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Development of a mESC line for studying the condensin II influence on interphase 3D genome organization

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Motivation and Aim: Study of the three-dimensional (3D) organization of chromatin became highly relevant recent years as we develop awareness of its great influence on the gene epigenetic regulation and the maintenance of a genome. The way in which DNA folded inside the nucleus heavily affects gene regulation and the development [1].

One of protein complexes responsible for the regulation of 3D genome is cohesin [2]. Besides the cohesion of sister chromatids during cell division, cohesin forms loops of chromatin and larger structures called topologically associating domains (TADs) in interphase. Both chromatin structuration and sister chromatids connection achieved due to ATP-dependent mechanism named loop extrusion (LE), which is literally a threading of DNA through the ring shaped cohesin complex.

Cohesin belongs to the structural maintenance of chromosomes (SMC) complexes family. Besides cohesin, it includes condensin I and condensin II. All of them have a similar structure: the core frame is a triangle “ring” made of two SMC proteins and one kleisin subunit. Condensins known as agents of chromosome condensation before cell division. Strikingly, mechanism of their work is the same as in case of cohesion – LE [3]. A condensed chromosome is a dense thread of loops which were stretched both by condensin I and II. The CTCF protein sets borders for loops made by cohesin. There are no such factors known for condensins, which gives reason to think that other agents regulate the work of these complexes.

One of such regulating factors is microcephalin 1 (MCPH1) protein. It prevents condensin II from binding and extruding chromatin during interphase. Mutations in *MCPH1* gene are found in patients with primary microcephaly. Their cells possess prophase-like chromatin structure due to absence of MCPH1 protein preventing condensin II from compacting it [4]. Chromosome conformation capture (Hi-C) in these cells shown a drastic change in interphase 3D genome architecture. Very likely, this affects gene regulation, which leads to the development of a disorder.

The simplest way to investigate the gene’s function is to knock it out and then observe resulting phenotype. Unfortunately, this approach can not be used in studies of proteins of SMC complexes because knockout of their genes is lethal. RNA interference is insufficient in this case, as it does not eliminate target protein completely. Therefore, the only option left is a direct depletion of a target protein. This achieved by using degron system. It consists of two elements: first is ligand receptor, which transmits signal to the ubiquitin ligase, and the second is amino acid sequence that is recognized by ubiquitin ligase and linked to the target protein which is wanted to be destroyed [5].

Our goal is to investigate the impact which every complex – cohesin and condensin II – has in the development of MCPH-dependent microcephaly. Since they are likely

interacting, it is hard to define which of them is responsible for each feature (if both of them bind chromatin in interphase). Thence, we decided to develop a mouse embryonic stem cell (mESC) line that can be depleted of cohesin using degron system and possesses a deletion in *Mcph1* gene, which allows condensin II to extrude loops in interphase.

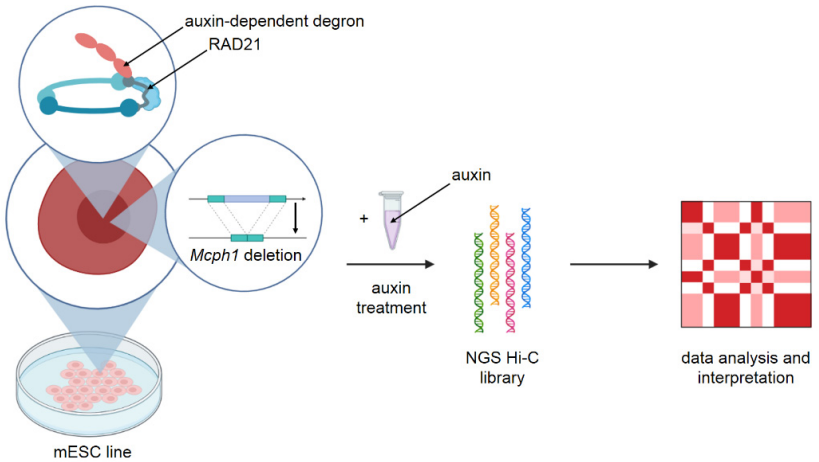

Fig. 1. Graphical abstract of the work and its planned continuation

Methods and Algorithms: We have previously developed mESC line with an auxin-inducible degron system (with auxin as a ligand) for depletion the RAD21 protein (kleisin subunit of cohesin) and with the eGFP tag. Efficiency of the system we have proved by flow cytometry: about 95 % of the RAD21 degrade after the addition of auxin. Next, we have made a deletion in *Mcph1* exon of these cells using CRISPR/Cas9 assay (guide design adopted from [4]).
Results: PCR genotyping and Sanger sequencing have proved the success of the modification, and the performance of degron system we verified with fluorescent microscopy and flow cytometry.
Conclusion: Developed cell line is suitable for further investigation of condensin II impact on 3D genome configuration and, potentially, for modelling of microcephaly on early stages of development.
After the development of a cell line, we performed a Hi-C analysis on control (auxin untreated) and treated cells and constructed a library for the next-generation sequencing. The results of sequencing are still awaited.
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