

sit at my desk and pull down almost any contemporary paper even before it is printed — I love that!

Fast communication, like media releases on an exciting discovery, often gets stuff wrong. Sorry media, but it is true and my advice to students and mentees is: if the media get 50% right that is pretty good. Media, do better, we can ignore you and do it ourselves these days. But as the old saying goes, even bad publicity is good and at least your thing got beyond the four walls of the laboratory. It is important not to worry about too many decimal places as a scientist, while by nature we need to stick to as accurate a picture as possible. Like any good marriage, there is a compromise but it may hurt finding it.

Social media should never be allowed to pass judgment on anything more than “did you like what I had for breakfast?” and should not, in my opinion, be viewed as a peer review process. In the Victorian era of science there was a magazine published called “*Science Gossip*” which contained observations from the Reverend Tottington-Bassett on the number of toads in his garden that year. Fascinating stuff! Scientific journals, like *Current Biology*, only allow words and ideas in print after an exhaustive and exhausting review process involving much hair-tearing and trying to get it right. More often than not, the work is rejected. A problem social media has introduced is that the innocent bystanders to the scientific process no longer distinguish “*Science Gossip*” from “*Current Biology*”. It is a challenge for both science and society to find this balance again using the new and exciting tools at our disposal.

For me, the answer lies in both reading books and keeping up to date with gizmos. Importantly the fast stuff, social media, needs to be taken as a first indication of the 50% truth and as a spark to getting out the peer reviewed stuff. Get interested, buy a book online, especially if it is one of mine.

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## Quick guide Shelterin

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### **What is shelterin and what does it do?**

Shelterin is a six-subunit protein complex (comprising TRF1, TRF2, POT1, TPP1, TIN2 and Rap1) that associates specifically with mammalian telomeres and allows cells to distinguish the natural ends of chromosomes from sites of DNA damage. Shelterin binds telomeres through TRF1 and TRF2, which interact with the double-stranded telomeric DNA, whereas the POT1 proteins (POT1a and POT1b in the mouse) associate with single-stranded (ss) telomeric DNA. POT1 is linked to TRF1 and TRF2 via an interaction between the POT1-binding protein TPP1 and TIN2, which binds both TRF1 and TRF2. The sixth shelterin subunit, Rap1, interacts solely with TRF2 (Figure 1A). All these factors are constitutively and ubiquitously expressed and the complex is highly abundant, potentially covering all telomeric DNA. In addition to the terminal telomeric repeats, shelterin proteins are found at certain interstitial telomeric sequences. Rap1 binding is also detected in the vicinity of several genes that are differentially regulated when Rap1 is lost. This suggests an additional role for Rap1 as a transcriptional co-regulator.

Shelterin function is crucial for telomere maintenance and genome integrity. Knockout experiments in mouse cells have revealed that shelterin protects telomeres from DNA damage signaling and DNA repair and also promotes the semi-conservative replication of the telomeric DNA. Moreover, shelterin regulates the telomerase-mediated maintenance of the telomeric DNA.

### **Do the different shelterin subunits work together or individually?**

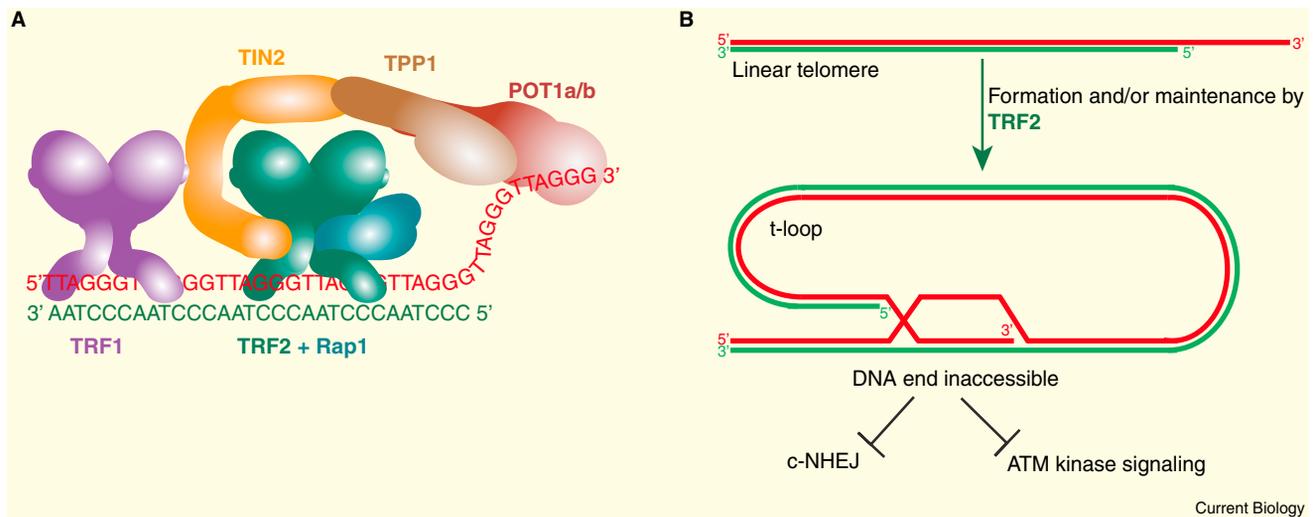
The protein–protein interactions between the different shelterin components are critical for its stable association with telomeric DNA. However, with regard to how shelterin represses the various aspects of the DNA damage response, there is substantial

division of labor amongst the different subunits. The TRF2 subunit is required for repression of the ATM-dependent DNA damage signaling pathway as well as classical non-homologous end joining (c-NHEJ). On the other hand, the ATR kinase signaling pathway is repressed by POT1. The repression of homologous recombination requires the concerted action of POT1 and Rap1. POT1 and TRF2 act independently to repress two different 5' end resection pathways at telomere termini. Finally, the TRF1 subunit of shelterin has a specialized role in promoting the semi-conservative replication of telomeres.

The regulation of telomerase-mediated telomere maintenance by shelterin appears to be complex and the molecular details have not been fully elucidated. Current models propose that the shelterin subunits POT1 and TPP1 play key roles in the regulation of telomerase action at individual telomeres. TPP1 is required for the recruitment of telomerase and thus acts as a positive regulator of telomere maintenance. However, telomerase is also negatively regulated by shelterin so that telomeres do not become inappropriately long and telomere length homeostasis is achieved. This negative regulation involves both TPP1 and POT1 but the molecular details of this interesting aspect of telomere biology are far from clear.

### **How does shelterin inhibit DNA damage signaling and repair?**

The mechanisms underlying the repression of DNA damage signaling and repair are not fully understood. One important mechanism involves the sequestration of the DNA ends into so-called t-loop structures (Figure 1B). T-loops are formed and/or maintained by TRF2. They are created by strand invasion of the single-stranded 3' overhang at the end of the telomere somewhere into the duplex telomeric DNA. These lariat structures provide an architectural solution to many aspects of end protection because they sequester the DNA end and make it inaccessible to DNA-end-binding proteins whose binding may initiate downstream DNA damage signaling and DNA repair. T-loop formation by TRF2 can explain



**Figure 1. The shelterin complex protects telomeres from DNA damage signaling and repair.**

At mammalian telomeres, the presence of shelterin (A) ensures genome integrity by repressing DNA damage signaling and repair, promoting semi-conservative replication of the telomeric DNA, and regulating telomerase-mediated telomere maintenance. The t-loop structure (B) is crucial for repression of c-NHEJ and ATM kinase signaling.

why the ATM kinase is oblivious to telomeres. The ATM kinase pathway is initiated by the recognition of a DNA end by the MRE11/RAD50/NBS1 (MRN) complex, which probably does not recognize the telomere end in the t-loop configuration. Similarly, c-NHEJ is dependent on the loading of a DNA-end-binding complex, which is incapable of gaining access to the telomere end in the t-loop structure.

The repression of ATR signaling by POT1 is thought to involve the protection of the single-stranded DNA from replication protein A (RPA), an abundant single-stranded DNA-binding protein that acts as the sensor in the ATR pathway. As long as POT1 covers the single-stranded DNA at telomeres, RPA is excluded from these sites and ATR will not be activated. Tethering of POT1 to the rest of the shelterin complex by TPP1 is necessary for POT1 to effectively exclude RPA from telomeres.

**Are other proteins involved in the functions of shelterin?** Lacking known enzymatic activity, shelterin exerts many of its functions through the transient recruitment of accessory factors. For instance, formation of the 3' overhang is tightly controlled by shelterin and involves the concerted action of the nucleases Apollo, which is recruited by TRF2, and exonuclease I, and C-strand fill-in synthesis by the

POT1-bound CST complex, a DNA polymerase  $\alpha$  primase accessory factor consisting of the proteins Ctc1, Stn1 and Ten1.

Another example is the promotion of replication fork progression at telomeres by TRF1. To facilitate lagging strand DNA synthesis, TRF1 associates with the Bloom's syndrome protein BLM, a RecQ helicase that can remove G-quadruplex DNA structures. These secondary structures can form at telomeres and present an obstacle to the replication machinery. For the proper synthesis of the leading strand, TRF1 may be assisted by the DNA helicase RTEL1, which is brought to telomeres by TRF2.

To ensure genome integrity, all of these DNA processing activities must be limited and tightly controlled by shelterin.

**What are the consequences of shelterin dysfunction?** Shelterin is critical for telomere maintenance and compromised shelterin function has deleterious consequences for the cell. Importantly, the telomeric DNA has to bind sufficient amounts of shelterin in order to fully suppress DNA damage signaling and repair. Because telomerase is silenced in most human somatic cells, telomeres shorten by 50 to 100 base pairs per cell division. This erosion is thought to eventually create telomeres that

are too short to bind enough shelterin for optimal telomere protection. As a result, the short telomeres activate a DNA damage signal that induces cell cycle arrest, as well as senescence or apoptosis. Moreover, the repair of the dysfunctional telomeres by various forms of NHEJ results in end-to-end fused dicentric chromosomes, which are unstable and generate genome instability.

**Can we live without shelterin?** No, shelterin is essential and we cannot live without shelterin-mediated telomere protection. Deletion of each shelterin component in mice, with the exception of Rap1, leads to embryonic lethality.

Mutations in shelterin have been linked to human diseases and to cancer. Mutations in TIN2 and TPP1 give rise to dyskeratosis congenita and related disorders, such as Hoyeraal-Hreidarsson syndrome and Revesz syndrome. These inherited telomere diseases are characterized by impaired telomere maintenance and bone marrow failure. In addition to TIN2 and TPP1, mutations in telomerase or in factors involved in telomerase biogenesis have been linked with dyskeratosis congenita. However, the exact nature of the telomere maintenance defects associated with the dyskeratosis congenita mutations of TIN2 is not

entirely clear, and it may also involve telomerase-independent mechanisms.

Recently, somatic mutations of POT1 were reported in 3.5% of cases of chronic lymphocytic leukemia. Most of these mutations clustered within the POT1-OB (oligonucleotide/oligosaccharide-binding) DNA-binding folds, and hence might compromise binding of POT1 to the single-stranded 3' overhang. Furthermore, rare germline variants of POT1 have been identified in familial cases of glioma and melanoma.

**Is shelterin conserved in other species?** All eukaryotes protect their chromosome ends with a telomere-binding protein complex. However, a shelterin-like complex is not always present. As in mammals, fission yeast telomeres are bound by a shelterin-like complex, consisting of a TPP1/POT1-like dimer, Tpz1–Pot1, and a TRF-like protein, Taz1. The Tpz1–Pot1 complex is connected to Taz1 via Rap1 and Poz1, establishing a link between the double-stranded and single-stranded telomeric DNA-binding factors. In contrast, the architecture of the telomere-binding complex and the proteins involved are quite distinct in budding yeast. Telomeres in budding yeast are bound by Rap1, which is the only structurally conserved shelterin component, although the mammalian and fission yeast Rap1 do not bind DNA. The single-stranded telomeric DNA in yeast is protected by the yeast CST complex.

#### Where can I find out more?

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## Primer Molecular clocks

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In the 1960s, several groups of scientists, including Emile Zuckerkandl and Linus Pauling, had noted that proteins experience amino acid replacements at a surprisingly consistent rate across very different species. This presumed single, uniform rate of genetic evolution was subsequently described using the term ‘molecular clock’. Biologists quickly realised that such a universal pacemaker could be used as a yardstick for measuring the timescale of evolutionary divergences: estimating the rate of amino acid exchanges per unit of time and applying it to protein differences across a range of organisms would allow deduction of the divergence times of their respective lineages (Figure 1).

In the 50 years since, leaps in genomic sequencing technology and new computational tools have revealed a more complex and interesting reality: the rates of genetic change vary greatly across the tree of life. The term ‘molecular clock’ is now used more broadly to refer to a suite of methods and models that assess how rates of genetic evolution vary across the tree of life, and use this information to put an absolute timescale on this tree. Modern molecular clocks are thus critical to inferring evolutionary timescales and understanding the process of genetic change. Analyses of genomic data using clock models that accommodate variation in evolutionary rates have shed new light on the tree of life, as well as the organismal and environmental factors driving genetic change along its branches. However, some major theoretical, empirical and computational challenges remain.

#### Evolutionary rate variation

Modern molecular clocks can handle various forms of evolutionary rate heterogeneity. Rates can vary across different parts of the genome (site effects), across taxa (lineage effects), and across time (here termed ‘epoch effects’). Site effects occur when

different parts of the genome evolve at distinct rates (Figure 2A). A widely recognized example involves protein-coding genes, which have a higher rate of evolution at the third position of codons than at the first and second. This is because changes at first and second codon sites are more likely to change the encoded amino acid, with potential consequences for protein function. In animals, mitochondrial DNA evolves faster than nuclear DNA, for reasons that are still debated. These site effects were the first major sources of rate heterogeneity to be characterized and accounted for during genetic analysis.

Lineage effects occur when different taxa exhibit distinct rates of molecular evolution (Figure 2B). For example, rodents have higher rates of genetic change than do other mammals, partly due to their short generation times. Likewise, parasitic plants evolve more rapidly than their free-living relatives. The importance of this form of rate variation took longer to be appreciated, but was confirmed in the 1970s when formal statistical tests of among-lineage rate variation were developed. This led to the introduction of ‘relaxed-clock’ approaches, which attempt to statistically model rate variation across branches of the evolutionary tree. These methods allow evolutionary timescales to be estimated using molecular clock approaches even when rates vary across lineages.

Epoch effects occur when rates of evolution differ across different time slices (Figure 2C). For instance, evolutionary rates in influenza were found to have undergone a sharp increase around 1990. Such temporal heterogeneity is harder to detect and model than either site effects or lineage effects. This is partly because it generates patterns of genetic divergence among living taxa that are very similar to those expected when rates have remained constant through time.

An extra layer of interest and complexity emerges when two or more sources of rate heterogeneity interact. Site and lineage effects interact when different genes have different patterns of rate variability across taxa (Figure 2D). Mitochondrial DNA has greatly accelerated rates of evolution in snakes and dragon lizards