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# RIF1: A novel regulatory factor for DNA replication and DNA damage response signaling☆

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ABSTRACT

DNA double strand breaks (DSBs) are highly toxic to the cells and accumulation of DSBs results in several detrimental effects in various cellular processes which can lead to neurological, immunological and developmental disorders. Failure of the repair of DSBs spurs mutagenesis and is a driver of tumorigenesis, thus underscoring the importance of the accurate repair of DSBs. Two major canonical DSB repair pathways are the non-homologous end joining (NHEJ) and homologous recombination (HR) pathways. 53BP1 and BRCA1 are the key mediator proteins which coordinate with other components of the DNA repair machinery in the NHEJ and HR pathways respectively, and their exclusive recruitment to DNA breaks/ends potentially decides the choice of repair by either NHEJ or HR. Recently, Rap1 interacting factor 1 has been identified as an important component of the DNA repair pathway which acts downstream of the ATM/53BP1 to inhibit the 5′–3′ end resection of broken DNA ends, in-turn facilitating NHEJ repair and inhibiting homology directed repair. Rif1 is conserved from yeast to humans but its function has evolved from telomere length regulation in yeast to the maintenance of genome integrity in mammalian cells. Recently its role in the maintenance of genomic integrity has been expanded to include the regulation of chromatin structure, replication timing and intra-S phase checkpoint. We present a summary of these important findings highlighting the various aspects of Rif1 functions and discuss the key implications for genomic integrity.



## 1. Introduction

### 1.1. Rif1 evolved from yeast to humans

Rap1 interacting factor 1 (Rif1) was identified in *Saccharomyces cerevisiae* as being important for the maintenance of telomeric length [1]. The repressor/activator protein 1 (Rap1) binds to telomeric repeat tract and maintain telomeric length by a negative feedback loop. Rap1 recruits Rif1 and Rif2 via its C-terminus, and the same domain facilitates the recruitment of the silencing protein Sir3p and Sir4p [1–3]. Later its orthologue was identified in *Schizosaccharomyces pombe*. Unlike budding yeast Rif1 (herein *ScRif1*), the fission yeast Rif1 (herein *SpRif1*) does not bind to Rap1 and is recruited to telomeres through a different telomeric protein Taz1, where it promotes telomere length homeostasis [2,4]. Rif1 is a part of the telomeric complex and its involvement in the inhibition of telomeric end resection has only recently been established [5,6]. Presence of Rif1 orthologues in vertebrates suggests important functions of Rif1 which has evolved in complex eukaryotic organisms [7].

High expression of mouse Rif1 was detected in totipotent and pluripotent cells, as well as in the testes [7]. Bioinformatic analysis reveals a characteristic HEAT repeat domain in Rif1 homologues found in yeast, invertebrates and vertebrates [8]. X-ray structural study of the yeast Rif1 has only recently been reported. This study reveals that Rif1 and Rif2 bind to Rap1 C-terminal domain via two independent Rap1 binding epitopes. Specifically, the C-terminal domain of Rif1 serves as Rap1 binding and tetramerisation sites [9]. However, a C-terminal protein interaction domain unique to the mammalian Rif1 is required for its interaction with BLM and the chromatin recruitment of BLM [10]. Unlike mammalian Rif1, drosophila Rif1 do not localize to sites of DNA damage, suggesting



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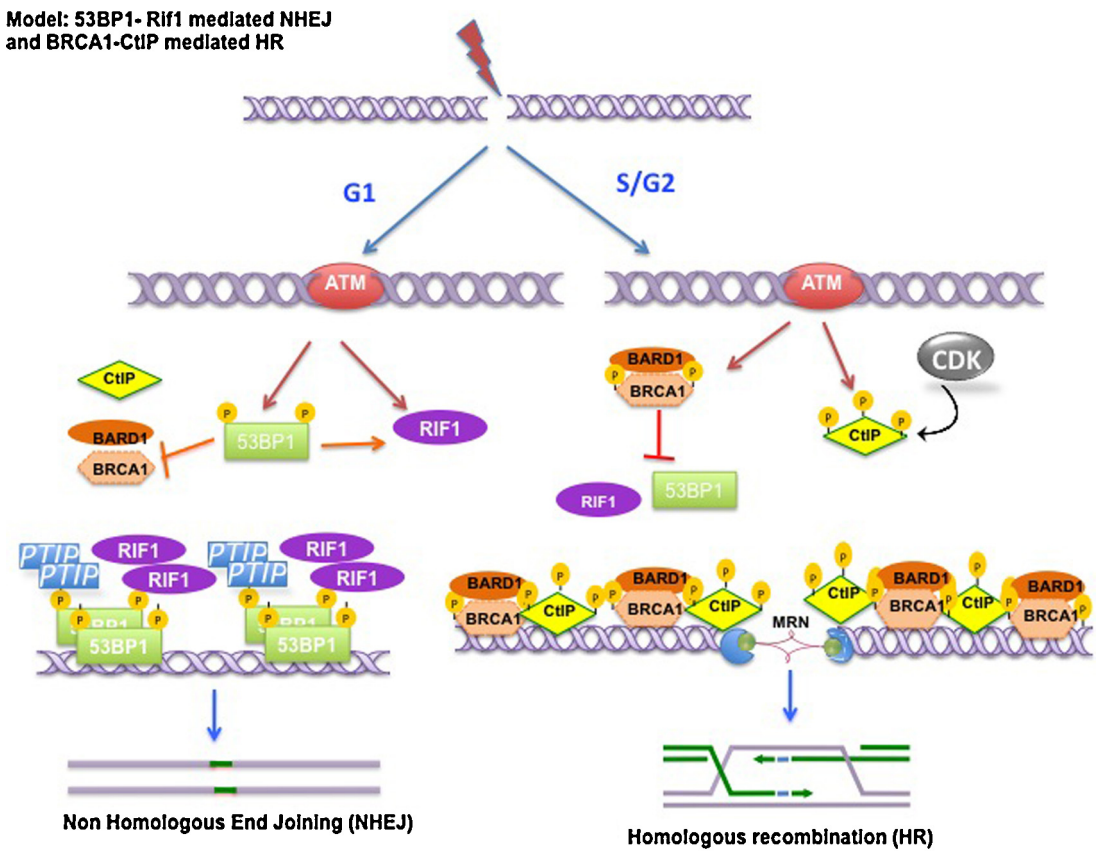


**Fig. 1.** 53BP1/Rif1 mediated NHEJ and BRCA1-CtIP mediated HR facilitates the DSBs repair during different phages of the cell cycle. In response to DNA double strand break, ATM dependent phosphorylation of checkpoint signaling molecules facilitate DNA double strand break repair by two main processes, NHEJ (favored in G1 cells) and HR (favored in S/G2 cells). Due to lack of a sister chromatid, 5′ end resection is suppressed and HR is inhibited in G1. RIF1 has been identified as a suppressor of BRCA1, a protein that facilitates the break resection. ATM phosphorylated 53BP1 recruits RIF1 which in turn inhibits the 5′–3′ end resection. ATM phosphorylated 53BP1 also binds to the RIF1 and PTIP via its C and N-terminus respectively. In BRCA1 deficient cells, in contrast to Rif1 depletion, PTIP ablation supports the continuous resection required to rescue the HR. During S/G2 phase of the cell cycle, ATM phosphorylated BRCA1/BARD1 complex is recruited at the sites of DNA DSBs, which in turn negatively regulates the RIF1 functions. CDK phosphorylation of CtIP promotes its interaction with BRCA1 and it also binds to the MRE11 complex to facilitate the nucleolytic resection of the 5′ end to generate the homology ends required for HR-mediated DNA DSBs repair.

a functional diversification of Rif1 in vertebrates and invertebrate [8]. Human Rif1 was identified by blast search where a significant similarity of RIF1 sequence was observed with *SpRif1* and *ScRif1* [1,4; Rif Gene Bank accession no. AY585745]. To understand the functional conservancy between *ScRif1* and human Rif1, Xu and Blackburn overexpressed human Rif1 in wild type and *rif2Δ* yeast cells which resulted in significant telomere elongation suggesting that the telomeric function of Rif1 is somewhat conserved from yeast to humans [11].

## 2. Rif1, an important component of DDR signaling

Unlike *ScRif1*, human Rif1 protein only binds aberrant telomeres. In human cells, uncapped telomeric ends are recognized as DNA damage site which promotes the recruitment of DDR factors including NBS1, ATM, 53BP1, Rad17 and γ-H2AX [12,13]. The pattern of foci generated at the aberrant telomeric ends is quite similar to DNA damage-induced foci and are referred to as telomere dysfunction-induced foci or TIFs [14]. Indeed, DNA damage-induced RIF1 foci formation was detected in cells treated with various DNA damaging agents including etoposide, hydroxyurea (HU) and ultraviolet light (UV), suggesting a potential role of human Rif1 in DDR signaling. Rif1 foci colocalise with 53BP1 foci but was completely abolished in 53BP1 depleted cells, attributing a significant role of 53BP1, a key player in the NHEJ pathway, to the regulation of Rif1 function [14]. Given the role of Rif1 in DDR signaling, recently many research groups are trying to understand the molecular mechanism of Rif1 function and so far their findings are quite encouraging.

### 2.1. Rif1 specifically acts in the ATM/53BP1 signaling pathway

Ataxia telangiectasia mutated (ATM) and ATM and Rad3-related (ATR) protein kinase signaling are central to the DNA damage response and repair pathways. DNA damage-induced Rif1 foci was reduced in ATM signaling defective cells, as demonstrated in two independent patient-derived AT cell lines. Moreover, Rif1 foci formation was severely reduced in cells treated with PIKKs (PI3 kinase-related family of kinases) inhibitors. Likewise, in the absence of 53BP1, IR induced Rif1 foci formation was reduced. However, in response to UV treatment, Rif1 foci formation was unaffected in ATRIP (an essential component of the ATR signaling) depleted cells suggesting an ATM dependent but ATR independent regulation of Rif1 function [14]. These findings clearly indicate that Rif1 DDR function is regulated by ATM and 53BP1 [14]. Rif1 depleted HeLa cells displayed normal levels of IR-induced ATM, Nbs1, Chk1, BRCA1, and p53 phosphorylation and IR-induced 53BP1 foci formation, however, these cells display increased radiosensitivity similar to ATM or 53BP1 deficient cells [15,16], suggesting that Rif1 do not directly affect the checkpoint signaling functions of the key DDR checkpoint proteins but may have a more direct impact on the repair processes. Fanconi anemia (FA) and ataxia telangiectasia (AT) share some common characteristics