Chemical and genetic interference

Summer School 2017

Silke Robatzek lab

Gildas Bourdais

How to determine the endocytic pathway of specific proteins?

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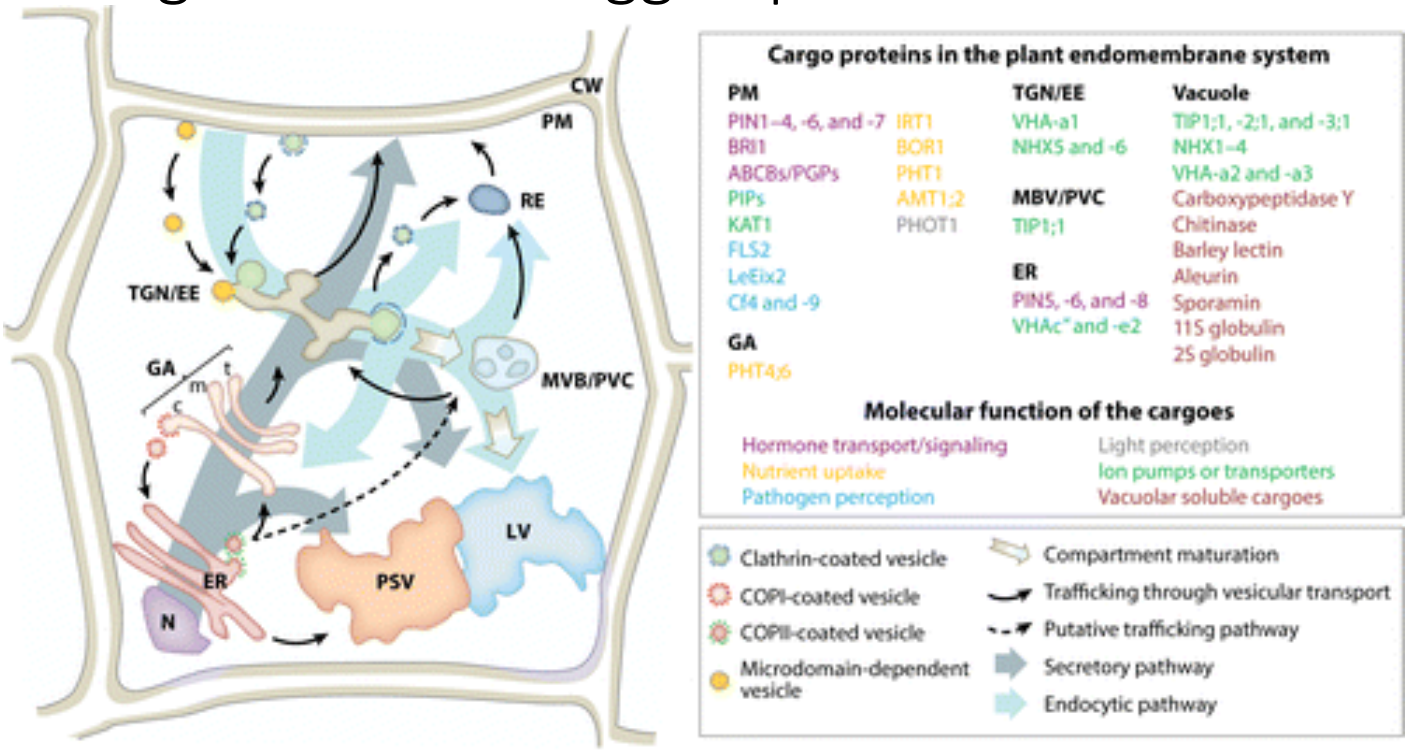
Monitoring of the GFP-tagged protein of interest in a cell



Norambuena L, Tejos R. 2017.
Annu. Rev. Plant Biol. 68:197–224

How to determine the endocytic pathway of specific proteins?

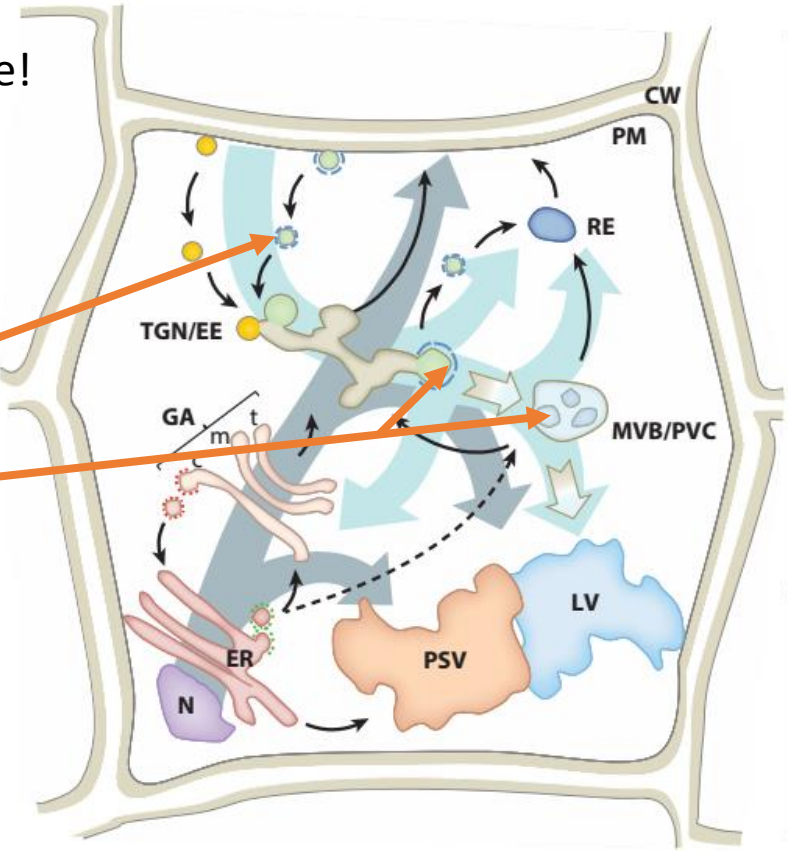
Monitoring of the GFP-tagged protein of interest in a cell

List not exhaustive!

RAB5 GTPase

ARA7

ARA6



Cargo proteins in the plant endomembrane system

PM		TGN/EE	Vacuole
PIN1–4, -6, and -7	IRT1	VHA-a1	TIP1;1, -2;1, and -3;1
BRI1	BOR1	NHX5 and -6	NHX1–4
ABCBs/PGPs	PHT1		VHA-a2 and -a3
PIPs	AMT1;2	MBV/PVC	Carboxypeptidase Y
KAT1	PHOT1	TIP1;1	Chitinase
FLS2			Barley lectin
LeEix2		ER	Aleurin
Cf4 and -9		PIN5, -6, and -8	Sporamin
GA		VHAc″ and -e2	11S globulin
PHT4;6			2S globulin

Molecular function of the cargoes

Hormone transport/signaling	Light perception
Nutrient uptake	Ion pumps or transporters
Pathogen perception	Vacuolar soluble cargoes

Clathrin-coated vesicle	Compartment maturation
COPI-coated vesicle	Trafficking through vesicular transport
COPII-coated vesicle	Putative trafficking pathway
Microdomain-dependent vesicle	Secretory pathway
	Endocytic pathway

Norambuena & Tejos 2017

How to determine the endocytic pathway
of specific proteins?

Monitoring of the GFP-tagged protein of interest in a cell

Specific alteration of the pathway using chemicals and genetic

Chemicals that perturb endomembrane trafficking

Table 1 Summary of compounds affecting endomembrane trafficking

Compounds	Organisms	Target	Mode of action	Source
BFA	Plant/animal	ARF-GEFs	Block exocytosis/endomembrane recycling	Dinter and Berger (1998), Robinson et al. (2008)
Wortmannin	Plant/animal	PI3Ks and PI4Ks	Block vacuolar trafficking, MVBs and endocytosis	Robinson et al. (2008), Takáč et al. (2012)
Tyrphostin A23	Plant/animal	Cargo recognition (YXXΦ) by μ subunit of AP complex	Blocks cargo recruitment into CCVs from PM, TGN	Ortiz-Zaoater et al. (2006), Robinson et al. (2008)
Concanamycin A	Plant/animal	V ATPase	Block trafficking at TGN, endosome acidification	Robinson et al. (2008)
Dynasore	Animal	GTPase activity of dynamin	Blocks endocytosis	Macia et al. (2006)
Sortin 1	Yeast/plant	Unknown	Affects vacuole biogenesis	Zouhar et al. (2004)
Sortin 2	Yeast/plant	Unknown	Interference with ESCRT complex components	Zouhar et al. (2004), Norambuena et al. (2008)
Sortin 3	Yeast	Unknown	Possibly target late endosomal compartments	Chanda et al. (2009)
Endosidin 1	Plant	Unknown (stablizes actin)	Blocks trafficking at the TGN	Robert et al. (2008), Toth et al. (2012)
Endosidin 3	Plant	Unknown	Blocks protein trafficking from the PM by affecting TGN/EE compartment	Drakakaki et al. (2011)
Endosidin 5	Plant/animal	Unknown	Blocks recycling	Drakakaki et al. (2011)
Endosidin 7	Plant	Unknown	Disturbs cell plate formation, cell wall maturation and expansion through the perturbation of secretory pathways	Drakakaki et al. (2011)
Gravicin	Plant	PGP19 and other unknown target	Inhibition of auxin transport and trafficking to the vacuole	Surpin et al. (2005), Rojas-Pierce et al. (2007)
LY294002	Plant/animal	PI3Ks	Blocks MVBs, endocytosis	Lee et al. (2008)

Li et al., 2012

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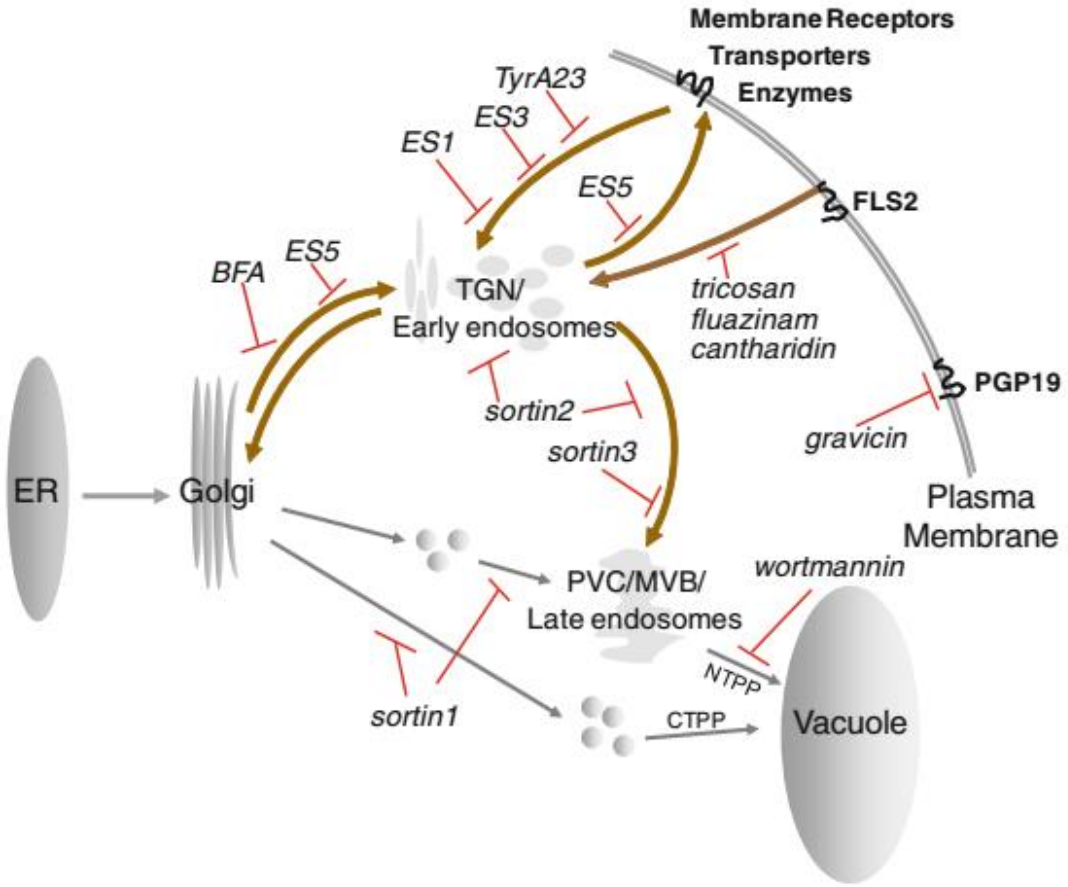
Chemicals that perturb endomembrane trafficking

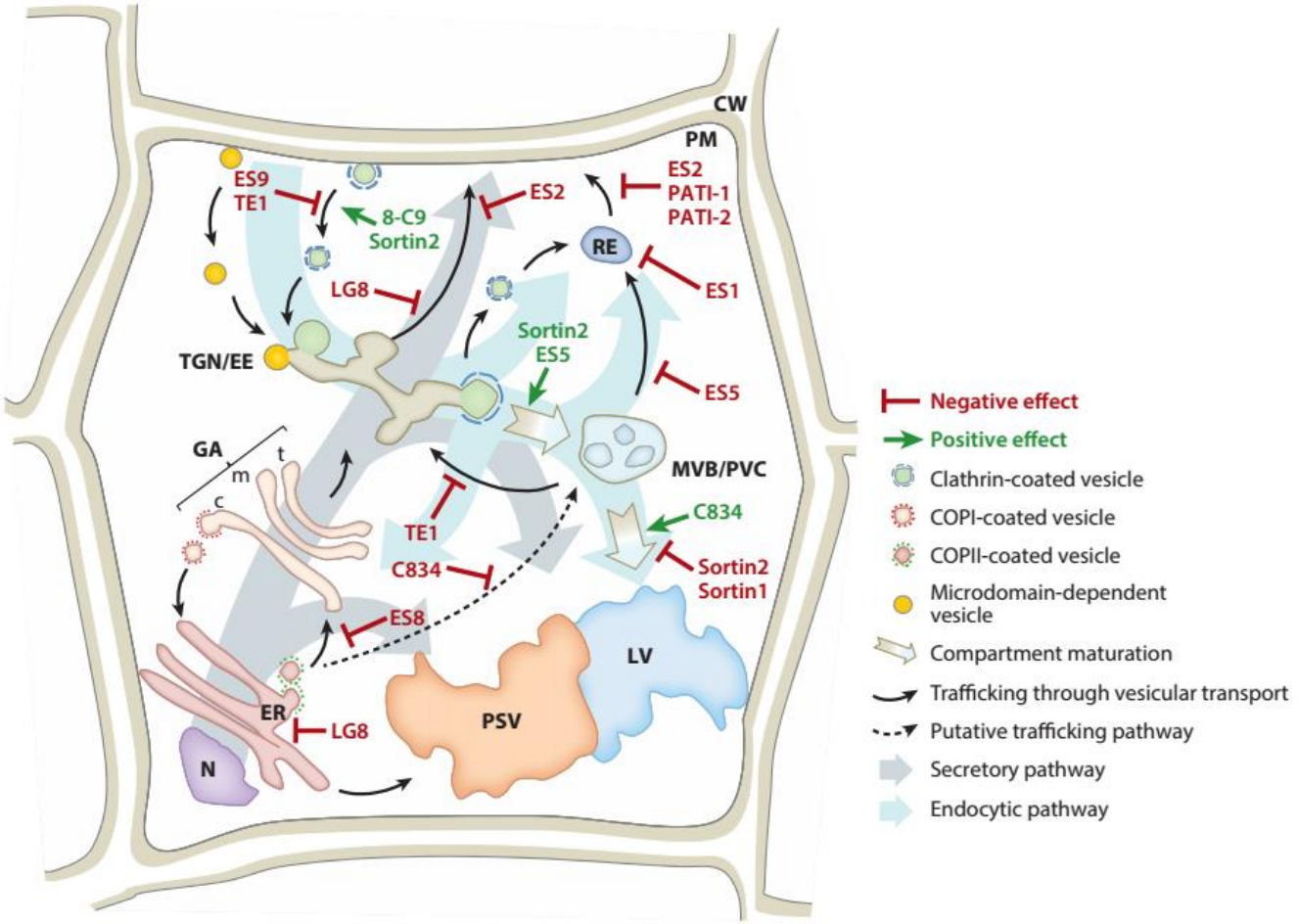
Table 1 (continued)

Compounds	Organisms	Target	Mode of action	Source
Bafilomycin A	Plant/animal	V ATPase	Blocks trafficking at the TGN, causes endosome acidification	Robinson et al. (2008)
Sulfonamide 16D10	Animal	V ATPase	Blocks endocytosis and exocytosis, causes endosome acidification	Nieland et al. (2004)
Monensin	Plant/animal	Ionophore	Blocks exocytosis, disrupts trafficking at Golgi	Diner et al. (1998)
Cantharidin	Plant/animal	PP2A-specific inhibitor	Blocks FLS2 endocytic trafficking	Serrano et al. (2007)
Triclosan	Plant	Enoyl-acyl carrier protein (acp) reductases (ENRs)	Blocks FLS2 endocytic trafficking, flg22-induced oxidative burst; inhibitor of fatty-acid synthesis	Serrano et al. (2007)
Fluazinam	Plant	Unknown	Blocks FLS2 endocytic trafficking, flg22-induced oxidative burst	Serrano et al. (2007)
Exo1and Exo2	Animal	Unknown	Block exocytosis, ER to Golgi transport	Nieland et al. (2004)
Cobtorin	Plant	Unknown	Affects cortical microtubule alignment	Yoneda et al. (2007)
Triclosan, Fluazinam and Cantharidin	Plant	PP2A (cantharidin)	Affect flg22-mediated FLS2 endocytosis	Serrano et al. (2007)
Morlin	Plant	Unknown	Affects cytoskeletal organization/ interaction with cellulose synthase	DeBolt et al. (2007)

Li et al., 2012

Chemicals that perturb endomembrane trafficking





Li et al., 2012

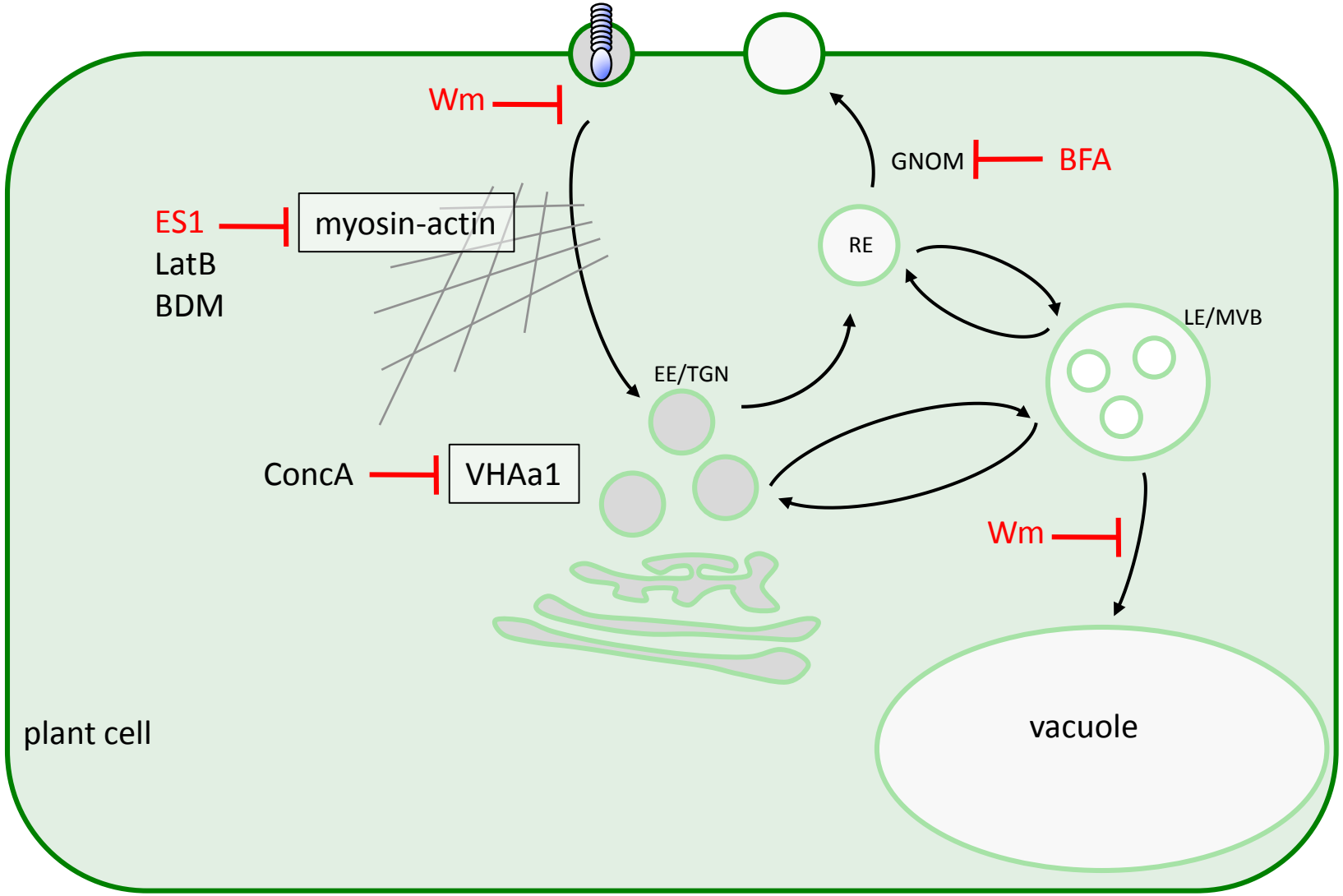
Norambuena & Tejos 2017

Inhibitors of the endocytic pathway

EE/TGN: Early Endosome/ Trans-Golgi Network

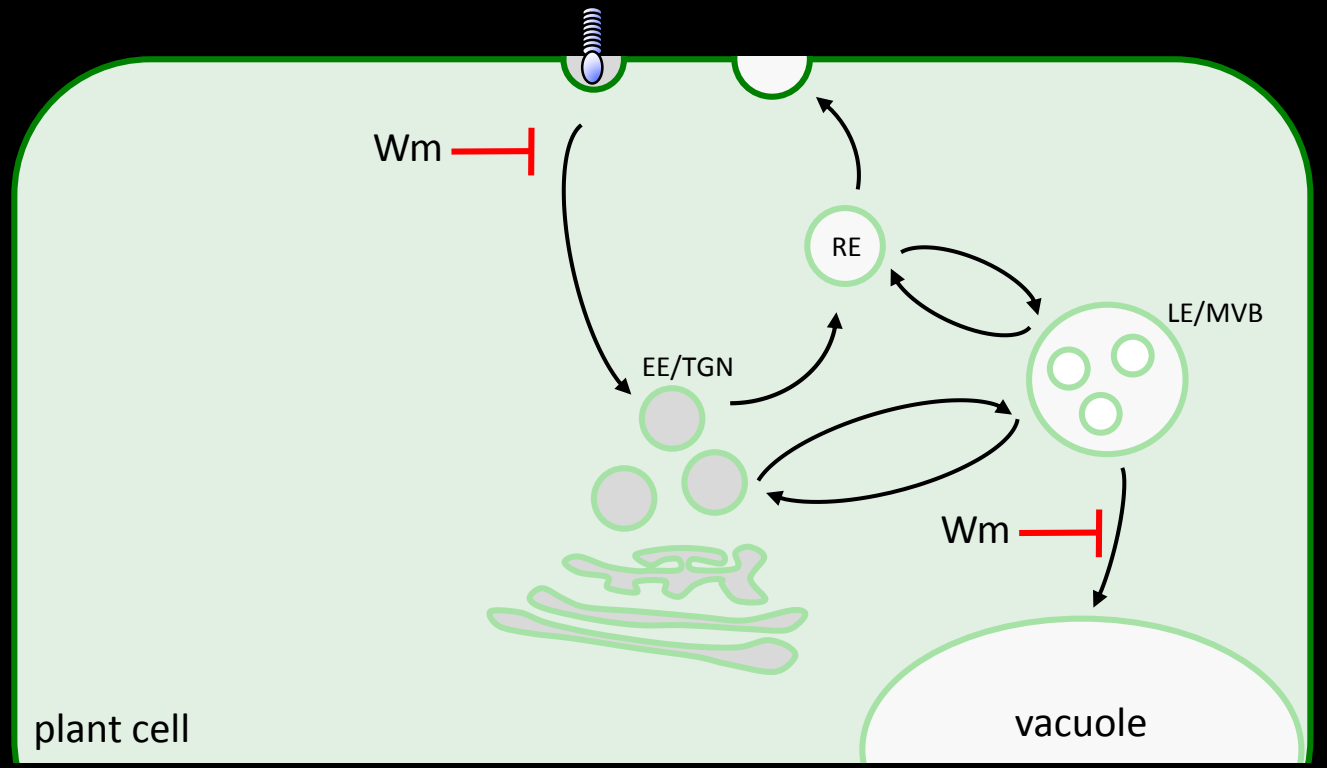
RE: Recycling Endosome

LE/MVB: Late Endosome/ Mulivesicular Body

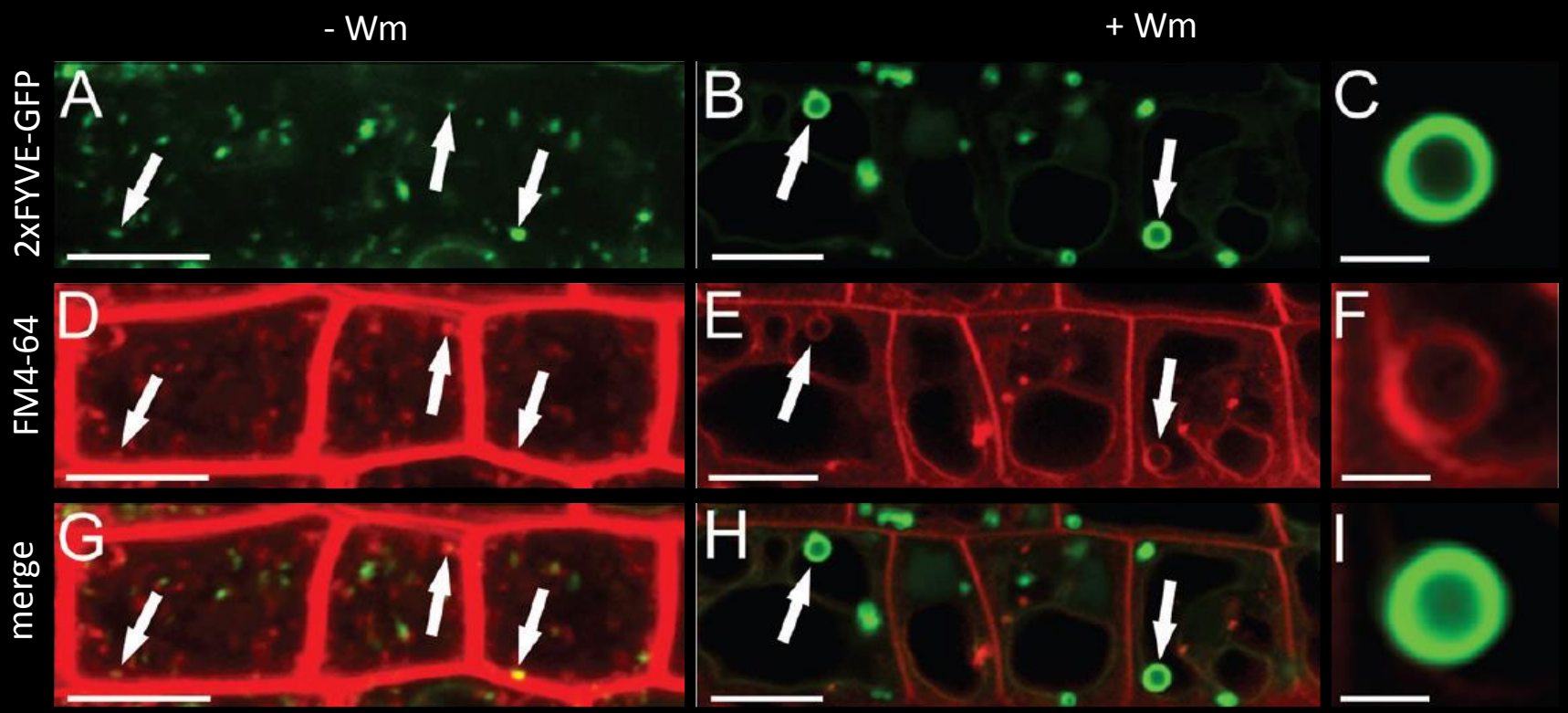


Wortmannin (Wm)

- A phosphatidylphosphate-3-kinase inhibitor that interferes with vesicle formation from the plasma membrane and the maturation of late endosomes (LEs) and multivesicular bodies (MVBs), resulting in their enlargement.



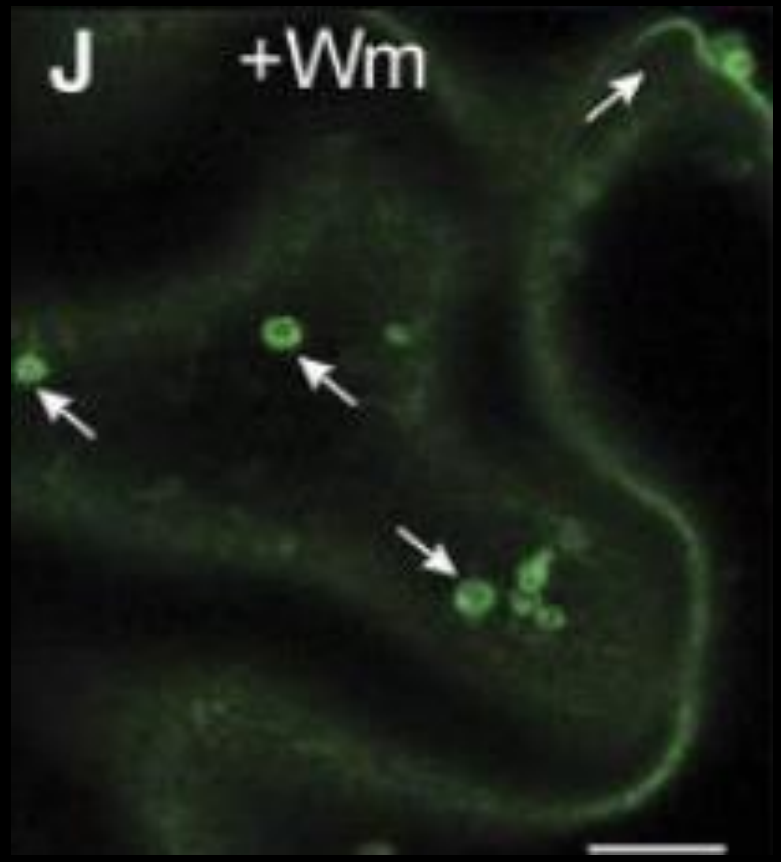
An effect of Wm on intracellular trafficking



Takáč et al., 2012

An effect of Wm on BRI1 trafficking

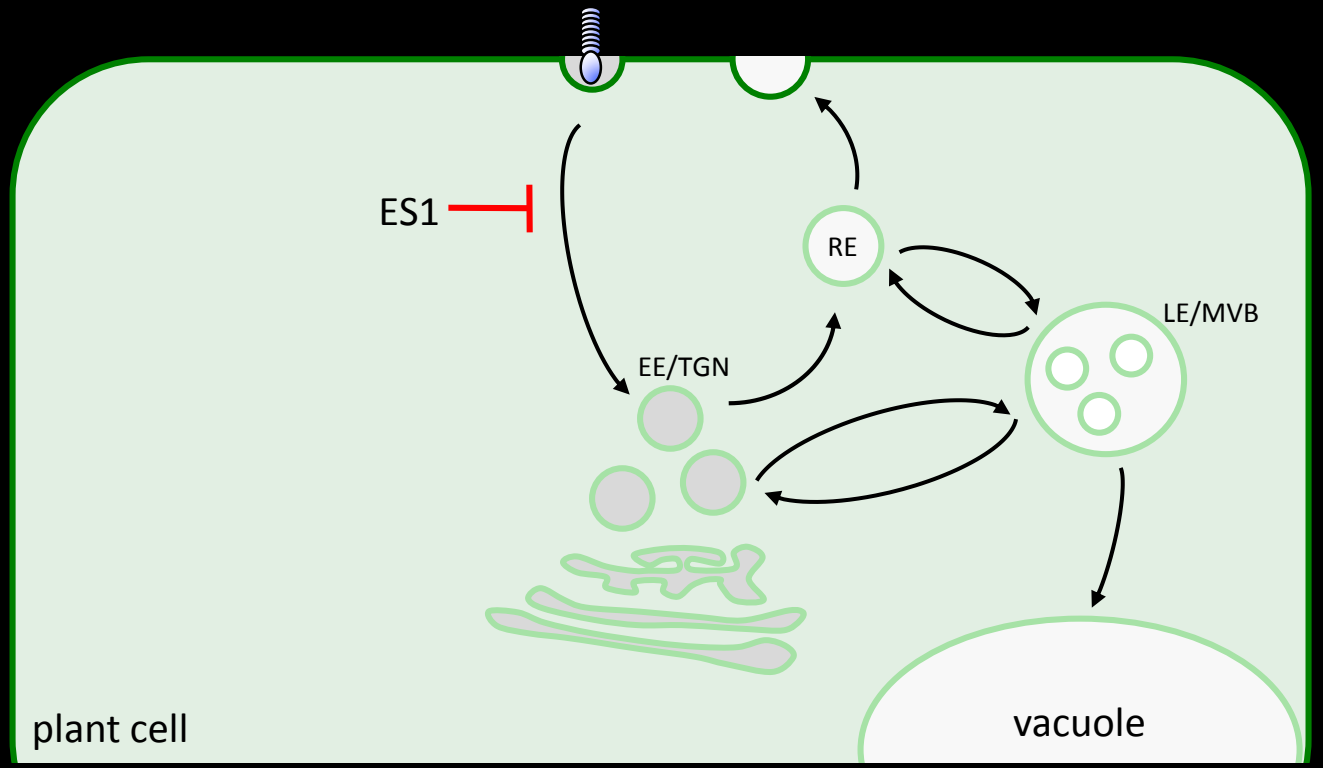
BRI1-sYFP



Irani et al., 2012

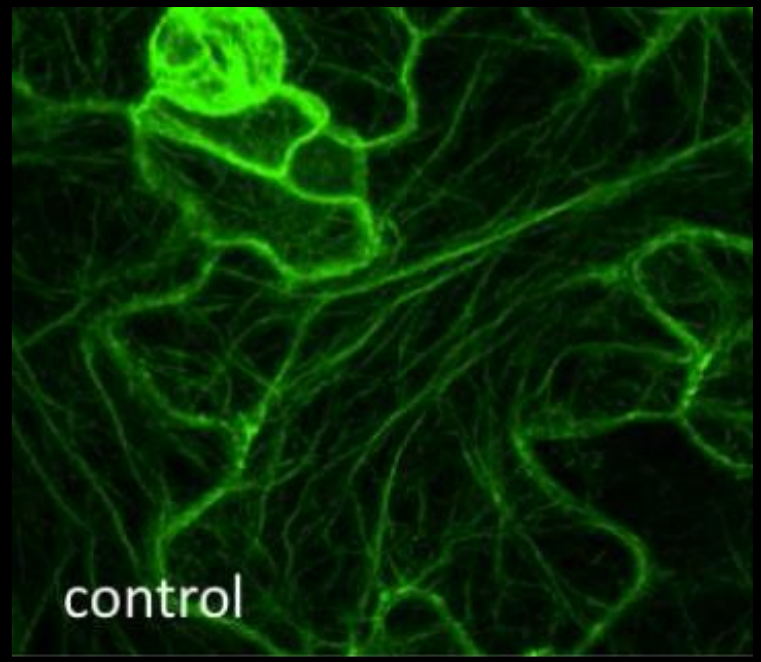
Endosidin 1 (ES1)

- A chemical compound, which selectively blocks specifically the endocytic trafficking of BRI1, PIN2, and AUX1 but not PIN1 and PIN7 at the TGN/EE. In *Arabidopsis* roots, treatment of ES1 leads to the formation of a TGN/EE aggregate.

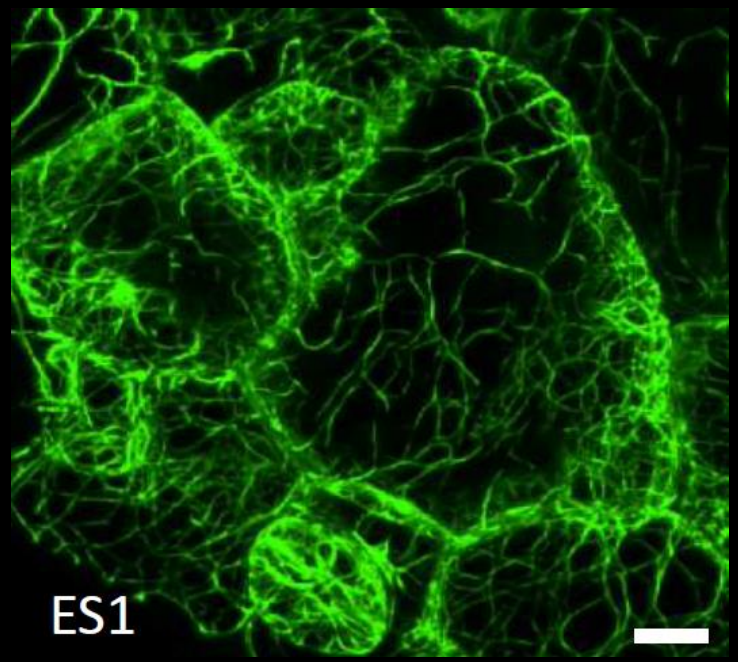


An effect of ES1 on cytoskeleton

GFP-ABD2



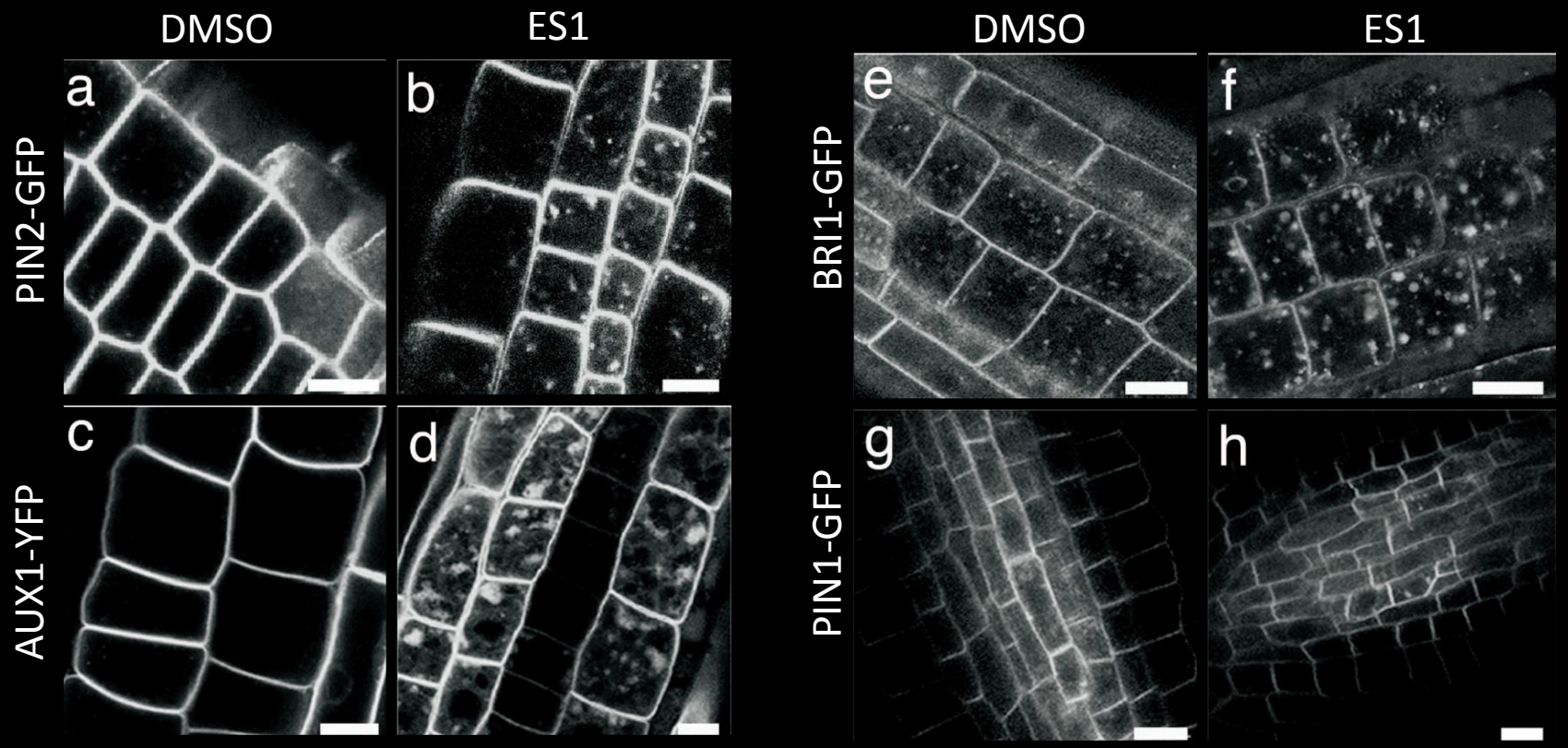
GFP-ABD2



GFP-ACTIN BINDING DOMAIN

Beck et al., 2012

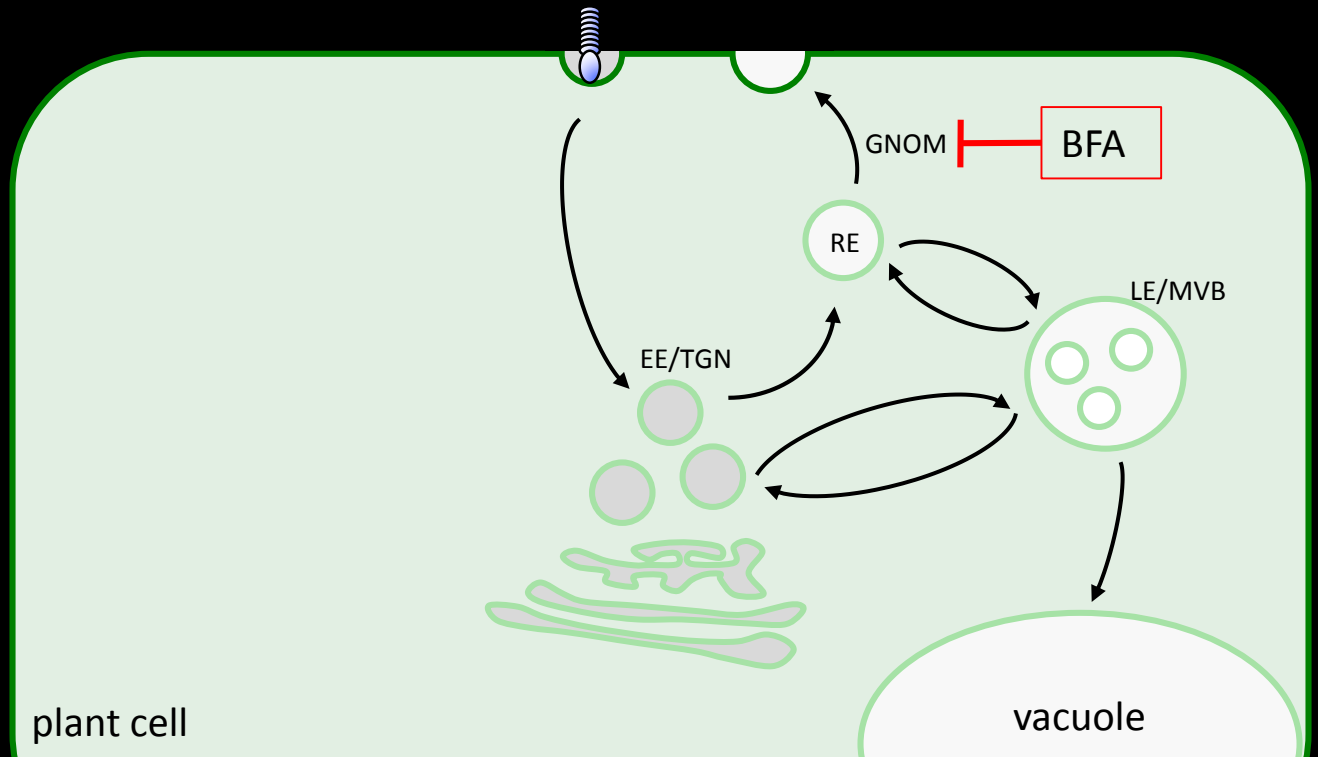
An impact of ES1 on different PM proteins



Robert et al., 2008

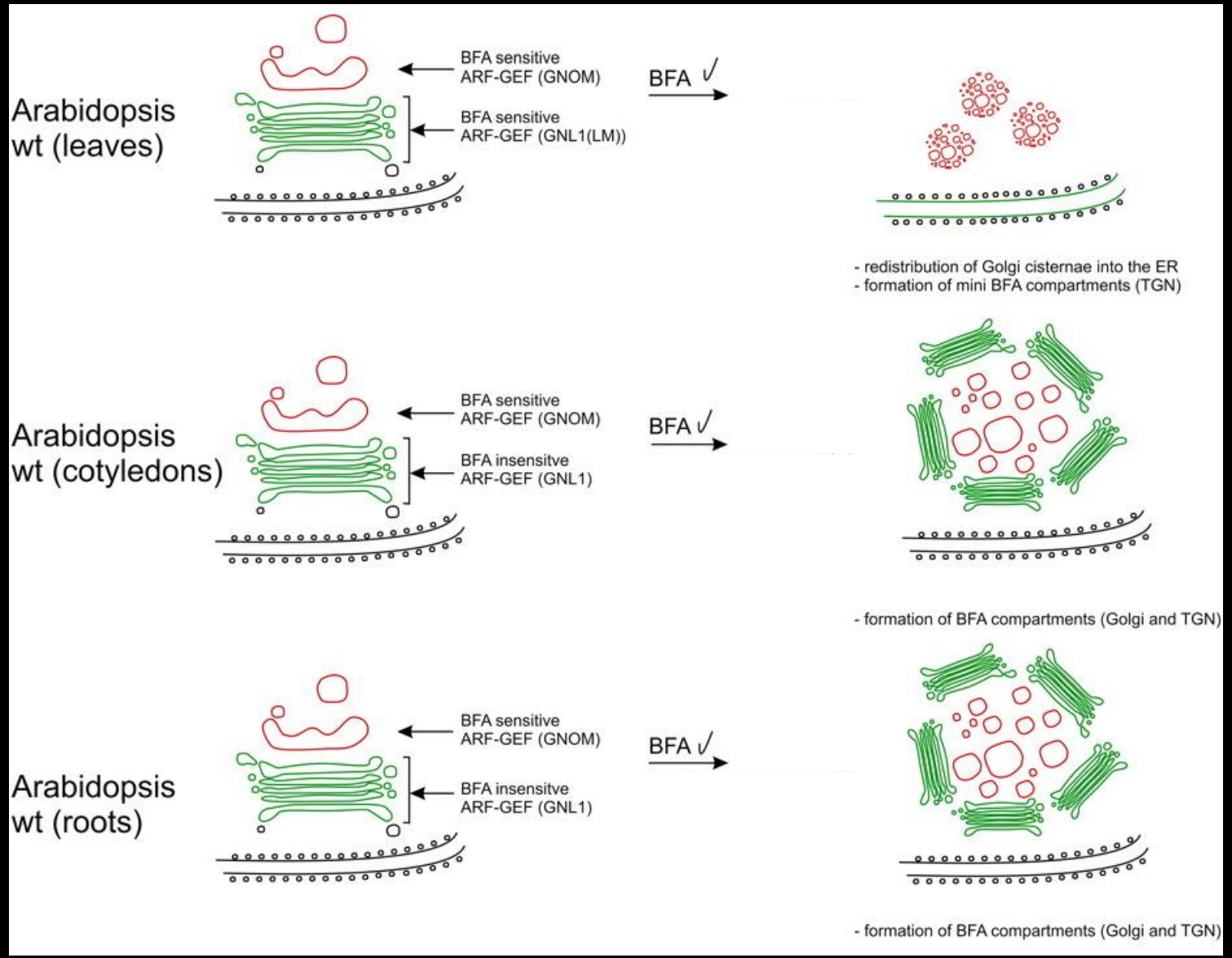
Brefeldin A (BFA)

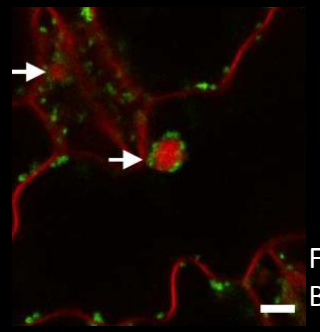
- A fungal macrocyclic lactone, which targets (guanine nucleotide exchange factors for ARF GTPases) ARF-GEFs, thus inhibiting the function of ARF GTPases, which leads to the formation of heterogeneous aggregations (so called BFA compartments) of early endosomal membranes in plants.



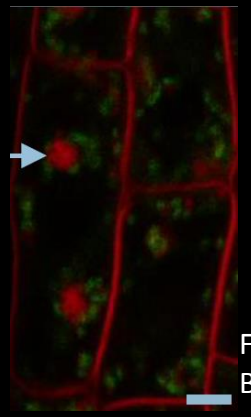
Klausner et al., 1992;
Gaynor et al., 1998;
Nebenführ et al., 2002

BFA resistance or sensitivity may vary from tissue to tissue within the same plant





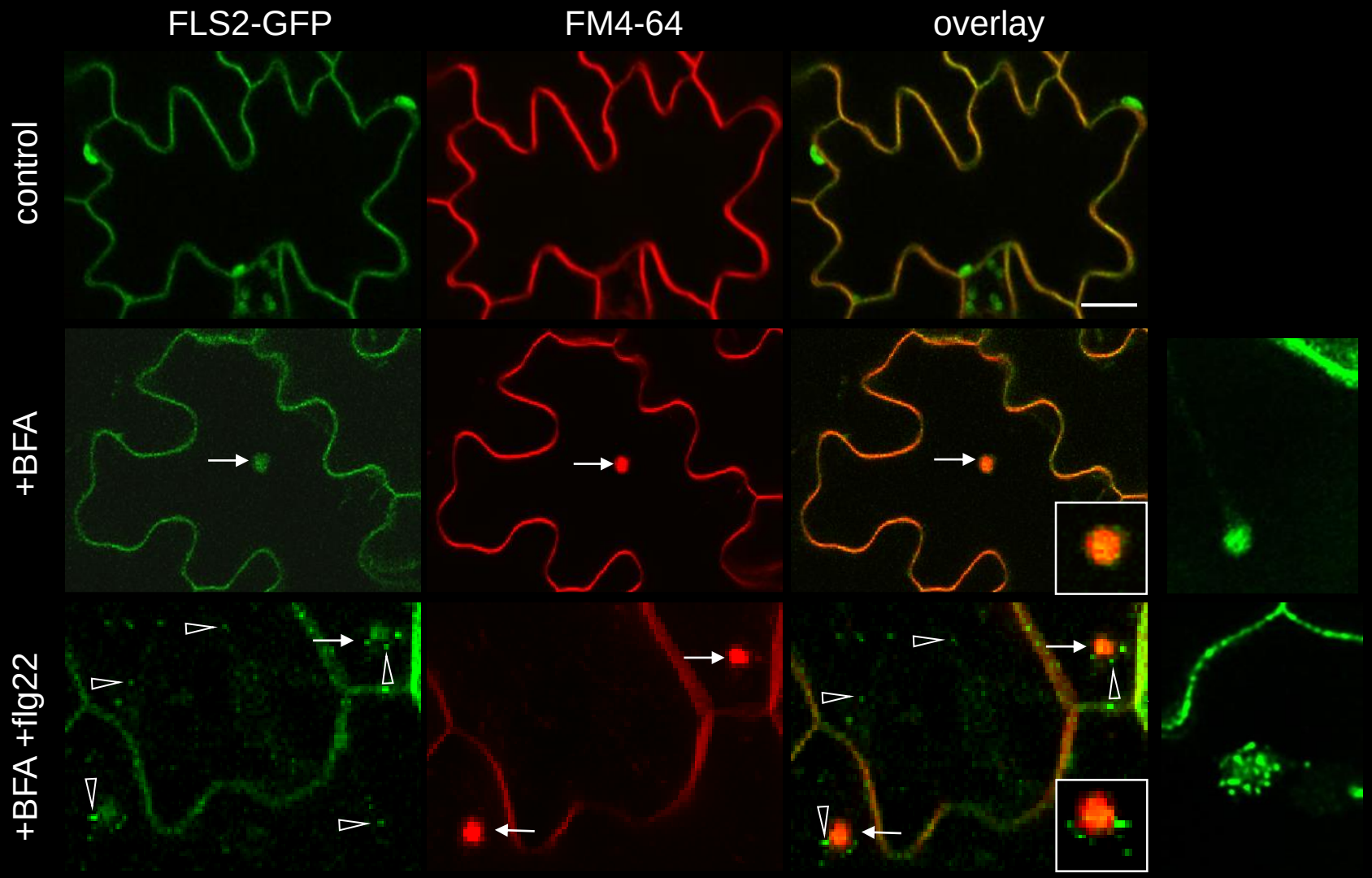
FM4-64, SYP32-YFP
Beck et al., 2012



FM4-64, SYP32-YFP
Beck et al., 2012

Langhans et al., 2011

BFA sensitivity of FLS2 endocytic compartments



Martina Beck

Conclusion

Viktor Žársky in 2016: “recent work show that there is no clear evidence for a recycling system of non-activated receptors”.

Ortiz-Morea *et al*., 2016 work demonstrate that the population of the PEPR1–GFP receptor captured in BFA bodies is coming from *de novo* biosynthetic anterograde membrane transport.

Jásik *et al*., 2016 work show that PIN-FORMED2 (PIN2)-containing BFA bodies are also mostly coming from *de novo* biosynthetic anterograde PIN2 membrane transport.

Specificity vs pleiotropic effect

Chemicals of the future

Small Molecules for Dissecting Endomembrane Trafficking: A Cross-Systems View

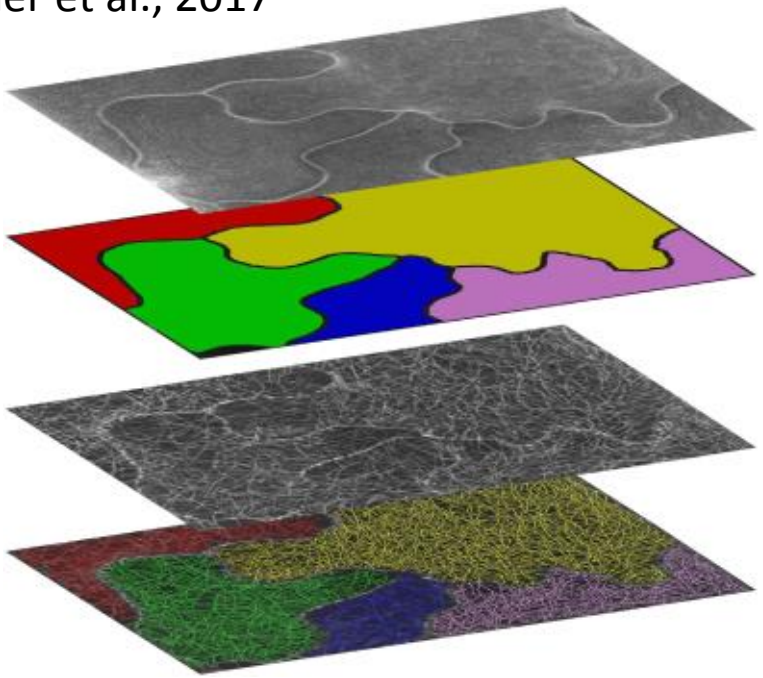
Kiril Mishev et al., 2013

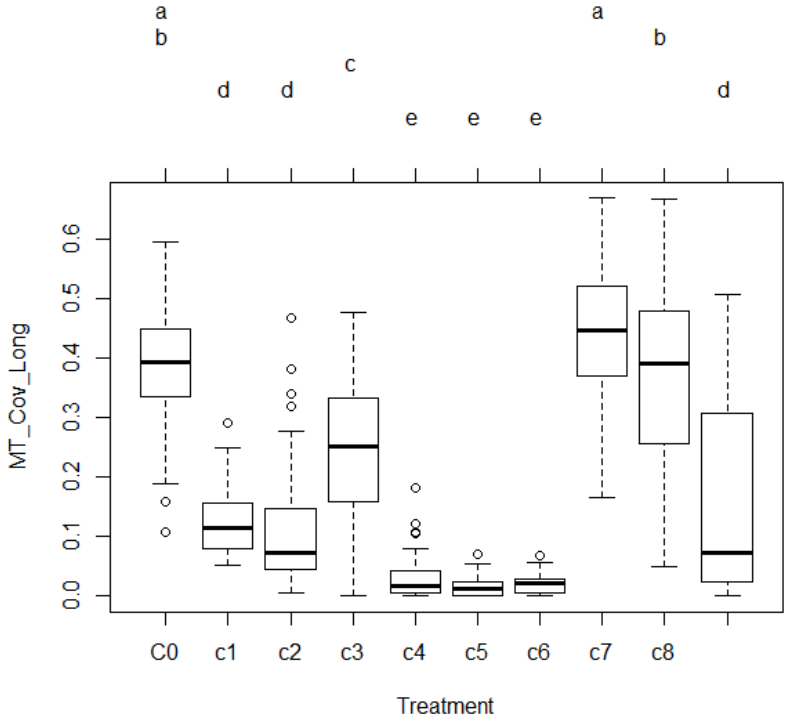
Plant Chemical Genetics

Wim Dejonghe and Eugenia Russinova 2017

An automated quantitative image analysis tool for the identification of microtubule patterns in plants

Faulkner et al., 2017





Genetic interference

T-DNA

EMS

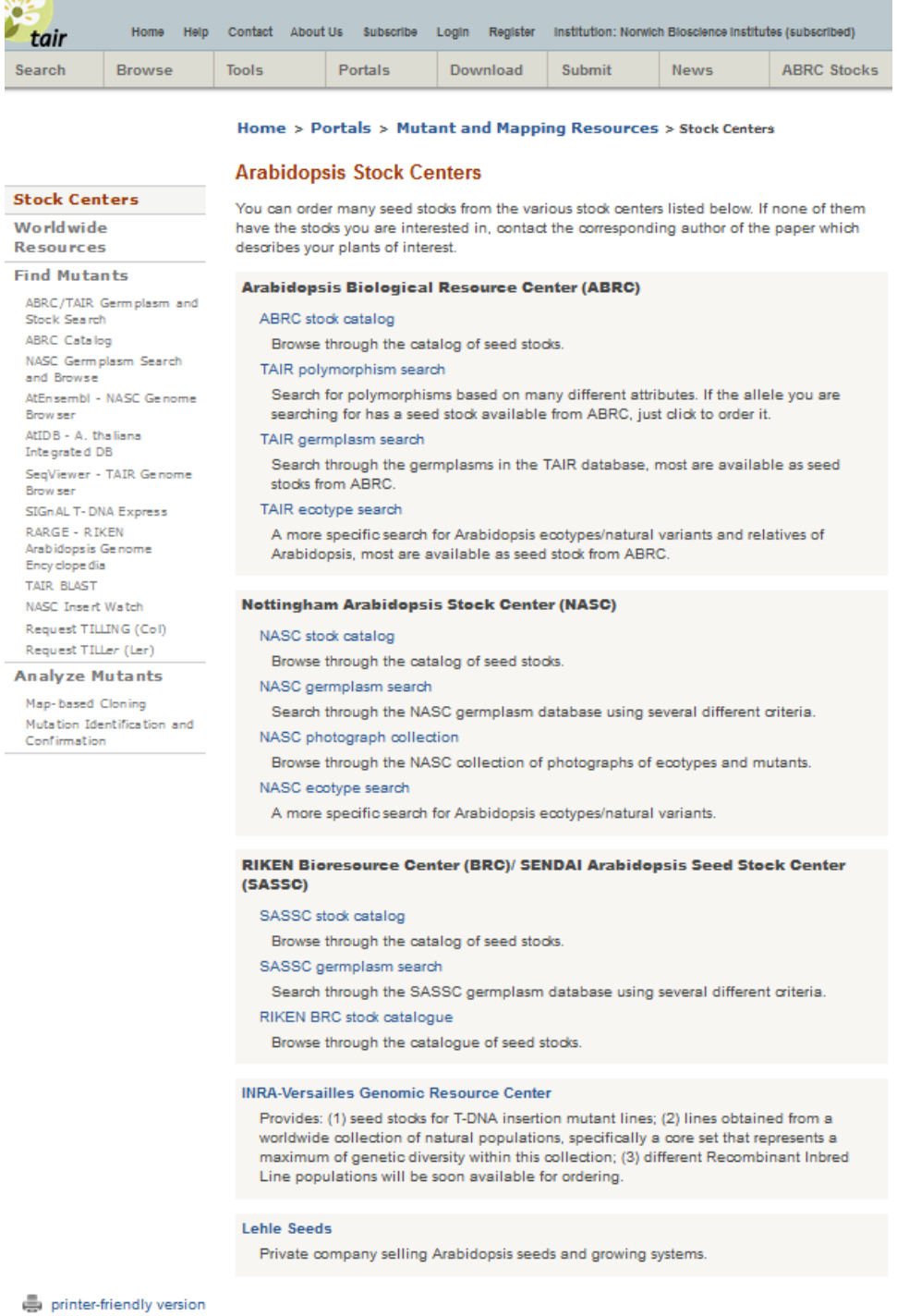
Dominant negative mutation

Genome editing (CRISPR site-specific double strand DNA breaks were restricted to zinc finger nucleases (ZFNs) and TAL effector nucleases (TALENs)

RNAi (microRNA and siRNA)

T-DNA

EMS



- Huge *Arabidopsis* database

- Exist for other crops (Tomato, Tobacco…)
(Gidoni et al., 2003; The Tobacco mutant Resources)

- Stable transformation

- Require selection, genotyping and several lines (Copy number)

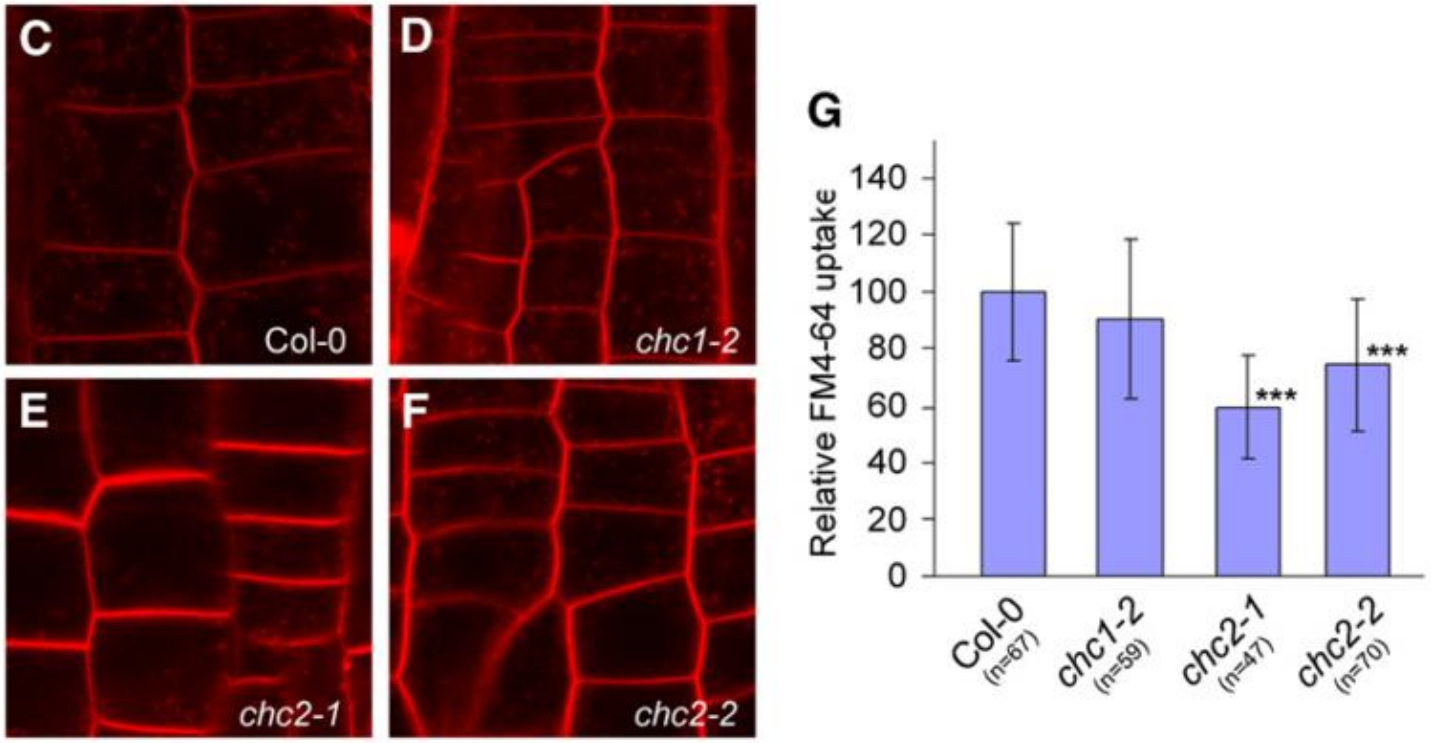
- Crosses required for multiple genes targeting

- Ds transposons can jump

https://www.arabidopsis.org

T-DNA

EMS



Kitakura *et al*., 2011

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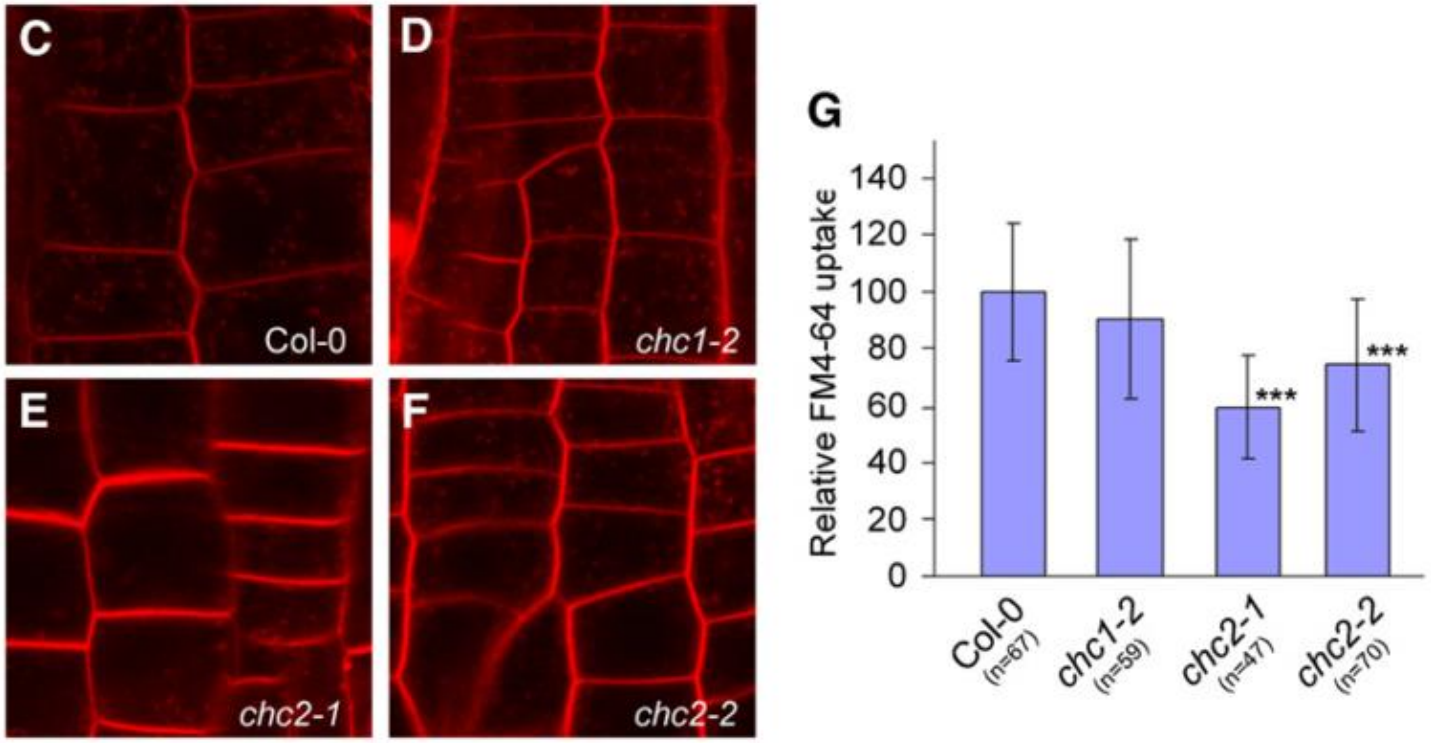
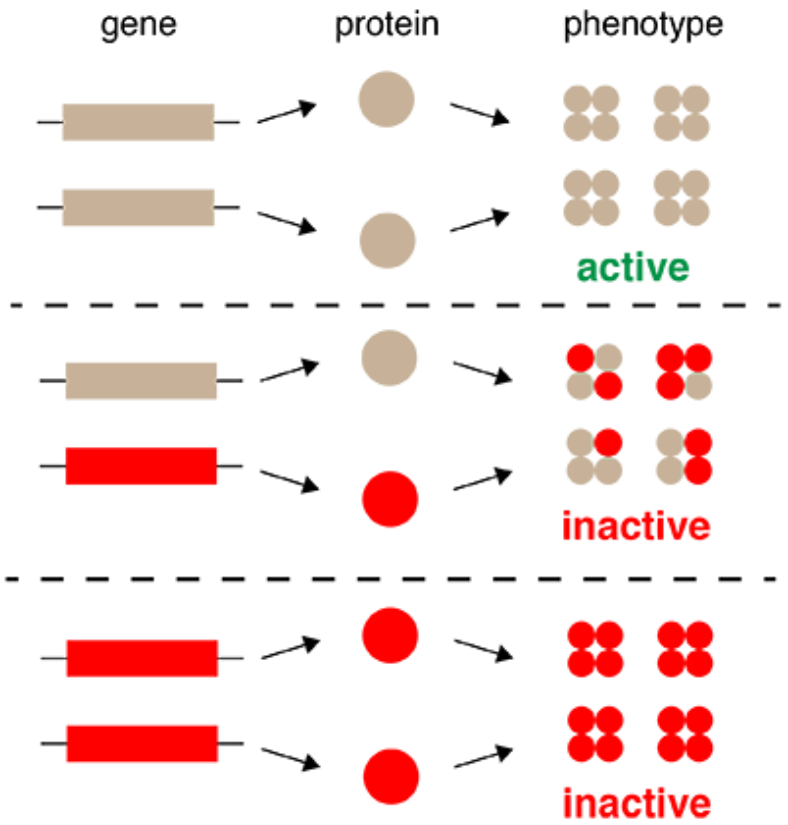


Double mutant is lethal

Kitakura *et al*., 2011

Dominant negative mutation



https://www.quora.com/What-makes-a-gene-dominant-co-dominant-or-recessive

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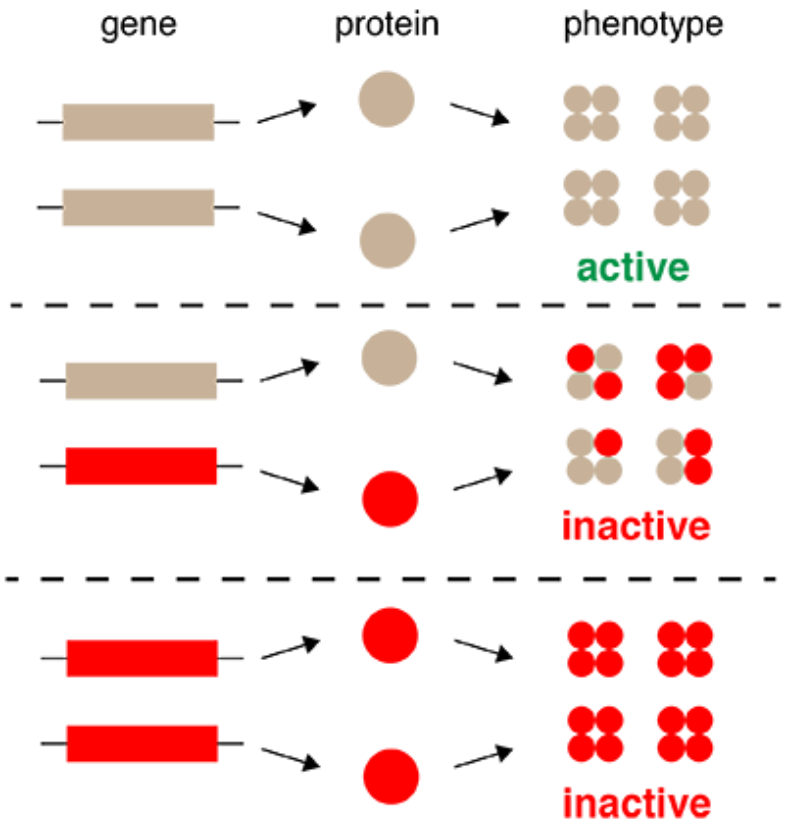

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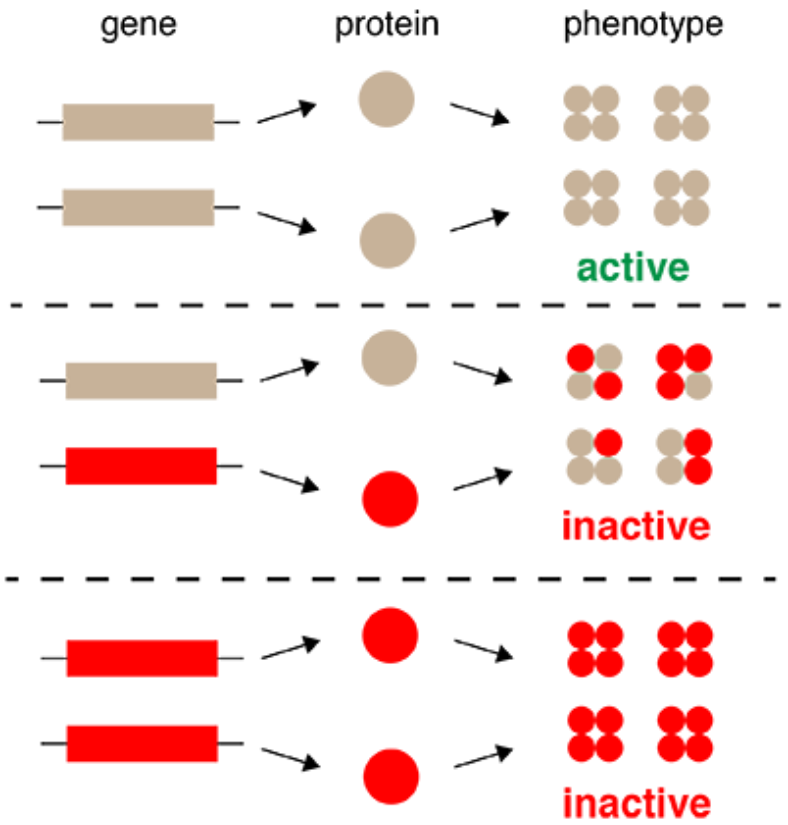
Li *et al.*, 2002

Dominant negative mutation



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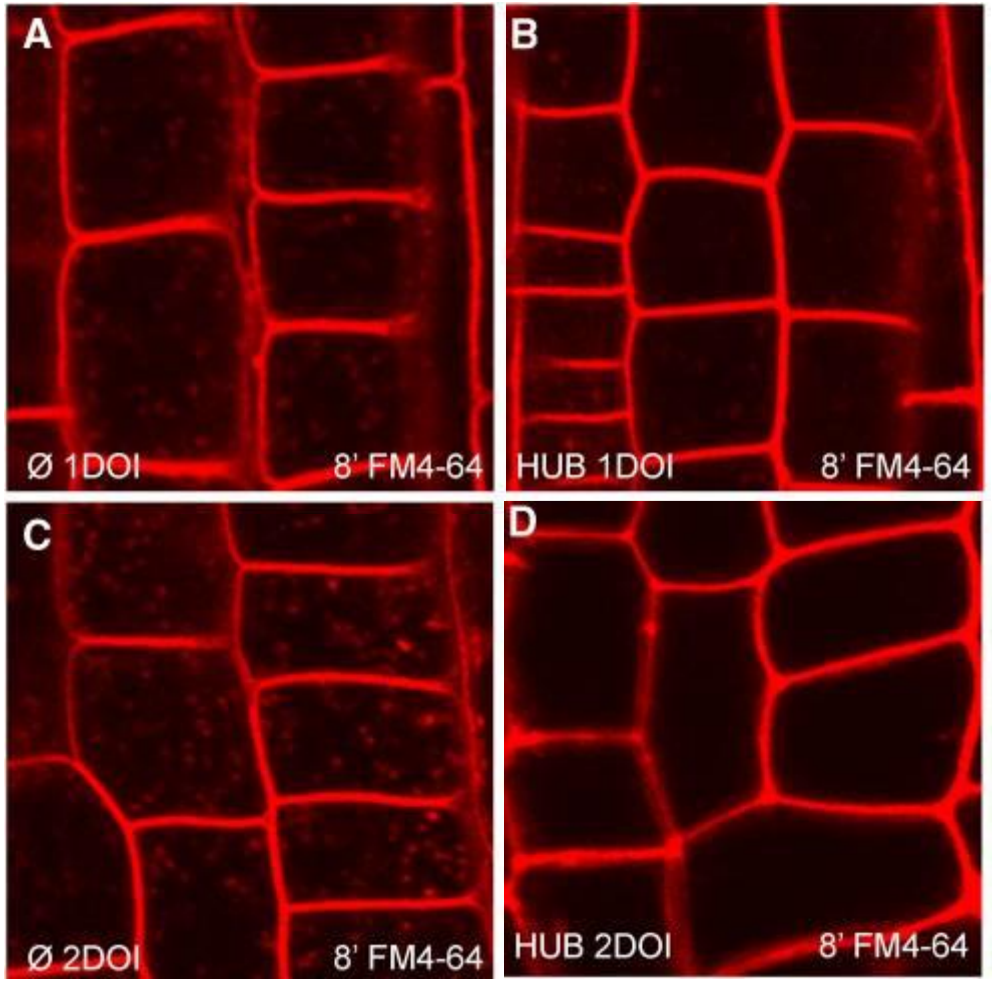
Conditionally overexpressing the C-terminal part of clathrin heavy chain (termed HUB1) that exerts a dominant negative effect on clathrin function by binding and consequently depleting clathrin light chains (Liu et al., 1995). Estradiol inducible construct.



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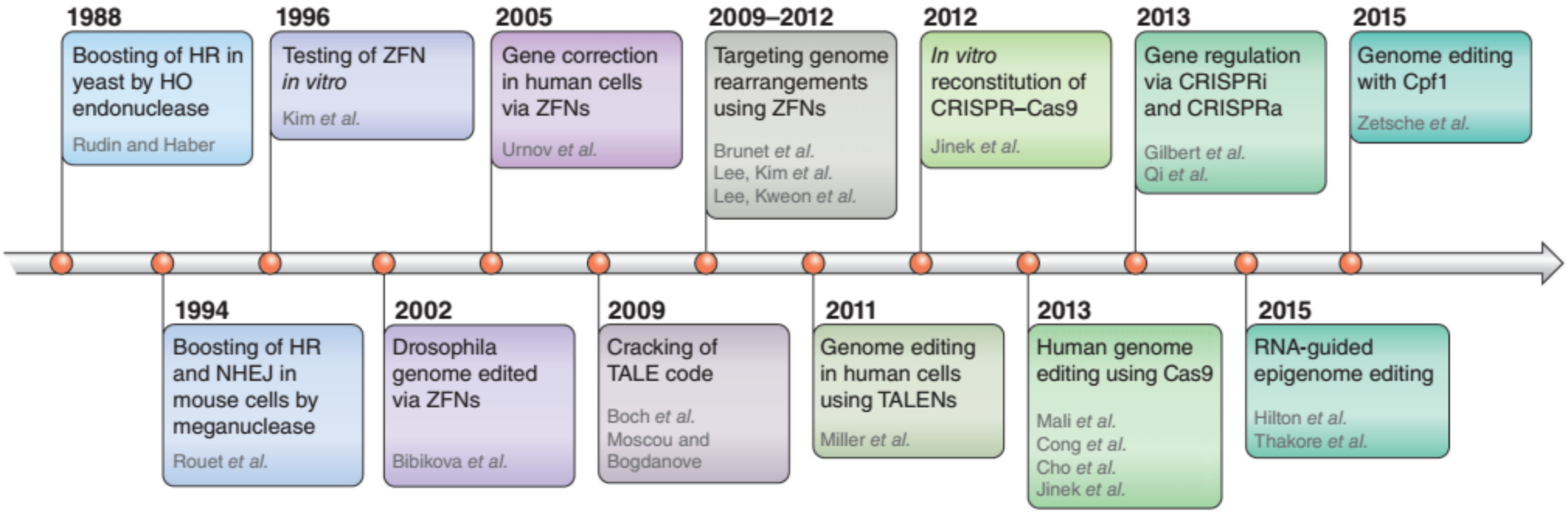
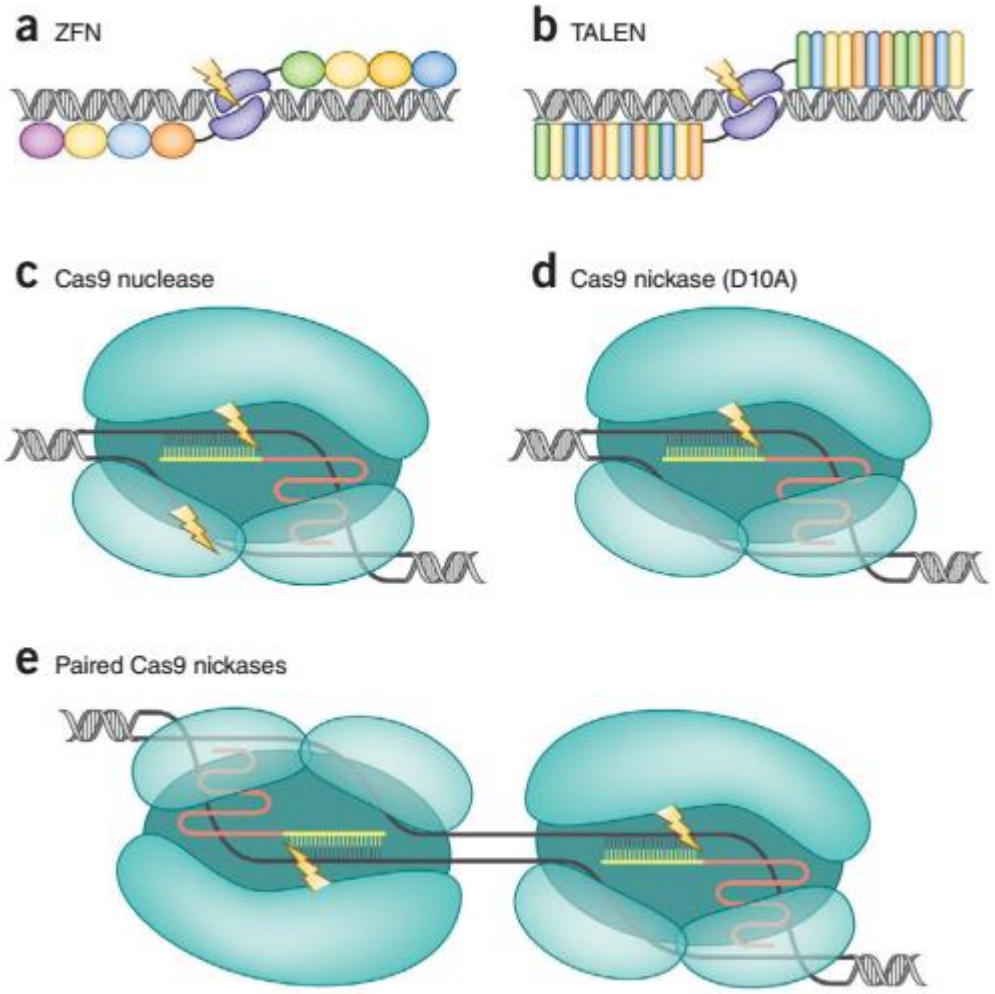


Jens Boch

Kim 2016

Genome editing



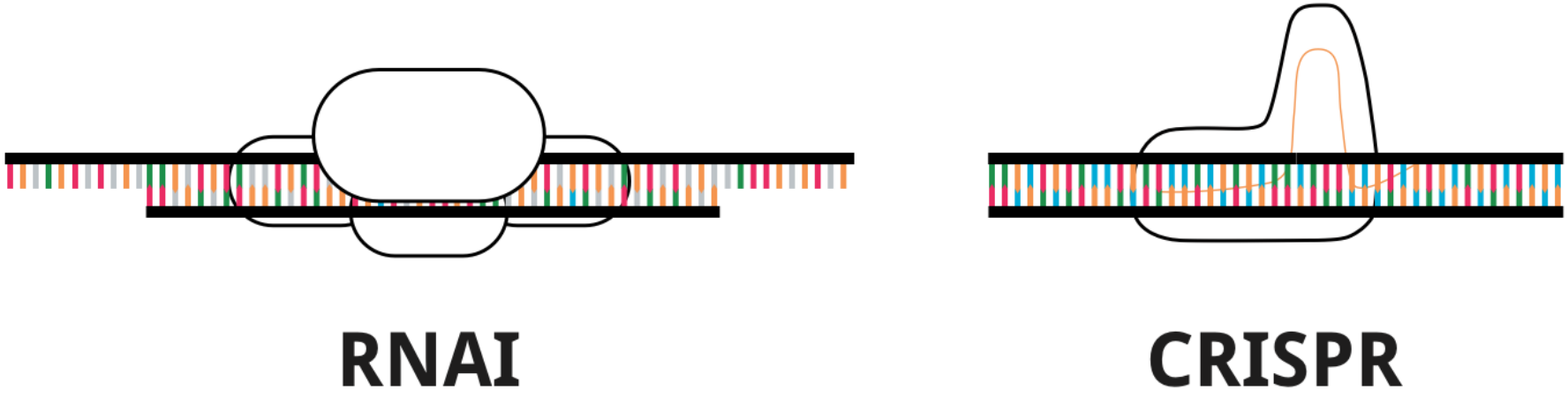
- Take advantage of error prone eukaryote DNA repair system

- Genome sequence known
- Off-target effects
- Very specific
- Target multiple genes
- Stable transformation
- Applicable to non eukaryotic

Jens Boch

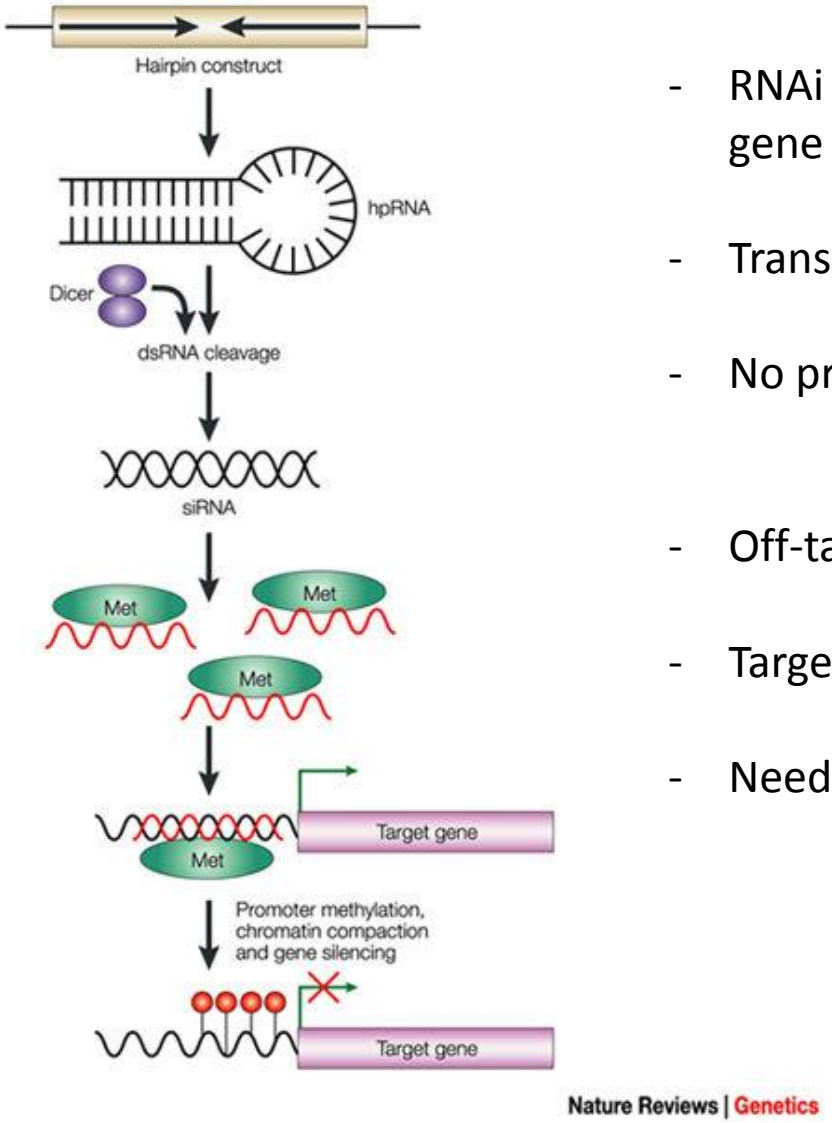
Kim 2016

RNAi



https://www.deskgen.com/landing/resources/rnai-crispr-functional-genomics-screen

RNAi



- RNAi offers the possibility of gene knockdown instead of gene knockout
- Transient and stable transformation possible
- No problem with chromatin structure
- Off-target effects
- Target only expressed genes (not non-coding sequences)
- Needs siRNA machinery

Waterhouse and Helliwell 2003

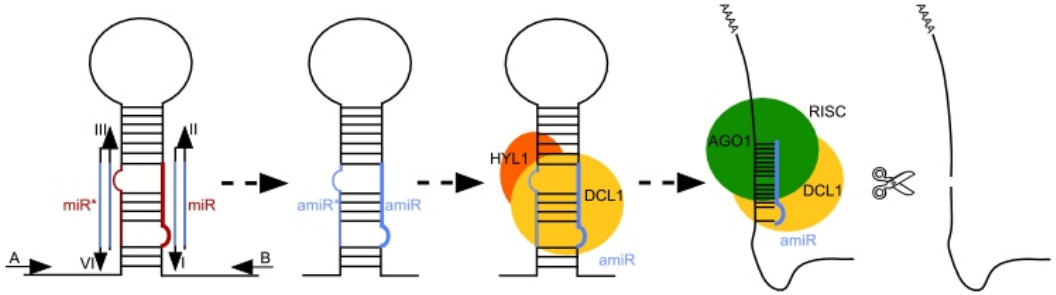
RNAi

WMD3 - Web MicroRNA Designer

Home | Target Search | Designer | Oligo | Hybridize | Blast | Downloads | About | Help

WMD3 - Web app for the automated design of artificial microRNAs.

Artificial microRNAs (amiRNAs) are 21mer small RNAs, which can be genetically engineered and function to specifically silence single or multiple genes of interest in more than 90 plants, according to the previously determined parameters of target gene selection. It uses your favorite gene(s), which you want to silence, and designs 21mer amiRNA sequences. You will retrieve oligo sequences to express the small RNA from endogenous miRNA precursors. Please read the Procedure before you use WMD3 for the first time! More information about miRNAs, design and application of amiRNAs and several guides on all the tools on this page can be found in the Help section. Cloning protocols are available in the Download section.
The artificial miRNA vectors pRS300 and pNW55 are available from Addgene. On their website, search for plasmids associated with the keyword "weigel".



Proof of concept studies and other related publications:

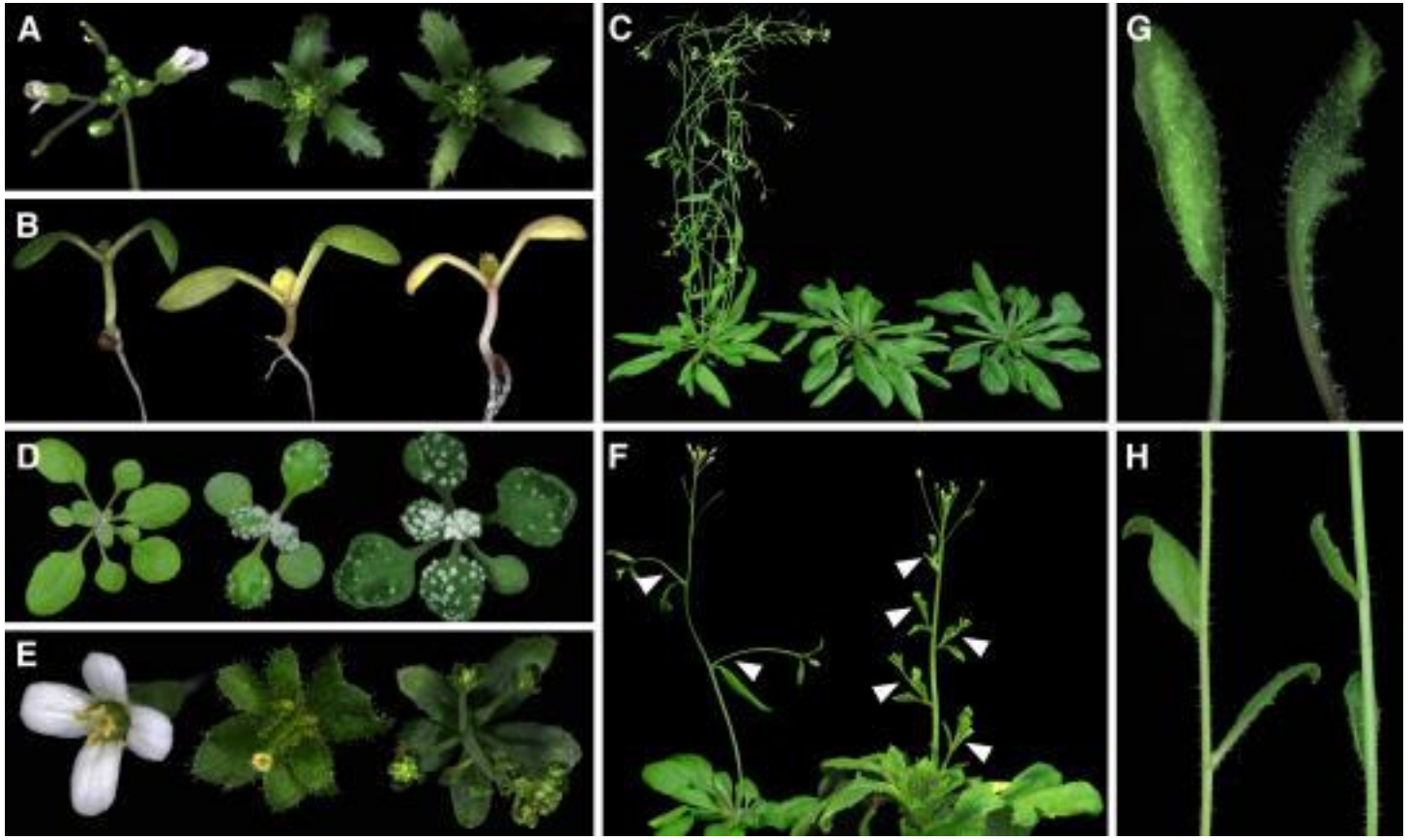
The systematic design of amiRNAs has first been described in:
Rebecca Schwab, Stephan Ossowski, Markus Riester, Norman Warthmann, and Detlef Weigel. (2006) **Highly Specific Gene Silencing by Artificial MicroRNAs in *Arabidopsis*** Plant Cell 18: 1121-1133. Link to PubMed

Detailed overview of WMD:
Stephan Ossowski, Rebecca Schwab, Detlef Weigel (2008) **Gene silencing in plants using artificial microRNAs and other small RNAs** The Plant Journal 53 (4) , 674-690 Link to PubMed

Oryza sativa:
Warthmann N, Chen H, Ossowski S, Weigel D, Hervé P. (2008) **Highly Specific Gene Silencing by Artificial miRNAs in Rice** PLoS ONE 3(3): e1829. Link to PLoS ONE

http://wmd3.weigelworld.org/cgi-bin/webapp.cgi

RNAi



(A) Ethanol-induced ubiquitous expression of amiR-white-1 3 and 5 d after induction. After 3 d, young leaves are all yellow; after 5 d, the youngest leaves are green again.
(B) Ethanol-induced ubiquitous expression of amiR-trichome (right) 3 d after induction. Clustered trichomes appear as white covering of youngest leaves (arrowhead).
(C) Inflorescences of plants expressing amiR-white-1 from the *AP1* promoter (middle) are pale yellow. Strong lines expressing amiR-mads-2 from the *AP1* promoter (right) resemble *ap1 cal* double mutants.
(D) Expression of amiR-lfy-1 from the *LFY* promoter (1) results in flowers resembling *lfy* mutants. amiR-mads-2 expressed from *AG* regulatory elements in the center of the flower (2) produces organ transformations in the central two whorls. Outer whorls remained unaffected. An opposite phenotype was seen after expression of amiR-mads-2 from the *AP1* promoter (3; weaker line), which didn't affect inner whorls but resulted in secondary flowers, resembling *ap1* mutants.
(E) Epidermal expression of amiR-white-1 from the *MERISTEM LAYER1* (*ML1*) promoter resulted in pale plants.

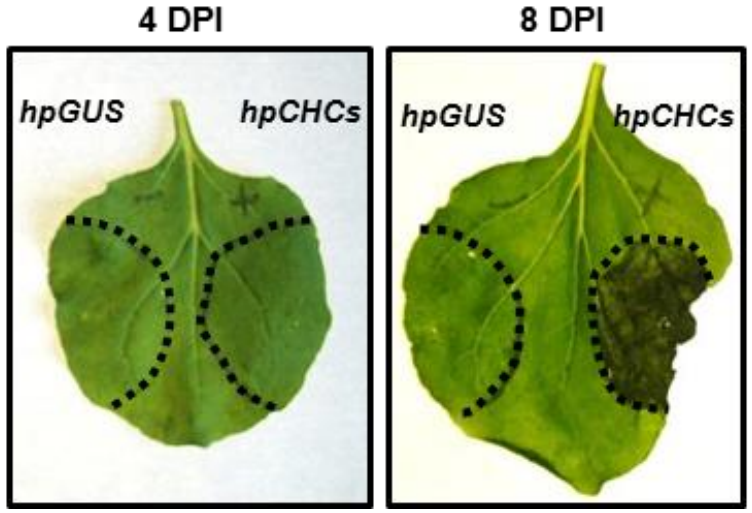
Schwab *et al*., 2006

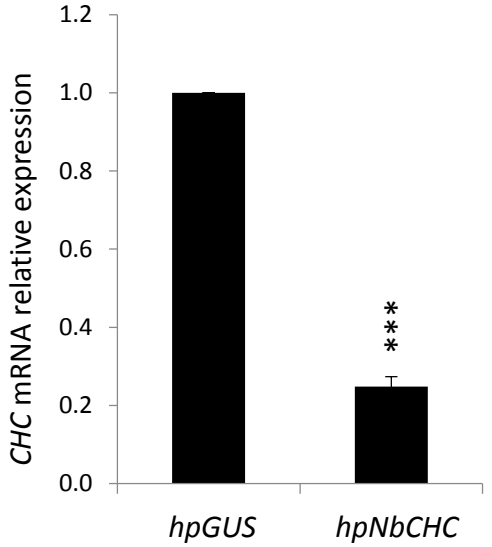
RNAi

2 CLATHRIN HEAVY CHAIN genes in *Arabidopsis thaliana*

Double mutant is lethal

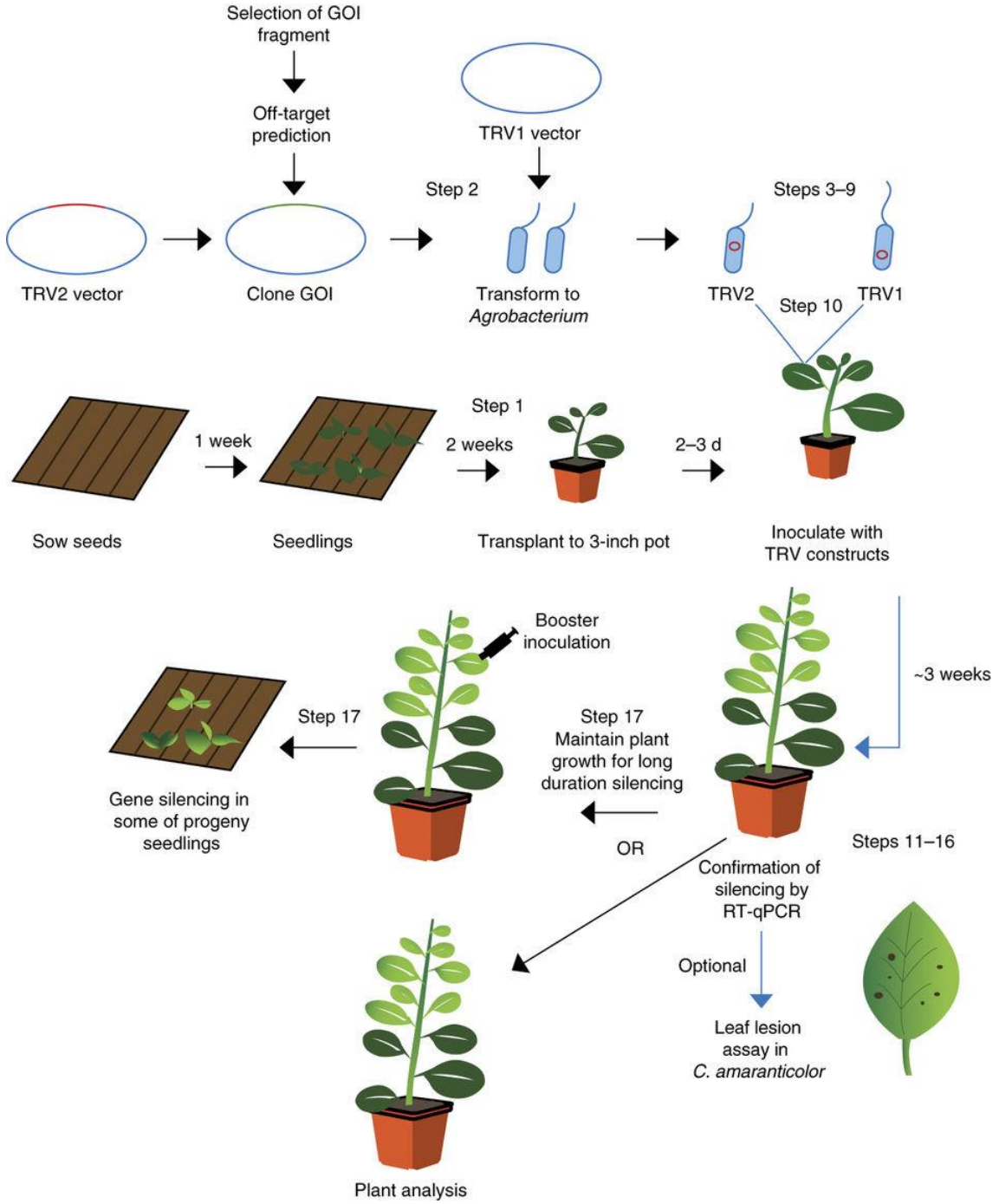
6 CLATHRIN HEAVY CHAIN genes in *Nicotiana benthamiana*





RNAi

VIGS



- Whole plant silencing
- Can be applied to other crops (tomato, eggplants,…)
- Not 100% and not uniform

Senthil-Kumar and Mysore 2014

RNAi in the future

Induction of Silencing in Plants by High-Pressure Spraying of *In vitro*-Synthesized Small RNAs

PERSPECTIVE
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nature plants

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Clay nanosheets for topical delivery of RNAi for sustained protection against plant viruses

Neena Mitter[1*], Elizabeth A. Worrall[1], Karl E. Robinson[1], Peng Li[2], Ritesh G. Jain[1], Christelle Taochy[1,3], Stephen J. Fletcher[1,3], Bernard J. Carroll[3], G. Q. (Max) Lu[2,4] and Zhi Ping Xu[2*]

No perfect technique

Controls controls controls

Combined approaches