

HUWE1 comes to the rescue at stalled replication forks

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HUWE1 is a multi-faceted E3 ubiquitin ligase of the HECT family with many confirmed substrates, but mechanistic understanding of its functional roles in signaling pathways remains limited. In this issue of *EMBO Reports*, Choe *et al* demonstrate a novel function for HUWE1 in promoting DNA damage tolerance mechanisms to bypass DNA lesions during replication stress, thereby preserving genome stability. The authors connect this role for HUWE1 with its function in maintaining H2AX monoubiquitination levels for efficient signaling at stalled replication forks [1]. Thus, this work highlights HUWE1 as a novel player in the replication stress response and prompts further investigation of its regulation during replication and other cellular processes.

See also: **KN Choe *et al*** (June 2016)

The process of DNA replication is inherently challenging, and even when conditions are ideal, obstacles to replication fork progression contribute to “replication stress”. Replication fork stalling and collapse resulting from such perturbations potentially compromise efficient replication completion, increasing genome instability and tumorigenesis. Fortunately, cells have multiple ways to cope with replication stress, including DNA damage tolerance mechanisms that promote the restart of stalled forks through bypass of DNA lesions. Despite much attention to the topic in recent years, mechanisms promoting fork restart during the replication stress response remain poorly understood in mammalian cells.

Recent findings from Choe *et al* [1] identify HUWE1 (also named ARF-BP1, HECTH9, MULE, and Lasu1) as a novel regulator of

replication fork restart following replication stress. HUWE1 has been previously shown to target a broad range of substrates for degradation, but its function in signaling pathway regulation has remained controversial [2–6]. It is interesting to note that the *Huwe1* gene is overexpressed in a significant proportion of lung, breast, and colorectal carcinomas and is required for the proliferation of a subset of tumor cells [3,5]. These findings make HUWE1 a highly attractive therapeutic target, but increased understanding of the mechanistic functions of HUWE1 in cellular signaling is still necessary to inform strategies toward manipulating this protein in cancer therapies.

As HUWE1 had already been reported to affect a number of genome stability mechanisms through degradation of proteins such as the replication licensing factor Cdc6 [6], and the DNA repair factors TopBP1 and BRCA1 [2], Choe *et al* [1] focused on identifying new roles for HUWE1 in controlling DNA replication dynamics in response to replication stress. The authors observed several striking phenotypes associated with dysregulated DNA replication upon HUWE1 knockdown or knockout in several mammalian cell lines. For instance, clonogenic growth assays and DNA fiber experiments revealed increased sensitivity of HUWE1-depleted cells to hydroxyurea (HU) and ultraviolet (UV) radiation, agents that induce replication fork stalling during S-phase. Other experiments demonstrated increased DNA breaks, reduced replication track length, and S-phase arrest in HUWE1-deficient cells [1]. Altogether, these results suggest a novel role for HUWE1 in regulating DNA damage tolerance to preserve genome stability.

The authors then interrogated the mechanistic basis by which HUWE1 controls

replication fork progression during replication stress. One of the central components of the replication fork promoting efficient fork restart following replication stress is proliferating cell nuclear antigen (PCNA), a homotrimeric processivity factor for DNA polymerases. PCNA serves as a docking platform for proteins involved in various processes, and most of these proteins interact with PCNA via a motif known as a PCNA-interacting peptide (PIP) box. Interestingly, the authors identified a PIP box motif (QPAVEAFF) within HUWE1, which mediates its interaction with PCNA both *in vitro* and *in vivo*. Binding of HUWE1 to PCNA was demonstrated to be important for the function of HUWE1 in DNA damage tolerance, since only wild-type and not a PIP box mutant form of HUWE1 could rescue the replication stress phenotypes of HUWE1 knockout cells. Accordingly, immunofluorescence experiments showed the co-localization of HUWE1 with PCNA, and iPOND approaches detected HUWE1 at replisomes [1]. Whether PCNA actively recruits HUWE1 to DNA damage sites, or HUWE1 simply marks sites for DNA repair synthesis, remains unknown, however. The authors observed that PCNA monoubiquitination, which activates the bypass of DNA lesions, is not a requirement for the interaction of HUWE1 with PCNA [1]. Thus, while there is solid evidence for an interaction with PCNA, how HUWE1 is recruited to PCNA during the replication stress response remains elusive.

To further investigate how HUWE1 influences fork restart of stalled replication forks, Choe *et al* [1] examined the effects of HUWE1 depletion on checkpoint signaling, which initiates downstream repair of damaged forks. The authors focused on

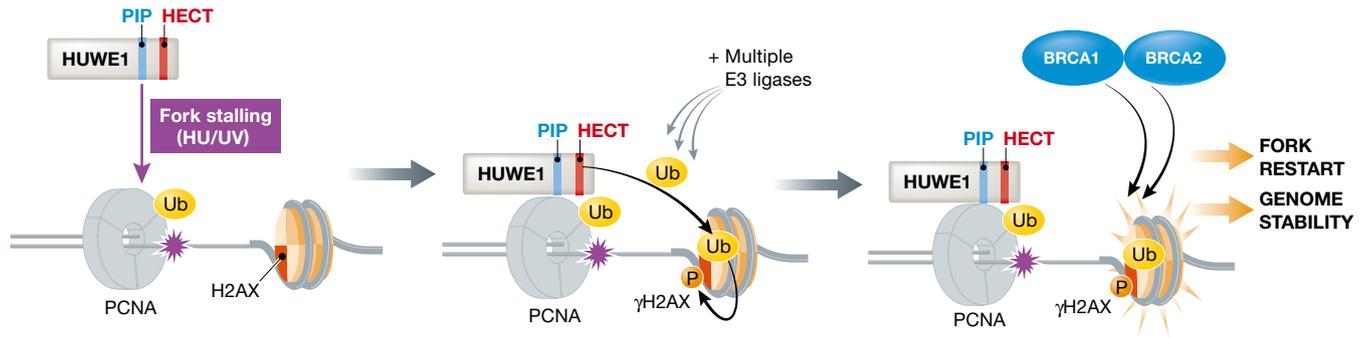


Figure 1. HUWE1 binds PCNA and promotes fork restart following replication stress.

Upon replication fork stalling induced by HU or UV treatment, HUWE1 interacts with PCNA via its PIP box motif. Once bound to PCNA, HUWE1 stimulates the monoubiquitination and subsequent phosphorylation of H2AX, which in turn recruits repair complexes, including BRCA1 and BRCA2, to chromatin to repair and/or restart the damaged fork. Star represents a DNA lesion.

modifications of the histone variant H2AX, which becomes monoubiquitinated [7] and subsequently phosphorylated (γ H2AX) [8] as an early step in signaling at stalled replication forks. Surprisingly, Choe *et al* [1] observed a significant reduction in levels of H2AX monoubiquitination in HUWE1 knockout cell lines. H2AX ubiquitination is also achieved by several other ubiquitin ligases including RNF168 and RING1B (RNF2)/BMI1, but these could not efficiently compensate for the loss of H2AX monoubiquitination upon HUWE1 depletion. As H2AX monoubiquitination is a prerequisite for phosphorylation of H2AX, the authors also observed lower γ H2AX levels in HUWE1 knockout cells. This phenotype was particularly evident on the common fragile site FRA3B, a difficult-to-replicate region particularly prone to fork collapse and breakage, indicating that HUWE1 is important for maintaining efficient γ H2AX signaling at sites of DNA damage during replication stress [1]. Finally, the authors linked this decrease in checkpoint signaling with inefficient recruitment of the recombination proteins BRCA1 and BRCA2, which had previously been implicated in initiating restart of stalled replication forks by homologous recombination-mediated mechanisms [9,10]. Taken together, these results indicate a model whereby HUWE1 binding to PCNA via its PIP box mediates ubiquitination and phosphorylation of H2AX, which in turn recruits repair proteins to restart stalled forks, summarized in Fig 1.

Significant questions still remain from this study regarding the function and regulation of HUWE1 at the replication fork during replication stress. For example, how does HUWE1 promote DNA damage tolerance? During replication fork stalling, PCNA becomes monoubiquitinated by the E3 ubiquitin ligase RAD18, leading to the recruitment of low-fidelity polymerases to bypass DNA lesions in a process known as translesion synthesis (TLS). However, Choe *et al* [1] show that HUWE1 does not regulate PCNA monoubiquitination levels. Furthermore, how does replication stress enhance HUWE1's activity in promoting fork restart? In their iPOND analyses, the authors show that the recruitment of HUWE1 to the replisome is enhanced during replication stress induced by UV treatment, but how this is regulated is still unclear. Importantly, does overexpression or deregulation of HUWE1 activity in cancer cells affect normal DNA replication? Finally, what are the relative contributions of HUWE1 and other E3 ubiquitin ligases in maintaining H2AX modifications during the replication stress response? The authors mention that the decreased γ H2AX signal observed during HUWE1 knockout experiments is a transient phenotype, and that HUWE1 knockout cells recover normal γ H2AX levels after several passages [1]. Therefore, do other E3 ligases compensate to restore DNA damage signaling after prolonged HUWE1 depletion?

Nevertheless, this study by Choe *et al* [1] has broadened our understanding of the role of HUWE1 in alleviating replication stress and maintaining genome stability. Cancer cells, in general, exhibit DNA replication defects and frequently experience chronic replication stress as a consequence. Considering that HUWE1 is also deregulated in human cancers, this work encourages further efforts toward understanding the complex regulation of this multi-faceted protein to improve strategies for pharmacologically targeting HUWE1 in cancer therapies.

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