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The role of Nucleoporin TPR in cell functioning and differentiation

Self-summary

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Abstract (English)

TPR is a large nucleoporin located in the nuclear basket of the nuclear pore that was proposed to form nucleoskeleton. It interacts with exported molecules, components of the nuclear lamina, and the nuclear interior, positioning itself strategically to facilitate connections between various cellular processes. Our findings offer detailed insights into the distribution of TPR within the nucleus and the nature of TPR nucleoskeletal fibers. Furthermore, we enhance our understanding of TPR's function in the context of nuclear pore complexes (NPCs) as central hubs for transcriptional regulation.

Through ChIP-seq analysis, we uncover TPR's association with lamina-associated domains within chromatin. Intriguingly, we demonstrate that unlike lamin, TPR exerts a positive influence on genes involved in myogenesis. We further report that LSD1 forms a complex with TPR and takes a part in the regulation of the TPR associated genes. Finally, our data demonstrate that depletion of TPR adversely affects myogenic differentiation, underscoring its crucial involvement in essential cellular processes.

Abstrakt (český)

TPR je velký nukleoporin, který tvoří košík jaderného póru, a který byl předchozím výzkumem spjat s tvorbou nukleoskeletu. Interaguje s exportovanými molekulami, jadernou laminou i s vnitřkem jádra a má tak strategickou pozici propojovat všemožné buněčně procesy. Naše data poskytují podrobné informace o distribuci TPR uvnitř jádra a o povaze TPR nukleoskeletálních vláken. Dále pak rozšiřují i naše ponětí o funkci TPR v kontextu jaderných pórů jako centrálních uzlů pro transkripční regulaci.

Skrze ChIP-seq analýzu odhalujeme vazbu TPR k doménám chromatinu asociovanými s laminem (LADs). Zároveň ale ukazujeme, že na rozdíl od Laminu, má TPR na expresi asociovaných myogenních genů pozitivní vliv. Ukazujeme, že TPR tvoří komplex s LSD1 a přispívá k regulaci exprese alespoň některých z asociovaných genů skrze tuto interakci. Deplece TPR pak ovlivňuje svalovou diferenciaci, což opět zdůrazňuje klíčovou roli TPR v buněčných procesech.

2. Background

Nucleoporins (NUPs) are proteins that comprise a nuclear pore complex (NPC), facilitating specific transport between the cytoplasm and the nucleus. Over the last decade, there has been extensive research on NUPs due to their pivotal role in gene expression. They alter chromatin condensation (Kuhn et al., 2019) and facilitate promoter-enhancer looping (Pascual-Garcia et al., 2017). Additionally, NUPs target genes, super-enhancer sequences, and transcription factors towards the regions surrounding NPCs (Pascual-Garcia et al., 2017; Su et al., 2018), which are linked to open, actively transcribing chromatin (Ibarra et al., 2016). This regulatory mechanism is especially crucial during differentiation, a process characterized by substantial alterations in gene expression profiles (Capelson et al., 2010; Ibarra et al., 2016; Raices et al., 2017).

Translocated promoter region (TPR) is a large nucleoporin weighing 267 kDa, forming part of the nuclear pore basket alongside NUP153 and NUP50 (Frosst et al., 2002; Krull et al., 2004). Apart from NPCs, TPR is localized also within the nucleoplasm (Krull et al., 2004), however, the significance of its nucleoplasmic localization has not yet been understood. Overall, TPR has been so far linked to various cellular regulation processes, including regulation of the TREX-2 dependent mRNA export (Aksenova et al., 2019; Lee et al., 2020) and scaffolding enzymes like ERK2 (Vomastek et al., 2008) and MYC (Su et al., 2018) at the NPCs. Moreover, TPR binds to chromatin in vitro (Agarwal et al., 2011) and is essential for the establishment of heterochromatin exclusion zones near NPCs (Krull et al., 2010). These NPC-adjacent areas are vital for transcriptional regulation and the expression of tissue-specific genes. However, the role of TPR in gene expression and cell differentiation remains unknown.

3. Aims

In our studies, we focused on broadening our understanding of the role of the nucleoporin TPR within the cell nucleus. Specifically, we aimed to clarify following questions:

1. What is the nuclear distribution of TPR? What is the structure of TPR in nucleoplasm? What process is the nucleoplasmic TPR associated with?
2. What is the epigenetic landscape associated with the TPR-defined HEZ? What type of chromatin TPR associates with? Does TPR affect the expression of the associated genes? By what mechanism?
3. Is TPR involved in the myogenesis? How?

4. Research papers

Chromatin organization at the nuclear periphery as revealed by image analysis of structured illumination microscopy data

Fišerová, J., Efenberková, M., Sieger, T., Maninová, M., Uhlířová, J., and Hozák, P., Journal of Cell Science 2017, 130(12), pp. 2066–2077. doi: 10.1242/jcs.198424.,

IF: 4.401 (2017), J. U. performed experiments (antibody testing, image analysis)

Nuclear pore protein TPR associates with lamin B1 and affects nuclear lamina organization and nuclear pore distribution

Fišerová, J., Maninová, M., Sieger, T., Uhlířová, J., Šebestová, L., Efenberková, M., Čapek, M., Fišer, K., Hozák, P., 2019. Cell. Mol. Life Sci. https://doi.org/10.1007/s00018-019-03037-0,

IF: 7.030 (2019), J. U. contributed to the experimental execution (shTPR cell line preparation)

Nucleoporin TPR regulates expression of specific genes during myogenic differentiation

Uhlířová, J., Šebestová, L., Fišer, K., Sieger, T., Fišerová, J., Hozák, P., 2021. Nucleoporin TPR Affects C2C12 Myogenic Differentiation via Regulation of Myh4 Expression. Cells 10, 1271. https://doi.org/10.3390/cells10061271

IF: 6.600 (2021), J. U. designed and performed experiments (fluorescence microscopy, qPCRs and western blottings, Chip and Chip-seq, data analysis), wrote and revised the manuscript.

Nucleoporin TPR forms baso-apical oriented fibers in nucleoplasm and affect the baso-apical nuclear polarity

Šebestová, L., Uhlířová, J., Efenberková, M., Sieger, T., Fišerová, J., and Hozák, P.,

Manuscript in preparation

J. U. performed experiments (Chip-qPCR, RT-qPCRs and western blotting, microscopy and image analysis), forma analysis, visualization and the manuscript review and edition.

5. Summary

Studying TPR provides valuable insights into its pivotal role in nuclear architecture and gene regulation. In this investigation, we sought to address several key questions:

What is the nuclear distribution of TPR? What is the structure of TPR in the nucleoplasm?

We reported TPR forms vertically oriented nucleoplasmic filaments of mean length 250-500 nm in C2C12 and MEF cells (Šebestová, unpublished). The decrease of the TPR nucleoplasmic pool in the differentiated MTs suggests that the TPR might play a specific role in certain nuclear types only. For instance, it might protect the nuclei from the external mechanical forces, that are generated by actin cap that is present in the proliferating and not differentiated cells, such as the C2C12 MTs. Our data support the exciting concept of nucleoskeleton, with functions in nuclear mechanics, transport and spatial distribution of various nuclear processes.

What is the epigenetic landscape associated with the TPR-defined HEZ? What chromatin TPR associates with? Does TPR affect the expression of the associated genes? By what mechanism?

We precisely defined HEZ in HeLa cells, as the areas characterized by reduced DAPI signal at NPCs, around ~360 nm towards the nuclear interior and 200 nm along the NP. The HEZ borders in our experiments overlapped with the NPC staining of TPR, further supporting the idea that TPR serves as a scaffold for their formation (Fišerová et al., 2017). We revealed that HEZ are void of majority of the epigenetic marks, and that the exclusion of the repressive histone marks H1 and H3K27me2 and H3K9me2 is larger than that of the active histone marks, H3K4me2, H4K5Ac or H3K36me3. We revealed that histone de-methylases LSD1 and KDM2a are accumulated at the nuclear pores and thus might contribute to the described organization. Our preliminary data further suggested TPR as the protein scaffolding LSD1 at the pores.

Our ChIP-seq data revealed that TPR binding to chromatin overlaps with LADs. We show that in the muscle progenitor cells, C2C12 MBs, TPR associates with genes involved in muscle function and to majority of the *Olfr* genes. However, in contrast to

lamin, TPR has a positive effect on the expression of associated genes. We present data disclosing LSD1 as a TPR interacting partner and a possible effector responsible for the activation of the TPR associated genes (Uhlířová et al., 2021).

Is TPR involved in the myogenesis? How?

Our data revealed that TPR affects myogenesis at the level of (i) proliferation of the myogenic precursors; (ii) cell cycle exit under the differentiation stimuli; (iii) and formation of mature multinucleated MTs (Uhlířová et al., 2021). Our data sugged that the aberrant phenotype of the TPR depleted MTs might be caused by the altered expression of myogenic genes, *MymK*, *MymX* and *Myh4*. The altered expression of *MymK* and *MymX* could be explained simply by the delayed differentiation onset, or by the TPRs involvement in the mRNA export. The expression of *Myh4*, however, seems to be affected directly by TPRs binding to the gene body before the differentiation onset. We have suggested that TPR affects the poised state of the *Myh4* gene, preparing it for the rapid initiation of the transcription once the differentiation is triggered. Overall, our data suggest that TPR plays a complex role in regulation of gene expression during the differentiation process.

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