DNA double-strand break repair: how to fix a broken relationship

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Abstract. DNA double-strand breaks (DSBs) arise in cells from endogenous and exogenous attacks on the DNA backbone, but also as a direct consequence of replication failures. Proper repair of all these DSBs is essential for genome stability. Repair of broken chromosomes is a challenge for dividing cells that need to distribute equal genetic information to daughter cells. Consequently, eukaryotic organisms have evolved multi-potent and efficient mechanisms to repair DSBs that are primarily divided into two types of pathways: nonhomologous end joining (NHEJ) and homologous recombination (HR). Here we briefly describe how eukaryotic cells sense DSBs and trigger cell cycle arrest to allow repair, and we review the mechanisms of both NHEJ and HR pathways and the choice between them. (Part of a Multi-author Review)

Keywords. DNA repair, genome stability, checkpoint, nonhomologous end joining, homologous recombination, repair pathway choice.

Introduction

Eukaryotic organisms have evolved multiple molecular mechanisms to ensure the integrity of their genetic information carried by the DNA molecule (reviewed by other authors in this issue). Among DNA lesions, the most harmful seems to come from the breakage of both DNA strands, since a single unrepaired DNA double-strand break (DSB) can induce cell death [1].

DSBs arise in cells from endogenous as well as from exogenous attacks on the DNA backbone. Inside cells, DNA faces nucleases and metabolic products such as reactive oxygen species. These products introduce chemical modifications in the DNA, including modified bases and sugars, DNA-protein adducts, base-free sites and tandem lesions. From the outside, ionizing radiation (IR) from the background and ultraviolet (UV) light alter the chemical composition of the DNA backbone. A large panel of chemical agents and DNA topoisomerase inhibitors used in anti-cancer therapy also modify DNA. If unrepaired, all these modifications can impede DNA replication in dividing cells and provoke DSB formation. Finally, it is believed that DSBs are created by physical stress when chromosomes are pulled to opposite poles during mitosis. Apart from DSBs arising ‘accidentally’, eukaryotic cells also produce DSBs by programmed expression of specific endonucleases for their own benefit. For instance, their repair is used for mating-type switching in yeast [2], for genetic mixing and proper chromosome segregation during meiosis in all eukaryotes [3] and for producing a diverse immune repertoire in the context of V(D)J and class-switch recombination in vertebrate cells [4].

Proper repair of DSBs is essential for genome stability. Repair of broken chromosomes is a challenge for dividing cells that need to distribute equal genetic information to daughter cells. Alteration or loss of chromosome fragments can lead to apoptosis but also to carcinogenesis with the activation of oncogenes or inactivation of tumor suppressor genes. Consequently, eukaryotic organisms have evolved multi-potent and
efficient DSB-repair mechanisms. DSB repair occurs primarily by two pathways: nonhomologous end joining (NHEJ) and homologous recombination (HR). Here we review the so-called checkpoints of eukaryotic cells that sense DSBs and trigger cell cycle arrest to allow repair, followed by a mechanistic description of both the NHEJ and HR pathways. Finally, the choice between the use of these pathways is discussed.

The cellular response to DSBs: DNA-damage checkpoints

Checkpoints were originally defined as a delay in cell cycle transition to allow time for repair to take place [5]. However, they have since been related to other functions such as transcriptional induction of DNA repair genes and post-translational modification of several other proteins. In multi-cellular organisms, DNA damage signalling can also induce apoptosis and prevent the dangerous proliferation of damaged cells, thus preserving genetic integrity in the whole organism. Among the different checkpoints, those that sense genome integrity are crucial in allowing eukaryotic cells safe progression through the cell cycle. Here we briefly review current knowledge on the factors and mechanisms of DNA-damage checkpoints.

Factors involved in the DNA-damage checkpoints

DNA-damage checkpoints consist of complex phosphorylation cascades in which DNA-damage sensors can detect unprocessed DSBs and recruit transducer kinases (Fig. 1). These are key players in the DNA damage response because they mediate the phosphorylation of several effector kinases and ultimately activate the appropriate effectors. Among the checkpoint players, the mammalian transducer kinases ATM (ataxia telangiectasia-mutated) and ATR (ataxia telangiectasia and Rad3-related) together with DNA-PKcs (DNA-dependent protein kinase catalytic subunit) play a central role in triggering the checkpoint response through activation of the effector kinases CHK1 and CHK2, which propagate the signal. Likewise, in yeast *Saccharomyces cerevisiae*, DNA lesions trigger the recruitment of ATM and ATR orthologs Tel1 and, more important, Mec1, responsible for the phosphorylation of the effector kinases Rad53, Chk1 and Dun1. In addition, several mediator proteins modulate the activity of the transducer kinases by interactions with multiple components of the DNA-damage response pathway. Thus, the *S. cerevisiae* Rad9 protein is a mediator of the checkpoint response for DNA damage occurring all over the cell cycle. Rad9 orthologs that act as ATM mediators in vertebrates include MDC1 and the breast and ovarian cancer-specific tumor suppressor BRCA1 (for an extended review on the DNA-damage response see [6]).

The checkpoint-signalling cascade

When a DSB occurs, it is primarily detected by the direct interaction of the DNA ends with a complex called MR(X)N, composed of Mre11, Rad50 and Xrs2 in yeast (Xrs2 is substituted by NBS1 in mammalian cells) (Fig. 1A) [7, 8]. This complex has multiple functions as it is involved in DSB repair and telomere maintenance. Further, being the first recognizer of the DSB, MR(X)N is involved in DNA-damage sensing. It recruits the Tel1/ATM transducer kinase triggering checkpoint activation (Fig. 1B) [9–12]. Tel1/ATM is thus recognizing and signalling unprocessed DSBs. In yeast, the signal generated by Tel1 seems sufficient to generate a checkpoint response and mediate a cell cycle arrest only when multiple unprocessed DSBs are present, but the persistence of a single unrepaired DSB leads to a G2 cell cycle arrest that depends on activation of the checkpoint-response pathway triggered by Mec1 [12]. The activation of Mec1/ATR relies on the formation of single-stranded DNA (ssDNA) [13]. DSB processing involves several nucleases (see below) that catalyze the generation of ssDNA (Fig. 1D). DSB processing is stimulated by Tel1/ATM [14–16], although it does not require the checkpoint to be activated. ssDNA is first coated by the ssDNA-binding factor RPA, which is recognized by the checkpoint transducer kinase Mec1/ATR via the cofactor Ddc2/ATRIP (Fig. 1E–F) [13, 17, 18]. In addition to Ddc2/ATRIP, full ATR activation requires RPA-mediated recruitment of a complex composed of RAD9-RAD1-HUS1 in humans and Rad17-Mec3-Ddc1 in *S. cerevisiae* (Fig. 1F) [19, 20]. This complex is structurally similar to the replication-sliding clamp PCNA (PCNA-like) and is loaded onto ssDNA by a replication factor C-like complex (RFC-like) that consists of yeast Rad24 (RAD17 in humans) in association with Rfc2, Rfc3, Rfc4 and Rfc5 replication proteins (Fig. 1F) [21].

The DNA-damage response

Once the checkpoint has been activated, transducer kinases convey a DNA-damage response through the