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CtIP: A DNA damage response protein at the intersection of DNA metabolism

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ABSTRACT

The mammalian CtIP protein and its orthologs in other eukaryotes promote the resection of DNA double-strand breaks and are essential for meiotic recombination. Here we review the current literature supporting the role of CtIP in DNA end processing and the importance of CtIP endonuclease activity in DNA repair. We also examine the regulation of CtIP function by post-translational modifications, and its involvement in transcription- and replication-dependent functions through association with other protein complexes. The tumor suppressor function of CtIP likely is dependent on a combination of these roles in many aspects of DNA metabolism.

1. Introduction

The CtBP (C-terminal binding protein) interacting protein (CtIP) was initially identified as part of the CtBP transcriptional corepressor complex that mediates repression of many genes and plays important roles in development and cancer [1,2]. In addition, CtIP was found to bind directly to the retinoblastoma (Rb) protein [3], as well as the tumor suppressor BRCA1 [4], suggesting the protein might function in tumorigenesis. Despite, an initial association with the regulation of gene expression, CtIP is now better known for its role in DNA double-strand break (DSB) repair and genome stability. CtIP is an interacting partner of the Mre11/Rad50/Nbs1 (MRN) DNA damage sensor protein complex, which recognizes DNA double-strand breaks (DSBs) and promotes the resection of 5′ strands to generate 3′ single-stranded intermediates that are necessary for homologous recombination [5–11]. CtIP is involved in these pathways supporting the initial steps of DNA processing as well as in the recruitment of additional DNA repair proteins [10,12–14]. There are no human conditions associated with complete CtIP deficiency, as it is lethal based on deletion experiments in the mouse [15]. Rare hypomorphic mutations in human CtIP have been identified, however, which lead to severe intrauterine growth retardation, profound microcephaly, dwarfism, mental retardation, and isolated skeletal abnormalities in patients with Seckel and Jawad syndromes [16,17].

CtIP is a known tumor suppressor. CtIP heterozygosity in the mouse generates a high frequency of tumorigenesis, indicating that CtIP haploinsufficiency is linked to cancer [15]. Mutations in CtIP are also found associated with endometrial, colorectal, breast, ovarian, and myeloid cancers in humans [18–21]. A homopurine repeat in CtIP is a hotspot for 1 bp deletions in colorectal cancers, which generates a truncated form of the protein [21]. A significant link also exists between CtIP levels, certain CtIP point mutations, and specific breast cancer types, while the absence of CtIP in breast cancer cells is associated with tamoxifen resistance [22,23]. Thus, CtIP has been suggested to be an important biomarker for breast cancer prognosis and clinical management [23].

CtIP interacts with a large number of proteins related to carcinogenesis. BRCA1 is a well known gene product linked to familial ovarian and breast cancers, while other interactors, such as the Rb protein, the LIM-only protein 4, CtBP, ZBRK1, and HMGA2, are also associated with cancer and regulate different branches of DNA metabolism including replication and transcription [2,24–27]. These diverse interactions may also explain the severe effects of CtIP deletion on mammalian cells.

Abbreviations: CtIP, C-terminal binding protein 1 (CtBP1) interacting protein; DSB(s), double-strand break(s); The MRN complex, Mre11/Rad50/Nbs1; ATM, ataxia-telangiectasia mutated kinase; ATR, ATM and Rad3 related kinase; IR, ionizing radiation; UV, ultraviolet; MMS, methanesulfonate; CDK, cyclin-dependent kinase; CPT, camptothecin; HR, homologous recombination; MMEJ, microhomology-mediated end joining; NHEJ, non-homologous end joining; The 9–1–1 complex, Rad9–Hus1–Rad1; LMO4, LIM domain transcription factor 4.

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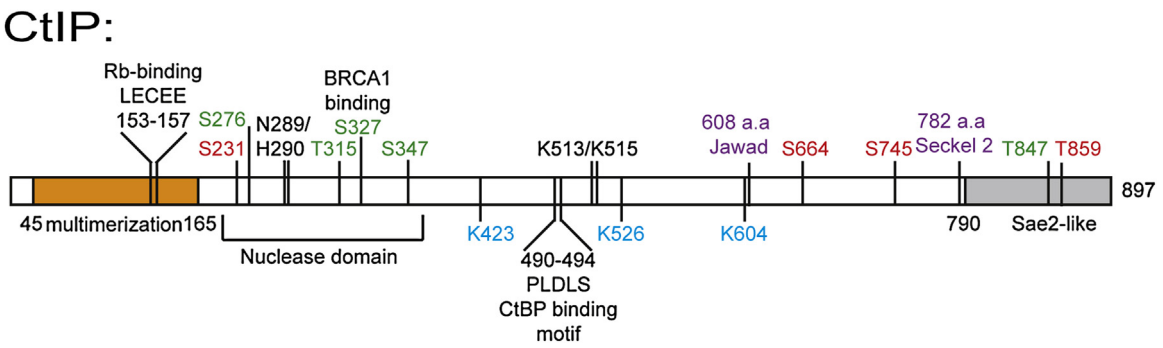

Fig. 1. Schematic diagram of CtIP showing known features. Orange and grey regions depict multimerization and MRN binding domains, respectively. Rb-, BRCA1-, and CtBP-binding motifs are shown at a.a. 153–157, 327, and 490–494, respectively [1,3,47,68]. S/TP (green) and S/TQ (red) phosphorylation sites are depicted at S276, T315, S327, S347, T847, and S231, S664, S745, T859, respectively [10,39,40,44–46,49]. K432, K526, K604 acetylation sites [41] are shown in blue. 608 a.a. Jawad, and 782 a.a. Seckel 2 (shown in purple) denote sites of truncations associated with Jawad and Seckel 2 syndromes (Note: C-termini of both truncations have alterations in sequences due to open reading frame shift) [17]. Nuclease domain includes residues from approximately 180 to 350 [10,12]. The K513/K515 residues highlight residues implicated in DNA binding [117].

2. CtIP is an endonuclease

The CtIP gene product is an endonuclease with specificity for 5′ flaps of splayed DNA, although it binds to different DNA structures with comparable affinity [10,12]. To cleave the 5′ overhang, CtIP requires both 3′ and 5′ flaps of a Y-DNA structure, and unlike its yeast functional homolog Sae2 it does not cleave single-stranded DNA adjacent to hairpin structures [28]. CtIP requires a divalent metal for its catalytic activity and shows the highest activity with Mn^{2+} ions, similar to the endo/exonuclease Mre11 [10]. However, unlike Mre11 [29], substituting Mg^{2+} for Mn^{2+} does not change either the polarity or pattern of DNA cleavage [10]. Recombinant CtIP co-purifies with an unknown transition metal, which in the presence of sodium ascorbate and hydrogen peroxide leads to cleavage of the CtIP protein in N-terminal region encompassing residues 181–290 [10]. Through limited sequence similarity searches and auto-proteolysis patterns, Makharashvili et al. and Wang et al. identified several groups of residues, N289/H290, N181/R185, and E267/E268, responsible for CtIP nuclease activity [10,12]. Recombinant CtIP protein with mutations in these residues shows severely diminished nuclease activity, but retains wild-type DNA binding ability, suggesting that these mutants can be used as separation of function alleles specific for loss of nuclease activity.

Analysis of these mutants reveals that CtIP has at least two distinct roles in the processes of DSB end resection. DSBs produced by restriction enzymes create simple broken ends that require the CtIP protein but not its nuclease activity [10,12]. In contrast, inverted DNA repeats, topoisomerase poisons, and ionizing radiation (IR) result in more complex DNA lesions with extruded DNA hairpins, protein–DNA adducts, or mixed types of DNA damage, respectively, and repair of these lesions requires not only the CtIP protein but also its nuclease activity. Similar patterns of sensitivity to DNA damaging agents and protein–DNA adducts were observed in CtIP deletion mutants in other organisms, suggesting a conserved mechanism of resolution of these lesions [30–34]. Thus, CtIP nuclease activity is directly implicated in the removal of covalently linked proteins and severely damaged bases from DNA, in addition to a role for the CtIP protein in DNA repair that likely occurs through CtIP-dependent recruitment of other DNA damage repair factors (Fig. 1).

3. CtIP and its regulation in cells

CtIP interacts with a large number of proteins involved in different branches of DNA metabolism, including DNA damage repair, DNA replication, and transcription regulation. The structure of the protein is not well defined; however, it is clear that the N-terminal half of CtIP and its orthologs contains domains responsible for protein's DNA binding and nuclease activities, as well as for multimerization and protein–protein interactions [10,12,35–38]. The C-terminus of CtIP is likely to have a regulatory function, since point mutations and partial deletions, but not its complete removal, render the enzyme inactive [10,12,39]. Despite extensive analysis of CtIP by many groups, however, a complete understanding of how CtIP is regulated remains elusive. Cell cycle- and DNA damage-dependent enzymes modify CtIP by phosphorylation, acetylation, ubiquitination, and proline isomerization, which affect protein's nuclease activity, interactions with CtIP's partner proteins, and proteasome-mediated degradation [10,39–46].

CtIP is extensively phosphorylated by cyclin- (CDK) and DNA damage-dependent kinases, which modulate different CtIP activities [10,39,40,44–50]. For example, the T847A and S327A CtIP mutants that cannot be phosphorylated by CDK are proficient in DNA binding and nuclease activity in vitro [10], but cannot complement the sensitivity of CtIP-depleted cells to DNA damaging agents, including ionizing radiation (IR), UV, cisplatin, methyl methanesulfonate (MMS), topoisomerase 1, and topoisomerase 2 poisons [39,40,48,51].

In contrast, a mutation in CtIP serine 347, also an SP site, yields a mutant that is nuclease inactive and supports restriction enzyme induced-, but not camptothecin- (CPT), etoposide-, or IR-induced DNA damage repair [10,40]. The S276A and T315A S/TP mutations, which abolish Pin1-mediated isomerization of the P277 and P316 proline residues to target CtIP for degradation, also render the protein nuclease-deficient, and make cells sensitive to DNA damage [10,42]. The S347 and S276 site are part of a group of CDK sites identified by Wang et al. that promote the binding of Nbs1 to CtIP and also promote phosphorylation by ATM [40], thus, some of the modifications of CtIP are clearly essential for multiple functions. Interestingly, the non-cyclin dependent kinase Plk3 was shown to phosphorylate the S327 and T847 sites in the G_0/G_1 cell cycle phases in response to DSBs [46], consistent with other reports suggesting roles for CtIP in G_1 [52–54]. However, in the absence of HR repair machinery these modifications engage CtIP and MRN into MMEJ-mediated repair [53].

Similar diverse effects are observed with the S/TQ sites modified by DNA-damage-dependent kinases, and in some cases CDK modification is required for ATM-mediated phosphorylation [40], similar to modifications of Sae2 in budding yeast [55]. The ATM phosphorylation sites on CtIP, S664, and S745, were originally reported as modifications of CtIP that blocked interaction of Brca1 after DNA damage [44]. However, these ATM-dependent sites, along with the S231 SQ site on CtIP are also essential for CtIP nuclease activity in vitro [10]. The ATR kinase has also been shown to phosphorylate CtIP, on the DNA damage-dependent TQ site T859 in human CtIP