



Lincoln's Inn Fields

## Telomere Biology

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The ends of eukaryotic linear chromosomes are potentially dangerous sites, as their resemblance to damage-induced DNA double strand breaks (DSBs) makes them vulnerable to DNA degradation and end-joining pathways. If left unchecked at chromosome ends, these pathways cause chromosome shortening and rearrangement, which in turn provoke genomic instability and cancer. Telomeres protect chromosome ends from these hazards. We study the components of telomeres, the spectrum and mechanisms of telomere function, and the events that follow telomere loss.

Telomeres are also critical for solving the 'DNA end replication problem', the inability of conventional DNA polymerases to replicate the extreme ends of linear DNA molecules. Telomeres solve this problem by engaging telomerase, a specialised reverse transcriptase containing an internal RNA subunit which templates synthesis of telomere repeats. In humans, telomerase is expressed in germ cells but not in most somatic cells. However, tumor cells must activate telomerase or an alternative mode of maintaining telomeres. While loss of telomere function promotes early tumorigenesis, the genomic instability that stems from telomere loss is incompatible with long-term cellular survival.

Therefore, regeneration of telomeres is critical for the eventual 'immortalisation' of cancer cells and suggests an intriguing universal target for anti-cancer therapy.

Fission yeast telomeres have a protein complement similar to that found in humans, but present substantial experimental benefits. Pot1 binds the terminal single strand telomeric overhang. Taz1, the only known ortholog of human TRF1 and TRF2, binds double strand telomeric DNA and regulates numerous functions including telomerase-mediated synthesis and nearby transcriptional silencing. It prevents DSB repair reactions from acting inappropriately on chromosome ends – loss of Taz1 leads to lethal chromosome end-fusions during G1 when nonhomologous end-joining (NHEJ) activities are elevated but not during G2 when NHEJ levels are low. Hence, telomere dysfunction yields strikingly different outcomes during G1 *versus* G2, conferring an advantage to studying telomeres in fission yeast, whose mainly G2 cell cycle allows cells lacking Taz1 to grow despite their dysfunctional telomeres.

### Control of telomerase through the cell cycle

Our recent studies shed light on how the telomere complex engages and regulates telomerase. Taz1 prevents accumulation of stalled telomeric replication forks (see below), which are themselves potent stimulators of telomerase activity. However, we find that telomerase engagement is possible only when Pot1 is phosphorylated during S-phase. We also find that the protein Ccq1 is crucial for telomerase recruitment. In the absence of Ccq1 or Pot1 phosphorylation, telomeres fail to restrain local checkpoint activation and telomeric recombination. These observations provide a foothold for deciphering the molecular underpinnings of cell cycle control of telomere accessibility to both telomerase and DNA damage response factors.

### Control of replication fork movement through telomeres

While telomerase synthesises the most terminal telomere

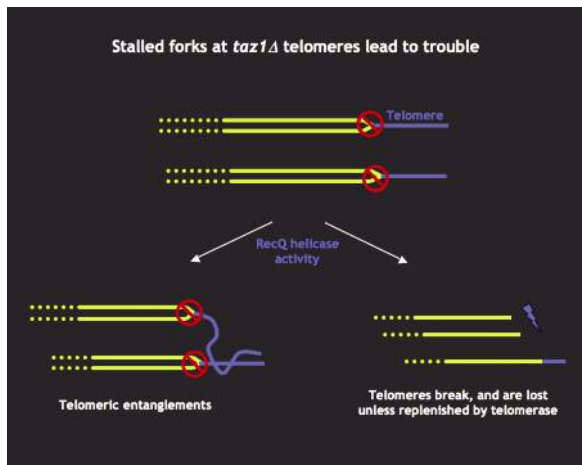


Figure 1. Stalled replication forks accumulate at *taz1* telomeres. The RecQ helicase appears to process these stalled forks aberrantly, leading to telomeric entanglements or, in the absence of telomerase, complete telomere loss.

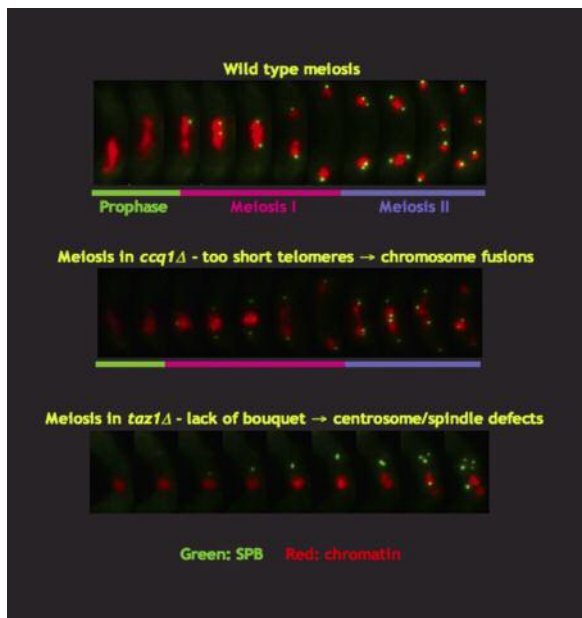


Figure 2. Spindle pole body (SPB) signals and chromatin segregate equally into 4 spores during meiosis in wild type cells. A fraction of *ccq1Δ* telomeres become too short, leading to chromosome fusion and missegregation even when bouquet formation and SPB division occur properly. In contrast, *taz1Δ* cells lack stable bouquet formation, leading to aberrant SPB division and chromosome missegregation.

repeats, the majority of telomere repeats are synthesised by semi-conservative DNA replication. We found that Taz1 is required for efficient replication fork movement through telomeres, as stalled replication forks accumulate at telomeres lacking Taz1. We find that such fork stalling is conferred by all repeated sequences and may have general relevance for genome maintenance. Replication fork stalling at telomeres leads to abrupt telomere loss in the absence of telomerase. It also leads to telomeric 'entanglements' that prevent chromosomes from segregating properly at mitosis. Our search for factors involved in resolving this telomeric

entanglement has uncovered two surprising key players. First, the RecQ helicase Rqh1 (ortholog of human Werner Syndrome helicase, whose mutation causes the eponymous premature aging disease) prevents resumption of fork movement through telomeres, triggering telomere loss and entanglement. Second, a non-canonical activity of the essential decatenation enzyme Topoisomerase II (Top2) prevents the entanglement that can result from stalled telomeric replication forks, heralding an unforeseen role of Top2 in promoting genome stability.

### A novel mode of survival in the absence of telomeres

When telomeres are lost in telomerase-minus cells, some cells acquire the ability to maintain telomeres *via* recombination. In addition, fission yeast can survive telomere loss by chromosome circularisation. These 'circular strains' are viable but exhibit several conspicuous defects, like slow growth and hypersensitivity to agents that induce DSBs. We have identified a DSB-resistant subclass of telomerase-minus cells that survive using a third strategy, in which telomere sequences are absent but large blocks of heterochromatin are amplified. This survival mode resembles that found in the fruit fly *Drosophila*, and may illuminate the universal 'stripped-down' requirements for chromosome end-maintenance.

### Telomeric control of meiotic spindle formation

When cells progress to the meiotic cell cycle, telomere function changes dramatically. Telomere clustering during early stages of meiosis, or 'bouquet formation', is observed throughout the Eukaryota. Our earlier studies showed that formation of this telomere bouquet depends on Taz1 and is required for successful meiosis. We find that telomeres not only associate with the centrosome during meiotic prophase, but also dissociate in a concerted manner, at a moment that immediately precedes centrosome division and the onset of meiosis I. In the absence of the telomere bouquet (e.g. in *taz1Δ* cells), the centrosome mislocalises at meiosis I and often dissociates from the nucleus. Moreover, meiotic spindle formation is aberrant. Thus, the highly conserved bouquet plays an unanticipated role in controlling spindle formation. Our data suggest that an ectopically engineered association between non-telomeric heterochromatin and the meiotic centrosome can confer proper meiotic spindle formation in the absence of a true 'telomere bouquet'. These data raise exciting new questions about the control of spindle formation by chromosomes.

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