



Faculty of Science



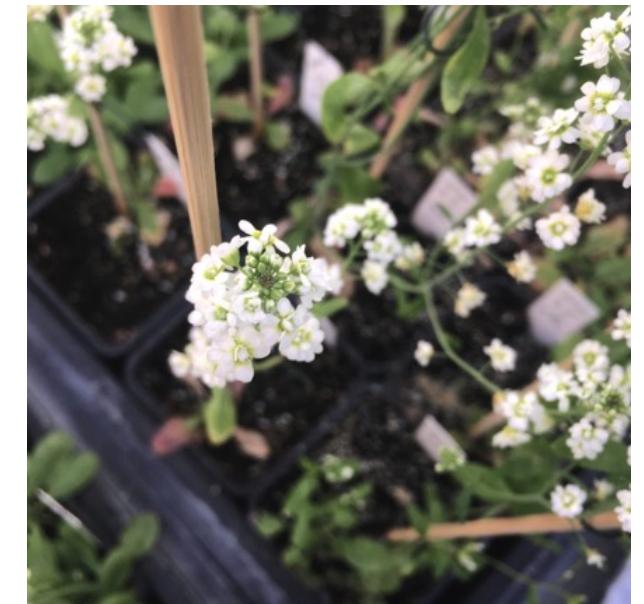
Unveiling the mechanisms of m⁶A-YTH RNA-binding proteins in *Arabidopsis thaliana* and their mRNA target identity

Carlotta Porcelli

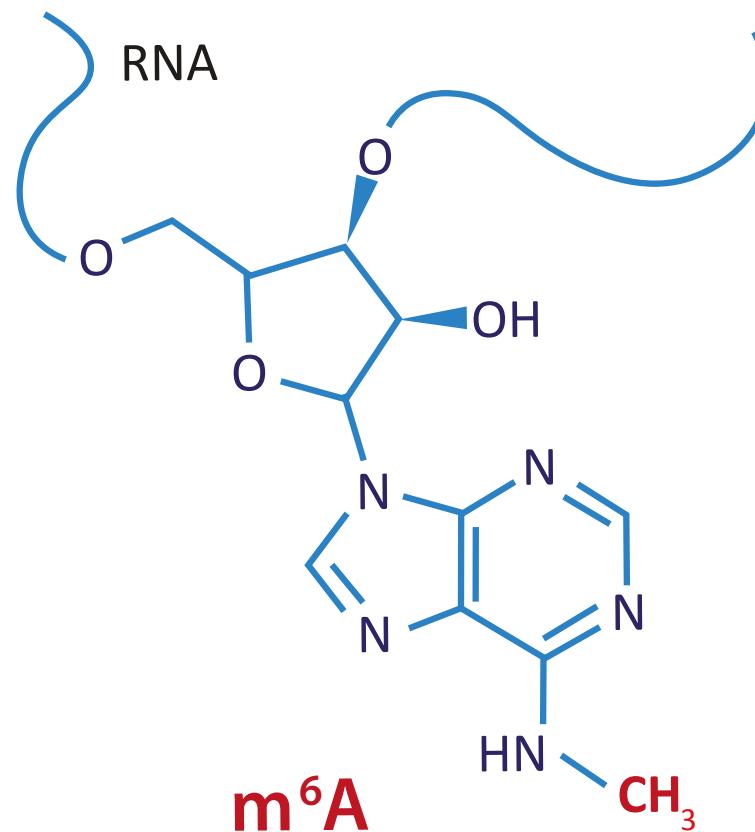
Peter Brodersen Group

University of Copenhagen,
Section for RNA and Computational Biology

6th ANNUAL DANISH BIOINFORMATICS CONFERENCE
19th November 2021 - Aalborg



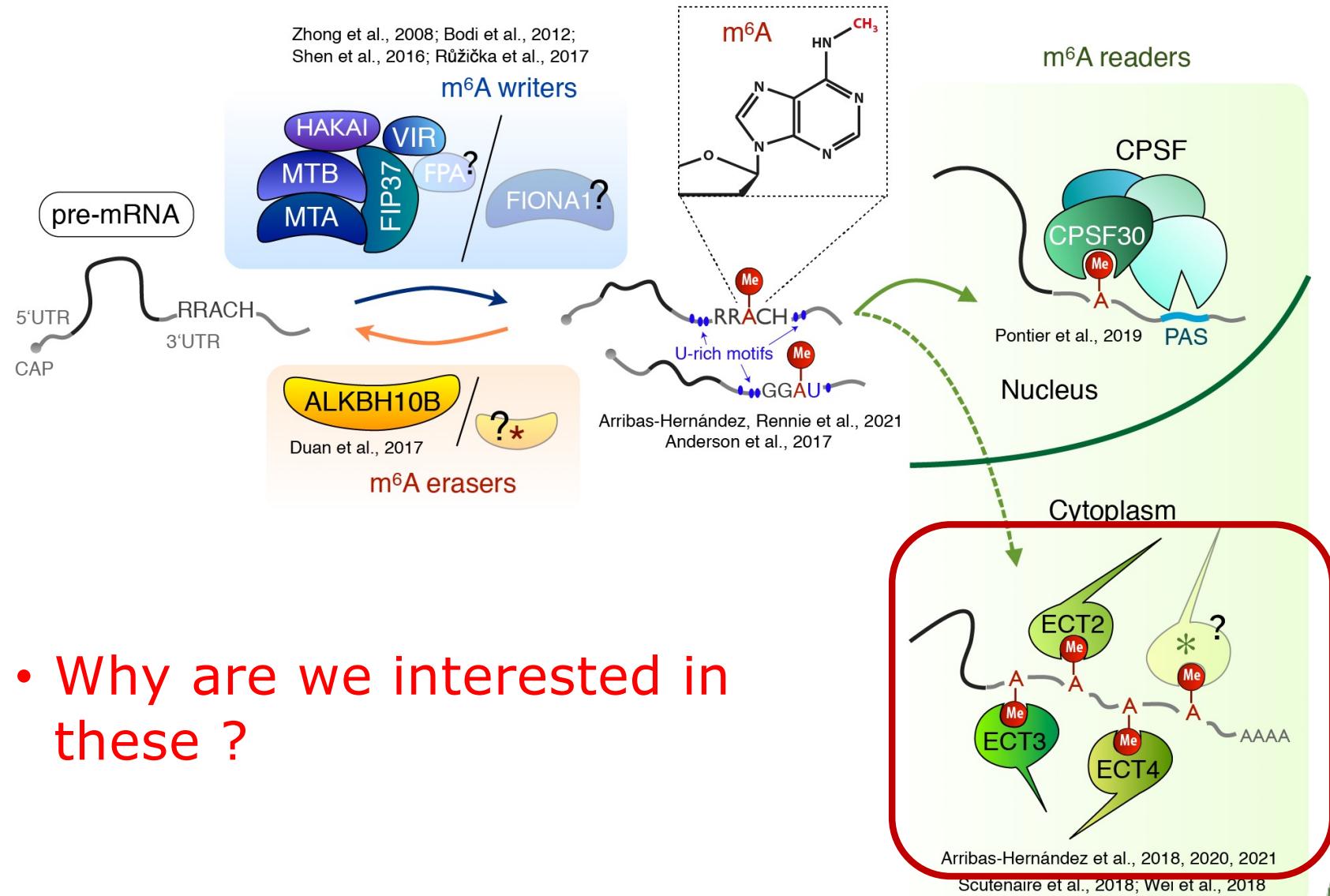
N6-Methyladenosine



is the most prevalent internal modification present in the mRNA of all higher eukaryotes



The m⁶A pathway



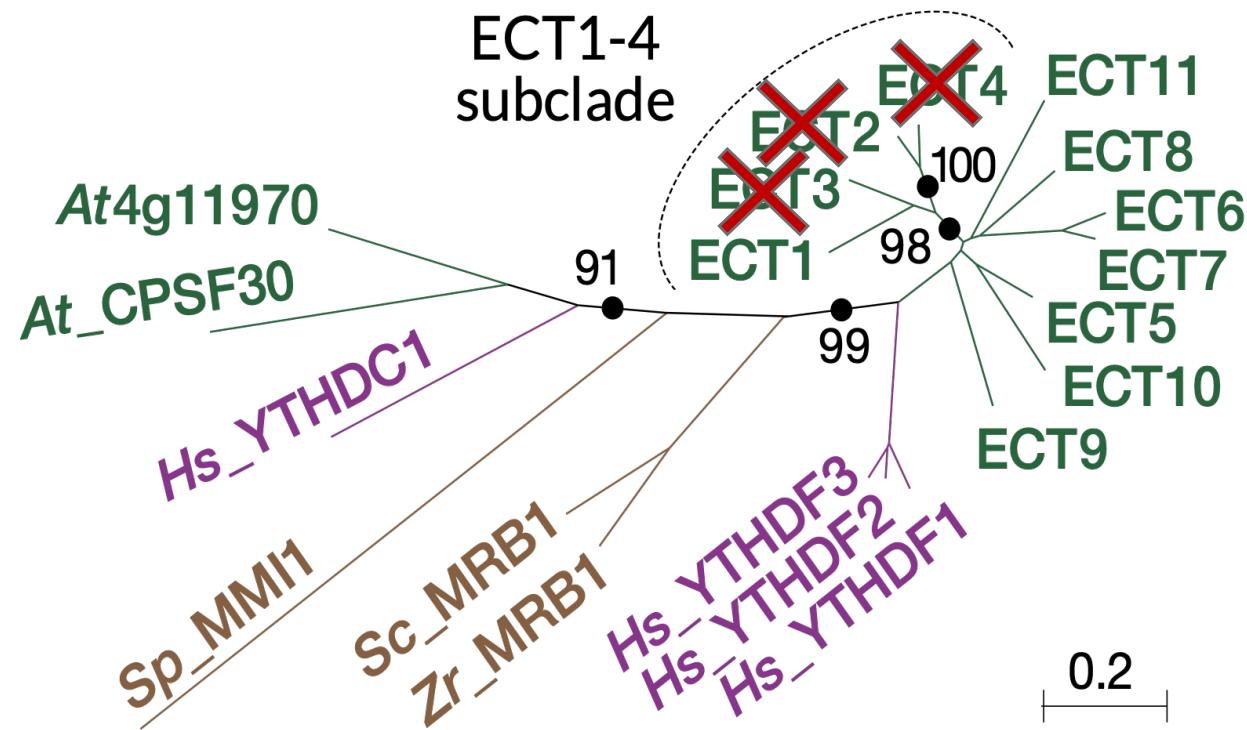
- Why are we interested in these ?

Arribas-Hernández, L., & Brodersen, P. (2020). Occurrence and Functions of m⁶A and Other Covalent Modifications in Plant mRNA. *Plant Physiology*



m⁶A RNA-binding proteins in *A. thaliana*

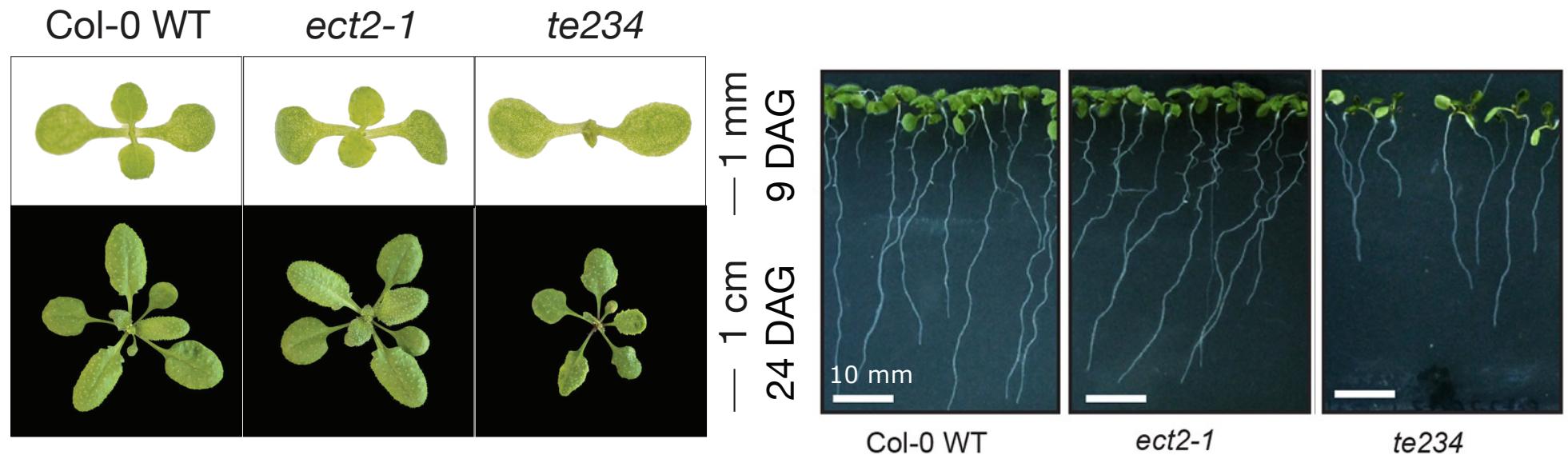
Arabidopsis YTH domain proteins (green)



Arribas-Hernández, L., Bressendorff, S., Hansen, M. H., Poulsen, C., Erdmann, S., & Brodersen, P. (2018). An m⁶A-YTH Module Controls Developmental Timing and Morphogenesis in Arabidopsis. *The Plant Cell*



ect2/ect3/ect4 mutants show a consistent delay of normal rate and directionality of primary root growth and of leaf formation



Research questions

TARGET IDENTIFICATION:

- Which are the mRNAs targeted by the ECTs ?
- What are the properties of their binding sites ?

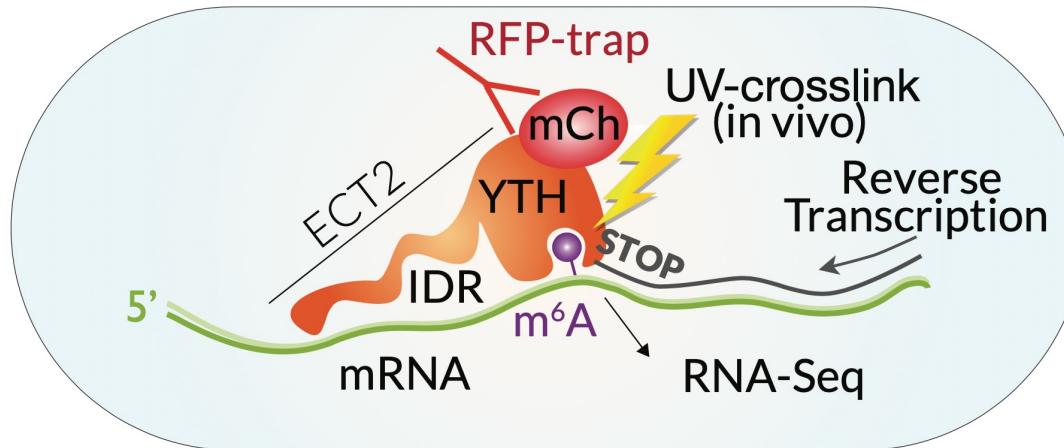
TARGET REGULATION:

- How are the ECTs targets regulated upon loss of ECT2/ECT3/ECT4 ?



Experimental methods – target identification

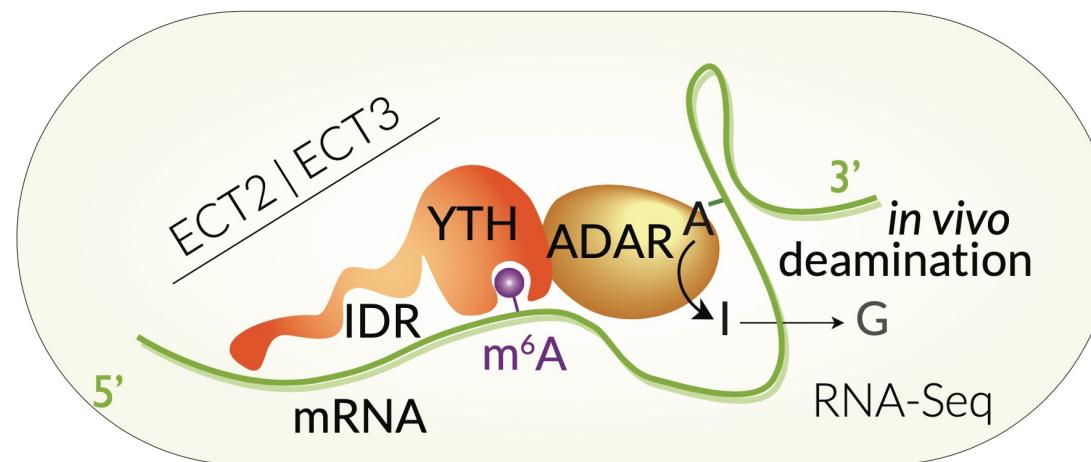
ECT2-mCherry iCLIP



iCLIP (individual nucleotide resolution crosslinking and immunoprecipitation)

Huppertz, I. et al., 2014 Methods

ECT2 & ECT3 HyperTRIBE

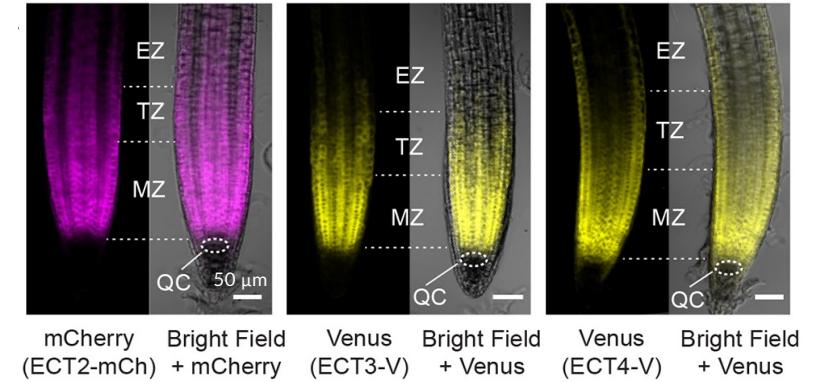
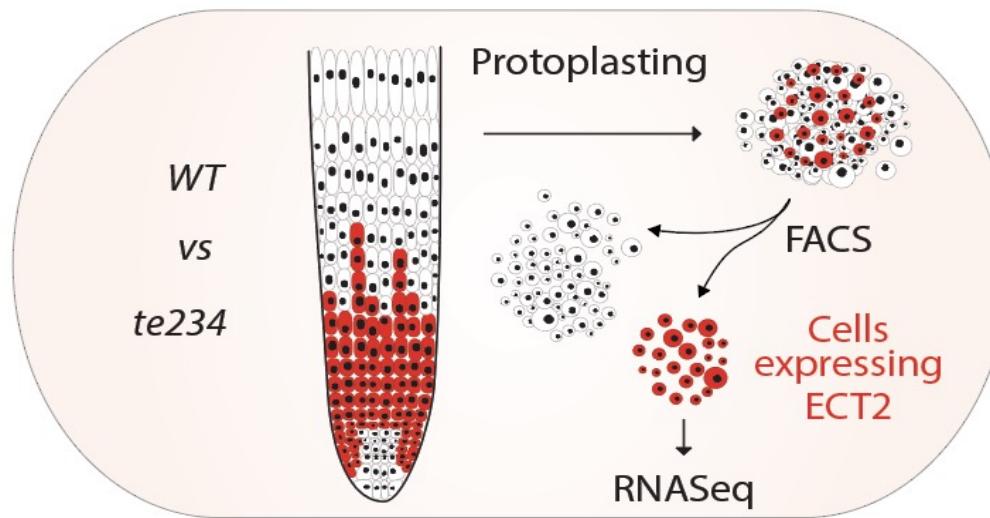


HyperTRIBE (targets of RNA-binding proteins identified by editing)

Rahman, R. et al., 2018
Nature Protocols



Experimental method – target regulation



**RNA-Seq from selected
cells expressing ECT2**



Bioinformatics data analysis

ECT2 iCLIP binding sites: PureCLIP pipeline

by Martin Lewinski using the PureCLIP pipeline [Krakau, S. et al., 2017, Genome Biology]

15960 called peaks, annotated to 2281 genes

ECT2 & ECT3 HyperTRIBE targets: hyperTRIBER pipeline

by Sarah Rennie et al. 2021 bioRxiv 2021.10.20.465108

6061 genes for ECT2 and 3898 genes for ECT3

RNASeq: Smart-seq2 libraries

by Michael Schon using the Smart-Seq2 pipeline [Picelli et al., 2014]

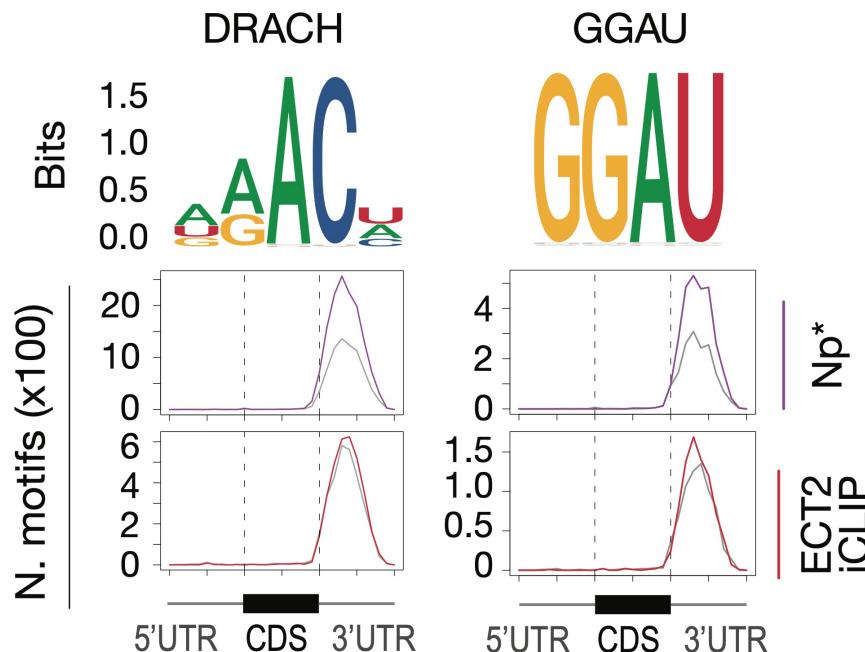
17427 expressed genes



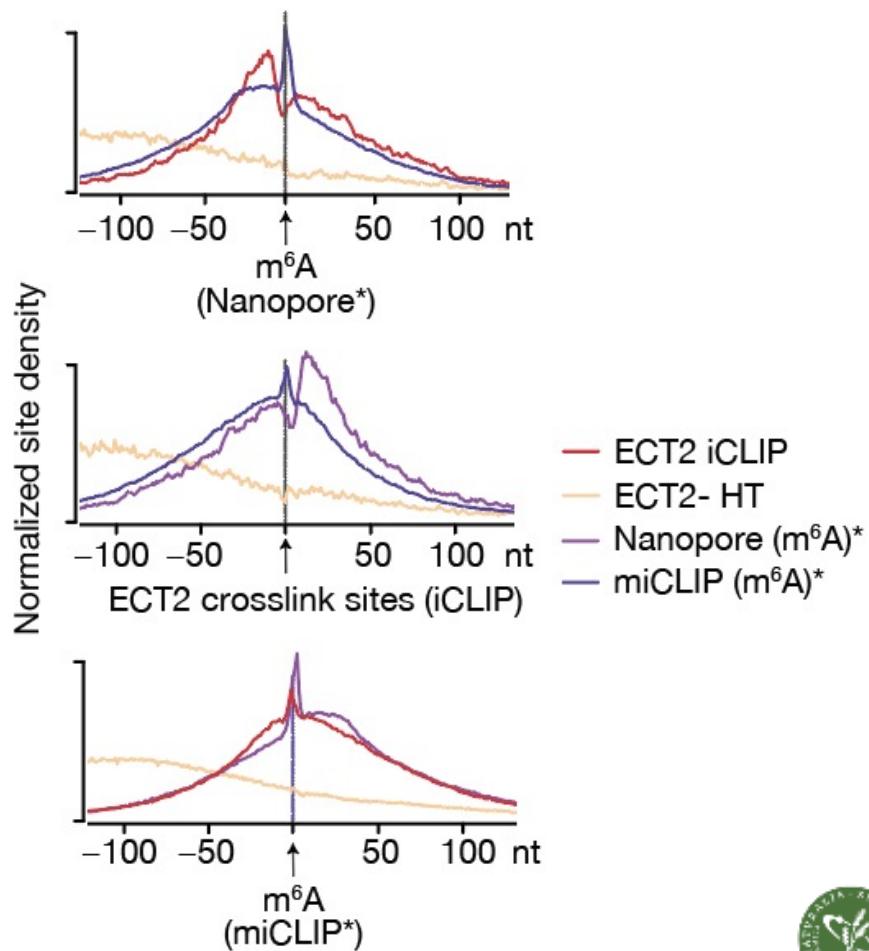
Results – ECT2 iCLIP binding sites

m^6A sites from Nanopore and miCLIP - Parker et al., 2020 eLIFE

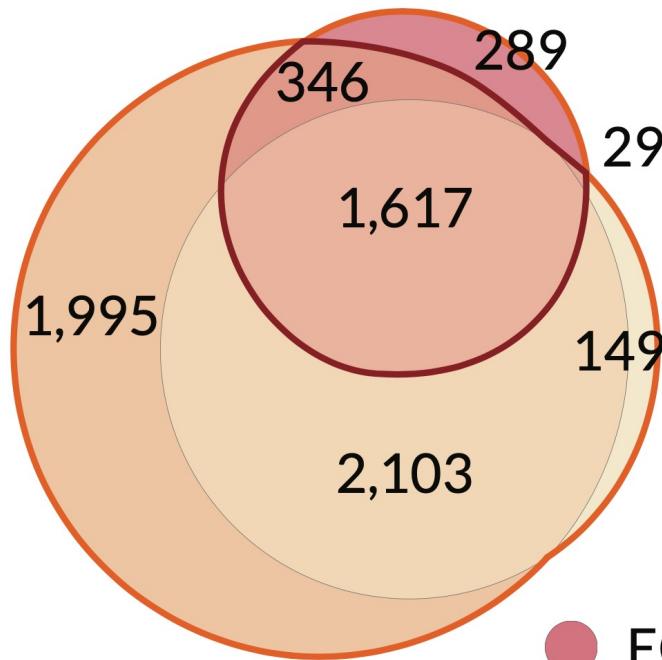
de novo motif search



Distribution of ECT2-binding sites relative to m^6A



Results – target identification



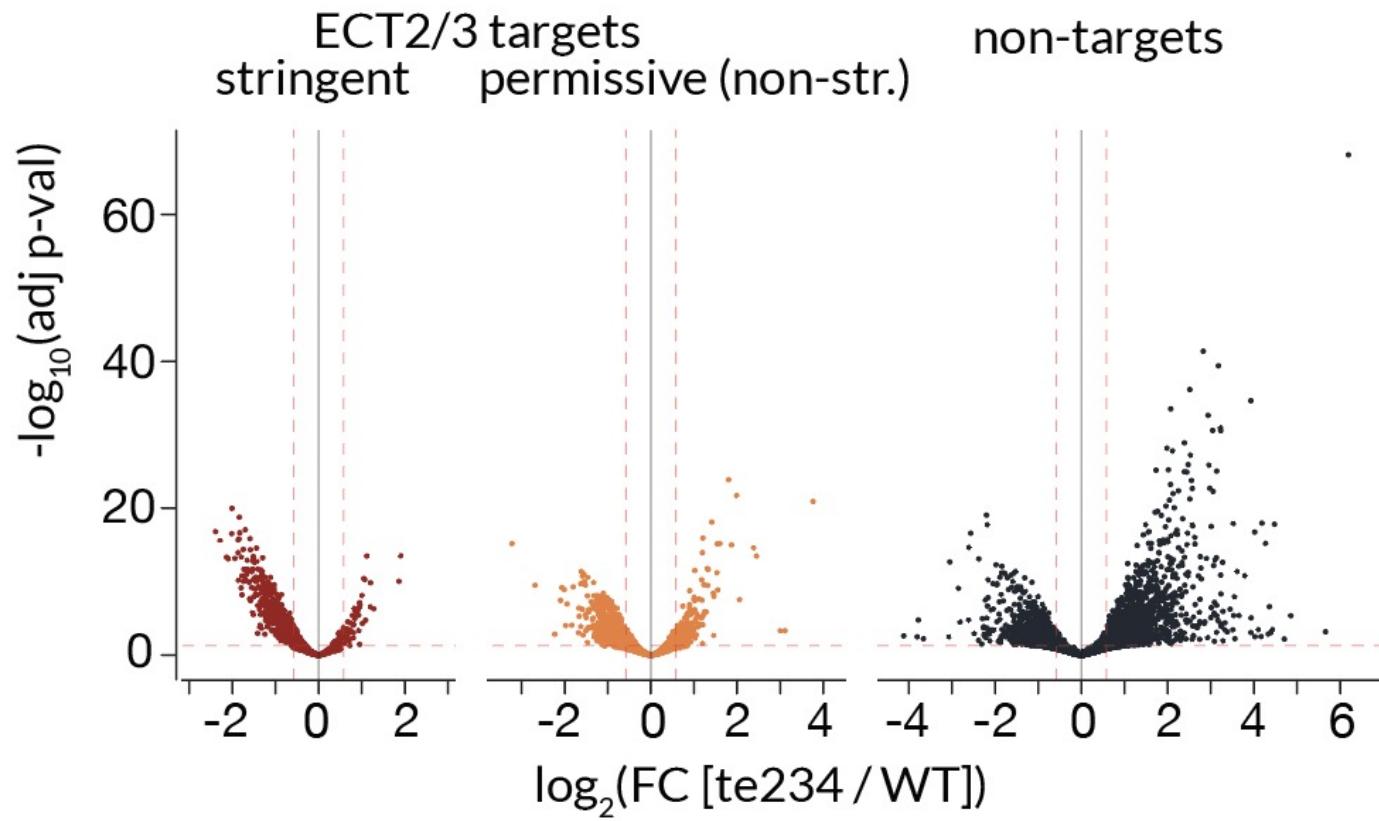
ECT2/ECT3 Target Sets

- **Permissive target set (6,528):** $[\text{ECT2-HT} \cup \text{ECT3-HT}] \cup \text{ECT2-iCLIP}$
 - **Stringent target set (1,992):** $[\text{ECT2-HT} \cup \text{ECT3-HT}] \cap \text{ECT2-iCLIP}$
 - **Non Targets (13,504)**
- ECT2-mCh iCLIP (2,281)
● ECT2-HT [roots U aerial] (6,061)
● ECT3-HT [roots U aerial] (3,898)



Results – target regulation

Differential Expression Analysis

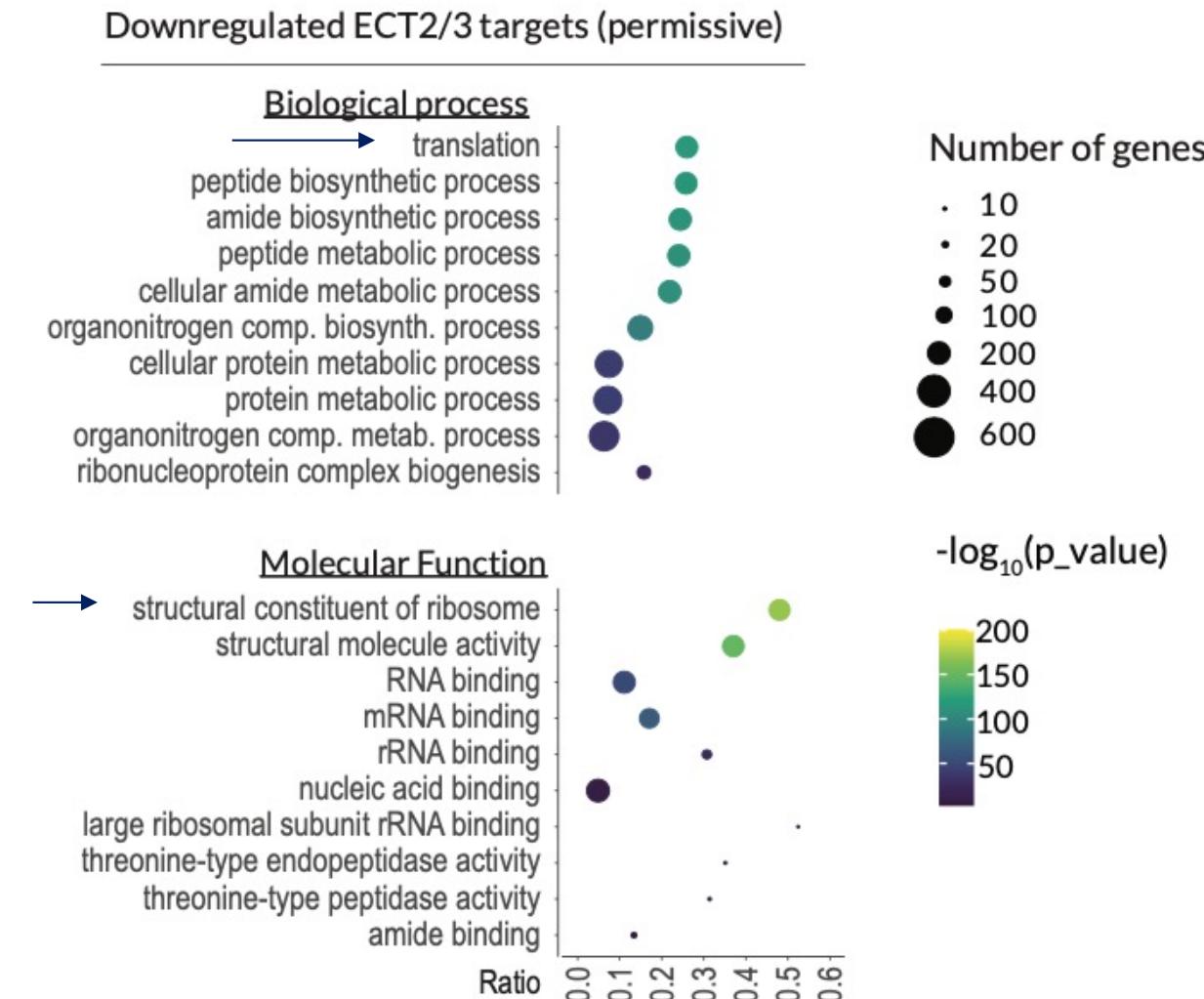


- ECT2/ECT3 targets show a tendency towards **downregulation** upon loss of ECT2/3/4 function



Results – target regulation

GO-Term Enrichment Analysis



Summary

- Identified ECT2 crosslink sites
 - Enriched in RRACH and GGAU motifs
 - Distributed around m⁶A-known sites
- Identified mRNA targets of ECT2 and ECT3 RNA-binding proteins
 - ECT2/ECT3 targets are **downregulated** upon loss of function of ECT2/ECT3/ECT4
 - Are enriched in **translation** and **ribosomal biogenesis** processes
 - Non-targets are **upregulated** upon loss of function of ECT2/ECT3/ECT4
 - Are enriched in **abiotic stress responses** and **transcription factors**



Acknowledgements



Peter
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Laura Arribas-
Hernández



Robin
Andersson



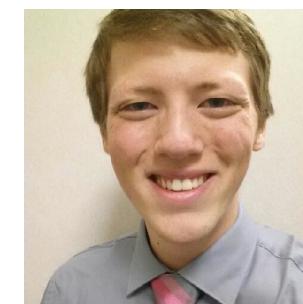
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Wageningen University, Netherlands)



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Peter Brodersen
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Robin Andersson
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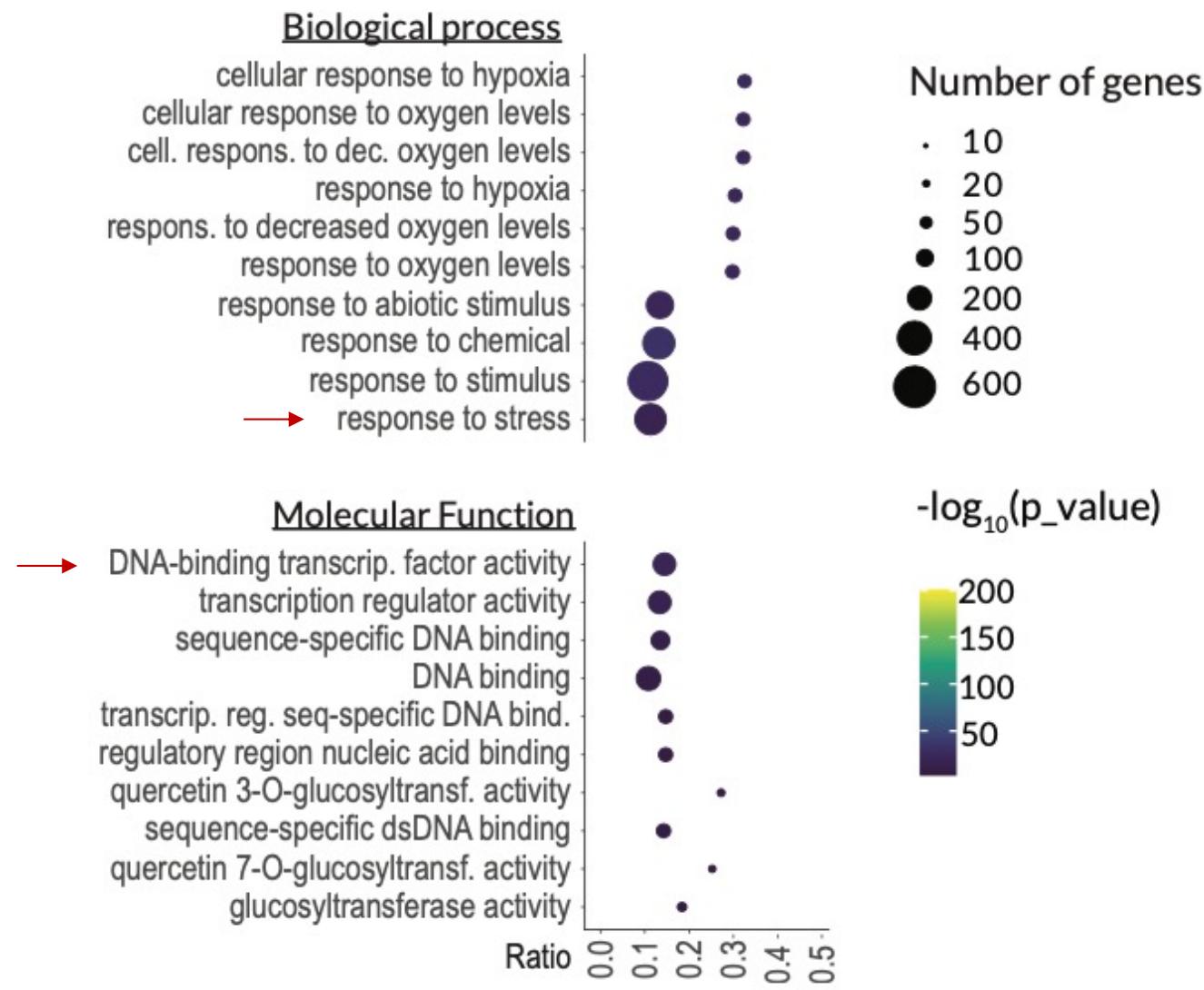
Thank you





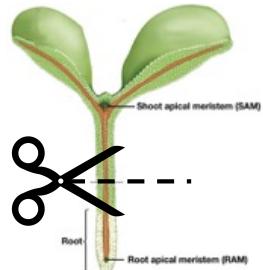
non-targets upregulated in ect2/3/4 vs WT

Upregulated non-ECT2/3 targets



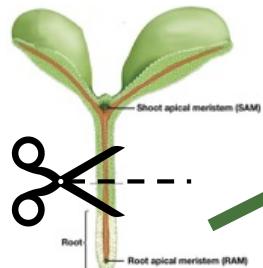
Experimental Design - what are protoplasts?

e2E2mCh



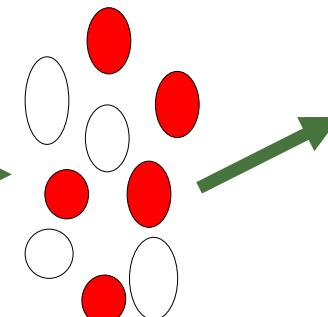
ect2
ECT2mCh
3 replicates

tE2WmCh



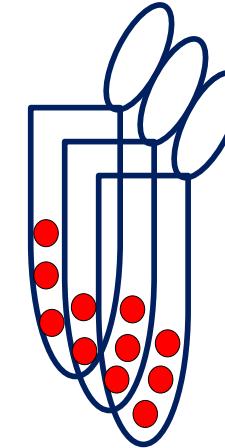
ect2
ect3
ect4
 $ECT2^{W464A}mCh$
3 replicates

cell-wall
digestion
enzymes

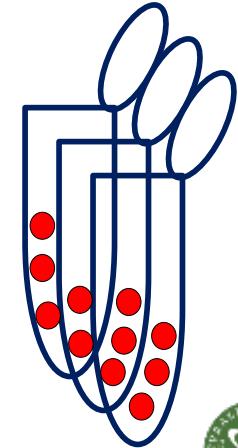


FACS:
Fluorescence-activated cell sorting

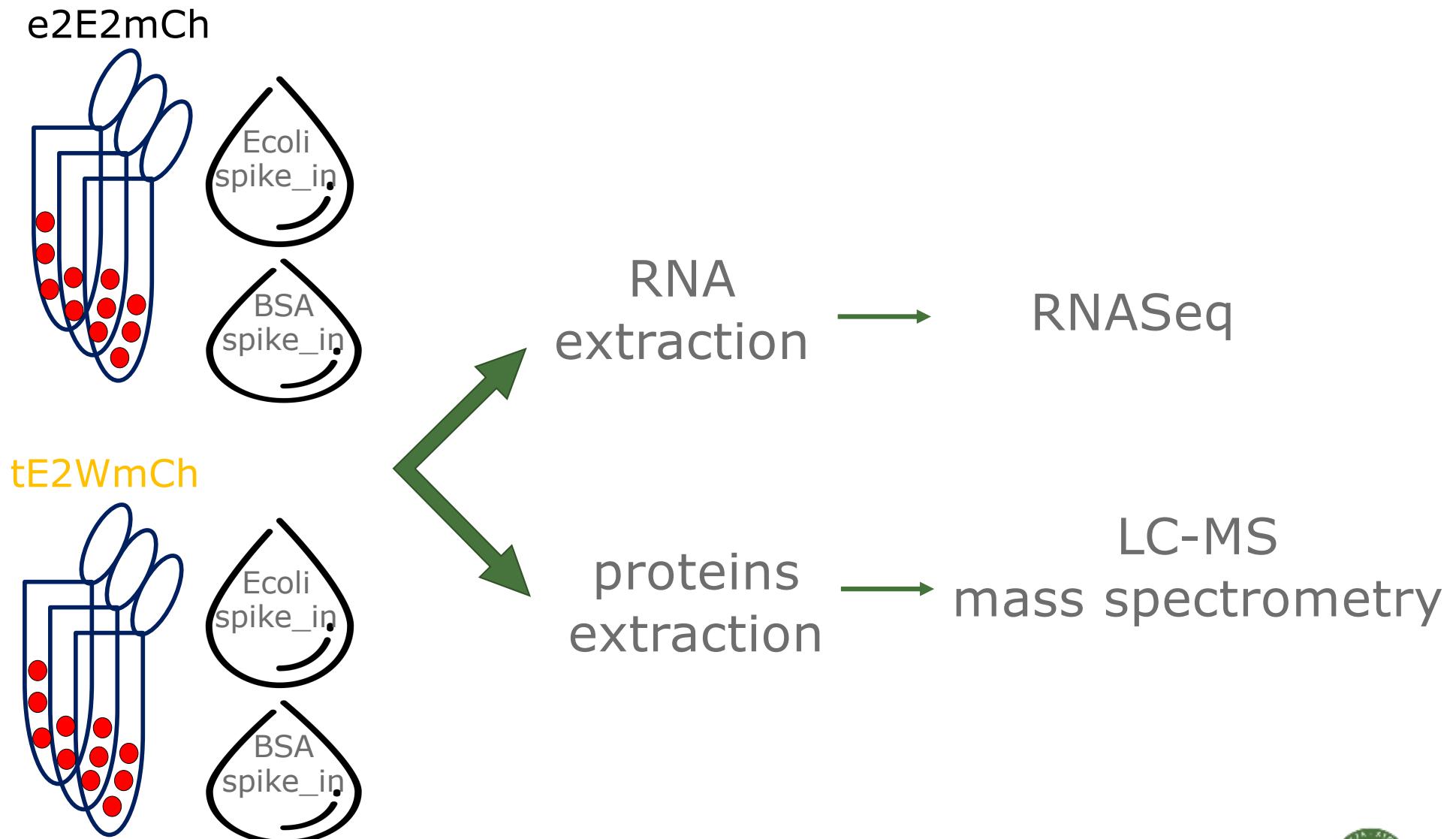
e2E2mCh



tE2WmCh



Experimental Design - what are protoplasts?



Why the ribosomal proteins?

- Overexpressed in targets sets
- Enriched in the top 10 GO Terms of downregulated genes
- Phenotype similarity:
 - Ribosomal mutant has a slow growth like the te234ECT2^{W464A}-mCherry mutants
- Genetic interaction:
 - When crossing ribosomal mutant with te234ECT2^{W464A}-mCherry mutant the result is not happy
- te234ECT2^{W464A}-mCherry mutants have fewer ribosomes, leading to delayed growth



Why the ribosomal proteins?



Why the ribosomal proteins?

