

Cancer Biology 1: Exercises week 6

1. Microhomology mediated end joining depends on polymerase theta (Polq, encoded by *POLQ*), which promotes annealing of the microhomologies and is responsible for the polymerization step. In addition to Polq, PARP1 (Poly[ADP-ribose] polymerase 1), ligase 3 and several other factors have been implicated in MMEJ. However, our knowledge of MMEJ remains limited and lacks a complete understanding of all the factors that cooperate with Polq to promote MMEJ repair.

A) How would you design a genetic screen in order to identify the full spectrum of MMEJ factors?

B) Describe also control experiments to validate your screening approach.

C) Compare your proposals with the recently published work of Sfeir and colleagues (see on moodle: Science 381, 653-660 (2023)). Figures 1A,B,C for question A)

2. The human telomerase RNA template contains the following sequence:
3'-CAAUCCCAAUC-5' specifying human telomeric repeat sequences
(5'-GGTTAG GGTAG GGTAG...-3').

A) To what sequence would you mutate the template in order to specify 5'-GGTTAC-3' repeats.

B) What consequences would you expect for cells which ectopically express this mutant version of telomerase.

3. A) Describe three experimental strategies how to overcome telomere-induced senescence?

B) Describe two strategies how to overcome cell crisis.

4. Upon loss of telomere capping, telomere fusions can occur before (G1) and after DNA

synthesis (G2). What will be the consequences for the involved chromosomal DNA molecules during M phase? Draw the involved chromosomes during the different cell cycle stages for a fusion that took place (A) in G1 and (B) in G2.

5. You identified a novel gene (*VLT1*; very long telomeres 1) whose deletion in HeLa cells gives abnormally long telomeres. How would you test the following hypotheses?

-VLT1 counteracts telomerase recruitment to telomeres.

-VLT1 enhances the end replication problem.

-VLT1 increases the length of the telomeric 3' overhang.

-VLT1 counteracts telomere repeat processivity of telomerase (the ability of telomerase to add multiple telomeric repeats before dissociating from the chromosome end; consult Figure 4 of the Nature paper present on moodle).

6. a) POT1 depletion can lead to checkpoint activation and cell cycle arrest. Draw a diagram showing all the steps and the involved molecules.
 b) Do the same for TRF2 depletion.
 c) How would you test if the ability of POT1 to bind TTAGGG-repeats is required to suppress checkpoint activation.

7. Give an example of a positive feedback-loop in the control of the mammalian R-point transition. Explain how the components you mention interact to produce this.

8. Wild type *S. cerevisiae* cells divide forever but deletion of telomerase leads to cellular senescence after 50-60 population doublings (due to *ever shorter telomeres*). Est1 is required for telomerase recruitment. Est2 corresponds to the yeast TERT telomerase subunit. Rad52 is involved in homologous recombination (*rad52-Δ* (delta means deletion of the gene) shows *per se* no growth defect). How do you interpret the results shown below indicating impairment of cell growth of yeast cells after 50 generations or even before when combining *est-Δ* with *rad52-Δ*? Please note that a single cell gives rise to a visible colony after having undergone 25 generations.

