Functional subdomains of the endoplasmic reticulum in cultured mammalian cells

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The endoplasmic reticulum (ER) is a single continuous network of multiple functional and structural subdomains. Proper ER operation requires an intricate balance within and in-between dynamics, morphology, and functions. List of known ER-associated functions is long, however, little is known about their distribution within the network and in respect to each other. To address this, we are determining the localization of some key ER structural proteins and enzymes involved in protein folding, lipid droplet biogenesis, cholesterol biosynthesis and esterification. Careful localization of these proteins helps to better understand the morphological and functional subdomains of the ER and to relate each protein function to a particular ER compartment.

For subcellular localization studies, immuno electron microscopy (IEM) is the method of choice. Lack of specific antibodies is often circumvented by over-expression of a tagged version of the protein of interest. However, in case of the ER subdomains, mistargeting is often a major challenge, as even after a mild overexpression the protein of interest has a tendency to spread throughout the ER network. When CRISPR/Cas9 system was used to tag the protein at its chromosomal locus with sfGFP, we could pinpoint the protein to its specific location.