

# The overexpression of nuclear envelope protein Lap2 $\beta$ induces endoplasmic reticulum reorganisation via membrane stacking

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## Summary

Some nuclear envelope proteins are localised to both the nuclear envelope and the endoplasmic reticulum; therefore, it seems plausible that even small amounts of these proteins can influence the organisation of the endoplasmic reticulum. A simple method to study the possible effects of nuclear envelope proteins on endoplasmic reticulum organisation is to analyze nuclear envelope protein overexpression. Here, we demonstrate that Lap2 $\beta$  overexpression can induce the formation of cytoplasmic vesicular structures derived from endoplasmic reticulum membranes. Correlative light and electron microscopy demonstrated that these vesicular structures were composed of a series of closely apposed membranes that were frequently arranged in a circular fashion. Although stacked endoplasmic reticulum cisternae were highly ordered, Lap2 $\beta$  could readily diffuse into and

out of these structures into the surrounding reticulum. It appears that low-affinity interactions between cytoplasmic domains of Lap2 $\beta$  can reorganise reticular endoplasmic reticulum into stacked cisternae. Although the effect of one protein may be insignificant at low concentrations, the cumulative effect of many non-specialised proteins may be significant.

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## Introduction

Eukaryotic cells are capable of adjusting the size, molecular composition and architecture of their organelles. Various mechanisms for the determination of organelle shape have been proposed (Voeltz and Prinz, 2007). Although the general organisation (macroscopic order) of different cellular organelles is relatively conservative, morphological details of any organelle (e.g. microscopic order) vary even between two neighbouring cells. The biological significance of this variability in morphological organisation is that it allows cells to adapt to environmental changes.

The highly dynamic and interconnected network that constitutes the endoplasmic reticulum (ER) is a classic example of an organelle with a high degree of morphological plasticity. The size and the structure of this compartment vary enormously between different cells (Friedman and Voeltz, 2011). Changes in the ER organisation can occur quickly in response to external cues; a well-known example of this is the rapid growth of smooth ER domains following phenobarbital-induced synthesis of the drug-metabolising enzyme system (Orrenius and Ericsson, 1966).

A striking example of ER reorganisation is the conversion of reticular ER into sheets of smooth ER that are tightly stacked into arrays. Such ER modifications have been reported to follow the overexpression of resident ER transmembrane proteins (Vergères

et al., 1993; Takei et al., 1994; Ohkuma et al., 1995; Gong et al., 1996; Yamamoto et al., 1996; Profant et al., 1999; Sandig et al., 1999; Snapp et al., 2003; Korkhov and Zuber, 2009). Here, we have described that a similar ER reorganisation can be induced by overexpression of the nuclear envelope protein Lap2 $\beta$ . Although stacked ER cisternae were highly ordered, Lap2 $\beta$  could diffuse readily into and out of these structures. It appears that low-affinity interactions between cytoplasmic domains of Lap2 $\beta$  can induce the reorganisation of reticular ER into stacked cisternae.

## Materials and Methods

HeLa cells were grown in Dulbecco's modified Eagle's medium supplemented with *L*-glutamine, 10% foetal calf serum (HyClone), and antibiotic/antimycotic solution (Sigma). The Lap2 $\beta$ -EGFP plasmid (Beaudouin et al., 2002) was used for transfection. The Lap2 $\beta$ -FLAG plasmid was constructed in two steps. (1) The region encoding Lap2 $\beta$  was amplified by the polymerase chain reaction with the Lap2 $\beta$  upstream primer (5'-TATCGGTACCATGCCGGAGTTCCTAGAGGACCCT-3') and the Lap2 $\beta$  downstream primer (5'-TATCGGATCCTGATTTCTTCAGTTGGATATTTAGTATCTTGAAGA-3'). The resulting PCR product was digested with BamHI and KpnI, gel purified, and cloned into the pEGFP-N1 vector (Clontech). (2) To obtain the Lap2 $\beta$ -FLAG plasmid, the oligonucleotides (5'-CTAGATGGACTACAAGGATGACGACGACAAGGGTAC-3' and 5'-CCTTGTGTCGTCATCCTGTAGTCCAT-3') were annealed and ligated into a plasmid digested with NheI and KpnI. Cell transfection was performed with the TurboFect reagent (Fermentas) according to the manufacturer's instructions.

Immunocytochemistry was carried out as previously described (Volkova et al., 2011). Sample preparations were observed with an Axiovert 200M microscope (Carl Zeiss) equipped with an ORCAII-ERG2 cooled CCD-camera (Hamamatsu).

Deconvolution was achieved by image processing with Axiovision 3.1 software (Carl Zeiss).

Correlative light and electron microscopy were performed as described elsewhere (Volkova et al., 2011). Briefly, the cells were grown on circular photo-etched coverslips (Electron Microscopy Sciences) and fixed in 4% glutaraldehyde (Pelco International) in 0.1 M Sorensen's phosphate buffer. Cells expressing the protein of interest were identified and photographed with a fluorescence microscope. The cells were embedded in Epon (Fluka), and, following Epon polymerisation, the cells of interest were selected under a phase-contrast microscope and sliced into ultrathin sections. The sections were then stained with lead citrate and examined using an LEO912 AB OMEGA electron microscope (Carl Zeiss).

For fluorescence recovery after photobleaching (FRAP) analysis, cells were grown on coverslips in 35-mm dishes. For FRAP experiments, four single scans were acquired and followed by a single bleach pulse. Laser power was decreased to 1.5% of the maximum intensity for all imaging. FRAP recovery curves were generated from background-subtracted images. The relative fluorescence intensity (RFI) was calculated as follows:  $RFI = T_0 I_t / T_t I_0$ , where  $T_0$  was total cellular intensity during prebleach,  $T_t$  was the total cellular intensity at timepoint  $t$ ,  $I_0$  was the average intensity in the region of interest during prebleach, and  $I_t$  was the average intensity in the region of interest at timepoint  $t$ .

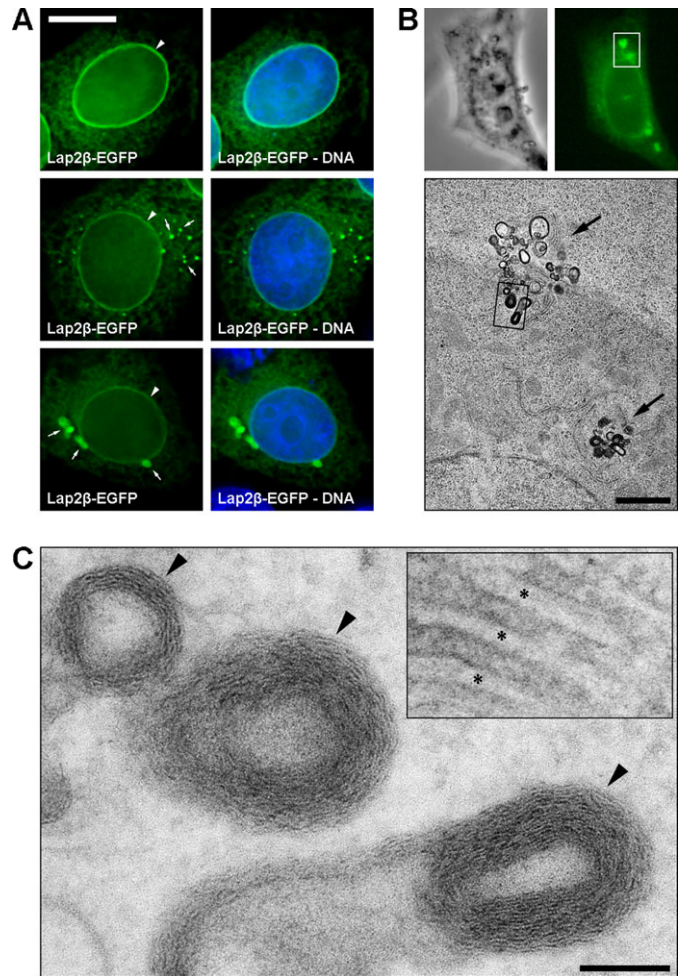
## Results

Cells with various levels of protein expression and different nuclear structures were observed in the cell population after the transient transfection of HeLa cells with the plasmid encoding Lap2 $\beta$ -EGFP. The protein expression level was approximately determined visually or estimated by the exposure time required for photographing. At low levels of Lap2 $\beta$ -EGFP expression, the protein was present in the nuclear envelope and in the ER appeared as a network of branching tubules, which is common for ER in cultured cells. Lap2 $\beta$ -EGFP was distributed uniformly throughout this system (Fig. 1A, top panels). Cells overexpressing Lap2 $\beta$ -EGFP contained cytoplasmic objects that fluoresced brightly (Fig. 1A, middle and bottom panels). The larger objects were round or oval vesicular structures of varying size (Fig. 1A, bottom panels). The preferential accumulation of overexpressed Lap2 $\beta$  in the ER rather than in the nuclear envelope may be due to the deficiency of binding sites in the nuclear envelope. In this situation, the redundant molecules are distributed also in the membranes which are bound with nuclear envelope membranes, i.e. in the ER membranes.

Correlative light and electron microscopy demonstrated that these vesicular structures were composed of series of closely apposed membranes that were mostly arranged in a circular fashion (Fig. 1B,C). Importantly, the membrane stacks were extremely dense; this was especially obvious compared to the sheets of unchanged ER localised near the multilamellar structures (Fig. 1C, insert).

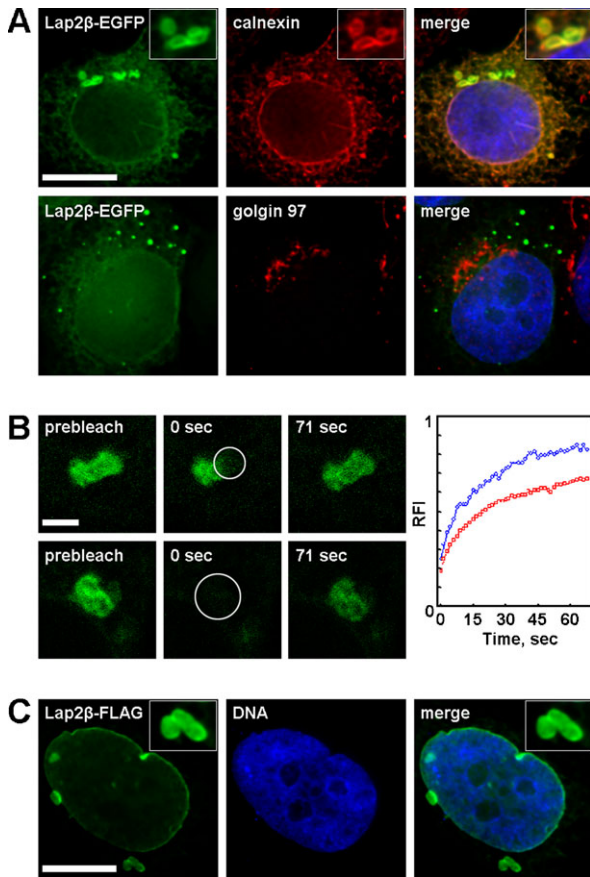
Immunofluorescent staining of cells overexpressing Lap2 $\beta$  revealed that cytoplasmic structures included the resident ER protein, calnexin (Fig. 2A, top panels). In contrast, cells labelled with anti-golgin 97, a Golgi marker protein, contained no colocalised structures (Fig. 2A, bottom panels).

One model of organised smooth ER biogenesis is that the cytoplasmic domains of overexpressed proteins on opposing membranes bind tightly to each other and fasten together the opposing membranes into stacked structures (Takei et al., 1994; Gong et al., 1996; Yamamoto et al., 1996). Such fastening should result in significantly restricted lateral mobility of membrane proteins. To test this assumption, fluorescence recovery after photobleaching (FRAP) was performed. In the first experiment, we bleached half of the Lap2 $\beta$ -containing vesicles. Fluorescence recovered rapidly into the bleached areas; this is shown in the fluorescent images and quantified in a plot of fluorescence



**Fig. 1. Structural organisation of Lap2 $\beta$ -EGFP-containing complexes in HeLa cells.** (A) Morphological organisation of Lap2 $\beta$ -EGFP-expressing cells. Green, Lap2 $\beta$ -EGFP; blue, DNA. When expressed at low levels, Lap2 $\beta$ -EGFP was localised in the nuclear envelope (arrowheads) and in an ER network (top panels). In cells expressing higher levels of Lap2 $\beta$ -EGFP, bright fluorescent structures (arrows) were observed in the cytoplasm (middle and bottom panels). The brightness of images is the same in both cases due to different exposures used for photographing. (B) Correlative light and electron microscopy of cells expressing high levels of Lap2 $\beta$ -EGFP. Phase contrast and fluorescence microscopic examination (top panels), and the ultrastructural organisation of cropped in the top panel region (bottom panel). Putative Lap2 $\beta$ -EGFP-containing complexes are indicated by large arrows. (C) The high magnification of cropped in B (bottom panel) region. The multilamellar structures are indicated by large arrowheads. Insert: the unmodified ER cisternae (luminal spaces are indicated by asterisks). Scale bars: 10  $\mu$ m (A), 1  $\mu$ m (B) and 0.1  $\mu$ m (C).

recovery (Fig. 2B, top panels). These results indicated that Lap2 $\beta$ -EGFP molecules were not immobilised within the induced structures. To ascertain whether Lap2 $\beta$ -EGFP was capable of moving into and out of induced structures, we bleached whole complexes and monitored their recovery dynamic. In the majority of cases, fluorescence recovered quickly, indicating that Lap2 $\beta$ -EGFP readily diffused into ER complexes from surrounding membranes (Fig. 2B, bottom panels). In 2 cells from 21 cells studied, the fluorescent signal did not recover; it seems that in these cases, such complexes were not connected to the surrounding ER network (data not shown).



**Fig. 2. Localisation and dynamics of Lap2 $\beta$ -EGFP.** (A) Lap2 $\beta$ -EGFP is colocalised with calnexin, a resident ER protein (top panels), but is not colocalised with golgin 97, a Golgi marker protein (bottom panels). Green, Lap2 $\beta$ -EGFP; red, calnexin or golgin 97; blue, DNA. (B) Lap2 $\beta$ -EGFP is highly mobile within the vesicular structures (top panels and blue line in graph) and is exchanged between these structures and a cytoplasmic ER network (bottom panels and red line in graph). (C) Localisation of Lap2 $\beta$ -FLAG in cells with high level of expression. The FLAG epitope was detected with specific antibodies (green), DNA with DAPI (blue). Scale bars: 10  $\mu$ m (A,C), 3  $\mu$ m (B).

It is important to note that GFP and GFP-like proteins tend to oligomerise (Chudakov et al., 2010). A previous study has shown that GFP-tagged transmembrane proteins, which normally do not induce organised smooth ER, can induce complex formation through GFP dimerisation (Snapp et al., 2003). To ascertain whether Lap2 $\beta$ -EGFP-containing complexes were induced by Lap2 $\beta$  itself or by the EGFP tag on the overexpressed protein, we examined the overexpression of Lap2 $\beta$  fused to a short tag (FLAG) containing mainly negatively charged amino acids. Dimerisation of the FLAG tag is unlikely to occur, in contrast to some GFP variants. However, Lap2 $\beta$ -FLAG induced ER complexes which were morphologically similar to those induced by Lap2 $\beta$ -EGFP (Fig. 2C).

## Discussion

Some nucleoporins and nuclear envelope proteins are not localised only to their specific structural domains (nuclear pore complexes or the inner membrane of the nuclear envelope, respectively), but small amounts of these proteins are also easily detectable in the cytoplasm (e.g. some nucleoporins (Rabut et al., 2004); LBR (Ma et al., 2007); emerin (Vaughan et al., 2001)). To

date, the presence of the aforementioned proteins in the ER has not been considered to be of biological significance. However, the robust and highly specific effects of overexpression suggest that these effects account for only the utmost realisation of morphogenetic activity. It appears that the effects of low concentrations of these proteins on the ER are similarly influenced. The weak influence of these effects did not lead to significant modification of ER morphology; however, there are many such proteins in the ER and the observable shape of the ER is partially influenced by these non-ER proteins.

Previously, we described that overexpression of lamins led to excess production of the nuclear envelope (Volkova et al., 2011). The overexpression of the nucleoporins, pom121 and ndc1, induced the modification of the ER and the formation of cytoplasmic structures consisting of ER tubules (Volkova et al., 2011). Also, it was shown that overexpression of lamin B receptor (LBR) causes membrane overproduction, inducing nuclear envelope invagination and membrane stack formation (Ma et al., 2007).

The nuclear envelope protein Lap2 $\beta$  can induce the formation of regular arrays of stacked ER membranes. The similar structures were induced by overexpression of cytochrome b(5) (Snapp et al., 2003), but the ultrastructural organisation of stacked membranes in the case of Lap2 $\beta$  was slightly different from that previously described for cytochrome b(5). The membranes inside Lap2 $\beta$ -containing structures were densely packed, and it was impossible to identify cytoplasmic or luminal space between them. In both cases, overexpression induced the formation of highly organised macroscopic complexes, and the proteins that induced such ER modifications were highly mobile and freely diffused inside and between structures and the surrounding ER.

Interestingly, the overexpression of the nucleoporins, pom121 and ndc1, led to the formation of tubules, indicating that these proteins function in a manner similar to the proteins of the reticulum and DP1/Yop1p families – specialised proteins that are necessary for membrane bending in ER tubules (Voeltz et al., 2006; Shnyrova et al., 2008). Overexpression of Lap2 $\beta$  led to ER membrane stacking; therefore, it is tempting to speculate that nuclear envelope proteins and nucleoporins contribute to ER formation, either by the tethering of opposing membranes or through membrane modification.

In conclusion, the experiments described here allow us to propose that small amounts of nuclear envelope proteins can influence the morphological organisation of cellular structures. The detailed mechanisms of the influence of individual proteins may be difficult to determine, but the cumulative effects of many of these proteins may influence ER organisation.

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## Competing Interests

The authors have no competing interests to declare.

## References

Beaudouin, J., Gerlich, D., Daigle, N., Eils, R. and Ellenberg, J. (2002). Nuclear envelope breakdown proceeds by microtubule-induced tearing of the lamina. *Cell* 108, 83-96.

- Chudakov, D. M., Matz, M. V., Lukyanov, S. and Lukyanov, K. A.** (2010). Fluorescent proteins and their applications in imaging living cells and tissues. *Physiol. Rev.* **90**, 1103-1163.
- Friedman, J. R. and Voeltz, G. K.** (2011). The ER in 3D: a multifunctional dynamic membrane network. *Trends Cell Biol.* **21**, 709-717.
- Gong, F. C., Giddings, T. H., Meehl, J. B., Staehelin, L. A. and Galbraith, D. W.** (1996). Z-membranes: artificial organelles for overexpressing recombinant integral membrane proteins. *Proc. Natl. Acad. Sci. USA* **93**, 2219-2223.
- Korkhov, V. M. and Zuber, B.** (2009). Direct observation of molecular arrays in the organized smooth endoplasmic reticulum. *BMC Cell Biol.* **10**, 59.
- Ma, Y., Cai, S., Lv, Q., Jiang, Q., Zhang, Q., Sodmergen, Zhai, Z. and Zhang, C.** (2007). Lamin B receptor plays a role in stimulating nuclear envelope production and targeting membrane vesicles to chromatin during nuclear envelope assembly through direct interaction with importin beta. *J. Cell Sci.* **120**, 520-530.
- Ohkuma, M., Park, S. M., Zimmer, T., Menzel, R., Vogel, F., Schunck, W. H., Ohta, A. and Takagi, M.** (1995). Proliferation of intracellular membrane structures upon homologous overproduction of cytochrome P-450 in *Candida maltosa*. *Biochim. Biophys. Acta* **1236**, 163-169.
- Orrenius, S. and Ericsson, J. L.** (1966). Enzyme-membrane relationship in phenobarbital induction of synthesis of drug-metabolizing enzyme system and proliferation of endoplasmic membranes. *J. Cell Biol.* **28**, 181-198.
- Profant, D. A., Roberts, C. J., Koning, A. J. and Wright, R. L.** (1999). The role of the 3-hydroxy 3-methylglutaryl coenzyme A reductase cytosolic domain in karmellae biogenesis. *Mol. Biol. Cell* **10**, 3409-3423.
- Rabut, G., Doye, V. and Ellenberg, J.** (2004). Mapping the dynamic organization of the nuclear pore complex inside single living cells. *Nat. Cell Biol.* **6**, 1114-1121.
- Sandig, G., Kärger, E., Menzel, R., Vogel, F., Zimmer, T. and Schunck, W. H.** (1999). Regulation of endoplasmic reticulum biogenesis in response to cytochrome P450 overproduction. *Drug Metab. Rev.* **31**, 393-410.
- Shnyrova, A., Frolov, V. A. and Zimmerberg, J.** (2008). ER biogenesis: self-assembly of tubular topology by protein hairpins. *Curr. Biol.* **18**, R474-R476.
- Snapp, E. L., Hegde, R. S., Francolini, M., Lombardo, F., Colombo, S., Pedrazzini, E., Borgese, N. and Lippincott-Schwartz, J.** (2003). Formation of stacked ER cisternae by low affinity protein interactions. *J. Cell Biol.* **163**, 257-269.
- Takei, K., Mignery, G. A., Mugnaini, E., Südhof, T. C. and De Camilli, P.** (1994). Inositol 1,4,5-trisphosphate receptor causes formation of ER cisternal stacks in transfected fibroblasts and in cerebellar Purkinje cells. *Neuron* **12**, 327-342.
- Vaughan, O. A., Alvarez-Reyes, M., Bridger, J. M., Broers, J. L. V., Ramaekers, F. C. S., Wehnert, M., Morris, G. E., Whitfield, W. G. F. and Hutchison, C. J.** (2001). Both emerin and lamin C depend on lamin A for localization at the nuclear envelope. *J. Cell Sci.* **114**, 2577-2590.
- Vergères, G., Yen, T. S., Aggeler, J., Lausier, J. and Waskell, L.** (1993). A model system for studying membrane biogenesis. Overexpression of cytochrome b5 in yeast results in marked proliferation of the intracellular membrane. *J. Cell Sci.* **106**, 249-259.
- Voeltz, G. K. and Prinz, W. A.** (2007). Sheets, ribbons and tubules - how organelles get their shape. *Nat. Rev. Mol. Cell Biol.* **8**, 258-264.
- Voeltz, G. K., Prinz, W. A., Shibata, Y., Rist, J. M. and Rapoport, T. A.** (2006). A class of membrane proteins shaping the tubular endoplasmic reticulum. *Cell* **124**, 573-586.
- Volkova, E. G., Kurchashova, S. Y., Polyakov, V. Y. and Sheval, E. V.** (2011). Self-organization of cellular structures induced by the overexpression of nuclear envelope proteins: a correlative light and electron microscopy study. *J. Electron Microsc. (Tokyo)* **60**, 57-71.
- Yamamoto, A., Masaki, R. and Tashiro, Y.** (1996). Formation of crystalloid endoplasmic reticulum in COS cells upon overexpression of microsomal aldehyde dehydrogenase by cDNA transfection. *J. Cell Sci.* **109**, 1727-1738.