ABSTRACT

Telomere and checkpoint regulation: conserved mechanisms

Maria Dahlén, Department of Cell and Molecular Biology, Göteborg University
Box 462, SE-405 30 Göteborg, Sweden

Checkpoint pathways are surveillance mechanisms that ensure proper order of cell cycle events, and delay the cell cycle in response to DNA damage or DNA replication defects, hence providing time for repair of DNA lesions. In fission yeast a group of six genes, called the checkpoint-*rad* group, which comprises *rad1*$^+$, *rad3*$^+$, *rad9*$^+$, *rad17*$^+$, *rad26*$^+$, and *hus1*$^+$, are thought to be involved in sensing DNA aberrations and generating a checkpoint signal that is transduced to the cell cycle regulators to prevent execution of the subsequent cell cycle event. Telomeres are the DNA-protein complexes found at the ends of chromosomes, which protect the chromosomal ends from degradation and fusion with other chromosomes.

We found that Hus1 is phosphorylated after DNA damage but not during hydroxyurea arrest. Rad1, Rad9, and Hus1 are all structurally related to PCNA, and form a protein complex *in vivo*, but the majority of each protein is not present in this complex. Rad 17 forms a distinct protein complex apart from the other checkpoint-Rad proteins. Two-hybrid interaction, in vitro association, and in vivo overexpression suggest a transient interaction between Rad1 and Rad17. Rad17 is also required for nuclear localization of Hus1 and Rad9.

The human homologue of *S. pombe rad1*$^+$, *hRAD1*, partially complements the hydroxyurea and ionizing radiation sensitive phenotype of a *rad1* mutant. This indicates a structural, and to some degree also a functional conservation of this checkpoint-*rad* gene throughout evolution. *hRAD1* is ubiquitously expressed and was chromosomally localized by fluorescence in situ hybridization (FISH) to 5p13.1.

We have identified a novel phenotype of a subset of the checkpoint-*rad* mutants. Mutants of *rad1*$^+$, *rad3*$^+$, *rad17*$^+$, and *rad26*$^+$ have a stable shorter telomere length than the wild-type strain. In contrast, mutants of *rad9*$^+$, *hus1*$^+$, *chk1*$^+$, *cds1*$^+$, and mutants affected in the regulation of Cdc2 have telomeres of wild-type length. This indicates that telomere regulation and checkpoint control are two separate functions of Rad1, Rad3, Rad17, and Rad26.

The replication checkpoint defect correlates with the telomere phenotype in previously characterized *rad1* mutant alleles. Overexpression of the *rad1*$^+$ gene causes slight telomere elongation. Telomeres gradually shorten when the *rad1*$^+$ gene is disrupted, and can be restored to wild-type length by reintroduction of the *rad1*$^+$ gene.

Cells with mutations of genes, the products of which are involved in lagging strand DNA replication, *pol*α^+/*pol1*$^+$, *spp1*$^+$, *spp2*$^+$, *pol*δ^+, and *cdc17*$^+$ (ligase), have longer telomeres compared to their respective parental wild-type strain. Mutants of the *pol*ε^+, and *rad2*$^+$ genes have slightly shorter telomeres. The telomere elongation seen in the *spp2-9* mutant is telomerase dependent. The telomere elongation seen in the *polats13*, *spp1-9*, and *spp2-9* mutants is a G-strand extension.

Polα (the catalytic subunit of the polα-primase complex) and Trt1 (the catalytic component of telomerase) associate in vivo already in early S phase, but the interaction is stronger in the G2 phase. The interaction is almost abolished in a *polats13* mutant, indicating that an interaction between the polα-primase and the telomerase complexes is required for proper telomere length maintenance.

Keywords: Cell cycle checkpoint, *rad* genes, *S. pombe*, PCNA, telomeres, Polα, primase.