with age in living organisms. Furthermore, the sensitivity and accuracy of STELA will likely find clinical applications. For example, the ability to use very small tissue samples should allow the analysis of premalignant lesions for telomere loss and subsequent chromosome instability.

A means to other ends

How far are we from applying this method to other chromosomes? The most significant barrier is finding chromosome end–specific sequence tags in the subtelomeric regions. This requires sequencing the ends of human chromosomes—a daunting task that is now being systematically attempted. Current data indicate that STELA will be feasible for 7q, 12q, 16p and 16q. Although the technique may ultimately prove impractical for all telomeres, data from even one or two additional telomeres is eagerly anticipated.

One drawback of STELA is that it reveals little information concerning the G-rich overhang (see figure), which is emerging as a critical determinant for telomere capping. However, used in combination with the telomere PCR method (Table 1), which generates products that correspond to the telomeric duplex plus the entire G-rich overhang, these two methods have the potential to provide a much clearer view of telomeric DNA architecture. □

1. Baird, D. *et al. Nat. Genet.* **33**,197–202 (2003).
2. Hemman, M.T. *et al. Cell* **107**, 67–77 (2001).
3. Counter, C.M. *et al. EMBO J.* **11**, 1921–1929 (1992).
4. Zijlmans, J.M. *et al. Proc. Natl. Acad. Sci. USA.* **94**, 7423–7428 (1997).
5. Rufer, N. *et al. Nat. Biotechnol.* **16**, 743–747 (1998).
6. Förstemann, K. *et al. Nucleic Acids Res.* **28**, 2690–2694 (2000).
7. Griffith, J.D *et al. Cell* **97**, 503–514 (1999).

Dissecting dyskeratosis

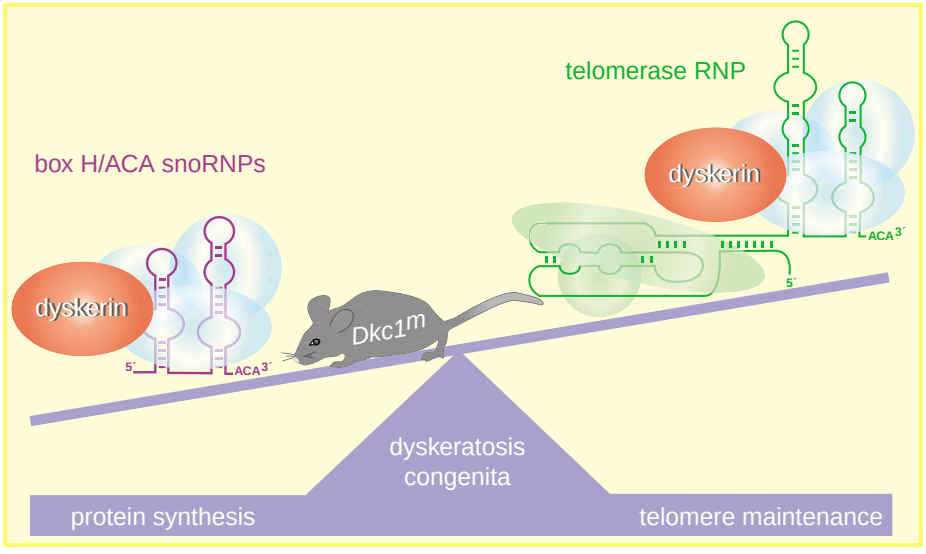
U. Thomas Meier

Department of Anatomy & Structural Biology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461, USA.
e-mail: meier@aecom.yu.edu

Dyskeratosis congenita is a rare but fatal syndrome characterized by bone marrow failure. A new mouse model informs the ongoing debate on its molecular pathogenesis.

The X-linked form of dyskeratosis congenita (DC) is caused by mutations in *DKC1*, encoding dyskerin[1], formerly known as NAP57 (ref. 2), or Cbf5 in yeast[3]. Dyskerin is an essential structural component of small nucleolar RNA-protein complexes (snoRNPs) and of the mammalian telomerase RNP[4,5]. Although DC was proposed to be caused by a telomerase deficiency, a new mouse model published in *Science* by Davide Ruggero and colleagues[6] sheds a different light on its origins.

Ruggero *et al.*[6] developed a mouse model of DC that has reduced expression of *Dkc1*. These hypomorphic $Dkc1^m$ mice reproduce the phenotype of human DC to a remarkable degree. The $Dkc1^m$ mice have severe anemia, lymphopenia, hypocellularity of the bone marrow, and reduced levels of erythroid and lymphoid colony-forming units, telltale signs of bone marrow failure, the predominant cause of death in people with DC. The hallmark of DC, dyskeratosis of the skin, is present in $Dkc1^m$ mice, along with abnormalities in the lungs and kidneys. Additionally, half of the $Dkc1^m$ mice develop tumors, mirroring the increased risk of malignancy in



Tipping the scales. The $Dkc1^m$ mouse tips the scales of the proposed molecular mechanism of DC from telomere maintenance to protein synthesis, represented by the telomerase RNP and the box H/ACA snoRNPs, respectively. purple, box H/ACA snoRNA; light blue, box H/ACA–specific core proteins; red, dyskerin; dark green, TR; light green, telomerase-specific proteins.

individuals with DC. Therefore, the mutant mice accurately and entirely duplicate the phenotypes of the human disease, a rare feat for an animal model.

Ribosomes and telomeres

How does this disease phenotype fit with what we know about dyskerin? Dyskerin functions as a pseudouridine synthase, isomerizing some hundred uridines in ribosomal and spliceosomal small nuclear RNAs[4]. To execute this function, dyskerin associates with an equal number of snoRNAs of the box H/ACA class, which, through site-specific base pairing, identify the nucleotides to be modified. In addition to its catalytic function, dyskerin also plays a structural role that is critical for the maturation and integrity of box H/ACA snoRNAs[4]. Though most of the snoRNAs serve as guides for pseudouridylation, some are also required for crucial steps in ribosomal RNA processing. Although apparently nonessential, a role for the ribosomal RNA pseudouridines in translation has been suggested based on their clustering in functionally important regions[7]. As a result, DC was originally recognized as a 'ribosomapathy'.

Not long after the identification of mutated *DKC1* as the culprit in DC, a box H/ACA motif was identified unexpectedly in the essential RNA component (TR) of telomerase[5]. Dyskerin associated with TR and seemed to be required for its maturation and stability. Telomerase is a specialized reverse transcriptase that uses its associated RNA as a template to add telomeric repeats to chromosome ends. Studies of TR-deficient mice demonstrated that telomere attrition can lead to chromosome instability, cancer and early onset of aging phenotypes, all features observed in human DC[8]. Owing to a peculiar feature of laboratory mice, however (their telomeres are three to four times longer than those of humans), these phenotypes are only manifested after four to six generations. Examination of cells from individuals with DC revealed drastically shortened telomeres, implicating the role of dyskerin in TR stability as the molecular basis for DC[5]. Although this idea initially met with some skepticism, it was supported by the identification of TR mutations in three families with an autosomal dominant form of DC[9]. As such, the scales of the proposed molecular mechanism for DC had tipped from ribosome to telomere dysfunction (see figure).

DC in the balance

One prediction from the TR-deficient mouse is that a disease caused by a failure to maintain telomere length should only manifest itself in later generations. The *Dkc1*^{m} mouse, however, displays the full DC phenotype within the first two generations when its telomeres are still of normal length. Although reduced levels of TR and telomere shortening could be detected in *Dkc1*^{m} mice of later generations, they could not account for the strong DC phenotype of the first two generations.

Clearly, telomere shortening did not correlate with the early generation phenotype, but what about pseudouridylation? Indeed, the authors demonstrated decreased levels of overall pseudouridylation and slowed processing of ribosomal RNA in B-lymphocytes from early-generation *Dkc1*^{m} mice. As a result, *Dkc1*^{m} ribosomes showed enhanced sensitivity to translation inhibitors as manifested by increased rates of apoptosis. Therefore, the DC phenotype of the early-generation *Dkc1*^{m} mice correlated with a dysfunction in protein synthesis, but not of telomere maintenance, adding the weight of the *Dkc1*^{m} mouse to the ribosome deficiency side of the DC scales.

Does this mean that telomerase plays no role in DC? Not necessarily—telomere shortening could exacerbate the DC phenotype, an issue that now can be addressed in late-generation *Dkc1*^{m} mice. What about the TR mutations in the autosomal dominant form of DC? These are not so easily reconciled with the *Dkc1*^{m} mouse and will obviously require further investigation. Along these lines, although mutations in TR initially were linked to the autosomal dominant form of DC, they may not be present in all families showing this mode of DC inheritance (I. Dokal, personal communication). Therefore, other factors may contribute to this milder form of DC.

The omnipresent dyskerin

The present studies of the *Dkc1*^{m} mouse are consistent with deficient ribosomal RNA pseudouridylation and ribosome dysfunction as the primary cause of DC. However, dyskerin has been implicated in additional functions that are likely to influence the complex phenotype of DC. In particular, dyskerin also pseudouridylates small nuclear RNAs, which is a prerequisite for their function in pre-mRNA splicing[10,11]. Further dyskerin functions have been suggested by genetic interactions in yeast—for example, with centromeres, cap-binding complex, transcription factors and localization of tRNA processing, implying roles in cell division, mRNA maturation, RNA polymerase I transcription and nuclear organization, respectively[3,12–14]. Interestingly, most of the dyskerin activities are required for cell growth and division and could thus explain why rapidly dividing cells are the primary targets in individuals with DC. How that translates into increased susceptibility to cancer will require further study of this multifaceted protein. With the *Dkc1*^{m} mouse in hand, it should now be possible to determine which aspects of the DC phenotype can be accounted for by the different functions of dyskerin. □

1. Heiss, N.S. *et al. Nat. Genet.* **19**, 32–38 (1998).
2. Meier, U.T. & Blobel, G. *J. Cell Biol. [correction appeared in* **140**, *447]* **127**, 1505–1514 (1994).
3. Jiang, W., Middleton, K., Yoon, H.-J., Fouquet, C. & Carbon, J. *Mol. Cell. Biol.* **13**, 4884–4893 (1993).
4. Lafontaine, D.L.J., Bousquet-Antonelli, C., Henry, Y., Caizergues-Ferrer, M. & Tollervey, D. *Genes Dev.* **12**, 527–537 (1998).
5. Mitchell, J.R., Wood, E. & Collins, K. *Nature* **402**, 551–555 (1999).
6. Ruggero, D. *et al. Science* **299**, 259–262 (2003).
7. Decatur, W.A. & Fournier, M.J. *Trends Biochem. Sci.* **27**, 344–351 (2002).
8. Blasco, M.A. *et al. Cell* **91**, 25–34 (1997).
9. Vulliamy, T. *et al. Nature* **413**, 432–435 (2001).
10. Yu, Y.T., Shu, M.D. & Steitz, J.A. *EMBO J.* **17**, 5783–5795 (1998).
11. Jady, B.E. & Kiss, T. *EMBO J.* **20**, 541–551 (2001).
12. Fortes, P. *et al. Mol. Cell. Biol.* **19**, 6543–6553 (1999).
13. Cadwell, C., Yoon, H.-J., Zebarjadian, Y. & Carbon, J. *Mol. Cell. Biol.* **17**, 6175–6183 (1997).
14. Kendall, A. *et al. Proc. Natl. Acad. Sci. USA* **97**, 13108–13113 (2000).