*J Cancer Sci Clin Ther 2021; 5 (4): 512-519* *DOI: 10.26502/jcsct.5079134*

**Review Article**

# Contemporary Perspective on a Foundational Paper in Role of Structural Maintenance of Chromosome Complex in Mediating DNA Damage Response Checkpoint

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**Received:** 29 October 2021; **Accepted:** 22 November 2021; **Published**: 06 December 2021

**Citation:** Wenfa Ng. Contemporary Perspective on a Foundational Paper in Role of Structural Maintenance of Chromosome Complex in Mediating DNA Damage Response Checkpoint. Journal of Cancer Science and Clinical Therapeutics 5 (2021): 512-519.

## Abstract

Forward is the direction to which research looks. But, scientists read prior literature for background and context, casting a retrospective glance to the past, albeit from a different vantage point. How do we evaluate the value of a scientific paper? Citations? Downloads? Or, more philosophically, the directions of inquiries it potentiated, and areas opened up? In retrospective analysis, counterfactual is the common tool in the social scientist's armamentarium. Natural scientists, on the other hand, glean prospectively. Retrospective in analysis but yet prospective in outlook in what appears as a dichotomy. But, on closer examination, which is in harmony. From the contemporary vintage and using a prospective angle, this commentary casts light on a foundational paper that illuminated hitherto unknown functions of structural maintenance of chromosome (Smc) complex in mediating chromosome integrity and potentiating entry into subsequent phase of the DNA damage response.

**Keywords:** Cell cycle; Chromosome integrity; Condensins; Cohensins, DNA damage response; Structural maintenance

## 1. Chromosomal binding aids DNA repair

Cell cycle regulation (or dysregulation) is important in many diseases, where aberrant cell proliferation and division manifests in clinically detectable phenotypes. Comprising G1, S, G2 and M phases, the cell cycle is tightly regulated through an assortment of enzymes, cofactors and molecular machines that partake in distinct checkpoints. Specifically, checkpoints serve as molecular decision mechanisms where presence/absence of specific molecular complexes or concentrations of effectors above specific thresholds control entry into the next cell cycle phase. Given the myriad processes of DNA condensation and chromosome shortening, assembly of chromatids at metaphase plate, formation of kinetochore and their attachment by microtubules from the Microtubule Organising Centre (MTOC), and finally, cytokinesis, M phase is tightly regulated: in particular, the spindle assembly checkpoint regulates entry into anaphase where the chromatids are pulled towards their respective cell poles.

DNA condensation is the initiation step of M phase, where remodelling and close packing poised DNA in defined structures suitable for subsequent formation of Anaphase Promoting Complex (APC) and kinetochore. From the structural perspective, adoption of specific secondary and tertiary structures by compacted DNA also aids microtubule attachment and the pulling of the chromatids through the fairly viscous cytoplasmic milieu. Comprising structural maintenance of chromosome Smc2/4 and non-Smc proteins, [1] condensin is a 5 subunit 0.5 MDa holoenzyme complex [2] that regulates DNA condensation, organisation and segregation in M phase, [1,3] and participates in gene expression and DNA repair (single strand breaks, SSB) during interphase [2,4] Specifically, condensins remove chromosomal proteins from DNA or RNA-DNA hybrids in its DNA repair role, [2] and mediates the folding of chromatin fibers into highly compacted chromosomes suitable for error-free segregation during mitosis [1] Identities and functions of many upstream regulators and downstream effectors of condensins, however, remain elusive [3] For example, putative condensin function has been assigned to several protein candidates, but their roles in loading condensin onto chromosome is poorly understood [2]. Writing in PNAS [5], Yanagida and coworkers reported the isolation of a new protein with important functions in DNA repair and condensation in the eukaryotic cell cycle model organism, Schizosaccharomyces pombe [6].

Using a then leading edge cell-based screening assay (yeast two-hybrid, with the hinge domain of Smc4 as bait [1]) for identifying protein-protein interactions, Chen et al. successfully identified a new essential nuclear protein, Cut-three interacting protein 1 (Cti1), that interact with the Smc subunits of condensin, in particular, Cut3 (Smc4), both in vivo and in vitro. Specifically, in vitro GST pull-down of Cti1-Cut3 is validated with in vivo immunoprecipitation of the protein-pair using polyclonal antibodies raised against Cti1 in rabbits. Sequence homology search reveals a close homolog in human, C1D, and in other organisms such as mouse, plant, fruit fly, worm and budding yeast. High conservation of the 133 aa (14 kDa) protein suggests probable functional essentiality, a point demonstrated in the study via spore viability assays where replacement of cti1 coding region with ura4+ led to spore non-viability. Functional consequence of cti1 null mutants emanated in spore germination assays, where a subset of spores germinated but were unable to divide; thereby, suggesting

possible roles of Cti1 in cell division. Interestingly, trans-complementation of Cti1 with a cnd2-1 (a non-SMC protein subunit of condensin complex) mutant alleviates deficiency in UV-induced DNA damage repair and temperature sensitivity. The effect is independent of promoters used (native or an inducible REP1 over-expression promoter); thus, suggesting the suppression effect is likely mediated only by the gene product (Cti1) rather than other nodes of the regulon. Different point mutational alleles of Cut3 and Cut14 are also used to assess the specificity of the amelioration effect of Cti1 overexpression on Cnd2-1 deficiency. Results reveal that the suppression effect is allele specific but they all point to close functional linkage between Cti1 and condensin in this rescue assay.

Stable association of condensin with chromosome in fission yeast requires the phosphorylation of the Cnd2 kleisin subunit by Aurora kinase Ark 1, in addition to cyclindependent kinase mediated phosphorylation of the Cut3 subunit [2] Mutations in the putative Cut14 binding site of the fission yeast γ-kleisin subunit Cnd2 result in cells being hypersensitive to hydroxyurea (HU)-induced replication fork stalling and UV-mediated thymidine dimer formation [2] Thus, using hypersensitivity of cnd2-1 null phenotype to UV irradiation and HU (a ribonucleotide reductase inhibitor) as reporters, experiments were conducted to examine possible detrimental impacts of Cti1 overexpression on cell physiology in general and cell cycle progression in particular. Surprisingly, the amelioration effect is strong (at low cti1- expression level) and almost equivalent to that available from the wildtype cnd2+ gene. Thus, the data suggests condensin and Cti1 might be part of the same DNA repair pathway and, [2] more important, Cti1 is likely to be a positive regulator of condensin's DNA repair function [1] Subcellular localisation study, using GFP as a fluorescence marker, reveals the protein accumulates in the nucleolus, which in addition to providing an address tag, also corroborates the protein's close association with the condensin complex.

Finally, confirmation of Cti1 chromatin localisation and function came from a disruption assay in which hydroxyurea (HU) is used to activate an intra-S-phase checkpoint. Specifically, imaging study using the same GFP-Cti1 reporter reveals significant Cti1 localisation change in cells exposed to HU. Genetic studies also reveal that Cti1 interacts with other members of condensin; thereby, raising possibilities that Cti1 may have broader roles beyond being a component of condensin. Specifically, the authors postulated that Cti1 functions as recruiter of the condensin complex to sites of DNA damage induced by HU treatment. Collectively, the study identifies and functionally characterised a protein that accumulates on chromosomes after DNA damage via binding to the hinge domain of Cut14 [2]. An immediate corollary of the paper would thus be the identification of other members of the protein family with similar or divergent functions. Nevertheless, at the broader level, the precise molecular function of condensin remains an open question; [2] specifically, the detailed molecular mechanism by which Smc protein mediate DNA repair is not understood [1].

More intriguing, it is unclear if the dual roles of condensin in DNA repair and condensin are functionally coupled at the molecular level through mediation by multi-functional protein domain [1] From an experimental perspective, this study's defining feature lies in the design of model systems with high physiological relevance; for example, in examining Cti1 localisation, the GFP-Cti1 fusion protein is expressed via genomic integration of the construct under a

native promoter. Such an approach significantly reduced possible artifacts and cross-talks common with expression of heterologous fusion proteins, and has been used in another study [7]

The scientific literature is a forum for exchanging evidence-based ideas. Closelysimilar studies do result in differing conclusions, and sometimes, controversies, but these could usually be explained by slight differences in experimental conditions or setup, and intangible factors such as experimenters' skill. A research paper's place in scientific history is partially determined by the lines of inquiries that it helps potentiate. Retrospective analysis of a paper thus provides a unique opportunity for examining the questions raised and topics opened up, with citations providing temporal and contextual information of the paper's influence. Broadly, the study has informed condensin research in organisms ranging from *Escherichia coli*, [7] *Tetrahymena thermophila* (cilitate), [8] *Sporisorium reilianum*, [9] *Drosophila melanogaster*, [10] *Pyrococcus furiosus* (thermophilic archaea), [11] and even *Arabidopsis*; [12] thus, reaffirming the functional importance of this evolutionarily conserved protein across all domains of life.

The article has also been cited in fields such as tumorigenesis, [13, 14] epigenetic regulation of condensin function, [15] condensin's emerging role of modulating chromosome 3D structure and participates in DNA SSB repair during interphase, [2,4,16] functional characterisation of the SMC hinge domain, [3,17,15] bacterial chromosomal segregation and structure, [10,18] chromosome condensation failure, [19] fundamental chromosome structure and organisation, [20,21] transcriptional activator like effector (TALE) regulation of mRNA translation, [22] and C1D-mediated repair of UV-induced DNA damage [23] In particular, emerging consensus that condensin partakes in chromosome maintenance during interphase [2] has opened up opportunities for understanding functional roles of Cti1 in DNA repair in physiological processes leading up to M phase. More specifically, follow-on studies have shown that in addition to the more stable association with non-Smc accessory subunits such as Cti1, PARP-1-XRCC1 and Wap I, [10] Smc also transiently interacts with other binding partners – the elucidation of which and characterisation of their functions are objectives of many studies.

Research opens up more questions than it answers. Several lines of follow-on inquiries that emanate from the above study can be divided into two broad categories. Firstly, given condensin comprises multiple subunits, possibilities exist that Cti1 interacts with hitherto unknown components of the complex; thereby, opening up the search space for using immumoprecipitation cum affinity purification strategies for isolating Cti1-mediated multiple subunit interactions. While yeast two-hybrid screening is a potent tool for identifying pairwise protein-protein interactions and GST-tag immune pull-down assays effective for isolating binary protein clusters, His-tag NTA pull-down affords the ability of isolating more subunits in a single cluster given the stronger binding affinity of the His-tag NTA system. Upon isolation and dissociation, various mass spectrometry tools such as matrix-assisted laser desorption/isolation time-of-flight (MALDI-TOF) and electrospray ionization (ESI) mass spectrometry could be used for characterising the peptides/proteins present. At a more mechanistic level, genome editing tools that allow single or few nucleotide resolution precision targeted mutations, such as CRISPR/Cas9 system, could be used to introduce specific point mutations for understanding the

importance of particular sequence motif in mediating Cti1 interactions with condensin subunits. Information gleaned would complement those available from protein domain or exon-based genetic engineering techniques such as homologous recombination, commonly used in the condensin field to replace specific regions of the three domain Smc protein. Such a combined approach could yield insights into more detailed "interactome" of Cti1 and condensin, of which a suitable question ripe for interrogation pertains to the hinge domains of Smc proteins since they are thought to exhibit other functions besides aiding Smc subunits dimerization [10]

Secondly, the dynamic nature in which Cti1 interacts with different condensin subunits is a more challenging question to answer; but one which would inform the functional roles and their possible temporal and spatial segregation as Cti1 mediates DNA repair or chromosome condensation. In the case of elucidating the DNA repair roles of Cti1, chromatin-immunoprecipitation chip (ChIP-chip) or ChIP-sequencing (ChIP-seq) or both could be used to identify the specific regions that Cti1 binds to, at different stages of the DNA repair process. On the other hand, similar techniques could offer insights into possible DNA-Cti1 interactions during DNA condensation. But, probing protein-protein interactions would likely yield more detailed information of chromosome organisation during mitosis since the intricate choreographed movement of different segments of the highly condensed DNA molecule requires mechanical forces only available through conformation changes of protein complexes. An in vitro re-constituted system using Cti1 as a "pulse" for "chasing" subsequent chromosome structural changes via Western blot would be a preliminary step in a broad survey of the multitude of proteins/peptides involved. Identities of the interacting partners could be resolved by mass spectrometry analysis of individual gel bands. Nevertheless, depending on the timescales over which different stages of chromosome condensation occurs, inhibitors may be needed to retard the process for more detailed functional characterisation. As with all in vitro assays, the degree of resemblance of the constituted system with physiological reality is an important caveat during data interpretation.

While always an inexact replica of actual cellular processes, in vitro experiments illuminate the assortment of molecular players involved and their putative importance. Such information is important for designing subsequent in vivo studies; specifically, selecting appropriate reporter systems for tracking the dynamic movements of particular entities through space and time. Immunofluorescence is one approach for following the dynamic movement of antibodylabelled proteins within the cell;1 however, the expense involved together with the introduction of motility artifacts given the significant mass of most antibodies relative to the target protein meant that the biological imaging field is constantly innovating. One example is the advent of super-resolution microscopy methods such as STORM (stochastic optical reconstruction microscopy) and PALM (photo-activated localisation microscopy) that enable molecular level details to be gleaned from a modified confocal microscope visualizing dye labelled biomolecules. Hence, by judicious choice of fluorophores and molecules for labelling, the dynamic interplay between Cti1 and various condensin subunits could be observed, in real-time, within the intracellular milieu with minimal interference.

Finally, epigenetics connects environmental influence on gene expression with observed phenotypes. While most

studies have focused on Cti1's roles at the genotype levels, it would be interesting (but a bit speculative) to examine possible interactions of the protein with DNA base modifications such as those associated with activation (acetylation) or inactivation (methylation) of gene expression. ChIP-chip or ChIP-seq would be the tools of choice for this endeavour but, given the capability of third-generation single Molecule Realtime Sequencing (SMRT) in resolving long repeat sequences and epigenetic modifications inaccessible to bisulphite sequencing, coupling the two in an integrated approach may provide clues to possible roles of Cti1 in mediating gene expression. If such an envisaged expansion of Cti1 functional repertoire is indeed realised, it speaks to the importance of methodological innovations in allowing continued dissection of molecular details enigmatic to more conventional approaches. And, more important, the multi-dimensional personalities of proteins in various context-dependent biological processes that emanate from a single conserved amino acid sequence. Such physiological manifestations are mediated in part by structural changes induced by conditions in subcellular localities, or the presence of interacting proteins or other biomolecules. Taken together, work done on elucidating new functions of Cti1 would fit into, and further inform, the broader picture of multi-functional (but sequence conserved) proteins exhibiting context-specific roles depending on particular cues present in molecular machines/structures or, more generally, the subcellular environment.

## Conflicts of Interest

The author declares no conflicts of interest.

## Funding

No funding was used in this work.

## Appendix

Important terms in chromosome condensation and DNA repair research

FEAR network: Cdc14 early anaphase release.

HEAT: Huntingtin-elongation a protein phosphatase 2A, subunit TOR multi-subunit complex.

NEBD: Nuclear Envelop Breakdown.

CCAN: Constitutively Centromere Associated Network.

SMC2-SMC4: Cut14-Cut3 in Schizosacchromyces cerevisiae baker's yeast.

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