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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.

Keywords

CtIP/Ctp1/Sae2; homologous recombination; resection; DNA bridging; intrinsically disordered proteins

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Conflicts of interest

The authors declare no conflicts of interest.

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 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 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 respective nomenclature.

2.2 A tetrameric oligomerization scaffold conserved from yeast to humans

Examination of primary sequence detected putative coiled-coil heptad repeats at the N-termini of CtIP [45], Ctp1 [11], and in a Sae2 self-association domain [46]. Consistent with the structural order predictions and circular dichroism analysis of protein fragments, the N-terminal 57 residues of Ctp1 were mapped as the sole ordered and protease stable domain in the protein [5]. Two recent studies unveiled X-ray crystal structures corresponding to this region in Ctp1 and CtIP, revealing a strong concordance in architecture [5,44]. Despite relatively low-level sequence identity, a similar four-helix bundle assembly is observed in these structures (Fig. 3A–B). This mode of coiled-coil quaternary structure in CtIP orthologs differs from other characterized tetrameric coiled-coil assemblies [47,48]. Whilst simplistic and compact, the structurally invariant four-helix bundle tetramer architecture has key implications for our understanding of CtIP function. Overall the tetramer is assembled from parallel coiled-coil dimers, which splay at their N-termini to build a homotetramer, also known as the **t**etrameric **h**elical **d**imer of **d**imers or THDD (Fig. 3A) [5,44]. In both the *H. sapiens* and S. *pombe* X-ray structures, aromatic ring stacking from opposing coiled-coils contributes to the stability of the tetramer (Fig. 3B). The structures can be closely superimposed, but the crossing angle of the tetrameric helices differs by ~17° (Fig. 3A), which can be attributed to a one-residue frame shift associated with the tetramer to coiled-coil transition, though the hydrophobic character of residues mediating tetramerization is maintained (Fig. 3B).

Structural conservation of the architecture of this assembly over millennia implies the tetramer performs a critical function. While structures of the Sae2 oligomerization domain are not yet known, Sae2 N-termini are predicted to have helical character. An L25P mutation within the predicted Sae2 coiled-coil that disrupts Sae2 self-association also impacts Sae2 influence on Mre11 nuclease and checkpoint function [46]. Similarly, mutational analyses

targeting the hydrophobic tetramer core showed that an L27E mutation renders CtIP dimeric *in vitro*. Interestingly, CtIP-L27E supports microhomology mediated end-joining, but not homologous recombination [44]. Thus, alternative oligomeric states may contribute to distinct CtIP functions *in vivo*. Complete deletion of the THDD region impairs all measured Ctp1 DSB repair activities *in vivo* in S. *pombe* [5]. Phosphorylation of Sae2 also regulates transitions between these oligomeric states, suggesting that regulation of assembly state controls may also modulate functions [49].

A direct DNA binding function has been ascribed to the isolated Ctp1 THDD helical bundle domain. The mode of Ctp1 DNA binding is likely to be distinct from that employed by coiled-coil basic leucine zipper (b-ZIP) transcription factors such c-Fos/Jun and GCN4, where the coiled-coil dimer scaffolds positively charged helicies that bind the DNA major groove in a sequence specific manner. [50–52]. Ctp1, however, likely employs a sequence independent mode of DNA interaction. Mutagenesis has identified a basic surface (a KKxR motif) on the exterior of the Ctp1 coiled-coil that is critical for supporting Ctp1 dsDNA binding functions *in vitro*, and chromosomal DNA repair following ionizing radiation *in vivo* [5]. An analogous surface is found on the human CtIP THDD domain, suggesting this DNA binding determinant is evolutionarily conserved (Fig. 3C) [44]. The molecular basis for Ctp1 interactions with nucleic acid remains undetermined. Additional structural and molecular characterizations of these interactions will undoubtedly shed important light on the nature of CtIP nucleic acid transactions in DSB repair.

2.3 Intrinsically disordered regions coordinate protein-protein interactions

For Ctp1, predictions of protein disorder have been validated by biophysical analyses. Circular dichroism and small angle X-ray scattering of the Ctp1 C-terminal region (aa 60–294) are consistent with this region adopting a random coil structure [5]. Intrinsically disordered regions (IDRs) contain low sequence complexity, with a reduced number of bulky hydrophobic residues and a high level of charged or hydrophilic residues [53,54]. The IDR of CtIP/Ctp1/Sae2 follows this trend with 28–33% of the IDR consisting of charged residues (D, E, R or K) and low hydrophobicity [55].

Intrinsically disordered proteins are often targeted by post-translational modifications and act as hubs for protein interaction networks that are involved in signaling, transcription, translation or cell-cycle regulation [42,43,53,54]. Interactions with other proteins or DNA can induce structural rearrangements (e.g. disorder to order transitions) in IDRs, facilitating reversible binding that is characteristic of regulatory scaffolds that require interactions to be transient and dynamic [53,54]. Indeed, CtIP orthologs have been reported to bind multiple DNA damage response protein cofactors via its IDR, positioning CtIP as a key regulatory hub in DSB repair. CtIP-protein interactions include BRCA1 [56–59], both Mre11 and Nbs1 of the MRN nuclease complex [10,60,61] and EXD2 nuclease [62]. Vertebrate CtIP has added roles in regulation of transcription. In fact CtIP was first identified through interactions with CtBP, a transcriptional repressor [63], and has been found to interact with Ikaros [64], Rb [65], and LMO4 [66]. All of these interactions occur via short peptide interaction motifs in the CtIP IDR.

An important functional interface of the Ctp1 IDR is the direct interaction with the N-terminal region of Nbs1 [60,61,67,68]. Genetic, biochemical, and structural analyses of the S. *pombe* Ctp1-Nbs1 interface are the most extensively characterized. Ctp1 was cloned as an MRN epistasis group protein, and as a high copy suppressor of an Nbs1 FHA domain mutant (nbs1-s10) [11,69]. These genetic interactions are explained by observations that Nbs1 directly binds a phosphorylated Casein Kinase 2 consensus pS-X-pT tandem repeat in Ctp1 [60,67]. Structural work showed that the S. *pombe* Nbs1 FHA domain directly engages Ctp1 (Fig. 3D), and that disruption of the Ctp1-Nbs1 interface blocks Nbs1 mediated recruitment of Ctp1 to DSBs *in vivo* (Fig. 3D) [60]. It is hypothesized that this direct tethering coordinates Ctp1-MRN activity proximal to DSBs [60,67].

2.4 The Conserved Sae2-like region

The second region of conservation amongst CtIP/Ctp1/Sae2 lies at the C-terminus of the protein, the Sae2-like domain (Fig. 2). Here, two motifs “RHR” (in CtIP/Ctp1, “RNR” in Sae2) and “CxxC” (in Ctp1/CtIP, absent in Sae2) are important for DSB resection and regulation. Deletion of the Ctp1 C-terminus encompassing the RHR renders cells sensitive to camptothecin and MMS, but can be rescued by deletion of Ku80 *in vivo*, indicating a role for Ctp1-dependent resection in displacing Ku70/80 during DNA repair [70,71]. RHR mutations also confer chromosomal repair defects *in vivo* [5], and abrogates clipping of Rec12 (Spo11)-DNA adducts by Mre11 in meiosis, highlighting the importance of Ctp1 in the repair of protein-DNA adducts [72]. These defects can be explained by *in vitro* observations that mutations to the RHR impair DNA binding and DNA bridging by Ctp1 in S. *pombe* [5]. C-terminal deletions of CtIP removing the Sae2-like domain impair MRN interactions, DNA-end resection, and activation of the G2-M checkpoint [10,44]. This region is further critical to the regulation of MRN nuclease activity *in vitro* [17,18]. The Ctp1 and CtIP CxxC motif is similar to a motif found in Rad50 that is associated with Zn^{2+} binding and assembly of the Rad50 coiled-coil-hook assembly [32,73]. Vertebrate CtIP has an additional N-terminal Zn^{2+}-binding CxxC (amino acids 82–92), which is proposed to stabilize structure [44]. Mutation of Ctp1 CxxC renders cells sensitive to multiple DNA damaging agents such as hydroxyurea, camptothecin and UV [11], however the precise function of the CxxC motif is unknown.

3. CtIP DNA transactions

A consensus view has emerged that CtIP orthologs are all DNA binding proteins. The overall architecture of CtIP orthologs and evidence that Ctp1, Sae2, and CtIP bind [5,44,74–77] and bridge [5,33] DNA have important implications for the function of these proteins. Recent data suggests three possible roles for these proteins participating in DNA transactions at double strand breaks: 1) DNA bridging and strand coordination, 2) acting as a cofactor for MRN nuclease end processing activities at DSBs and 3) possessing intrinsic nuclease activity (Fig. 4A).

3.1 DNA binding and strand coordination (bridging) activities

All three homologs bind DNA [5,44,74–77], and display a slight preference for forked DNA structures over ssDNA and dsDNA [5,76,77]. DNA binding activity in Ctp1 has been

mapped to both the THDD tetrameric core and Sae2-like regions (the RHR motif) [5]. In the context of a tetramer this indicates that Ctp1 DNA interactions are multivalent, with at least eight DNA interaction sites, all tethered together by flexible polypeptide linkers. Xenopus CtIP [75] bears an additional central DNA binding region, suggesting increased complexity of CtIP DNA binding capacity in vertebrates. In isolation, the purified N- and C-terminal DNA binding regions of S. *pombe* Ctp1 show weak interactions with nucleic acid, with binding affinities in the micromolar range. The combination of these low affinity sites evidently contributes to the high nanomolar affinity of Ctp1-DNA interactions [5].

Interestingly, early work uncovered a role for Sae2 in the intra-chromosomal bridging of HO-endonuclease generated DSBs in budding yeast [33]. Examination of the role of Sae2 in resection of IR induced breaks of a circular chromosome revealed that Sae2 is important for coordination of two-ended resection on each side of a DSB [79]. Consistent with a strand coordination activity, the architecture of Ctp1 appears appropriate to mediate DNA bridging (Fig. 4B) [5,44,80]. *In vitro*, purified Ctp1 can link DNA molecules in bridging reactions [5]. An additional striking feature of the Ctp1-DNA interaction is its assembly as multimeric complexes on DNA when viewed by electrophoretic mobility shift analysis, indicative of higher order protein-nucleic acid structures [5]. Mutational analyses established that integrity of the intrinsically disordered region (IDR), RHR motif, and THDD are all required for DNA bridging [5]. It is possible that Ctp1 coordination of two duplex molecules requires the flexibility imparted by the IDR, and direct protein-DNA interactions of both the THDD and RHR. Ctp1 DNA bridging interactions may involve a host of DNA binding surface combinations within a Ctp1 tetramer. Further, internal deletion studies have indicated that the length of the IDR is critical for Ctp1-mediated DNA bridging *in vitro* [5]. The localization of the Nbs1 binding site to the IDR also suggests that Nbs1 interactions with phosphorylated Ctp1 may regulate Ctp1 DNA transactions, or vice-versa [5,60]. However, the precise molecular basis for DNA bridging and strand coordination remains unknown.

It is well established in multiple systems that the Mre11-Rad50 (MR) core complex can also bridge DNA [23,30–32,81,82]. Maintaining close proximity of DNA ends may play a role in determining DNA pathway choice during DSB repair. The complexity of the needs to coordinate both ends of a DSB before, during, and after end processing, as well as the sister chromatid template during end resection might explain multiple strand coordination activities during the initiation of HR. Furthermore, it has been proposed that sequential modes of DNA tethering may be required to facilitate DSB sensing (e.g. MR mediated bridging) followed by DSB processing (e.g. Ctp1-mediated tethering) [5]. Deficiency in DNA strand coordination and intra-chromosomal bridging of DSB ends could account for specific mutant Sae2 deficiencies in single-strand annealing [33].

3.2 CtIP is a cofactor for MRN DNA end processing activity

CtIP [10,68], Ctp1 [11,69] and Sae2 [18,33,83] all promote resection by the Mre11-Rad50-Nbs1 complex during DSB repair. Ctp1 facilitates the removal of the 5′-protein adducts Rec12/Spo11 during the initiation of meiotic recombination [15,16,84,85]. This function may be akin to the roles played by Ctp1 and Mre11 in Topoisomerase II removal from 5′ termini that arise during poisoned topoisomerase reactions [15,86]. In humans, CtIP also

interacts with and requires BRCA1 to promote Topoisomerase II adduct repair [14,87,88]. In reconstituted *in vitro* reactions, CtIP and Sae2 behave as co-factors for the MRN nuclease complex stimulating Mre11-dependant endonuclease incision of oligonucleotides harboring 5′-protein-blocked (biotin-streptavidin blocked) DNA ends [17,18]. The precise mechanics of how CtIP controls the Mre11 endonuclease activity requires further investigation; whether this activity facilitates removal of biologically pertinent (e.g. Ku70/80, Top2, or Spo11) protein-blocked ends is not yet known. CtIP might act as a molecular ruler or spacer to direct an endonucleolytic cleavage proximal to protein-blocked termini (Top2, Spo11 or Ku70/80) (Fig. 4C). Endonuclease cleavage would precede Mre11 3′-5′ exonucleolytic activity in the two-step nuclease reaction to clear the 5′ protein blocked end, and create the 3′ overhanging end required for downstream strand invasion (Fig. 1) [25,26].

3.3 CtIP and Sae2 nuclease activity

Recombinant purified CtIP and Sae2 have also been reported to harbor DNA structure-specific intrinsic nucleolytic activity [74,76,89]. Intriguingly, mutagenesis and oxidative cleavage studies of human CtIP have identified a putative metal binding active center within the low complexity and weakly conserved region of human CtIP [74], suggesting CtIP has a unique active center compared to other known nuclease folds [90]. However, nuclease free preparations of Sae2 [18,91], Ctp1 [5] and CtIP [17] have also been reported. Additional studies are needed to clarify the significance and mechanism for intrinsic CtIP nuclease activity.

4. CtIP is a regulatory hub for DSB repair Pathway Choice

DSB repair by HR is limited by its requirement for a sister chromatid to serve as template for repair synthesis, a condition fulfilled during S and G2 phases of the cell cycle (Fig. 5A). Here, HR must compete with NHEJ activity [92], where Ku competitively binds DNA ends to promote NHEJ [5,60,70,71,93]. The channeling of DSB repair intermediates to HR requires CtIP. That CtIP stimulates Mre11 endonuclease activity at DNA-protein crosslinks [17,94] suggests a mechanism for releasing Ku from DNA ends, thereby promoting resection and HR repair over DNA end protection and NHEJ repair [71,95].

CtIP ortholog functions are further regulated at the transcriptional level and through posttranslational modifications. Phosphorylation is a key regulator of CtIP/Sae2/Ctp1 activity, with CDK-mediated phosphorylation playing a central role. These modifications may be indirect, as is the case for Ctp1, where CDK phosphorylation of transcription factor MBF regulates Ctp1 transcription and thus protein levels during S/G2 (Fig. 5A) [11]. Direct phosphorylation of the Sae2-like region in Sae2 (Ser267) and CtIP (Thr847) activates resection (Fig. 5B) [83,96]. The integrity of these sites is critical to cell survival of DNA damage during G2 [49,83], while mutation of CtIP Thr847 causes genomic instability [96]. CtIP localization to chromatin during S phase also depends on Thr847 phosphorylation [97]. Interestingly, conservation of these CDK phosphorylation sites is widespread, but does not extend to fission yeast Ctp1 (Fig. 5B) [83]. This otherwise well-conserved CDK site in the Sae2-like domain is an isoleucine in *S. pombe* Ctp1, highlighting an important divergence in regulation. Additional levels of post-translational modifications control human CtIP.

Constitutive lysine acetylation inhibits CtIP during G1 (Fig 5A), but deacetylation by SIRT6 following DNA damage [98] in combination with Thr847 phosphorylation initiates HR repair [96]. As cells transition out of mitosis CtIP levels are controlled by PIN1, a prolyl isomerase that regulates CtIP proteasome-mediated degradation, along with the Cullin3 E3 ligase substrate adaptor Kelch-like protein 15 (KLHL15) thus keeping CtIP levels low in G1 [99–101].

Post-translational modifications also mediate protein-protein interactions that regulate Ctp1/Sae2/CtIP activity and thus promote HR over NHEJ. For example, Mec1/Tel phosphorylation sites of Sae2 are not only required for nuclear localization of the repair complex, but are also critical for interactions with Xrs2, the S. *cerevisiae* Nbs1 homolog [102,103]. Furthermore phosphorylation of the human ortholog CtIP allows for binding both the FHA and BRCT domains of Nbs1 [61]; these interactions create the link between Ctp1/Sae2/CtIP and MRN, targeting the repair complex to DNA damage.

5. Conclusions and outlook

Evidence from CtIP, Sae2 and Ctp1 structural, biochemical and biological results highlight the multifunctional roles of CtIP architecture in directing DSB repair pathway choice and coordinating repair activities. This work has important implications for understanding the underlying mechanisms of DNA strand break repair, and individual genetic vulnerabilities to environmentally linked DNA damaging agents and resistance to commonly employed cancer chemotherapeutics. Seckel and Jawad syndromes are also associated with abnormal CtIP expression [104]. These genetic diseases are typified by impaired neurodevelopment and microcephaly [105] and result from homozygous mutations in CtIP linked to alternative splicing (Seckel) or frameshift mutations (Jawad) [104]. CtIP mutations produce truncated protein [104] lacking the conserved CxxC-RHR motifs in the Sae2-like region mediating resection and MRN and DNA binding in CtIP [5,10,44,61,96], DNA bridging [5] and the promotion of MRN protein-DNA adduct removal by CtIP/Sae2 [17,18]. Truncated CtIP protein in SCKL2 may exert the dominant negative phenotype by mutant CtIP forming tetramers with wild type protein, poisoning normal CtIP activities [104]. Moreover, mouse CtIP is a tumor suppressor [106]. In humans, CtIP deficiency has been associated with breast cancer and decreased abundance of CtIP mRNA correlates with a poor therapeutic response and lower survival rate [107–109]. The roles of CtIP in removing covalent protein-DNA modifications suggests that therapeutics generating protein-DNA adducts combined with Parp1 inhibitors could be effective in treating breast cancers [108]. Thus, monitoring CtIP status could inform upon strategies for individual chemotherapeutic drug interventions. CtIP structure-activity relationships are forming the basis for a deeper understanding of its biological functions, and the mechanisms of human CtIP inactivation in human disease. Further dissection of the mechanics of how CtIP coordinates DNA strand breaks and orchestrates the action of Mre11 nuclease will require additional investigation.

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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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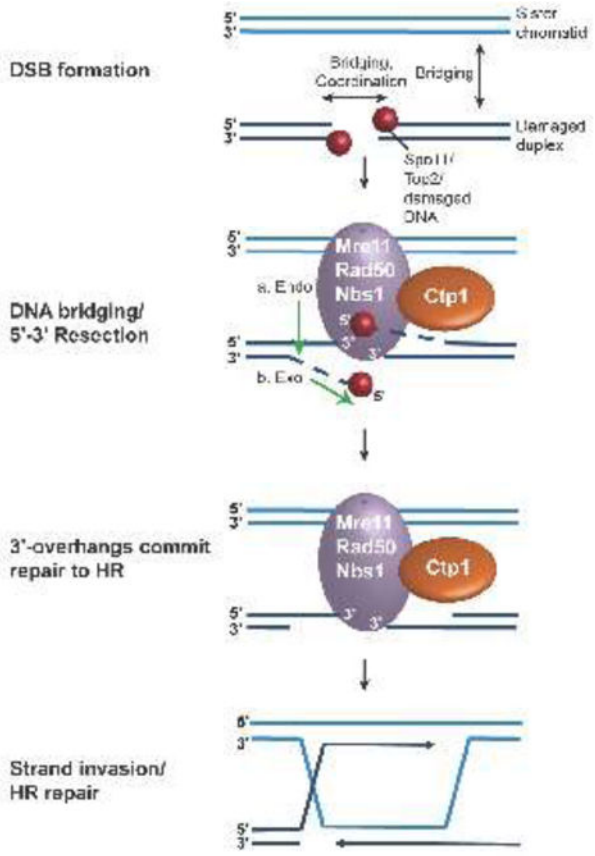

Figure 1.

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.

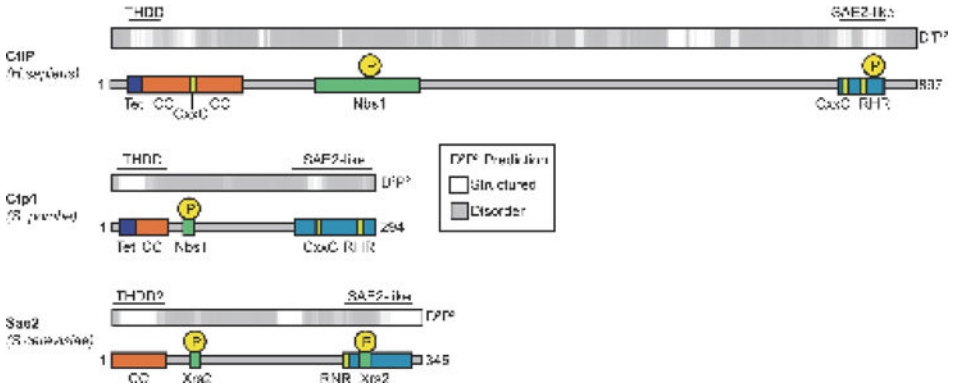

Figure 2.

Alignment of predicted structural and conserved domains of Ctp1 (*S. pombe*), CtIP (H. *sapiens*) and Sae2 (*S. cerevisiae*). Predictions of protein structure (white) and disorder (grey) were generated by D^2P^2 [40], with regions corresponding to the THDD and SAE2-like domain noted above. Corresponding regions of conserved function and structure (colored boxes) are aligned below the structural prediction. Key phosphorylation sites of Ctp1 and its homologs are denoted as yellow circles.

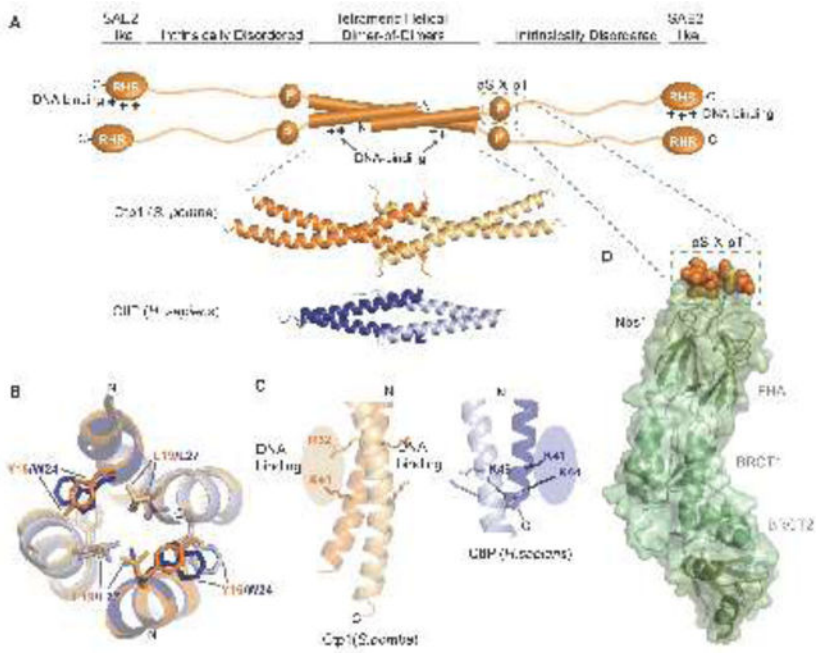

Figure 3.

Structural features of Ctp1 and CtIP. (A) Domain organization of Ctp1 (*S. pombe*) and CtIP (*H.sapiens*). The domains of Ctp1 (orange schematic) highlight the core N-terminal tetrameric helical dimer of dimers (THDD), an intrinsically disordered region that contains both the phosphorylated pS-x-pT motif for Nbs1-binding (green dotted box) and the C-terminal SAE2-like domain. Crystal structures of Ctp1 (orange; PDB 4X01) and CtIP (blue; PDB 4D2H) show the conserved tetramerization core formed by interlocking alpha-helices. (B) Comparison of Ctp1 and CtIP tetramerization. Overlay of Ctp1 (orange/yellow) and CtIP (blue/gray) tetramerization interface, mediated by leucine and aromatic amino acids. (C) DNA binding surfaces. Ctp1-DNA interactions map to the exposed surface of Ctp1 dimers (orange). A similar basic surface in CtIP also exists. (D) Ctp1-Nbs1 complex. The phosphorylated Ctp1 (orange) binds a positively charged, surface-exposed phosphoprotein recognition pocket of the Nbs1 FHA domain (green) (PDB 3HUF).

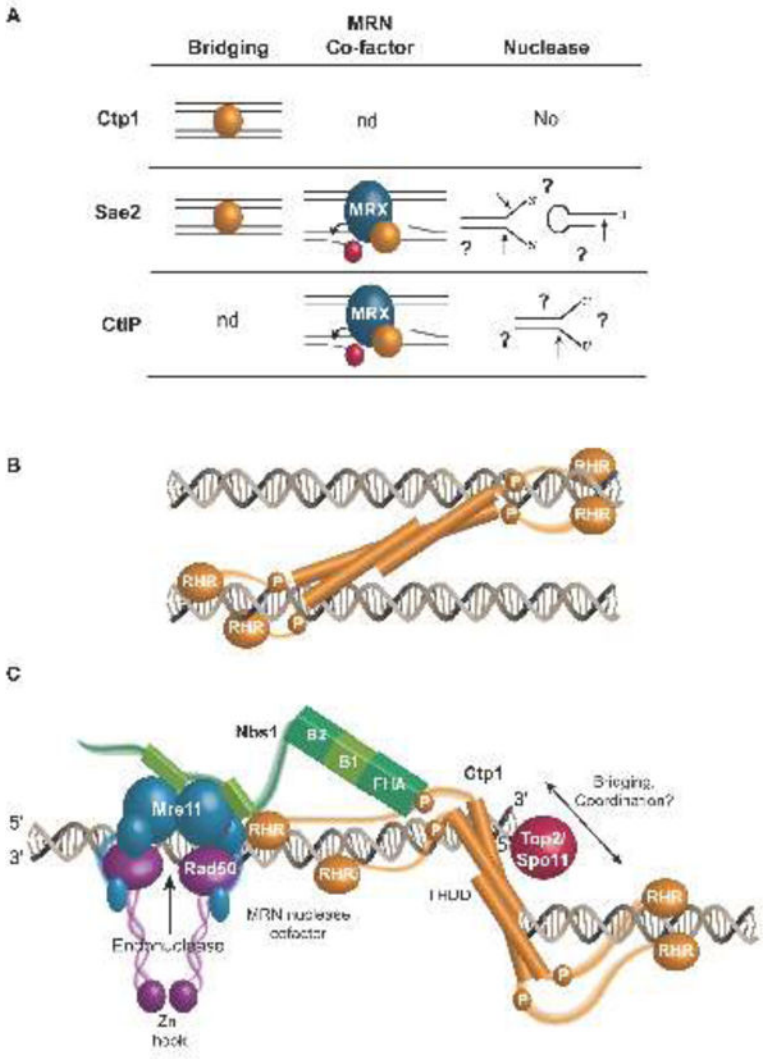

Figure 4.

Functional roles for Ctp1 in homologous recombination repair. (A) Biochemical activities of Ctp1 and Ctp1 homologs. Ctp1 and Sae2 act as bridging factors between two DNA molecules, while Sae2 and CtIP are co-factors of MRN, which stimulate Mre11 endonuclease activity at protein blocked DNA ends. Ctp1 lacks nuclease activity [5], and reports of endonuclease activity on forked and hairpin DNA structures by Sae2 and CtIP are inconsistent [17,18,74,76,89,91]. Arrows mark reported endonuclease cut sites. (B) Model of Ctp1 bridging DNA. Ctp1 contains multiple DNA binding sites and contains inherent flexibility in its intrinsically disordered region. Different bridging architectures are possible, with one potential bridging mode depicted here. (C) Model of MRN and Ctp1 at a protein-blocked DNA DSB. Mre11 harbors endonuclease activity, while Nbs1 links Ctp1 to the MRN complex. Ctp1 bridges across the DNA double-strand break, facilitating coordinated resection.

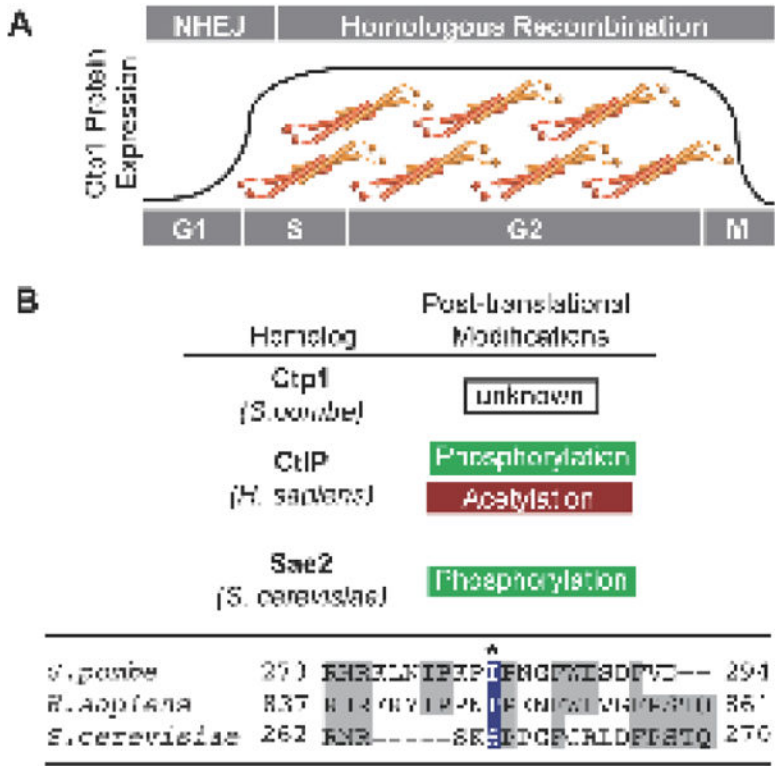

Figure 5.

Regulation of Ctp1/CtIP/Sae2. (A) Protein expression levels of Ctp1 and its homologs are upregulated during S and G2 phase of the cell cycle, coincident with active homologous recombination repair. Regulation of Ctp1/CtIP/Sae2 activity during repair also relies on post-translational modifications. Sae2 and CtIP phosphorylation is required for HR, while acetylation targets CtIP for degradation. (B) Alignment of conserved C-terminal SAE2-like domain. Ser267 in S. *cerevisiae* is critical for cell-cycle regulation of DSB repair, yet is not conserved in S. *pombe*. Conserved residues are highlighted grey.