Cellular communication: unlocking the secrets of ER-NE junctions

Essential junctions within our cells.

Eukaryotic cells contain membrane-bound organelles, which enable them to compartmentalise biochemical and biological processes. Cells maintain a constant exchange and communication between organelles, which need to be tightly controlled to respond to environmental cues and coordinate homeostasis. A key organelle for inter-organelle communication is the endoplasmic reticulum (ER). The ER is the largest continuous membrane-bound organelle in the cell and the site of synthesis and turnover of a major fraction of lipids and membrane proteins.

The ER membrane is directly connected to the nucleus via junctions with the outer nuclear membrane of the nuclear envelope (NE) (Craig and Staehelin, 1988; West *et al.*, 2011) (Figure 1). Considering that the surface area of the NE is about 2–10 per cent compared to that of the entire ER (Griffiths *et al.*, 1984; Heinrich *et al.*, 2021), the majority of NE proteins are expected to be synthesised on the ER and subsequently transported to the NE through ER-NE junctions. The NE proteins supplied via ER-NE junctions play important roles in regulating gene expression, genome organisation, lipid metabolism, the formation of nuclear pore complexes, nuclear membrane repair, nuclear shape and positioning (Gauthier and Comaills, 2021). Hence, nuclear function and morphology depend on the proper communication via ER-NE junctions. Nevertheless, it remains poorly understood which proteins shape and stabilise ER-NE junctions, how these junctions are formed and maintained during the cell cycle, and how they regulate lipid and protein traffic.

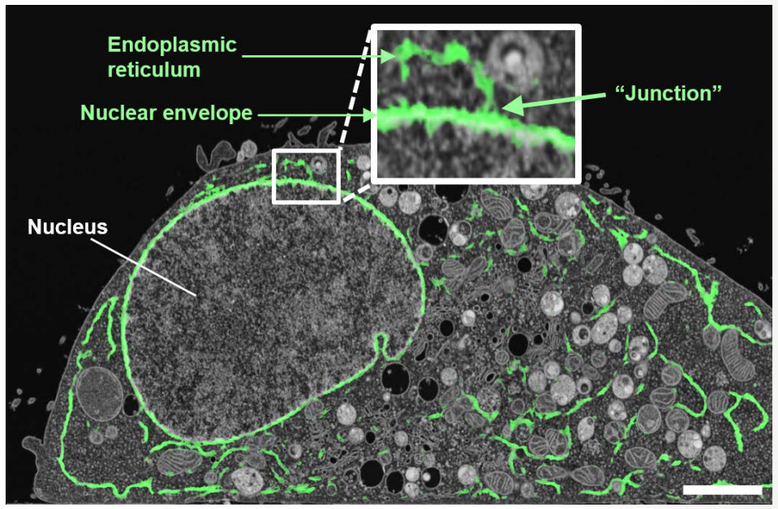

Figure 1: An electron micrograph of a HeLa cell (Hennies et al., 2020). The endoplasmic reticulum and nuclear envelope are highlighted in green (Tischer and Pepperkok, 2019). The enlarged is the junction connecting the endoplasmic reticulum and nuclear envelope. Scale bar; 2 mm.

The challenge of investigation

While ER-NE junctions were discovered more than 60 years ago (Watson, 1955), their precise structure remains obscure.

Typically, ER-NE junctions are illustrated in the same way as junctions within the ER. This view is based on electron microscopy (EM) studies carried out in yeast, and chemically-fixed mammalian and plant cells, where the morphology of ER-NE and ER-ER junctions are comparable (Watson, 1955; Whaley *et al.*, 1960; West *et al.*, 2011). However, it has been observed that ER-NE junctions in plant root tip cells are constricted (25–30 nm in diameter) when the samples are high-pressure frozen but not when chemically fixed (Craig and Staehelin 1988; Staehelin, 1997). Thus, techniques that preserve native intracellular structures to allow rigorous and quantitative analysis of the junctions in various cell types are required to understand the functional architecture of ER-NE junctions.

The conNEctoER project

We have recently systematically elucidated the ultrastructure of ER-NE junctions in human cells at defined cell-cycle stages by correlating live imaging with three-dimensional (3D) EM (focused ion beam scanning EM and electron tomography) (Bragulat-Teixidor *et al.*, 2024). The time-resolved 3D-EM observation revealed that ER-NE junctions form narrow hourglass-shaped structures (~10 nm in inner diameter), distinct from the ER junctions (Figure 2a). This structural feature of ER-NE junctions was observed in both HeLa cells and human macrophage and mouse pancreatic islet cells. By contrast, in yeast, ER-NE junctions were similar to the junctions in the ER and an hourglass-shaped morphology was not observed (West *et al.*, 2011; Bragulat-Teixidor *et al.*, 2024). These observations strongly suggest that ER-NE junctions are shaped by a functionally distinct set of proteins that have yet to be identified in mammalian cells. We have also found that when ER-NE junctions are newly built during NE assembly at late anaphase, their morphology resembles ER-ER junctions. In contrast, ER-NE junctions are constricted at early telophase, indicating that their constricted shape is rapidly formed after mitosis and maintained for the rest of the cell cycle.

Photo: Max Kropitz for Max Perutz Labs

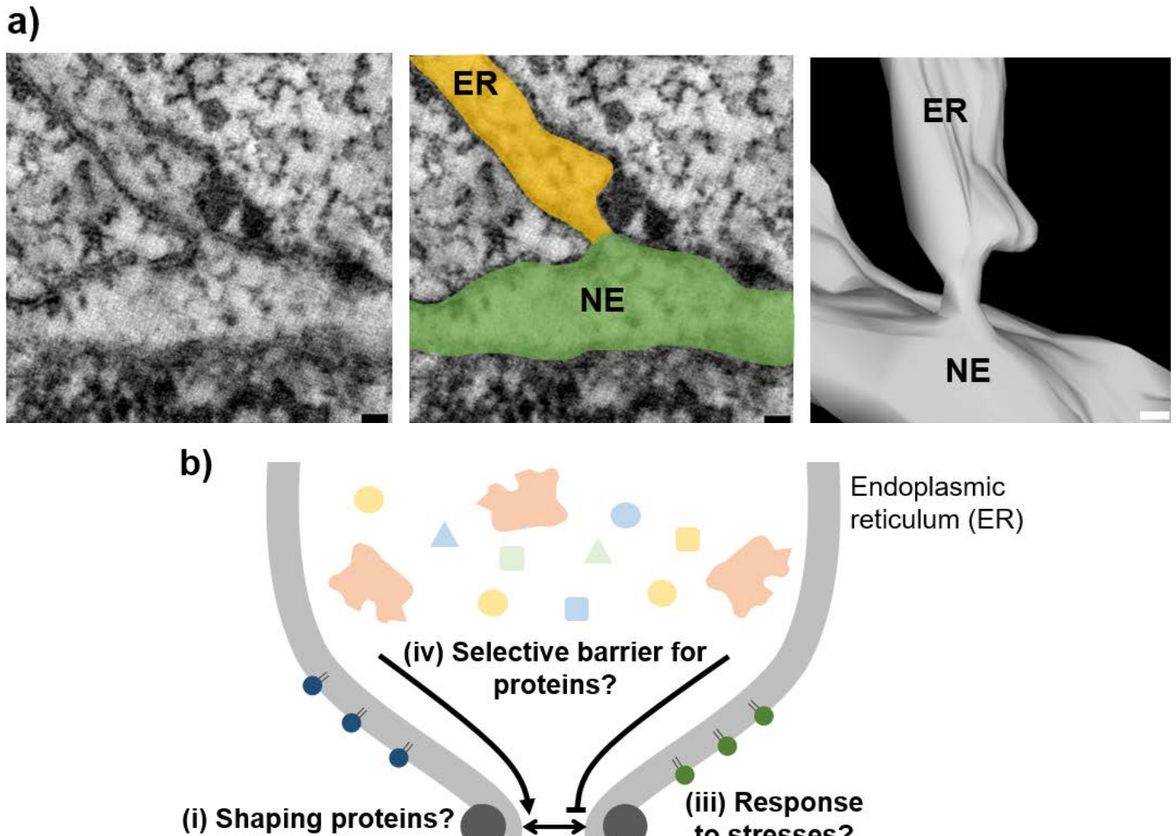

Figure 2: a) An example of the ER-NE junction visualised by electron tomography (Bragulat-Teixidor et al., 2024). Scale bars; 20 nm. b) Illustration of working hypotheses.

Based on our finding that ER-NE junctions form a narrow and constricted shape, we formulate the following hypotheses (Figure 2b):

i. the junctions are formed and maintained by a complex of specific membrane-shaping/stabilising proteins

ii. these proteins are crucial for proper nuclear growth and nuclear membrane repair

iii. the junctions change their nature in response to environmental stimuli

iv. the narrow junctions function as a barrier that restricts the ER-to-NE diffusion of large protein aggregates

v. misregulated ER-NE junctions would cause an aberrant lipid transfer and protein supply from the ER to the NE, which may lead to abnormal nuclear growth and function.

In the conNEctoER project, we aim to test the proposed hypotheses by combining time-resolved 3D EM and quantitative live imaging with molecular perturbations and ultimately generate a biophysical model for gaining a mechanistic understanding of the regulatory mechanisms of ER-NE junctions. The biophysical modelling will profit from an established collaboration with Dr Sara Merino at the University of Vienna.

Way forward

The ER-NE junctions are supposed to play a critical role as a 'supply chain' for the NE lipids and proteins that are synthesised in the ER. The NE proteins supplied via ER-NE junctions have essential roles in gene expression, nuclear organisation and nuclear pore biogenesis, as well as in differentiation, development, and disease (Gauthier and Comaills, 2021). Therefore, elucidating the molecular regulation and function of ER-NE junctions will provide a new conceptual framework that will open lines of investigation in other fields beyond ER/NE biology.

Dysregulated nuclear size, mislocalised NE proteins, and dysfunction of ER-shaping proteins have been associated with many diseases, including cancer and neurodegenerative disorders (Jevtic and Levy, 2014; Öztürk *et al.*, 2020; Rose *et al.*, 2022; Deolal *et al.*, 2024). However, few physiological processes have been linked to ER-NE junctions, possibly due to the lack of knowledge on this essential junction. The outcome of this conNEctoER project will lead to a better understanding of these associated diseases.

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PROJECT NAME

conNEctoER

PROJECT SUMMARY

The conNEctoER project aims to reveal the mechanism governing essential junctions within our cells, the junctions that supply proteins and lipids to the cell nucleus. By combining electron microscopy and live imaging, we plan to elucidate the junction formation and function in human cells, shedding light on gene expression, nuclear organisation and disease mechanisms.

PROJECT PARTNERS

The project benefits from the world-class facilities of mass spectrometry and light and electron microscopy at Max Perutz Labs and Vienna BioCentor, as well as a collaboration with Dr Sara Merino Aceituno in the Faculty of Mathematics at the University of Vienna.

PROJECT LEAD PROFILE

Shotaro Otsuka was born in Tokyo, Japan. He obtained a PhD in Biophysics at Kyoto University. In 2011, he moved to Germany to carry out postdoctoral work at the European Molecular Biology Laboratory. Since 2019, he established his own lab at the Max Perutz Labs in Vienna.

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