Homologous recombination (HR) is central to the repair of double-strand DNA breaks that occur in S/G2 phases of the cell cycle. HR relies on the CtIP protein (Ctp1 in fission yeast, Sae2 in budding yeast) for resection of DNA ends, a key step in generating the 3'-DNA overhangs that are required for the HR strand-exchange reaction. Although much has been learned about the biological importance of CtIP in DNA repair, our mechanistic insight into its molecular functions remains incomplete. It has been recently discovered that CtIP and Ctp1 share a conserved tetrameric architecture that is mediated by their N-terminal domains and is critical for their function in HR. The specific arrangement of protein chains in the CtIP/Ctp1 tetramer indicates that an ability to bridge DNA ends might be an important feature of CtIP/Ctp1 function, establishing an intriguing similarity with the known ability of the MRE11-RAD50-NBS1 complex to link DNA ends. Although the exact mechanism of action remains to be elucidated, the remarkable evolutionary conservation of CtIP/Ctp1 tetramerisation clearly points to its crucial role in HR.

**Introduction**

The appropriate choice of DNA double-strand break (DSB) repair mechanism is critical for maintenance of genomic stability. During the S and G2 phases of the cell cycle, when sister chromatids become available due to ongoing or completed DNA replication, cells can repair DSBs by HR. Extensive resection of DNA ends commits cells to HR, by generating 3’-DNA overhangs that are the required substrate for the strand-exchange reaction promoted by the RAD51 recombinase.

CtIP (CtBP-interacting protein), also known as RBBP8 (Retinoblastoma-binding protein 8), is an evolutionarily conserved DNA-repair factor with a critical role in HR. It is now well established that a shared function of CtIP and its orthologues in HR-mediated DSB repair is to promote DNA-end resection in a cell-cycle dependent fashion. Accumulating evidence from studies in budding yeast supports a model where the CtIP ortholog, Sae2, is required for effective resection of DNA ends that are blocked by a lesion or the presence of bound proteins. The critical function of CtIP/Ctp1/Sae2 at DNA ends is carried out in close functional and physical cooperation with the DNA damage sensor and repair complex MRE11-RAD50-NBS1 (MRN, where NBS1 is Xrs2 in budding yeast).

The central role of CtIP/Ctp1/Sae2 in DSB repair is further highlighted by its complex regulation, mediated by an array of post-translational modifications that include phosphorylation by cyclin-dependent and DNA-damage activated kinases, acetylation, ubiquitylation, NEDDylation and proline isomerisation.

Despite its importance to DSB repair, our mechanistic understanding of how CtIP promotes resection remains incomplete. Inspection of its amino acid sequence offers little insight into the molecular details of its possible function, as regions of high sequence conservation are limited to its N- and C-terminal ends and display no similarity to known protein domains.
diverged radically in evolution relative to its orthologues in lower eukaryotes; this is highlighted by its increased size in metazoans, a phenomenon shared with other mediators of DNA repair such as BRCA2. These observations suggest that CtIP might function to promote repair via multiple, regulated protein-protein interactions. However, nuclease activity has also been reported for Sae2 and CtIP, indicating that CtIP might have both catalytic and non-catalytic roles in processing of DNA ends. The complexity of the available evidence highlights the need for additional work to further define the biochemical roles of CtIP in DNA repair.

Here, we comment on two recent structure-function studies that have provided fresh insight into CtIP function. The new evidence uncovers a remarkable oligomerisation mechanism that is shared by human CtIP and its fission-yeast ortholog Ctp1: juxtaposition of their conserved N-terminal regions results in a specific tetrameric structure that is necessary for effective DSB repair by HR.

**Structural basis of CtIP/Ctp1 tetramerisation**

Although published reports had already provided evidence that CtIP could self-associate, the precise oligomeric state of CtIP and orthologues had remained undefined. Our own new data, together with the work by Andres and colleagues, has now solved this issue for human CtIP and fission yeast Ctp1, respectively. Both reports provide biophysical and structural evidence that CtIP and Ctp1 exist as constitutive tetramers and share a mode of self-association that has remained remarkably conserved over a billion years of evolutionary history. Furthermore, the crystallographic analyses reveal that tetramerisation is mediated by short sequence motifs present at the start of parallel coiled-coil segments located in the amino-terminal regions of both CtIP and Ctp1. The splayed ends of 2 coiled-coil dimers come together in an interlocking interaction, generating a tetrameric arrangement of protein chains that is best described as a dimer-of-dimers architecture.
CtIP18–145 and full-length Ctp1 exist pre-heterogeneous oligomeric mixtures, both measurements show that, rather than forming angle laser scattering (SEC-MALS) measures.

The biophysical evidence coming from size-exclusion chromatography - multi-angle laser scattering (SEC-MALS) measures shows that, rather than forming heterogeneous oligomeric mixtures, both CtIP18–145 and full-length Ctp1 exist predominantly as tetrameric species in solution, in agreement with their extensive tetramerisation interfaces revealed by the crystallographic analysis. This tetrameric architecture must therefore be considered as the constitutive oligomeric state for both CtIP and Ctp1. However, it remains possible that their tetrameric arrangement may be altered by the regulated intervention of other proteins, and/or by post-translational modifications of the CtIP/Ctp1 N-terminal region. The striking similarity between the tetrameric architectures of CtIP and Ctp1 strongly implies that their presence must be widespread among their eukaryotic counterparts. Indeed, the functional ortholog of CtIP in budding yeast, Sae2, has been reported to exist in multimeric form16,24 and a single-point mutant in its N-terminal region, L25P, abolishes its ability to self-associate.25

Although the crystallographic analysis of the CtIP N-terminus was limited to the first 52 amino acid residues, secondary structure prediction of the CtIP sequence shows that its parallel coiled-coil structure extends to include the first ~150 residues. Intriguingly, the coiled-coil region is interrupted in its middle by a zinc-binding motif comprising conserved cysteines 89 and 92.19 The tetrahedral coordination of zinc is presumably satisfied by shared coordination of one metal atom between 2 CtIP polypeptides. The likely structural alterations induced by zinc binding on the regular coiled-coil structure, as well as the functional role of the zinc-binding motif, are currently not understood. Zinc binding by the N-terminal domain appears to be an exclusive feature of vertebrate CtIP sequences, as the cysteines that serve as ligands for the metal are absent in Ctp1 orthologues from simpler eukaryotes.

**Functional implications of CtIP tetramerisation**

The best-characterized cellular phenotype of human CtIP deficiency is a profound defect in DNA-end resection, resulting in impaired DNA repair for all pathways that require generation of single-stranded DNA overhangs at a DSB site. These include HR by gene conversion5 or single-strand annealing,26 as well as microhomology-mediated end-joining (MMEJ; also known as alternative end-joining59). In yeast, Ctp1 or Sae2 mutants are also hypersensitive to DNA-damaging agents27,28 and show defective resolution of meiotic recombination intermediates.29,30

We exploited our structural insight into CtIP oligomerisation to design a single-residue substitution, replacing leucine at position 27 with glutamate, which prevented tetramer formation while preserving CtIP’s ability to dimerize. Remarkably, we found that the L27E CtIP mutant phenocopies CtIP deficiency in terms of defective resection and gene conversion,19 highlighting the critical role of the tetrameric CtIP architecture for recombinational DNA repair. Interestingly, the L27E CtIP mutant also shows a strong defect in accumulating at DNA damage sites,19 providing a molecular explanation to the functionally-null phenotype observed in the complementation assays.

Why would CtIP’s accrual at DNA damage sites depend on its tetrameric state? CtIP localization to DSBs is thought to occur in 2 different ways: through recruitment by the NBS1 component of the MRN complex31,32 and by direct interaction with the Fanconi Anemia related protein FANCID2,33,34 However, L27E CtIP shows no defect in its interaction with either NBS119 or FANCID2 (our unpublished data). We note that the conserved C-terminal domain (CTD) of CtIP and Ctp1 can bind DNA19,20 and that mutation of Ctp1 residues critical for DNA binding causes hypersensitivity to DNA-damaging agents.20 The tetrameric architecture adopted by CtIP/Ctp1 thus appears well suited to simultaneously position multiple CTDs on distinct DNA molecules. Although a CtIP tetramer could in principle locate its CTDs on 4 different DNA molecules, we favor the possibility that a ‘dimer of dimers’ CtIP could locate pairs of CTDs at the 2 ends of a DNA DSB,20 in preparation for simultaneous processing of DNA ends (Fig. 2). Thus, tetrameric CtIP would help link DNA ends while at the same time.
time providing 2 copies of its CTD to promote end-resection, a task shared with the MRN complex, which also contains dimeric versions of its core components MRE11 and RAD50. Failure to connect CtIP dimers, as in the case of the L27E CtIP mutant, could result in weaker DNA binding and improper retention of CtIP at DNA-damage sites. Interestingly, a requirement for CtIP tetramerisation does not seem to be equally important to all forms of CtIP-dependent DNA repair, as CtIP dimers appear to retain some functionality in MMEJ.

This opens the possibility of functional regulation of CtIP oligomeric states, a feature already described for budding yeast Sae2, even though tetrameric oligomerisation seems to be CtIP’s natural state in unperturbed cells.

The newly uncovered architectural parallelism between CtIP and the MRN complex is functionally intriguing, although the similarity breaks down in one important respect: the size of CtIP, unlike that of RAD50 (the architectural component of the MRN complex), is not evolutionarily conserved. Thus, human CtIP is about 3 times larger than Ctp1 (Fig. 1A), and in general a direct correlation is observed between organismal complexity and size of the CtIP ortholog.

In addition, the coiled-coil region of CtIP comprises only one sixth of its length, whereas most of its remaining sequence shows no clear signs of structural conservation. Consequently, although the ability of CtIP to operate concomitantly at the 2 ends of a DNA break is clearly important as suggested by its ‘dimer-of-dimers’ structure, it is unlikely that CtIP will have the principal mechanical role in bridging DNA ends. Important aspects of the biochemical function of the tetramerisation region might also differ between human CtIP and its orthologues, as highlighted by the observation that the N-terminal domain of fission yeast Ctp1 possesses DNA-binding abilities that have not so far been observed for the NTD of human CtIP.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
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