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ABSTRACT BOOK

GENETICS GSA G3 Genes | Genomes | Genetics

1 **Deciphering mechanisms of centriole elimination during oogenesis** Alexander Woglar, Keshav Jah, Fabian Schneider, Marie Pierron, Coralie BussoEPFL

Centrioles are nine-fold symmetrical microtubule-based organelles that template the formation of cilia and flagella, and form the core of the centrosome that organizes cytoplasmic microtubules, including during cell division. The number of centrioles must be tightly controlled: like chromosomes, each of the two centrioles present early in the cell cycle is licensed to seed the formation of a new centriole once, and only once during S-phase. However, during reproduction, centriole numbers must be controlled differently. Here, sperm and egg cells fuse to form a single cell. Here, the sperm contributes two centrioles, while centrioles are eliminated during oogenesis, thus avoiding doubling the number of centrioles with each generation.

We present mechanistic insights into this evolutionarily conserved and thus far enigmatic process by employing the gonad of *C. elegans* as an optimal model system, using ultrastructure expansion coupled with STED microscopy, electron microscopy and tomography, live-imaging and novel genetic and acute pharmacological inhibition methods. We find that oogenesis centriole elimination begins when nuclei enter late meiotic prophase I (late pachytene) and is characterized by several ultrastructural and compositional changes. First, the microtubule-binding and -bundling factor SAS-1 is eliminated from centrioles at that stage during oogenesis. This is followed by widening of the centriole and progressive decoration of the core centriolar components microtubules and SAS-4 with ubiquitin. Thereafter, the proteasome becomes enriched at centrioles, which coincides with loss of the ubiquitinated centriolar microtubules and SAS-4, followed by a complete loss of centriolar integrity. Importantly, these processes happen prematurely in *sas-1* mutant animals.

We propose that centriole elimination during oogenesis involves differential access of the proteasome to the centriole, which is controlled by an alteration in centriolar architecture imparted by the loss of SAS-1.

2 **Determining the mechanism of Kinesin-1 dependent translocation of the meiotic spindle to the cortex** Alma Martinez Peraza[1], Francis J McNally[21]University of California, Davis - Davis, CA, [2]MCB, University of California, Davis - Davis, CA

Cortical positioning of the meiotic spindle within an oocyte is required to expel chromosomes into polar bodies to generate a zygote with the correct number of chromosomes. In *C. elegans* the prophase nucleus migrates to the oocyte cortex and the metaphase spindle moves further toward the cortex, both in a kinesin-1 dependent manner. In contrast, yolk granules, mitochondria and kinesin-1 are packed inward, away from the cortex in a kinesin-dependent manner. The kinesin-dependent inward packing of yolk granules and mitochondria suggests the existence of microtubules with minus ends at the cortex and plus ends extending inward. Thus, the mechanism of outward translocation of the spindle has remained a mystery. We first generated a germline null allele of *unc-116* which encodes the kinesin-1 heavy chain by complementing the *unc-116(gk5722)* lethal deletion with an integrated *unc-116::GFP* array, duIs1, that is silenced in the germline. Time-lapse imaging of *unc-116(gk5722) duIs1* worms revealed a stronger phenotype than previously reported for viable alleles or RNAi depletions, with the meiotic spindle positioned in the center of the embryo. Kinesin-1 has been reported to bind to multiple different cargoes through its C-terminal tail domain. To identify the direct cargo of kinesin that mediates movement of the meiotic spindle to the cortex, we are taking an optogenetic approach to couple tailless kinesin-1 directly to ER, nuclear envelope, mitochondria, or yolk granules to determine if this restores spindle translocation in *unc-116(gk5722) duIs1* embryos. K420 is the first 420 aa of UNC-116 which does not include the cargo-binding tail. TMCO-1 is an integral membrane protein of the ER. iLID and SSPB bind when illuminated. Attaching K420::mKate::iLID tailless kinesin specifically to TMCO-1::GFP::SSPB labeled ER has restored the localization of the meiotic spindle to the cortex in an *unc-116(gk5722) duIs1* background in 6/6 time-lapse sequences. This result suggests that the ER, which envelopes the meiotic spindle after nuclear envelope breakdown, may be the direct cargo of kinesin-1 in meiotic spindle translocation. TMCO-1 is in the nuclear envelope as well as the ER whereas the nuclear pore protein NPP-24 is only in the nuclear envelope. To determine whether kinesin-1 acts before or after nuclear envelope breakdown, we are attempting the same experiment with NPP-24::GFP::SSPB.

3 **Repurposing the Chromosome-Microtubule Coupling Machinery as a "Tuner" of Actin for Dendritic Branching.** Dhanya Cheerambathur[1], Mattie Green[2], Henrique Alves Domingos[2], Vasilis Ouzounidis[21]School of Biological Sciences, University of Edinburgh, [2]University of Edinburgh

Dendrite branching is an essential process for building complex nervous systems. A neuron's dendritic patterns govern the number, distribution, and integration of inputs. Though significant progress has been made in understanding the signalling pathways that pattern the dendrite, little is known about the intrinsic mechanisms involved in sculpting the branches. The actin & microtubule cytoskeleton are critical to provide structure and exert force during dendrite branching. Our study reveals an unexpected role for the kinetochore, the chromosome-microtubule machinery, in shaping the dendrites of the mechanosensory neuron, PVD in *C. elegans*. The kinetochore is a highly conserved multiprotein complex whose canonical function is to connect chromosomes to microtubules during cell division. Kinetochore proteins are enriched in the PVD dendrites where they associate

(TRPV1). Prolonged treatment with vanilloids triggered the desensitization of the TRPV1 leading to analgesic or antinociceptive effects. Following *C. elegans* genome sequencing, several genes encoding TRP ion channels, including TRPVs, were identified. Furthermore, several studies have shown that *C. elegans* TRPV orthologs (OSM-9 and OCR-2) are associated with behavioral and physiological processes, including sensory transduction. We have already shown capsaicin and eugenol targets *C. elegans* TRPV orthologs. The objective of this study was to perform proteomics to identify the proteins and pathways responsible for the induced phenotype.

Methods

N2 (Bristol) and other strains were obtained from the Caenorhabditis Genetics Center (CGC), University of Minnesota (Minneapolis, MN, USA). Strains were maintained and manipulated under standard conditions. Capsaicin or eugenol was dissolved in Type 1 Ultrapure Water at a concentration of 25 µM. *C. elegans* was isolated and washed and then expose to capsaicin or eugenol for 60 min. Then after, the nematodes were isolated, carefully washed, homogenized and protein were extracted, normalized, denature, reduced, alkylated, and digested with trypsin. Ultra-high-performance liquid chromatography/Quadrupole-Orbitrap mass spectrometry analysis operating in Data-Dependent Acquisition (DDA) mode was performed to identify and quantify proteins. Pathway analyses were performed using Metascape and the Reactome database.

Preliminary data

Capsaicin and eugenol can impede nocifensive response of *C. elegans* to noxious heat (32°C – 35°C) and the effect was reversed 6h post exposition. Additionally, we have identified the capsaicin target, OCR-2 and eugenol act redundantly with both OSM-9 and OCR-2. After we use proteomic investigations to performed *C. elegans* exposed to vanilloids. Preliminary results demonstrate that several specific processes were modulated following the pharmacological manipulation of *C. elegans* with capsaicin and eugenol. The inflammatory signaling pathways and the regulation of translation stand out from our bioinformatics analyses. These two processes are intimately linked to cell protection and survival mechanisms.

Novel aspect

Proteomics reveals inflammatory signaling pathways are triggered by the agonistic effects of capsaicin and eugenol on *C. elegans* vanilloid receptors.

1038V **The Ketone Body β-hydroxybutyrate ameliorates neurodevelopmental deficits in the GABAergic system of *daf-18/PTEN Caenorhabditis elegans* mutants.** Sebastián Giunti[1,2], María Gabriela Blanco[1,2], María José De Rosa[1,2], Diego Hernán Rayes[1,21]Instituto de Investigaciones Bioquímicas de Bahía Blanca, Departamento de Biología, Bioquímica y Farmacia (CONICET - UNS), [2]Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur

An increased Excitation/Inhibition (E/I) ratio in the brain is a hallmark of neurological disorders such as Autism Spectrum Disorder (ASD). Mutations in *PTEN,* a gene that encodes for the main negative regulator of the conserved PI3K/AKT pathway, are strongly associated with ASD. However, it is unclear how *PTEN* deficiencies can lead to E/I disequilibrium. The *C. elegans* neuromuscular system, where both excitatory (Cholinergic) and inhibitory (GABAergic) motor neurons regulate muscle activity, provides a proven simple model for studying E/I balance. We found that mutants in *daf-18* (ortholog for *PTEN)* exhibit phenotypes typical of animals with deficient GABAergic signaling. While cholinergic neuron morphology is normal, we observed defects that occur specifically in GABAergic neurites. This selective impairment accounts for the disruption of the E/I balance in *daf-18* mutants. In addition, we showed that the low activity of the transcription factor DAF-16 (ortholog for FOXO3A) during GABAergic neurodevelopment arises for the behavioral defects in *daf-18* mutants. Ketogenic Diets (KGDs), in which the production of ketone bodies (KBs) is forced, have been established as an effective treatment for disorders associated with E/I imbalances. The mechanisms underlying its effect are not understood. We found that exposure to the KB hydroxybutyrate (βHB) during early development improves GABAergic neurodevelopment in *daf-18* mutants. This effect depends on DAF-16/FOXO. Since the PI3K/AKT pathway is highly conserved, this study may provide universal information on the proven link between PTEN mutations and neurodevelopmental defects and, equally important, the mechanisms underlying KGDs positive effects on neuronal disorders characterized by E/I imbalance.

1039V **Reconstruction of *C. elegans* locomotion by optimal fluid control** Yongxing Wang, Thomas Ranner, Netta CohenUniversity of Leeds

C. elegans lives in a 3D environment whose locomotion however has been studied primarily through a microscope on a flat dish. There has been progress in developing a full 3D model of the worm, such as OpenWorm [1] or body midlines modelled as 3D curves [2,3]. Recent advances in imaging and midline reconstructions of worm's locomotion in 3D have opened up questions about the underpinning mechanics and neuromuscular control in 3D [4]. This dataset provides accurate body-midlines but