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SPOTLIGHT

ETAA1 ensures proper chromosome segregation: A matter of S phase or mitosis?

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ETAA1 activates the master checkpoint kinase ATR. Bass and Cortez (2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201810058) recently reported an intra-mitotic function of ETAA1 that safeguards chromosome stability. In this issue, Achuthankutty et al. (2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201905064) describe a mechanism controlling the ATR-activating potential of ETAA1 in S phase to preserve chromosome stability.

ATR is a master regulator of DNA replication stress responses that protects the genome from endogenous and exogenous threats. After DNA damage in S phase, ATR delays cell cycle progression, protects replication forks, and promotes DNA repair (1). During mitosis, ATR is activated at centromeric R-loops to promote faithful chromosome segregation (2). After being recruited to RPA-coated single-stranded DNA (RPA-ssDNA), ATR kinase activity is stimulated by the ATR activation domains (AADs) of TopBP1 (topoisomerase II binding protein 1) or ETAA1 (Ewing tumor-associated antigen 1; 1; Fig. 1). The central role of TopBP1 in checkpoint activation is highlighted by the fact that TopBP1-knockout mice are embryonic lethal (3). In contrast, ETAA1-knockout mice exhibit only mild phenotypes (4). ETAA1-ablated cells are viable though sensitive to DNA damaging agents such as camptothecin, hydroxyurea, and mitomycin C (5, 6, 7, 8). Notwithstanding this, four papers published in 2016 have unequivocally demonstrated that ETAA1 is a direct activator of ATR (5, 6, 7, 8). Concomitant loss of TopBP1 and ETAA1 results in synthetic lethality and genomic instability, indicating that ETAA1 and TopBP1 cooperate to promote full ATR activation (5, 6, 8). ETAA1 and TopBP1 converge at the phosphorylation of the ATR target RPA and, depending on the cellular context, of the ATR effector Chk1 (Checkpoint Kinase 1; 5, 6, 7, 8). However, by controlling specific subsets of ATR targets, ETAA1 and TopBP1 regulate parallel, independent branches of ATR signaling (9). Another important distinction between TopBP1 and ETAA1 is the way in which both ATR activators are recruited to DNA. While TopBP1 senses junctions between ssDNA and double-stranded DNA (dsDNA), ETAA1 interacts directly with RPA-ssDNA (1). Hence, ETAA1 might be more important than TopBP1 in unstressed cells because, while there is always availability of ssDNA at replicating forks, ss/dsDNA junctions increase only after replication stress (9, 10). In that sense, recent work demonstrated that ETAA1, but not TopBP1, controls basal ATR activity to keep FOXM1 in a hypo-phosphorylated inactive state (10). FOXM1 is a transcription factor that becomes activated by CDK1-dependent phosphorylation at late S to promote entry into G2. Once ssDNA decreases as DNA replication finishes, ETAA1-dependent ATR activity decreases, enabling FOXM1 activation and so the S-to-G2 transition. If the S/G2 checkpoint fails, cells undergoing replication accumulate phospho-H3 and such untimely initiation of mitosis culminates in the accumulation of ultra-fine bridges (UFBs) in anaphase cells (Fig. 1). While it was clear that the aberrant finalization of S phase is prevented by ETAA1, in this issue, a new study from Achuthankutty et al. sheds light on the biological relevance of ETAA1 functions during S phase.

Achuthankutty et al. (11) conducted a CRISPR-Cas9 screen to identify genes whose ablation is synthetically lethal with ETAA1 loss. Gene ontology analysis indicated a notable enrichment of terms associated with DNA replication among the genes identified. Interestingly, synthetic lethality was associated with the accumulation of the DNA damage marker γH2AX in G2/M cells, premature entry into mitosis, and increased mitotic DNA synthesis (MiDAS). Such a dysregulated and accelerated entrance in M phase culminated in chromosome instability (CIN), revealed as UFBs, anaphase chromatin bridges, lagging chromatin, and chromosome breaks. Importantly, by using mutants that preclude key phosphorylation events on ETAA1's AAD, the authors showed that ETAA1-mediated activation of ATR during S phase promotes chromosome stability and cell fitness after replication stress (Fig. 1). Such results may complement published results (10), ascribing a central role of ETAA1 in restraining mitotic entry. Furthermore, the data provide exciting insights into the biological relevance of the mechanisms that fine-tune the ATR-activating potential of ETAA1 during S phase. Importantly, while the ETAA1-ATR-CDK1-FOXM1 pathway is TopBP1-independent but Chk1-dependent (10), the contribution of TopBP1 and Chk1 to ETAA1 phosphorylation-mediated prevention of CIN remains to be tested. Also, whether CIN is avoided by ETAA1 mainly through the regulation of FOXM1 or involves other ATR targets as well requires further investigation.

Intriguingly, a recent *JCB* article from Bass and Cortez also associated ETAA1 to CIN (9). In this case, however, CIN in ETAA1-deficients cells was linked to a dysfunctional spindle assembly checkpoint (SAC), rather than to a

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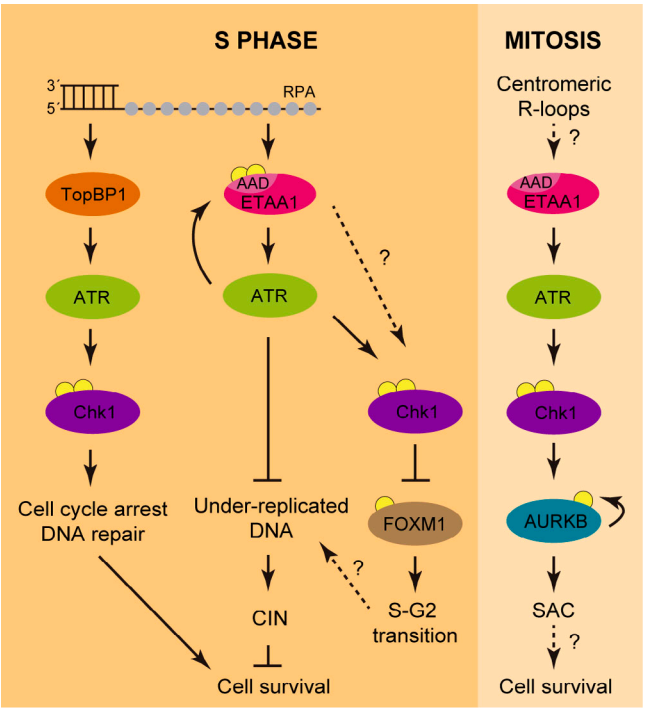

Figure 1. **ETAA1 safeguards chromosome stability independently of TopBP1.** Upon replication stress during S phase, TopBP1-dependent activation of ATR drives Chk1-dependent cell cycle arrest and DNA repair. In addition, S phase–specific stimulatory phosphorylation of ETAA1 at its AAD activates ATR to avoid premature mitotic entry, CIN, and cell death. It is still unclear whether this function of ETAA1 requires Chk1 and whether ETAA1 phosphorylation (yellow circles) regulates the ETAA1-ATR-Chk1-FOXM1 axis, which prevents an untimely transition to G2. During mitosis, ETAA1 activates ATR, possibly at centromeric R-loops, leading to Chk1 and Aurora B activation to prevent chromosome misalignment. The contribution to cell survival of this intra-mitotic role of ETAA1 remains undefined.

defective function in S phase (Fig. 1). Unlike Achuthankutty et al. (11), whose screening approach focused on identifying synthetic lethal interactions with ETAA1, Bass and Cortez (9) used quantitative phosphoproteomics to identify factors functioning downstream of ETAA1. This approach unveiled a prominent role of ETAA1 during mitosis, as many kinetochore- and spindle-localized proteins were phosphorylated in an ETAA1-dependent but TopBP1-independent manner. Bass and Cortez (9) confirmed this notion by showing that the loss of ETAA1, but not of TopBP1, compromises the ability to sustain mitotic arrest. In agreement, ETAA1 was required for the Chk1-dependent phosphorylation of the master M phase kinase Aurora B, which prevents cell division until all chromosomes are properly attached via kinetochores to the spindle. To directly link CIN to the role of ETAA1 in promoting a fully functional SAC via ATR–Chk1–Aurora B signaling, Bass and Cortez (9) showed that specific removal of ETAA1 in mitosis results in mitotic defects.

The Mailand and Cortez laboratories have provided exciting insights into the molecular details around the ATR activator ETAA1. It is now clear that ETAA1 has important, independent functions in S phase and in mitosis. Both studies report similar chromosome segregation defects in ETAA1-deficient cells, but propose a different origin for those abnormalities. MiDAS and UFBs are clear signals of conflicts in S phase, supporting the notion that ETAA1 deficiency in S phase triggers CIN. Aurora B dysregulation is a known cause of chromosome misalignment, supporting the notion that the intra-mitotic function of ETAA1 contributes to CIN. It should be mentioned that the Mailand laboratory reported no modulation of Aurora B phosphorylation under their experimental settings. However, other ETAA1-dependent events in M phase reported by Bass and Cortez (9), such as Chk1 activation, were not tested by Achuthankutty et al. (11). Another point to consider is that, while both studies use HCT116 cells, Achuthankutty et al. (11) use knockout expression cell lines, whereas Bass and Cortez (9) use cells expressing a mutant version of ETAA1, incapable of activating ATR but proficient in RPA binding. In the future, it would be of interest to combine the complementation approach taken by the Mailand laboratory and the phase-specific degradation experiments performed by the Cortez laboratory to clarify the relative contribution of S and M phase ETAA1 functions to CIN and cell fitness.

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