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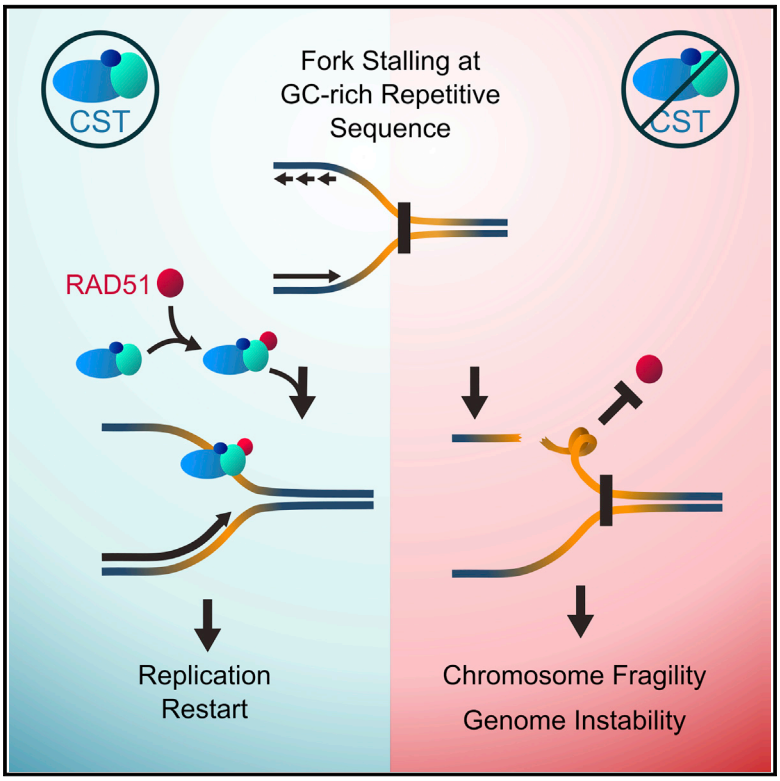
Human CST Facilitates Genome-wide RAD51 Recruitment to GC-Rich Repetitive Sequences in Response to Replication Stress

Graphical Abstract



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In Brief

Chastain et al. find that under replication stress, the telomeric complex CST interacts with RAD51 and is enriched at GC-rich repetitive fragile sites. CST suppression inhibits RAD51 recruitment to fragile sites, resulting in genome instability.

Highlights

- STN1 is enriched at GC-rich repetitive sequences in response to replication stress
- STN1 suppression exacerbates the fragility of these sequences under replication stress
- CST interacts with RAD51 in an ATR-dependent manner
- CST deficiency diminishes RAD51 foci formation and recruitment to fragile sequences

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Human CST Facilitates Genome-wide RAD51 Recruitment to GC-Rich Repetitive Sequences in Response to Replication Stress

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SUMMARY

The telomeric CTC1/STN1/TEN1 (CST) complex has been implicated in promoting replication recovery under replication stress at genomic regions, yet its precise role is unclear. Here, we report that STN1 is enriched at GC-rich repetitive sequences genome-wide in response to hydroxyurea (HU)-induced replication stress. STN1 deficiency exacerbates the fragility of these sequences under replication stress, resulting in chromosome fragmentation. We find that upon fork stalling, CST proteins form distinct nuclear foci that colocalize with RAD51. Furthermore, replication stress induces physical association of CST with RAD51 in an ATR-dependent manner. Strikingly, CST deficiency diminishes HU-induced RAD51 foci formation and reduces RAD51 recruitment to telomeres and non-telomeric GC-rich fragile sequences. Collectively, our findings establish that CST promotes RAD51 recruitment to GC-rich repetitive sequences in response to replication stress to facilitate replication restart, thereby providing insights into the mechanism underlying genome stability maintenance.

INTRODUCTION

Faithful and complete duplication of chromosomal DNA is vital for avoiding detrimental replication errors and preserving genome stability. Replication stress, induced by exposure to environmental agents, oncogenic stress, or partial inhibition of DNA replication, results in fork stalling at fragile sites (FSs) that may lead to fork collapse, thereby generating DNA breaks that trigger unwanted repair or rearrangement activities and driving genome instability (Debacker and Kooy, 2007; Debatisse et al., 2012; Durkin and Glover, 2007; Tercero et al., 2003). FSs are frequently involved in sister chromatid exchanges, deletions, translocations, and intra-chromosomal gene amplifications (Durkin and Glover, 2007). Important genes, including certain tumor suppressors, have been identified within FSs (Arlt et al., 2006; Barlow et al., 2013; Debacker and Kooy, 2007; Durkin and Glover, 2007; Ozeri-Galai et al., 2012). Therefore, pathways have evolved to prevent fork stalling and to facilitate the restart of stalled replication to preserve genome stability.

Successful rescue of stalled replication requires coordination of multiple proteins that stabilize stalled forks and promote reinitiation of DNA synthesis (Franchitto and Pichierri, 2014; Zeman and Cimprich, 2014). Crucial genome maintenance proteins, including RAD51, MRE11, XRCC3, SLX1-SLX4-MUS81-EME1, BLM, WRN, RTEL1, SMARCAL1, and FANCD2, play important roles in this process (Bétous et al., 2012; Bryant et al., 2009; Davies et al., 2007; Franchitto and Pichierri, 2004; Hanada et al., 2007; Hashimoto et al., 2010; Pepe and West, 2014; Petermann and Helleday, 2010; Petermann et al., 2010; Sarbajna et al., 2014; Schlacher et al., 2012; Sidorova et al., 2008; Tittel-Elmer et al., 2009; Vannier et al., 2013). In addition, TIMELESS, TIPIN, CLASPIN, and AND1 form the replication protection complex that stabilizes stalled forks and keeps helicases connected to polymerases, thus preventing excessive DNA unwinding (Chini and Chen, 2003, 2004; Errico et al., 2007, 2009; Gotter et al., 2007; Kemp et al., 2010; Kumagai and Dunphy, 2000; Lee et al., 2003; Unsal-Kaçmaz et al., 2007; Zhu et al., 2007). Fork restart also requires reinitiation of DNA synthesis mediated by various replication factors, including MCM2-7, PCNA, CDC45, and POLδ (Heller and Marians, 2006), and the PrimPol primase that is important for priming DNA synthesis at stalled forks (Bianchi et al., 2013; García-Gómez et al., 2013; Mourón et al., 2013).

The human CST complex (hCST), composed of the three proteins CTC1, STN1, and TEN1, has emerged as an important player in counteracting replication stress. CST is an RPA-like complex that binds non-specifically to single-stranded DNA (ssDNA) with high affinity (Miyake et al., 2009). Originally discovered as a telomere maintenance factor (described later), the hCST complex also promotes efficient replication of difficult-to-replicate sequences in the genome (Kasbek et al., 2013; Stewart et al., 2012). Deficiency in CST components reduces cell viability after exposure to reagents stalling replication forks,