The endoplasmic reticulum (ER) is the largest membrane-bound organelle spreading throughout the cytoplasm as a continuous membrane-enclosed network in mammalian cells. It comprises of the nuclear envelope along with a dynamic network of peripheral interconnected tubules and membrane sheets [1]. Sheets are predominant over tubules in the central area of the cell and long interconnected tubules close to the plasma membrane (PM) at the periphery [2]. The ER in mitotic cells has been observed to undergo both spatial reorganization and structural transformation of sheets towards a more fenestrated and tubular form [2,3]. ER subdomains are believed to play a crucial role in a variety of functions ranging from Ca2+ handling to protein translocation [4]. The Reticulons (Rtns) are membrane bound proteins found in all eukaryotic forms. In addition to the ER, the Rtns are known to insert to other cellular membranes including the PM and the Golgi apparatus. Rtn4B’s close neighbour Rtn4A is known to give rise to ER tubule structures in association with DP1 [5].

The aim of this project is to screen interacting partners for Rtn4B. We first narrowed down our search for interacting partners within the proteins of the Rtn family, and then broadened our scope towards other potential candidates. The screening was performed using the BiFC (Bimolecular fluorescence complementation) method [6] (Fig 2). These interacting partners will then be studied for their effects on ER structure maintenance and dynamics using a variety of imaging techniques such as immuno EM, electron tomography and serial block face scanning EM [3]. First, we showed that Rtn4B was forming dimers and/or homo-oligomers as biochemical data has suggested [7], as BiFC signal was detected between two Rtn4B-constructs (Fig. 1A). The BiFC signal (Green) in Fig. 1B shows to co-localize with the signal from immunolabelled endogenous Rtn4b (Red) suggesting that the oligomerization was not restricted to certain subdomain of the rtn4B-positive ER or rtn4B molecules. In addition, using CLEM, we narrowed down the BiFC-GFP positive signal to pin-point the morphological changes on ER upon the overexpression of locked Rtn4b dimers/oligomers at an EM level (Fig. 3). Electron tomography revealed the induction of tight network of narrow tubules that were connected to ER sheets and tubules in cells over-expressing BiFC-Rtn4b dimers/oligomers.


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Fig. 1: Huh-7 cells expressing constructs with positive BiFC signal post 24hr incubation; A. 20X image of GFP signal from dimerization of RTN4b. B. 63X image of cells expressing GFP from RTN4b dimerization & antibodies against RTN4B(in red). C&D. 63X images of GFP from RTN4b dimerization. GFP(green),Nucleus(Blue) & anti-RTN4b(Red)

Fig. 2: Overview of BiFC technique

Fig. 3: A. Images from the same region of interest at different levels of microscopy of the positively expressing BiFC cells for Rtn4b dimers. B. Micrograph showing ER morphology from overexpression of Rtn4b dimers/oligomers. C. Serial tomography generated model from overexpression of Rtn4b dimers