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functional significance of the coiled-coil domain for human (h) and *Saccharomyces cerevisiae* (y) Gle1. Both yGle1 and hGle1 self-associate via their coiled coil domain *in vitro* to form higher order homo-oligomeric complexes. Strikingly, using electron microscopy, the hGle1 and yGle1 oligomers form ~26 nm in diameter disk-shaped structures that were malformed with the h-gle1-Fin_{Major} protein. Because LCCS1 is a homozygous recessive condition, we established an RNAi knockdown and add-back system to test for functional defects. Reduction of *GLE1* activity in HeLa cells resulted in nuclear accumulation of poly(A) $^{+}$RNA. Co-expressing siRNA-resistant wild-type *hGLE1B*^{R} rescued the mRNA export defect. However, co-expression of *hgle1B*^{R}-*Fin*$_{Major}$ did not. Live cell microscopy studies found that GFP-hgle1B-Fin_{Major} had altered nucleocytoplasmic shuttling dynamics. A parallel series of genetic studies were conducted with *y-gle1* loss-of-function mutants that mimic the *h-gle1-Fin*$_{Major}$ allele. Growth defects of yeast mRNA export mutants were exacerbated when combined with *y-gle1-Fin* alleles; whereas, translation initiation and termination mutants were not impacted. We conclude that proper Gle1 self-association is specifically required during mRNA export, revealing a new model for controlling rounds of Dbp5 activity at NPCs. This work also provides the first evidence for the molecular mechanism causing the human LCCS1 disease, and impacts the global understanding of the role for altered mRNA transport and gene expression in other human diseases.

245

Nuclear target search at the single molecule level: protein interactions define the exploration landscape

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Gene regulation relies on highly mobile transcription factors (TFs) exploring the nucleoplasm in search of their targets. Our view of the nucleus has evolved from that of an isotropic and homogenous reactor to that of a highly organized yet very dynamic organelle. However important questions remain on how these regulatory factors explore the nuclear environment in search of their DNA or protein targets, and how their exploration strategy affects the kinetics of transcriptional regulation.

We implemented a single-molecule tracking assay to determine the TFs dynamics using photoactivatable tags in human cells. We investigated the mobility of several nuclear proteins, including the transcription factor c-Myc and the elongation factor P-TEFb. We found that, while their diffusion speed was comparable, these proteins largely differed in terms of their exploration geometry. We discovered that c-Myc is a global explorer diffusing in the nucleus without spatial constraints. In contrast, the positive transcription elongation factor P-TEFb is a local explorer that oversamples its environment, constrained by a fractal nuclear architecture. Consequently, each c-Myc molecule is equally available for all nuclear sites while P-TEFb reaches its targets in a position-dependent manner. We also measured the mobility of a P-TEFb mutant in which the interaction with the CTD of the RNA Pol II was truncated. In this case, the single-molecule experiments suggested a global exploration of the P-TEFb mutant, consistent with free diffusion.

Our observations are in line with a model in which the exploration geometry of TFs is constrained by their interactions and not by exclusion properties. Our findings have strong implications on how proteins react in the nucleus and how their function can be regulated in space and time.

246

PNCs associate with structure and function of the nucleolus and Pol III

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