A genetic approach to understand nuclear envelope assembly enabled by a unique yeast

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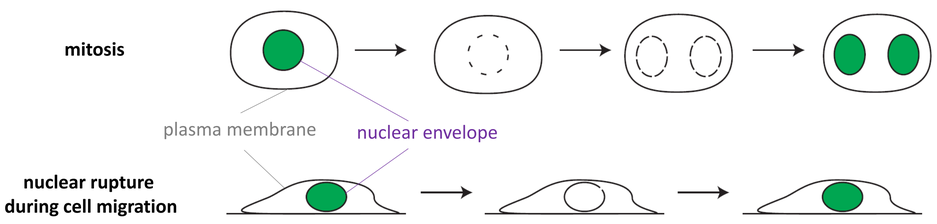
Introduction

• Why is nuclear envelope assembly important?

The nuclear envelope (NE) is the double membrane bilayer that separates the chromatin from the rest of the cell in eukaryotes. The integrity of the NE is critical to the function and stability of the genome. Defects in NE result in various diseases including cancers, myopathies, progeria, and are also frequently observed in ageing cells.

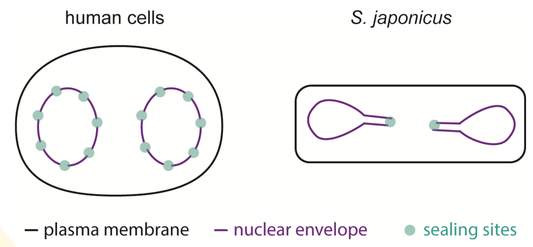
In the cell, NE integrity is being challenged frequently. The figure below shows two examples. Whenever the NE is damaged or disassembled, it needs to be repaired or reassembled. Intriguingly, despite its importance, our understanding of how NE assembles in the cell is limited.



• What do we already know about NE assembly?

In the final stage of NE assembly and repair, small holes on the NE are closed via membrane fusion. This step is called "nuclear envelope sealing". Previously, the only pathway proposed to mediate NE sealing was the ESCRT (Endosomal Sorting Complex Required for Transport) machinery. Although the existence of additional pathways has been implied, the lack of a model system for NE assembly precluded their identification.

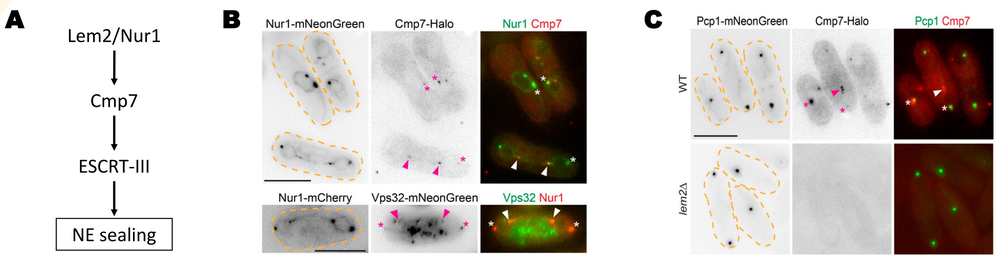
• How do we establish a genetic system for NE assembly?



We utilized a unique fission yeast called *Schizosaccharomyces japonicus*. Unlike other established yeast model systems, *S. japonicus* undergoes partial NE disassembly during mitosis and therefore requires NE sealing during reassembly.

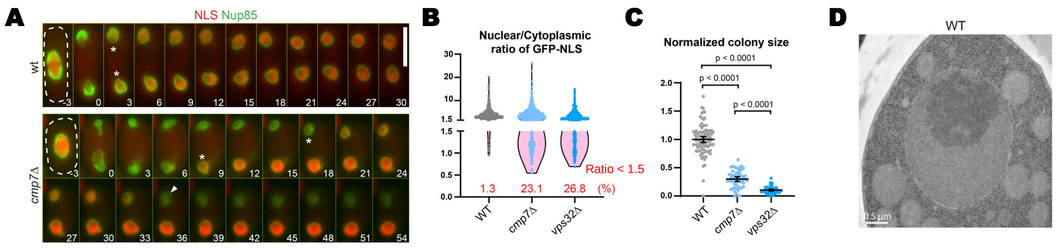
Results

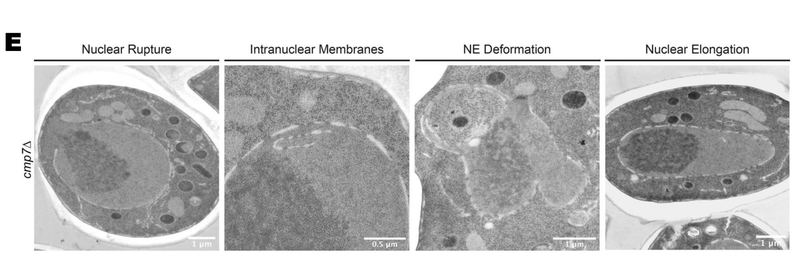
• The ESCRT pathway for NE sealing is conserved in *S. japonicus*.



(A) The canonical pathway for NE sealing. **(B)** The ESCRT adaptor Cmp7 and ESCRT-III protein Vps32 localize to the NE sealing sites (arrowheads) and the spindle pole body (asterisks). **(C)** Localization of Cmp7 depends on Lem2.

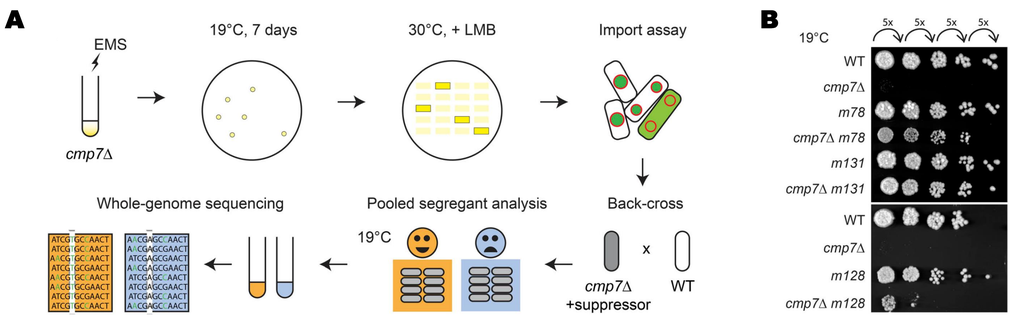
• Cmp7 is important but not essential for NE sealing.





(A) *cmp7*Δ cells exhibit delays in NE reassembly after mitosis (asterisks) and undergo nuclear rupture (arrowhead).
(B-C) Defects in NE integrity (B) and cell growth (C) in cells lacking Cmp7 or Vps32.
(D-E) Electron microscopy images of WT (D) and *cmp7*Δ (E) cells.

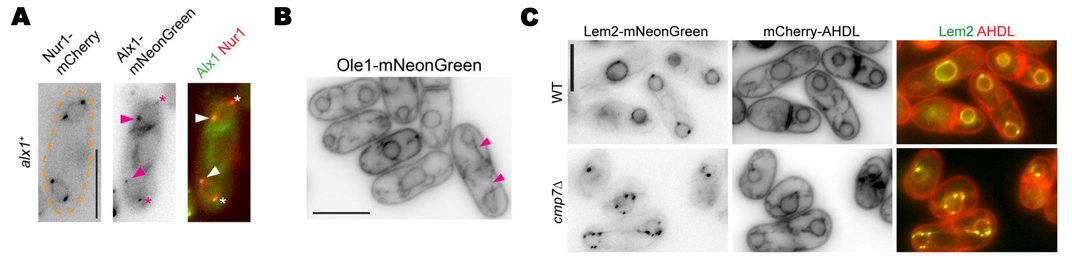
• We designed a forward genetic screen to identify factors that can promote NE assembly in the absence of Cmp7.



(A) Workflow of the screen. *cmp7*Δ cells mutagenized by ethyl methanesulfonate (EMS) were plated and selected against cold- and leptomycin- (LMB) sensitivity, two conditions that would kill *cmp7*Δ cells. Colonies that pass the selections were visually screened for improved NE integrity, and mutations were identified using genetic analysis followed by next-generation sequencing. **(B)** Validation that mutations identified in the screen were sufficient to rescue cold-sensitivity of *cmp7*Δ cells.

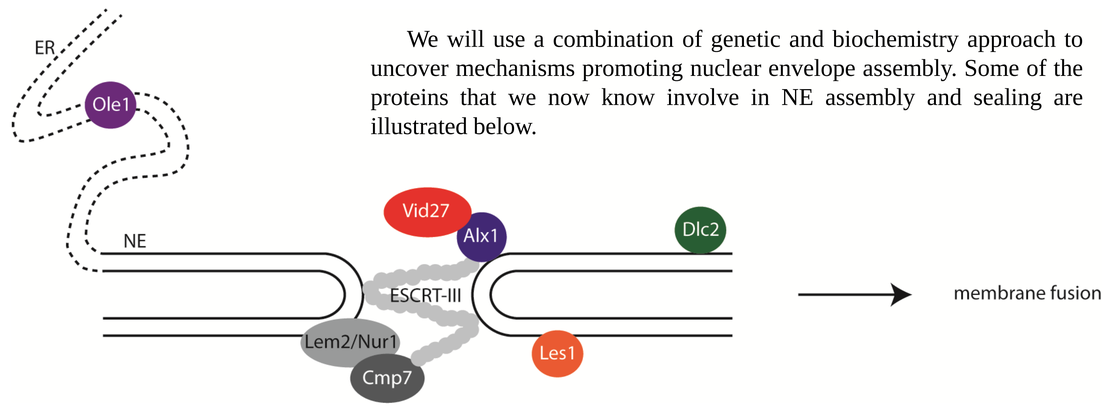
• What did the screen identify?

In summary, our forward genetic screen has resulted in the most extensive list of proteins participating in NE sealing in any organisms studied to date. These include several proteins that exclusively localize to the NE sealing sites (e.g. Alx1 in the figure below, panel **A**, asterisks), and NE and ER proteins (e.g. Ole1 in panel **B**). Intriguingly, in the absence of Cmp7, NE aggregates containing Lem2 (panel **C**) appear to further impair with NE integrity and are toxic to the cell. These abnormal structures have also been found in higher eukaryotes recently.



Future directions in Dr. I-Ju Lee's Laboratory at NTHU

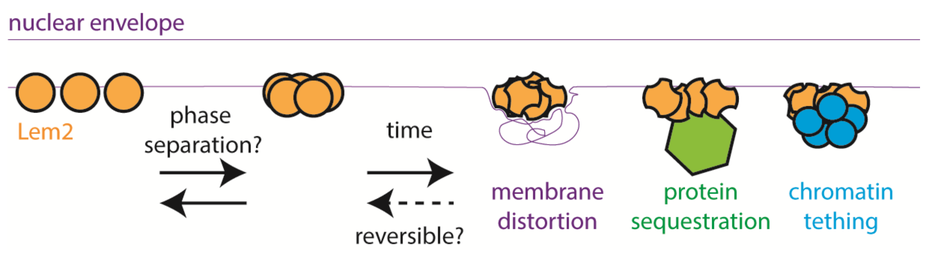
1. What are the functions of these NE assembly proteins?



We will use a combination of genetic and biochemistry approach to uncover mechanisms promoting nuclear envelope assembly. Some of the proteins that we now know involve in NE assembly and sealing are illustrated below.

2. How do the toxic NE aggregates form? Are they reversible?

Previous studies on NE aggregates provided limited temporal resolution. To introduce time as a parameter in our system, we will use degron-mediated protein depletion to induce the formation of NE aggregates. We will then determine (1) whether liquid-liquid phase separation plays a role in aggregates formation, (2) how do the aggregates impair with NE integrity and genome stability, (3) do the aggregates exhibit increasing toxicity over time, and (4) are the aggregates reversible.



3. What else may participate in NE assembly?

Next-generation sequencing
- Many mutants in the screen have not been sequenced yet.

Candidate-based approach
- Other membrane fusion proteins.
- Preliminary data suggest a role for endoplasmic reticulum shaping proteins.

Proximity labeling
- TurboID followed by mass spectrometry.

4. How conserved are NE assembly in different organisms?

Human cell lines
- Localization and function of homologs.

Fission yeast *S. pombe*
- Function of NE assembly proteins in closed mitosis.

• For more information

Lee I-J, Stokasimov E, Dempsey N, Varberg J, Jacob E, Jaspersen SL, and Pellman D. 2020. Factors promoting nuclear envelope assembly independent of the canonical ESCRT pathway. *J. Cell Biol.* 219:e201908232. PMID: 32243490

• Join us!

Please send an email to Dr. I-Ju Lee 李以如 ijuleelab@gmail.com

• Acknowledgement

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