The role of homologous recombination in mitotic and meiotic double-strand break repair

Vries, Femke Adriana Theodora de

Summary

Summary

All organisms are composed of cells, the basic units of structure capable of performing the activities of life. Every cell's nucleus contains DNA, the heritable material that directs the many functions of the cell. In eukaryotic cells, the DNA is organized within the nucleus along with certain proteins into structures called chromosomes. In humans, each somatic cell (any cell other than reproductive cells) has 46 chromosomes. The chromosomes are found in pairs and we inherit one chromosome of each pair from each parent. The 46 chromosomes in our somatic cells are therefore actually two sets of 23 homologous chromosomes, a set from our mother and a set from our father. The chromosome is divided in hereditary units called genes. Genes are segments of DNA that encode a specific hereditary trait. DNA is a double-stranded helical polymer of four different kinds of monomers called nucleotides. Each nucleotide consists of three components: a nitrogen-containing base, a sugar (deoxyribose), and a phosphate group. The base can be adenine (A), thymine (T), guanine (G) or cytosine (C). A–T and C–G bases are paired in the interior of the double helix. Inherited information is passed on in the form of each gene's specific sequence of nucleotides. Cells are programmed to transcribe and translate genes and doing so cells synthesize specific enzymes and other proteins. It is the cumulative action of these proteins that produces an organism's inherited traits.

The transmission of hereditary traits has its molecular basis in the precise replication of DNA, which produces copies of genes that can be passed along from parent to offspring. When a cells divides normally, during a process called mitosis, two daughter cells are formed that are genetically identical to the parent cell. The reproductive cells, or gametes, that transmit genes from one generation to the next are sperm and ova (unfertilized eggs). Sperm and ova are made by meiosis. Meiosis, like mitosis, is preceded by the replication of chromosomes. However, this single replication is followed by two consecutive cell divisions, called meiosis I and meiosis II. These divisions result in four daughter cells (rather than the two daughter cells of mitosis), each with only half as many chromosomes as the parent cell. After meiosis, each of the gametes has a single set of 23 chromosomes. After a sperm cell unites with an ovum, chromosomes from both parents are present in the nucleus of the fertilized egg.

The induction of DNA damage is a threat to all organisms. First, the correct functioning of the cell is assured by gene expression by transcription and translation. DNA damage possibly leads to blockage of transcription and delay of gene expression. Second, during nuclear division (mitotic and meiotic division) the DNA of the mother cell is first replicated before it is equally distributed over two new nuclei. In this way damaged DNA can be passed on to daughter cells. Accumulation of damaged DNA in cells can lead to mutations and chromosome aberrations. Proteins encoded by mutated DNA can be altered leading to lack of

or a modified function, the genetic basis of hereditary diseases. In addition, mutations and chromosome aberrations can lead to unlimited cell growth (carcinogenesis). Signalling of DNA damage and subsequent repair is of substantial importance to all organisms. This importance is underlined by the existence of various damage signalling and DNA repair pathways in all prokaryotic and eukaryotic cells.

Chapter 1 of this thesis introduces one of the most genotoxic lesions: the DNA double-strand break (DSB), which affects both DNA strands of the double helix. DSBs can be induced by ionizing radiation and other exogenous DNA damaging agents or free radicals generated during cellular metabolic processes or collapsed forks during normal replication. DSBs also arise as intermediates in several endogenous cellular processes such as the initiation of recombination and assurance of proper chromosome segregation during meiosis, mating-type switching in yeast and the assembly of immunoglobulins and T cell receptors from variable (V), diversity (D), and joining (J) gene segments (V(D)J rearrangement). Persisting or incorrectly repaired DSBs in DNA can result in chromosomal rearrangments (including chromosomal fragmentation, translocations and deletions). Genetic instability resulting from unrepaired DSBs can lead to programmed cell death via apoptosis. In multicellular organisms chromosomal rearrangements can lead to or contribute to carcinogenesis by activation of oncogenes, inactivation of tumor suppressor genes or loss of heterozygosity. The importance of DSB repair is underlined by the evolution of several DSB repair pathways. The two major pathways are non-homologous endjoining (NHEJ) and homologous recombination (HR). By non-homologous endjoining, broken ends of DNA are sealed together, irrespective of their sequence in a not necessarily error-free way. Due to resection of the broken ends, genetic information can be lost resulting in inaccurate DNA repair. HR, in which damaged chromosomes are restored using intact sister chromatids or homologous DNA molecules as a template, accurately repairs DSBs.

Genes involved in HR are introduced in **Chapter 1.3**. These genes belong to the RAD52 epistasis group and are well characterized in *S. cerevisiae*. Key proteins in the RAD52 epistasis group are Rad51, Rad52 and Rad54. In HR, Rad51, stimulated by the activities of Rad52 and Rad54, mediates the pairing of homologous DNA molecules and strand exchange. In this thesis we mainly focus on the Rad52 protein. Rad52 is a key protein in recombinational repair in *S. cerevisiae*. In the fission yeast *S. pombe*, two *RAD52* homologs, *rad22A*$^+$ and *rad22B*$^+$, are present. *S. pombe rad22A* mutants resemble *S. cerevisiae* rad52 mutants in radiosensitivity, while inactivation of *rad22B*$^+$ does not lead to such obvious repair defects. This demonstrates that Rad22A is crucial in recombination and repair of vegetative *S. pombe* cells, while Rad22B has an auxiliary role in DSB repair. Meiosis in *S. pombe* is dependent on the presence of either Rad22A or Rad22B. Interestingly, the level of Rad22B protein is strongly enhanced in *S. pombe* cells from 5 to 7 hours after induction of meiosis in comparison to vegetatively dividing cells and cells in early and late meiosis. We

wondered whether differences in phenotypes of *rad22A* and *rad22B* mutants can be clarified by differences in biochemical activities. In mammals, Rad52 deficiency has no obvious effect on cells and the mild phenotype of *rad52* mutants suggests the existence of additional mammalian proteins functionally redundant with Rad52. Recently, it was demonstrated that the biochemical function of Rad52 can probably be compensated for by the breast cancer-associated factor BRCA2.

In **Chapter 1.4** we stress the importance of recombination between homologous chromosomes during meiosis. This recombinational behaviour of homologous chromosomes during meiosis is responsible for genetic variation and gives rise to the process of evolution. During prophase of the first meiotic division, the duplicated chromosomes pair with their homologues, a process called synapsis. During part of prophase I, a protein "zipper", the synaptonemal complex (SC), holds the homologous chromosomes tightly together all along their lengths. When the SC disappears in late prophase, chiasmata can be seen, the physical manifestations of recombination between homologous chromosomes also called crossing overs. SCs are assembled from two axial elements (AEs), one along each homolog, which are connected by numerous transverse filaments (TFs). Meiotic HR, in contrast to recombination in mitotically dividing cells, is actively induced in high frequencies (100 to 1000 fold higher than in mitotic cells). During meiosis, the homologous chromosome is the preferred template, whereas in mitotically dividing cells the sister chromatide is used predominantly as a template molecule. In most organisms meiotic recombination is initiated by the formation of a double-strand break by the Spo11 protein. In addition to Spo11, the formation and resection of breaks during meiosis requires factors belonging to the RAD52 group and a number of meiosis-specific recombination proteins.

In **Chapter 1.5** we descibe post-translational modification as a way to alter the function of a protein. We discuss peptide modifications, post-translational modifications of target proteins by attaching other peptide groups to it. Ubiquitination is the most common mechanism to regulate the properties of a protein by marking target proteins for degradation. Also covalent attachment of the small ubiquitin-like modifier, SUMO, to proteins plays a major role in regulating cellular functions through changes in cellular localization, biochemical activation, or through protection from ubiquitin-dependent degradation. SUMO modification of a specific target occurs in three enzymatic steps: first E1 enzymes, known as activating enzymes, modify SUMO so that it is in a reactive state, then E2 enzymes, known as conjugating enzymes, catalyze the attachment of SUMO to the substrate protein. E3 enzymes, or ligases, play a role in recognizing the subtrate protein.

In **Chapter 2** we analyse the biochemical properties of the two Rad52 homologs in *S. pombe*, Rad22A and Rad22B. *S. cerevisiae* Rad52 is a key protein in the repair of DNA double-strand breaks by homologous recombination. *In vitro*, Rad52 displays DNA binding and strand annealing activities and promotes

Rad51-mediated strand exchange. To obtain insight in the functions of the Rad22A and Rad22B proteins, both proteins are overexpressed in *E. coli* as his-tagged proteins and purified to near homogeneity. Rad22A and Rad22B are tested for DNA binding by gel retardation and filter binding assays and we demonstrate that both proteins are capable of binding to short single-stranded DNAs. Rad22A and Rad22B do not bind to duplex oligonucleotides or linearized plasmids containing blunt ends or 5' single-strand overhangs. In contrast to Rad22A, Rad22B promptly binds to DNA fragments containing 3' overhangs. As expected, Rad22A as well as Rad22B efficiently promote strand annealing of complementary oligonucleatides. In a reaction containing Rad22A, almost 90% of the complementary DNAs is annealed to duplex molecules, whereas in reactions containing Rad22B, the maximum level of annealing is 60%. We demonstrate that the presence of duplex DNA inhibits the annealing activity of Rad22B. The presence of Rad22A can overcome this inhibitory effect. Rad22A and Rad22B are self-associating proteins and exist as multimeric structures as was shown by electron-microscopy.

In **Chapter 3** we illustrate that combined mutations in *RAD52* and *RAD54* homologs (*rad22A*$^+$, *rad22B*$^+$ and r*hp54*$^+$) in *S. pombe* results in identical sensitivities to X-rays, cis-diammine-dichloroplatinum and hydroxyurea. In this respect, these fission yeast homologs resemble the epistatic interaction (*i.e.* single, double and triple mutant strains are equally sensitive to DNA damaging agents) of their counterparts in *S. cerevisiae*. To verify whether inactivation of *RAD52* affects the DNA damage sensitivities of Rad54 deficient mice, we have studied double mutant mice and bone marrow cells derived from these animals. Haemopoietic depression in bone marrow and the formation of micronuclei after *in vivo* exposure to mitomycine C (MMC), is not increased in either single or double mutants in comparison to wildtype mice. The induction of sister chromatid exchanges in splenocytes is slightly reduced in *rad54* single and double mutants. We show that the MMC survival of *rad54*$^{-/-}$ mutant mice and the survival of bone marrow cells after exposure to X-ray is aggravated by a deficiency of Rad52. These findings demonstrate that double mutants have additive defects in HR. Possibly, the single-strand annealing activity of Rad52 may be more prominent in the absence of Rad54.

In **Chapter 4** we present the identification of novel proteins involved in DSB repair by homologous recombination. Using *Drosophila* DmRad51 as bait protein in yeast two-hybrid studies, we have identified Uba2, Ubc9 and Su(Var)2-10, the SUMO E1 activating, E2 conjugating and E3 ligating enzymes, respectively, as associating factors. Su(Var)2-10, which is originally identified as a dominant suppressor of position-effect-variegation, is essential for viability and is involved in the regulation of chromosome structure. We observe an increased X-ray sensitivity in heterozygous *Su(Var)2-10* flies, which implies that sumoylation has a role in DNA damage responses to X-rays. Both Rad22A and Rad22B display interaction with Hus5, the E2 component of the SUMO conjugation pathway in *S. pombe*. To investigate the importance of sumolyation of Rad22A, we have

changed the single SUMO consensus motif of Rad22A. This alteration does not influence DSB repair by Rad22A after X-ray. We have screened the *S. pombe* database for proteins belonging to the PIAS family of SUMO ligases en identified the SP-RING domain protein Pli1. Although, the protein-protein interactions suggest a connection between SUMO modification and DSB repair by HR, *pli1* fission yeast mutants are not hypersensitive to X-rays and hydroxyurea. Defects in chromosome structure in *su(var)2-10* mutants imply an essential role for SUMO in maintaining proper nuclear organization, suggesting that the role for SUMO in DNA repair may be indirect.

In **Chapter 5** we introduce *sycp1*$^{-/-}$ mice. Sycp1 is a component of the transverse filament of the mouse synaptonemal complex, which tightly connects homologous chromosomes during meiotic prophase and assures meiotic recombination. *Sycp1*$^{-/-}$ mice are infertile, but otherwise healthy. The axial elements of the SC are normally formed in *sycp1*$^{-/-}$ spermatocytes, but synapsis does not occur. Most *sycp1*$^{-/-}$ spermatocytes arrest in pachynema, whereas a small proportion reaches diplonema, or, exceptionally, metaphase I. γH2AX foci (indicative of double-strand breaks) appear during leptotene in both *sycp1*$^{-/-}$ and wildtype spermatocytes. Significant numbers of discrete γH2AX foci are found along each chromosome in pachynema *sycp1*$^{-/-}$ spermatocytes, whereas γH2AX disappears from autosomes in wildtype spermatocytes. Rad51/Dmc1, RPA and Msh4 foci (which mark early and intermediate steps in pairing/recombination) appear in similar numbers as in wildtype, but do not all disappear in *sycp1*$^{-/-}$ mutants. Mlh1 and Mlh3 foci (which mark late steps in crossing over) are not formed. Unexpectedly, *sycp1*$^{-/-}$ spermatocytes do not form XY bodies. Crossovers were rare in metaphase I of *sycp1*$^{-/-}$ mice. We propose that Sycp1 has a coordinating role and ensures formation of crossovers.