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SPOTLIGHT

ISG15 fast-tracks DNA replication

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In this issue, Raso et al. (2020. *J. Cell Biol.* https://doi.org/10.1083/jcb.202002175) uncover a novel replication fork speed regulatory network controlled by the ubiquitin-like modifier interferon-stimulated gene 15 (ISG15), which plays a central role in the innate immune response and regulates tumorigenesis as well as chemotherapy response.

DNA replicates by unzipping its two interwoven strands and making copies of each. As the DNA strands separate and copy, they form a "replication fork." The speed at which replication forks progress along the DNA must be tightly controlled to ensure genome stability and faithful duplication of the genome. In this issue, Raso et al. (1) uncover a novel replication fork speed regulatory network controlled by the ubiquitin-like modifier interferon-stimulated gene 15 (ISG15). They propose that higher expression of ISG15 induced by interferons or commonly detected in many types of cancer cells drives deregulated fork speed, leading to DNA damage and genome instability.

ISG15 is as ubiquitin-like modifier that plays a central role in the innate immune response, which is the first line of host defense against a vast array of pathogens, including bacteria, fungi, viruses, and parasites. The innate immune response promotes the production of interferons (IFNs) that in turn stimulate the production of ISG15 (2). ISG15 acts by modifying cellular proteins in a process called ISGylation consisting on the conjugation of a small-size polypeptide similar to ubiquitin to target proteins. The exact function of ISG15 and its ubiquitin-like activity in the innate immune response is poorly understood. Interestingly, ISG15 is highly expressed in many tumor types, but how its higher expression levels correlate with tumorigenesis and cancer cell response to chemotherapeutics remains largely unexplored.

Raso et al. provide a fresh view into the function of ISG15 by showing that its higher expression levels increase replication fork speed, leading to DNA damage and genomic instability. The idea that increasing replication fork speed above a certain threshold leads to DNA damage and genome instability is an emerging theme in the replication field, whereby faster replicating forks have insufficient time to recognize damaged DNA in need of repair. This leads to accumulation of DNA damage and increased cellular sensitivity to DNA damaging agents, as observed by Raso et al. A central post-translation modification for replication speed is poly-(ADP-ribosyl)ation mediated by the PARP family of proteins, whose inhibition also leads to faster replication forks (3). Raso et al. suggest that PARP inhibitors and ISG15 affect replication fork speed by similar mechanisms and show that increasing ISG15 levels significantly enhances cancer cell sensitivity to PARP inhibitors. PARP inhibitors are used to treat different tumor types, particularly those carrying mutations in key DNA repair genes (4). The findings of Raso et al. reveal that the ISG15-dependent increase in fork speed is an important and previously unappreciated modulator of cancer cell response to PARP inhibitors and DNA-damaging chemotherapy.

To uncover the pathway by which ISG15 speeds up replication forks, Raso et al. ran a mass spectrometry analysis searching for ISG15-interacting proteins and found a number of chromatin-associated factors, some of which were previously reported to interact with replication forks. Among these, they focused on the human RECQ1 helicase, a protein that promotes the restart of stalled replication forks (5). In particular, RECQ1 promotes restart by facilitating the resolution of reversed fork structures. Fork reversal is a mechanism that allows replication forks to cope with DNA damage encountered ahead of their path by reversing their course (6). The results of Raso et al. suggest that ISG15 may regulate RECQ1 function by unleashing its reversed fork restart activity. Interestingly, this mechanism depends on the functional interaction of ISG15 with the RECQ1 helicase but seems to be largely independent of ISG15 conjugation activity. These findings have several important implications. First, they point to a potential and previously unappreciated role of ISG15 in replication fork reversal, even though the exact mechanism by which ISG15 promotes the reversed fork restart function of RECQ1 remains elusive. Second, the effect of PARP inhibitors on replication fork restart also relies on the deregulation of RECQ1 activity (5), suggesting that ISG15 and PARP inhibitors might affect replication fork progression by a similar mechanism. Third, the authors' findings point to a novel link between deregulated fork progression induced by the ISG15-PARP inhibitor-RECQ1 axis, increased DNA damage, and cancer cell response to chemotherapy. These findings also suggest that increased RECQ1 activity caused by high ISG15 levels or PARP inhibition might represent an important vulnerability

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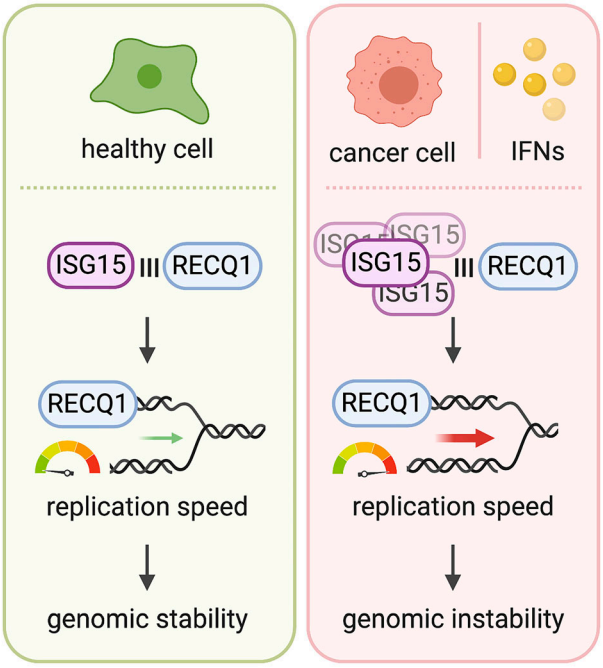

Figure 1. **The ISG15-RECQ1 network controls replication fork speed.** High levels of ISG15 expression, which are triggered by the production of interferons and are commonly detected in many human tumors, stimulate the replication fork start activity of RECQ1, leading to accelerated fork speed and increased genomic instability.

to be exploited in cancer treatment. As increased ISG15 levels sensitize cancer cells to DNA damaging chemotherapeutics and PARP inhibitors, monitoring ISG15 or RECQ1 levels in tumor samples may represent a novel predictive parameter to stratify patients in personalized cancer therapy.

The innate immune system operates under the assumption that no free DNA should be present in the cytosol and that any cytosolic DNA linked to bacterial or viral infection is recognized as nonself (7). Cytosolic DNA can be recognized by the cGAS-STING pathway, which induces type I IFNs and other cytokine genes (8). Recent studies demonstrated that, in addition to pathogens, defects in replication fork processing can also lead to the accumulation of cytosolic DNA and transactivate innate immune response genes, leading to higher expression of ISG15 (9). These studies, along with the findings of Raso et al., suggest that ISG15 might be at the center of a feedback loop where the increased replication fork speed linked to higher ISG15 expression leads to increased replication stress, in turn activating the innate immune response.

In summary, Raso et al. provide a fresh view on the function of a central immune response factor in genome stability and outline a previously unknown network influencing replication fork speed (Fig. 1). Their work opens several new areas of investigation that will revolutionize current models of how innate immune response genes regulate tumorigenesis and chemotherapy response, but it also raises several questions. For example, the authors' observation that the ISG15 function in replication is largely uncoupled from its conjugation activity begs the question of what might be the role of this activity in the context of DNA damage and replication stress. In particular, the mass spectrometry analysis revealed several other ISG15 interactors whose function might be regulated by ISGylation activity. A key challenge for future studies will be to understand if ISG15, and its interaction with these protein partners, play additional roles in genome maintenance besides the observed regulation of RECQ1's fork restart function.

Another area of interest for future research is to explore why cells evolved a system that is potentially harmful to cell survival to counteract host infection. Raso et al. speculate that the detrimental effects of ISG15 might arise only when its increased expression is uncoupled from its conjugation function, but this would need to be demonstrated. Finally, the findings of Raso et al. will no doubt prompt many research groups to study why cancer cells choose to increase ISG15 expression. How does higher ISG expression give an advantage to cancer cell growth? Recent studies suggest that ISG15 has either oncogenic or tumor-suppressive effects depending on the context (10), and the findings of Raso et al. will undoubtedly spark new research to understand the connection between deregulated fork speed caused by higher ISG15 expression and tumorigenesis.

Acknowledgments

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