Signed details of the excellence in research work for which the Sun Pharma Research Fellowship is claimed

Impaired nucleo-cytoplasmic transport (NCT), miRNA-mediated post-transcriptional gene regulation and ER-mitochondria contact site functions are independently implicated in multiple neurodegenerative disorders such as Amyotrophic Lateral Sclerosis (ALS), Alzheimer's, Parkinson's and Huntington's diseases (Ding & Sepehrimanesh, 2021; Rodríguez-Arribas *et al*, 2017; McCann *et al*, 2011). However, whether there exist any interconnections between these processes has not been explored.

Nuclear transport of macromolecules across the nuclear envelope through nuclear pore complexes (NPCs) is critical for cellular homeostasis. Interestingly, a subset of nucleoporins is present at the ER subdomains called annulate lamellae (AL). The cellular functions of this organelle are unclear.

AL are characterized by stacked ER membranes having pore-like structures, much similar to the nuclear pore complexes (NPCs) on the nuclear envelope. Many nucleoporins that are components of NPCs are also present at AL. However, the functions of this organelle remain underexplored. The term 'annulate lamellae' was coined in 1956, and over the last 6 decades or so 422 publications have been documented in PubMed. Very few of these have attempted to address the functional role of this organelle. Most of the reports present the electron microscopic structures of AL from different organisms, perturbations of AL under different conditions such as in viral infections and speculative functions derived from these observations. One such speculated function of AL is that they act as storehouses of nucleoporins. This notion has been supported by a few recent studies, wherein, AL were shown to be incorporated into the newly made nuclear envelope as precursor platforms for the NPC assembly during early embryonic cell divisions in Drosophila. Is that the only function for AL? As AL are present in different organisms ranging from plants to animals, and even in non-embryonic cells, these structures could be involved in other unexplored and conserved functions. However, a systematic investigation to shed light on the cellular functions of this organelle has been lacking. Our laboratory is focused on understanding the role of AL in the cellular processes.

ER-mitochondria contact sites (ERMCSs) regulate processes, including calcium homoeostasis, energy metabolism and autophagy. Previously, it was shown that during growth factor signalling, mTORC2/Akt gets recruited to and stabilizes ERMCSs. Independent studies showed that GSK3β, a well-known Akt substrate, reduces ER-mitochondria connectivity by disrupting the VAPB-PTPIP51 tethering complex. However, the mechanisms that regulate ERMCSs are incompletely understood. Here we find that annulate lamellae (AL), relatively unexplored

subdomains of ER enriched with a subset of nucleoporins, are present at ERMCSs. Depletion of Nup358, an AL-resident nucleoporin, results in enhanced mTORC2/Akt activation, GSK3β inhibition and increased ERMCSs. Depletion of Rictor, an mTORC2-specific subunit, or exogenous expression of GSK3β, was sufficient to reverse the ERMCS-phenotype in Nup358-deficient cells. We show that growth factor-mediated activation of mTORC2 requires the VAPB-PTPIP51 complex, whereas, Nup358's association with this tether restricts mTORC2/Akt signalling and ER-mitochondria connectivity. Expression of a Nup358 fragment that is sufficient for interaction with the VAPB-PTPIP51 complex suppresses mTORC2/Akt activation and disrupts ERMCSs. Collectively, our study uncovers a novel role for Nup358 in controlling ERMCSs by modulating the mTORC2/Akt/GSK3β axis (**Figure 1**) (Kalarikkal *et al*, 2024, 2021).

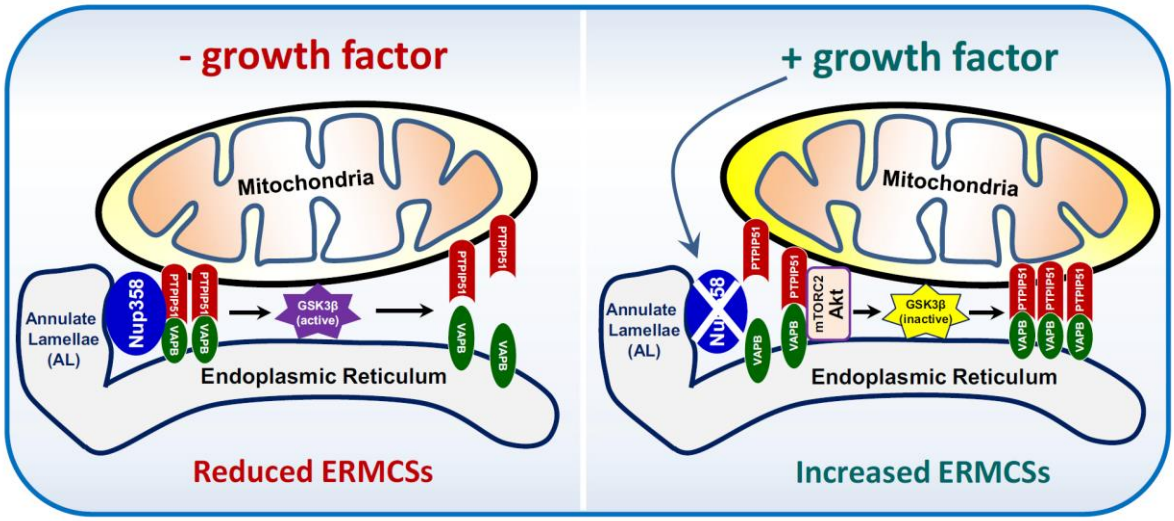

Figure 1. ***Model for the role of Nup358 in ERMCS remodelling during growth factor signalling***. ***Left***: *In the absence of growth factor signalling, presence of Nup358 at the ERMCSs due to its interaction with VAPB and PTPIP51 prevents the recruitment and activation of mTORC2 at the ERMCSs, leading to inhibition of Akt, activation of GSK3β and thereby decreased ER–mitochondria connectivity.* ***Right***: *In the presence of growth factors, through an unknown (but PI3 kinase-independent) mechanism, interaction of Nup358 with VAPB/PTPIP51 is reduced. This allows the recruitment of mTORC2/Akt complex to the ERMCSs through interaction with preformed VAPB–PTPIP51 tether. This results in activation of mTORC2 and its downstream target Akt which in turn phosphorylates and inhibits GSK3β. This prevents GSK3β-mediated disruption of VAPB–PTPIP51 interaction which subsequently increases ER–mitochondrial contacts. Thus, a reciprocal binding of Nup358 and mTORC2 with VAPB–PTPIP51 complex, ultimately determines the extent of ER–mitochondria connectivity by modulating the activity of GSK3β (Kalarikkal et al, 2024).*

MicroRNA (miRNA)-guided mRNA repression, mediated by the miRNA-induced silencing complex (miRISC), is an important component of post-transcriptional gene silencing. However, how miRISC identifies the target mRNA in vivo is not well understood. Here, we

show that the nucleoporin Nup358 plays an important role in this process. Nup358 localizes to the nuclear pore complex and to the cytoplasmic annulate lamellae (AL), and these structures dynamically associate with two mRNP granules: processing bodies (P bodies) and stress granules (SGs). Nup358 depletion disrupts P bodies and concomitantly impairs the miRNA pathway. Furthermore, Nup358 interacts with AGO and GW182 proteins and promotes the association of target mRNA with miRISC A well-characterized SUMO-interacting motif (SIM) in Nup358 is sufficient for Nup358 to directly bind to AGO proteins. Moreover, AGO and PIWI proteins interact with SIMs derived from other SUMO-binding proteins. Our study indicates that Nup358-AGO interaction is important for miRNA-mediated gene silencing and identifies SIM as a new interacting motif for the AGO family of proteins. The findings also support a model wherein the coupling of miRISC with the target mRNA could occur at AL, specialized domains within the ER, and at the nuclear envelope (**Figure 2**) (Sahoo *et al*, 2017).

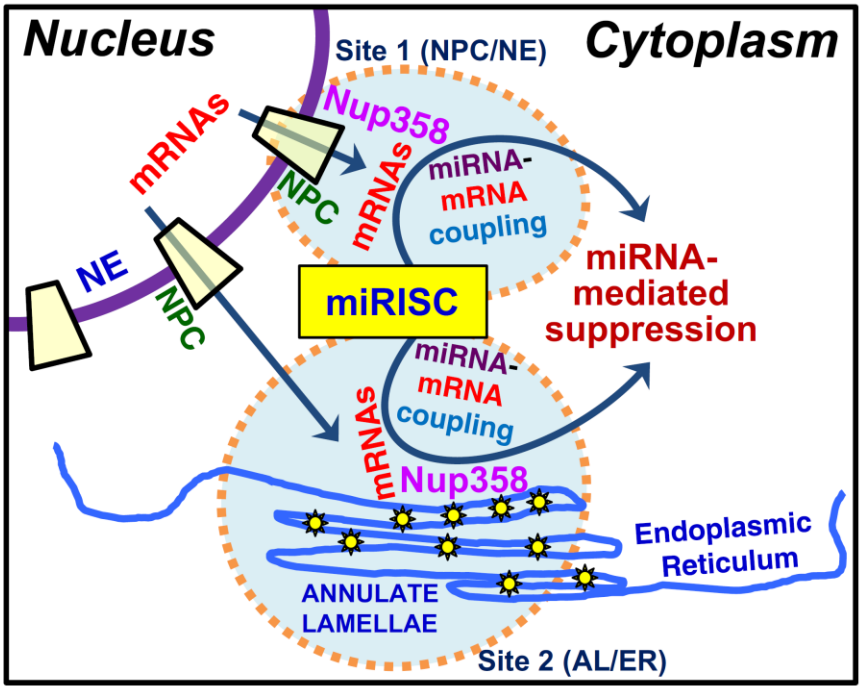

Figure 2. Model for the role of Nup58 in miRNA-mediated translational suppression. *Exported mRNAs could be sorted for miRNA-mediated suppression at two potential sites in the cytoplasm: site 1-the nuclear pore complex (NPC) as a part of nuclear envelope (NE); and/or site 2-annulate lamellae (AL) as a part of endoplasmic reticulum (ER). Nup358, a component of the NPC and AL, plays an important role in the coupling of target mRNA with miRISC (Sahoo et al, 2017).*

In another study from our lab, it is shown that P-bodies, the post-transcriptional gene regulation platforms, are localized to ERMCSs and disruption of ERMCSs leads to

disappearance of P-bodies. These results imply that both ERMCS functions are linked with P-body functions (More and Joseph, J.C.S., under revision)

Collectively, these findings highlight the interconnection between NCT, miRNA-mediated post-transcriptional gene regulation and ERMCS functions. Nup358, an AL-associated nucleoporin appears to be at the heart of this connection. In other words, Nup358 links ERMCS functions and gene expression through NCT.

Ding B & Sepehrimanesh M (2021) Nucleocytoplasmic transport: Regulatory mechanisms and the implications in neurodegeneration. *Int J Mol Sci* 22: 4165

Kalarikkal M, Saikia R, Oliveira L, Bhorkar Y, Lonare A, Varshney P, Dhamale P, Majumdar A & Joseph J (2024) Nup358 restricts ER-mitochondria connectivity by modulating mTORC2/Akt/GSK3β signalling. *EMBO Rep*: doi: 10.1038/s44319-024-00204-8

Kalarikkal M, Saikia R, Varshney P, Dhamale P, Majumdar A & Joseph J (2021) Nup358 regulates remodelling of ER-mitochondrial contact sites and autophagy. *bioRxiv*: 2021.10.01.462723

McCann C, Holohan EE, Das S, Dervan A, Larkin A, Lee JA, Rodrigues V, Parker R & Ramaswami M (2011) The ataxin-2 protein is required for microRNA function and synapse-specific long-term olfactory habituation. *Proc Natl Acad Sci U S A* 108: E655–E662

Rodríguez-Arribas M, Yakhine-Diop SMS, Pedro JMBS, Gómez-Suaga P, Gómez-Sánchez R, Martínez-Chacón G, Fuentes JM, González-Polo RA & Niso-Santano M (2017) Mitochondria-Associated Membranes (MAMs): Overview and Its Role in Parkinson's Disease. *Mol Neurobiol* 54: 6287–6303

Sahoo MR, Gaikwad S, Khuperkar D, Ashok M, Helen M, Yadav SK, Singh A, Magre I, Deshmukh P, Dhanvijay S, *et al* (2017) Nup358 binds to AGO proteins through its SUMO -interacting motifs and promotes the association of target mRNA with miRISC. *EMBO Rep* 18: 241–263

Jomon Joseph