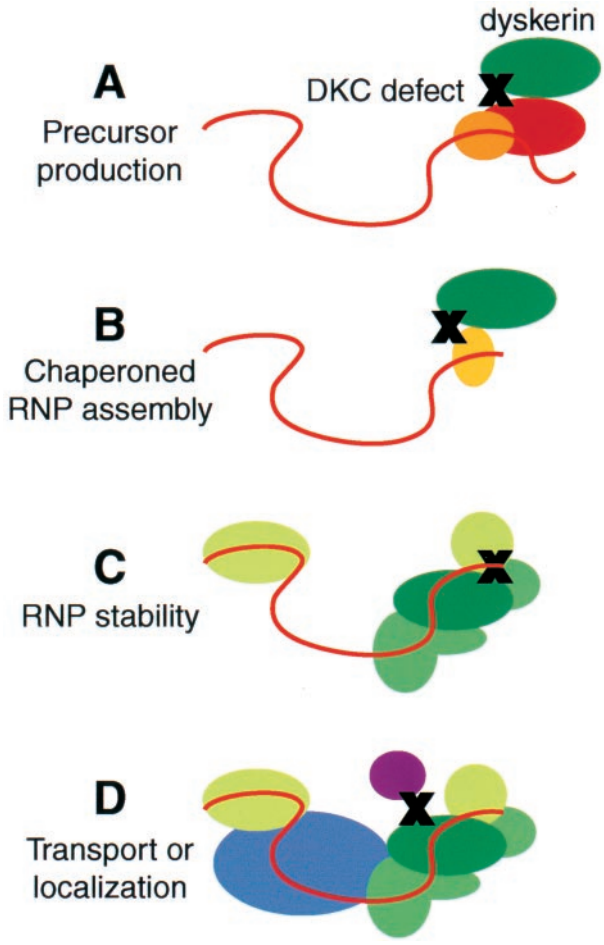

Figure 4 Models for dyskerin functions compromised by DKC dyskerin gene mutations. Proteins colored orange are transiently associated with telomerase RNA. Proteins in green represent dyskerin (darkest green), other H/ACA proteins (intermediate green) and non-H/ACA hTR binding proteins required for RNA accumulation (lightest green). Proteins required for catalytic activation (blue) or regulation (purple) are also depicted. 'X' indicates an interaction altered in DKC (see text for details). (**a**) Dyskerin may interact with proteins required for proper precursor production. Defective interaction could result in aberrant transcription termination or failure to deposit RNA processing proteins on precursor-specific sequences. (**b**) Dyskerin may interact with chaperones of H/ACA RNP assembly. Defective interaction could slow the kinetics of stable H/ACA RNP assembly, favoring an increase in RNA degradation before or after the completion of precursor processing. (**c**) Dyskerin structure may be important in maintaining the stability of telomerase RNP. Altered dyskerin conformation could affect the architecture of the RNP complex in some manner that leads directly to a reduced cellular half-life. For example, altered dyskerin could affect the binding affinity of the protein associated with the distal stem-loop of the H/ACA motif 3′ stem, thereby enhancing telomerase RNP turnover directly. (**d**) Dyskerin may interact with factors involved in directing telomerase RNP nuclear or nucleolar tethering, targeting or transport. Defective interaction could affect the telomerase RNP in a manner that leads to altered regulation and therefore indirectly reduces RNP half-life

difference in the extent of cellular renewal required in a mouse versus human life span. Differences between mouse and human physiology would have to be considered in comparative analysis of phenotypes in young humans versus those that could arise after several generations of germline passage of DKC dyskerin alleles in mice.

Telomerase and human health

DKC is a genetically determined human telomerase deficiency. X-linked gene mutations are the most frequent, with AD and probably AR gene mutations as well. In addition, not all X-linked, AD or AR inheritance patterns necessarily derive from mutations in the same gene. This implied diversity of genetic lesions is likely to be a valuable resource for further discovery of factors that influence the cellular biogenesis and function of telomerase. Numerous exciting directions for future research are evident. For the great number of X-linked DKC cases with dyskerin gene mutations, the generality of a defect in hTR accumulation can be examined: do all dyskerin mutations affect hTR accumulation or do some affect catalytic activity or telomere recruitment instead? For AD DKC cases, similar questions about the molecular basis of the telomerase defect(s) can be pursued. For DKC cases without associated dyskerin

or hTR gene mutations, and also for proliferative renewal deficiencies other than DKC, it should prove very interesting to explore possible inadequacies of telomere length maintenance and their underlying genetic lesions.

It would appear that human telomerase deficiency can severely limit life span, with death in childhood or at middle age imposed by only a fivefold or twofold reduction in maximal telomerase activation, respectively (Mitchell *et al*., 1999b; Vulliamy *et al*., 2001b). This haunting observation implies that the telomerase insufficiency inherent in normal human somatic cell telomerase regulation could also impact longevity. DKC patients and late generation mTR−/− mice do share some signatures of the aged, for example poor quality of hair and skin and decreased function of the immune system (reviewed by Marciniak *et al*., 2000). The incidence of mortality from bone marrow failure in humans is low, but there is substantial evidence for a general decline in hematopoietic response in the elderly (reviewed by Globerson, 1999). The tissue specificity of telomere-imposed restrictions on cellular renewal should differ somewhat comparing DKC patients, in whom maximal telomerase activation is reduced, to normal individuals at old age, in whom maximal telomerase activation is unaffected but more cell divisions have occurred. It also seems plausible that various forms of disease could tip the demand for cellular renewal to beyond what is available in the proliferative budget, even before old age. Individuals challenged by chronic infection or inherited predisposition to cellular damage may suffer some symptoms as the consequence of telomere-derived proliferative limitation, imposed by a greater rate of proliferation rather than a reduced budget of cell division cycles. Evidence for chronologically premature telomere shortening can be found in T-cell subsets of patients with human immunodeficiency virus infection (reviewed by Effros, 2000) or in cells from individuals carrying mutations in genes involved in DNA damage repair (e.g., ataxia telangiectasia (Metcalfe *et al*., 1996) or Fanconi's anemia (Leteurtre *et al*., 1999)).

DKC is an excellent setting in which to test the therapeutic value of artificially enhanced telomerase activation in the human soma. Clinical treatment for DKC is a standard bone marrow transplant, which is ineffective. In other studies, telomere length in a donor bone marrow cell population has been shown to experience a rapid decrease immediately following transplant (Brümmendorf *et al*., 2001; Notaro *et al*., 1997), which may be exaggerated in DKC patients by their pancytopenia. Rather than expressing wild-type dyskerin or hTR in DKC patient hematopoietic cells as a gene therapy, which would not reverse the extant telomere loss from embryogenesis onward, the best therapy is likely to be accomplished by over-expression of full-length TERT. Only if sufficient telomere length is gained in these cells will they be capable of supporting a sustained repopulation of the hematopoietic system. Expression of recombinant TERT is sufficient to immortalize cultured lymphocytes and promote telomere lengthening (Hooijberg *et al*., 2000; Rufer *et al*., 2001). TERT gene introduction and expression could be accomplished transiently, to return somatic cells to a telomerase-negative state after sufficient telomere lengthening but before transplantation. Preliminary experiments suggest that long term TERT over-expression in DKC cells is sufficient for proliferative immortalization and may have the secondary consequence of 'rescuing' the hTR accumulation defect (J Wong and K Collins, unpublished data).

In DKC patients, reintroduced hematopoietic cells should eventually replace those with shorter telomeres due to their proliferative advantage, as apparently occurs in the maternal carriers of X-linked DKC by the age of their testing for skewed X-inactivation (Devriendt *et al*., 1997; Vulliamy *et al*., 1997). We note that if this strategy for gene therapy improves the health and longevity of DKC patients, it could be equally useful for treatment of other proliferative deficiencies imposed by disease or aging.

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