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Review

CtIP/Ctp1/Sae2, molecular form fit for function

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ABSTRACT

Vertebrate CtIP, and its fission yeast (Ctp1), budding yeast (Sae2) and plant (Com1) orthologs have emerged as key regulatory molecules in cellular responses to DNA double strand breaks (DSBs). By modulating the nucleolytic 5′-3′ resection activity of the Mre11/Rad50/Nbs1 (MRN) DSB repair processing and signaling complex, CtIP/Ctp1/Sae2/Com1 is integral to the channeling of DNA double strand breaks through DSB repair by homologous recombination (HR). Nearly two decades since its discovery, emerging new data are defining the molecular underpinnings for CtIP DSB repair regulatory activities. CtIP homologs are largely intrinsically unstructured proteins comprised of expanded regions of low complexity sequence, rather than defined folded domains typical of DNA damage metabolizing enzymes and nucleases. A compact structurally conserved N-terminus forms a functionally critical tetrameric helical dimer of dimers (THDD) region that bridges CtIP oligomers, and is flexibly appended to a conserved C-terminal Sae2-homology DNA binding and DSB repair pathway choice regulatory hub which influences nucleolytic activities of the MRN core nuclease complex. The emerging evidence from structural, biophysical, and biological studies converges on CtIP having functional roles in DSB repair that include: 1) dynamic DNA strand coordination through direct DNA binding and DNA bridging activities, 2) MRN nuclease complex cofactor functions that direct MRN endonucleolytic cleavage of protein-blocked DSB ends and 3) acting as a protein binding hub targeted by the cell cycle regulatory apparatus, which influences CtIP expression and activity via layers of post-translational modifications, protein–protein interactions and DNA binding.

1. Introduction

DNA double-strand breaks (DSBs) pose a serious threat to genomic integrity, and arise from multiple exogenous and endogenous sources. These lesions are frequently characterized by complex chemical modifications including covalently-linked proteins, such as Spo11 blocked ends that are formed during meiotic recombination [1], Topoisomerase 2 cleavage complexes [2,3], and chemically adducted ends created by ionizing radiation induced free radicals that are incompatible with ligation [3–5]. The repair of DSBs relies on three available pathways: non-homologous DNA end joining (NHEJ) [6], homologous recombination (HR) [7] and microhomology-mediated end joining (MMEJ) [8]. Both NHEJ and MMEJ are potentially mutagenic, due to processing of the DNA ends to remove chemical modifications (NHEJ) or deletion of nucleotides in order to align small regions of microhomology (MMEJ) prior to ligation. For comprehensive discussion on these topics, the reader is directed to recent reviews [6,8,9].

Homologous recombination facilitates error-free repair by utilizing a DNA template, typically a sister chromatid. The Mre11-Rad50-Nbs1 (MRN) complex initiates HR and DNA damage response signaling to halt cell cycle progression [7]. MRN activities are regulated by a protein known as CtIP in mammals [10], Ctp1 in *Schizosaccharomyces pombe* [11], Sae2 in *Saccharomyces cerevisiae* [12], and Com1 in *Arabidopsis thaliana* [13]. CtIP and MRN are also important for resolution of complex protein blocked ends [14–18] and critical for 5′-3′ DNA strand resection proximal to DSBs [9–11,18,19].

Mre11 is the catalytic subunit of the complex and possesses Mn^{2+}-dependent endonuclease and 3′-5′ exonuclease activities *in vitro* [20–24]. Recent studies of human and *S. cerevisiae* MRN and CtIP/Sae2 have shown that a bi-directional resection event takes place, whereby the CtIP-stimulated Mre11 endonuclease first cuts proximal to the 5′-end of the break, and is followed by the 3′-5′ exonucleolytic removal of DNA towards the break site (Fig. 1) [17,18,25,26]. The two-step endonuclease, then reverse exonuclease process resolves a historical polarity paradox associated with the Mre11 exonuclease activity that catalyzes nucleolytic resection with 3′-5′ polarity, but generates ends

Abbreviations: MRN, Mre11-Rad50-Nbs1; HR, homologous recombination; THDD, tetrameric helical dimer of dimers; DSB, double-strand break; IR, ionizing radiation; NHEJ, non-homologous DNA end joining; MMEJ, microhomology-mediated end joining; IDR, intrinsically disordered region

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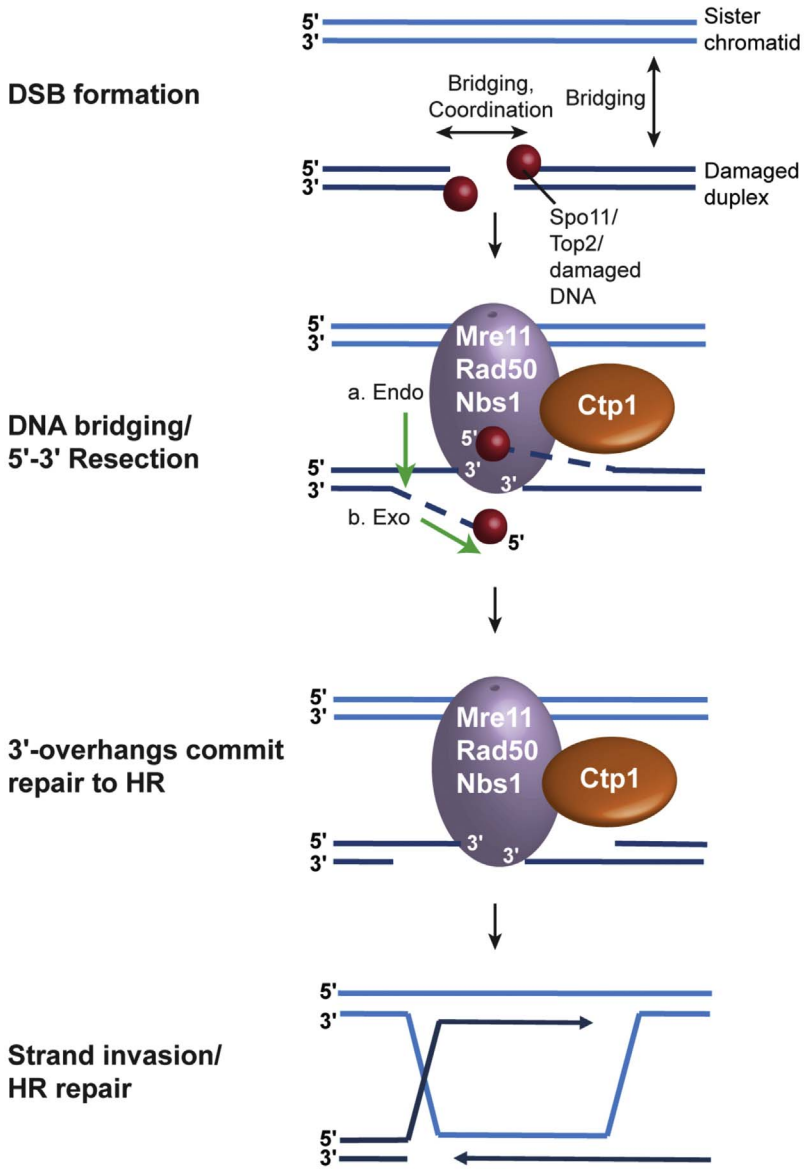

Fig. 1. Initiation of homologous recombination. DNA double-strand breaks (DSBs) often contain "dirty" ends, with secondary DNA structure, protein and chemical adducts. Mre11-Rad50-Nbs1 (MRN) recognizes DNA breaks, bridging both across the DSB and to the sister chromatid. Mre11 is stimulated by Ctp1 and carries out a two-step resection, utilizing first endonuclease activity, then 3′-5′ exonuclease activity, generating single-stranded 3′-overhangs. These ssDNA overhangs are further resected and then bound by Rad51, forming a nucleoprotein filament for invasion of the sister chromatid, initiating homologous recombination repair.

expected from a 5′-3′ polarity nuclease. MRN-CtIP mediated strand incision further primes the damage site for extensive 5′-3′ DSB resection by additional helicases and nucleases including Sgs1, Dna2 and Exo1 [27–29]. Together these reactions create 3′-overhanging ssDNA required for strand invasion and recombination repair. In addition to DNA end processing nucleolytic reactions, a second key requirement of DSB repair is the ability to coordinate and bridge DNA ends. This is achieved through the deployment of MRN complex and CtIP/Ctp1/Sae2 architectural DNA scaffolding activities [5,23,30–33].

The current state of knowledge on the structural biology of Mre11/Rad50/Nbs1 has been reviewed and discussed [7,34–39]. New discoveries from integrated structural, biophysical and biological studies are illuminating novel functional roles for CtIP orthologs in controlling DSB repair DNA transactions. We provide a survey of recent work on CtIP with a focus on implications of structural studies for our understanding of CtIP function.

2. CtIP architecture assembles a flexible DNA and protein-binding scaffold for the regulation of DSB repair

2.1. Molecular architecture of CtIP family proteins

At first glance, the primary sequences of CtIP orthologs are unremarkable. Their functions are not revealed by the presence of readily identifiable structured enzymatic domains (*e.g.* nuclease folds). The most conspicuous feature of these proteins from yeast to human is the high abundance of low complexity sequence throughout the length of the protein (Fig. 2) [5,40,41]. Computational calculations of protein disorder using the database of protein disorder predictions [40] places CtIP orthologs as highly disordered proteins on the spectrum of protein disorder [42,43]. Additional regions that are predicted to contain structural motifs and which show a moderate level of homology across phyla from yeast to human map to the extreme N- and C- termini of CtIP (Fig. 2). Structural characterizations have identified an amino-terminal oligomerization fold that assembles a functionally critical minimalist tetramer [5,44]. Additional low complexity protein sequence confers a dynamic DNA strand coordination and protein binding regulatory scaffold. For discussion herein, we will explicitly refer to studies based on vertebrate CtIP, budding yeast Sae2 and fission yeast Ctp1 with their