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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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It has been established in yeast that transcriptional induction can be accompanied by a repositioning of the induced genes to the nuclear periphery. In this context, the authors have previously shown that upon heat shock (HS), the heat shock response (HSR) genes form clusters in a heat shock transcription factor HSF1 dependent manner. HSF1 forms condensates involved in the cis and trans interaction between HSR genes and in HSR gene coalescence. Here, the authors study the role of nuclear pore proteins (Nup145, Nup1, Nup2, and Mlp1) in this 3D chromatin reorganization of HSR genes. They show that only the two nuclear pore basket proteins Nup2 and Mlp1 are involved concomitantly in cis- and trans-interaction between HSR genes. However, these proteins are not required for the formation of HSF1 condensates.

This study is interesting and well-written and raises a novel role for NPC proteins in the response to HS. However, some experiments deserve to be consolidated, and some minor points addressed to enhance the robustness of this nice study.

1. The authors show that the concomitant depletion of Nup2 and Mlp1 decreases cis- and trans-interaction between HSR genes (Fig. 4F-H). The most important missing experiment is the validation of 3C by microscopy. It is necessary to demonstrate the same by microscopy using the system in Fig. 1 and Fig. S2B. In the latter figure, single deletions of Nup2 or Mlp1 have no effect, but double deletion must be tested.
2. Based on the delocalization of Nup2 and Mlp1 after an HS (Fig. 5A) and the observation that the coalescence between HSP12 and HS104 is mainly nucleoplasmic (Fig. 1B), the authors argue that these nuclear basket proteins mediate their topological effect in their nucleoplasmic form. It will be important to quantify the foci of coalescence at the periphery in the double mutant Nup2 and Mlp1; if foci are still found at the periphery, it will be interesting to discuss their significance.

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3. All over the manuscript, clearly explicate how statistics are done and what defines an N? As known, to correctly estimate a standard deviation, a population from three different independent experiments (N=3) would be required.
4. To show that Nup2 and Mlp1 are recruited temporarily at the level of the HSP104, HSP82, and SSA4 genes, the authors carry out ChiP-qPCR with HS of 3, 15, and 60 minutes (Fig. 5). At the same time, the authors observe a relocation of the Nup2-mNG and Mlp1-mNG proteins, for a HS of 30 min.: the authors should show the localization of these proteins after a HS of 60 minutes as well.
5. Can the authors explain how it can be concluded that the depletion of Nup2 and Mlp1 has no effect on the viability of cells that have undergone an acute HS (Fig. S3C), when plating is done at 30°C and the response to HS is reversible?

Minor points

1. To study coalescence in vivo within cells, the authors cleverly use a variable number of LacO repeats under the control of different HS promoters, HSP12 and HSP104. To exclude that the coalescence between HSP12 and HSP104 is not related to the formation of GFP dimers, also show the coalescence between HSP82 and HSP104, where a distinct couple of bacterial repeats labeled mCherry are used.
2. Specify in the legend of Figure 1 that the counting of coalescent foci is based on selected focal planes.
3. Define the x and y axes more clearly in Fig. 2D
4. Explain the TaqI-3C technique in detail in materials and methods, even if it has already been published.
5. Avoid citing a personal communication; rather, show the data if possible.

Declarations

Potential competing interests: No potential competing interests to declare.