Downloaded from www.genesdev.org on February 9, 2007

DNA breaks are masked by multiple Rap1 binding in yeast: implications for telomere capping and telomerase regulation

Simona Negrini, Virginie Ribaud, Alessandro Bianchi, and David Shore[1]

Department of Molecular Biology and National Center for Competence in Research (NCCR) Program 'Frontiers in Genetics,' University of Geneva, Geneva 4, 1211 Switzerland

Eukaryotic cells distinguish their chromosome ends from accidental DNA double-strand breaks by packaging them in a protective structure referred to as the telomere "cap." Here we investigate the nature of the telomere cap by examining events at DNA breaks generated adjacent to either natural telomeric sequences (TG repeats) or arrays of Rap1-binding sites that vary in length. Although DNA breaks adjacent to either short or long telomeric sequences are efficiently converted into stable telomeres, they elicit very different initial responses. Short telomeric sequences (80 base pair [bp]) are avidly bound by Mre11, as well as the telomere capping protein Cdc13 and telomerase enzyme, consistent with their rapid telomerase-dependent elongation. Surprisingly, little or no Mre11 binding is detected at long telomere tracts (250 bp), and this is correlated with reduced Cdc13 and telomerase binding. Consistent with these observations, ends with long telomere tracts undergo strongly reduced exonucleolytic resection and display limited binding by both Rpa1 and Mec1, suggesting that they fail to elicit a checkpoint response. Rap1 binding is required for end concealment at long tracts, but Rif proteins, yKu, and Cdc13 are not. These results shed light on the nature of the telomere cap and mechanisms that regulate telomerase access at chromosome ends.

[*Keywords*: DNA double-strand break; telomere capping; telomerase; DNA damage checkpoint; telomere length regulation]

Supplemental material is available at http://www.genesdev.org.

Received July 12, 2006; revised version accepted December 4, 2006.

The ends of linear eukaryotic chromosomes require special mechanisms to assure their complete replication (Lingner et al. 1995) and to prevent them from being either degraded or joined with other telomeres or accidental DNA breaks (for review, see Smogorzewska and De Lange 2004). An elaborate and dynamic protein–DNA complex referred to as the telomere has thus evolved to cope with these problems. In most eukaryotes, chromosome end replication is assisted by the telomerase enzyme, which can synthesize de novo TG-rich repeat sequences at chromosome 3′ ends (Greider and Blackburn 1985), thus reversing the loss of information resulting from the 5′ exonucleolytic degradation (5′ end resection) observed at telomeres in all organisms examined to date. How telomerase action is regulated so as to bring about a fixed average TG-tract length at telomeres is still unknown. The second problem, end protection or "capping," is resolved by proteins that bind to telomeres through mechanisms that are still poorly understood. In mammalian cells, capping requires a protein complex called "shelterin" (de Lange 2005), which may act by helping to form a protective structure, the t-loop, wherein the chromosome 3′ single-stranded terminus is buried in a more internal telomere repeat sequence (Griffith et al. 1999). In budding yeast, capping requires a protein complex consisting of Cdc13, Stn1, and Ten1 that localizes to the 3′ TG-rich single-stranded DNA at telomeres by virtue of Cdc13 DNA binding (Bertuch and Lundblad 2006). The loss of Cdc13 function in the temperature-sensitive *cdc13-1* mutant results in rapid 5′ end resection, activation of the DNA damage checkpoint, and a disruption of telomere structure (Weinert and Hartwell 1993; Garvik et al. 1995).

Both telomerase action and telomere capping are tightly linked to the length of the TG-repeat tract at individual telomeres. [In budding yeast, telomeric repeats take the form $G_{2-3}(TG)_{1-6}$, but will be referred to hereafter simply as "TG repeats."] Duplex TG-repeat sequences are bound by specific proteins (Rap1 in budding yeast, Taz1 in fission yeast, and Trf1/Trf2 in mammals)

[1]Corresponding author.
E-MAIL David.Shore@molbio.unige.ch; FAX 41-22-379-6868.
Article is online at http://www.genesdev.org/cgi/doi/10.1101/gad.400907.