

Cloning, Expressing, and Purifying Transient Receptor Potential Mucolipin (TRPML-1)



SURF Program, Summer 2012
Addison Goodley



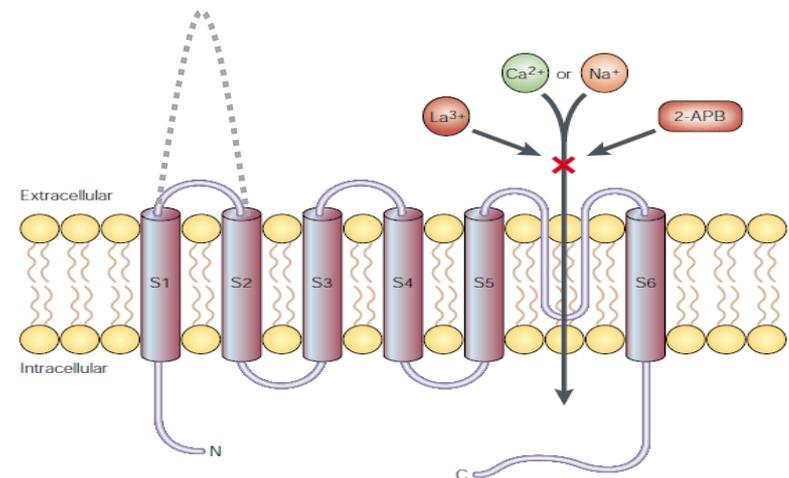
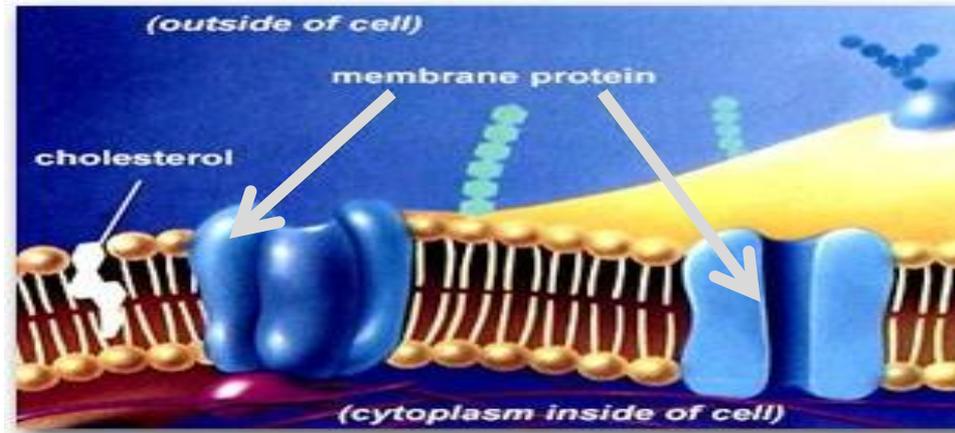
Advisors: Joe Blasic, Ella Mihailescu

GOAL

- Clone human TRPML-1 gene into yeast vector
- Express and purify TRPML-1 protein from yeast
- After purifying the protein, its structure and function can be studied

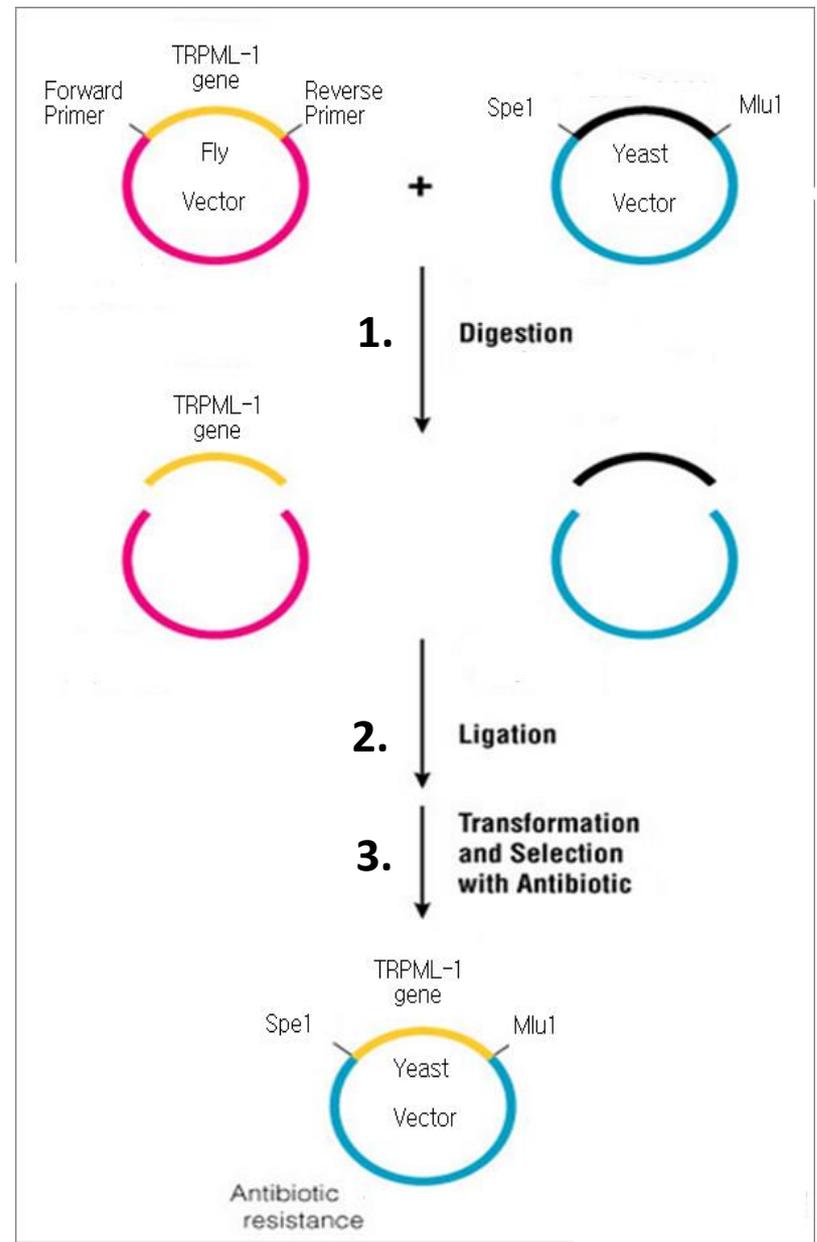
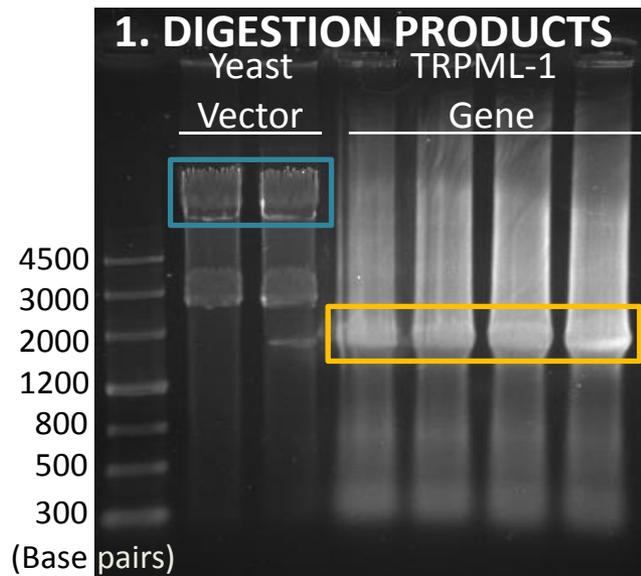
TRANSIENT RECEPTOR POTENTIAL (TRP) FAMILY

- Transmembrane proteins attributed to:
 - lysosomal signaling
 - intracellular trafficking
 - sensory nervous system function
 - calcium regulation
- Prefer lipid bilayer environment
- No solved structure



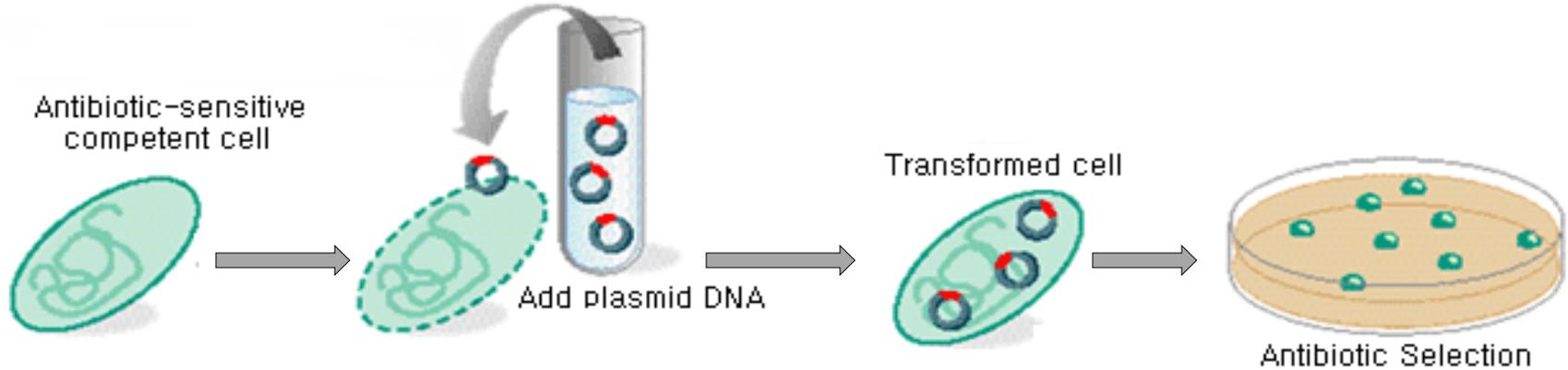
CLONE

DNA $\xrightarrow[\text{(in cells)}]{\text{translation}}$ Protein

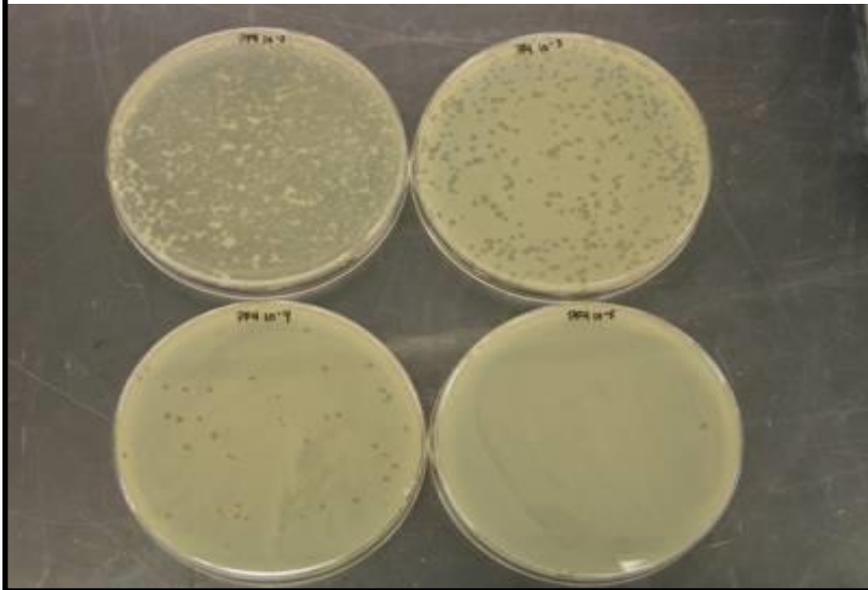


>> TRPML-1 gene in yeast vector, \therefore TRPML plasmid DNA

TRANSFORMATION

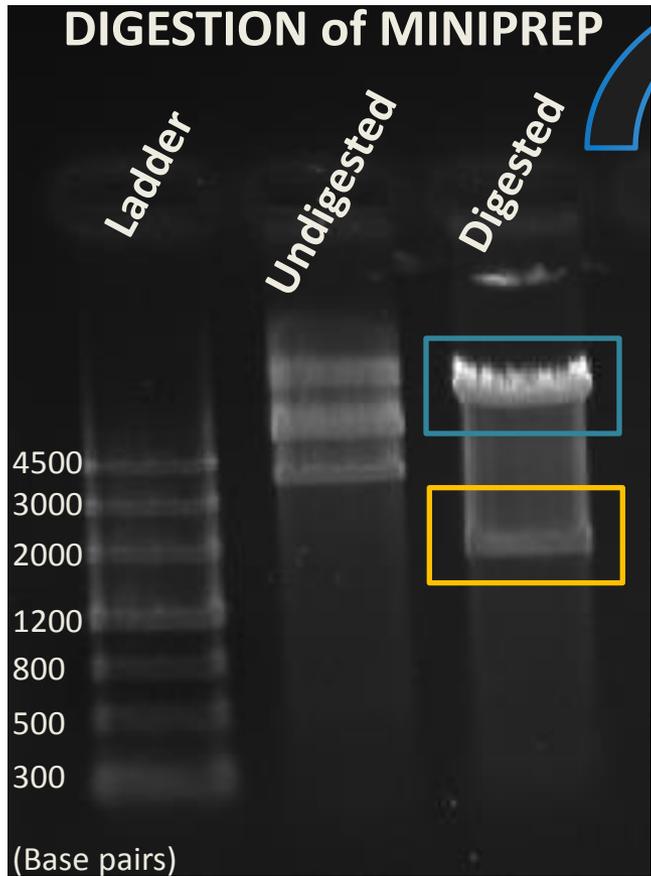


COLONY SELECTION (+AMPICILLIN PLATES)

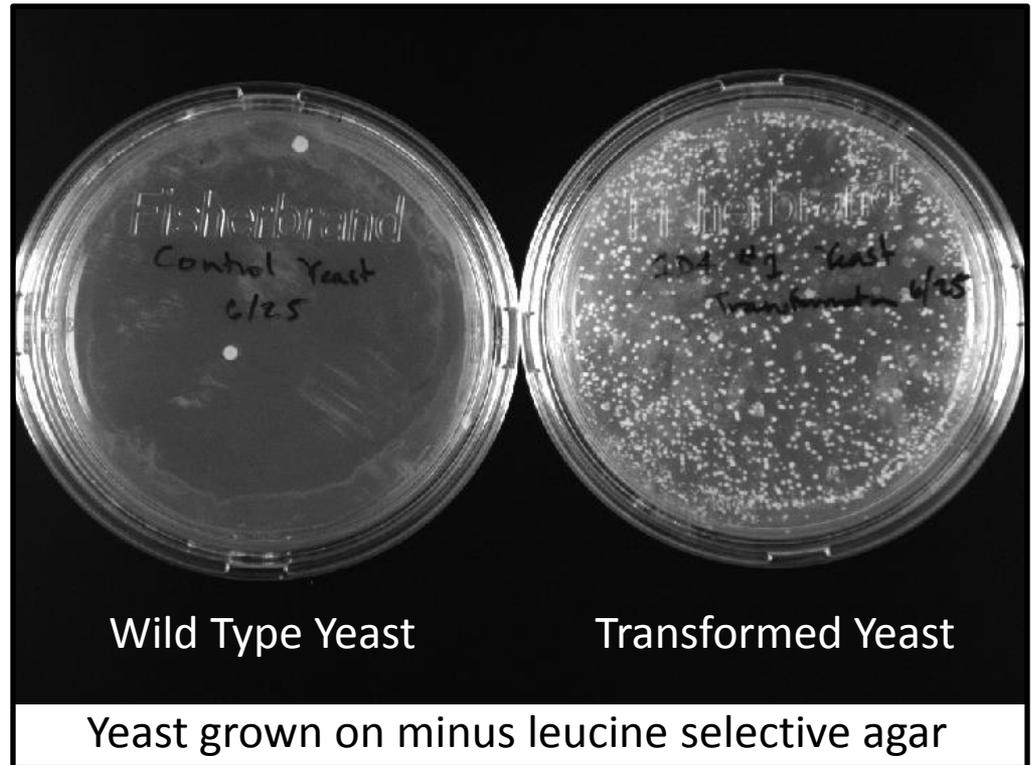


>> Antibiotic resistance effect,
∴ TRPML plasmid in cells

YEAST TRANSFORMATION



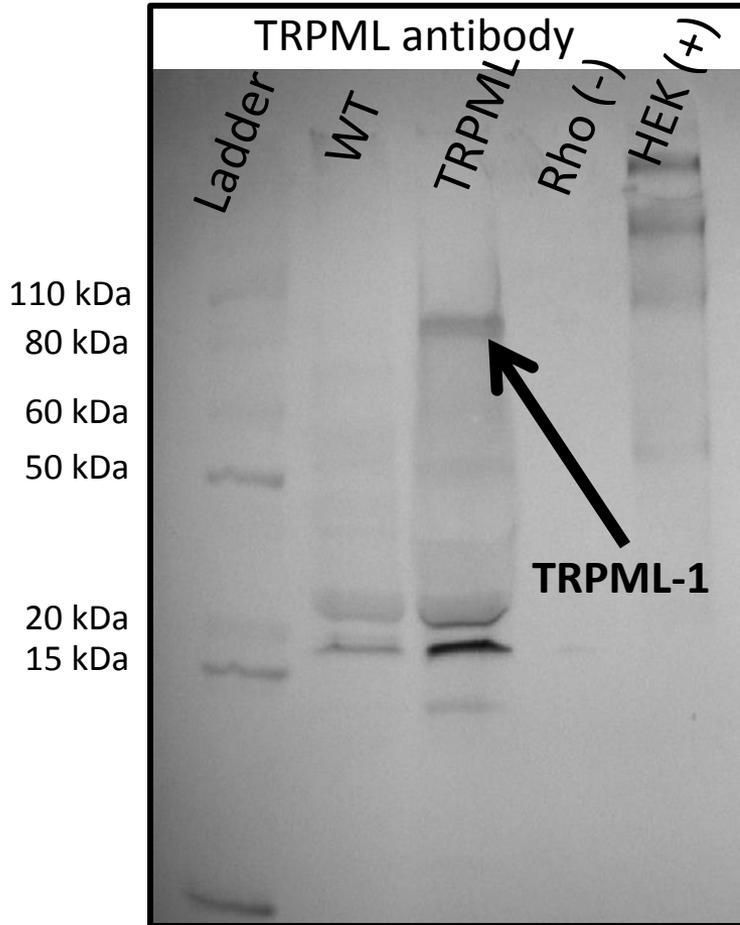
Miniprep plasmid used to transform yeast



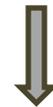
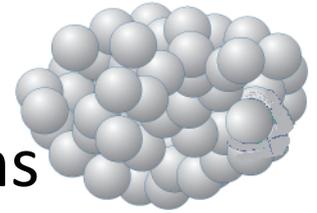
>> TRPML-1 plasmid in yeast, \therefore protein can be made

EXPRESS

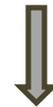
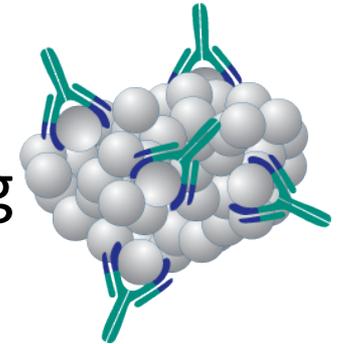
1. Western Blot



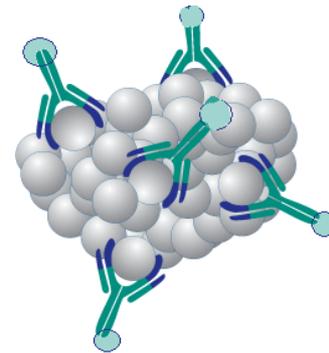
A. Electric current separates proteins by size



B. Antibody binding



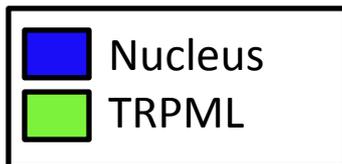
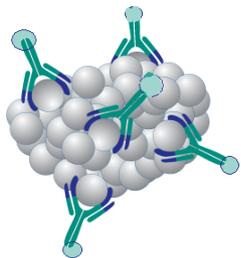
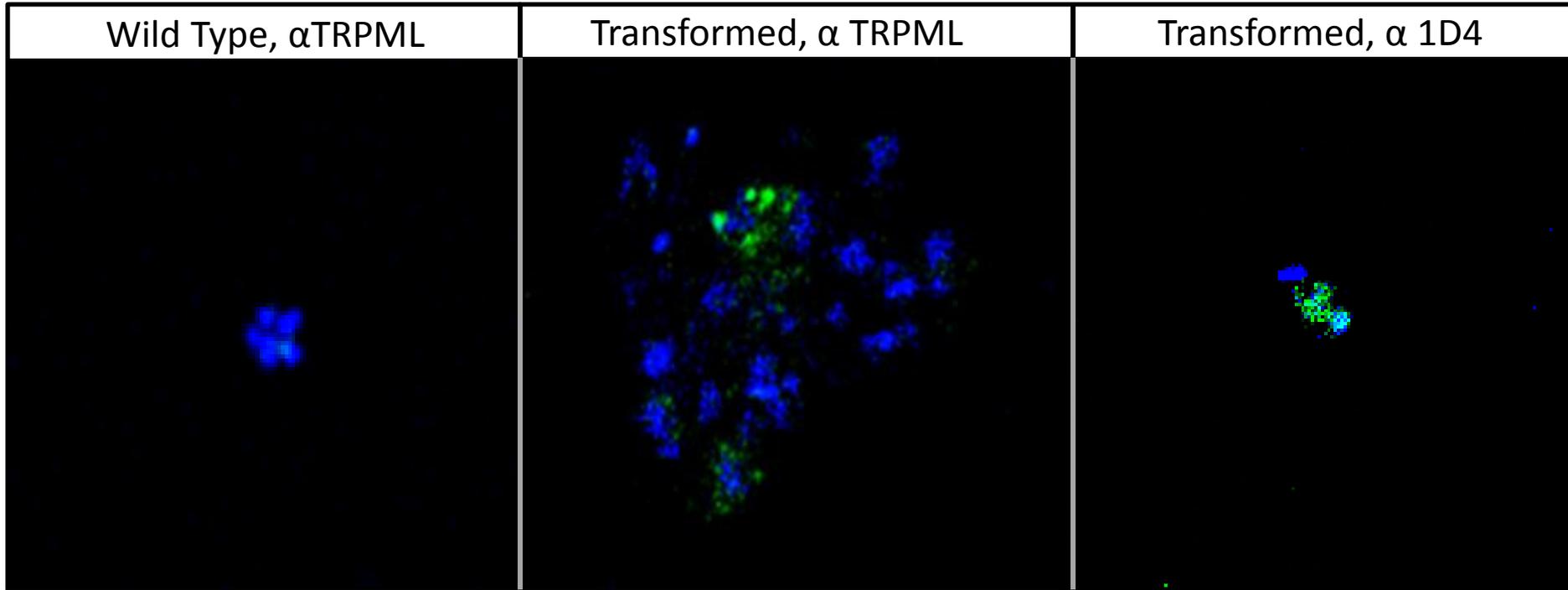
C. Dye



>> Band has proper size and antibody interaction, \therefore TRPML

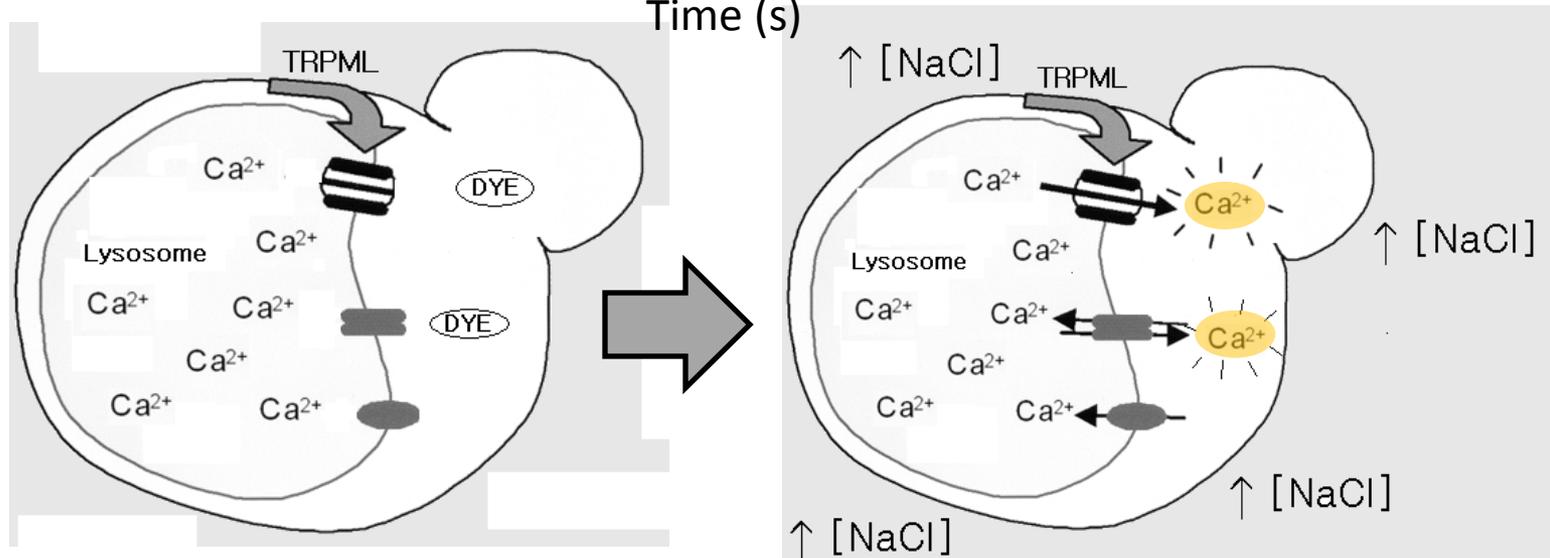
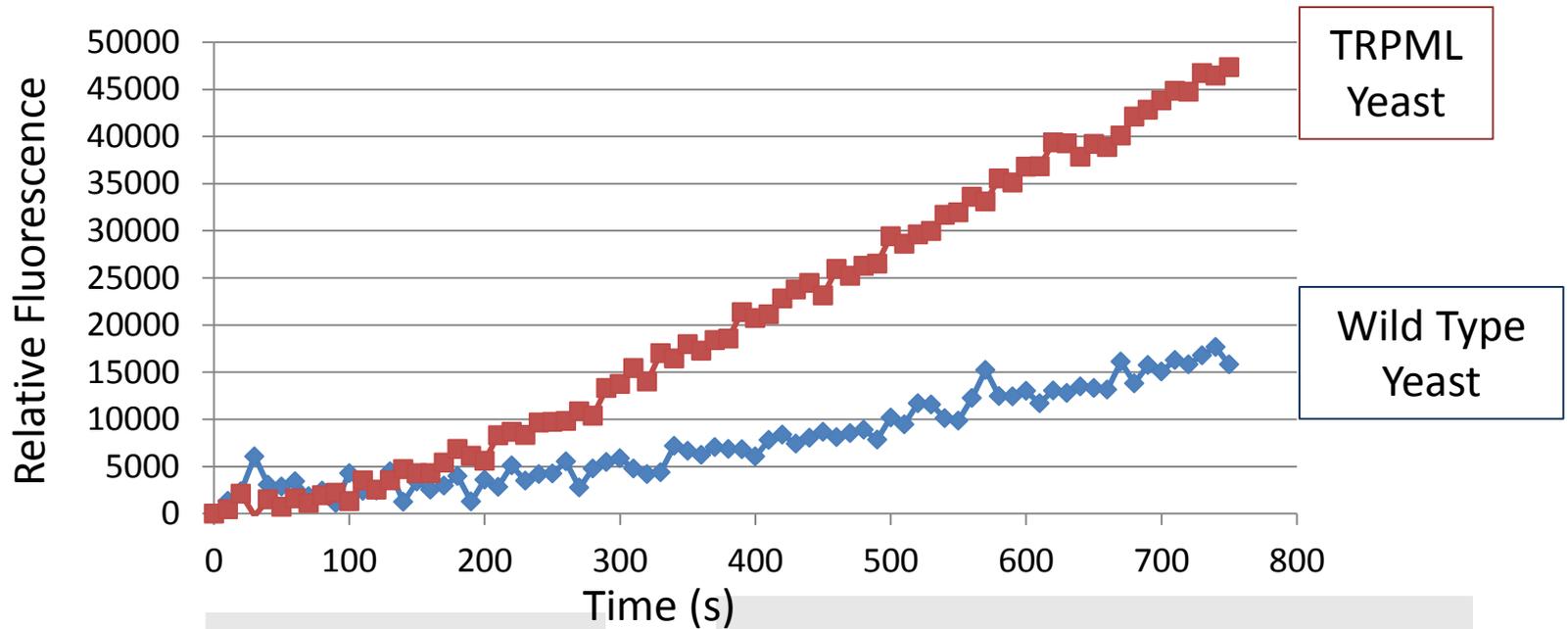
EXPRESS

2. Yeast Immunofluorescence

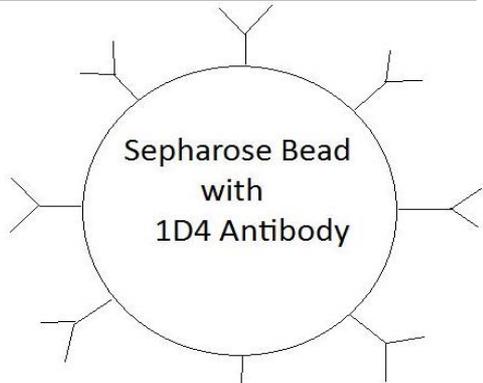


>> TRPML protein in yeast

FUNCTIONAL ASSAY



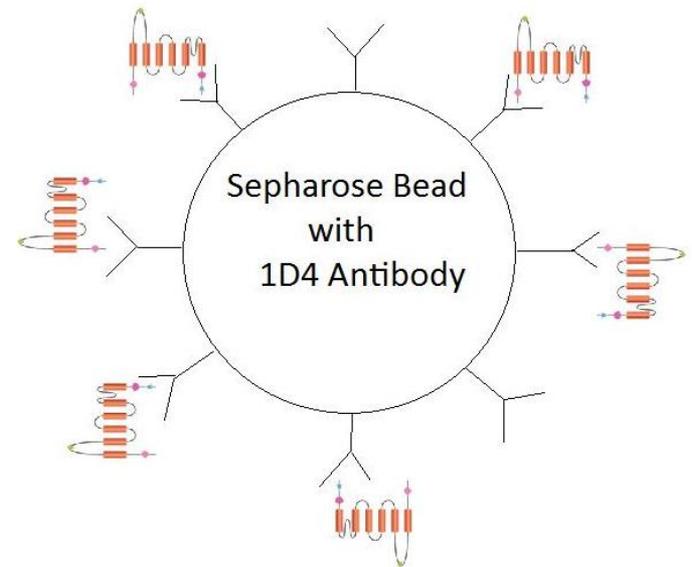
PURIFY



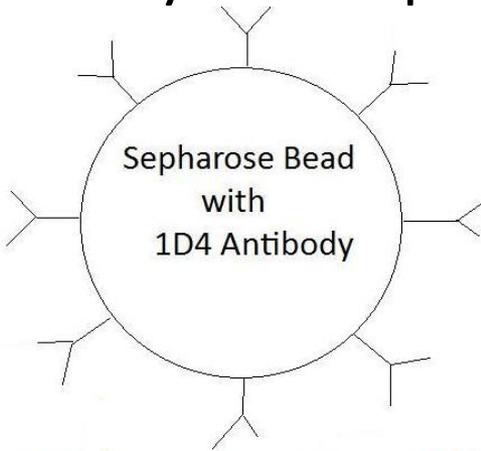
Solublized yeast protein run on column of 1D4-bound sepharose beads.

1D4 protein tag and bead antibody interact to bind TRPML to beads.

Thorough wash to remove all non-specifically bound protein.

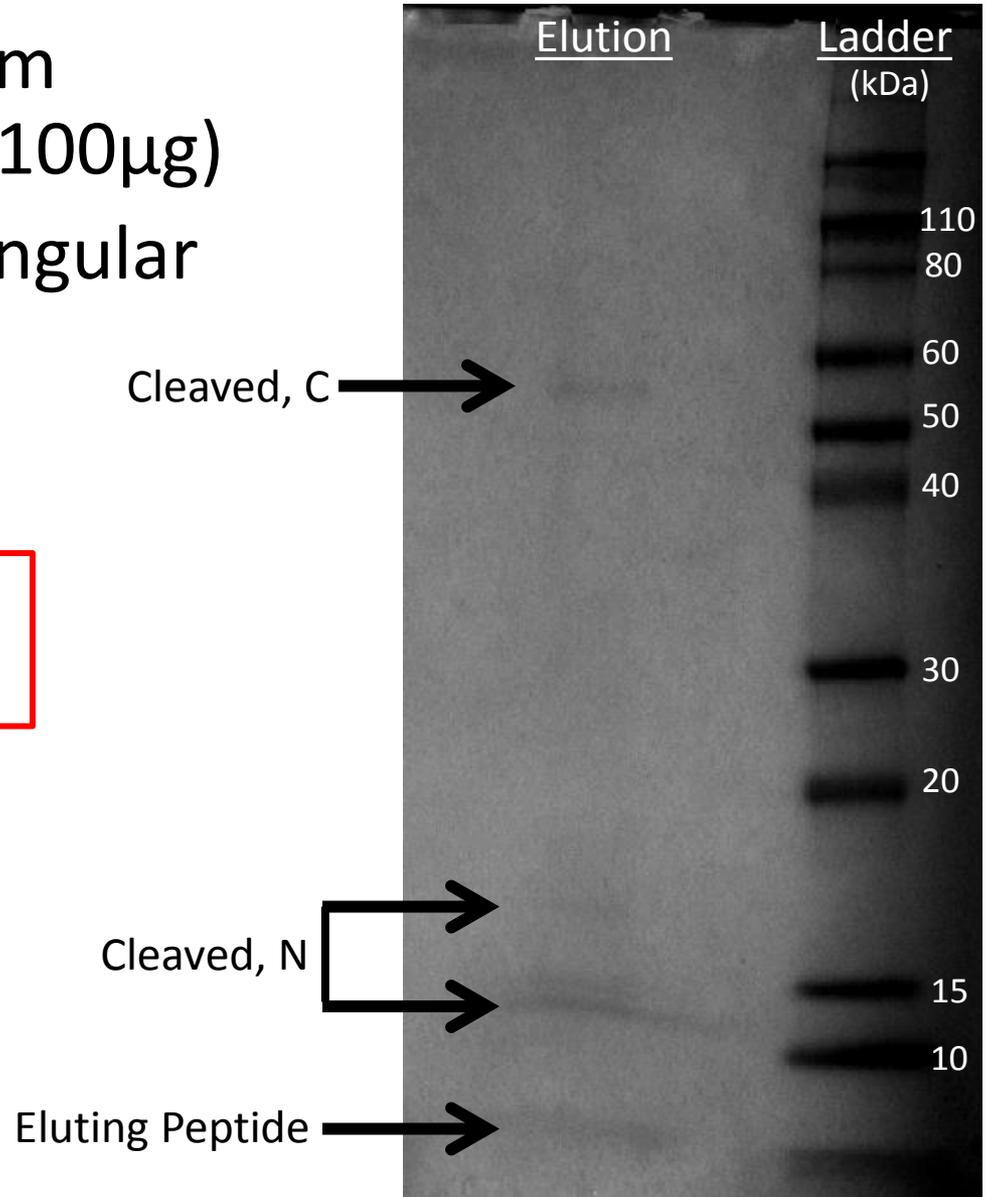
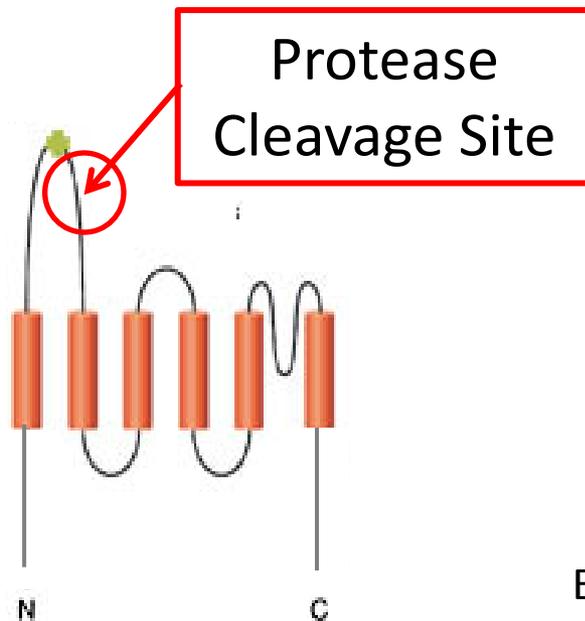


Elute with peptide to bind 1D4 antibody and collect TRPML protein in solution.



PURIFY

- Protein stain to confirm purification elution (~100μg)
- Cleaved protein, no singular band around 80kDa
- Quality and quantity



CONCLUSIONS

- Able to clone and express TRPML-1 in yeast
- Continue attempts to purify
- Future directions
 - Structure
 - Neutron scattering
 - Crystallography
 - Functional assays
 - Calcium ion channel

ACKNOWLEDGEMENTS

IBBR:

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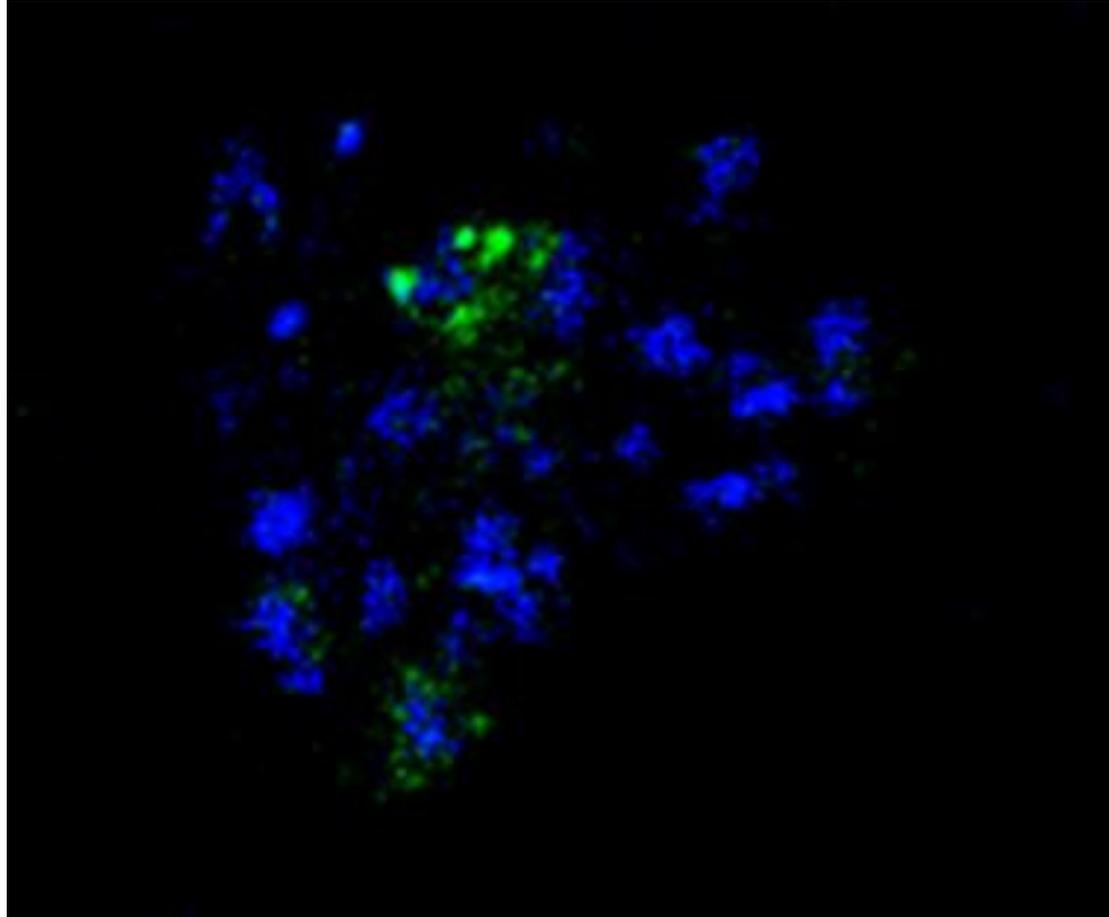
SURF Program



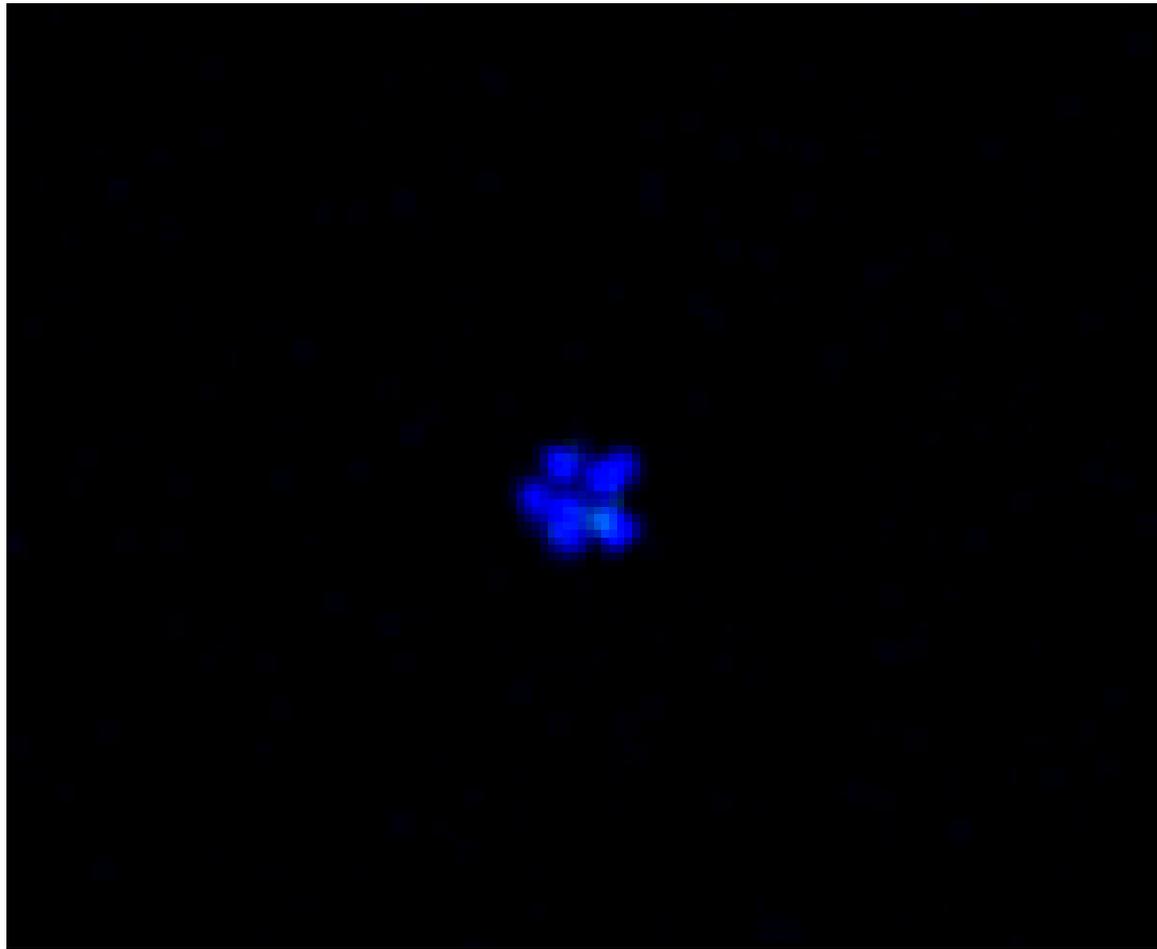
Questions?



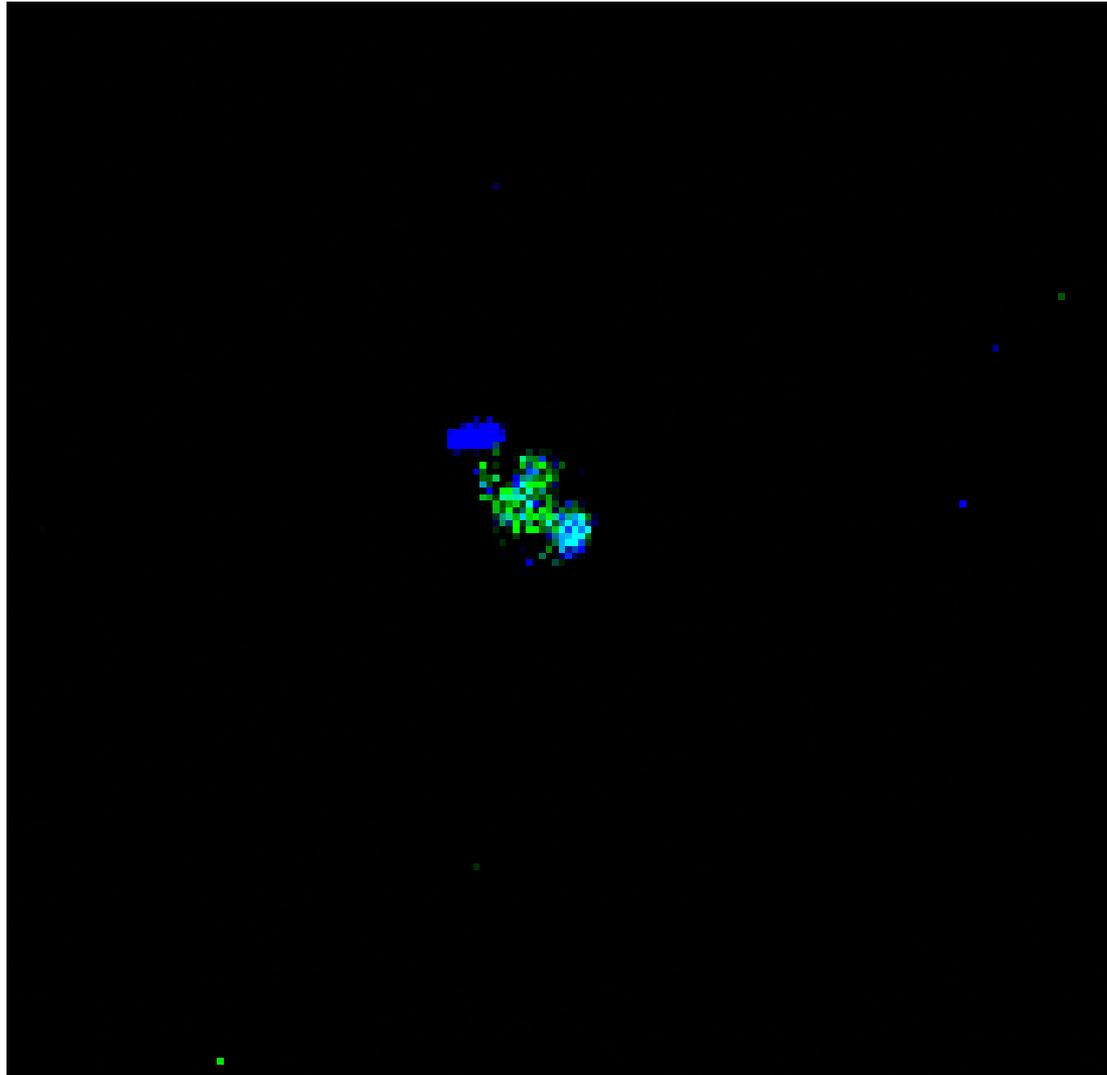
Transformed yeast, TRPML antibody

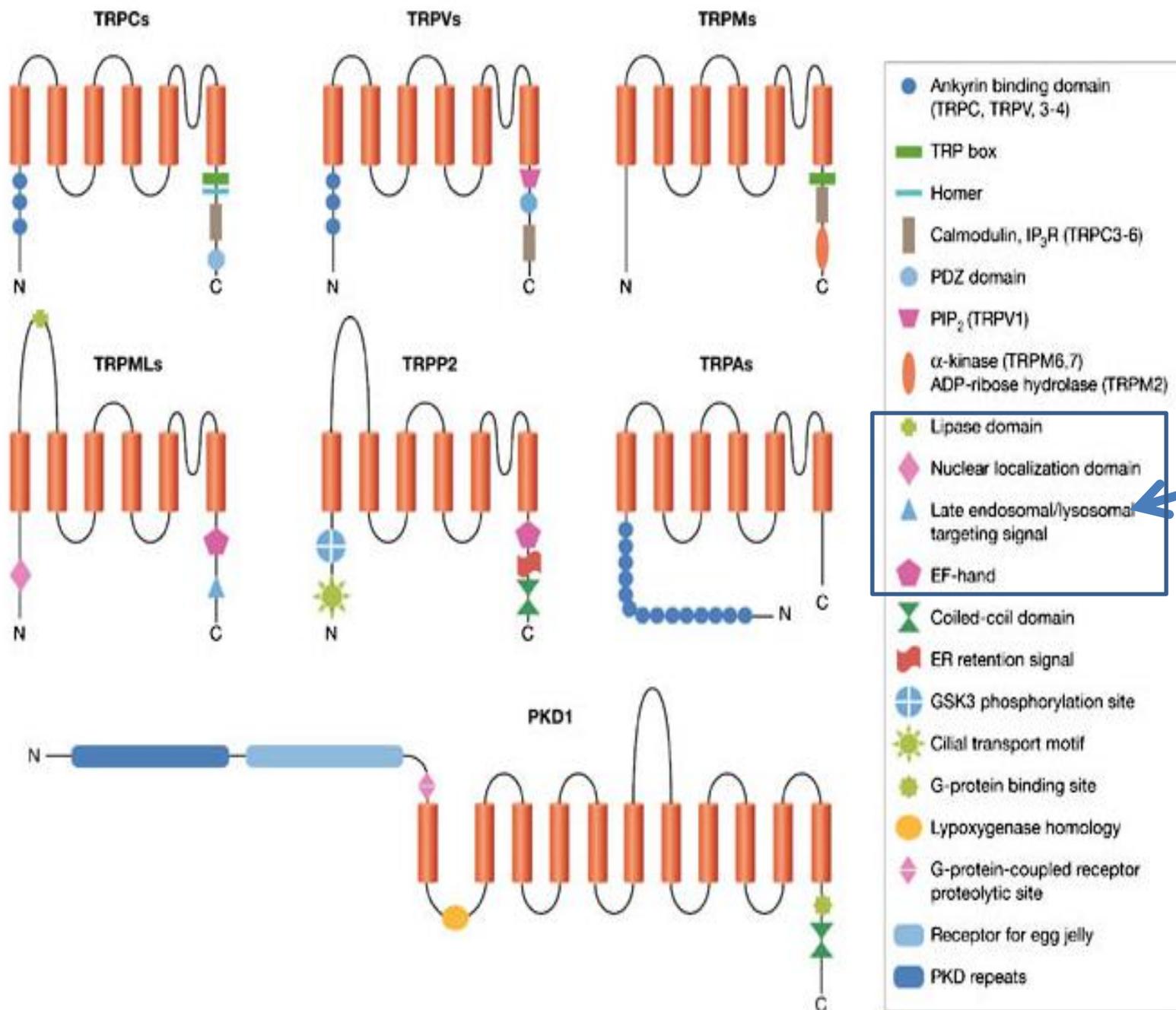


Wild type yeast, TRPML antibody



Transformed yeast, 1D4 antibody





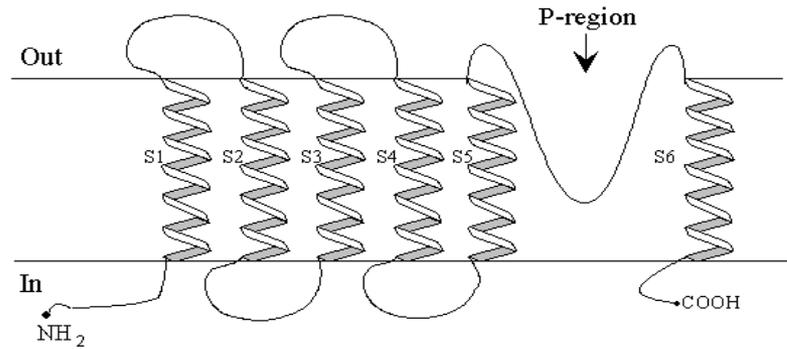
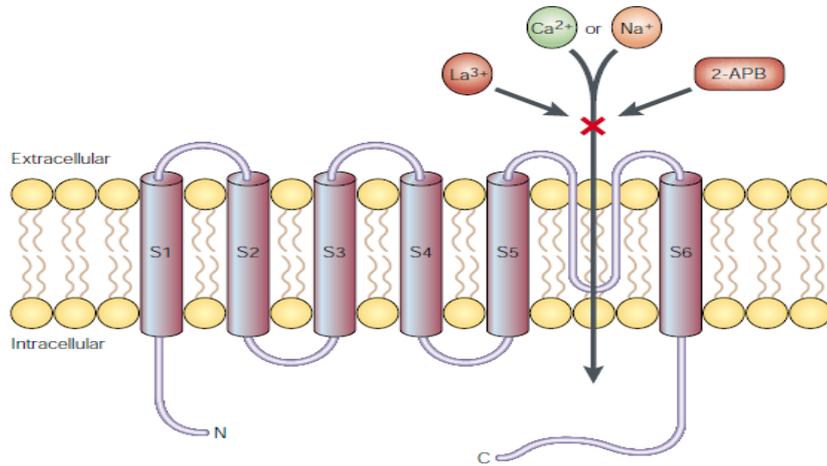
STRUCTURE

TRP channels are thought to resemble the structure of K⁺ channels

TRP

vs.

K⁺ channels



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