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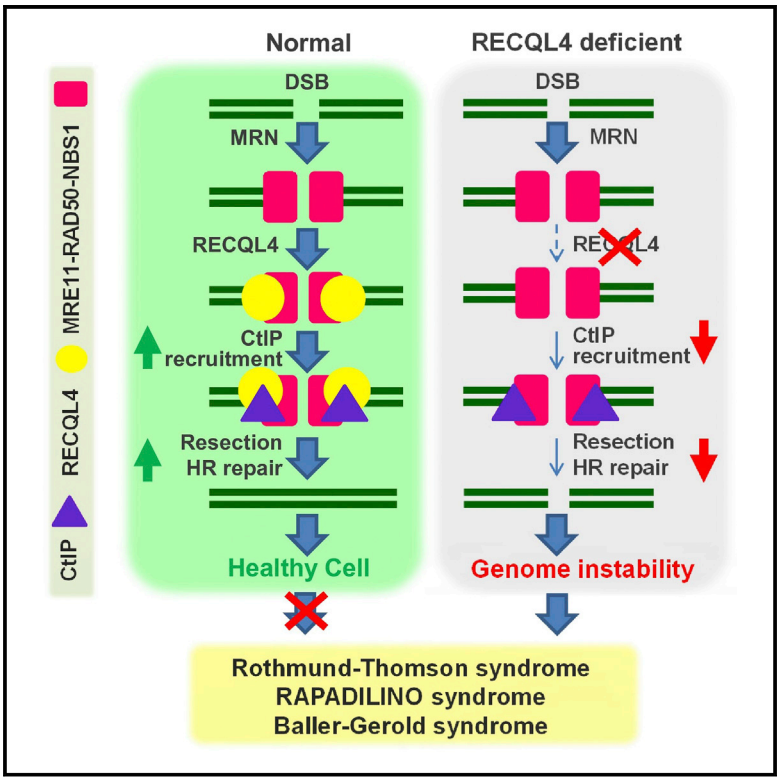
RECQL4 Promotes DNA End Resection in Repair of DNA Double-Strand Breaks

Graphical Abstract



Authors

Huiming Lu, Raghavendra A. Shamanna, Guido Keijzers, ..., Petr Cejka, Deborah L. Croteau, Vilhelm A. Bohr

Correspondence

vbohr@nih.gov

In Brief

RECQL4, a RecQ helicase mutated in Rothmund-Thomson syndrome, is a guardian of genome stability and repairs DNA, but the underlying mechanisms remain unclear. Lu et al. show that RECQL4 plays a role in homologous recombination repair of DNA double-strand breaks (DSBs). RECQL4 promotes 5′ DNA end resection through the MRE11-RAD50-NBS1 and CtIP complexes.

Highlights

- RECQL4 promotes 5′ end resection at DSBs
- RECQL4 recruitment to DSBs depends on MRE11
- RECQL4 promotes recruitment of CtIP to DSBs
- RECQL4 helicase activity is required for 5′ DNA end resection

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RECQL4 Promotes DNA End Resection in Repair of DNA Double-Strand Breaks

Huiming Lu,[1,4] Raghavendra A. Shamanna,[1,4] Guido Keijzers,[2] Roopesh Anand,[3] Lene Juel Rasmussen,[2] Petr Cejka,[3] Deborah L. Croteau,[1] and Vilhelm A. Bohr[1,2,*]
[1]Laboratory of Molecular Gerontology, National Institute on Aging, National Institutes of Health, Baltimore, MD 21224, USA
[2]Center for Healthy Aging and Department of Cellular and Molecular Medicine, University of Copenhagen, 2200 Copenhagen, Denmark
[3]Institute of Molecular Cancer Research, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland
[4]Co-first author
*Correspondence: vbohr@nih.gov
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SUMMARY

The RecQ helicase RECQL4, mutated in Rothmund-Thomson syndrome, regulates genome stability, aging, and cancer. Here, we identify a crucial role for RECQL4 in DNA end resection, which is the initial and an essential step of homologous recombination (HR)-dependent DNA double-strand break repair (DSBR). Depletion of RECQL4 severely reduces HR-mediated repair and 5′ end resection in vivo. RECQL4 physically interacts with MRE11-RAD50-NBS1 (MRN), which senses DSBs and initiates DNA end resection with CtIP. The MRE11 exonuclease regulates the retention of RECQL4 at laser-induced DSBs. RECQL4 also directly interacts with CtIP via its N-terminal domain and promotes CtIP recruitment to the MRN complex at DSBs. Moreover, inactivation of RECQL4's helicase activity impairs DNA end processing and HR-dependent DSBR without affecting its interaction with MRE11 and CtIP, suggesting an important role for RECQL4's unwinding activity in the process. Thus, we report that RECQL4 is an important participant in HR-dependent DSBR.

INTRODUCTION

DNA double-strand breaks (DSBs) are generated by exogenous stress, endogenous replication, and programmed recombination events. Improperly repaired DSBs can lead to genome instability, chromosomal rearrangements, and/or cell death (Symington, 2014). DSBs are usually repaired by one of two major pathways: homologous recombination (HR) and non-homologous end joining (NHEJ) (Aparicio et al., 2014). HR-dependent DSBR is mostly error free, but it requires a sister or non-sister chromatid as template and is only active during the S and G2 phases of the cell cycle. In contrast, NHEJ-dependent DSBR is error prone, DNA template-independent, and active during all phases of the cell cycle.

HR-dependent DSBR is initiated by 5′ end resection of the DSBs, which generates 3′ protruding single-strand DNA (ssDNA) tails (Chen et al., 2013; Zhu et al., 2008). RPA coats the ssDNA, and then RAD51 replaces RPA to promote strand invasion. This is followed by repair synthesis, dissolution, and resolution of Holliday junctions and ligation of the ends (Prakash et al., 2015). It is generally considered that DNA end resection occurs in two steps (Cejka et al., 2010; Gravel et al., 2008; Mimitou and Symington, 2008; Nimonkar et al., 2011; Niu et al., 2010; Zhu et al., 2008). The first step is the initial resection by Mre11-Rad50-Xrs2 (MRX) and Sae2 at the DSB in yeast (Cannavo and Cejka, 2014; Mimitou and Symington, 2008) or by MRE11-RAD50-NBS1 (MRN) and CtIP (CtBP-interacting protein) in human cells (Sartori et al., 2007; You et al., 2009). This is followed by extensive resection by either exonuclease1 (EXO1) or DNA2/BLM/TOP3/RMI1/2 (Dna2/Sgs1/Top3/Rmi1 in yeast) (Cejka et al., 2010; Gravel et al., 2008; Mimitou and Symington, 2008; Nimonkar et al., 2008, 2011; Niu et al., 2010; Zhu et al., 2008).

RECQL4 is one of five RecQ helicase proteins in mammalian cells. Defects in human RECQL4 are associated with three genetic diseases: Rothmund-Thomson syndrome (RTS), RAPADILINO, and Baller-Gerold syndrome (Siitonen et al., 2009) as well as several cancers (Fang et al., 2013; Lu et al., 2014b; Su et al., 2010). It is well established that RECQL4 is required for the assembly of the DNA replication initiation machinery (Im et al., 2009; Sangrithi et al., 2005; Xu et al., 2009). However, the role of RECQL4 in DNA repair is less clear (Croteau et al., 2014). Lack of RECQL4 increases persistent DNA damage and triggers cellular senescence in human and mouse primary fibroblasts (Lu et al., 2014a). RECQL4 is recruited to laser-induced DSBs and RTS fibroblasts are sensitive to ionizing radiation (IR), suggesting that RECQL4 plays a role in DSBR (Singh et al., 2010). Recently, we showed that depletion of RECQL4 inhibits NHEJ in U2OS cells (Shamanna et al., 2014). Nevertheless, RECQL4 is highly expressed during S phase (Singh et al., 2012; Xu et al., 2009), when HR-dependent DSBR dominates. Thus, we explore the possibility that RECQL4 also plays a role in HR-dependent DSBR. We find that RECQL4 promotes DNA end resection and HR-dependent DSBR by stimulating the association of CtIP with MRN at DSBs and that the helicase activity of RECQL4 is necessary for DNA end resection. Together, these