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The moonlighting functions of the NON3 protein in *Drosophila melanogaster*

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Motivation and Aim: It was previously shown that a number of proteins, which are components of the nucleolus and necessary for the biogenesis of ribosomes, are also involved in the mitotic spindle assembly in Drosophila S2 cells [1]. The molecular mechanism of the latter process currently is not fully understood. Our aim was to clarify the role of *D. melanogaster* NON3 (Novel nucleolar protein 3) protein on kinetochore-driven microtubule growth.

Methods and Algorithms: We generated polyclonal antibodies specific to NON3. Using *P*-element imprecise excision, we obtained a set of new *Non3* mutations. They were sequenced and analyzed by complementation analysis. To study microtubule regrowth from kinetochores in cultured S2 cells, we performed RNA-interference (RNAi) to deplete NON3 and used colcemid treatment.

Results: We describe the viability and fertility of generated *Non3* alleles: null-mutations are early embryonic recessive lethal, hypomorphic mutations survive to adult stage and are semi-sterile, precise excisions (control) are fully viable and fertile. We found that NON3 protein is a component of nucleolus and pericentric regions of chromosomes. NON3 depletion after RNAi in S2 cells results in (1) a short mitotic spindle, (2) formation of metaphases with disorganized spindle and (3) appearance of pseudo-anatelophases (anatelophases, in which the spindle looks like at the last stages of division, but sister chromatids didn't separate to the poles of the spindle). The colcemid treatment of NON3-depleted S2 cells affects microtubule regrowth from kinetochores. A lack of NON3 protein (due to mutations or RNAi) affects the loading of drosophila CENP-A protein (CID) at centromeric regions of chromosomes, which may be a reason of the short spindle in NON3-depleted S2 cells.

Conclusion: Our results suggest that the NON3 protein affects kinetochore-driven microtubule growth due to the disruption of CID loading to centromeric chromatin.

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References

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