logical strain rates of past processes. By isotopic dating of the growing crystal fibers, it should be possible to constrain the geometric data in a time frame that enables the strain rates at differing times within the evolution of rock deformation to be calculated. Field geologists, in collaboration with isotope chemists, now have to seek rocks appropriate to undertake the type of analysis set out in the report.

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PERSPECTIVES: MOLECULAR BIOLOGY

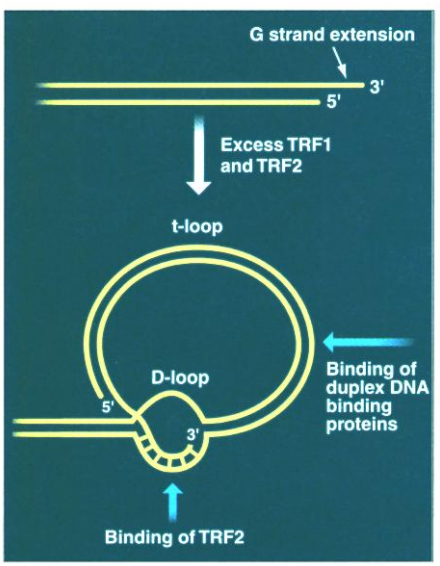
Telomeres Keep On Rappin'

Victoria Lundblad

Many fundamental cellular processes have been conserved throughout evolution, and the biology of telomeres (the ends of chromosomes) is no exception. In most eukaryotes, chromosome ends consist of tandem reiterations of a closely related G-rich repeat sequence, with the G-strand protruding as a single-strand extension. The enzyme telomerase, which synthesizes these telomeric repeats, is similarly highly conserved from unicellular eukaryotes to human cells. Studies in both yeast and human cells also point to a central role for proteins that bind the duplex regions of telomeres in maintaining telomere length homeostasis. But, a missing piece of the telomeric puzzle has been the apparent lack of conservation between human duplex DNA binding proteins and those of budding yeast. Now, in a recent issue of *Cell*, de Lange and colleagues (*1*) describe a human counterpart of the well-characterized yeast duplex DNA binding protein, Rap1, illustrating an additional point of convergence between telomere biology in unicellular and vertebrate organisms.

In budding yeast, Rap1 is a major regulator of telomere length. It binds with high affinity to tandem GGTGT sites present within the irregular $G_{1\text{-}3}T$ telomeric repeat tracts found in yeast telomeres (*2*). The carboxyl terminus of Rap1 interacts with two different sets of proteins, thereby exerting effects on transcriptional repression of subtelomeric genes (through binding of the Sir proteins) and telomere length (through binding of the Rif proteins). Targeting additional copies of this protein interaction domain to the telomere is sufficient to reset telomere length. This has led to the proposal that a protein-counting mechanism can discriminate the number of Rap1 proteins bound to the telomere (*3*). This establishes a negative-feedback loop that is thought to modulate telomere length by inhibiting access of telomerase to the chromosome terminus. As Rap1 is capable of bending DNA, one mechanism proposed for this length-sensing process is a number-dependent switch that flips the telomere between a telomerase-accessible "open" structure and a "closed" conformation that is refractory to telomerase action.

Duplex binding factors also modulate telomere length in human cells, but the picture is more complex. Not one, but two related proteins, TRF1 and TRF2, bind the TTAGGG repeats found at human chromosome termini (*4*). The amount of TRF1 and TRF2 bound to chromosome ends inversely correlates with the length of the telomere (*5*). Increased expression of either TRF1 or TRF2 in a telomerase-positive cell line results in a gradual decline in telomere length (*5*, *6*). Conversely, removal of endogenous TRF1 from telomeres leads to telomere elongation (*6*). These observations indicate that a longer telomere recruits more TRF1 and TRF2 protein, thereby blocking the ability of telomerase to act on that terminus. Thus, results in both yeast and human cells suggest that the telomere itself inhibits further elongation by telomerase when telomere length exceeds a certain threshold.



Loop the loop. A t-loop structure forms when the 3′ G-strand extension at the end of a chromosome (telomere) invades duplex telomeric repeats, thereby forming a displacement loop (D-loop). Embedding the 3′ end of the chromosome not only has the ability to sequester the telomere from telomerase action, but also can protect the terminus from exposure to DNA damage checkpoints and repair activities. Consistent with a role for t-loops in chromosome end protection, loss of TRF2 from telomeres induces rapid telomere dysfunction and subsequent chromosome end-to-end fusions (*11*).

Although the properties of duplex telomere binding proteins in yeast and humans mirror each other, it has been perplexing that the vertebrate TRF proteins do not bear any notable sequence similarity to the budding yeast Rap1 protein, nor have orthologs of the vertebrate TRF proteins been identified in the sequenced yeast genome. The current report from de Lange's group potentially supplies a missing link by demonstrating that a human ortholog of Rap1 is indeed present at human telomeres, although in somewhat unfamiliar territory (*1*). Like its yeast counterpart, hRap1 overexpression results in telomere elongation, showing that hRap1 contributes to the length-sensing mechanism that sets human telomere length. However, one intriguing difference distinguishes the budding yeast and mammalian Rap1 proteins: hRap1 associates with telomeric chromatin through an interaction with TRF2, rather than by binding directly to the DNA.

The identification of hRap1 strengthens the argument for a common mechanism for negative length regulation, but it raises a new question: Why does budding yeast lack TRF proteins? De Lange and co-workers suggest an evolutionary model. They propose that the ancestral telomeric complex, still represented in human cells, consisted of a TRF-like protein that bound telomeric DNA and tethered a Rap1-type protein to the telomere. During the evolution of budding yeast, loss of TRF function and concomitant acquisition of Rap1's ability to bind DNA—perhaps arising as the result of a divergence in telomeric sequence (*7*)—may have changed the specific players involved, but preserved the key components that regulate telomere extension.

Does the proposed protein counting mechanism for telomere length proceed by the same mechanism in yeast and human cells? A framework for modeling telomere length regulation comes from the recent

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discovery of a novel structural conformation that can be assumed by mammalian telomeres. Electron micrographs of psoralen cross-linked telomeric DNA preparations has revealed that telomeres can fold back such that the single-strand terminus of the G-rich strand of the telomere invades duplex DNA to form a duplex "lariat" structure, called the t-loop (*8*). The biochemical properties of TRF1 and TRF2 suggest a role for both proteins in t-loop formation (see the figure). TRF1 most likely promotes t-loop formation through its ability to bend and otherwise alter DNA conformation. TRF2 is localized to the junction where the 3′ end invades the duplex tract, suggesting that TRF2 and associated proteins aid in strand invasion. Formation of a t-loop thereby provides a means by which telomere replication could be regulated by a higher order structure—in this "closed" conformation, the tucked away 3′ terminus should be inaccessible to telomerase.

Does budding yeast Rap1 promote telomere length homeostasis through a similar t-loop mechanism? The functional similarities between budding yeast and human telomere length regulation certainly argue in favor of this possibility. Furthermore, the presence of large duplex loops at the chromosome termini of a ciliated protozoan (*9*) suggest that this type of end structure is a feature that is not necessarily restricted solely to mammalian chromosomes. If negative regulation of telomerase is indeed conserved, this raises the expectation that human orthologs of positive regulators of yeast telomere replication, such as Cdc13 and Est1 (*10*), are waiting to be discovered. Although the identification of the human Rap1 protein ties up one loose end, the story is far from over.

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PERSPECTIVES: DEVELOPMENT

A Degrading Way to Make an Organ

Jeff Hardin

Building an organ is a precise business. Organ rudiments must often navigate through a complex, three-dimensional terrain as their shape is transformed, and they must do so in a way that is carefully orchestrated with other concurrent developmental events. Many organ rudiments develop as tubular structures that are elaborated as the rudiment grows. In such cases, cells at the tip of the growing tube can be key regulators of organogenesis. Examples include gut formation in echinoderms, the tracheal system of *Drosophila*, and branching morphogenesis in vertebrates (*1*). As such growing organ rudiments extend, they must contend with a variety of extracellular matrices (ECMs), some of which may serve as barriers to migration. It has long been presumed that matrix metalloproteases, the major family of proteolytic enzymes responsible for degrading ECM components, play a key role in remodeling the ECM during cell migration (*2*). On page 2205 of this issue, Nishiwaki *et al.* (*3*) provide evidence that just such a protease, encoded by the *mig-17* gene, is required for directional migration of another well-studied tubular organ rudiment, the gonad of the nematode *Caenorhabditis elegans*. Together with other recent experiments in *C. elegans*, these results provide clear evidence that metalloproteases are important regulators of organogenesis.

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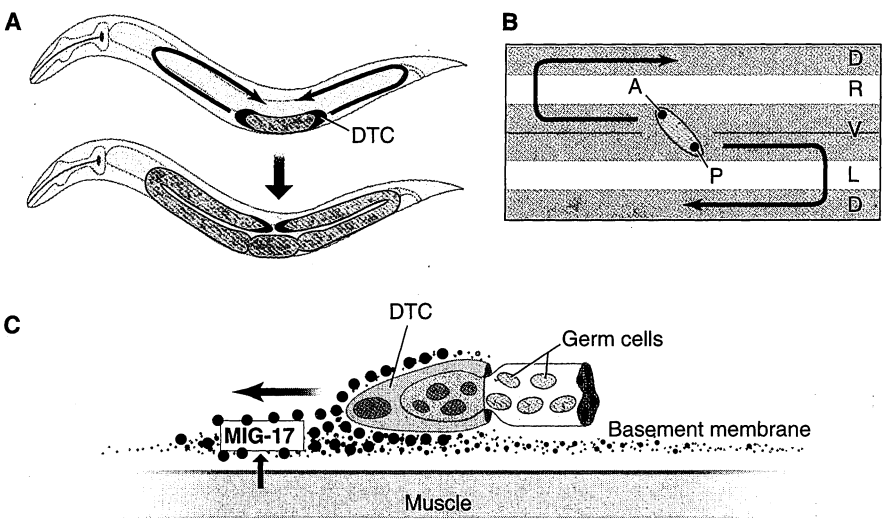
The hermaphrodite gonad of *C. elegans* has two U-shaped arms (see the figure). Both laser ablation (*4*) and genetic analyses (*5, 6*) indicate that the shape of the developing gonad is largely determined by the migration of somatic cells at the tips of the growing arms known as distal tip cells (DTCs). DTCs are born during the first larval stage, and they begin to migrate during the second larval stage. Normally, they migrate in opposite directions: the anterior DTC migrates anteriorly and the posterior DTC migrates posteriorly. During the third larval stage, the anterior DTC turns toward the right and the posterior DTC turns toward the left side of the animal, as each migrates across lateral epidermal cells toward muscle cells that lie in two dorsal quadrants. Upon reaching the dorsal muscle cells, the two DTCs turn again, this time leading their respective gonad arms back toward the center of the animal. This labyrinthine migration comes to an end in the fourth larval stage, after the tip of each arm has migrated hundreds of micrometers.

What role does the ECM play in the



Gonads on the go. (**A**) Mid-sagittal views of a *C. elegans* hermaphrodite larva showing the growth of the two gonad arms (dark gray). The two distal tip cells (DTCs) are shown in red. (**B**) A "filet" of a hermaphrodite at the first larval stage, opened up along the dorsal midline. Dorsal (D) and ventral (V) muscle quadrants are shown in purple; right (R) and left (L) lateral epidermal cells are shown in beige. The migratory routes of anterior (A) and posterior (P) DTCs in subsequent stages are shown (black arrows). (**C**) Hypothetical mechanism of MIG-17 release and activity. MIG-17 is produced by muscle cells, but can move to cover the surface of the migrating gonad primordium. It then acts on the extracellular matrix to support DTC migration. [(A) adapted from (*9*); (B) and (C) adapted from (*5*)]