RESEARCH HIGHLIGHT

Chromatin compartments at DNA double-stranded breaks

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In a recent article published in *Nature*, Arnould and colleagues reveal that DNA double-stranded breaks (DSBs) strengthen chromatin compartmentalization near the breaks. They show that "D" compartments arise from clustering of Topologically Associated Domains harboring DSBs to facilitate the expression of R-loop-rich genes involved in DSB repair, thereby aiding in their efficient repair at the expense of specific chromosome translocations.

DNA transactions: transcription, replication and repair take place within nuclear subdomains, called foci in the case of DNA repair. Clustering of repair reactions within a dedicated domain is thought to facilitate efficient repair.[1]

Topologically Associated Domains (TADs), the structural units of genome organization associated with transcription regulation, have also been associated with megabase γH2AX-positive chromatin domains using multiple chromosome conformation capture approaches. Neighboring damaged TADs can associate in a single domain[2] or can cluster from distant DNA double-stranded breaks (DSBs).[3] Using U2OS cells expressing an inducible AsiSI-ER transgene (DIvA cells), Arnould at al.[4] now show that ATM reinforces TAD clustering as previously reported following irradiation.[5] TAD clustering is occurring within the same or different chromosomes. Furthermore, DNA-PK inhibition significantly increases damaged TAD clustering, presumably due to the accumulation of unrepaired, resected DNA ends.[3] Notably, concomitant inhibition of ATM and DNA-PK did not rescue TAD clustering. Locally, loop extrusion increases following DNA damage,[3,6] which the authors found to also be ATM dependent.[4] This indicates that following DSB induction, DSB-bearing TADs marked by γH2AX, 53BP1, and ubiquitin chains physically interact within nuclear domains, in line with previous studies.[3,5,7]

Seeing is believing; thus the authors further investigate clustering using live-cell microscopy techniques including half-FRAP with fluorescently tagged 53BP1. Live-cell imaging previously established that DNA repair foci cluster[1] and form biomolecular condensates with liquid-liquid behavior.[8] Arnould et. al. use half-FRAP experiments to measure 53BP1 diffusion within partially bleached 53BP1 foci. They detect liquid-liquid phase separation (LLPS) early; however, upon clustering and fusion, 53BP1 foci no longer display this LLPS behavior: interfacial diffusion barrier dampens, fusion kinetics is slow and yields irregularly shaped clustered foci. This suggests that clustering is driven by self-interactions among chromatin-bound 53BP1 molecules via polymer-polymer phase separation (PPPS). It will be interesting to test whether this sequential assembly of 53BP1 foci with distinct biophysical properties reflects the assembly of increasingly complex 53BP1 nano- and micro-domains.[2]

Microscopy provides increasingly detailed views of DNA repair domains or foci. However, a genomics view of these domains is lacking. The authors identify a DNA damage compartment through computational analysis of Hi-C contact maps. Chromatin compartments are defined in Hi-C studies by principal component analysis (PCA) of contact matrices. PCA allows for the identification of loci that share similar interaction patterns. The A compartment corresponds to active, accessible euchromatin within which the majority of cleaved AsiSI sites are located. In their study, the authors defined a "D" compartment using a PCA of differential Hi-C maps. They first establish differential Hi-C matrices with and without DSBs, namely (+DSB/–DSB), to characterize the DSB-dependent characteristics of the D compartment in response to DNA damage. Because AsiSI breaks form primarily within the A compartment, this D compartment arises from the A compartment and could be considered as strengthened A compartment. Indeed, damaged and non-damaged loci segregating within D compartment are enriched in chromatin marks associated with transcription. D compartment provides a very useful tool to study the genomics characteristics of DSB repair domains (Fig. 1).

R-loops are transient intermediates arising during transcription composed of the nascent RNA hybridized to DNA and the displaced non-template single-strand DNA. R-loops accumulate in the vicinity of DSBs.[9] This study reveals that the genes targeted to the D compartment (D genes) are enriched in R-loops compared to those not in the D compartments (non-D genes). Experimental resolution of R-loops following RNaseHi expression or accumulation of R-loops in senataxin-depleted cells resulted in decreased or enhanced D compartment, respectively. This indicates that R-loops facilitate the establishment of the D compartment following DNA damage. R-loops are associated with genome instability and promote rearrangements to distal DSBs.[10] It is conceivable that the propensity of R-loops to interact with DSBs facilitates the formation of the D compartment.

Notably, the D compartment is enriched in genes regulating the DNA damage response (DDR), including damage-dependent p53-target genes, and this enrichment is independent of the distance from the closest AsiSI site. Of note, a fraction of B compartments harboring a cleaved AsiSI DSB occasionally flip to A. A similar switch was reported in MEFs.[3] Intriguingly, R-loop enrichment within D compartment is also observed upon etoposide

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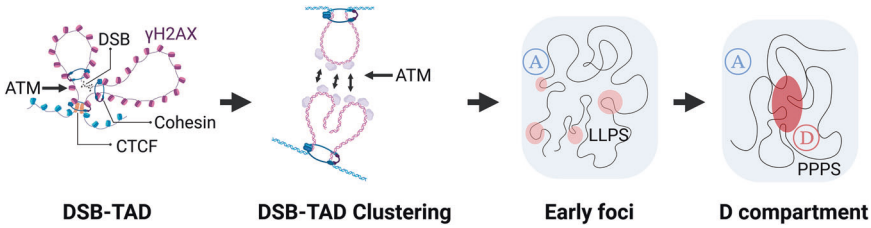

Fig. 1 Stepwise assembly of the D compartment. From left to right: DSB-TAD arises from loop extrusion and concomitant phosphorylation of H2AX by ATM yielding γH2AX-decorated TADs. These TADs cluster via protein–protein (53BP1), ubiquitin and poly-ADP ribose chain (PAR) interactions. At a larger scale, DSB-TADs corresponding to early microscopic foci (pink domains) form within the A compartment (blue compartment) by LLPS and are converted into larger domains — D compartment — in dark red via PPPS. D compartment is enriched in R-loops and active DDR genes. Created with BioRender.com.

treatment, which induces Top2-DNA adducts leading to DSBs and RNF4-mediated DNA–protein crosslink repair at PML bodies, which promotes clustering.[11] It will be important to elucidate the mechanisms by which the "D" compartment gets enriched in DDR genes and facilitates their expression.

Bringing DSBs in close proximity within repair foci/D compartments poses the risk of facilitating illegitimate repair, namely translocations.[3] The authors now show that strengthening or weakening the D compartment increase or decrease chromosome translocations.

In summary, this study provides further important insights into the mechanisms regulating chromatin compartmentalization into damaged domains and how these compartments influence repair and potentially contribute to genomic instability.

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ADDITIONAL INFORMATION

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