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Smc5/6 AS A NOVEL MODULATOR OF HEPATITIS B VIRUS INFECTION

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ABSTRACT

The Smc5/6 protein complex inhibits the replication of hepatitis B virus (HBV) by an unknown mechanism. HBx, the regulatory protein of HBV, binds Smc5/6, promotes its ubiquitination by recruiting it to DDB1-CRL4 E3 ubiquitin ligase and mediates proteasomal degradation. In absence of HBx, Smc5/6 complex binds to cccDNA of HBV and inhibits transcription and replication of HBV. Thus Smc5/6 complex acts antagonistically to HBV infection. This could aid in the development of potential therapeutics against HBV infection. In this minireview, the role of Smc5/6 in replication and its interaction with HBx protein of HBV is discussed.

Key words: HBV, Smc5/6 complex, DDB1, HBx, replication

Abbreviations: SMC, Structural maintenance of chromoosmes; HBV, Hepatitis B virus, HBx, HBV protein X, CRL4, CUL4-ROC1 ligase, DDB1, DNA damage binding protein1

1. INTRODUCTION

The Smc (Structural maintenance of chromosomes) proteins are a highly conserved class of proteins, required for the structural organization and function of chromosomes (Hirano, 2002). These proteins are central regulators of many chromosomal transactions such as sister chromatid cohesion, condensation, replication, transcription, dosage compensation and recombinational repair (Jeppsson *et al.*, 2014). Smc proteins are large proteins having 1000-1500 amino acids and share a common domain structure comprising of five distinct domains: Walker A and Walker B nucleotide-binding globular domains at N'- and C'-terminus, respectively, two coiled-coil domains separated by a moderately conserved hinge domain in the middle [Fig. 1]. These proteins fold at the hinge in a manner that both N' and C'-terminal domains come together and the coiled regions exhibit anti-parallel arrangement (Melby *et al.*, 1998; Haering *et al.*, 2002). Smc proteins were first identified during genetic analysis of chromosome loss mutants in budding yeast (Strunnikov *et al.*, 1993) as well as during composition analysis of chromosome scaffolds in mammalian cells (Earnshaw and Laemmli, 1983). Prokaryotes have one Smc protein while eukaryotes possess six types of Smc proteins (Smc1-6). Each Smc protein complex comprises of an Smc protein dimer (homodimer in prokaryotes and heterodimer in eukaryotes). In eukaryotes, the six Smc proteins form three heterodimers via hinge domain which along with non-Smc proteins give rise to three Smc complexes. Smc1 and Smc3 along with non-Smc proteins form cohesion complex. Similarly, Smc2 and Smc4 with non-Smc proteins form cohesin complex while Smc5 and Smc6 associate with six non-Smc subunits to form the yet unnamed Smc5/6 complex. In present mini-review, the role of Smc5/6 complex in replication has been discussed along with its novel role as a restrictive factor in HBV replication.

2. Smc5/6 COMPLEX AND REPLICATION

Replication of genome is a highly complex process that ensures the correct duplication of genetic information as well as preserves the genomic stability. Although there are differences in the

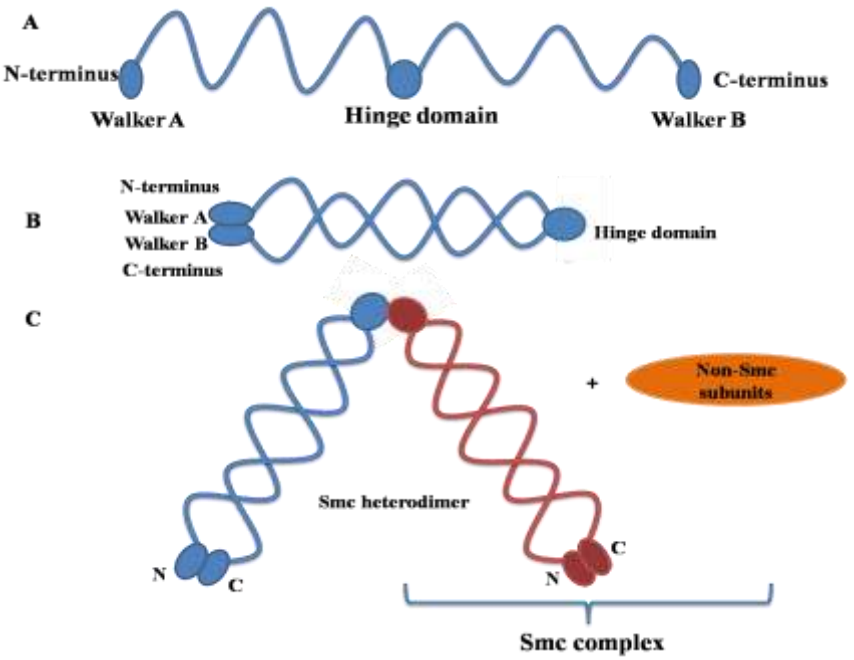

Fig. 1: Schematic of the Smc complex formation in eukaryotes. A) Basic arrangement of domains in a Smc protein monomer. B) Folding of Smc monomer occurs at the hinge domain, such that Walker A and Walker B motifs come close together to form the ATP-binding head domain and give rise to a rod-shaped molecule. C) Two Smc monomers undergo dimerization via hinge domains to form a Smc heterodimer, which along with other non-Smc proteins leads to the formation of Smc complex.

complexity of factors governing the process in different eukaryotes, the sequence of events and many proteins are highly conserved (Kearsey and Cotterill, 2003; Schwob, 2004). Initiation of replication occurs from multiple regions along the chromosomes. In budding yeast, *Saccharomyces cerevisiae*, such regions represent the origins of replication and are called 'autonomously replicating sequences' where replication forks are formed by unwinding of dsDNA (Branzei and Foiani, 2010). Various defects pertaining to the maintenance of replication forks have been observed in Smc5/6 mutants. In budding yeast *smc6-9* mutant cells, an increase in replication fork barrier-arrest, recombination and termination structures was detected in S-phase (Torres-Rosell *et al*., 2007). Additionally, there was a delay in replication of rDNA and premature mitotic entry before completion of rDNA replication in *smc6-9* cells (Torres-Rosell *et al.*, 2007). Also, during replication stress in presence of hydroxy urea (HU) various hypomorphic mutants of Smc5/6 components show hypersensitivity thus indicate a role of Smc5/6 complex during replication fork stalling (McDonald *et al*., 2003; Pebernard *et al*., 2004, 2008). In fission yeast there is evidence for the recruitment of Smc5/6 complex to stalled replication forks (Ampatzidou *et al.*, 2006). Similarly, a defect in the recruitment of Smc5/6 complex to the stalled replication forks has been observed in *nse5-ts1* in budding yeast (Bustard *et al.*, 2012). Also, a significant decrease in polymerase association with early firing origins has been observed in *nse5-ts1* (Bustard *et al.*, 2012). In addition to recruitment at stalled forks, the Smc5/6 complex is important for the rescue of collapsed replication forks (Lindroos *et al.*, 2006).

The Smc5/6 complex has also been implicated in a chromosome length-dependent role in replication. Delay in replication of longer chromosomes has been observed in *smc6-56* (Kegel *et al.*, 2011). The association of Smc6 is increased on longer chromosomes thus indicate that the binding sites are more frequent. Further, the binding also increased in a *top2*Δ strain. As Top2 is involved in relieving the sister chromatid inter-twinning, this reveals that the function of Smc5/6 is influenced by chromosome topology (Kegel *et al.*, 2011). Interestingly, in a hypomorphic *smc6-P4* mutant a

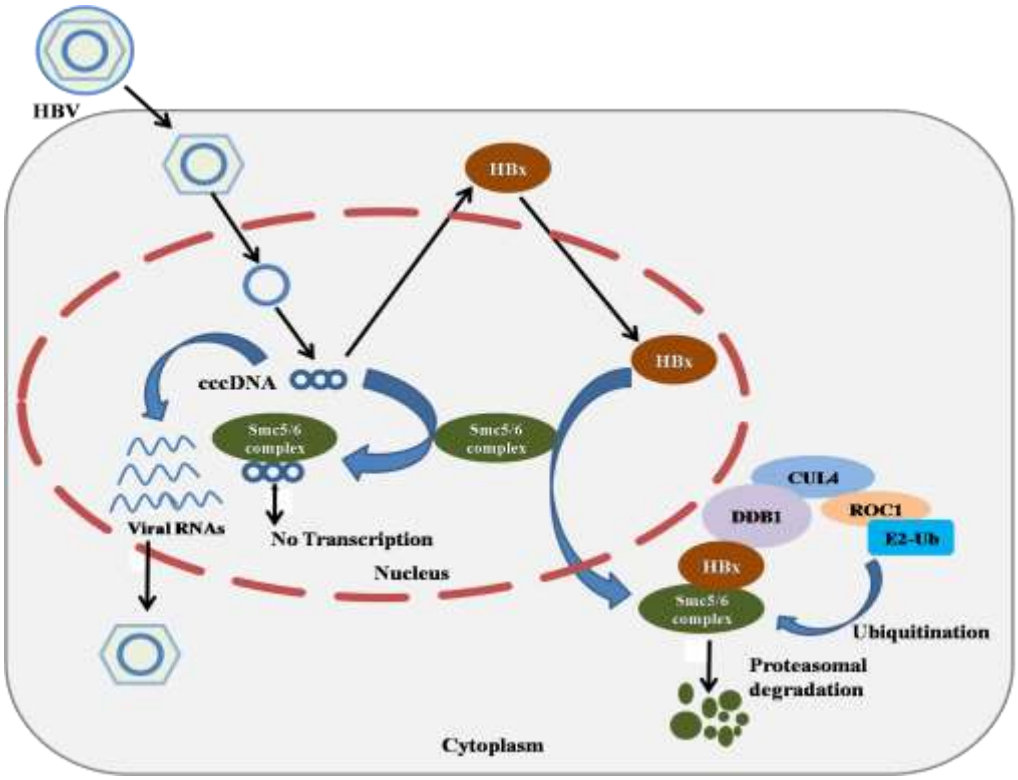

Fig. 2. A Schematic diagram depicting the interaction of Smc5/6 complex with HBx and its effect on viral replication. HBx, the regulatory protein of HBV, binds Smc5/6 complex that is recruited to DDB1-CRL4 E3 ubiquitin ligase and ubiquitinated that is subjected to proteasomal degradation. This leads to viral transcription and production of viral particles. Smc5/6 complex acts antagonistically to the viral transcription and hence viral replication.

faster progression of replication fork during replication stress by HU was noticed thus suggesting that the complex has a role in preventing the movement of replication fork which may be detrimental to cells (Peng and Feng, 2016). Menolfi *et al*. (2015) have revealed the role of Smc5/6 complex in replication through replication fork pause sites and suggested that Smc5/6 complex components may lead to defect in replication fork progression.

3. INTERACTION OF Smc5/6 COMPLEX WITH HBV

The identification of Smc5/6 as a binding partner of regulatory protein, HBx, in HBV replication has revealed a rather obscure function of Smc5/6 in cells. In contrast to its role in replication, it has recently been observed to be an anti-replication factor for HBV (Murphy *et al.*, 2016; Decorsière *et al.*, 2016). By using a covalently-linked HBx to a mutant DDB1 that cannot get incorporated into E3 ubiquitin ligase, CRL4, Decorsière *et al.* (2016) were able to identify Smc5/6 complex as substrate for DDB1-CRL4 ubiquitin ligase. HBx, the regulatory protein X of HBV, interacts with Smc5/6 complex and mediates its proteasomal degradation by targeting it to the CRL4 E3 ubiquitin ligase for ubiquitination (Murphy *et al.*, 2016; Niu *et al.*, 2017) [Fig. 2]. Conversely, the binding of Smc5/6 complex to cccDNA is prevented by HBx so facilitating the viral transcription. Additionally, the knockdown of Smc5/6 rescued HBV replication and transcription of HBx-deficient cells (Murphy *et al.*, 2016; Livingston *et al.*, 2017). It is unclear how the Smc5/6 complex affects the transcription process. It would be interesting to know what other proteins are involved in the process at this point. A possibility could be that the Smc5/6 complex prevents the accessibility of RNA polymerase or other transcription factors to cccDNA (Livingston *et al.*, 2017). As Smc5/6 complex harbours Nse1, a ubiquitin ligase (Doyle *et al.*, 2010), and Mms21, a SUMO ligase (Andrews *et al.*, 2005; Zhao and

Blobel, 2005) as its components, another possibility could be that the post-translational modifications [i.e. SUMOylation or ubiquitylation of RNA polymerase or other proteins by this complex] may inflict inhibitory effect on transcription from HBV genome. This would provide insight into deeper understanding of the mechanism by which Smc5/6 functions in restricting the transcription of HBV and, therefore, aids in potential therapeutic intervention against HBV infection.

Till date, the Smc5/6 complex was known to play a role in replication process. For the first time Smc5/6 complex has been reported to act as a negative modulator of HBV replication; thereby adding one more function to the diverse roles of this complex i.e. providing protection against HBV or as an anti-viral replication factor. The studies exploring the role of Smc5/6 in preventing the replication of any foreign entity would further our understanding about the mechanism of action as well as may provide information whether it provides protection against other viruses also or specifically acts on HBV. These findings unveil a novel role for the Smc5/6 complex as a restrictive factor by selectively blocking the transcription of extrachromosomal DNA.

4. FUTURE PERSPECTIVE

The Smc5/6 complex is unique in having a SUMO E3 ligase, MMS21, and a ubiquitin ligase, NSE1, as its components. It would be interesting to explore whether the post-translational modifi-cations, attributed to these proteins i.e. SUMOylation and ubiqitination, do have any influence on the modulation of viral transcription. It has been revealed that knockdown of either cytoplasmic or nuclear forms of E3 ligase i.e. CUL4A and CUL4B, respectively, block Smc5/6 degradation (Murphy *et al.*, 2016). The studies are desired that aim at the understanding of viral particle assembly in cytoplasm. This may help in devising the strategies against this HBV at multiple levels. As a global effort to cure and eliminate HBV, a clear understanding of the exact mechanism by which HBx mediates degradation of the Smc5/6 complex or how the complex mediates viral repression will pave the way for the development of novel therapeutics against HBV infection.

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