ReepA is Required for Endoplasmic Reticulum Clearance from Chromosomes but not Endoplasmic Reticulum Partitioning to Spindle Poles in Dividing *Drosophila* Cells

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BACKGROUND

Endoplasmic Reticulum (ER) is the the largest membrane-bound organelle in the cell. The ER regulates lipid and protein synthesis and transport, calcium metabolism and protein stress response[1,2]. ER cannot be formed *de novo* and thus must be inhered during cell division[3]. During cell division ER and microtubules (MT) undergo dramatic reorganization (Figure 1).

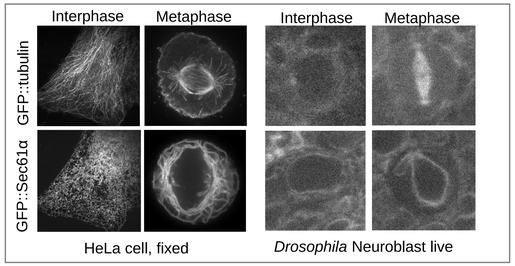


Figure 1. Changes in ER and microtubule morphology during cell division

The molecular mechanisms and components of ER-MT association are still unknown and it's unclear if ER-MT is required for proper execution of cell division or to ensure functional ER partitioning to progeny cells.

Our goal is to uncover the mechanisms and molecules that ensure proper ER partitioning during cell division in live, intact tissue

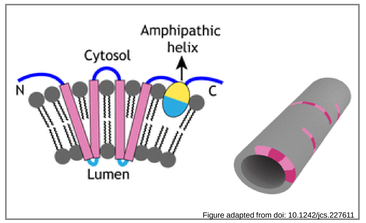


Figure 2. Structure of REEPs in the ER membrane. Membrane is in gray, shape bending components are in pink.

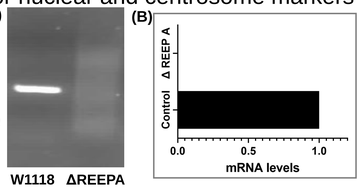
REEPs (Receptor Expression Enhancing Proteins) is a group of proteins that maintain ER structure by introducing hairpin motifs to generate curvature[4,5]. (Figure 2). Recently it has been shown that REEP3 & 4 play a role in ER morphology during cell division in mammalian cultured cells by organizing ER around spindle poles and keeping ER away from chromosomes during metaphase[5,6].

We investigated whether REEP has similar roles in organizing ER morphology in live intact tissues in *Drosophila*

METHODS

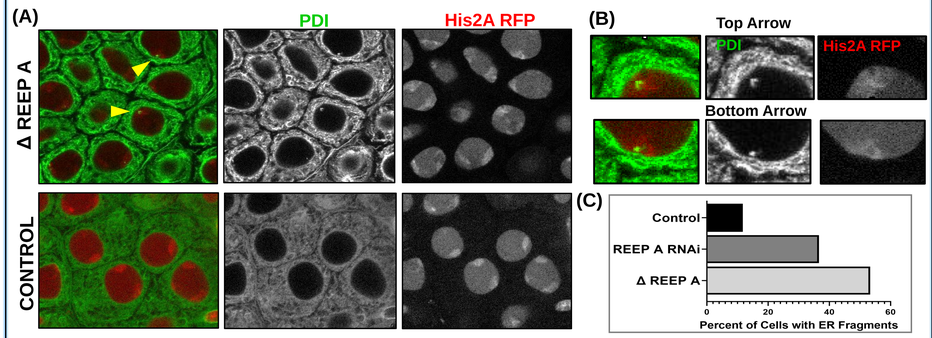
We have developed a method that allows us to image various ER reporter proteins in conjunction with either tubulin or nuclear and centrosome markers in a number of *Drosophila* tissues[7].

We have made a fly with REEP A deletion (A). The homozygous ΔREEPA fly is fully viable and fertile despite showing absence of REEP A mRNA (B)



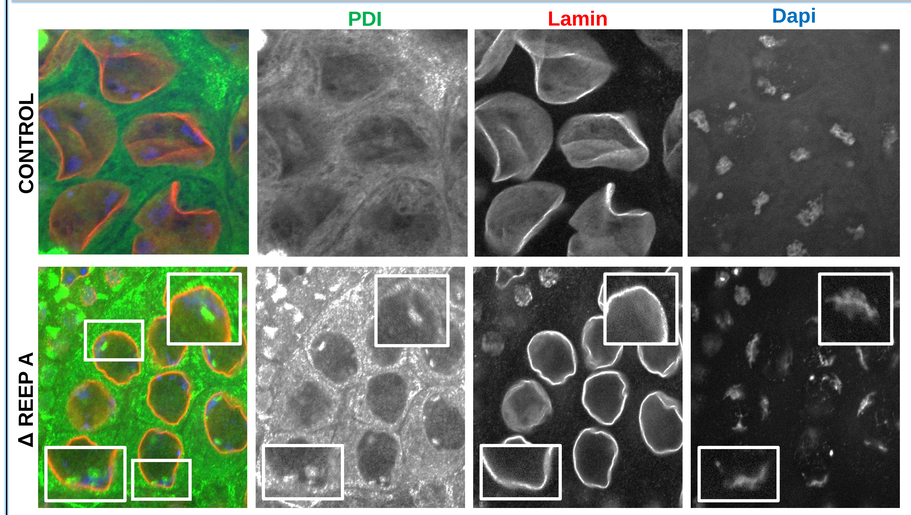
RESULTS & CONCLUSIONS

ER Fragments are Present in the Nucleus of REEP A Depleted Spermatocytes



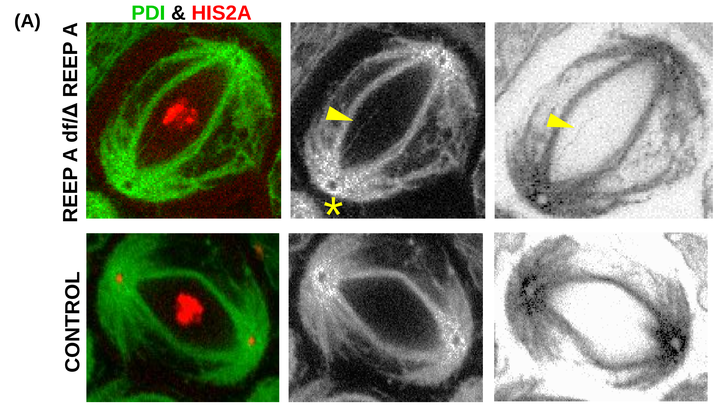
(A) Interphase spermatocytes with depleted REEP A contain ER fragments in the nucleus (yellow arrows). (B) Magnified inset of interest shows that ER fragments are near chromosomes. (C) Quantification of number of cells with ER fragments in the nucleus.

Lamin Morphology and Localization is Not Affected in REEP A Depleted Cells



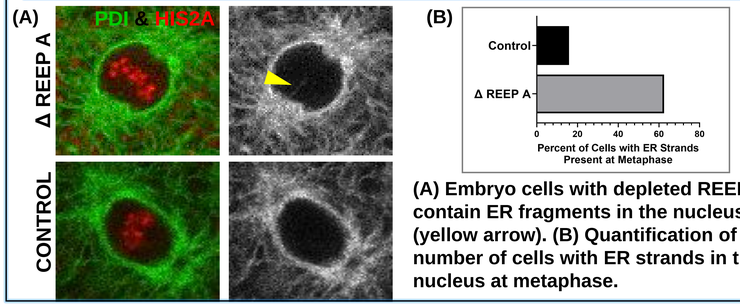
LaminA morphology appears unaltered in spermatocytes depleted of REEP A. The ER fragments are next to chromosomes as indicated by dapi staining (magnified insets). Similar results were obtained for Lamin C (data not shown)

Depleting REEP A Causes an Increase in ER Strands in the Spindle during Metaphase



(A) REEP A depleted spermatocytes display ER strands (yellow arrows) in the spindle. ER morphology at the centrosomes (yellow asterisk) is unaffected. (B) Number of spermatocytes with ER strands during metaphase is increased.

Similar Phenotype is Observed in the Drosophila Embryo



(A) Embryo cells with depleted REEP A contain ER fragments in the nucleus (yellow arrow). (B) Quantification of number of cells with ER strands in the nucleus at metaphase.

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