Regulated transport and positioning of mitochondria are essential for providing ATP to power nerve cell function and calcium buffering.[1,2] In *C. elegans* touch receptor neurons, the mitochondrial number is very tightly regulated and positioned at a constant inter-mitochondrial distance throughout the process.[3] *C. elegans* genome contains three Miro orthologs but mitochondrial trafficking and distribution is independent of Miro proteins (unpublished data). This suggests that in addition to classical Kinesin/Trak/Miro complex to position mitochondria, an alternative pathway exists in *C. elegans*.[4] We hypothesized that this alternative pathway might involve a Ca^{2+} mediated mechanism. In fact, in hippocampal neuron cultures, it is shown that increasing mitochondrial calcium can alter mitochondrial trafficking. It has been also shown that mitochondrial motility is decreased increased Ca^{2+} levels and mitochondria can be localized in region with high Ca^{2+} fluctuations e.g synapses.[2,5,6] While the effect of Ca^{2+} on mitochondrial trafficking is well characterized in vertebrates, this mechanism remained poorly understood in worms.

Part (i): Spontaneous Ca^{2+} transients in *Caenorhabditis elegans* affect mitochondrial positioning

Using genetically encoded calcium sensors, I showed that Posterior Lateral Microtubule (PLM) neurons exhibit spontaneous calcium transients. These transients are present on both cell body (CB) and axonal processes. Quantification of the Ca^{2+} transients in the cell body revealed no specific pattern or intervals, instead, they showed a purely stochastic behaviour. Around one-third of the worms show these transients with typically 1.16±0.2 transients per 100s. Duration of these transients are 17.08±1.41s long with a mean amplitude of 1.34±0.03 fold from the baseline. To test whether these transients are arising from touch-induced stimulation, we carried out similar experiments in animals carrying mutation in *mec-4* gene that exhibit loss of gentle touch sensation. *mec-4* codes for an ion channel from Na^{+} channel of the DEG/ENaC superfamily which is known to allow Na^{+} influx after gentle touch which in turn allow Ca^{2+} influx from Voltage-Gated Calcium Channels.[7] Interestingly, in *mec-4* animals, we observed Ca^{2+} transients similar to wildtype animals. This suggests that Ca^{2+} transients are not arising from the depolarization of the membrane (Due to touch-induced effects) rather originating from internal sources. Similar to *mec-4*, *mec-7* (β-tubulin) mutants are also insensitive to gentle touch.[8] Mutation in *mec-7* alters microtubule assembly and resulting in reduced number of protofilaments (~11 from classic 15 protofilaments in wildtype).[8] To test if Ca^{2+} transients are conserved in all animals that are defective in gentle touch sensation, we carried out Ca^{2+} imaging in worms carrying a mutation in *mec-7* gene. Surprisingly, I did not observe any transients (>20 animals) in *mec-7* animals. This possibly indicate that spontaneous Ca^{2+} transients in *C. elegans* do not depend on MEC-4 DEG/ENaC channel rather coming from internal stores which are affected by mutation in β-tubulin gene.

In addition to altering protofilament numbers, *mec-7* mutation also shows an increased mitochondrial number in touch receptor neurons (unpublished data). Since mitochondria are strongly correlated with Ca^{2+} in cultured neurons from rodents, I thought of checking correlation of mitochondria with spontaneous Ca^{2+} transients. First, we proposed the hypothesis that mitochondria are either act as a source or merely recruited at a high calcium region because of calcium buffering capabilities. This hypothesis was further examined in a strain (*ric-7;jsIs1073;NBR-19*) where mitochondria are arrested at the cell body.[9] When I carried out dual-color imaging of Ca^{2+} and mitochondria in the *ric-7* mutant animals, they showed spontaneous Ca^{2+} transients. These transients are similar in frequency and duration when compared to wildtype animals suggesting that mitochondria do not initiate these transients rather they are recruited in a highly spiking region. If this argument is true, then mitochondrial positioning should be overlapped with the actively spiking region. When we mapped all the stationary mitochondria on top of kymograph of a spiking neuron, I observed that in wildtype animals, Ca^{2+} transients are highly overlapped with mitochondrial positions. I masked the mitochondrial position and measure the frequency of the transients around mitochondria and compared with a region that lacks mitochondria. I observed that regions with mitochondria showed preferential calcium transients (1.5 times higher probability than region devoid of mitochondria) with respect to a region devoid of any mitochondria. My data also revealed that ~64% of the transients occur in a region that has at least one mitochondria present. *unc-16* mutation is known to increase the mitochondrial number and alter its distribution in the neuronal processes. In contrast to *mec-7*, *unc-16* animals showed persistent Ca^{2+} transients in the cell body. Upon quantification, *unc-16* animals showed frequency and duration of these transients similar to wildtype animals. Together with experiments in *ric-7* mutant, data from *unc-16* mutant revealed that mitochondria do not initiate spontaneous Ca^{2+} transients and most likely they are mobilized at an actively spiking region. This indicates the level of calcium can immobilize mitochondria and their distribution might change in response to higher or lower cytoplasmic calcium levels. I tested this by using two complementary

experiments. First, I expressed calcium sponge (Where cytoplasmic Ca^{2+} level is decreased due to blocking ER mediated Ca^{2+} release by overexpressing IP3 binding peptides)[10] to decrease cytoplasmic calcium level in touch receptor neurons and in a second experiment I used an optogenetic method to artificially increase cytoplasmic calcium level. When I analysed inter-mitochondrial distances from PLM neuron, I found that in calcium sponges inter-mitochondrial distances are significantly decreased from the control animals. I found 50% mitochondria show inter-mitochondrial distances around 7.5 μm while in control this increased to 9.5 μm. Inversely, growing Chr-2::YFP worms under blue light (that increases cytoplasmic Ca^{2+})[11] for at least 22h (L2 to L4/1-day adult molt) significantly increased inter-mitochondrial distances than animals grown in the dark. Both of these experiments suggested that mitochondrial distribution is strongly correlated to calcium levels. Decreasing cytoplasmic Ca^{2+} might allow more mitochondria to come out in the axonal processes thus reducing inter-mitochondrial distances. On the other hand, higher calcium most likely increases mitochondrial calcium which in turn reduce its trafficking into the processes thus increasing inter-mitochondrial distances. I believe this study will potentially indicate the existence of an intrinsic mechanism of mitochondrial positioning in response to changes in cellular calcium levels and may help to extend our current understanding of mitochondrial trafficking and function.

Part (ii): Role of a G-protein coupled receptor *str-2* on intracellular calcium levels

AWC neurons exhibit stochastic calcium transients in response to change in the temperature gradient.[12] It is also known that GPCR SRTX-1 expressed in AWC^{off} is involved in this thermosensation which in turn affects secretion and inhibition of signal transmission to AIY neurons.[13] Both AWC^{off} and AWC^{on} showed thermosensation and change in cytoplasmic calcium level in response to increase in environmental temperature.[14] We hypothesized that STR-2 which is a GPCR, might respond to temperature in AWC^{on} neuron as similar to SRTX-1 expressed in AWC^{off}. To test if STR-2 affects calcium responses in AWC^{on}, we expressed GCaMP5 under *str-2* promoter, and measured the change in basal Ca^{2+} level from animals cultivated at 20 ºC and 25 ºC. We observed a significant decrease in Ca^{2+} level in worms cultivated at 25ºC for >12h compared to the worms grown at 20 ºC. In contrast, *str-2(ok3148)* mutants grown at 20 ºC showed a marginal decrease in calcium levels than WT animals. Surprisingly, *str-2(ok3148)* mutants grown at 25 ºC did not show any significant decrease in Ca^{2+} levels compared to WT animals or *str-2(ok3148)* mutants grown at 20 ºC. When we compared the basal Ca^{2+} level in *str-2(ok3148)* mutants grown in either 20 ºC or 25 ºC with respect to WT animals cultivated at 25 ºC, we found Ca^{2+} level is significantly higher in *str-2(ok3148)* mutants. This suggests that in *str-2(ok3148)* mutants there is no change in Ca^{2+} level at different temperatures and overall Ca^{2+} level remains high than WT animals even at a higher temperature. Previous literature showed that AWC neurons respond to odor removal by decreasing cytoplasmic Ca^{2+} which is in excellent agreement with our observations from quantifying Ca^{2+} levels in WT animals grown at 20 ºC or 25 ºC. We believe, in addition to the response against odor removal, AWC neurons also respond to temperature removal and most likely through *str-2* as the adaptation response to increasing temperature gradients is abolished in *str-2* mutants.

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