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Review

The role of SMARCAL1 in replication fork stability and telomere maintenance

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ABSTRACT

SMARCAL1 (SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily A-Like 1), also known as HARP, is an ATP-dependent annealing helicase that stabilizes replication forks during DNA damage. Mutations in this gene are the cause of Schimke immune-osseous dysplasia (SIOD), an autosomal recessive disorder characterized by T-cell immunodeficiency and growth dysfunctions. In this review, we summarize the main roles of SMARCAL1 in DNA repair, telomere maintenance and replication fork stability in response to DNA replication stress.

1. Introduction

SNF2 (Sucrose Non Fermenting 2) is a family of ATP-dependent chromatin-remodeling enzymes conserved from yeast to humans. Members of this family are involved in various processes, such as cell cycle regulation, gene transcription, DNA recombination, DNA methylation and DNA damage repair. SNF2-family proteins contain a helicase-like ATPase domain which consists of 7 conserved motifs, similar to the ones found in many DNA and RNA helicases [1,2].

SMARCAL1, a member of the SNF2 family, was first isolated from calf thymus in 1986 [3]; it exists in different species, from *Caenorhabditis elegans* to humans, but has no apparent homologue in yeast. SMARCAL1 was shown by different groups to have roles in the maintenance of genome stability and reactivation of stalled DNA replication forks [4–6]. At its N-terminus, SMARCAL1 contains an RPA (replication protein A)-interacting motif, followed by 2 tandem HARP domains [2,7,8]; whereas, at its C-terminus lies the helicase domain, which has ATPase activity and is split into two RecA-like domains (DEXDc and HELICc) linked by a 115 amino acid long sequence (Fig. 1).

2. Role of SMARCAL1 in the DNA damage response

2.1. SMARCAL1 is recruited to sites of DNA damage through its interaction with RPA

One of the best-studied functions of SMARCAL1 is its involvement in the DNA damage response. SMARCAL1 is phosphorylated by ATR, ATM and DNA-PK checkpoint kinases that are activated in response to DNA damage. Initial studies suggested a role of SMARCAL1 at sites of DNA double-stranded breaks (DSBs). Thus, in irradiated U2OS cells, foci of SMARCAL1 are present at DNA damage sites, and these foci colocalize with a subset of γH2AX and RAD51 foci [6]. Biochemical assays also indicated that SMARCAL1 recognizes DNA ends. Specifically, using linearized plasmids bound to streptavidin-coated beads, one can extract proteins bound to DNA and then identify these proteins by mass spectrometry. Linear DNA molecules biotinylated only on one end present a free DNA end, resembling a DNA DSB, after attachment to streptavidin beads. On the contrary, if the DNA is biotinylated on both ends, both these ends are masked by the beads. By using this technique with *Xenopus laevis* egg extracts, it was found that SMARCAL1 is strongly recruited to DNA when only one DNA end is biotinylated, but not when both ends are biotinylated [6,9]. However, an alternate interpretation of these findings is that free DNA ends are processed in the Xenopus extracts, leading to the formation of single-stranded DNA (ssDNA), to which SMARCAL1 is recruited. Consistent with this interpretation, in direct binding assays, SMARCAL1 does not have high affinity for blunt DNA ends [10].

Indeed, the main function of SMARCAL1 may be in the response to DNA replication stress. Cells depleted for SMARCAL1 show hypersensitivity to agents that induce replication stress, such as hydroxyurea (HU), aphidicolin or camptothecin [4]. In gel mobility shift assays, SMARCAL1 has higher affinity for forked DNA structures, as

Abbreviations: SIOD, Schimke immune-osseous dysplasia; DSB, double-stranded break; ssDNA, single-stranded DNA; RPA, replication protein A; HU, hydroxyurea; BIR, break-induced replication; ALT, alternative lengthening of telomeres

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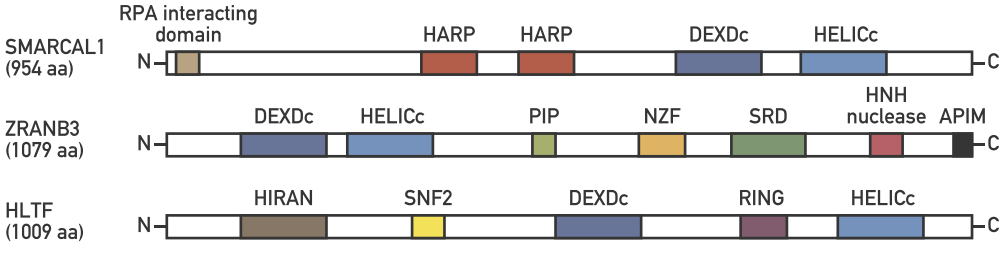

Fig. 1. Schematic representation of the protein domains of SMARCAL1, ZRANB3 and HLTF. The helicase domains of SMARCAL1, ZRANB3 and HLTF contain DEXDc and HELICc motifs. SMARCAL1 contains an RPA interacting motif at its N-terminus region and 2 HARP domains that are needed for its annealing activity. ZRANB3 contains a PIP (PCNA-interacting protein) motif, a structure recognition domain (SRD), an HNH nuclease domain and an AlkB homolog 2 PCNA interaction-motif (APIM). HLTF contains a HIRAN domain, which promotes binding to DNA, an SNF2 amino-terminal domain (SNF2), and a RING domain that possesses ubiquitin-ligase activity.

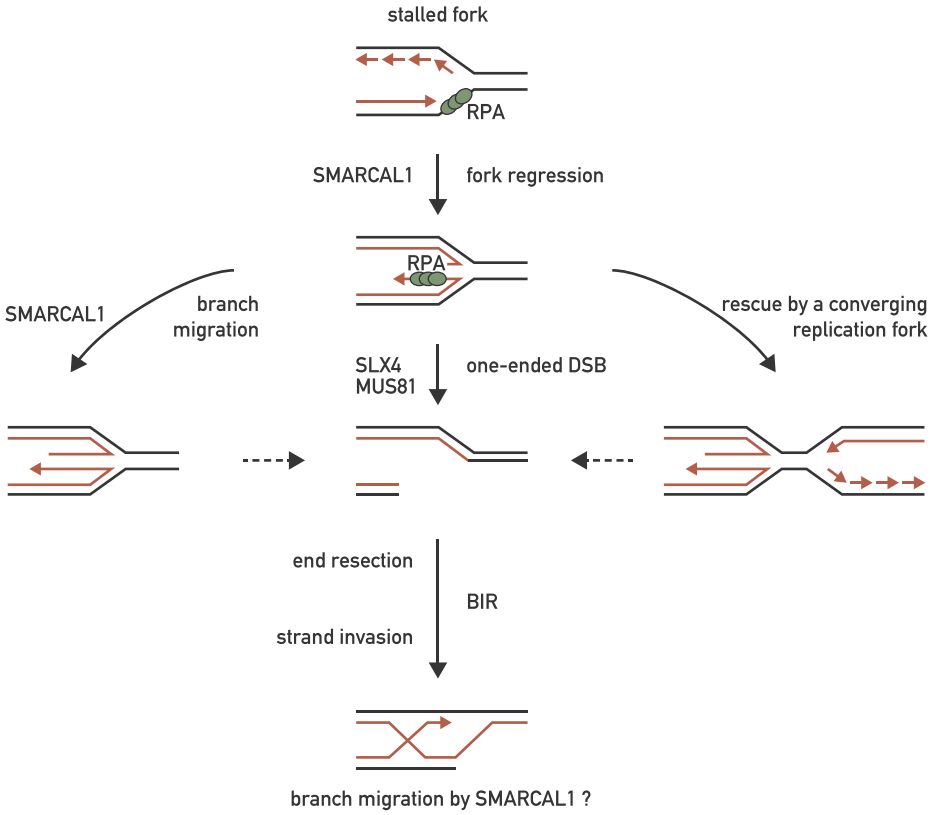

Fig. 2. The role of SMARCAL1 in the repair of damaged DNA replication forks. SMARCAL1 is recruited at stalled forks by its interaction with RPA and promotes fork regression and possibly, further branch migration of the Holliday junction present in regressed forks. Regressed forks can be rescued by nearby newly-fired converging replication forks or can be processed by the SLX4-MUS81 complex to generate one-ended DNA DSBs, which can then serve to initiate repair by BIR.

compared to ssDNA or double-stranded DNA. The ATPase activity of SMARCAL1 was also stimulated when the protein was bound to forked DNA. Based on these findings, SMARCAL1 was proposed to be an ATP-dependent helicase that unwinds DNA. However, in a helicase assay in which a partial duplex DNA was used as substrate, SMARCAL1 failed to show unwinding activity. In contrast, in an annealing helicase assay where partially unwound plasmid DNA was used as substrate in the presence of RPA and ATP, SMARCAL1 was able to rewind the ssDNA strands. Altogether, these results demonstrate that SMARCAL1 is an ATP-driven annealing helicase that can anneal complementary RPA-bound ssDNA (Fig. 2) [11].

RPA is a heterotrimeric complex, formed by RPA1, RPA2 and RPA3, which binds ssDNA during replication, recombination and repair to prevent the formation of DNA secondary structures [12]. RPA coats and protects ssDNA both in the presence and absence of DNA damage. When RPA is recruited at sites of DNA damage it serves as a scaffold protein for the recruitment of DNA repair enzymes. By microirradiation techniques and immunofluorescence, SMARCAL1 was shown to colocalize with RPA at DNA damage sites, while coimmunoprecipitation studies demonstrated that SMARCAL1 directly interacts with RPA [4–6,13]. This interaction was evident independent of DNA damage. Indeed, SMARCAL1 possesses an RPA2 interaction motif at its N-terminal region. This motif corresponds to an α-helix that can bind RPA2 [14]. A truncated construct of SMARCAL1 (31-954 aa), lacking this N- terminal motif failed to interact with RPA2. Similarly, RPA2 coimmunoprecipitation with SMARCAL1 was greatly reduced when conserved residues (RQK) at the RPA2 interaction motif were mutated [5]. Interestingly, SMARCAL1's RPA-binding sequence is very similar to the one found in other DNA repair proteins including TIPIN, a member of the replication fork protection complex, and RAD52, an important player in the replication stress response [15–20]. Importantly, impairment of the interaction between SMARCAL1 and RPA abolishes recruitment of SMARCAL1 to sites of DNA damage.

Elegant biochemical experiments have shown that SMARCAL1 can mediate the regression of stalled replication forks by annealing the nascent DNA strands [21]. Similar activity has been observed by its homologue RecG in bacteria. RecG is a DNA helicase with 3′ to 5′ polarity, which can regress stalled replication forks and perform branch migration of Holliday intermediates. Similarly to SMARCAL1, RecG interacts with SSB (single-stranded DNA binding protein), an interaction that stabilizes RecG on the DNA and promotes RecG's fork regression activity [22]. By using the iPOND procedure, the group of Cortez isolated active and stalled replication forks and showed that SMARCAL1 could be purified with nascent DNA at elongating replication forks, as well as at stalled forks induced by HU treatment [10].

A regressed DNA replication fork is a 4-way DNA structure that resembles Holliday junctions. These DNA structures can be cleaved to produce one-ended DNA DSBs that can then be used to initiate