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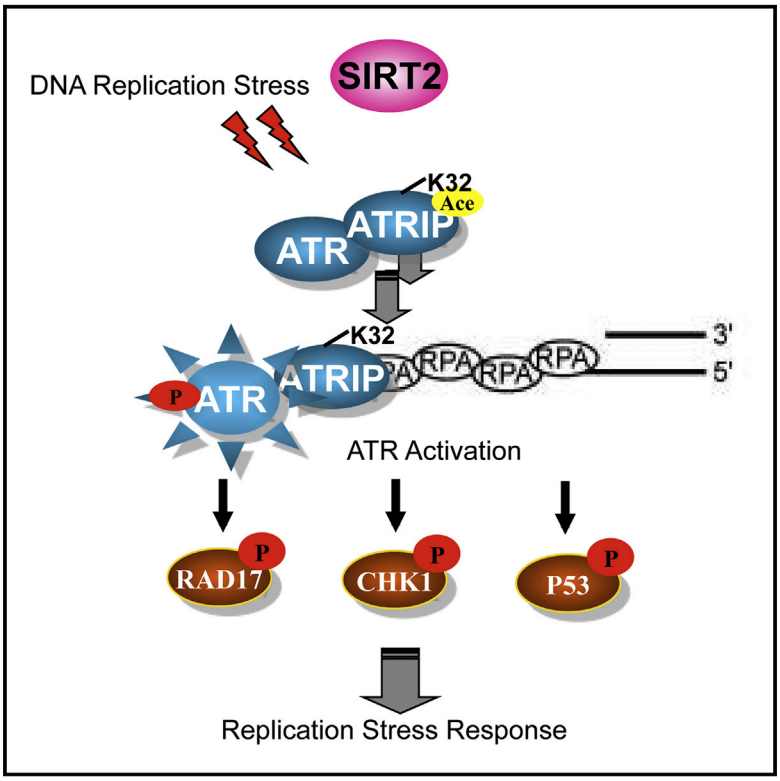
ATRIP Deacetylation by SIRT2 Drives ATR Checkpoint Activation by Promoting Binding to RPA-ssDNA

Graphical Abstract



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In Brief

Zhang et al. demonstrate that ATRIP deacetylation at conserved lysine 32 by SIRT2 promotes ATR-ATRIP binding to RPA-ssDNA to drive ATR activation and thus facilitate recovery from replication stress.

Highlights

- SIRT2 interacts with and deacetylates ATRIP at K32 in vitro and in cells
- ATRIP deacetylation by SIRT2 promotes ATR activation and replication stress recovery
- ATRIP deacetylation by SIRT2 promotes accumulation to sites of DNA damage
- SIRT2 deacetylation of ATRIP at K32 promotes its direct binding to RPA-ssDNA

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ATRIP Deacetylation by SIRT2 Drives ATR Checkpoint Activation by Promoting Binding to RPA-ssDNA

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SUMMARY

The ataxia telangiectasia-mutated and Rad3-related (ATR) kinase checkpoint pathway maintains genome integrity; however, the role of the sirtuin 2 (SIRT2) acetylome in regulating this pathway is not clear. We found that deacetylation of ATR-interacting protein (ATRIP), a regulatory partner of ATR, by SIRT2 potentiates the ATR checkpoint. SIRT2 interacts with and deacetylates ATRIP at lysine 32 (K32) in response to replication stress. SIRT2 deacetylation of ATRIP at K32 drives ATR autophosphorylation and signaling and facilitates DNA replication fork progression and recovery of stalled replication forks. K32 deacetylation by SIRT2 further promotes ATRIP accumulation to DNA damage sites and binding to replication protein A-coated single-stranded DNA (RPA-ssDNA). Collectively, these results support a model in which ATRIP deacetylation by SIRT2 promotes ATR-ATRIP binding to RPA-ssDNA to drive ATR activation and thus facilitate recovery from replication stress, outlining a mechanism by which the ATR checkpoint is regulated by SIRT2 through deacetylation.

INTRODUCTION

The precise replication of the genome and the continuous surveillance of its integrity are essential for cell survival and the avoidance of various diseases, including cancer and premature aging. The genome is constantly exposed to environmental and endogenous genotoxic insults that challenge DNA replication. To cope with this challenge, the replication stress response (RSR), a subset of the DNA damage response (DDR), coordinates diverse DNA repair and cell-cycle checkpoint signaling pathways necessary to maintain genome integrity. The ataxia telangiectasia mutated and Rad3-related (ATR) checkpoint kinase and its regulatory partner, ATR interacting protein (ATRIP), function at the apex of the RSR (Cimprich and Cortez, 2008; Marechal and Zou, 2013; Zeman and Cimprich, 2014). *ATR* deficiency causes embryonic lethality (Brown and Baltimore, 2000) and premature aging in adult mice (Ruzankina et al., 2007), and hypomorphic mutations in *ATR* and *ATRIP* are associated with Seckel syndrome (O'Driscoll et al., 2003; Ogi et al., 2012). Furthermore, cells lacking *ATR* have defects of DNA replication (Brown and Baltimore, 2000; Cortez et al., 2001), chromosomal instability (Casper et al., 2002), and expression of fragile sites (Casper et al., 2002). The ATR checkpoint pathway promotes genome integrity following replication stress through a kinase signaling cascade that mobilizes DNA repair, causes cell-cycle arrest, or induces apoptosis or senescence; however, the precise mechanisms by which the pathway is regulated, including through acetylation, are not well understood.

ATR is activated by single-stranded DNA (ssDNA), resulting from stalled replication forks (Byun et al., 2005; Sogo et al., 2002) or processing of DNA double-strand breaks (DSBs) (Garcia-Muse and Boulton, 2005; Jazayeri et al., 2006), which is then bound by the single-stranded DNA binding protein replication protein A (RPA) (Costanzo et al., 2003). RPA-ssDNA recruits ATR-ATRIP through direct and indirect interactions with ATRIP mediated by RPA-ssDNA ubiquitylation (Maréchal et al., 2014; Zou and Elledge, 2003), leading to the autophosphorylation of ATR at Thr-1989 (Liu et al., 2011; Nam et al., 2011). RPA-ssDNA also recruits the RAD17 clamp loader (Zou et al., 2003), which loads the RAD9-HUS1-RAD1 (9-1-1) clamp complex onto DNA (Bermudez et al., 2003) and recruits the MRE11-RAD50-NBS1 (MRN) complex (Oakley et al., 2009; Olson et al., 2007). Both the 9-1-1 complex and MRN interact with topoisomerase II beta binding protein 1 (TopBP1), which enables it to activate ATR and stimulate checkpoint signaling (Delacroix et al., 2007; Duursma et al., 2013; Kumagai et al., 2006; Lee et al., 2007). The activation of ATR is facilitated by the interaction of TopBP1 with ATRIP (Kumagai et al., 2006; Mordes et al., 2008). ATR activation is also potentiated by the sumoylation of ATRIP, which promotes multiple interactions in the ATR pathway (Wu et al., 2014). Once activated, ATR phosphorylates numerous downstream substrates including the CHK1 kinase, which helps to disperse the signal.