**C. elegans** survivors without telomerase

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In most eukaryotic organisms with a linear genome the telomerase complex is essential for telomere maintenance and, thus, for genomic integrity. Proper telomerase function in stem and germ cell populations counteracts replication-dependent telomere shortening. On the other hand, repression of telomerase expression in most somatic tissues limits the proliferative potential of these cells through the induction of a permanent cell cycle arrest termed senescence upon critical telomere erosion. Thus, senescence, induced by telomere shortening and subsequent DNA damage signaling, is an essential tumor-suppressive mechanism, emphasized by the fact that repression of telomerase is lost in about 90% of cancers, endowing them with unlimited proliferative potential. In 10% of cancers, telomeres are maintained using the recombination-based alternative mechanism of telomere lengthening (ALT). To date, ALT and ALT-like mechanisms have only been described in the context of individual cells such as cancer cells and yeast. Now, several “survivor” strains of the nematode *Caenorhabditis elegans* have been generated that can propagate despite mutations of the telomerase gene. These nematode strains represent the first multi-cellular organism with canonical telomerase that can survive in the absence of a functional telomerase pathway.

Telomeres are the physical ends of linear chromosomes and are essential for conserving the integrity of the genome. They usually consist of several kilobases of double-stranded G-rich repetitive DNA sequence [(TTAGGG)\textsubscript{n}] in mammals and [(TTAGGC)\textsubscript{n}] in the nematode *Caenorhabditis elegans* ending in a shorter single-stranded overhang. Telomeres serve two main functions in the cell: first, they act as protective structures to ensure that the ends of linear chromosomes are not being recognized as points of DNA damage. This protection is achieved in conjunction with specific telomere-binding proteins that bind to the double-stranded or single-stranded portion of the telomere. In mammals, the core telomeric protein complex is called shelterin and is comprised of six members.1 In *C. elegans*, to date only two telomere-binding proteins have been identified and they both have been shown to bind to the single-stranded overhang portion of the telomere.2

Second, telomeres act as a buffer to counteract replication-dependent chromosome shortening. Due to the limitations of DNA polymerases in combination with end processing, telomeres shorten during each replication cycle, since the very 3' end of the template cannot be replicated (“the end-replication problem”).3 Once telomeres become critically short they induce a DNA damage response that can ultimately induce an irreversible cell cycle arrest called senescence. As senescence limits the growth proliferation of human somatic cells, this is considered as an important tumor-suppressive mechanism.4,5

In germ and stem cells, the telomerase enzyme counteracts telomere shortening by using its own RNA template and reverse transcriptase to add telomeric repeats to the ends of the chromosomes.6,7 Thus, telomerase provides these cells with an infinite replicative lifespan. As such, it is not surprising that telomerase, the expression of which is repressed in somatic tissues, is highly upregulated in about 90%
of cancer cells. However, in about 10% of cancers, telomerase remains shut-off and telomeres are maintained using ALT (alternative lengthening of telomeres). Whereas ALT was initially referred to as any alternative telomere maintenance mechanism not involving the telomerase enzyme, it is now more commonly used to describe a specific mechanism in mammalian cells. ALT is based on recombination between telomeres, but the exact mechanism is still elusive. Thorough understanding of how ALT works on a molecular level is highly relevant in the context of the potential use of telomerase inhibitors as anticancer drugs, as these drugs will not be efficient in the 10% of ALT-positive cancers. Furthermore, there is mounting evidence that targeting of telomerase might actually induce ALT in certain cases, emphasizing the need to fully understand both telomere maintenance pathways.

Until now, ALT and ALT-like mechanisms have only been described for cellular systems, such as in the aforementioned tumor cells and yeast. However, recent work from the Ahmed and Karlseder laboratories have now demonstrated that C. elegans strains can survive in the absence of a functional telomerase pathway, implicating the involvement of ALT-like mechanisms. While there are some differences in the details of these two studies, the emerging overall picture is very similar: mutations in the C. elegans telomerase gene (trt-1) have no initial impact on the organisms due to a sufficient telomeric sequence buffer. Eventually, progressive telomere erosion over several generations leads to DNA damage signaling and genomic instability, which ultimately results in sterility. However, using differing approaches, the Ahmed and Karlseder labs have now generated nematode strains with mutations in the telomerase gene that have managed to propagate for more than 200 generations and still thrive. These nematode strains represent the first multicellular model for ALT.

In our approach, we exploited the phenotype of a C. elegans strain with a mutation in the telomere-binding protein CeOB2/POT-1, since mutation of pot-1 has been shown to result in increased telomere length heterogeneity reminiscent of human ALT cells. Furthermore, in pot-1 mutants, we found enriched levels of a species of single-stranded (ss) C-rich telomeric circles, which has been described as a marker of ALT activity in human cells, suggesting increased telomeric recombination. When pot-1/trt-1 double mutant strains were tested for long-term survival by transferring five to six worms every two generations, several survivor lines could be established that have now been propagated for more than 200 generations. All trt-1 single mutants became sterile during the experiment.

The Ahmed lab initially set out to find suppressor mutations in trt-1 mutants that would allow for long-term survival in the absence of a functional telomerase pathway. They initially mutagenized early generation trt-1 mutants and then tested for long-term survival by transferring chunks of agar containing hundreds of worms over a long period of time. Cheng et al. discovered that after chunking the worms for more than 260 generations, there were many survivor lines in the non-mutagenized trt-1 negative control (5) than in the mutagenized trt-1 animals (only one line).

It is important to point out that in both cases the majority of trt-1-deficient lines still became sterile after a finite number of generations, most likely due to telomere erosion and genomic instability, and that the emergence of telomerase-negative survivor strains is a rare event. This suggests a mechanism where an adaptation process, likely in the form of additional mutations, takes place that allows the survivor strains to propagate. These mutations might arise through a crisis-like process, which in mammalian cells often precedes the transition of a healthy to a cancerous cell devoid of proper regulation of proliferation. During crisis, cells with critically short telomeres continue to divide, resulting in telomere-driven breakage fusion cycles and ultimately genomic instability. Eventually, cells emerge that have lost checkpoint controls and proliferate rapidly. Accordingly, in our study, we have observed that survivor lines often become almost sterile and then display a sudden recovery over the next generations, pointing toward a selection mechanism where strains emerge that can propagate in the absence of a functional telomerase pathway. The mutation of pot-1 facilitated this selection process in our hands, since telomeres were already rendered more recombinogenic. In the Ahmed study, the much higher number of nematodes used might have aided the selection of single trt-1 mutant survivors.

The Ahmed laboratory report a massive increase of telomerase-negative survivors in a strain mutated for the second C. elegans telomere-binding protein CeOB1/POT-2, which had initially been suggested to be responsible for regulation of telomerase access to telomeres, since mutations in this gene result in extremely long telomerase-dependent telomeres, which had initially been suggested to be responsible for regulation of telomerase access to telomeres, since mutations in this gene result in extremely long telomerase-dependent telomeres (data not shown). The results by the Ahmed lab suggest the possibility that mutation of pot-2 renders telomeres not only longer, but also much more recombinogenic. Indeed, when we tested a pot-2 mutant strain for C-circles, we found much more circles as compared with pot-1 mutants, proposing even higher recombination potential, which could facilitate ALT (unpublished data).
It is tempting to speculate that alternative mechanisms of telomerase maintenance have evolved in this clade independently of mammalian ALT, both as main telo-
mer maintenance mechanism (D. melano-
gaster) and as back-up mechanism in case of telomerase de-activation (C. ele-
gans). It will therefore be necessary to
describe the telomere maintenance me-
chanisms for many other yet uncharac-
terized model systems and potentially point
out that various ALT mechanisms are
more prevalent than previously thought.
Alternatively, one could conclude that
C. elegans is evolutionarily so far removed
from mammals that it might not be a use-
ful model system to study such complex
mechanisms as telomere length mainte-
nance. However, despite the evolutionary
distance between mammals and nema-
todes, more work is necessary to identify
molecular players that are essential for the
ALT-like survival in C. elegans, and it will
be exciting to see if there is overlap with
some of the few identified players of ALT
in mammals.

As for ALT in general, it will be excit-
ing to uncover whether ALT is a mecha-
nism that can simply be “switched on”
as a back-up when telomerase-mediated
telomere maintenance fails, whether ALT
could even work in conjunction with
telomerase, or if ALT happens seren-
dipitously as a combination of additional
mutations in telomerase-negative strains.

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