



Phase separation as a dynamic regulatory mechanism of DNA end resection

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Abstract

DNA end resection is a pivotal process that governs repair pathway choice following double-strand breaks and is essential for maintaining genomic stability. Traditionally considered an enzyme-driven cascade regulated by post-translational modifications, recent insights have revealed an additional layer of control mediated by liquid-liquid phase separation (LLPS). Our recent findings identify LLPS as a key organizing principle in DNA end resection through the ERCC6L2-RNF138-CtIP axis, in which ERCC6L2-driven condensates stabilize CtIP and modulate the extent of resection. This perspective discusses the emerging concept of LLPS as a regulatory mechanism in DNA repair, highlighting key mechanistic questions regarding condensate formation, spatial coordination, and pathway choice. We further explore the broader implications of dysregulated phase separation in aging and cancer, and consider how pharmacological modulation of LLPS could be leveraged to enhance therapeutic responses to genotoxic stress.

Keywords: DNA end resection, liquid-liquid phase separation, ERCC6L2-RNF138-CtIP axis, DNA damage response

DNA end resection serves as a critical process that determines the cellular choice between the error-prone non-homologous end joining pathway and the high-fidelity homologous recombination (HR) repair pathway following double-strand breaks^[1]. Through the concerted actions of nucleases such as MRE11, EXO1, DNA2, together with accessory factors including CtIP and BRCA1, the damaged DNA ends are processed to generate 3' single-stranded DNA (ssDNA) overhangs^[2]. The timing, precision, and extent of resection critically impact DNA repair pathway choice and fidelity, thereby maintaining genomic stability.

Traditional models describe DNA end resection as a linear, enzyme-centric cascade orchestrated by post-translational modifications and protein-protein interactions^[3]. While this framework has been instrumental in elucidating individual enzymatic steps, it fails to fully explain the remarkable efficiency, speed, and spatiotemporal coordination of resection reactions within the densely packed nuclear environment. Recent conceptual advances in cell biology have introduced an additional layer of regulation: liquid-liquid phase separation (LLPS).

The concept of LLPS originated as a fundamental physicochemical principle, with its biological significance first established through early observations of membraneless organelles, particularly the nucleolus. A landmark 2009 study on *C. elegans* P granules provided definitive experimental validation of LLPS as a fundamental mechanism in cell biology^[4]. These included observing droplet formation and fusion, assessing molecular dynamics through fluorescence recovery after photobleaching, employing targeted mutagenesis of critical intrinsically disordered regions, and *in vitro* protein purification^[5]. Beyond its well-established role in DNA repair, LLPS has been demonstrated to serve essential functions in diverse biological processes, including the assembly of transcriptional activation complexes, formation of neuronal postsynaptic densities, and dynamic regulation of stress granules^[6-9].

An increasing number of DNA damage response (DDR) factors, including 53BP1, TopBP1, RPA80, and RAD52, exhibit the capacity to undergo phase separation, which helps assemble repair complexes and improves repair efficiency^[10-14]. These findings collectively suggest that LLPS may serve as a general organizing principle for the DDR, integrating spatial organization with molecular signaling.



Yet whether DNA end resection, the gateway to HR, is similarly governed by phase separation has remained an open question.

Recent work has uncovered compelling evidence supporting this view through the identification of an ERCC6L2–RNF138–CtIP axis^[15]. ERCC6L2, a chromatin remodeler implicated in the resolution of DNA damage, undergoes phase separation via its intrinsically disordered C-terminal region to form dynamic nuclear condensates that selectively enrich CtIP (Figure 1A,B). Under conditions of ataxia telangiectasia mutated (ATM) inhibition, these ERCC6L2 condensates sequester the E3 ubiquitin ligase RNF138, thereby protecting CtIP from ubiquitin-mediated degradation. The resultant stabilization of CtIP drives hyper-resection and leads to apoptotic cell death. In contrast, ERCC6L2 deletion abolishes this protective compartment, rendering CtIP susceptible to RNF138-mediated degradation and conferring resistance to ATM inhibitors (Figure 1C). These findings suggest a context-specific interplay between kinase signaling and condensate-mediated resection control.

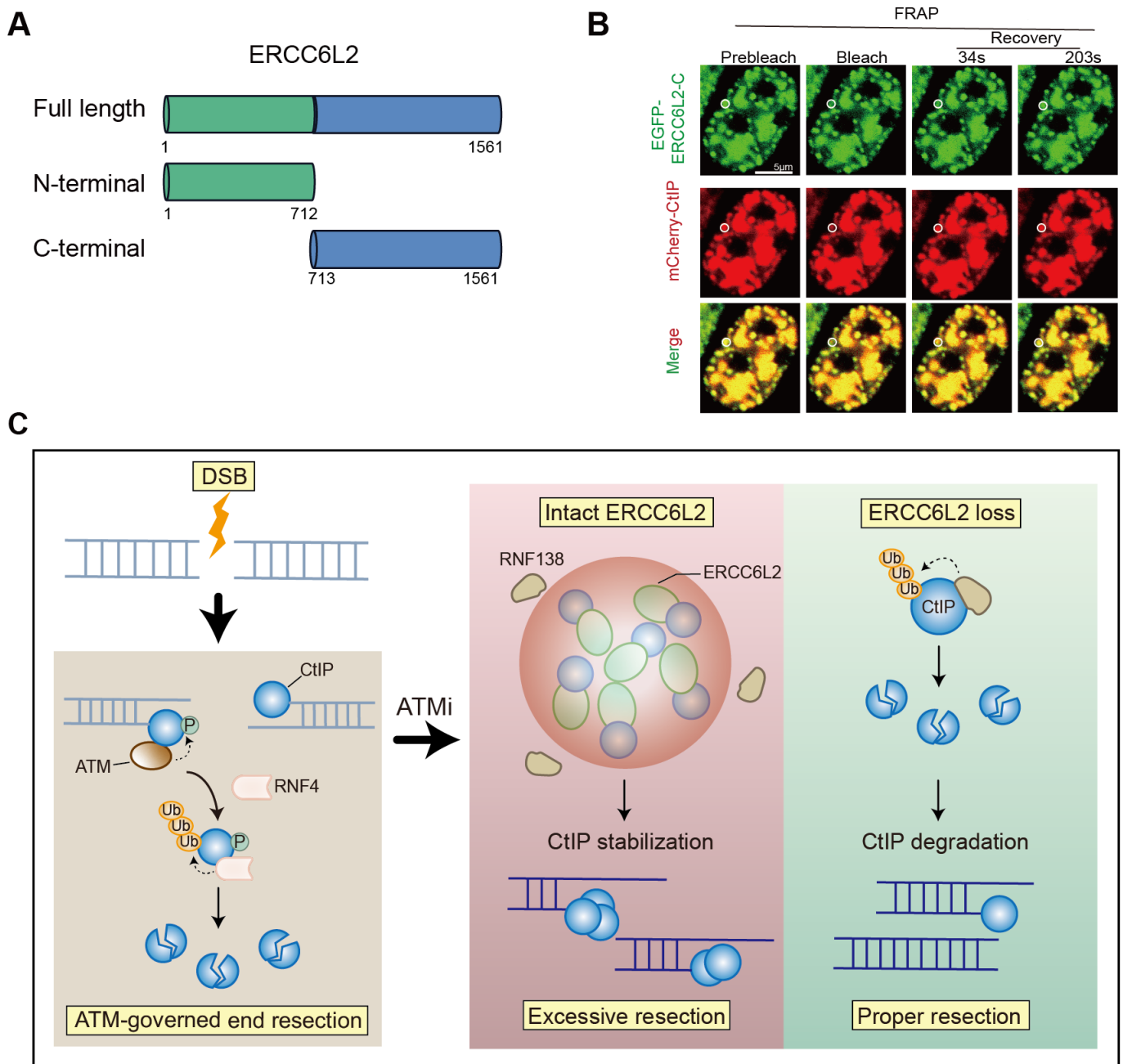


Figure 1. ERCC6L2 forms biomolecular condensates that recruit CtIP to govern DNA end resection. (A) Schematic diagram of truncated mutants of ERCC6L2; (B) HEK-293T cells were co-transfected with EGFP-ERCC6L2-C and mCherry-CtIP. Representative images from the FRAP experiments of EGFP-ERCC6L2-C and mCherry-CtIP condensate. The white box highlights the puncta undergoing targeted bleaching. Scale bars: 5 μ m; (C) A mechanistic model depicting how ATM and the ERCC6L2–RNF138–CtIP axis govern the extent of DNA end resection, with or without ATM inhibition. FRAP: fluorescence recovery after photobleaching; ATM: ataxia telangiectasia mutated.

While this conceptual framework is rapidly gaining traction, several key questions remain unresolved (Table 1). Do biomolecular condensates actively recruit and activate the resection machinery, or do resection intermediates such as ssDNA or R-loops nucleate condensate formation? Beyond the ERCC6L2–CtIP axis, do core nucleases like EXO1 and DNA2 participate in similar condensate-driven assemblies? Is resection organized through a hierarchical network of condensates, coordinating early and long-range processing steps? Furthermore, how do pro-resection condensates (e.g., BRCA1–CtIP) and anti-resection condensates (e.g., 53BP1–Shieldin) achieve spatial segregation and temporal alternation to ensure precise repair pathway choice?

Table 1. Key scientific questions and future research directions in LLPS-regulated DNA damage response.

Key Open Question	Experimental Approaches	Potential Implications
Nucleation Mechanism: Do condensates recruit repair machinery, or do repair intermediates nucleate condensates?	- <i>In vitro</i> reconstitution with purified components - High-resolution live-cell imaging	Define the initiating event and regulatory logic of DDR condensates
Composition&Hierarchy: Do distinct condensates coordinate different repair steps (e.g., short-range vs. long-range resection)?	- Proximity labeling & proteomics - Single-molecule tracking	Uncover the full resection network and its functional organization.
Spatiotemporal Control: How do pro- and anti-resection condensates achieve spatial segregation?	- Super-resolution microscopy - Optogenetics to manipulate condensates	Understand the precision of repair pathway choice.
Pathophysiological Impact: Does dysregulated LLPS contribute to genomic instability in aging and cancer?	- Analyze patient-derived mutations in LLPS domains - Develop LLPS-modulating compounds	Identify new therapeutic targets and strategies.

LLPS: liquid-liquid phase separation; DDR: DNA damage response.

From a mechanistic perspective, a major challenge lies in reconciling the seemingly “coarse” nature of phase separation with the nanometer-scale precision required for DNA processing. Post-translational modifications may act as molecular switches that modulate condensate dynamics, enabling fine control of resection extent and timing.

The pathophysiological implications of this emerging paradigm are equally compelling. Dysregulation of phase separation properties in DNA repair proteins may contribute to genomic instability observed in aging and cancer. Targeting LLPS-dependent repair processes offers a potential therapeutic strategy by selectively disrupting aberrant condensates in cancer cells, and it may be possible to sensitize tumors to genotoxic therapies or overcome resistance to DDR inhibitors.

Moving forward, advancing this field will require methodological innovation capable of visualizing condensate dynamics in living cells with high spatiotemporal resolution. Differentiating functional LLPS from nonspecific aggregation remains a key technical hurdle. Integrating single-molecule imaging, biophysical modeling, and genome-editing approaches will be essential to define how condensate behavior translates into DNA repair outcomes in physiological and pathological contexts.

By situating DNA end resection within the broader framework of phase separation biology, we are beginning to uncover a new regulatory logic in genome maintenance—one that merges the physical organization of the nucleus with the molecular choreography of DNA repair.

Declarations

Authors contribution

Cai M: Conceptualization, methodology.
Yin Y, Lin J: Investigation, writing-original draft.

Conflicts of interest

The authors declare no conflicts of interest.

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