

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/22970> holds various files of this Leiden University dissertation.

Author: Roerink, Sophie Frederique

Title: Alternative polymerases in the maintenance of genome stability in *C. elegans*

Issue Date: 2014-01-09

ADDENDUM



Summary
Nederlandse samenvatting
Dankwoord
Curriculum Vitae
List of publications

SUMMARY

DNA - the carrier of genetic information in the cell - is a chemically reactive molecule. It is constantly attacked by various sources of damage, from endogenous as well as exogenous origin. Base damage interferes with cellular processes such as replication and transcription and may result in incorporation of incorrect nucleotides in the genome. Accumulation of mutations may threaten viability of the cell, or even worse, result in tumorigenesis if the 'brake' on cellular proliferation is also mutated. However, a limited source of genetic variability is desirable, because it also allows for evolutionary changes.

The key question in this thesis is how organisms ensure genome stability - how do they protect their DNA from mutations caused by base damage? At the same time, this system should not be infallible, as mutations are also needed for evolutionary adaptation to take place.

In my thesis I am studying the contribution of DNA polymerases on the maintenance of genome stability. DNA polymerases copy the DNA content of a cell during replication. In most cases, this is done by the very precise replicative polymerases, that contain proofreading domains which correct occasional misinsertions. However, in the case of base damage, 'normal' replicative polymerases cannot pass the damaged template strand. The cell employs an alternative strategy by means of specialized translesion synthesis (TLS) polymerases. These enzymes can bypass damaged bases, and prevent replication fork stalling. The downside is that TLS polymerases are error-prone; they are less precise and can occasionally result in incorporation of the wrong nucleotide.

Chapter 2 and 3 focus on the contribution on genome stability of two members of the Y-family of TLS polymerases, Pol η and Pol κ , while chapter 5 focuses on a third member, REV1. In chapter 2 I introduce two new mutant alleles for Pol η and Pol κ in *C. elegans*, and study their function in the protection against various exogenous sources of DNA damage. I conclude that Pol η has a key role in protection of a developing *C. elegans* embryo against different damaging agents, such as the cytostatic cisplatin, UV-irradiation and X-rays. In some cases Pol κ functions redundantly to Pol η . Furthermore I compare results of a screen for sensitivity against a methylating agent, to find new interactors with Pol η and Pol κ in the cell.

In chapter 3 I ask to which extent Pol η and Pol κ contribute to genome stability in the absence of exogenous sources of base damage, thus to the effects of endogenous damage. To study this, we studied mutation accumulation in the mutants isolated in chapter 2. We kept these strains in culture for many successive generations and then determined the profile of the spontaneous mutations that arose.

Strikingly, we observed a very characteristic pattern of mutagenesis in TLS deficient animals: deletions of ~100 basepairs. Two other characteristics were shared by many deletions: microhomology of a single nucleotide on two sides of the deletion, or insertion of small stretches of duplicated DNA from the flanks. This mutational pattern suggested an error-prone mechanism to repair breaks that are the result of stalled replication in the absence of TLS. Insertion of DNA predicted the involvement of another DNA polymerase.

We identified this polymerase to be the A-family member Pol θ . In the absence of Pol θ , large stretches of DNA are resected, resulting in loss of DNA, checkpoint activation and cell death.

In chapter 4 I use a direct source of DNA breaks to study the function of Pol θ : activation of transposons in the germline. Transposons are DNA fragments that can under certain conditions be excised from the DNA, resulting in DNA breaks. Normally, this process is silenced in the germline; however if so-called mutator genes are knocked out, transposition results in breaks in DNA of germ cells. To analyze Pol θ - mediated repair at a molecular level, I study transposons in the muscle gene *unc-22* with two different sequence contexts: either surrounded by microhomology or without any microhomology. In both cases efficient repair is largely dependent of functional Pol θ , demonstrating a direct role for Pol θ in repair of double strand breaks.

REV1 - described in chapter 5 - plays also a role in genome protection both against endogenous and exogenous damage, and protects against spontaneous deletions, analogous to the other Y-family members Pol η and Pol κ . However, its cellular function may be even broader, as Rev1 knockout worms also suffer from progressive loss of maturing germ cells, resulting in sterility.

In conclusion, this study presents the first functional analysis of the Y-family polymerases in genome protection against endogenous and exogenous sources of DNA damage in *C. elegans*. Unexpectedly, this study lead to identification of a new error-prone pathway to repair double stranded breaks, mediated by the A-family polymerase Pol θ and hence termed Pol θ -mediated end joining (TMEJ). Traces of mutagenesis by TMEJ are abundant in evolutionary separated *C. elegans* strains and bear also resemblance to mutational patterns identified in cancers, highly encouraging further analysis of Pol θ function under physiological and cancerous conditions.