Moonlighting proteins in mitosis: new roles for splicing and nucleolar proteins

At beginning of mitosis, the nucleolus disassembles and the nuclear envelope breaks down. As a result, many nuclear proteins are released in the cytosol. These proteins include those involved in cellular processes such as transcription and splicing that are strongly reduced during cell division. In the course of evolution, this situation might have favored the acquisition of direct mitotic functions by some nuclear proteins, making them “moonlighting proteins” that play both a primary role in interphase nuclei and an additional function during mitosis.

Some years ago, we performed a large RNAi screen in Drosophila S2 cells to identify new mitotic functions. We identified 104 mitotic genes, and among these 42 appeared to be required for chromosome segregation. Unexpectedly, almost a half of these 42 genes encodes conserved splicing factors (SFs). To assess whether “mitotic SFs” control mitotic mRNA splicing or play direct moonlighting mitotic functions, we have recently performed a detailed study on two highly conserved SFs, Sf3A2 and Prp31. We demonstrated that these SFs play direct mitotic functions in both Drosophila and human mitosis. Depletion of either SF affects spindle formation and disrupts chromosome segregation. Inhibition of Sf3A2 or Prp31 in fly embryos by specific antibody injections resulted in a strong and characteristic mitotic defect occurring within 1-2 minutes after the injection, a finding that argues strongly against an indirect mitotic role of these SFs. Consistent with these results, Sf3A2 and Prp31 bind microtubules and the Ndc80 complex that mediates kinetochore-MT attachment. In Sf3A2- and Prp31-deficient Drosophila and human cells Ndc80/HEC1 failed to accumulate properly at kinetochores and displayed different degrees of dispersion in the mitotic cytoplasm. Our working model is that SF3A2 and PRP31 are required to anchor Ndc80/HEC1 to the kinetochores and that, in the absence of these SFs, Ndc80/HEC1 tends to diffuse along the k-fibers, weakening the connection between the spindle MTs and the chromosomes. Collectively, these results indicate that at least a fraction of the SFs has acquired moonlighting mitotic roles.

In metaphase, both Sf3A2 and Prp31 concentrate around the chromosomes and appear to be part of a compartment defined as perichromosomal layer, which contains proteins (mostly nuclear in interphase) and chromosome-associated RNA molecules whose function is poorly understood. We plan to gain insight into the roles of the proteins included in this compartment by isolation and characterization of mitotic complexes containing specific SFs and nucleolar proteins. Our studies will provide new insight into the mechanisms orchestrating the mitotic machinery. A detailed understanding of this machinery might be instrumental to devise new cancer therapies.

References:
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Keywords: mitosis; moonlighting proteins; splicing factors.

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