Revisiting the role of heterochromatin protein 1 in DNA repair

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Heterochromatin protein 1 (HP1) is a conserved factor critical for heterochromatin organization and gene silencing. It is recruited to chromatin by its direct interaction with H3K9me (methylated lysine 9 residue of histone H3), an epigenetic mark for silenced chromatin. Now, Luijsterburg et al. (Luijsterburg, M.S., C. Dinant, H. Lans, J. Stap, E. Wiernasz, S. Lagerwerf, D.O. Warmerdam, M. Lindh, M.C. Brink, J.W. Dobrucki, et al. 2009. *J. Cell Biol.* 185:577–586) reveal a new H3K9me-independent role for HP1 in the DNA damage response, which is distinct from the one recently reported by Ayoub et al. (Ayoub, N., A.D. Jeyasekharan, J.A. Bernal, and A.R. Venkitaraman. 2008. *Nature.* 453:682–686).

HP1 was originally identified in *Drosophila melanogaster* as a nonhistone chromosomal protein involved in heterochromatin-mediated gene silencing (Eissenberg et al., 1990). This protein has two conserved domains: the chromodomain (CD) and the chromo shadow domain (CSD) connected by a linker/hinge region (Fig. 1 A). The CD directly interacts with H3K9me, a hallmark of transcriptionally repressed chromatin, and this binding is crucial for the maintenance of the heterochromatic state (for reviews see Maison and Almouzni, 2004; Lomberk et al., 2006; Fanti and Pimpinelli, 2008). The linker/hinge region interacts with RNA and DNA, and the CSD is involved in multiple protein–protein interactions, including homo- and heterodimerization. Since its discovery, a variety of functions have been described for HP1 (for reviews see Maison and Almouzni, 2004; Lomberk et al., 2006; Fanti and Pimpinelli, 2008). HP1 associates with numerous proteins, including transcription factors, chromatin regulators, and DNA replication and repair factors, as well as components of the nuclear envelope (for a comprehensive list of interacting proteins see Lomberk et al., 2006). This protein also recruits the cohesin complex to pericentromeric heterochromatin for centromeric sister chromatid cohesion. Recent studies have shown that HP1 does not always act in the context of heterochromatin and functions in gene activation and telomere maintenance (for review see Fanti and Pimpinelli, 2008). In mammalian cells, there are three HP1 variants (i.e., HP1-α, HP1-β, and HP1-γ) that exhibit distinct subnuclear localization patterns and associate with centromeric and other heterochromatic regions to varying extents (for reviews see Maison and Almouzni, 2004; Lomberk et al., 2006; Fanti and Pimpinelli, 2008). However, their functional distinction is not well understood.

The involvement of HP1 in DNA repair was recently described. The checkpoint kinase ataxia telangiectasia mutated (ATM) was found to facilitate DNA double-strand break (DSB) repair in heterochromatic regions (Goodarzi et al., 2008). Depletion of all three HP1 variants by siRNA was shown to alleviate the requirement for ATM in heterochromatic DSB repair, presumably because loss of HP1 results in the loosening of compacted chromatin regions, allowing easier access and processing of DNA damage by repair factors. It was also found that HP1-β was rapidly phosphorylated at threonine 51 (T51) in the CD by casein kinase 2 (CK2) in response to DSB damage (Fig. 1 A; Ayoub et al., 2008). This phosphorylation reduces the affinity of HP1-β for H3K9me, resulting in its transient mobilization from chromatin as detected by FRAP analysis at both heterochromatic and euchromatic regions in the nucleus, which are easily discernible in mouse cells. Although what signals CK2 to phosphorylate HP1 at the damage sites is currently unknown, this phosphorylation and mobilization of HP1 appears to be important for efficient H2AX phosphorylation, the DSB-specific histone modification important for checkpoint signaling and repair (Ayoub et al., 2008).

Although the studies of Goodarzi et al. (2008) and Ayoub et al. (2008) suggest a negative effect of H3K9me-bound HP1 on DSB repair (and the likely benefit for its removal), a study in this issue (see Luijsterburg et al. on p. 577) supports an apparently active and positive role for HP1 in DNA repair. These authors demonstrate that HP1 is specifically recruited to both DNA cross-links and DSB damage sites as an early event of DNA damage recognition in mammalian cells. Using both endogenous and fluorescent-tagged HP1 variants and multiple DNA-damaging methods, the study by Luijsterburg et al. (2009) is convincing. However, the apparent discrepancy between this study and that of Ayoub et al. (2008), which documents the transient mobilization of HP1 from DNA damage sites, cannot be easily reconciled (Fig. 1 B). Both groups used FRAP/fluorescence loss in photobleaching analyses using similar doses of γ irradiation. In addition,

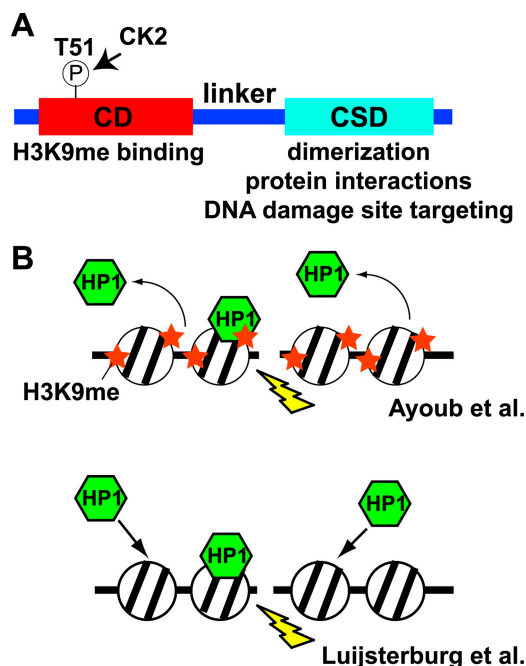Correspondence to Kyoko Yokomori: kyokomor@uci.edu

Figure 1. HP1 and DNA repair. (A) A schematic diagram of HP1. The CD binds to H3K9me, whereas the CSD is involved in dimerization and multiple protein interactions (for reviews see Lomberk et al., 2006; Fanti and Pimpinelli, 2008). T51 is phosphorylated by CK2 in response to DNA damage, which reduces the affinity of HP1 for H3K9me (Ayoub et al., 2008). The CSD, but not the CD, is required for DNA damage site targeting (Luijsterburg et al., 2009). P, phosphorylation. (B) Summary of HP1 behaviors at the damage sites according to Ayoub et al. (2008) and Luijsterburg et al. (2009). According to the observation made by Ayoub et al. (2008), HP1 transiently dissociates from H3K9me in response to damage. In contrast, Luijsterburg et al. (2009) found that HP1 is actively recruited to DNA damage sites in an H3K9me-independent manner.

despite the use of a similar laser system (405 nm) with Hoechst sensitization, Ayoub et al. (2008) did not observe the accumulation of HP1-β at the damage sites, whereas Luijsterburg et al. (2009) did. There is evidence suggesting that slight differences in laser parameters (e.g., total energy input, irradiance, etc.) may affect protein detection at the damage sites (Kong et al., 2009). Subtle differences in experimental design, reagents, and measurement sensitivities may also contribute to conflicting experimental outcomes. Interestingly, however, phosphorylation of HP1 at T51 by CK2 was specifically observed at the damage sites, suggesting that although HP1 may lose its affinity for H3K9me, it is not completely dispersed from the damaged region (Ayoub et al., 2008). Furthermore, the recruitment of HP1 to the damage sites appears to be mediated by the CSD and is independent of both its CD (and T51 phosphorylation) and H3K9me (Luijsterburg et al., 2009). Thus, one possibility that reconciles these two studies is that HP1 may undergo a change in chromatin binding within the DNA damage region; although HP1 dissociates from H3K9me (triggered by phosphorylation by CK2) in response to DSB damage, it is actively recruited to the damage sites by an H3K9me-independent mechanism to promote DNA repair.

How does HP1 recognize DNA damage? It is surprising that HP1 can be recruited to two different types of DNA damage: UV-C–induced damage enriched for cross-links and damage enriched for DNA breaks induced by soft x rays, α-particles,

and by a 405-nm laser with Hoechst sensitization. The CSD was shown to interact with the histone chaperone chromatin assembly factor-1 (CAF-1; Murzina et al., 1999). CAF-1 is recruited to UV-C–induced DNA cross-linking damage sites in a nucleotide exchange repair (NER)-dependent manner, which is required for postrepair chromatin recovery and restoration of epigenetic marks (Green and Almouzni, 2003; Polo et al., 2006; Zhu et al., 2009). However, using mutant cells, Luijsterburg et al. (2009) demonstrated that HP1 recruitment to the UV damage sites does not depend on NER factors. Similarly, cells defective for Ku, which recognizes DSBs, did not affect HP1 recruitment to the DSB sites. Future work to decipher the mechanism of HP1 recruitment may reveal the earliest molecular event that is common to different types of DNA damage.

What is the function of HP1 at the damage sites? Luijsterburg et al. (2009) provide in vivo evidence for the functional significance of HP1 in DNA repair in *Caenorhabditis elegans*, which has two HP1 variants. Mutation of one or both of these variants revealed that both proteins seem to redundantly participate in UV damage repair, but only one appears to be important for DSB repair. These results suggest that HP1 variants function differently in DNA repair. However, this is somewhat puzzling because all three mammalian HP1 variants are recruited to DSB sites equally efficiently in Luijsterburg et al. (2009). Furthermore, siRNA-mediated depletion of all three proteins in mammalian cells has no effect on DSB repair unless ATM is codepleted to reduce the efficiency of heterochromatin repair (Goodarzi et al., 2008). However, the study by Goodarzi et al. (2008) focused primarily on DSB repair in G0/G1 phase, which occurs most likely through nonhomologous end joining. Thus, the involvement of mammalian HP1 in other repair pathways may still be possible. Studies designed to assess the effect of inactivation of either individual and/or a combination of HP1 variants on the cellular response to different types of DNA damage in mammalian cells would be of great interest. The availability of the HP1-β knockout mice should facilitate this endeavor (Aucott et al., 2008). However, phenotypic analyses of the global depletion of HP1 by siRNA may be complicated by the aforementioned multiple functions of HP1 in the cell, including its role in gene regulation and the apparent dual behaviors of HP1 (i.e., removal from heterochromatin and recruitment to the damage sites; Fig. 1 B). Furthermore, the three mammalian HP1 variants can heterodimerize, which may add an extra level of complexity to the analysis (Nielsen et al., 2001). Eventually, separation of function mutants or in vitro reconstitution experiments may be necessary to address this issue. Nonetheless, the original findings by Luijsterburg et al. (2009) will no doubt open up a new and exciting direction of research to understand the role of the enigmatic HP1 in DNA damage response and repair.

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