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Peer Review

Review of: "Nuclear Basket Proteins Mlp1 and Nup2 Drive Heat Shock–Induced 3D Genome Restructuring"

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This manuscript provides an insightful exploration into the role of the nuclear pore complex (NPC), particularly its nuclear basket components, in regulating the spatial genome architecture of Heat Shock Responsive (HSR) genes in yeast during thermal stress. The study presents compelling evidence that the nuclear basket proteins Mlp1 and Nup2 contribute significantly to 3D genome reorganization, independently of their structural role in NPC integrity. The authors have successfully uncovered a distinct function for these proteins in mediating chromatin clustering, adding a valuable dimension to our understanding of gene regulation under stress conditions.

A major strength of the work lies in its clear focus on dissecting the specific roles of different NPC components. By methodically demonstrating that Mlp1 and Nup2, but not other scaffold-associated NPC proteins such as Nup1 and Nup145, are essential for HSR gene clustering, the authors contribute to a more nuanced view of nuclear organization. The use of double depletion experiments adds rigor to their conclusions and reinforces the assertion that chromatin clustering can be mechanistically separated from transcriptional activation processes.

Importantly, the study differentiates between chromatin clustering and transcriptional condensate formation, showing that Mlp1 and Nup2 are not required for the assembly of Hsf1-driven transcriptional condensates or for Pol II recruitment and mRNA abundance. This distinction is critical, as it shifts the paradigm from viewing nuclear architecture solely as a passive reflection of transcriptional activity to a more active, structural role in genome reorganization. These findings could pave the way for future studies exploring how chromatin topology and transcriptional machinery are decoupled or interdependent.

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Methodologically, the manuscript appears well-constructed, though it would benefit from more detailed descriptions of imaging techniques and the spatial resolution used to detect gene clustering within the nucleoplasm. For instance, the resolution limits of the microscopy approach and any controls employed to confirm that clusters are biologically meaningful and not technical artifacts should be clearly stated. Clarifying whether super-resolution methods or fluorescence in situ hybridization (FISH) were used would help readers assess the reliability of the 3D genome positioning claims.

One opportunity for improvement is the limited discussion on potential downstream consequences of chromatin clustering on genome stability, epigenetic regulation, or transcriptional memory. While the authors rightly emphasize that Mlp1 and Nup2 are not essential for mRNA output during heat shock, the broader functional relevance of HSR gene clustering is not fully explored. Speculating on whether such clustering confers advantages in genome repair, reactivation kinetics, or nucleosome remodeling could add depth to the biological implications of the findings.

The study could also benefit from more extensive comparisons to higher eukaryotes. Although the focus is on yeast, many of the components discussed have homologs in mammalian cells. Discussing whether similar clustering behaviors have been observed in human cells during stress responses would elevate the translational relevance of the work. Furthermore, exploring evolutionary conservation could help situate these findings in the broader context of nuclear organization.

In terms of data presentation, figures related to gene clustering could be improved for clarity. Providing quantification of cluster number, size, and dynamics across time points of thermal stress would strengthen the argument that Mlp1 and Nup2 actively drive this process. Time-lapse data or single-cell variability in clustering behavior would also help illustrate the robustness and reproducibility of the observed phenomena.

Another area of potential enhancement is the mechanistic insight into how Mlp1 and Nup2 mediate gene clustering. Are they acting as scaffolds that tether genomic loci, or are they involved in altering chromatin states conducive to clustering? Incorporating chromatin immunoprecipitation sequencing (ChIP-seq) or proximity ligation assays (e.g., Hi-C or Micro-C) might offer further granularity in understanding the molecular underpinnings of this process.

The manuscript is generally well-written, with a strong command of the relevant literature and clear articulation of its scientific rationale. Nevertheless, a few sections, particularly in the Results and Discussion, could benefit from improved flow and logical transitions. Ensuring that each paragraph

builds on the previous one and avoiding overly technical language in key interpretive sections would help broaden the manuscript's accessibility to interdisciplinary readers.

In conclusion, this manuscript offers a valuable and novel contribution to the field of nuclear architecture and stress biology by revealing a unique role for nuclear basket proteins in 3D genome repositioning. The distinction between transcriptional activation and genome restructuring is particularly impactful and highlights the potential for further discovery at the interface of spatial genome dynamics and cellular stress responses. With minor clarifications in methodology and more extensive contextualization of the results, this study stands to significantly enhance our understanding of nuclear function during environmental challenges.

Declarations

Potential competing interests: No potential competing interests to declare.