Qeios

Peer Review

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

Leonor Margalha[1]

1. Universidade Nova de Lisboa, Portugal

The work from Mohajan *et al*., unveils unprecedented roles for the nuclear basket proteins Mlp1 and Nup2 regarding 3D genome restructuring in response to heat shock stress. The work is well organized and structured, providing relevant new data.

My comments mainly address aspects that could be more thoroughly analyzed/discussed. Congratulations on the work developed.

1. Although the authors ascribe a role for Mlp1 and Nup2 in 3D genome restructuring in response to heat shock, the nuclear processes that those topological changes may impact remain unclear, since no alterations could be detected at the HSR gene expression level/replication upon double depletion of Mlp1/Nup2. Could there be putative roles for Mlp1/Nup2 in other processes such as DNA repair and/or mRNA export in the heat shock stress context?
2. The authors show that the nuclear basket proteins Mlp1 and Nup2 don't seem to impact NPC and NE integrity, but do drive HSR genes into coalesced chromatin clusters. Conversely, the authors show that Nup1 and Nup145 are important for maintaining NPC integrity and that they alter the subnuclear localization of Nup2 and Mlp1, with minimal effect on HSR gene 3D restructuring. The latter results should be more discussed/revisited. By altering Nup2 and Mlp1 localization, couldn't Nup1 and Nup145 also have an indirect role in Nup2 and Mlp1-dependent 3D genome restructuring? In Figure 3, there is a clear trend of the effect of Nup1 and Nup145 concerning some inter-chromosomal interactions, despite there not being statistical significance.
3. In the different charts, it would be better to visualize both the upper and lower parts of the error bars.

doi.org/10.32388/VUVOJF

In all the experiments, biological replicates are done up to n=2. n=3 biological replicates would increase the robustness of the work presented as well as of the subsequent statistical analysis.

4. Moreover, the spatial distribution of Nup2/Mlp1 may impact their role in HSR, since Nup2 and Mlp1 re-localize in cells exposed to heat shock (Figure 5). Is there causality between Nup2/Mlp1 localization and their impact on HSR gene coalescence upon heat stress? How does the absence of Nup1 and Nup145 affect the redistribution of Nup2/Mlp1 upon heat shock? For instance, Nup2 has a predominant presence at the nucleoplasm after heat shock; could it be compromised in the Nup1 Nup145 double mutant? And concerning Mlp1 distribution after heat shock, could it be less affected by Nup1 and Nup145? Could Nup2/Mlp1 redundancy be important in response to heat stress in the Nup1 Nup145 double mutant?

5. Nup2 and Mlp1 are rapidly recruited to HSR genes, but the association is transient, as occupancy peaked at 15 min and diminished thereafter. How can this be interpreted? Is it possible to have a scenario in which they have a role in nuclear processes in response to a transient but not to a more prolonged stress?

6. How do you reconcile HSR gene coalescence mainly at the nucleoplasm driven by Nup2 and Mlp1 with previous reports where these proteins target transcriptionally active genes to the NPC? Can the localization of HSP104-HSP12 coalescence be considered representative of what happens with other HSR genes? And could nuclear periphery vs. nucleoplasm gene coalescence mediated by Nup2 and Mlp1 be differentially regulated?

7. On Page 7, a personal communication is referred to; this should not be considered/presented as scientific evidence since it does not allow for critical analysis of the data. Its removal is suggested.

8. Typos:

On Page 17, there is a typo in the word immunoprecipitation.

In the first line on page 22, there is a typo and it should be Nup2 and Mlp1 (not Mlp2).

Declarations

Potential competing interests: No potential competing interests to declare.