HDM2 ERKs PCNA

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In this issue, a study by Groehler and Lannigan (2010. *J. Cell Biol.* doi:10.1083/jcb.201002124) sheds light on the regulation of proliferating cell nuclear antigen (PCNA) turnover and how it is counteracted by the small chromatin-bound kinase ERK8 (extracellular signal-regulated kinase 8). Importantly, inactivation of ERK8 results in genome instability and is associated with cell transformation.

Almost 30 yr ago, proliferating cell nuclear antigen (PCNA) was first identified in dividing cells using sera derived from patients suffering from systemic lupus erythematosus (Takasaki et al., 1981). A few years later, the "mother" of all cancer markers had been associated with DNA synthesis (Madsen and Celis, 1985), but it wasn't until 1988 that Bauer and Burgers (1988) and Prelich and Stillman (1988) discovered that the homotrimeric clamp served as a processivity factor for DNA polymerases. In 1992, Shivji et al. (1992) showed that PCNA was required for DNA repair, and 10 yr later, it was identified as a target of ubiquitin and SUMO (small ubiquitin-like modifier) conjugation after exposure to ultraviolet light (Hoege et al., 2002). For a protein that has been in the spotlight of modern biochemistry, it is quite remarkable that almost nothing is known about its normal cellular turnover.

Insight into this process comes now from the study of an unlikely regulator. In this issue, Groehler and Lannigan (2010) demonstrate that the relatively poorly characterized ERK8 (extracellular signal-regulated kinase 8) takes center stage in the regulation of PCNA stability in primary mammary epithelial cells. The ERK family of kinases belongs to the mitogen-activated protein kinase superfamily and carries a Thr-Glu-Tyr (T-E-Y) activation motif that needs to be phosphorylated to enable kinase activity (Abe et al., 2002). Interestingly, ERK8 also needs to bind to chromatin to become active. The authors identified a highly conserved PXXXP motif in the C-terminal half of ERK8 that appeared to confer autoinhibition, an activity which is relieved upon chromatin binding. Relatively close by, in the middle of ERK8, resides a PCNA-interacting peptide (PIP) box required for the interaction with PCNA (Warbrick, 1998). Curiously, only the chromatin-bound fraction of ERK8 bound to the chromatin-bound fraction of PCNA. However, a functional PIP box was not required for ERK8 to associate with nuclear DNA in the cell. These results argue that ERK8 is not anchored to chromatin by PCNA but associates with it independently. Moreover, they strongly suggest that ERK8's PIP box binds to PCNA only when the kinase is associated with chromatin. Importantly, overexpression of an ERK8 PIP box mutant resulted in destabilization of PCNA. The effect on PCNA stability seemed to be highly specific, as depletion of ERK8 caused codepletion of PCNA but did not lead to a decrease in steady-state levels of a variety of other cell cycle regulators.

Why is the interaction with PCNA confined to chromatin? The reason is likely due to the fact that ERK8's PIP box is buried in the middle of the protein. Most PCNA-interacting proteins carry their PIP box either at the N or C terminus (Vivona and Kelman, 2003). One other well-studied example for a protein with an internal PIP box is the essential replication factor MCM10 (minichromosome maintenance protein 10). MCM10 undergoes cell cycle–regulated modification, which probably induces a conformational switch that is necessary for the PIP box–mediated interaction with PCNA (Das-Bradoo et al., 2006). In the same vein, it is conceivable that chromatin association and the accompanying relief of autoinhibition of ERK8 cause the middle portion of the kinase to change its configuration, thereby assuming a functional PIP box domain that can be recognized by PCNA. In situations in which the rapid unloading of PCNA is required, regulation of ERK8 may be the most effective way to dispose of chromatin-bound PCNA, which is known to have an exceedingly low exchange rate (Sporbert et al., 2002). Despite the fact that interaction with ERK8 is necessary to stabilize chromatin-bound PCNA, it remains unclear whether PCNA is a direct target of ERK8-mediated phosphorylation.

The next goal of Groehler and Lannigan (2010) was to dissect the mechanism underlying the ERK8-regulated degradation of PCNA. Based on the consideration that physical contact between the kinase and PCNA was an integral part of the protection, they hypothesized that ERK8 might compete with an E3 ubiquitin ligase that may target PCNA via its own PIP box. This turned out to be a smart guess because the only candidate to test was the E3 ligase HDM2, the human homologue of murine double minute 2 (Momand et al., 1992). In a set of well-controlled experiments, the authors not only demonstrate that HDM2 interacts directly with and degrades PCNA when ERK8 is absent, but they also exclude indirect effects by p53 and retinoblastoma (Rb) on this process. p53 is a direct target of HDM2 and is stabilized when their interaction is inhibited (Tao and Levine, 1999). Elevated levels of p53 trigger cell cycle arrest concomitant with hypophosphorylation of Rb, but none of these changes affect the stability of PCNA.

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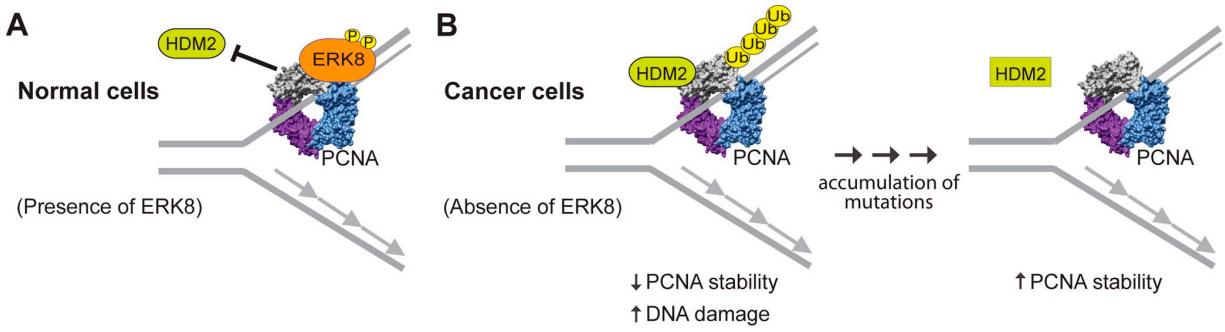

Figure 1. **Role of ERK8 in maintaining genome stability.** (A) In normal cells, chromatin-bound ERK8 interacts with the chromatin fraction of PCNA, which resides at the replication fork (here just shown at the leading strand for simplicity). ERK8 binding inhibits the E3 ubiquitin ligase HDM2 from interacting with PCNA. (B) In cancer cells, inactivation of ERK8 enables HDM2 to interact with and ubiquitinate PCNA, targeting it for degradation. A decrease in PCNA levels causes an increase in DNA damage, resulting in the accumulation of new mutations. These new mutations may render HDM2 nonfunctional (rectangular form), which ultimately results in an increase of PCNA stability and facilitates cell proliferation. The homotrimeric PCNA structure (Protein Data Bank ID 2OD8) was generated using the Chimera software program (Pettersen et al., 2004).

It is not hard to imagine that the loss of chromatin-bound PCNA has severe consequences for the functionality of DNA replication and repair, resulting in chromosome breakage. The authors argued that a similar level of genome instability should be visible in ERK8-depleted cells. This was indeed the case as visualized by the accumulation of γ-H2AX foci and broken DNA (Rogakou et al., 1998). Importantly, Groehler and Lannigan (2010) observed similar effects in the ERK8 PIP box mutant, further lending credence to their model. It is worthwhile pointing out that the turnover of PCNA expands the spectrum of replication factors whose degradation is tightly linked to chromatin. CDT1, a member of the prereplication complex (Cook, 2009), is rapidly degraded in the face of DNA damage. Its degradation occurs exclusively on the chromatin-associated fraction of the protein pool and is dependent on CDT1 binding to PCNA (Arias and Walter, 2005; Hu and Xiong, 2006; Senga et al., 2006).

An important question that this study raises is of course to what extent, if at all, is PCNA turnover deregulated in cancer cells? The commonly high levels of PCNA in transformed cells would be most compatible with a deregulation of ERK8 and/or HDM2 to provide a significant growth advantage. Indeed, the authors show in the last part of their study that in at least two transformed cell lines, PCNA is rendered inert to the presence of ERK8. They speculate that the underlying reason is a defect in HDM2, and although this is the most likely explanation, it still needs to be validated. It will be interesting to see how common the misregulation of PCNA turnover is in cancer tissues. At this point, it is intriguing to envision a dynamic scenario in which a two-step mechanism facilitates cell transformation (Fig. 1). Initially, deregulation of ERK8 may cause PCNA levels to decrease. This would contribute to genome instability and the accumulation of new mutations, including those affecting proper function of HDM2. In step two, deregulation of HDM2 may turn things around and result in an increase of PCNA, supporting rapid proliferation.

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