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USP37 deubiquitinates Cdt1 and contributes to regulate DNA replication

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ABSTRACT

DNA replication control is a key process in maintaining genomic integrity. Monitoring DNA replication initiation is particularly important as it needs to be coordinated with other cellular events and should occur only once per cell cycle. Crucial players in the initiation of DNA replication are the ORC protein complex, marking the origin of replication, and the Cdt1 and Cdc6 proteins, that license these origins to replicate by recruiting the MCM2-7 helicase. To accurately achieve its functions, Cdt1 is tightly regulated. Cdt1 levels are high from metaphase and during G1 and low in S/G2 phases of the cell cycle. This control is achieved, among other processes, by ubiquitination and proteasomal degradation. In an overexpression screen for Cdt1 deubiquitinating enzymes, we isolated USP37, to date the first ubiquitin hydrolase controlling Cdt1. USP37 overexpression stabilizes Cdt1, most likely a phosphorylated form of the protein. In contrast, USP37 knock down destabilizes Cdt1, predominantly during G1 and G1/S phases of the cell cycle. USP37 interacts with Cdt1 and is able to de-ubiquitinate Cdt1 *in vivo* and, USP37 is able to regulate the loading of MCM complexes onto the chromatin. In addition, downregulation of USP37 reduces DNA replication fork speed. Taken together, here we show that the deubiquitinase USP37 plays an important role in the regulation of DNA replication. Whether this is achieved via Cdt1, a central protein in this process, which we have shown to be stabilized by USP37, or via additional factors, remains to be tested.

Abbreviation: DUB, deubiquitinating enzyme.

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1. Introduction

Replicating the genome is an essential process for living organisms. DNA replication needs to be tightly regulated and monitored in order to preserve cellular genomic stability. First, because the genome needs to be replicated only once per cell cycle to avoid differences in genome content between the mother and daughter cells and second, because while DNA synthesis occurs, the genome is particularly vulnerable to damage and errors. In eukaryotic cells, complex mechanisms control and monitor DNA replication. A critical regulated step to avoid DNA replication associated instability occurs at the initiation of DNA replication. In eukaryotes, the control of DNA initiation requires the coordination of several proteins/protein complexes (reviewed in Costa et al., 2013; Fragkos et al., 2015). The origin recognition complex (ORC), a heterohexamer with DNA-dependent ATPase activity, directly recognizes and binds origins of replication. Subsequently, Cdc6 and Cdt1 are recruited to the origins and are able to load the minichromosome maintenance protein (MCM) complex MCM2–7, a heterohexamer that has ATPase-dependent DNA helicase activity, onto the replication origin. The binding of the helicase to the DNA starts the licensing of replication origins and forms the so-called prereplicative complex (pre-RC). In this loading process, ATP hydrolysis by Cdc6 helps the stable association of MCM2-7 with the DNA, after which the interaction with two additional factors enhance its helicase activity: the heterotetrameric GINS complex (formed by Psf1, Psf2, Psf3 and Sld5 proteins) and CDC45, forming the CMG complex. This complex is subsequently activated by S phase CDKs and CDC7–DBF4. Then, the replication protein A complex (RPA) binds to and stabilizes the single-stranded DNA, interacts with the DNA polymerase-α-DNA primase complex (Pol α complex) and acts as a "fidelity-clap" for the polymerase (Bochkareva et al., 1998; Fanning et al., 2006; Maga et al., 2001). Polymerization by the Pol α complex marks the start of DNA replication in the cell that continues with the recruitment of other DNA polymerases and the help of associated factors.

Cdt1 is a major regulatory factor during the initiation of DNA replication. Cdt1 is inhibited by Geminin, but regulation of Cdt1 protein levels also critically depends on ubiquitin-mediated degradation by the proteasome during cell cycle progression (Saxena and Dutta, 2005). Cdt1 levels are high during mitosis and G1 phase of cell cycle and low during S and G2 phases (Nishitani et al., 2001; Rialland et al., 2002; Wohlschlegel et al., 2000). DNA origin licensing starts during metaphase (Dimitrova et al., 1999), which coincides with drop of Geminin levels that continue to be low during G1 and rise again during S/G2 phases, thereby avoiding origin licensing after G1 (Clijsters et al., 2013; McGarry and Kirschner, 1998). Moreover, Cdt1 was shown to be degraded in the presence of DNA damage as Cdt1 levels drastically drop after a genotoxic insult (Higa et al., 2003; Hu et al., 2004). Together these regulatory mechanisms help to restrict the replication licensing to only once per cell cycle and/or avoid DNA replication in the presence of damage.

Several E3 ligase complexes were described to target Cdt1 for proteasomal degradation in different conditions. Cdt1 degradation during the cell cycle was shown to depend on the SCF–Skp2, the CRL4-Cdt2 E3 ubiquitin ligase complexes and on SCF-FBXO31 (Chandrasekaran et al., 2011; Higa et al., 2006; Jin et al., 2006; Johansson et al., 2014; Nishitani et al., 2006; Sansam et al., 2006). Moreover, CRL4-Cdt2 dependent degradation of Cdt1 depends on DNA-bound PCNA, which occurs during S phase and after DNA damage (Arias and Walter, 2006; Senga et al., 2006).

Although relatively much is known about the ubiquitination of Cdt1, the reverse process or Cdt1 deubiquitination is less studied. In this article we find USP37 as a ubiquitin hydrolase for Cdt1. USP37 was first identified as key enzyme that stabilizes Cyclin A counteracting the ubiquitination by the anaphase-promoting complex APC/CCdh1 (Huang et al., 2011). USP37 itself is a substrate of the APC/CCdh1 and its regulated during the cell cycle as USP37 levels increase at the G1/S boundary, and remain high during S and G2 phases (Huang et al., 2011). USP37 is degraded at the G2/M boundary in a SCF-β-TRCP and Plk1-dependent manner and remains low in G1 phase of cell cycle (Burrows et al., 2012). Since its first description in 2011, USP37 function has been linked to a number of different proteins. These include important pro-division regulators (Cyclin A, c-Myc or 14-3-3γ) but also USP37 is able to regulate genomic stability by controlling DNA double strand break repair by homologous recombination or proper mitotic progression by controlling WAPL, a negative regulator of chromatin cohesion (Huang et al., 2011; Kim et al., 2015; Pan et al., 2014; Typas et al., 2015; Yeh et al., 2015).

In this article we describe a new role of USP37 controlling Cdt1 and DNA replication. USP37 overexpression increases Cdt1 protein levels and knock down of this deubiquitinating enzyme (DUB) leads to lower Cdt1 levels. Importantly, USP37 depletion impacts on the loading of the MCM2-7 replication helicase and on the replication fork speed demonstrating its critical role in regulating DNA replication.

2. Materials and methods

2.1. Cell lines and plasmids

U2OS and 293T cells were grown using standard procedures.

The Addgene expression plasmid #22602 for Flag-HA-USP37 was obtained from JW Harper (Harvard Medical School, Boston, USA) (Sowa et al., 2009). A catalytic inactive version of USP37 was obtained by changing the Cysteine 350 to Serine into the Flag-HA-USP37 expressing plasmid, using the QuickChange Site-Directed Mutagenesis Kit (Agilent Technologies). The same kit was used to generate an siRNA#1 resistant version of the Flag-HA-USP37 by introducing the following 4 silent mutations (shown in lower case) in the cDNA: CAGCTgtcTCAcAACATT. Cdt1 cDNA was cloned into the pEXPR-IBA103 (Novagen) vector to obtain a Strep-Cdt1 expressing plasmid. An expression plasmid for His-Ubiquitin was a gift from D. Bohmann (Rochester, New York, USA) (Salghetti et al., 1999).

2.2. Cell synchronizations

Cells in G1 or mitosis were synchronized with a single thymidine block (2.5 mM thymidine for 24 h) and release. Also to