The RAD5-dependent Postreplication Repair Pathway is Important to Suppress Gross Chromosomal Rearrangements

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Genome instability is characteristic of cancer cells. Although it frequently occurs during carcinogenesis, the mechanism underlying genome instability is not clearly understood. Recent extensive genetic analyses from different organisms have begun to reveal mechanisms for the suppression of genome instability in general DNA metabolisms including DNA replication, recombination, DNA repair, and signal transduction. One DNA repair pathway called postreplication repair (also known as DNA damage bypass) has been highlighted for its role in genome stability. Central to DNA damage bypass, proliferating cell nuclear antigen (PCNA) directs different pathways through its mono- or polyubiquitination and sumoylation. In this review, we will discuss template switching dictated by the PCNA polyubiquitination and its roles in the suppression of genome instabilities.

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Genetic changes occurring in cancer cells known as genome instability could initiate the inactivation of tumor suppressor genes and the activation of proto-oncogenes (1,2). Small changes such as base substitutions and frameshift mutations, as well as gross chromosomal rearrangements (GCRs) including translocations, the large changes of chromosome region copy number through amplification or deletion, and aneuploidy have been documented in many cancer cells by loss of heterozygosity and comparative genome hybridization studies (3,4). It is generally accepted that the accumulation of genetic changes is required for the development and progression of cancer cells, although a single genetic alteration could produce dramatic effects causing significantly increased cancer predisposition or progression (5).

Certain mutations affecting normal DNA metabolisms as well as enhancing spontaneous DNA damage could act as mutator mutations and accelerate GCR formation during carcinogenesis (1,2). In addition, GCRs themselves could cause more mutations and facilitate further carcinogenesis. Identification of many inherited cancer-susceptibility syndromes associated with GCRs strongly suggest that defects in the repair of DNA damage generated during DNA replication as well as telomere erosion could be major sources of GCR formation (1,6).

GCR Formation by Misrepair of DNA Damage From Replication Errors

Mutations in GCR mutator genes could accelerate GCR formation by either increasing DNA damage itself or abrogating proper DNA repair of DNA damage. Both cases leave unrepaired DNA damage and consequently lead to misrepair of DNA damage to GCR formation (1).

A major source of DNA damage transformed to GCR formation is produced during DNA replication. DNA damage that blocks and stalls DNA replication ultimately results in double-strand breaks (DSBs). These strand breaks are a major source for GCR formation.

DNA damage caused by errors in DNA replication is ultimately modified to DNA DSBs and can be used as intermediates of GCR formation. This model of the DNA DSB as an intermediate for GCR formation is strongly supported by the high levels of GCR formation observed upon γ-ray irradiation, treatment with bleomycin, and from induction of a site-specific DSB (7).

Several other DNA-damaging agents also enhance GCR formation through indirect DNA DSB generation. Camptothecin enhances GCR formation by inhibiting DNA topoisomerase I resulting in a replication block, which then creates a DNA DSB (7,8). Methyl methanesulfonate (MMS) also enhances GCR formation by methylating DNA and subsequently causing a DNA DSB (7).

Interestingly, when surveying the different DNA-damaging agents for GCR enhancement capability, it was observed that UV irradiation could also be included in this group (7). The major form of DNA damage generated by UV irradiation is the thymidine–thymidine (T-T) dimer (9), but it is still not clear how these dimers are eventually converted to GCR. However, T-T dimers could persistently stall DNA replication resulting in DNA DSB generation.

Proliferating Cell Nuclear Antigen as a Central Molecule for the DNA Damage Bypass Decision

DNA damage such as a T-T dimer formed from UV irradiation or a chemically modified base can interfere with replicative DNA polymerases causing DNA replication machinery to stall. Persistent stalled replication forks can collapse, ultimately leading to genome instability or cell death. In Saccharomyces cerevisiae, stalled replication forks are resolved by bypassing the damage either with translesion synthesis (TLS) or template switching (TS) to the nascent strand of DNA.
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**Is There a TS Pathway in Mammals?**

Numerous attempts to discover the RAD5-dependent TS pathway for genomic stability in mammals have been made, including homologous RAD5 protein searches and yeast complementation screening looking for human cDNA complementation of the UV-sensitive phenotype of the yeast rad5 strain. Unfortunately, the results of those searches have been disappointing. Recently, our group utilized a different search engine called simple modular architecture research tool search (http://smart.embl-heidelberg.de/) and identified a protein called SHPRH that shares yeast Rad5 domain structures (17). We proved that SHPRH shares all known biologic activities of the yeast Rad5 protein. SHPRH and yeast Rad5 both polyubiquitinate PCNA at K164 and both enzymes build polyubiquitin chains at the RAD18 monoubiquitinated K64 of PCNA. SHPRH as well as yeast Rad5 both interact with PCNA, UBC13, and RAD18 and multimerize themselves. In addition to biologic activities, the inactivation of SHPRH in human cell lines increased sensitivity to MMS and enhanced chromosomal breakage upon MMS treatment, similar to what has been observed in the yeast rad5 strain (17). Independently, in vivo and in vitro PCNA polyubiquitination were observed (18,19). Intriguingly, DNA damage bypassed by TLS DNA polymerases results in many point mutations.

The monoubiquitinated lysine residue (K164) of PCNA has been shown to be further polyubiquitinated after the treatment of yeast cells with an alkylating agent, MMS, at a low concentration (0.02%) (11). The PCNA polyubiquitination does not occur via the canonical lysine 48 (K48)-linked polyubiquitin chain, which typically triggers protein degradation (12), but is instead through a noncanonical lysine 63 (K63)-linked polyubiquitin chain catalyzed by yRad5 (E3) and the yUbc13–yMms2 (E2 and E2 variant) heterodimeric complex (Figure 1, C), This modification of PCNA presumably promotes the error-free mode of bypass, which is thought to utilize a TS type of recombination (10) (Figure 1). However, the exact molecular mechanisms of the RAD5-dependent TS pathway are largely unknown. In addition to mono- and PCNA polyubiquitination, the same lysine residue (K164) of PCNA is differentially modified with a small ubiquitin-like modifier by a sumo ligase Siz1 when cells are exposed to a lethal level of MMS (0.3%) (11). The sumoylation of PCNA appears to recruit an anti-recombination helicase Srs2 for suppression of homologous recombination during DNA replication (13,14).

A genome-wide screen in *S. cerevisiae* developed to identify additional pathways that function to suppress GCR formation uncovered the DNA damage bypass as a putative GCR suppression pathway (15). Further analysis confirmed that the yeast Rad5-dependent TS pathway is a major pathway to suppress GCR formation (16). In this study, we suggested that the absence of the Rad5-dependent TS pathway caused aberrant homologous recombination at stalled DNA replication forks, ultimately causing DNA DSBs and GCR formation. In the same study, the PCNA sumoylation and subsequent recruitment of Srs2 were discovered to be a GCR promoting pathway.
four different mutations of SHPRH were discovered in several cancer cells including breast cancer and melanoma cells (20).

The Fork Reversion Model as an Explanation of the TS Pathway

In spite of accumulating evidence suggesting the important role of the TS pathway for genomic stability and possibly for suppression of carcinogenesis, the detailed molecular mechanisms of the TS pathway and proteins participating in this pathway are not clearly understood even in yeast. The requirement of the Rad5 protein but not the Rad51 protein in the TS pathway (21) suggests that the TS pathway might not require Rad51-coated single-stranded DNA as an intermediate structure (Figure 2). One intriguing model called “fork reversion” could explain the ability to bypass DNA damage without using Rad51 and strand invasion (Figure 2). In this model, the stalled replication forks would be reversed until the stalled replicating strand and its replicated sister strand could be hybridized. This fork reversion would allow the DNA polymerase to use the newly replicated sister strand as a template to extend the stalled replicating strand. When the DNA replication machinery reached the end of the sister strand, an X-shaped structure would then be resolved and the replication fork would be reestablished. Because the shape generated during fork reversion resembles that of a chicken foot, the fork reversion model is also known as the chicken foot model. Although the fork reversion model seems to be a very attractive molecular mechanism for the TS pathway, there is no evidence that can exclude that the template is simply switched without fork reversion to initiate the pathway. Alternatively, stalled forks are simply marked by the PCNA modification, and another replication origin in eukaryotes could be fired to continue DNA replication (22).

We characterized the initiation of the TS pathway through the PCNA polyubiquitination by Rad5/SHPRH; however, it is still unclear what the next step in this process could be. K63-mediated PCNA polyubiquitination has not been linked to the proteasomal degradation of PCNA and therefore appears to be a signal for pathway switching. If this is the case, there might be specific proteins recruited to polyubiquitinated PCNA. The discovery of proteins interacting with polyubiquitinated PCNA will help clarify these downstream events.

What could then facilitate the next step of fork reversion, which is proposed to be a change in DNA structure? Yeast Rad5 and SHPRH both have Swi2/Snf2 ATPase–helicase domains, which could promote the modification of DNA (Figure 3). Therefore, besides its role for PCNA polyubiquitination, SHPRH could also facilitate the modification of DNA structures or the remodeling of chromatin structure for fork reversion. Proteins specifically recruited through their interaction with polyubiquitinated PCNA could directly or indirectly participate in this step.

When the DNA replication fork has been reversed and the sister strand has been annealed to the reversed strand, the replicative DNA polymerase could then function in normal DNA replication mode (Figure 2). However, when DNA replication approached the end of the sister strand, the chicken foot–like DNA structure would have to be resolved. The resolution of this structure could occur through simple branch migration or perhaps a helicase could facilitate this event.

Polyubiquitinated PCNA at the reestablished DNA replication fork should be removed for normal DNA replication. This could be achieved by the degradation of the ubiquitin chain by a deubiquitinating (DUB) enzyme. A mammalian DUB enzyme, USP1, was discovered and found to deubiquitinate monoubiquitin from PCNA (23). Recently, RAP80 was identified as a DUB enzyme to deubiquitinate K63-linked polyubiquitin chain (24). It is possible that USP1 or RAP80 functions to remove the polyubiquitin chain from PCNA after the reestablishment of the DNA replication fork.

Lastly, it is still unclear whether the TLS and TS pathways are equally or specifically used in different situations. In yeast studies, the inactivation of the TS pathway by the rad5 mutation enhanced TLS-dependent mutagenesis (25–27). Therefore, at least in yeast, both pathways are somewhat in competition. It is still unclear what could trigger the activation of the different pathways. Although

Figure 2. The hypothetical molecular model of the TS pathway includes DNA replication fork reversion to create a chicken foot–like DNA structure. Several important steps are described in the clockwise direction. X and Y represent unknown interacting proteins such as resolvase or helicase.
thorough studies are necessary, it could be possible that different DNA adducts, different DNA sequences, different phases of the cell cycle, or leading vs lagging strands could affect this choice.

Emerging studies have begun to uncover the importance of the RAD5-dependent TS pathway for the suppression of genomic instability and its putative role for the suppression of carcinogenesis. Further studies that will answer many questions posed in this review and new animal models that can demonstrate its direct role in tumorigenesis will be beneficiary for understanding at least a specific role of DNA damage bypass for the suppression of cancer development.

References


Notes

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