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

Gabriele A. Fontana and Ulrich Rass

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.
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 by 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

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**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.

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