# Nuclear envelope rupture in Drosophila D11 cells inhibits mitosis

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## Introduction

The nuclear envelope (NE) is the largest endoplasmic reticulum (ER) compartment which consists of the outer and inner membranes clamped together with nuclear pore complexes and attaches to the lamina. It is a dynamic structure that reorganizes during the cell cycle. The outer nuclear membrane normally interacts with ER membranes through local fusion (Fig. 1, a). The abnormal ER and NE membranes aggregation as well as their incorrect fusion in cells have been previously observed by many authors and usually correlate with cell pathology and the disruption of mitosis. Three types of atypical membrane interactions are described: ER-ER stacks [1,2], ER-NE (ER sticking to the NE) [3,4] and NE-NE folds sticking (Fig. 1, b, c, d, respectively).

The atypical NE-NE outer membranes fusions were previously observed when lamina proteins were overexpressed in intestinal stem cells and enterocytes of *D. melanogaster* [5]. In particular, overexpression of Lam led to extra NE membranes formation with small fragments of quadruple membranes formed by the inner nuclear membrane contacts with itself.

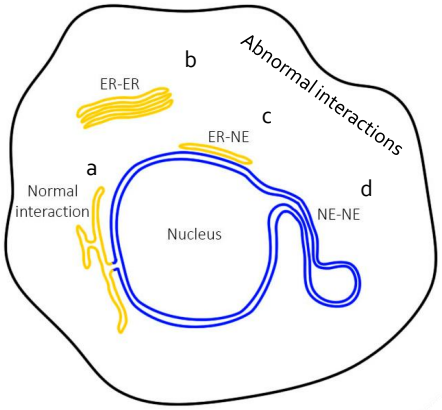


***Figure 1**. Different types of intracellular membranes contacts:* a – *typical ER-NE interaction observed in interphase cells; abnormal close interactions:* b – *ER-ER*, c – *ER-NE*, d – *NE-NE.*

## Object

ML-DmD11 (D11) cell line derived from the eye-antennal disk of *D. melanogaster*. These cells show very low mitotic index (about 3%).

## Aim

To analyze the NE structure in D11 cells by TEM and to check whether it has an abnormal organization, which could be a reason for the low mitotic index.

## Methods

- Cell culture was fixed with 2,5% glutaraldehyde, then with 1% $OsO_4$, and embedded to Epon 812 [6].
- Ultrathin sections were analyzed in TEM(JEM-1400, Japan)

## Results

TEM analysis revealed long quadruple (4-layered) membranes formed by NE folds in 40% of all examined D11 cells (Fig. 2, a-a'). These NE fragments form long protrusion into the cytoplasm suggesting the extra NE growth and show close contacts between fragments of the inner nuclear membrane.

The presence of numerous microtubules in the nucleoplasm of these cells, as well as slightly compacted chromosomes (Fig. 2, a'), may indicate that they are paused at the interphase or early prophase.

We also found loop-like structures in a few cells at the interphase stage (Fig. 2, b-b').

It was previously demonstrated that similar structures can be the result of the lamina protein Lam or the insect-specific protein Kugelkern overexpression [5]. Presumably, some lamina proteins are overexpressed in D11 cells causing extra NE membranes growth and as a consequence folds of the NE additional fragments due to the self-fusion of the inner membranes.

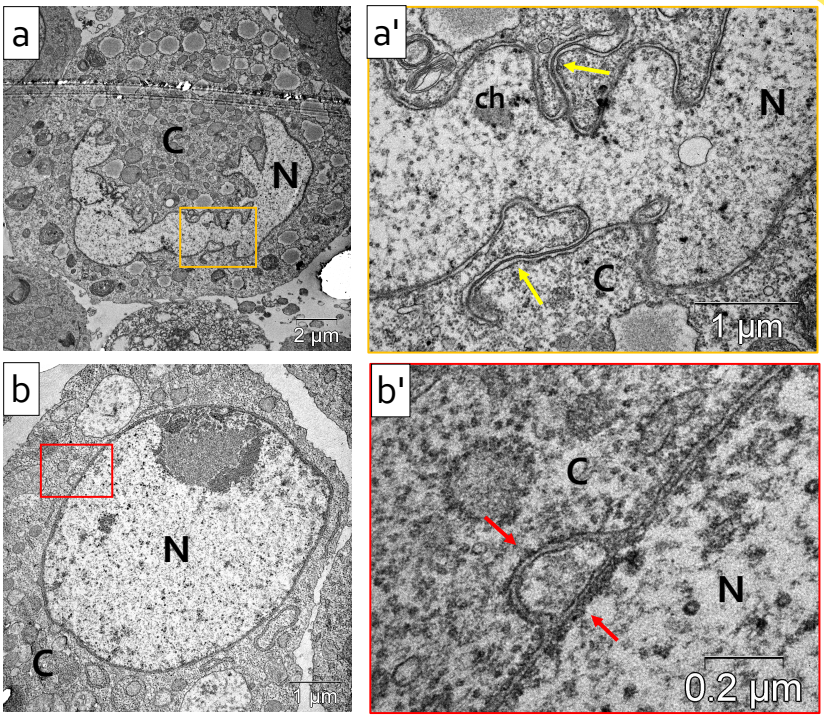


***Figure** 2: a, b – D11 cells in prophase and interphase, respectively; a', b' – magnified parts of a and b. Red arrows show a loop-like structure, yellow arrows indicate NE-NE contacts formed by excess NE growth. N – nucleus, C – cytoplasm, ch – compacted chromosome.*

## Conclusions

1. Nuclei of *D. melanogaster*'s ML-DmD11 cells form continuous protrusions with NE-NE inner membranes contacts caused by the extra NE growth (in 40% of observed cells).
2. We suggest that the low mitotic index of ML-DmD11 cells is probably caused by misshaped nuclei and NE-NE sticks as one of the possible reasons.
3. Abnormal NE formation can be the consequence of lamina proteins defective expression as was previously shown in intestinal stem cells and enterocytes of *D. melanogaster* [5].
4. To verify this hypothesis, we are going to measure the expression level of genes encoding lamina proteins in D11 cells.

References:
[1] – L. Orci et al, 2009
[2] – T. Kuijpers et al, 2013
[3] – S. Bahmanyar et al, 2014
[4] – A. Strunov et al, 2018
[5] – R. Petrovsky, G. Krohne and J. Großhans, 2018
[6] – A. Strunov et al, 2016

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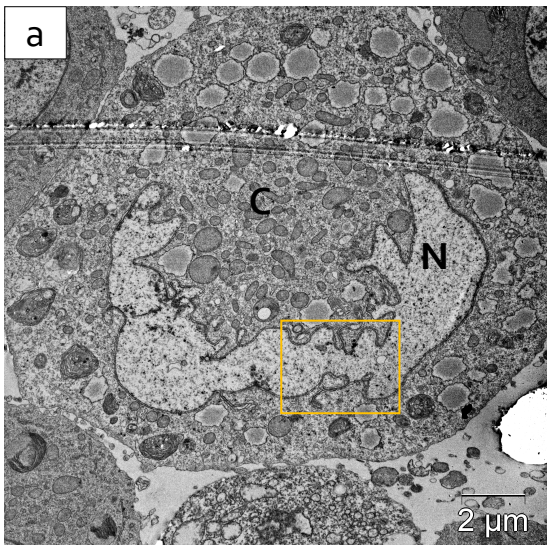
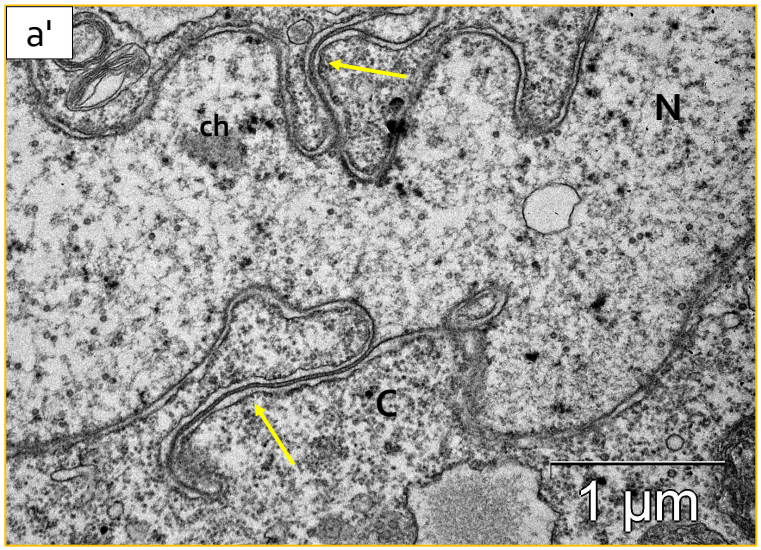
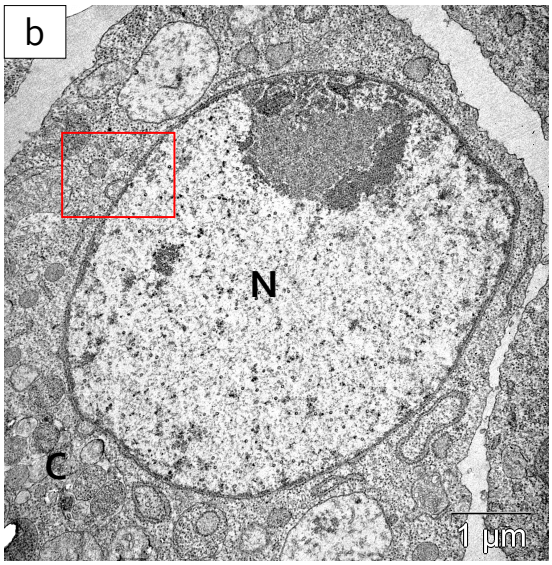
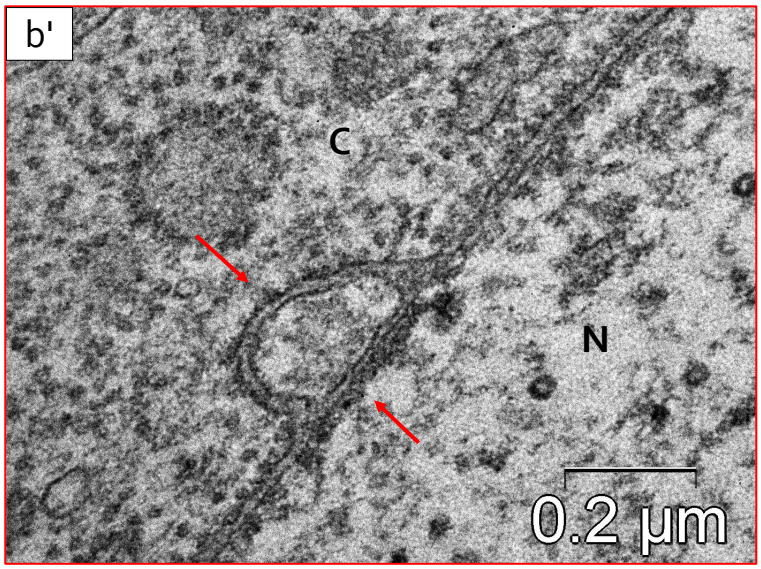
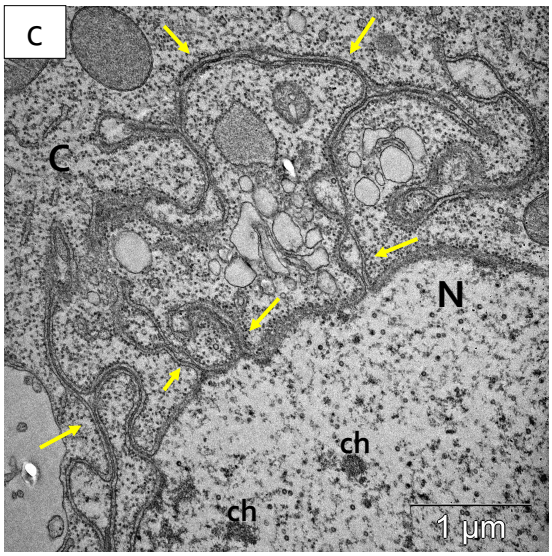
## Appendix

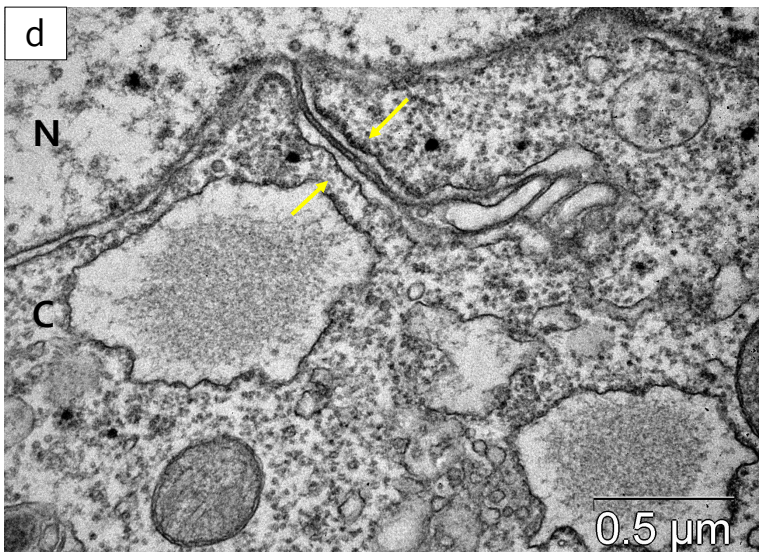



Ultrastructure of *D. melanogaster* D11 cells in prophase (a, c, d) and interphase (b); a', b' – magnified parts of a and b. c - numerous long membrane protrusions,. d - four-layered membrane at low magnification. Red arrows show a loop-like structure, yellow arrows indicate NE-NE contacts formed by excess NE growth. N – nucleus, C – cytoplasm, ch – compacted chromosome.