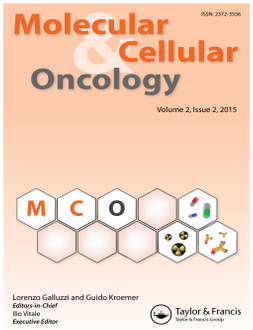

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AUTHOR'S VIEW

Novel mechanism of PCNA control through acetylation of its sliding surface

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ABSTRACT

Recent findings revealed a new unexpected regulatory mechanism that controls the proliferating cell nuclear antigen (PCNA). Multiple positively-charged lysine residues located on the ring inner surface are neutralized by acetylation and required for cellular resistance to Desoxyribonucleic acid (DNA) damage. Here, we summarize the key observations, discuss implications, and perspectives linked to cancer, as well as challenges for future work.

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PCNA has a ring-shaped structure highly similar in the 3 kingdoms of life, which has evolved to maximize its contacts with the DNA double-helix during sliding movements. Critical post-translational modifications, such as SUMOylation and ubiquitination, occur at the external surface of the PCNA ring to regulate its interaction with trans-acting factors without affecting its structure.[1] The inner surface of the ring, enriched in lysine and arginine residues, was previously suggested to merely act as a positively-charged surface involved in sliding along the negatively-charged DNA, thereby enabling PCNA function as a processivity factor for DNA polymerases (Fig. 1A). However, recent work has challenged this model not only by demonstrating that the sliding surface is heavily modified through acetylation of conserved lysine residues[2,3] but also by uncovering unexpected regulatory function of these modifications for resistance to DNA damage.[2] Finally, the observation that acetylation affects the structure of PCNA suggests that transient conformational changes of the trimeric ring may be coupled to the sliding motion on the DNA molecule.

PCNA inner surface is highly conserved as its sliding is essential for DNA polymerase processivity during replication and repair.[4] Even though the detailed molecular interactions are still not precisely defined, it is predicted that the positively-charged residues align with the negatively-charged phosphates of the DNA.[5] This suggests that their neutralization by acetylation may be an elegant mechanism to transiently modify the interaction between PCNA and the DNA molecule during movement. Interestingly, we found that the conserved lysines lining the inner surface of the ring have different functional importance in the response to DNA damage.[2] For instance, only two lysine residues (K20 and K77) sensitize cells to DNA damaging agents when mutated as acetyl-mimic glutamine residues (K20Q and K77Q). The difference in sensitivity suggests that these two acetylated lysines act in distinct DNA damage bypass/repair mechanisms and have differential effects on PCNA sliding. PCNA acetylation on K20 has been associated with a striking suppression of the DNA damage sensitivity to cells deficient in the mechanisms to bypass DNA lesions during replication. This occurs by promoting Rad52-dependent homologous-recombination mechanisms.[2] The effect of K77ac is not yet determined but preliminary observations suggest a function independent of K20ac (Billon and Cote, unpublished observations). Importantly, we demonstrated the existence of an intricate regulation of the sliding surface through multiple acetylated lysine residues with regard to the cellular resistance to the alkylating agent methyl-methanesulfonate (MMS). We showed that the presence of at least one lysine mutated in acetyl-mimic glutamine residue is important for the phenotypes observed, suggesting that an initial single acetylation event needs to occur at PCNA sliding surface.[2] It will be interesting to determine whether acetylation of single sites or specific combinations play distinct roles in DNA damage-induced mechanisms. An intriguing observation is that mutations of individual lysine residues into non-acetylable positively-charged arginine residues do not create any phenotype. This is likely explained by a level of functional redundancy between the different acetylation sites on PCNA sliding surface. On the other hand, mutating all six conserved lysine residues into arginines does lead to weak but significative DNA damage sensitivity without affecting normal DNA replication.

Interestingly, most of these lysine residues—with the specific exception of K20—have also been found acetylated in the absence of exogenous DNA damage in different species. Although blocking acetylation does not lead to growth

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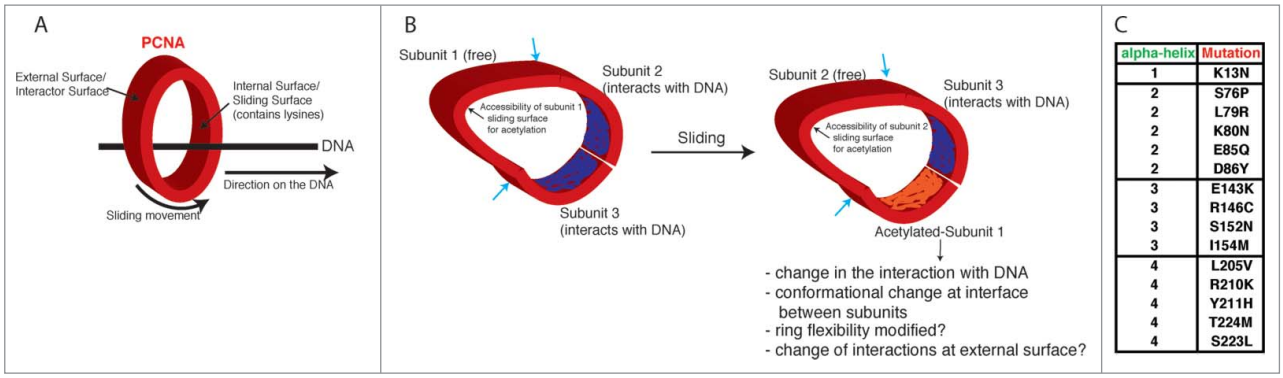

Figure 1. Importance of specific residues lining up the sliding surface of PCNA. (A) The PCNA ring during sliding motion on the DNA molecule. The two different surfaces of the ring are indicated. (B) Mechanism and consequences of acetylation at the inner surface of the PCNA ring. Subunits 1, 2, and 3 of homo-trimeric PCNA are arranged in a ring-shaped structure (in red) and a snapshot of potential conformations during the sliding motion is shown. In this scenario subunits 2 and 3 favor interactions with the DNA phosphates while subunit 1 is more flexible (see text). Blue and orange inner ring surfaces represent either positively-charged lysine residues (blue) or lysines neutralized by acetylation (orange). Blue arrows represent potential regions of contraction in the subunit interface to favor the interaction with DNA during sliding. (C) PCNA mutations found in cancer samples that are located at the inner sliding surface.

defect in the absence of DNA damage in budding yeast,[2] it is reasonable to believe that acetylation of PCNA influences DNA replication and repair/bypass at endogenous impediments such as RNA-DNA hybrids, repetitive sequences, DNA structures, and collision of the replication fork with other machineries. Alternatively, a DNA damage independent role of the sliding surface acetylation may exist during PCNA loading and unloading on the DNA.[6] The positively-charged residues have been proposed to play a role during PCNA loading, but mixed results were obtained.[7-9] It is likely that the combination of multiple acetylation sites can affect these important mechanisms. Thus, further studies are required to determine the functional significance of PCNA acetylation in the absence of exogenous DNA damage.

Preliminary observations suggest that acetylation occurs when PCNA is in the chromatin fraction, most likely coupled with its sliding or loading on DNA[3] (Billon and Cote unpublished observations). This raises an important question as to how an acetyltransferase can get access to the internal surface when these residues are contacting the DNA. An interesting hypothesis emerges based on a simulation of the dynamics of the three protomers of the PCNA ring in complex with DNA. It was proposed that only two PCNA subunits associate with the DNA to maximize their interactions, while the third is more mobile.[5] The mobility of the third subunit potentially provides the opportunity for an acetyltransferase to get access to the lysine residues at its inner surface, resulting in acetylation to tightly control motion of PCNA on DNA (Fig. 1B). Notably, PCNA differs in its multimeric association of subunits during the course of evolution, supporting the hypothesis that the ring has evolved as a trimer to better control its conformational flexibility during sliding, and access to the sliding surface for regulation. Moreover, our observation that PCNA-K20ac crystal structure shows long-range structural differences at the interface between protomers in the ring adds an unexpected mechanism of regulation of PCNA sliding surface through acetylation. It is possible that acetylation, along with the loss of interaction with the DNA phosphate, can induce conformational changes during sliding-coupled transactions. One consequence of a transient conformational change of the ring may be the accommodation of different polymerases. It can also affect the modification and affinity of interactors at the external surface of the ring. An important challenge will be to determine the precise molecular interactions of PCNA lysines, in their acetylated or unacetylated forms, with the DNA phosphate backbone, to reveal the impact of acetylation during PCNA movement on DNA. A first step would be to perform molecular simulations of acetylated PCNA dynamics.[5]

PCNA functions during DNA replication and repair have been recently explored as powerful targets to block cancer progression. This led to the development of specific molecules targeting PCNA external surface.[10] The description of new regulatory mechanisms of PCNA occurring at its inner sliding surface presents an exciting opportunity to develop molecules targeting, specifically, this region. Furthermore, about 30% of the PCNA mutations, listed on the cBioPortal repository of large-scale cancer genomic data sets, are located at its sliding surface, including conserved lysine residues that have been found acetylated (Fig. 1C). Collectively, the demonstration that PCNA sliding surface is highly regulated to control DNA damage resistance is a new unexpected concept that opens up new opportunities to develop tools to target fast replicating cancer cells, influencing the genome instability observed in human cancers to block their progression.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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