Microtubule nucleation controlled by testis-specific γ-TuRC in Drosophila spermatogenesis

PhD Thesis

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Introduction

Drosophila melanogaster spermatogenesis is an ideal model to study the assembly and regulation of conserved protein complexes, related to the cytoskeletal organization in somatic and germ cells. In addition, spermatogenesis and sperm structure show a high degree of conservation among different organisms. In the developing spermatids, we can investigate the rearrangement and formation of cytoskeletal elements, changes in cellular organelles, the nuclear remodeling with protamine containing chromatin, formation of specialized mitochondria after meiosis and the process responsible for the centrosome and basal body formation.

MTs are an essential part of the eukaryotic cytoskeleton, playing crucial roles in cell division, and intracellular transport between organelles and cellular compartments. MTs are mainly formed *de novo* at specific positions in the cell, termed MT organization centers (MTOCs). Depending on their location in the cell, centrosomal and non-centrosomal MTOCs can be distinguished. The nucleation of MTs from α and β-tubulin dimers is an essential cellular process; however, they are operating with a distinct set of

participating proteins in the different MTOCs. There is one common factor likely required for MT nucleation in all different locations, γ-tubulin. This protein is conserved throughout eukaryotes and shares high homology with α-tubulin and β-tubulin. γ-tubulin is a member of γ-TuRC, a complex with conserved composition and function. γ-tubulin small complex (γ-TuSC) is a heterotetrameric protein complex with a molecular weight of ~300 kDa, composed of γ-tubulin, GCP2 and GCP3 (γ-Tub23C, Grip84 and Grip91 in Drosophila). The γ-TuSC incorporates into a larger complex, the γ-TuRC. In addition to multiple γ-TuSC, γ-TuRC contains additional three GCP proteins, GCP4, GCP5 and GCP6 (Grip75, Grip128 and Grip163 in Drosophila, respectively). γ-TuRC structure resembles a conical open left-handed spiral, with 14 spokes, each is a single GCP protein associated with γ-tubulin. The γ-TuRC might contain other proteins in addition to the related GCP2, 3, 4, 5 and 6, such as NEDD1/Grip71, NME7/nmdyn-D7, Mozart1 and Mozart2.

In Drosophila γ-TuRC is composed of γ-tubulin, Grip84, Grip91, Grip128, Grip75, Grip163 and Grip71. Grip84 and Grip91 with γ-tubulin form the γ-TuSC, the core of the ring complex. Mutants of the γ-TuSC proteins, Grip91 and Grip84 are

lethal, with defects in the spindle assembly. Grip75 and Grip128 mutants are viable with defects in both female and male germ cell development.

A recent study shows non-centrosomal MTOC-specific recruiter protein, CnnT on the surface of the giant elongated mitochondria of the spermatids. It was also found recently that the conserved γ-TuRC interacting protein, Mzt1 also accumulates in the male germline, and localizes to the centrosome and basal body of the developing spermatids during spermatogenesis. Mzt1 was found to interact with the N-terminal regions of Grip91 and Grip128 in Drosophila; however, the precise molecular function and localization pattern was not tested rigorously.

Aims

Drosophila melanogaster is a good model to study various biological processes, due to the wide variety of available classical and molecular genetic tools. Drosophila spermatogenesis is a suitable model to study basic cellular processes, such as MT organization. It was shown recently that besides the known classical MTOC, such as centrosome and basal body there are alternative ones on the surface of the mitochondria of the developing spermatids.

Our general aim was to explore the molecular components, and function of the different MTOCs during spermatogenesis by studying the predicted testis-specific γ-TuRC members and interacting partners.

Specific aims:

- We aimed to characterize genetically the predicted testis-specific paralogous of the γ-TuRC proteins, t-Grip84, t-Grip91 and t-Grip128, by describing the phenotype of their classical mutants.
- We wanted to describe the subcellular localization of t-Grip84, t-Grip91 and t-Grip128 and also the precise

localization of Mzt1, the testis-specific γ-TuRC associated protein.

- Our goal was to identify the molecular composition and interacting partners of the predicted testis-specific γ-TuRC (t-γ-TuRC).

Applied methods

1. Classical Drosophila genetics: fertility assay, genetic characterization of classical transposon mutants of t-Grip84, t-Grip91, complementation analysis.
2. Designing and creating null mutant of t-Grip128 using CRISP-Cas9 technology.
3. Molecular characterization of the t-Grip mutants, quantitative RT-PCR.
4. Cloning of transgenes in Drosophila transformation vectors and establishing transgenic lines with tagged proteins (t-Grip84, t-Grip91, t-Grip128, Mzt1, gamma-tubulin).
5. Cloning of t-Grips into yeast transformation vectors.
6. Yeast transformation and yeast-two hybrid analysis.
7. Cloning of t-Grips into pJET1.2 vector.
8. Transfection of Drosophila Dmel2 tissue culture cells.
9. Immunostaining of Drosophila Dmel2 cells and testis samples.
10. Fluorescent and confocal microscopy.
11. Image processing.
12. Statistical analysis of data

Results and conclusion

Drosophila melanogaster spermatogenesis harbor various MTOCs, which makes it a good model to study MTOCs and their components, like γ-TuRC. In addition, spermatogenesis and sperm structure show a high degree of conservation among different organisms. In this study, we identified 3 testis-specific γ-TuRC component proteins, t-Grip84, t-Grip91 and t-Grip128. Transcripts of t-γ-TuRC genes accumulate in the middle-basal part of the testis with a high testis specificity index. Transcripts of the ubiquitous *Grip84*, *Grip91* and *Grip128* accumulate at the apical region of the testis with a low testis-specificity index. The mutant lines of *t-Grip84, t-Grip91* and *t-Grip128* were analyzed in detail in this study. Mutants of *t-Grip84* were male sterile in homozygous, hemizygotes and transheterozygous, while *t-Grip91* mutants were male semi-sterile in homozygous and hemizygotes. *t-Grip128* mutant *t-Grip128*$^{\Delta 65}$ was male-fertile, with fertility comparable to the wild type. We found that transcripts of *t-Grip84*, *t-Grip91* and *t-Grip128* were highly reduced in testis extracts of *t-Grip84*^{ms}, *t-Grip91*^{ms} and *t-Grip128*$^{\Delta 65}$ mutants respectively, which indicates that those mutants are null alleles. Since t-Grip84 has a typical Grip protein

structure we checked t-Grip84 localization to the centrosome of the Drosophila Dmel2 cells. We found that t-Grip84-GFP was able to localize to the centrosome of Drosophila Dmel2 cells. We established transgenic lines for the t-γ-TuRC proteins with different tags to test their subcellular distribution. We did not find t-Grip84-GFP, t-Grip84-mCh, HA-t-Grip91 or HA-t-Grip128 proteins in epithelial tissue or ovaries. t-γ-TuRC proteins appeared only in the testis during spermatogenesis. t-Grip84-mCh (GFP), HA-t-Grip91 and HA-t-Grip128 have a dynamic localization pattern during spermatogenesis, the proteins were not present during the early stages of spermatogenesis in GSCs, mitotic or meiotic cells. t-γ-TuRC proteins start to localize after meiosis at the centriole adjuncts of the round spermatid and their localization persists during elongation at the centriole adjuncts but disappears in the elongated spermatids. Later during elongation, the t-γ-TuRC proteins appear at the anterior tip of the elongated nucleus. Moreover, a third localization focus of the proteins starts to appear on the surface of the mitochondria. We tested the colocalization between the t-γ-TuRC proteins, γ-tubulin and the t-γ-TuRC interacting protein Mzt1. t-Grip84, t-Grip91 and t-Grip128 all colocalize with each other, γ-tubulin and Mzt1 at the centriole adjuncts during elongation. The anterior tip

localization was shared between all t-γ-TuRC proteins, t-Grip84-mCh (GFP) HA-t-Grip91 and HA-t-Grip128. In the case of GFP-Mzt1, the signal starts to localize at the beginning of spermatogenesis to the centrosome of spermatocytes. Later at meiotic spermatids, GFP-Mzt1 signal was visible at the centrosome and the mitochondria. During later stages of elongation GFP-Mzt1 and γ-Tub23C-GFP colocalized with the t-γ-TuRC proteins at the centriole adjunct, the apical tip of the nuclei and the surface of the elongating mitochondria. t-γ-TuRC proteins colocalized with the PCM proteins PACT and Ana1 in addition to the centrosomal protein Asl and the ubiquitous γ-TuRC protein Grip163. In *t-Grip84*^{ms} and *t-Grip91*^{ms} mutants, early stages of spermatogenesis were normal. The axonemes of *t-Grip84*^{ms} and *t-Grip91*^{ms} mutants were normally developed and elongated. During elongation, the basal bodies detached from the nuclei where the nuclei were scattered in the cytoplasm. The ICs were normally formed but their movement was disturbed as they start their migration. ICs became scattered in *t-Grip84*^{ms} and *t-Grip91*^{ms} mutants. Neither t-Grip84-GFP nor HA-t-Grip91 were able to localize in *t-Grip91*^{ms} and *t-Grip84*^{ms} respectively. The same γ-tubulin and GFP-Mzt1 were not localized to the elongating spermatids in the mutants of *t-Grip91*^{ms} and

t-Grip84^{ms}. We divided the t-γ-TuRC proteins into two parts N- and C- terminal parts to test their interactions biochemically using yeast two-hybrid and IVTT. All t-Grip84, t-Grip91 and t-Grip128 bind to each other and γ-tubulin, while only the N-terminal part of t-Grip91 binds to Mzt1.

In conclusion, we identified three testis-specific γ-TuRC proteins, which have dynamic localization throughout spermatogenesis. They start to localize to the centriole adjunct after meiosis, then simultaneously localize to the apical tip of the nucleus and the basal body and finally during the late elongation stages they localize near the surface of the mitochondria. t-γ-TuRC proteins bind to each other and γ-tubulin while only t-Grip91-N binds to Mzt1. This all suggests that Drosophila spermatogenesis functions with two different γ-TuRC, the ubiquitous γ-TuRC and the newly identified t-γ-TuRC. The ubiquitous γ-TuRC is involved in MTOCs organization till the end of the meiosis, whereas t-γ-TuRC is involved in MTOCs after meiosis. Since the majority of Drosophila and human genes share high homology, studying and modelling the organization and composition of different MTOCs in flies could have great potential to understand better several human diseases such as microcephalies, ciliopathies and even cancer.

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Publication List (MTMT ID: 10084128)

Number of scientific publications: **3**

Total impact factor: **19,56**

Conference abstracts: **2**

Journal articles used for attaining the PhD degree

Alzyoud, E., Vedelek, V., Réthi-Nagy, Z., Lipinszki, Z., and Sinka, R. (2021). Microtubule Organizing Centers Contain Testis-Specific γ-TuRC Proteins in Spermatids of Drosophila. Front. cell Dev. Biol. 9, 727264. doi:10.3389/fcell.2021.727264 **(I.F: 6.68 (2020))**

Vedelek, V., Kovács, A. L., Juhász, G., Alzyoud, E., and Sinka, R. (2021). The tumor suppressor archipelago E3 ligase is required for spermatid differentiation in Drosophila testis. Sci. Rep. 11, 8422. doi:10.1038/s41598-021-87656-3. **(I.F: 4.38(2020))**

Other publications

Ibragimova, S., Szebenyi, C., Sinka, R., Alzyoud, E. I., Homa, M., Vágvölgyi, C., et al. (2020). CRISPR-Cas9-Based Mutagenesis of the Mucormycosis-Causing Fungus Lichtheimia

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CONFERENCES

Oral presentations:

Elham Alzyoud[1,2], Viktor Vedelek[1], Zsuzsanna Réthi-Nagy[2,3], Zoltán Lipinszki[3], Rita Sinka[1]

Microtubule organizing centers contain testis-specific γ-TuRC proteins in spermatids of Drosophila melanogaster.

2nd conference of the Visegrád Group Society for developmental Biology 2021 (Szeged, Hungary)

Elham Alzyoud[1,2], Viktor Vedelek[1], Zsuzsanna Réthi-Nagy[2,3], Zoltán Lipinszki[3], Rita Sinka[1]

Alternative microtubule organizing centers in spermatids of Drosophila

Hungarian Molecular Life Sciences Conference 2021 (Eger, Hungary)

DECLARATION

I declare that the contribution of Elham Alzyoud was significant in the below listed publications and the doctoral process is based on the publications listed. The results reported in the PhD dissertation were not used to acquire any PhD degree in the past and will not be used in the future either.

Alzyoud, E., Vedelek, V., Réthi-Nagy, Z., Lipinszki, Z., and Sinka, R. (2021). Microtubule Organizing Centers Contain Testis-Specific γ-TuRC Proteins in Spermatids of Drosophila. Front. cell Dev. Biol. 9, 727264. doi:10.3389/fcell.2021.727264
(I.F: 6.68 (2020))

Vedelek, V., Kovács, A. L., Juhász, G., Alzyoud, E., and Sinka, R. (2021). The tumor suppressor archipelago E3 ligase is required for spermatid differentiation in Drosophila testis. Sci. Rep. 11, 8422. doi:10.1038/s41598-021-87656-3.
(I.F: 4.38(2020))

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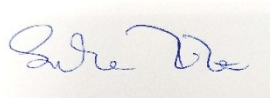

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