# Nuclear envelope breakdown induces OMMP acetylation

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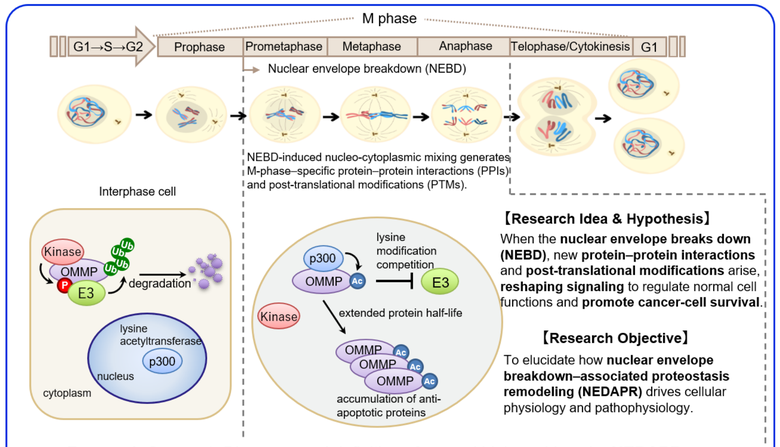
## Objectives and hypothesis

➢ **Background**

- OMMP is an outer mitochondrial membrane protein, has an anti-apoptotic function, it determines cell fate between survival and cell death and frequently overexpressed in a variety of cancers.
- p300-mediated acetylation of Lys40 (K40) stabilizes OMMP by preventing its proteasomal degradation.

➢ **Problems need to be solved**

✓ The specific cellular conditions under which nuclear-localized p300 engages with OMMP are yet to be elucidated?




**Research Concept:** Discovery and definition of a novel biology driven by **NEDAPR (nuclear envelope breakdown–associated proteostasis remodeling).**

## Results 1: Nuclear envelope breakdown drives OMMP acetylation

**Figure 1 | OMMP acetylation increases during M phase.**
**(A)**Immunofluorescence images showing acetylated OMMP (AcK–OMMP) in 293T cells over-expressing p300.
**(B)** Representative proximity ligation assay (PLA) images detecting the interaction between OMMP and p300. Nuclei are stained with DAPI.

**(C)** Quantification of %PLA signal positive cells per area in M phase.**(D)** Immunoprecipitation of OMMP followed by acetyl-lysine immunoblotting showing increased OMMP acetylation in M phase after nocodazole treatment.**(E, F)** Immunofluorescence analysis showing elevated AcK–OMMP signal in phospho-H3–positive cells and in cells with reduced Lamin B1 expression, indicating increased OMMP acetylation during M phase and nuclear envelope breakdown.**(G, H)** Quantification of AcK–OMMP intensity in phospho-H3–positive and Lamin B1–degraded cells. Data are presented as mean ± SEM (*n*=4) and analyzed by unpaired two-tailed t-test (*$p$ < 0.05; **$p$ < 0.01; ***$p$ < 0.001; ****$p$ < 0.0001).

## Results 2: OMMP expression correlates with acetylation and undergoes proteasomal degradation during M phase.

**Figure 2: OMMP expression level increase with acetylation and it subjected to proteasomal degradation during M phase.**
**(A)** Immunoprecipitation of OMMP and acetylated OMMP (AcK–OMMP) showing increased OMMP acetylation in M phase after sequential thymidine and nocodazole treatment. **(B)** Densitometric quantification of AcK–OMMP/OMMP band intensity from the western blot in **(A)**. **(C)** HeLa cells were treated with thymidine and nocodazole with or without the proteasome inhibitor MG132 and analyzed by western blotting. Data are presented as mean ± SD ($n$ = 3) and analyzed by one-way ANOVA followed by Tukey's post-hoc test (*$p$ < 0.05; **$p$ < 0.01; ***$p$ < 0.001; ****$p$ < 0.0001).

## Results 3: Acetylation of OMMP extends OMMP stabilization in M phase

**Figure 3: OMMP protein half-life is prolonged during M phase.**
**(A, B)** Immunoblot (IB) analysis of whole-cell lysates (WCLs) from HeLa and C42 cells treated with thymidine and nocodazole for 3 h, followed by addition of the protein-synthesis inhibitor cycloheximide (CHX; 100 μg mL$^{-1}$) for the indicated times.
**(C, D)** Quantification of OMMP band intensities from IB replicates in **(A)** and **(B)**. Data represent mean ± SD ($n$ = 3 independent experiments; *$p$ < 0.05; **$p$ < 0.01; ***$p$ < 0.001; ****$p$ < 0.0001, unpaired two-tailed $t$-test).
**(E)** Immunofluorescence analysis showing that OMMP signal is more stable in cells expressing AcK–OMMP.

## Results 4: OMMP acetylation confers resistance to mitotic apoptosis.

**Figure 4: OMMP exerts anti-apoptotic effects during M phase and OMMP acetylation confers resistance to apoptosis during mitosis by stabilizing OMMP expression.**
**(A)** C42 *Ommp*-KO cells re-constituted with *Ommp* WT or GFP control were treated with nocodazole and assayed for viability for the indicated time using a Cell Titer Glo assay.
**(B)** Parental C42 *Ommp* WT and C42 *Ommp* KO cells were analyzed in parallel under the same conditions.
**(C, D)** IB analysis of WCLs from C42 *Ommp*-KO cells re-constituted with *Ommp* WT or GFP control treated with nocodazole or with thymidine plus nocodazole for the indicated times. **(E)** IB analysis of WCLs from C42 cells treated with thymidine and nocodazole for the indicated times with or without A485 (a p300 inhibitor). **(F, G)** Reconstituted *Ommp*-WT, *Ommp*-KQ, and *Ommp*-KR C42 and HeLa cell lines were treated with nocodazole + $H_2O_2$ (C42) or with dimethylenastron (HeLa) for 24 h. Cell viability was measured using the Cell Titer Glo assay. **(H)** IB analysis of WCLs from C42 cells representing four genotypes: *Ommp*-KO reconstituted with *Ommp* WT, *Ommp*-KO reconstituted with GFP, parental *Ommp* WT, and *Ommp* KO were treated with paclitaxel for 24 h at the indicated concentrations. Data are presented as mean ± SD ($n$ = 4) and analyzed by one-way ANOVA followed by Tukey's post-hoc test (*$p$ < 0.05; **$p$ < 0.01; ***$p$ < 0.001; ****$p$ < 0.0001).

## Results 5: Adriamycin-induced senescence increasing OMMP acetylation and cytoplasmic translocation of p300

**Figure 5: Adriamycin-induced senescence is associated with increased OMMP acetylation and p300 cytoplasmic translocation.**
**(A)** Western blot analysis showing that Adriamycin (ADR) treatment induces cellular senescence, as indicated by increased levels of p21 and p27 in HeLa cells.
**(B)** Immunofluorescence images showing cytoplasmic translocation of p300 in senescent cells following ADR treatment.
**(C)** Immunofluorescence analysis showing elevated AcK–OMMP signal in p21-positive senescent HeLa cells treated with ADR.

## Conclusions

➢ OMMP acetylation increases during M phase, following nuclear envelope breakdown (NEBD).

➢ OMMP expression correlates with acetylation and appears to undergo proteasomal degradation during mitosis.

➢ OMMP acetylation extends its half-life there by leading to resistance to mitotic apoptosis during M phase.

➢ Adriamycin-induced senescence is associated with cytoplasmic translocation of p300 and enhanced acetylation of OMMP in senescent cells.