MOLECULAR & CELLULAR ONCOLOGY
2019, VOL. 6, NO. 6, e1648025 (3 pages)
https://doi.org/10.1080/23723556.2019.1648025

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AUTHOR'S VIEW

Compartmentalized DNA repair: Rif1 *S*-acylation links DNA double-strand break repair to the nuclear membrane

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ABSTRACT

DNA double-strand breaks (DSBs) disrupt the structural integrity of chromosomes. Proper DSB repair pathway choice is critical to avoid the type of gross chromosomal rearrangements that characterize cancer cells. Recent findings reveal *S*-fatty acylation and membrane anchorage of Rap1-interacting factor 1 (Rif1) as a mechanism providing spatial control over DSB repair pathway choice.

ARTICLE HISTORY
Received 15 July 2019
Revised 19 July 2019
Accepted 23 July 2019

KEYWORDS
NHEJ; HR; chromosome stability; protein palmitoylation; DHHC palmitoyl transferases; Pfa4

DNA double-strand breaks (DSBs) pose a direct threat to chromosome stability and must be repaired in a timely manner to avoid chromosomal translocations or loss. Cells have two competing DSB repair pathways at their disposal: non-homologous end-joining (NHEJ) and homologous recombination (HR).[1] NHEJ is a DNA splicing mechanism that entails DNA end-protection, end-bridging, alignment of DNA ends, and ligation. The pathway is very effective but associated with the risk of DNA sequence alterations at the repair joint and even gross chromosomal aberrations by ligation of DNA ends that did not originate from the same DSB. HR is a more elaborate DSB repair pathway that uses an intact donor DNA with sequence homology to the break site. In a process called DNA end-resection, nucleases first generate 3′-single-stranded DNA. This is followed by homology search and strand invasion of a suitable donor duplex, where the invading 3′-single-strand initiates DNA repair synthesis. Resolution of the joint molecule, consisting of the broken molecule and the intact donor, ultimately restores the DSB site to its original sequence. DSB repair by HR is considered an error-free process, although non-allelic recombination can result in chromosome rearrangements. It is perhaps not surprising then that DSB repair pathway choice is highly regulated to ensure faithful repair at chromosome breaks. NHEJ or HR are used in a context-specific manner dependent on cell-cycle stage, chromatin status, and subnuclear location of DSBs.[1,2]

At the nexus of DSB repair pathway choice lies the antagonism between DNA end-protection, which preserves the option of NHEJ, and DNA end-resection, which commits to HR repair.[1] In mammalian cells, DNA end-resection is promoted by breast cancer type 1 susceptibility protein (BRCA1) in conjunction with C-terminal binding protein (CtBP)-interacting protein (known as CtIP). BRCA1-CtIP interactions are cell-cycle-regulated and promoted in the S and G2 phases, ensuring that end-resection and HR operate at a time when sister chromatids in close proximity can serve as homologous DSB repair templates for one another. DNA end-resection is counteracted along an axis defined by tumor protein 53 (TP53)-binding protein 1 (TP53BP1, better known as 53BP1) and Rap1-interacting factor 1 (RIF1). Thus, 53BP1-RIF1 limit the formation of microscopically visible BRCA1 foci at DSBs, and, with downstream effectors that constitute the so-called shieldin complex, preserve DSB ends for NHEJ.[3] Much of the current interest in the molecular underpinnings of DSB repair pathway choice arises from the success of poly (ADP-ribose) polymerase inhibitors (PARPi) in cancer therapy.[4] BRCA1-deficent tumors are exquisitely sensitive to PARPi, reflecting an accumulation of DNA lesions that would normally be repaired by HR. However, acquired PARPi resistance occurs in the clinic. In BRCA1-defective cells, PARPi resistance is conferred by mutations affecting end-protection along the 53BP1-RIF1 axis, which restores DNA end-resection and DSB repair by HR. A detailed understanding of these pathways is therefore of biomedical importance.

53BP1 and RIF1 are recruited to DSBs through interactions between 53BP1 and DNA damage-induced histone modifications around chromosome breaks. RIF1's mode of action relates to its ability to bind shieldin, providing a bridge between 53BP1 and downstream effector proteins that physically interact with DNA ends to attenuate end-resection.[3] Crystal structure analysis of the conserved N-terminal domain of Rif1 from *Saccharomyces cerevisiae* has revealed an elongated, all-α-helical fold resembling the shape of a shepherd's crook.[5] The "hook", located at its N-terminal tip, contains a high-affinity DNA-binding site that is required for Rif1 to mediate NHEJ, and also to fulfill a yeast-specific role in telomere length control.[5] Thus, in budding yeast, direct engagement with DNA ends enables Rif1 to function in

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distinct biological processes by gating access of end-processing factors including telomerase and the end-resection machinery.[6]

Recently, we found that Rif1-mediated NHEJ not only depends on Rif1's DNA-binding activity, but surprisingly, also its posttranslational *S*-fatty acylation.[7] Rif1 *S*-acylation is mediated by protein fatty acyltransferase 4 (Pfa4), a member of the conserved DHHC family of palmitoyl acyltransferases, but the functional significance of this post-translational modification had remained unclear.[8] Palmitoylation, the addition of 16-carbon fatty acid moieties to cysteine residues, is the most common type of protein *S*-acylation with hundreds of confirmed and putative protein targets.[9] The increased hydrophobicity of *S*-acylated proteins promotes protein–membrane interactions and impacts protein trafficking, compartmentalization, stability, and function. A well-characterized example is provided by the HRas and KRas GTPases whose palmitoylation is required for plasma membrane localization and effective signal transduction. Very little is currently known about the importance of *S*-acylation of nuclear proteins and the functional role of *S*-acylation-mediated inner nuclear membrane interactions. Interestingly, protein *S*-acylation has not previously been implicated in DNA repair reactions.

Systematic mutation of surface-exposed cysteine residues to alanine identified Rif1 cysteine residues 466 and 473 (C466 and C473) as alternative *S*-acylation sites required for the accumulation of Rif1 at endonuclease-induced DSBs, the attenuation of DNA end-resection, and efficient DSB repair by NHEJ.[7] Using a method we termed acyl-carbamidomethyl exchange (ACE) for the replacement of *S*-fatty modifications with a chemical moiety more amenable to detection by mass spectrometry, we verified *S*-acylation of C466 and C473 *in vivo*. C466/C473 *S*-acylation promoted Rif1-membrane interactions and proved essential for the formation of microscopically visible Rif1 foci in response to DSBs induced by ionizing radiation or radiomimetic drug Zeocin. These DNA damage-induced Rif1 foci located exclusively to the nuclear periphery, consistent with enhanced membrane interactions of Rif1 through *S*-fatty acylation. In contrast to Rif1 foci, HR repair foci – marked by the recombinase Rad52 (encoded by radiation-sensitive gene *RAD52*) – are usually observed at luminal positions, away from the inner nuclear membrane. These findings are consistent with an element of spatial control in DSB repair pathway choice, where *S*-acylation-mediated residency of Rif1 at the inner nuclear membrane establishes a subnuclear compartment geared towards NHEJ (Figure 1).[7]

The compartmentalization of DSB repair raises interesting possibilities of designating repair pathway choices through nuclear positioning of chromosomal regions. Rif1 *S*-acylation would conceivably bias DSB repair within nuclear envelope-associated heterochromatin or near telomeres towards NHEJ, which could protect from non-allelic recombination within repetitive DNA sequences. It is also interesting to consider that the ability to effectively tether DSB ends to the inner nuclear membrane might have a direct impact on the efficiency of NHEJ by assisting the coordination of DNA ends for ligation. In a thought-provoking parallel to our findings in yeast, NHEJ is favored within nuclear lamina-associated chromatin in human cells.[10] It is currently not known whether these observations relate to inner nuclear membrane interactions of human RIF1. Going forward, it will be important to determine the *S*-acylation status of mammalian RIF1, and to address the possibility and potential

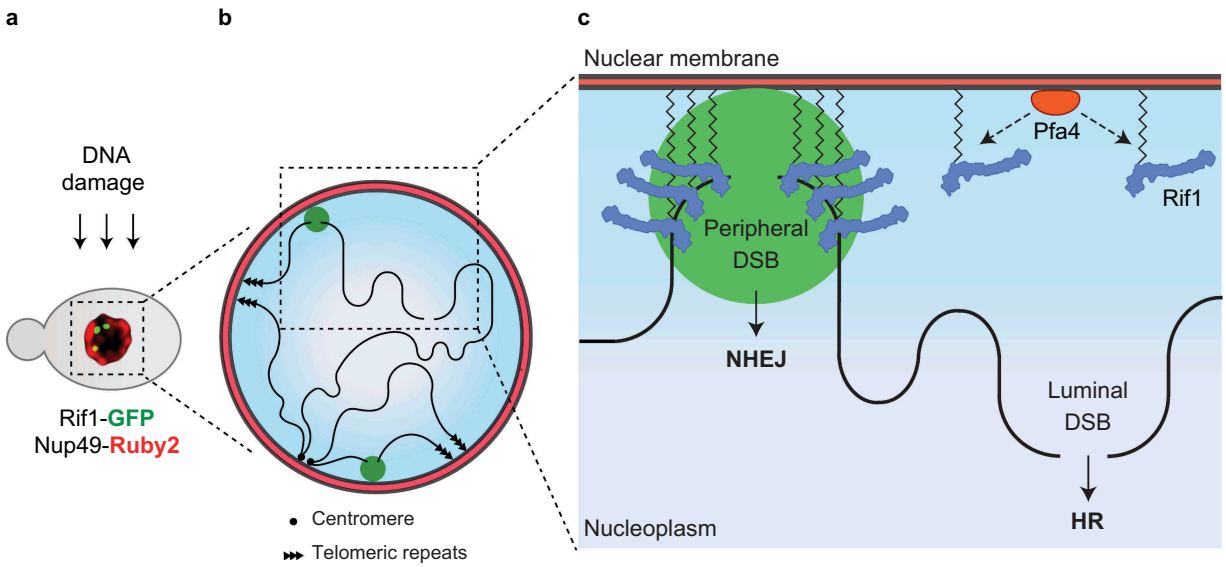

Figure 1. Compartmentalized DNA double-strand break (DSB) repair pathway choice mediated by *S*-acylated Rap1-interacting factor 1 (Rif1). (a) *Saccharomyces cerevisiae* Rif1 forms nuclear-peripheral foci in response to DSB-inducing agents. Z-projected confocal microscopy image shows the nuclear envelope labeled by nuclear pore protein 49 (Nup49) fused to a red fluorescent protein tag (Ruby2). A version of Rif1 that is proficient for DSB repair but devoid of telomere interaction motifs is expressed as fusion with a green fluorescent protein tag (GFP).[7] (b) Rif1 foci are strongly biased towards the nuclear periphery. This indicates an accumulation of Rif1 at DSBs within chromosomal regions attached to or near the inner nuclear membrane, and its absence from more luminal DSBs. (c) Membrane anchorage of Rif1 by protein fatty acyltransferase 4 (Pfa4)-dependent *S*-acylation of cysteine residues 466/473 (indicated as a zig-zag line). High local concentration of Rif1 at the inner nuclear membrane sets up a nuclear-peripheral zone in which DNA end-resection and homologous recombination (HR) is attenuated, favoring DSB repair by non-homologous end-joining (NHEJ).

biomedical implications of RIF1 promoting DSB repair pathway choice at the nuclear periphery in human cells.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

For the work described here, the authors have received support by the Novartis Research Foundation.

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