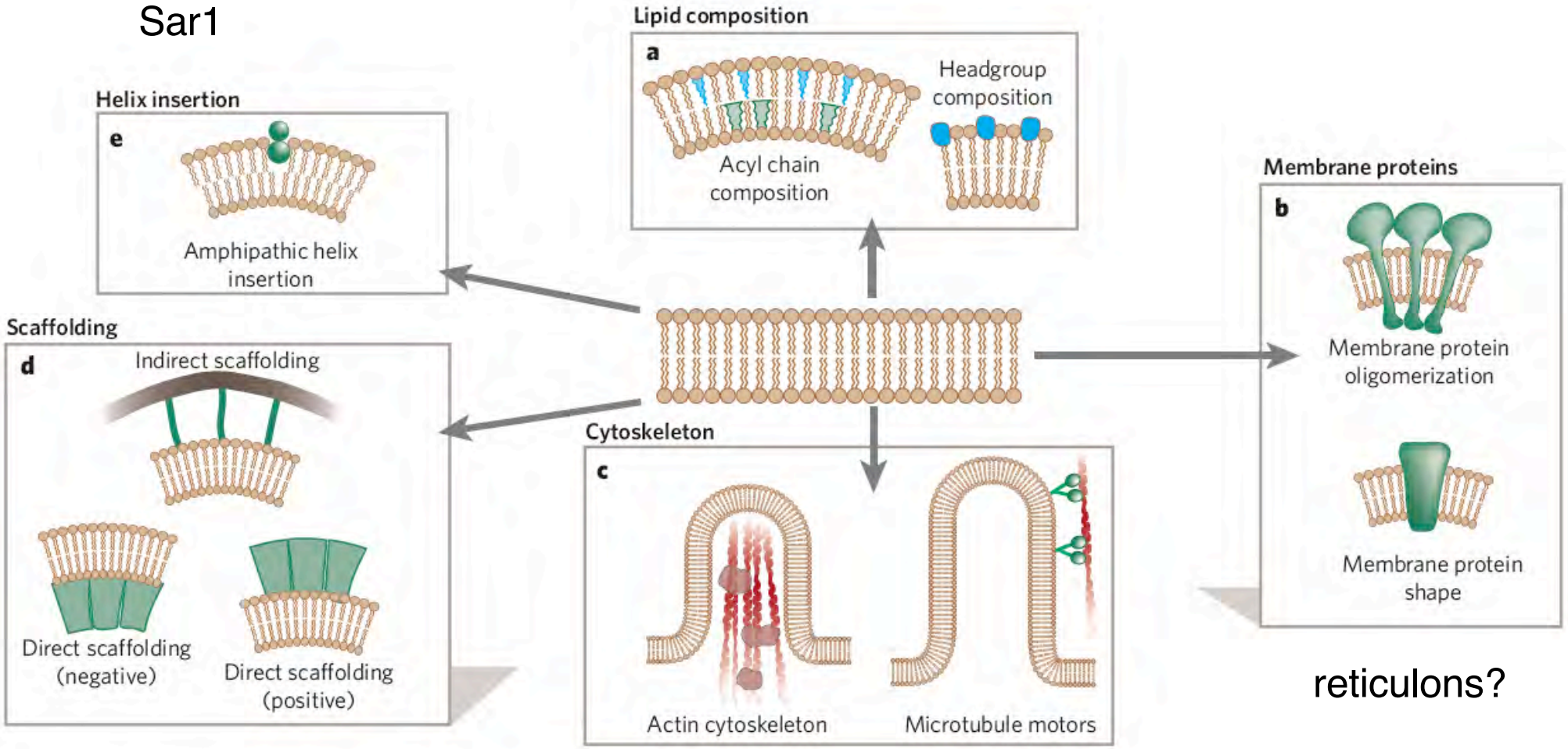
Way to impose membrane curvature

Sar1

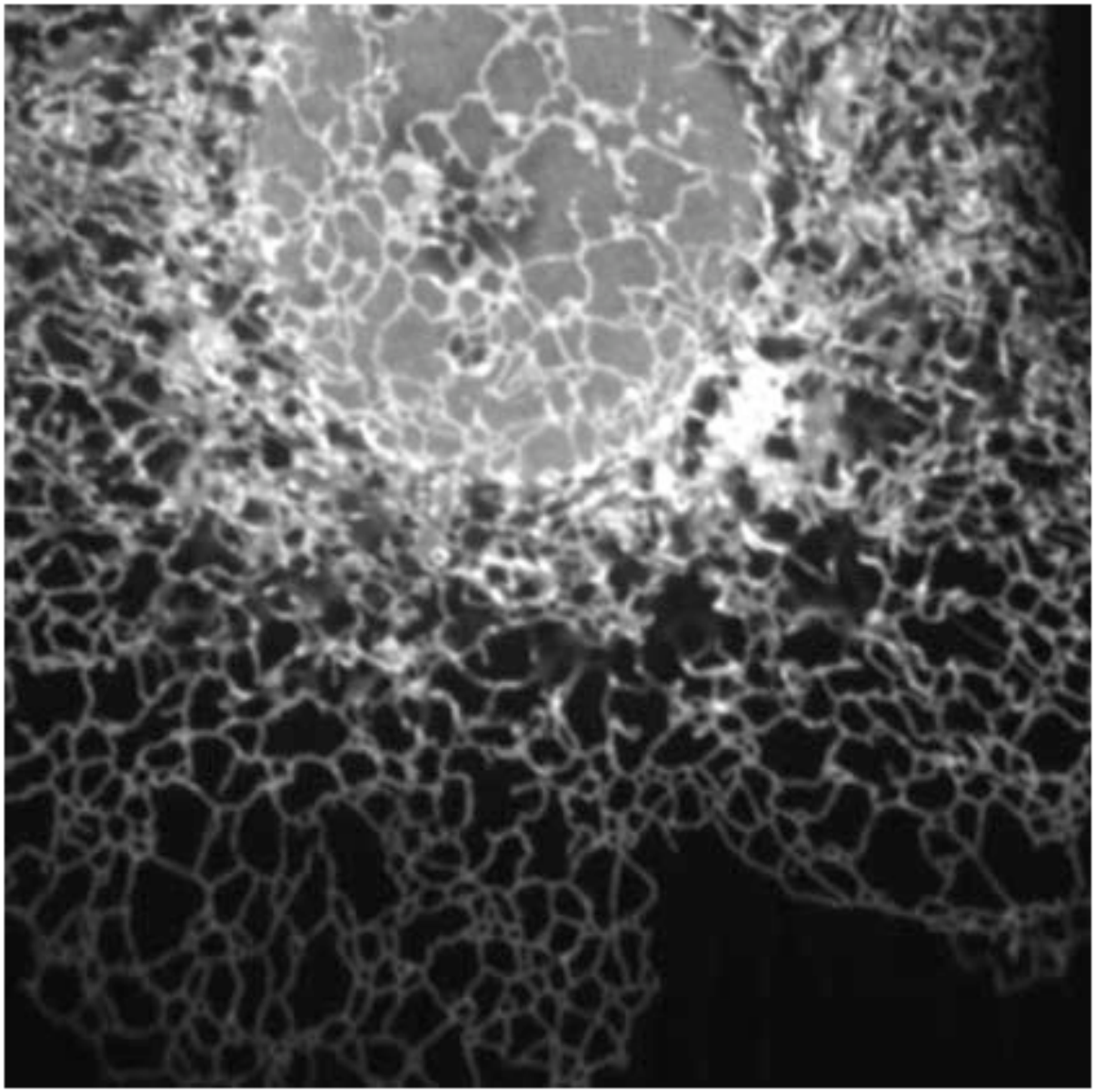


reticulons?

clathrin and other vesicle coats
dynamin
BAR domains

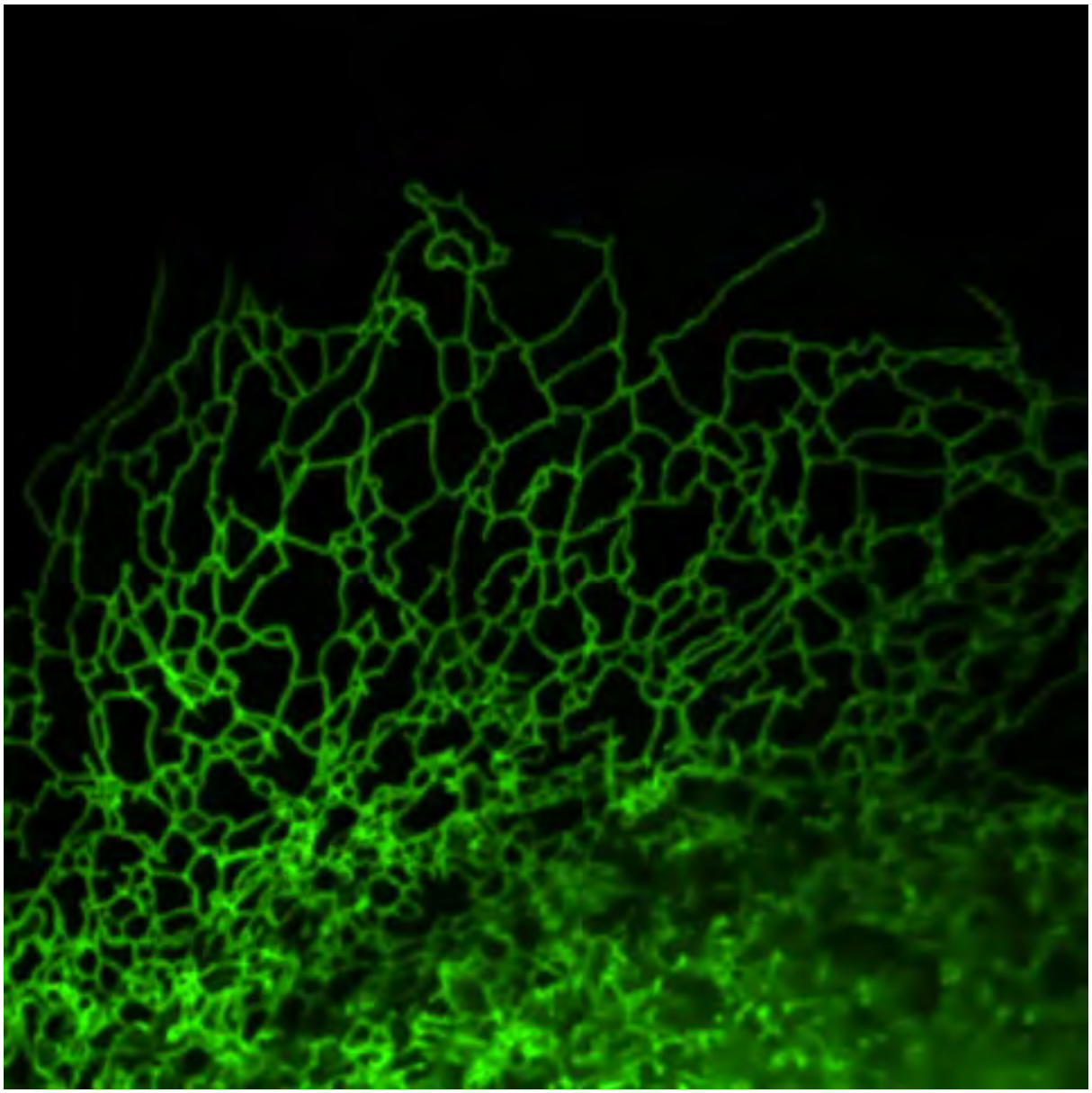
McMahon, 2005

The ER is continuous with the nuclear envelope and contains tubules and sheets



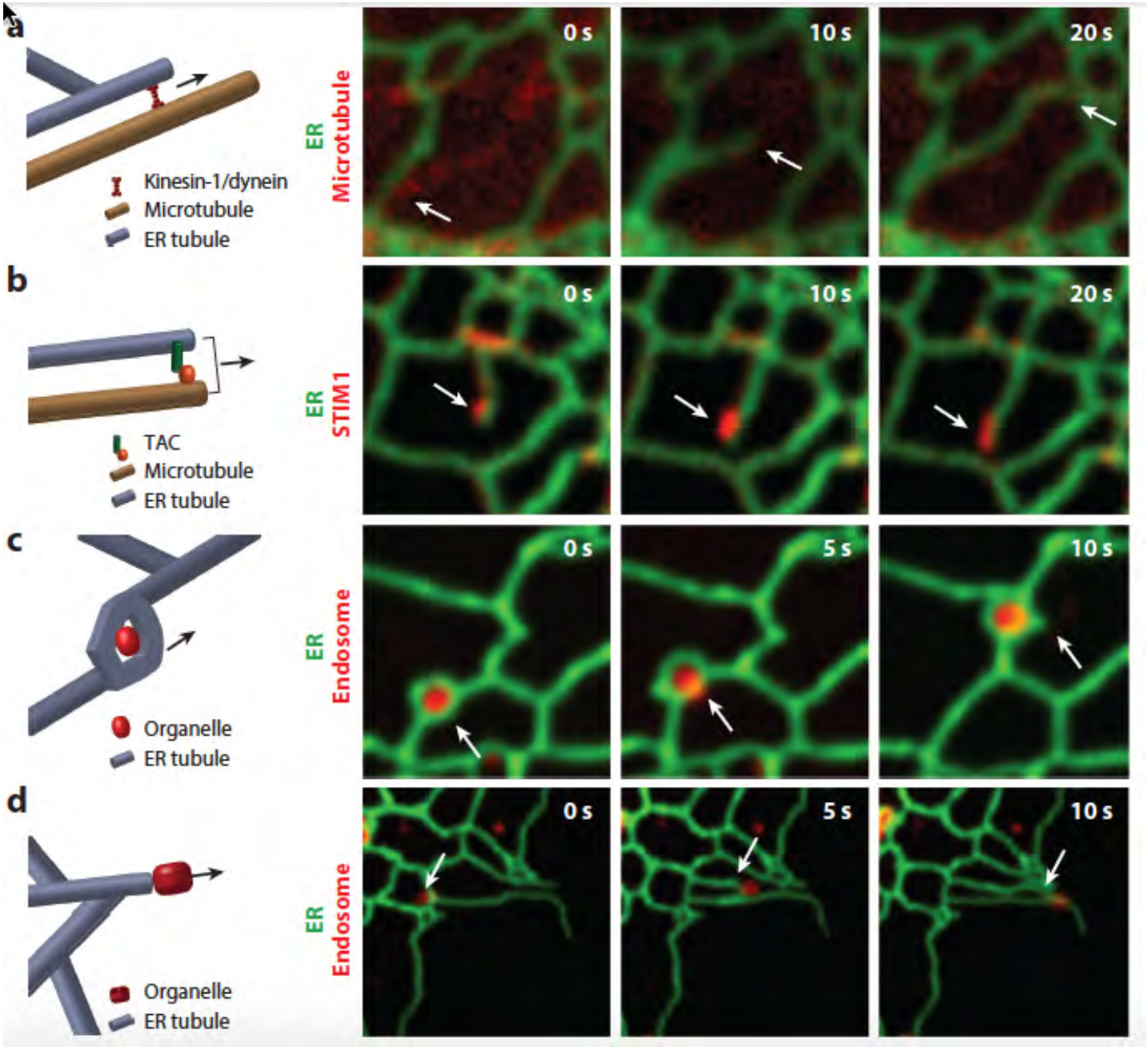
Gia Voeltz

The ER network is dynamic



Friedman et al. (2010) JCB

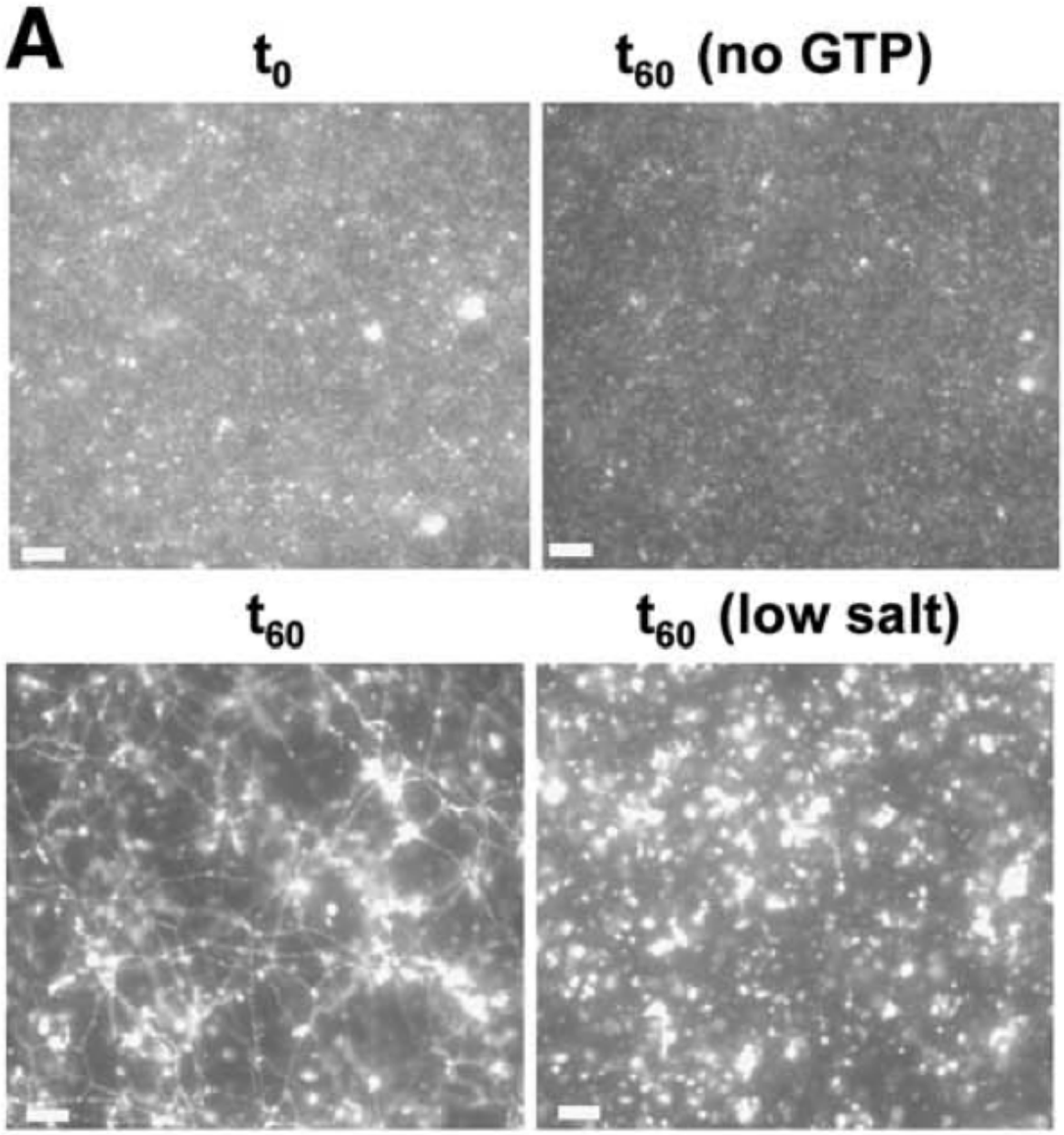
Examples of ER movement



- ER "sliding" along (acetylated) microtubule; depends on kinesin/dynein
- ER carried on the growing/shrinking tip (plus end) of microtubule
- ER ring rearrangements where ER wraps around organelles
- ER tubules "pulled" behind organelle.

Westrate et al (2015) AR Biochem

Fusion and tubulation of ER vesicles *in vitro*



- ER vesicles from Xenopus fuse into a network when incubated with GTP, 25˚C, 200 mM salt.

- requires GTP, and inhibited by GTPγS

- at 50 mM salt, get large vesicles

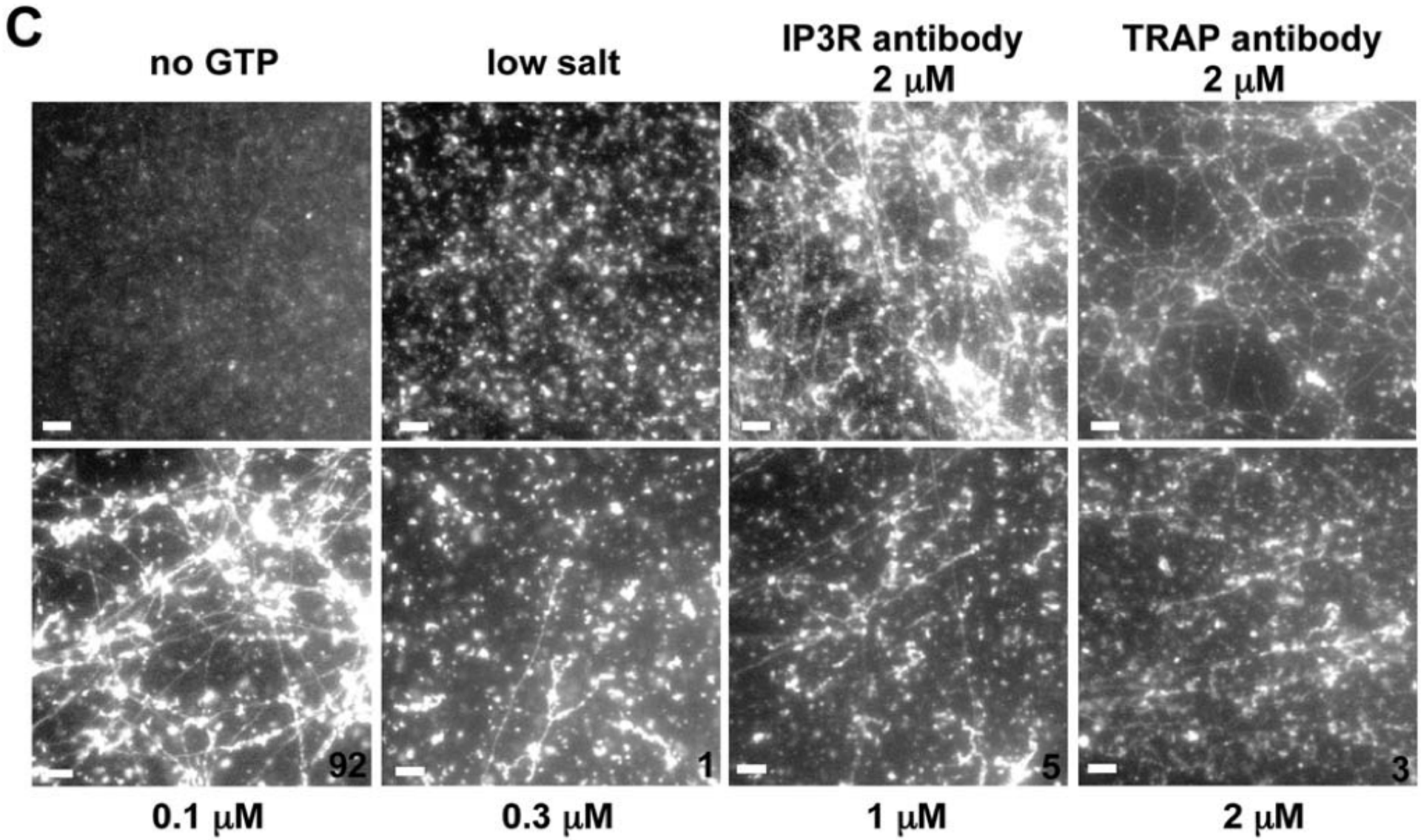
- Sulfhydryl reagents like maleimide inhibited network formation

- Sulfhydryl-biotin used to affinity purify the targets

- Identified reticulon 4a and 4b

Voeltz et al (2006) Cell

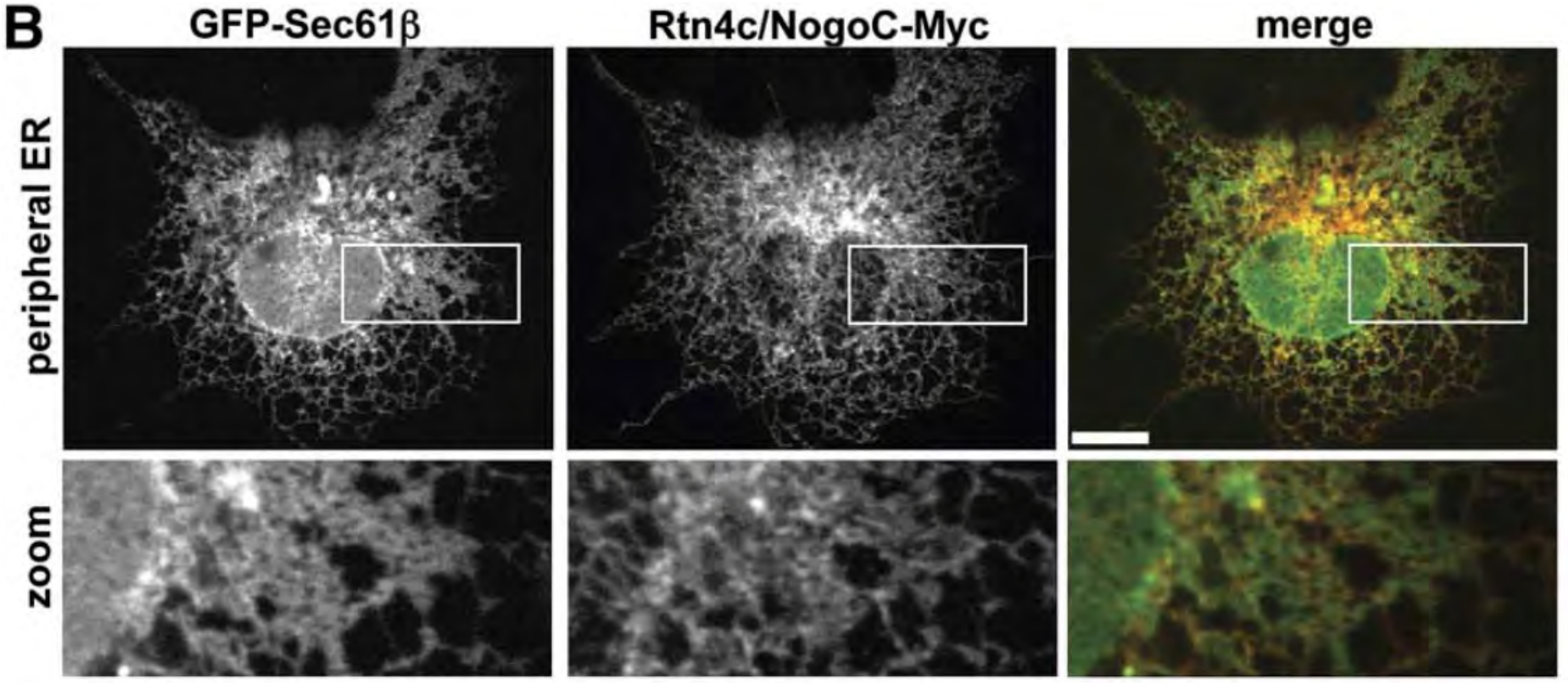
Antibodies to reticulon 4a inhibit tubule formation



- anti-IP3R and anti-TRAP are controls.
- anti-Rtn4b inhibited tubules but not large vesicles (fusion).

Voeltz et al (2006) Cell

Reticulons localize to tubular ER (versus sheets or nuclear envelope)

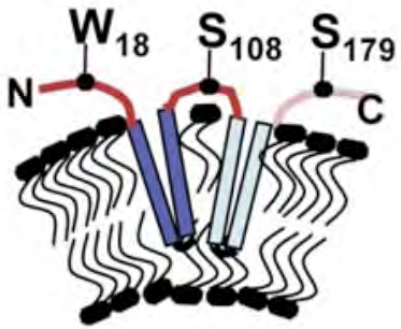


In addition:
• Overexpression increases length of ER tubules and reduces amount of sheets
• Yeast lacking reticulons (Rtn1, Rtn2) have normal ER; but under high osmolarity, converted to sheets (abnormal).

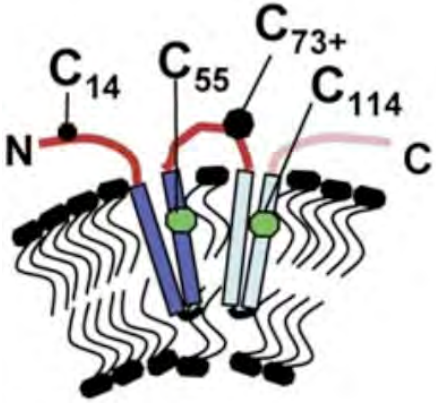
Voeltz et al. (2006) Cell

Schematic of reticulons and related proteins suggest a wedging mechanism

Rtn4c/NogoC



DP1



- DP1 (deleted in polyposis) identified as a Rtn binding partner.

- DP1 also localizes to tubular ER only.

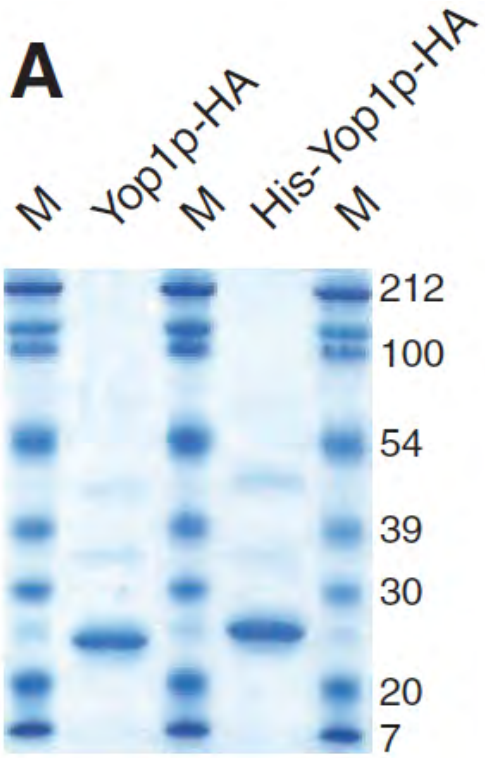
- Yeast DP1=Yop1; triple mutant *Δrtn1 Δrtn2 Δdp1* showed a defect in ER morphology under normal growth conditions (peripheral ER had mostly sheets, few tubules.

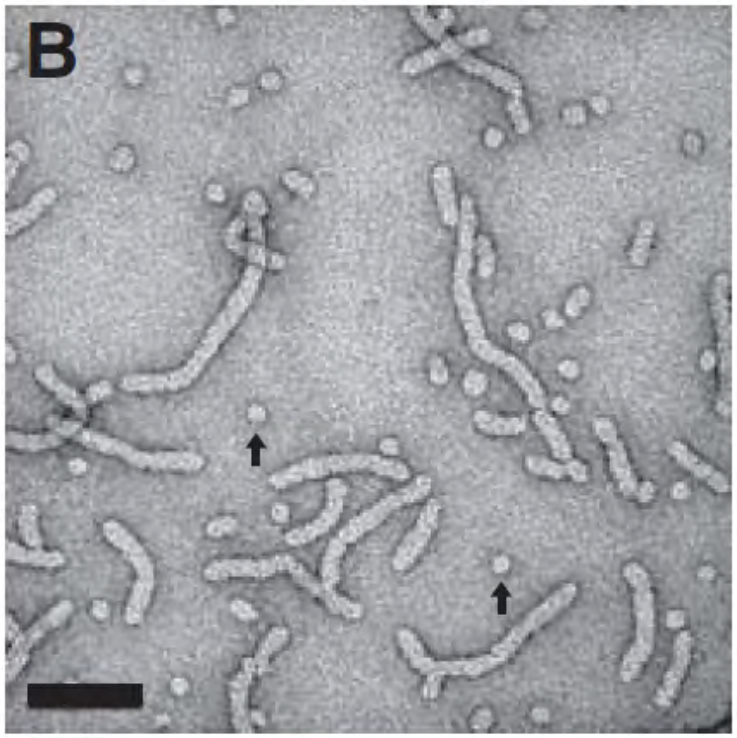
- All these proteins have hydrophobic segments of 30-35, instead of normal 20. Experimental evidence for hairpin formation.

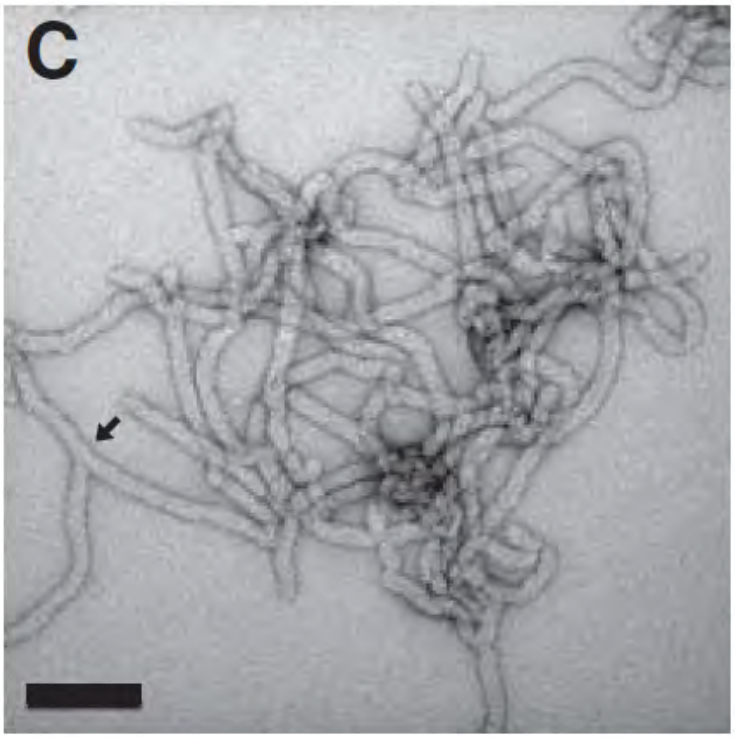
Model: Rtns and DP1 form hairpins in the membrane that cause high membrane curvature in tubules, compared to sheets; high abundance of these proteins may be important.

Voeltz et al. (2006) Cell

Tubulation of liposomes by Yop1





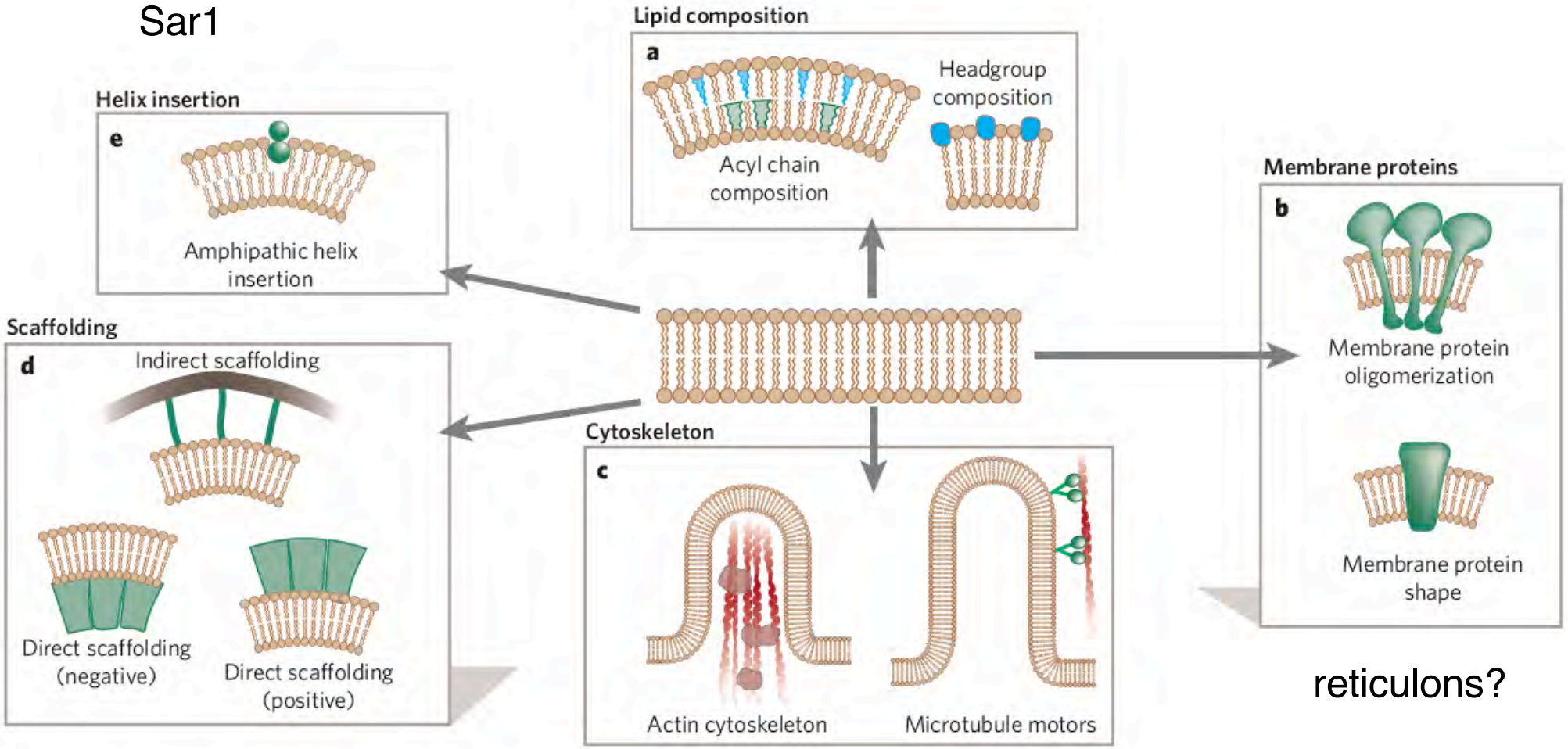


(A) His-tagged yeast Yop1 purified from E. coli.
(B) Addition of cleaved Yop1 to liposomes resulted in small vesicles and short tubules, diameter ~17 nm.
(C) Over time, tubules elongated with same diameter.

Hu et al. (2008) Science

Way to impose membrane curvature

Sar1



reticulons?

clathrin and other vesicle coats
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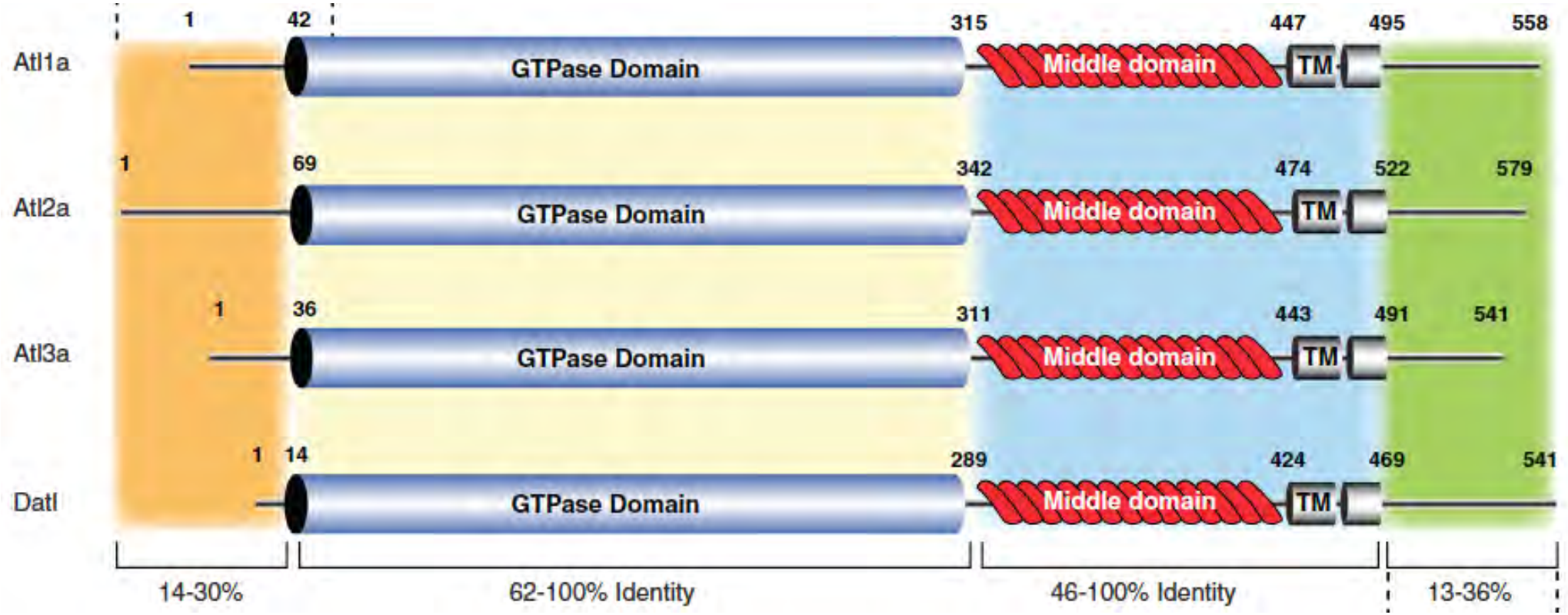
BAR domains

McMahon, 2005

Atlastins are involved in ER morphology

- Atlastins co-immunoprecipitate with reticulons and DP1.
- Atlastins are found in the tubular ER.
- Overexpression or depletion of atlastins cause ER morphology defect.

Atlastins are important for ER structure

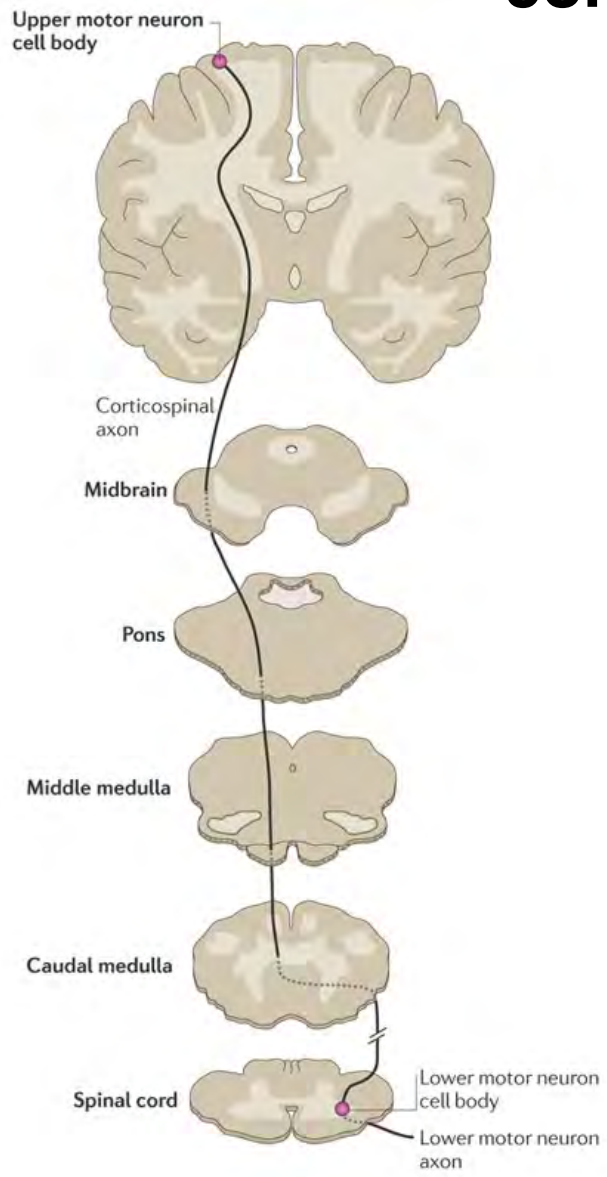


• Atlastin 1 is mutated in autosomal dominant hereditary spastic paraplegia (HSP); Atl1=SPG3A.

comparison of 3 human isoforms with Drosophila atlastin (Datl)

Moss et al. (2011) TICB

Hereditary spastic paraplegia: degeneration of the corticospinal tract



- HSP is characterized by stiffness, contraction, spasticity, and weakness of the lower extremities.

- Pure and complex (other neurological problems) forms.

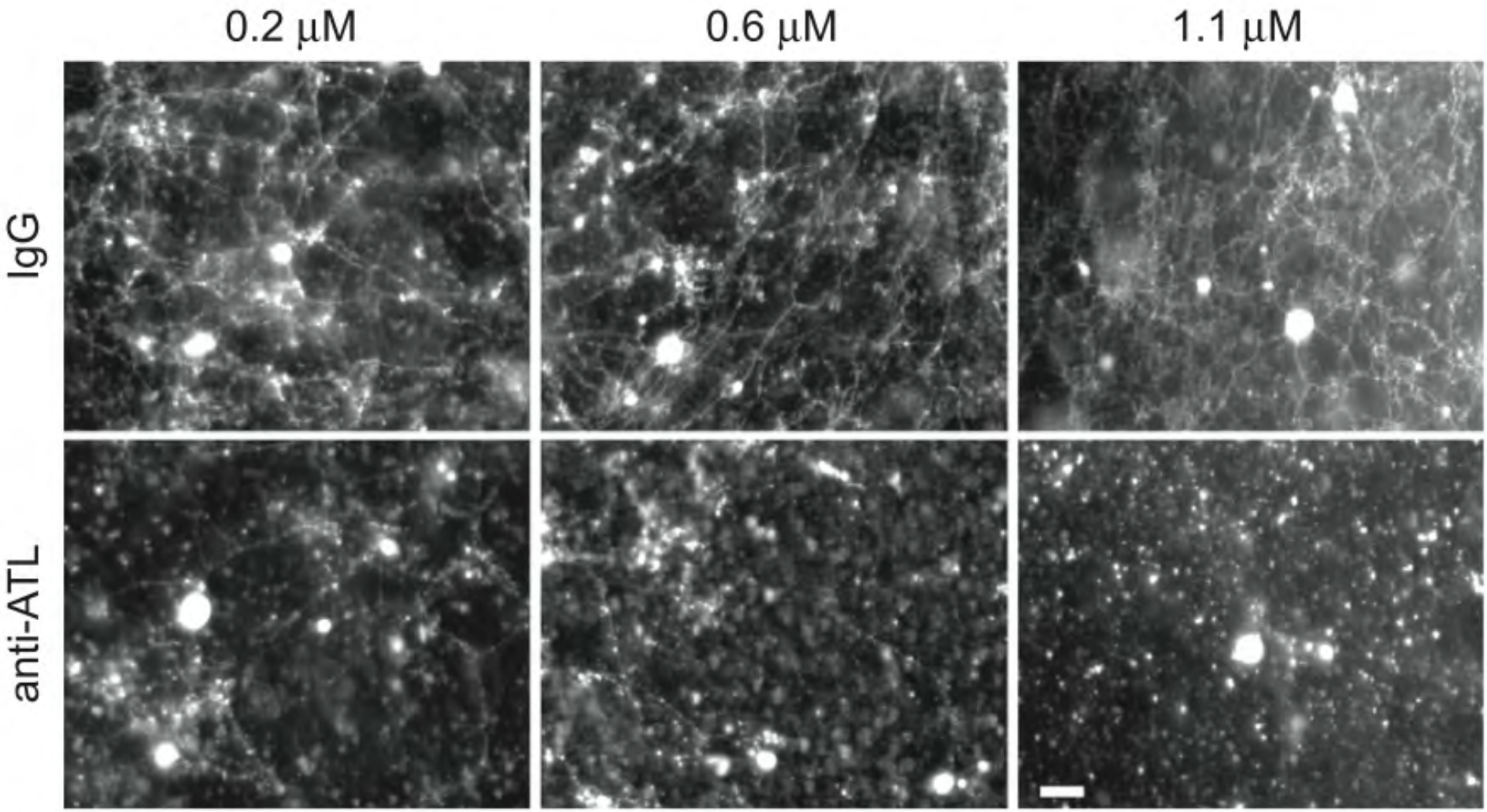
- Due to distal degeneration of the corticospinal tracts (not much cell death). Appears to be length-dependent.

- Over 40 loci linked to HSP. Includes spastin, atlastin, KIF5A, REEP1, HSP60, paraplegin.

- Processes involved: intracellular trafficking, ER structure, mitochondrial quality control.

Blackstone et al. (2011) Nat Rev Neuroscience

Atlastin antibodies inhibit ER network formation in vitro

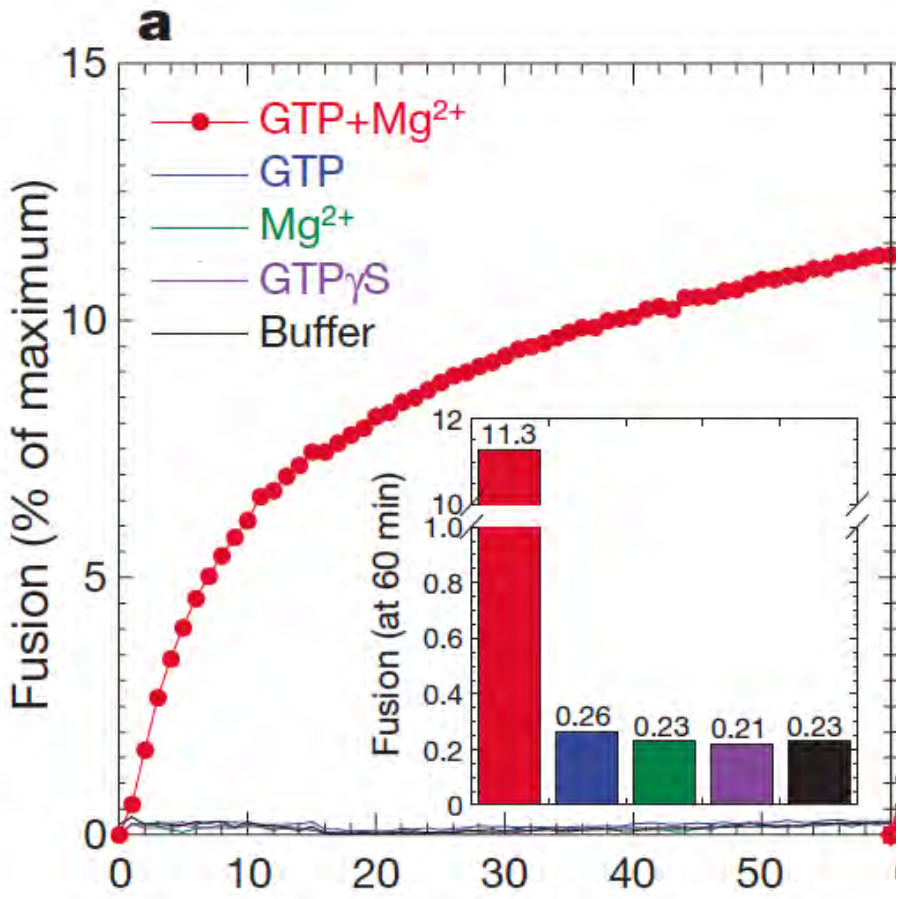


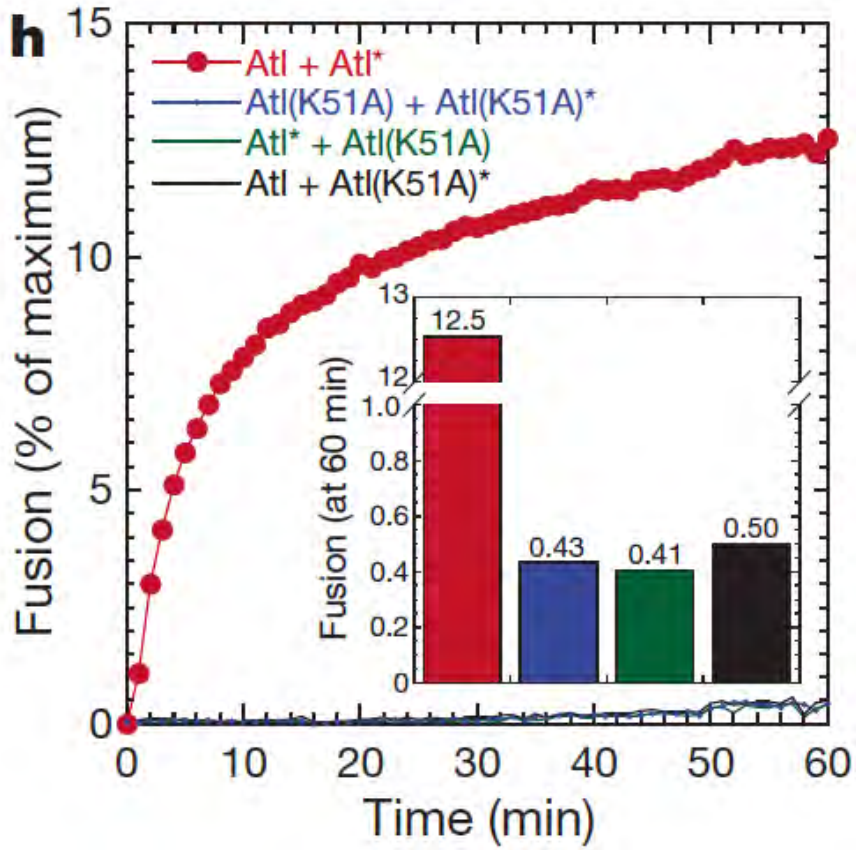
in vitro ER network assay from Xenopus
control antibodies versus pan anti-atlastin antibodies

Hu et al. (2011) Cell

Atlastins can mediate ER fusion in vitro

Could atlastins influence ER morphology by mediating homotypic ER fusion?





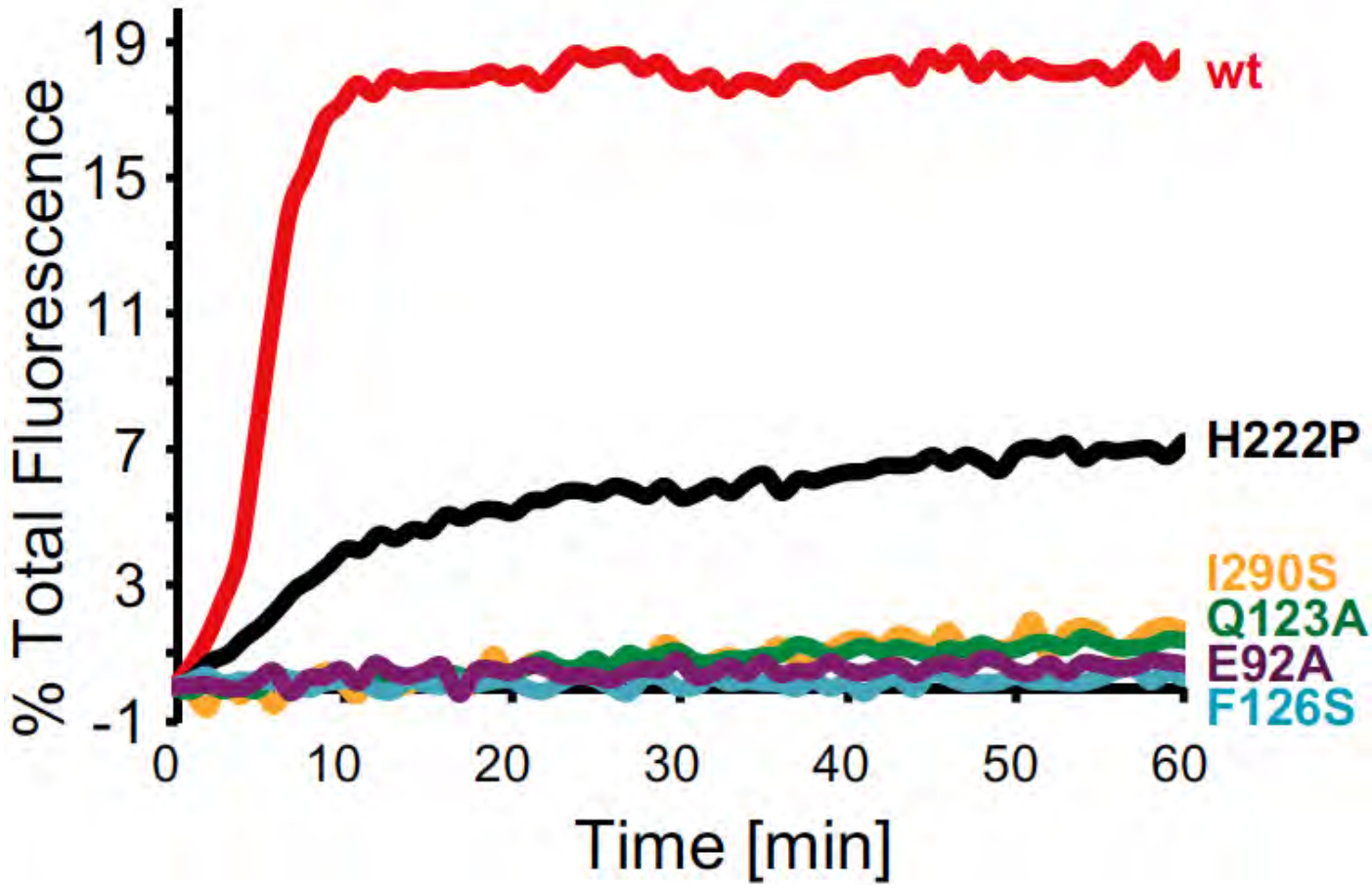
Unlabeled liposomes containing atlastin were mixed with NBD-labeled liposomes containing atlastin.

Detergent was added to determine max fluorescence.

Atlastin alleles associated with HSP

Orso et al. (2009) Nature

Disease alleles of atlastin are defective in fusion assay

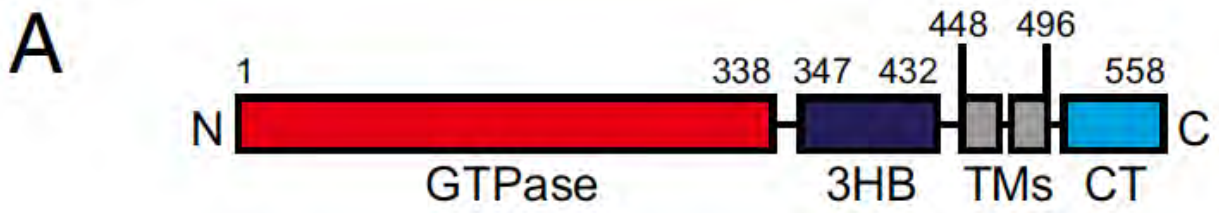


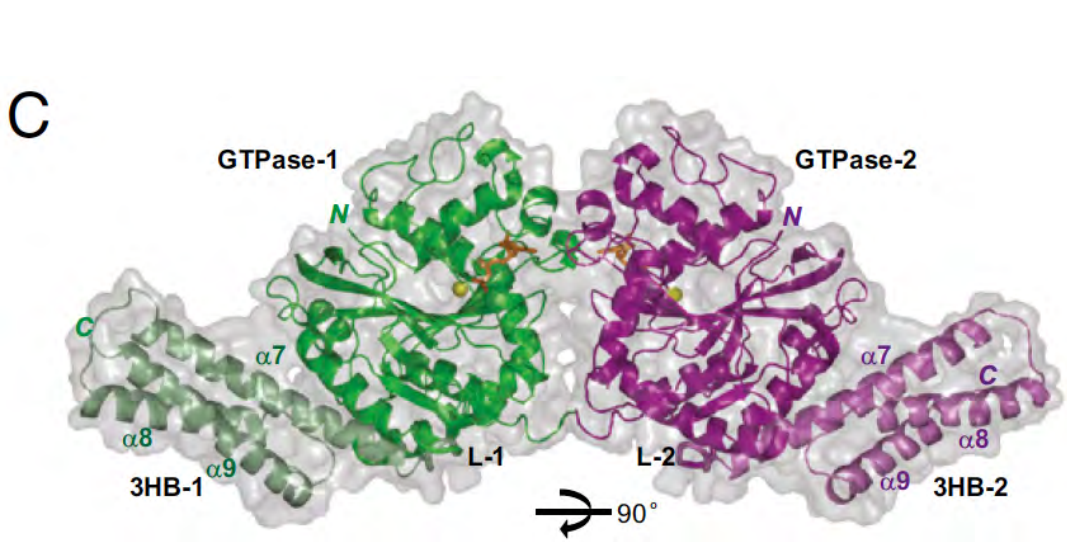
F126S, H222P, and 290S are disease alleles.

The others are GTPase mutants.

Bian et al. (2011) PNAS

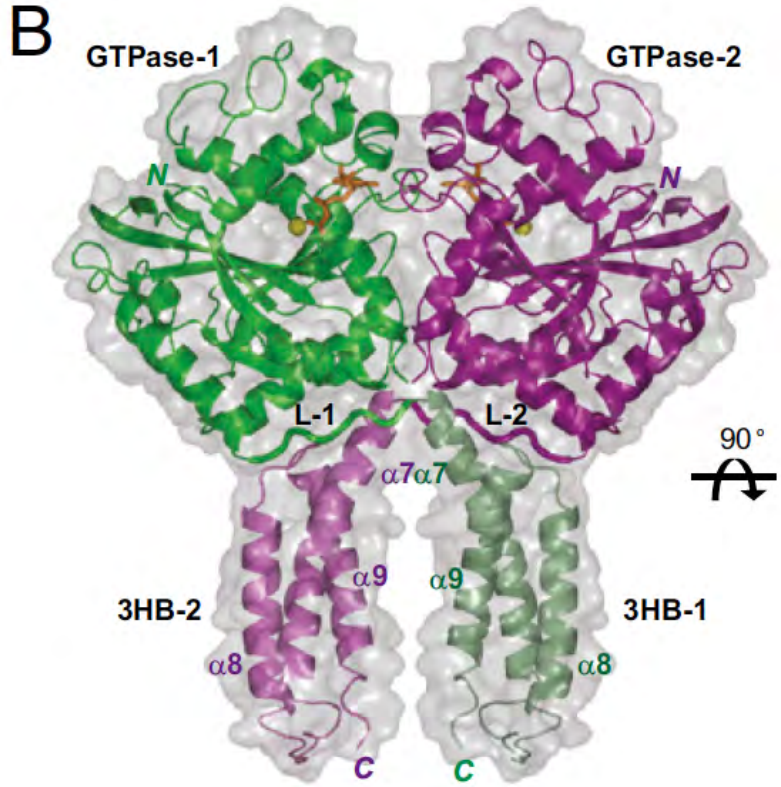
Structures of atlastin in 2 conformations





GDP + P_i

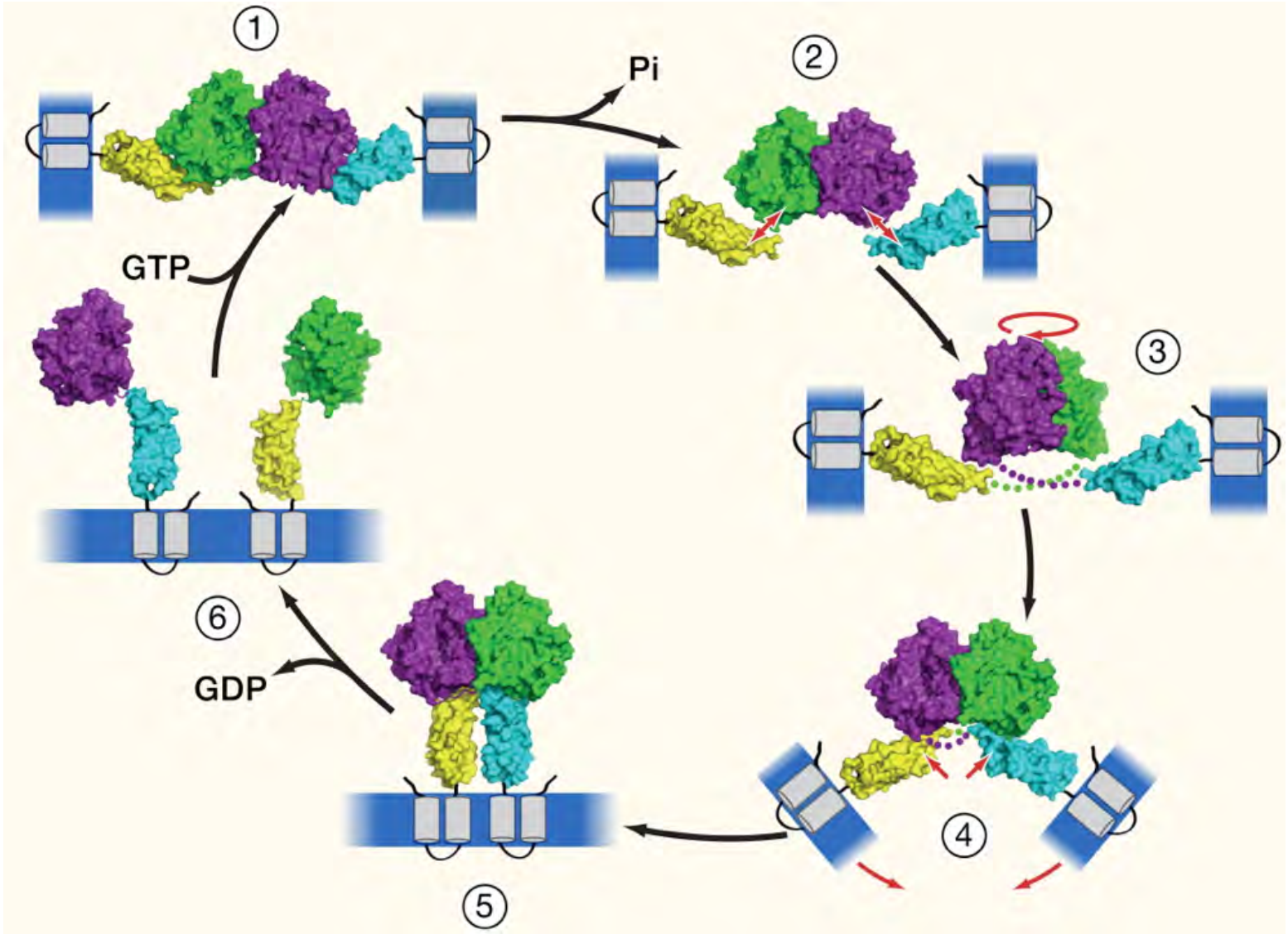
Pre-fusion state



GDP-bound, post-fusion state

Bian et al. (2011) PNAS

Model for atlastin-mediated ER fusion



Hu et al. (2011) Cell

Targeting of integral membrane proteins

• Hydrophobic transmembrane (TM) domains are synthesized in cytosol and must be transported to more energetically favorable lipid bilayer. Several problems to solve:

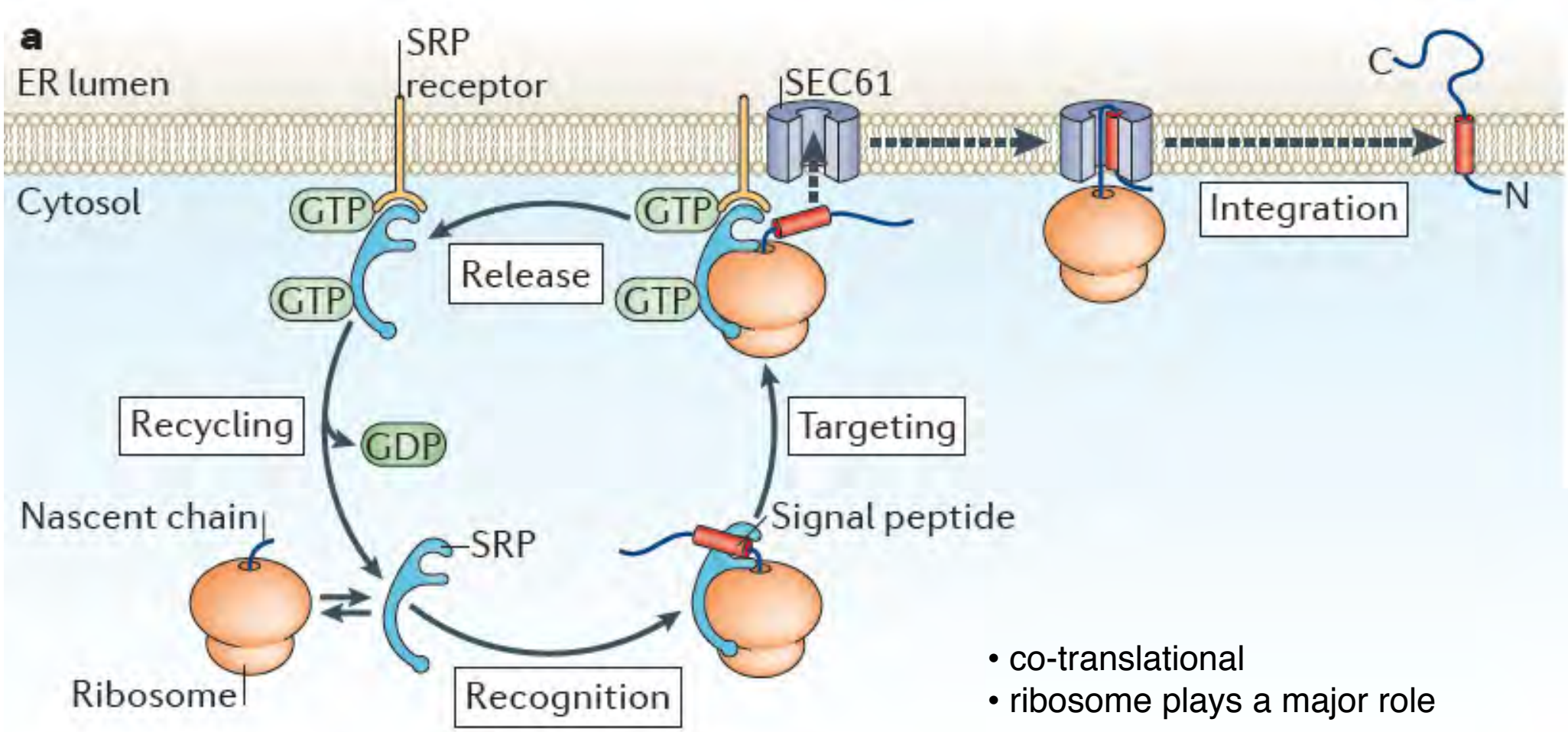
• Recognize TM domains

• Shielding/stabilizing TM domains in the cytosol

• Targeting to the correct membrane

• Integration of the TM domain into the membrane in the correct topology

Standard co-translational transport into the ER



- co-translational
- ribosome plays a major role

- SRP recognizes signal peptide in nascent polypeptide chain
- SRP receptor/SRP interaction is GTP dependent and targets ribosome to ER surface
- Translation provides force to translocate polypeptide through Sec61

Hegde & Keenan (2011) NRMCB

Standard co-translational transport into the ER

• Recognize TM domains: SRP recognizes signal sequence through hydrophobic domain

• Shielding/stabilizing TM domains in the cytosol: SRP

• Targeting to the correct membrane: SRP/SRP receptor interactions

• Integration of the TM domain into the membrane in the correct topology: subsequent TM domains are shielded and inserted without exposure to cytosol, due to action of Sec61.

•Translation by ribosome provides directionality.

• Most large or multi-TM proteins are targeted via the co-translational mechanism.

Some ER proteins cannot use the co-translational mechanism

• Some ER proteins have TM domain near the C-terminus (termed **tail-anchored** proteins, or **TA** proteins). Examples: v-SNAREs, t-SNAREs, components of the Sec61 complex.

•Incompatible with co-translational mechanism and classical signal hypothesis, because the ribosome tunnel is 80-100 angstroms long and shields the last ~40 amino acids.

•Consistent with this idea, synaptobrevin targeting does not require Sec61 and SRP.

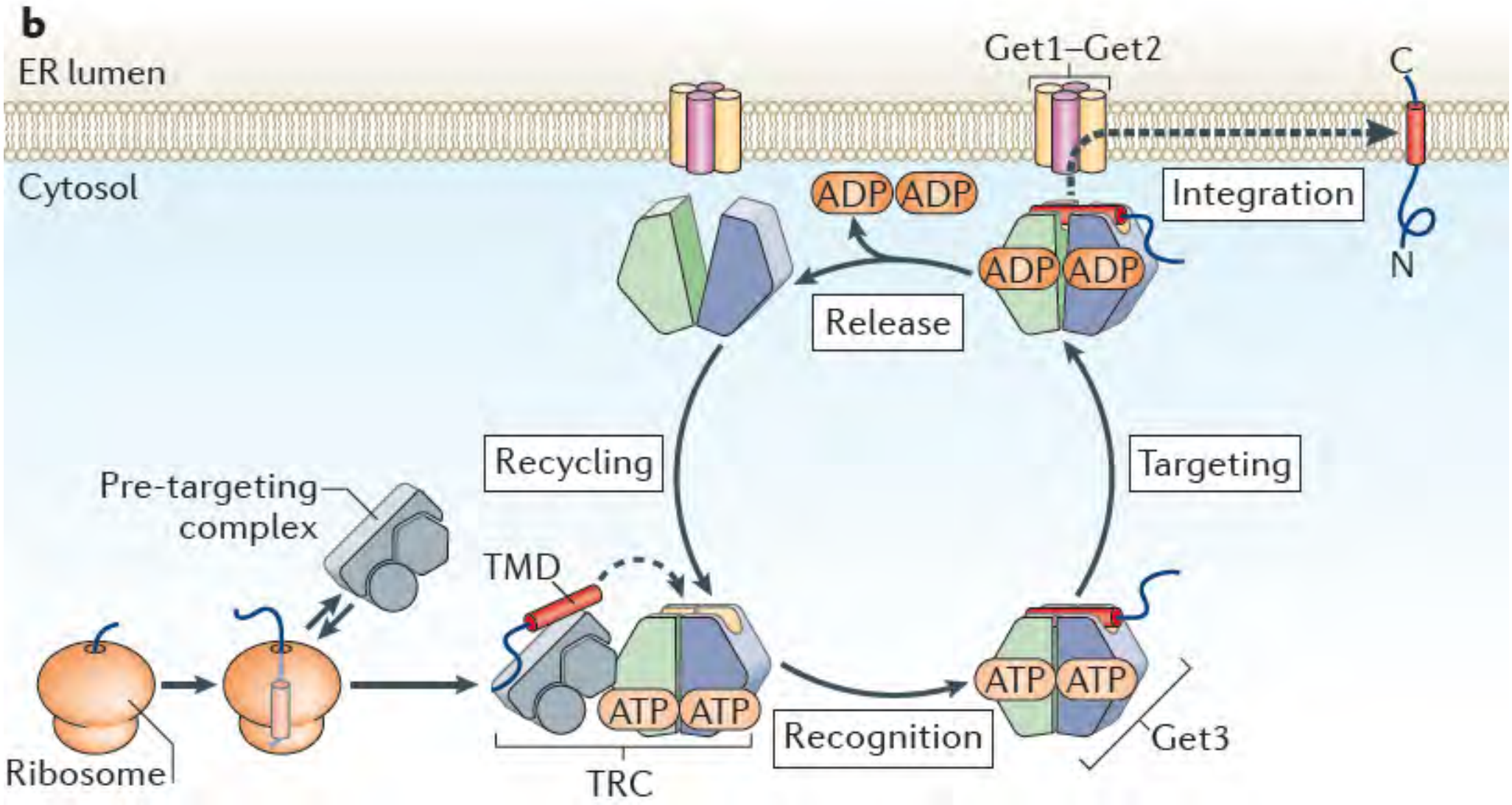
• TA proteins comprise 3-5% of membrane proteins.

• An ATPase complex (containing Get3) found to be involved in TA transport

• Get pathway: "guided entry of TA proteins"

• Genetic studies in yeast:
    early steps: Get3, Get4, Get5, Sgt2
    late steps: Get3, Get1, Get2

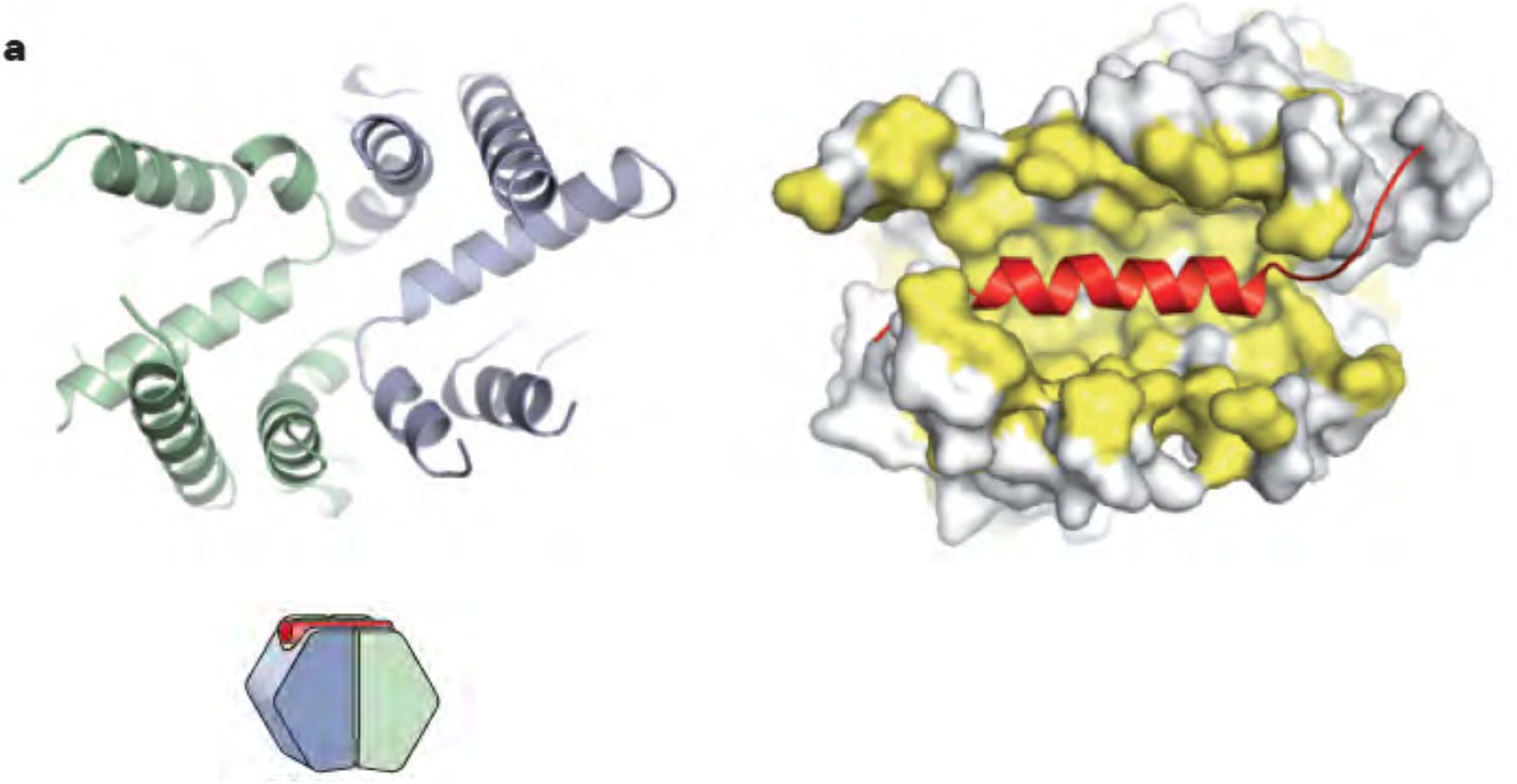
Import of tail-anchored proteins into the ER



- post-translational
- soluble pre-targeting complex captures polypeptide
- polypeptide loaded to Get3 (ATPase)
- Get3 targets to the membrane for interaction with Get 1/2

Hegde & Keenan (2011) NRMCB

The ATP bound form of Get3 has a hydrophobic groove for binding to TM domains

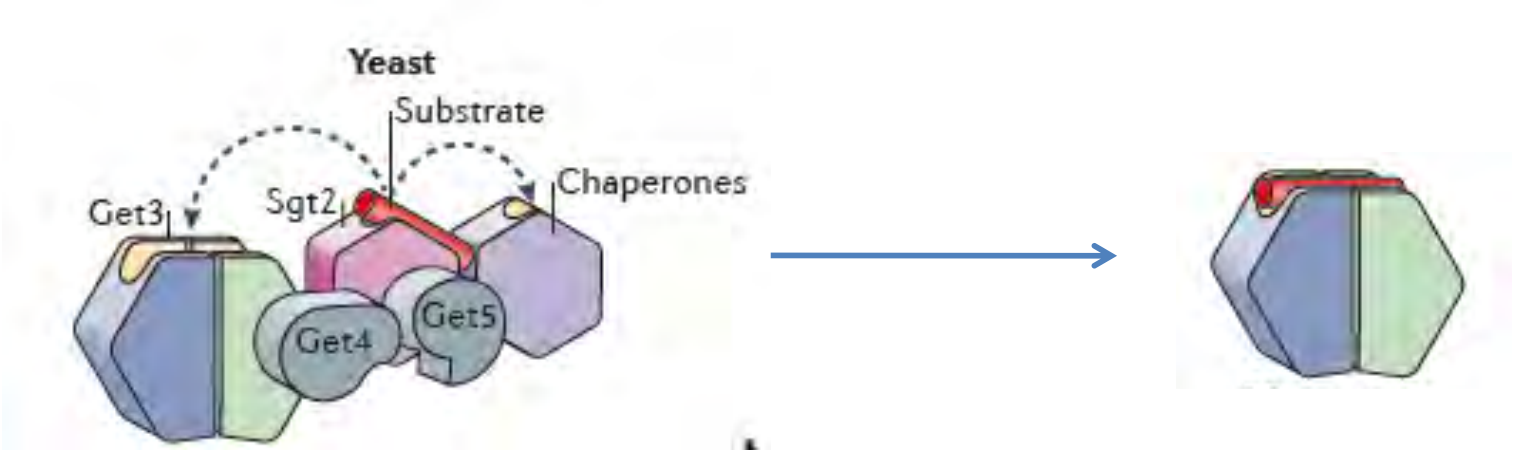


Hegde & Keenan (2011) NRMCB

Transport of TA proteins into the ER

• Recognize TM domains: the pretargeting complex of Get4,Get5, Sgt2

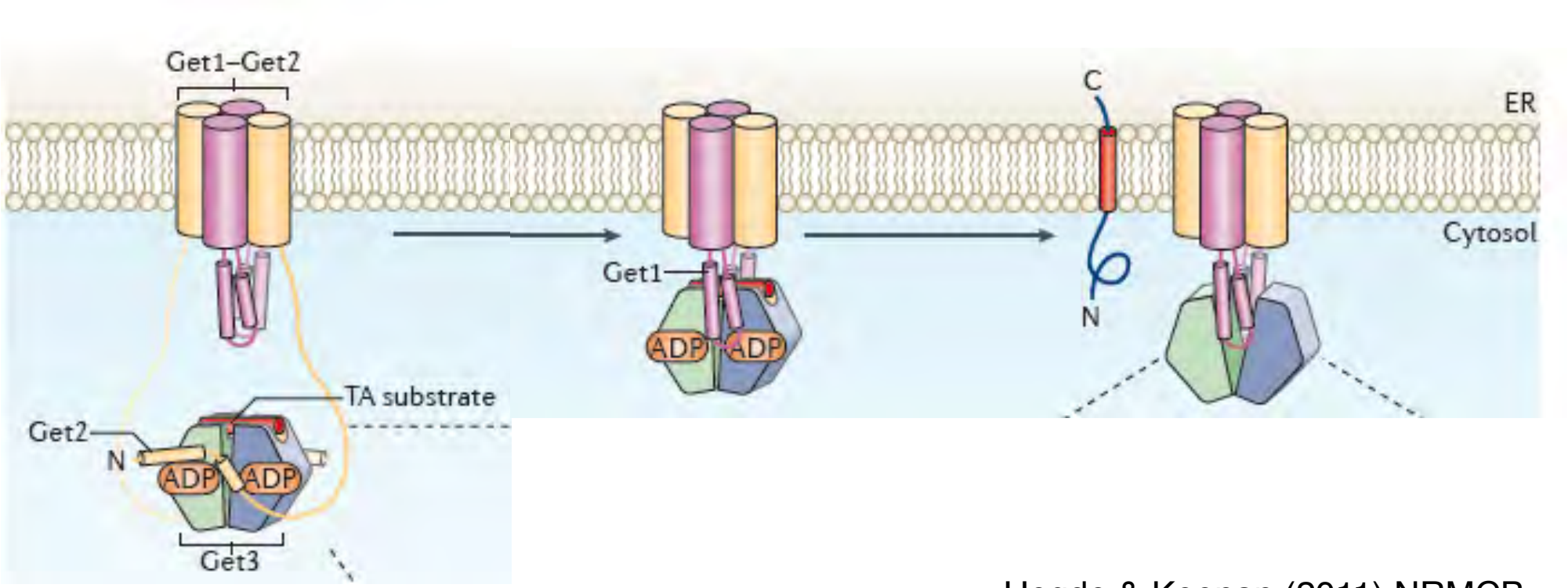
• Shielding/stabilizing TM domains in the cytosol: the pretargeting complex of Get4,Get5, Sgt2; followed by transfer to Get3 ATPase



Hegde & Keenan (2011) NRMCB

Transport of TA proteins into the ER

- Targeting to the correct membrane: Get3 interactions with Get1/Get2
- Integration of the TM domain into the membrane in the correct topology: unclear
- The timing of these events are likely controlled by the ATPase cycle of Get3



Hegde & Keenan (2011) NRMCB

ER protein quality control

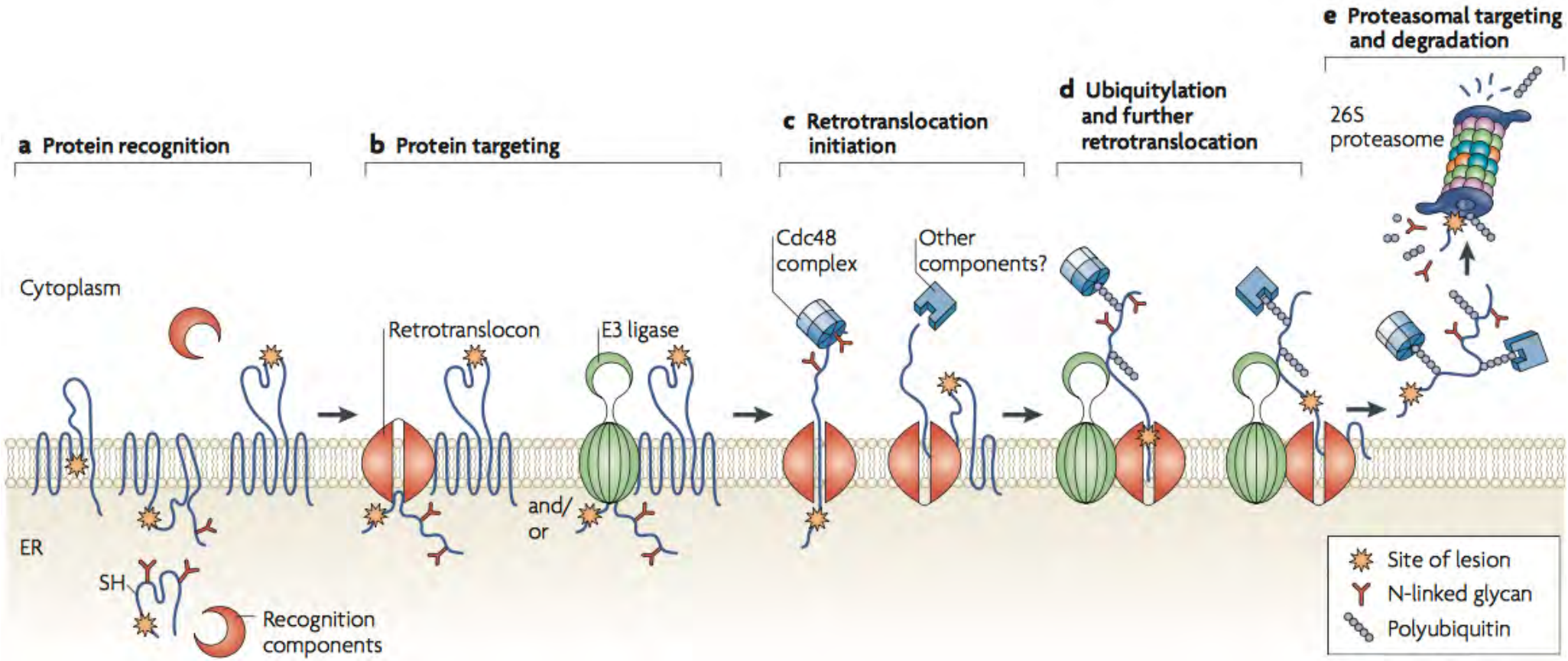
• Misfolded, damaged, or improperly modified proteins in the ER need to be degraded. This included soluble and TM proteins.

• Many of these proteins are removed from the ER for degradation in the cytosol.

• **ER associated protein degradation (ERAD)**

  • Several pathways: ERAD-L, -M, -C (depending on misfolded domain being in the lumen, within the membrane, or on cytosolic side.

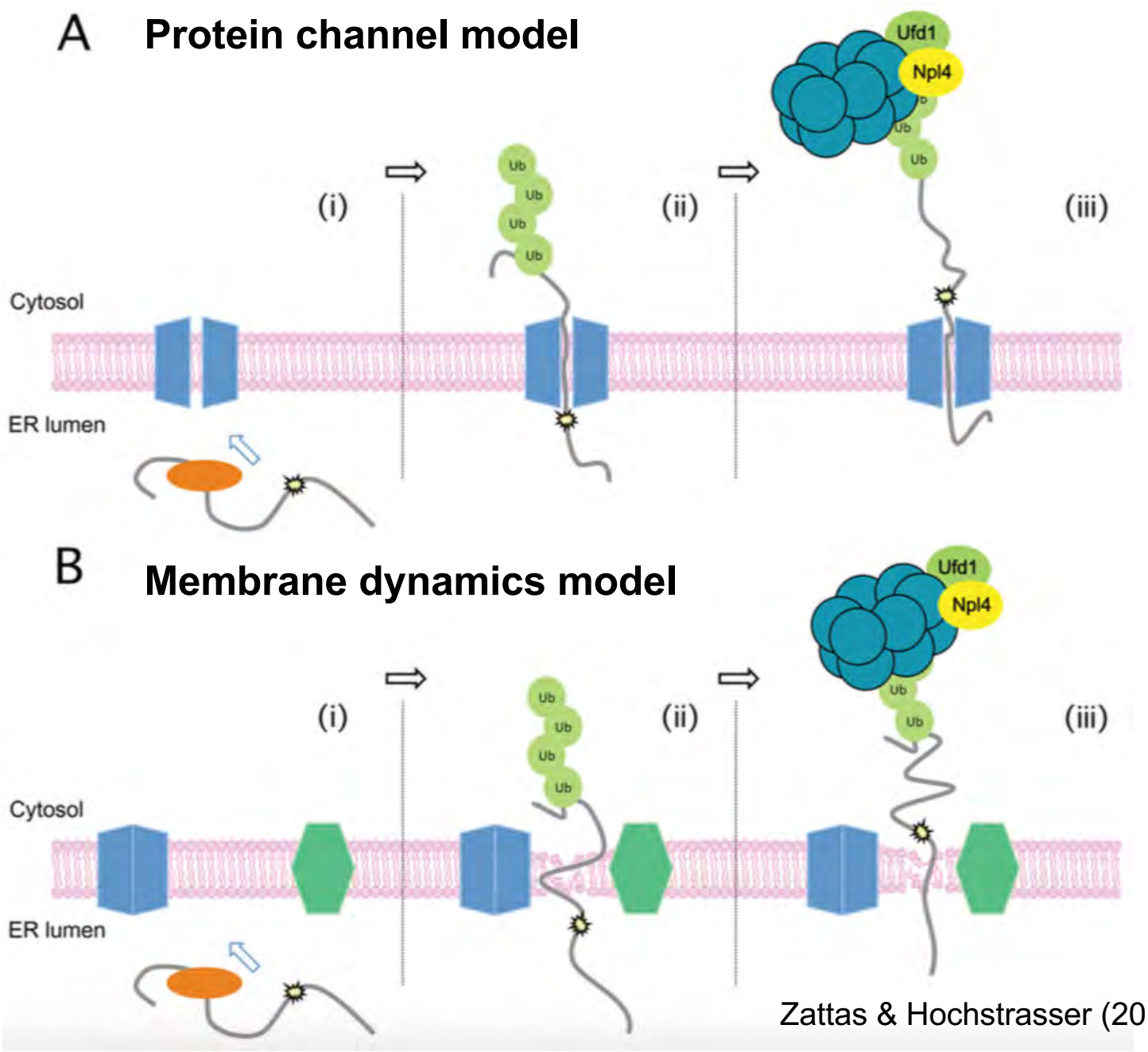
ER associated degradation (ERAD)



- **Substrate recognition**: only misfolded/unfolded proteins are targeted
- **Retrotranslocation**: misfolded protein reverse-translocated across ER
- **Polyubiquitination:** occurs on cytosolic side (E3 ligase)
- **Membrane extraction**: the p97 AAA ATPase
- **Degradation**: the 26S proteasome (UPS)

Vembar & Brodsky (2008) Nat Rev MCB

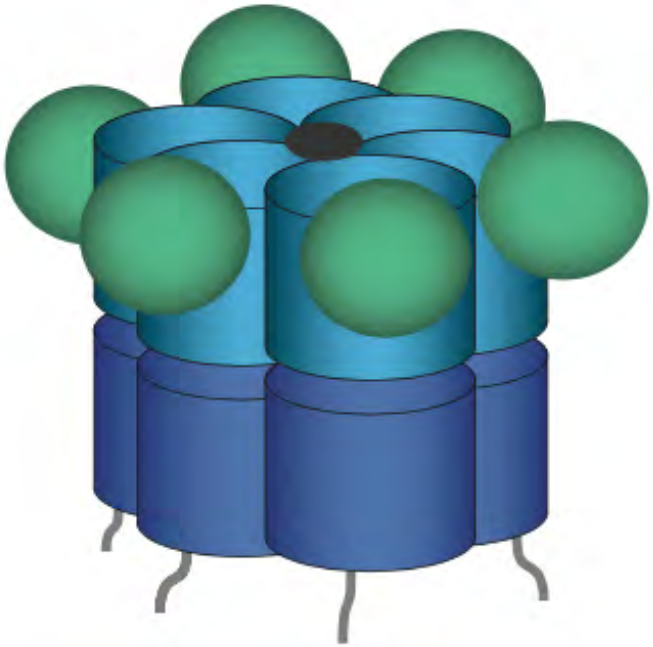
Models of retrotranslocation

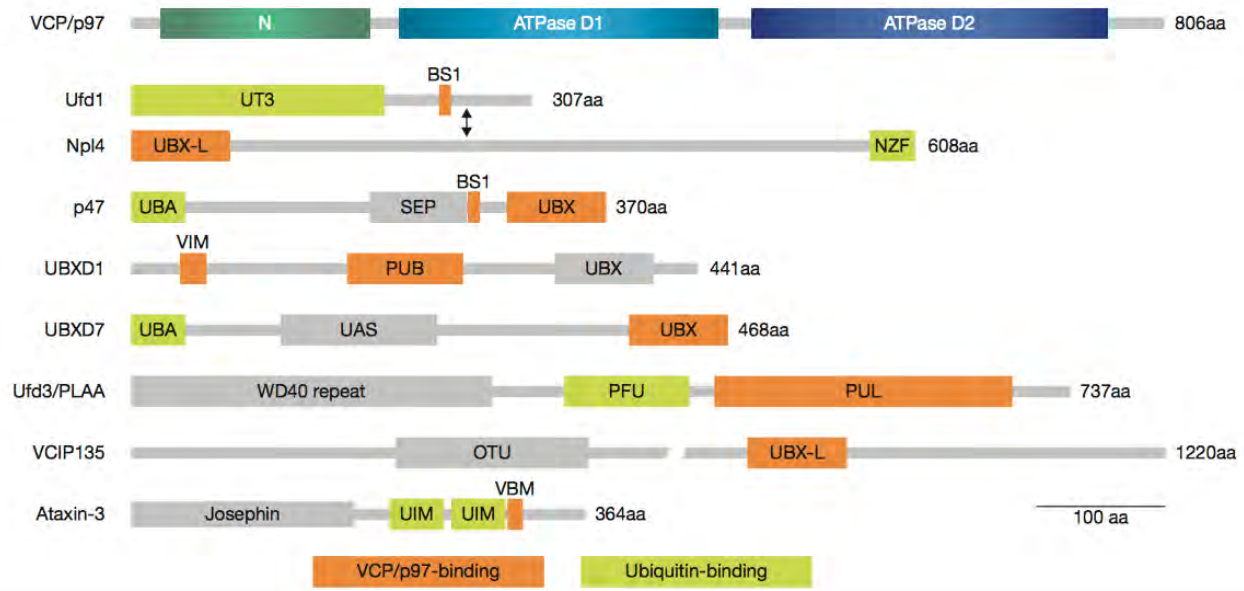


- Recent experiments suggest in yeast that the transmembrane E3 ligase Hrd1 (6 TMs) can function in retrotranslocation.
- Retrotranslocation and membrane extract may be distinct steps
- Roles for Ub (Brownian racket model) and p97

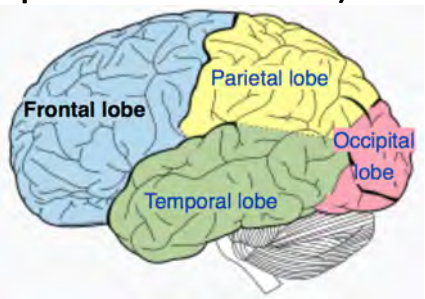
Zattas & Hochstrasser (2015) Crit Rev Biochem Mol Biol

p97/VCP is a AAA ATPase with many functions



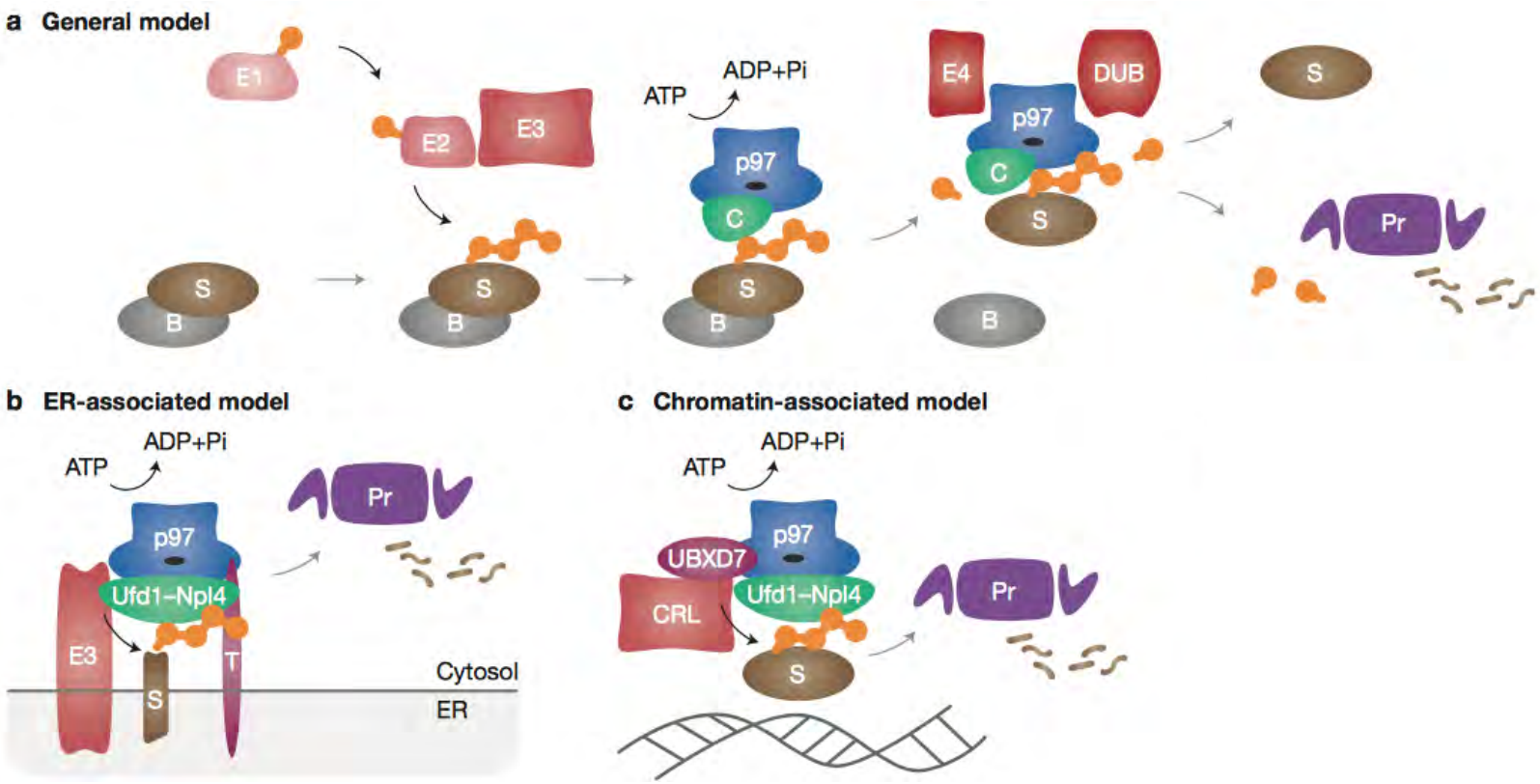


- p97/VCP (valosin-containing protein), Cdc48 in yeast
- Highly homologous to NSF.
- D2 has higher ATPase activity than D1.
- p97 operates with many adaptors/co-factors.
- Mutations in p97 cause IBMPFD (inclusion body myopathy associated with Paget disease of the bone and frontotemporal dementia)



Meyer et al (2012) Nat Cell Biol

p97 often functions between ubiquitylation and the 26S proteasome



- p97 associates with ubiquitylated substrates that need remodeling before delivery to the 26S proteasome.
- Substrates might be in stable complexes or associated with surface.

Meyer et al (2012) Nat Cell Biol