

RETICULON VARIATION AND CORRELATION TO NUCLEAR SIZE REGULATION

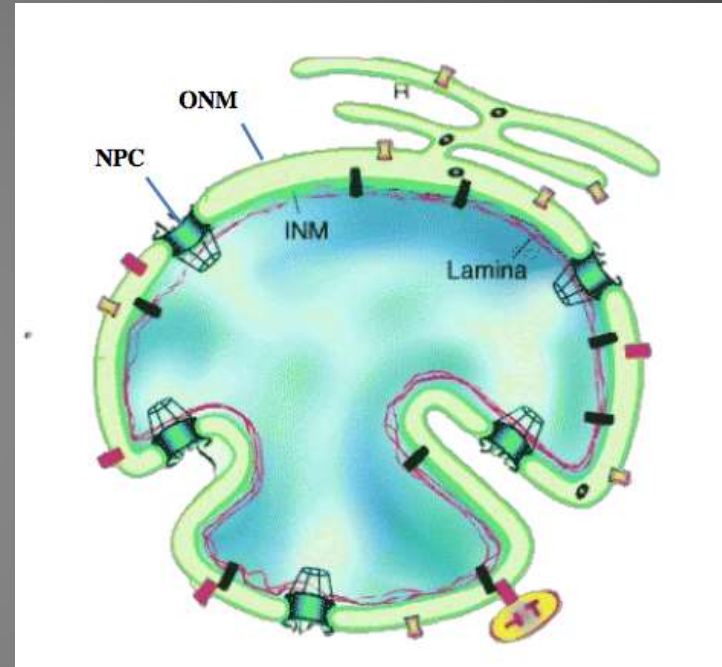
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University of Wyoming

BACKGROUND:



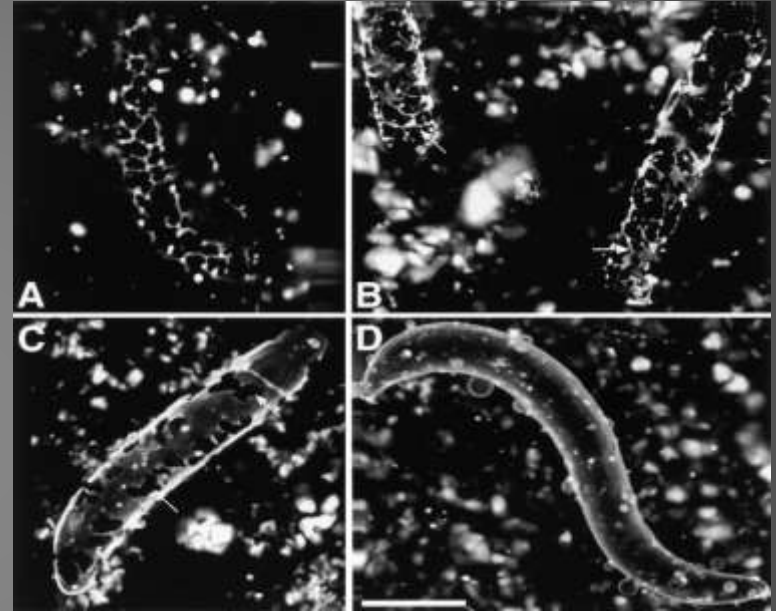
The nuclear envelope: form and reformation

Amy J Prunuske, Katharine S Ullman

MODEL ORGANISM: *XENOPUS LAEVIS*



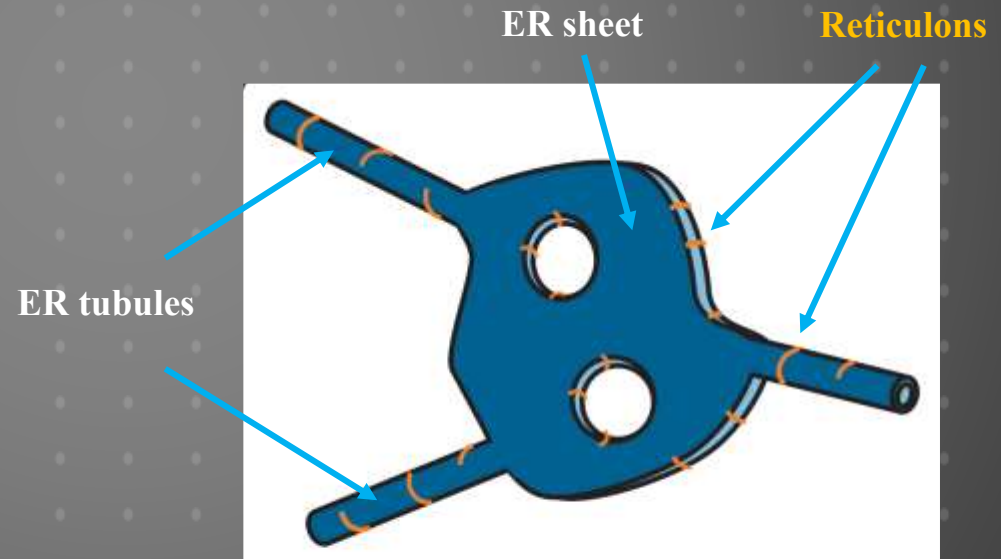
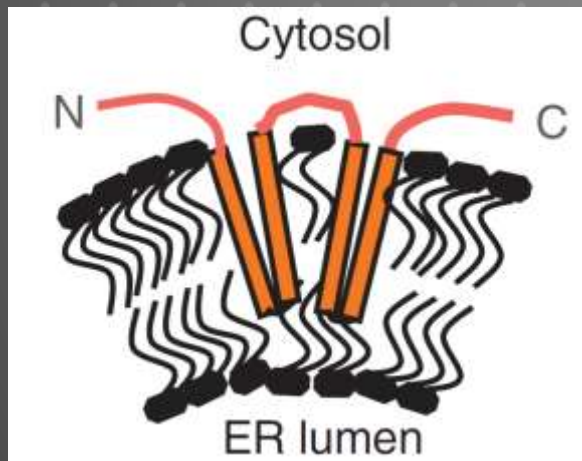
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Formation of a nuclear envelope within egg extracts following the addition of *Xenopus* sperm.

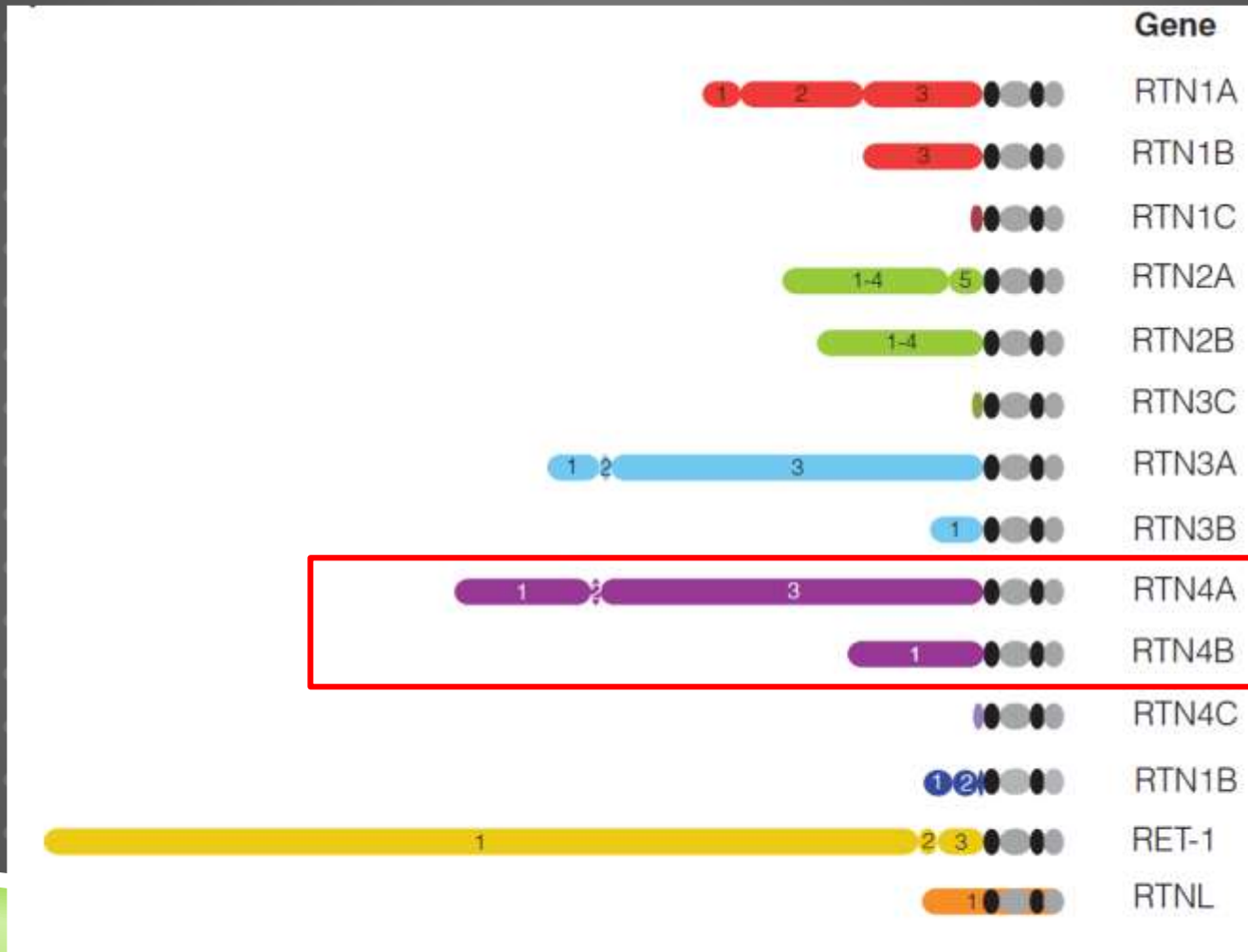
RETICULONS:

- ▶ Set of proteins exclusively in the ER tubules
- ▶ Contribute to ER bending and shaping
- ▶ Shift the balance from flat membrane sheets towards tubular network



(Friedman et al. Trends in Cell Biology, December 2011)

RETICULON ISOFORMS



RESEARCH OBJECTIVE:

- ▶ To evaluate the effective properties of various reticulon proteins on nuclear size regulation.
- ▶ To prompt further investigation on this area of research
 - ▶ How altering the concentrations of these proteins might effect nuclear size in:
 - ▶ embryonic development
 - ▶ cancer progression

EXPERIMENTAL OUTLINE:

- ▶ Transformation of chemically competent *E. coli* BL21 cells to introduce the following plasmids with their specific reticulon proteins and isoforms:
 - ▶ pET-30b Rtn4b (Kan resistance)
 - ▶ pKW 197 Rtn4a #6
 - ▶ pKW 197 Rtn4a #8
 - ▶ pKW 197 Rtn4a #10
- } (Amp resistance)

EXPERIMENTAL OUTLINE:

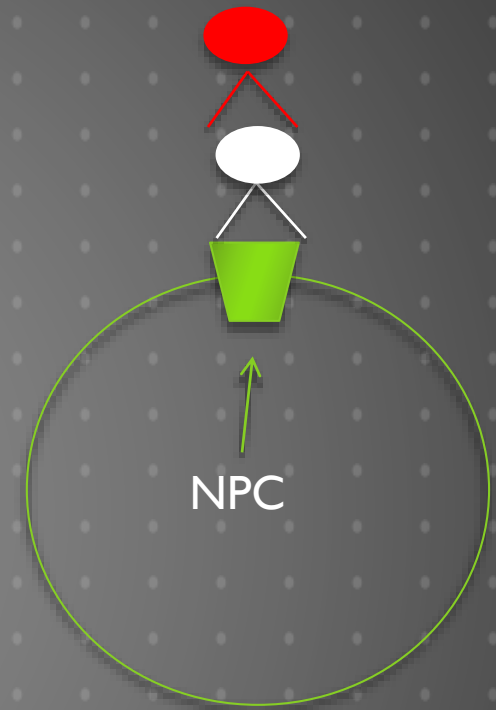
- ▶ Overnight expression cultures (using IPTG) grown for each of the bacterial cultures
 - ▶ 16° C
 - ▶ 25° C ← Eliminated background on Western blots
 - ▶ 37° C
- ▶ Gel electrophoresis and Western blotting to determine the presence and purity of the proteins
 - ▶ Rtn4a size is around 130-135 kD
 - ▶ Rtn4b size is around 39-45 kD

EXPERIMENTAL OUTLINE:

- ▶ Protein purification using His-binding resin
- ▶ Dialysis to further purify and concentrate desired reticulon proteins
- ▶ Quantification of protein (Rtn4b and Rtn4a #8) concentrations
 - ▶ Image J
- ▶ *Xenopus* egg extract preparation

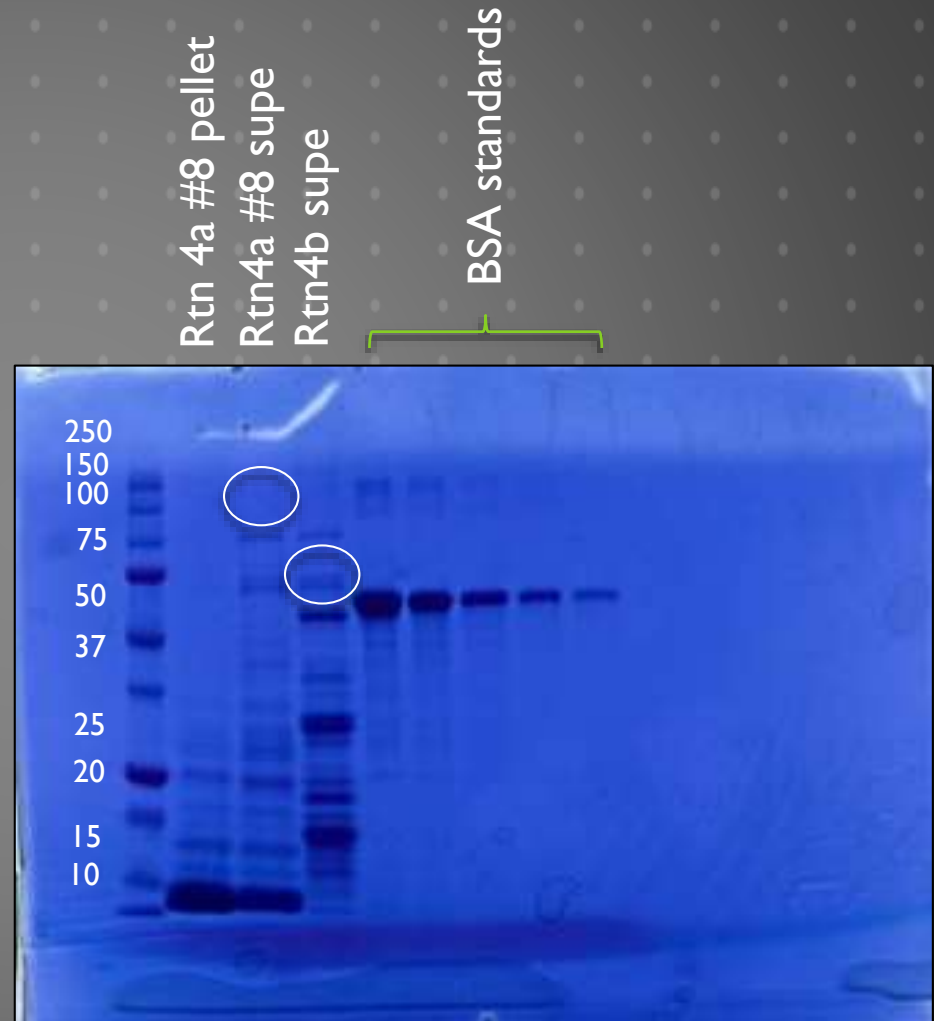
EXPERIMENTAL OUTLINE:

- ▶ Nuclear assembly in vitro using *Xenopus laevis* egg extract
- ▶ Addition of Rtn4b or Rtn4a #8 at varying concentrations
- ▶ Nuclear Immunofluorescence
 - ▶ 1° antibody binds nuclear pore complexes
 - ▶ 2° anti-mouse antibody binds 1° antibody
- ▶ Nuclear quantification
 - ▶ Metamorph



QUANTIFYING RTN CONCENTRATION USING IMAGE J

- ▶ Gel used to quantify concentrations
 - ▶ Post-dialysis
 - ▶ Rtn4a #8: 130kD (w/out tag)
 - ▶ Rtn4b: 46kD (w/out tag)



QUANTIFYING RTN CONCENTRATION USING IMAGEJ

Concentration:

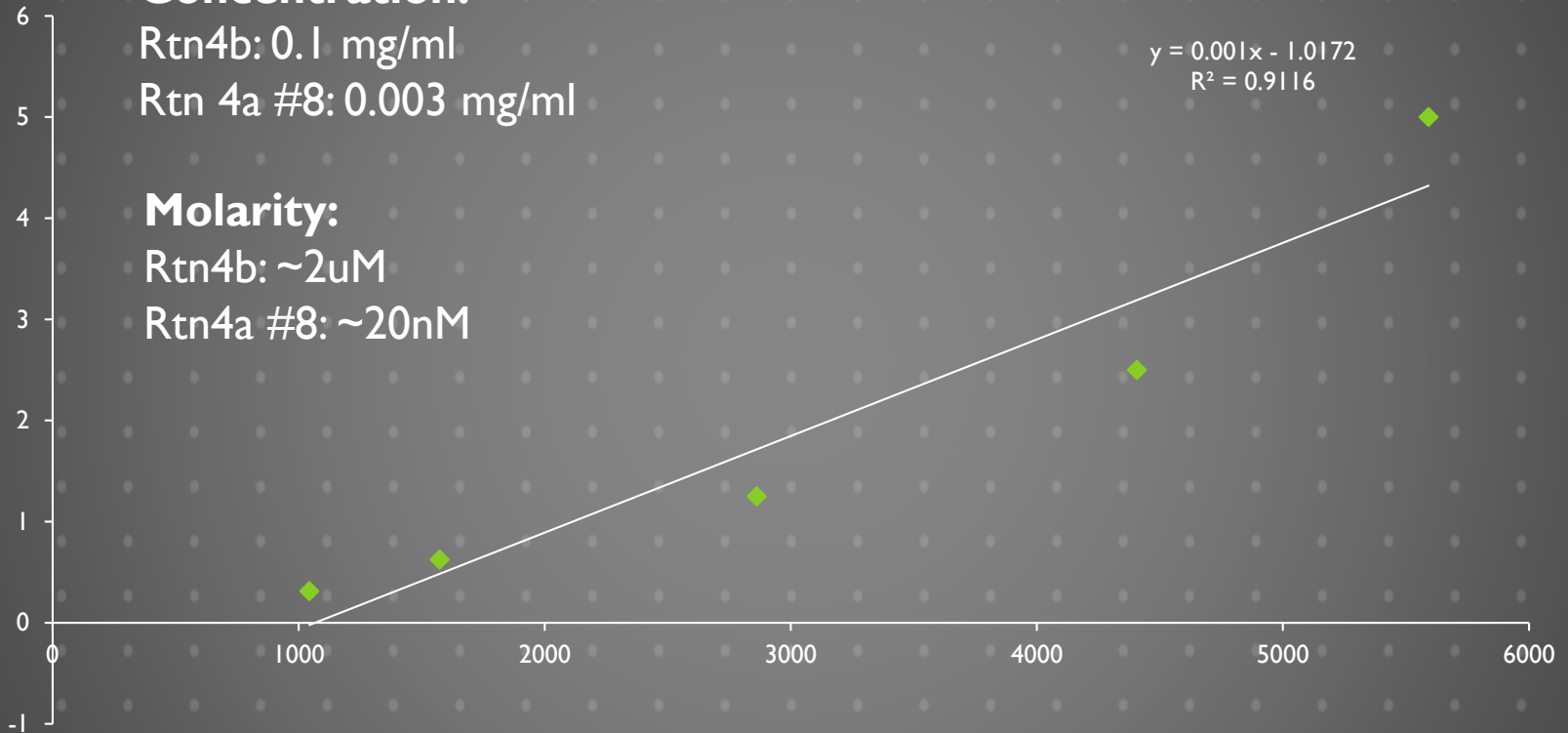
Rtn4b: 0.1 mg/ml

Rtn 4a #8: 0.003 mg/ml

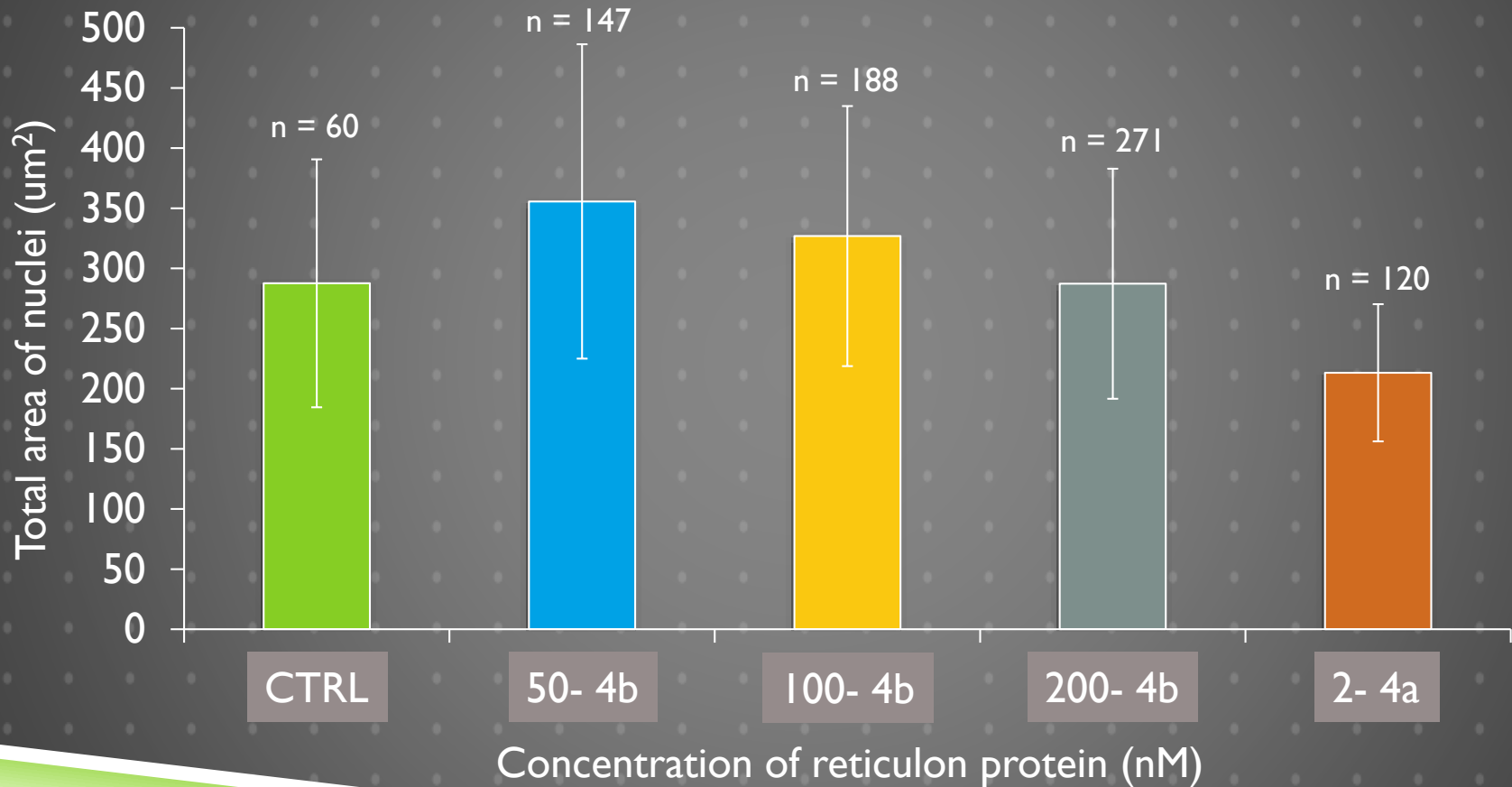
Molarity:

Rtn4b: ~2uM

Rtn4a #8: ~20nM

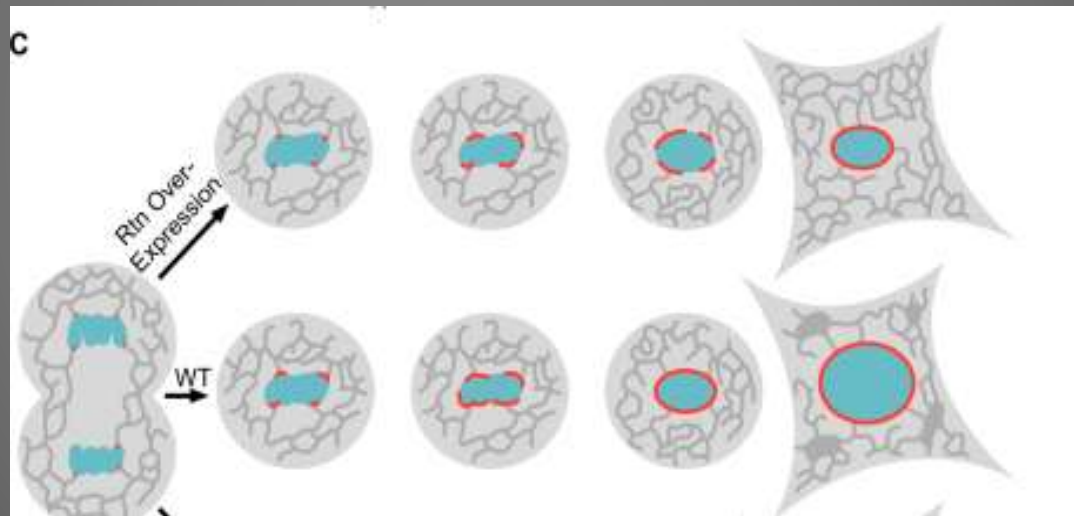


RTN OVEREXPRESSION DECREASES NUCLEAR SIZE



CONCLUSION:

- ▶ The overexpression of reticulons (either Rtn4a or Rtn4b) in the cell has a direct effect on nuclear size. The increase in reticulon proteins causes a decrease in nuclear size because of the decreased ER sheet availability.



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