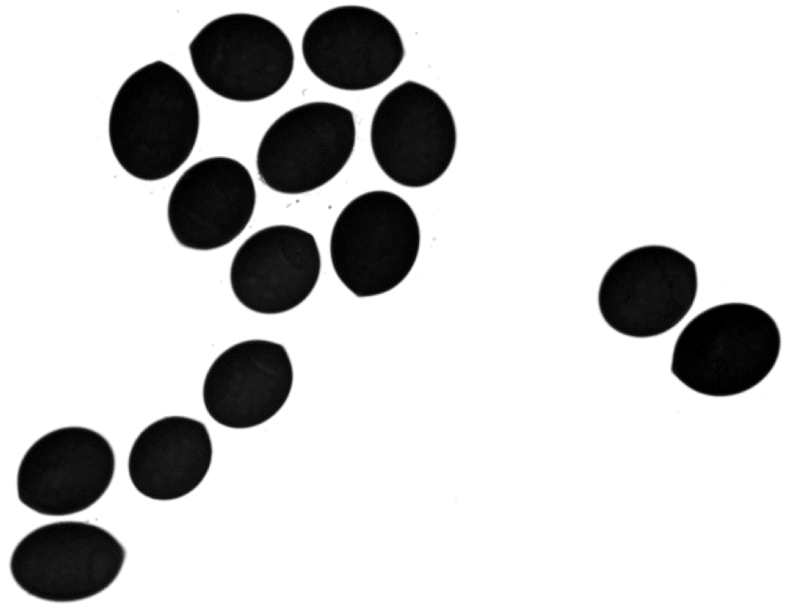
A-to-I mRNA editing: Diversification of the fungal proteome during sexual propagation

Ines Teichert



mRNA Editing

The central dogma

DNA

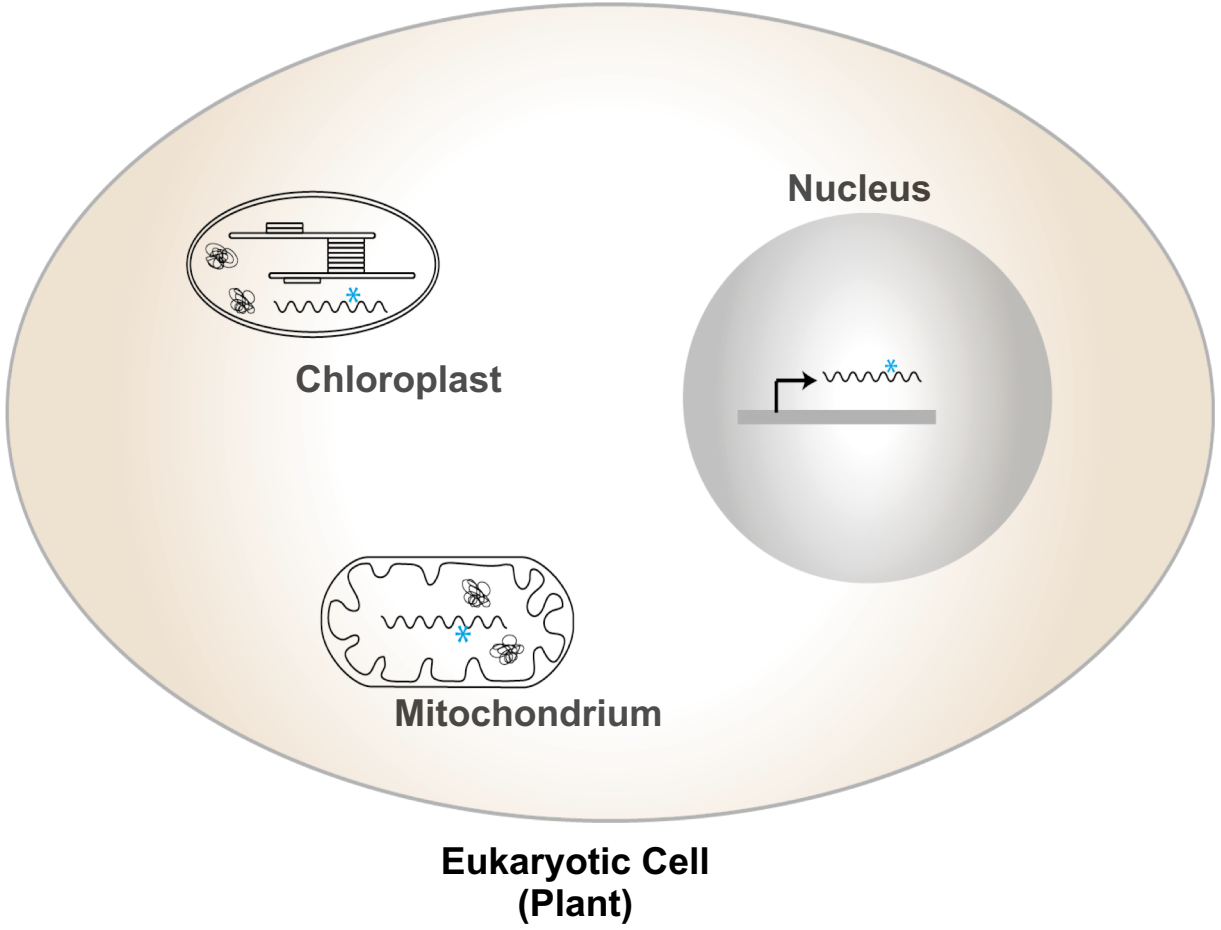
↓

mRNA

↓

protein

- Induces changes at the RNA level that could already be encoded at the DNA level
- Can lead to changes at the protein level



Plastids
- C → U
- U → C
- Land plants, moss

Mitochondria
- C → U
- U Insertions
- Diverse Eukaryotes

Restoration of proteins

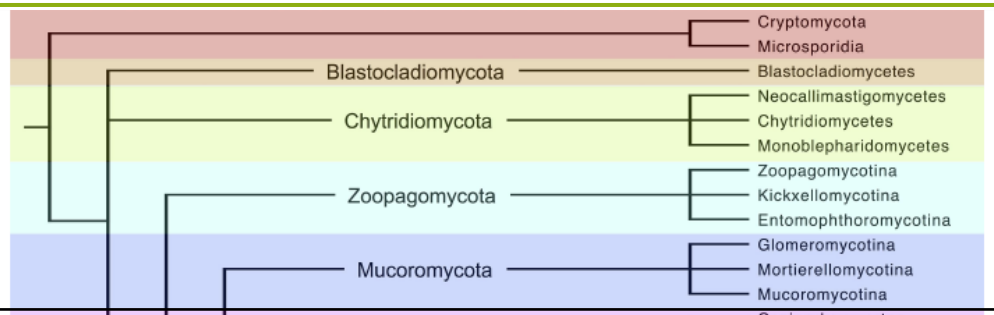
Nucleus
- C → U
- A → I
- Metazoa, Fungi

Diversification of proteins

A, adenosine; C, cytidine; G, guanosine; I, inosine; U, uridine

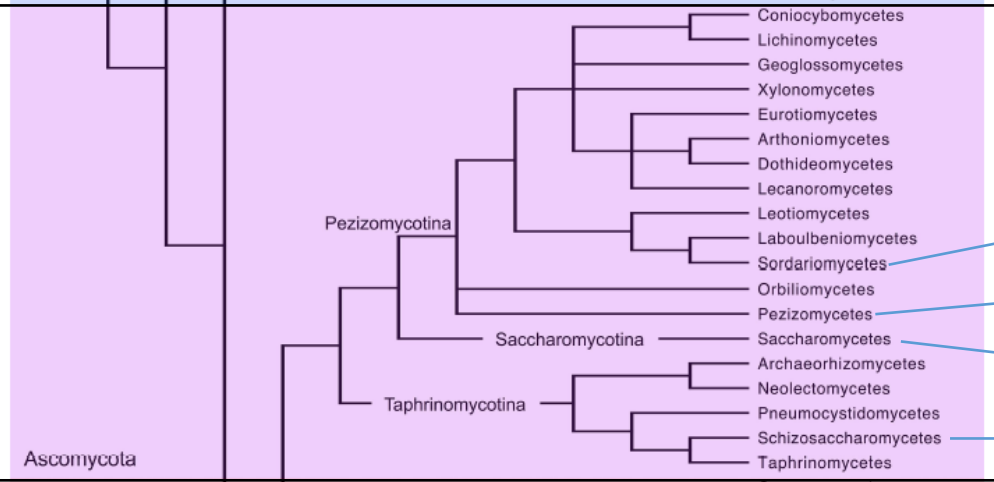
Fungal mRNA editing

Basal fungi

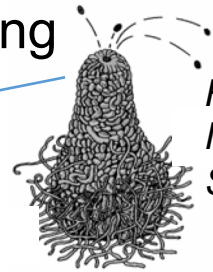


No editing described

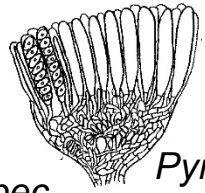
Ascomycetes



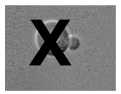
A-to-I mRNA editing in fruiting body formation



Fusarium spec.
Neurospora spec.
Sordaria spec.



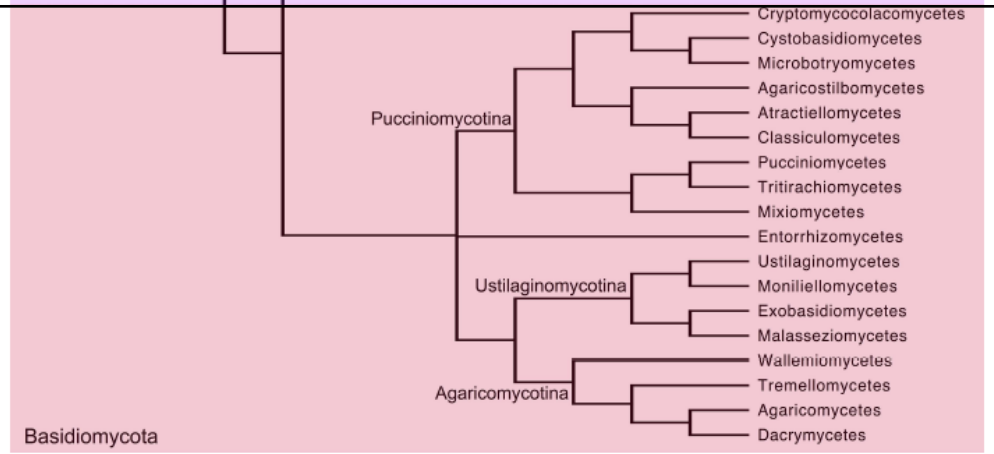
Pyronema spec.



Saccharomyces spec.

Schizosaccharomyces spec.

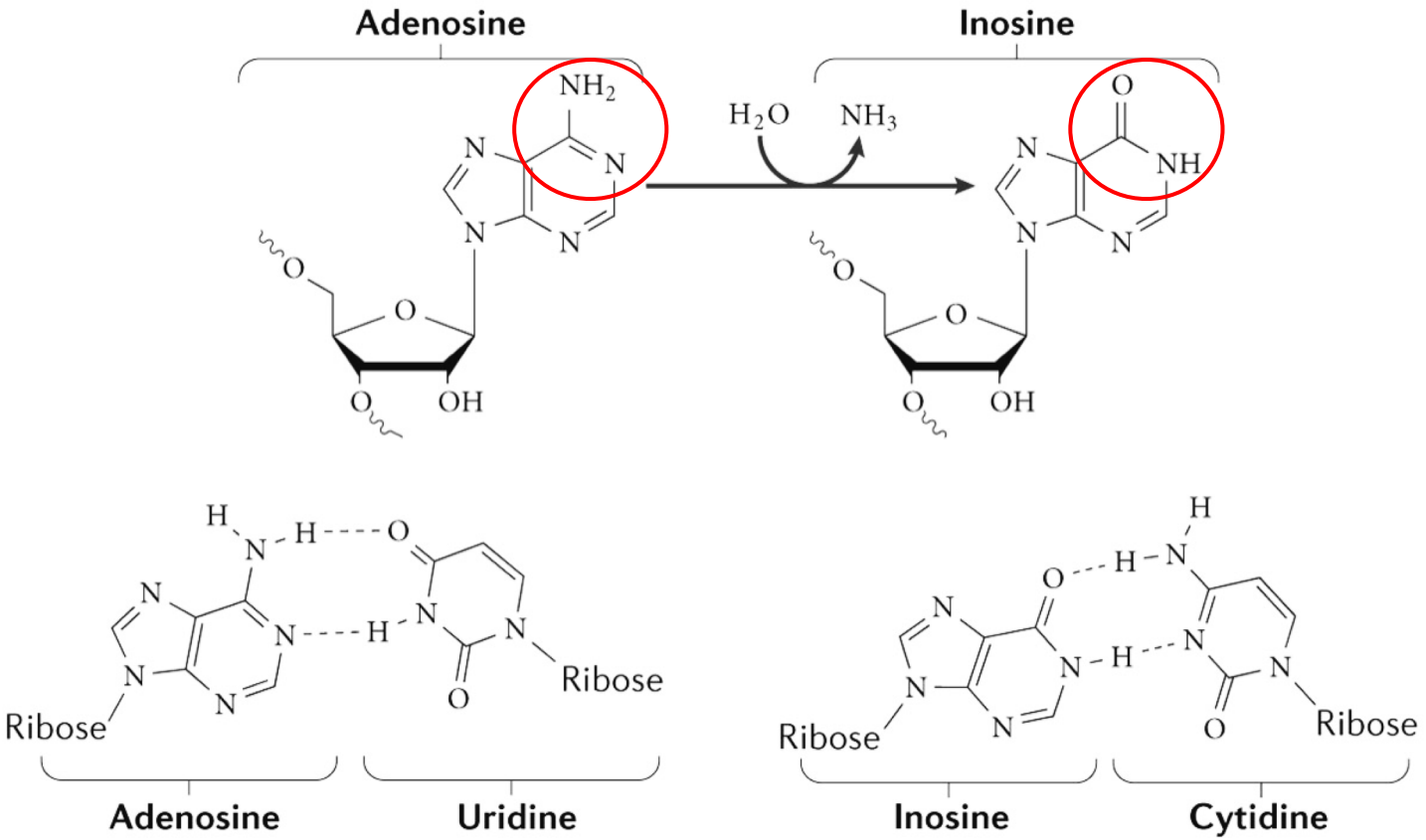
Basidiomycetes



Different forms of mRNA editing, basal level, no correlation with development
Polyporales: adaptation to substrates

Liu et al. 2016, 2017, Spatafora et al. 2017, Teichert et al. 2017, Wu et al. 2018, Wu et al. 2019, Zhu et al. 2014

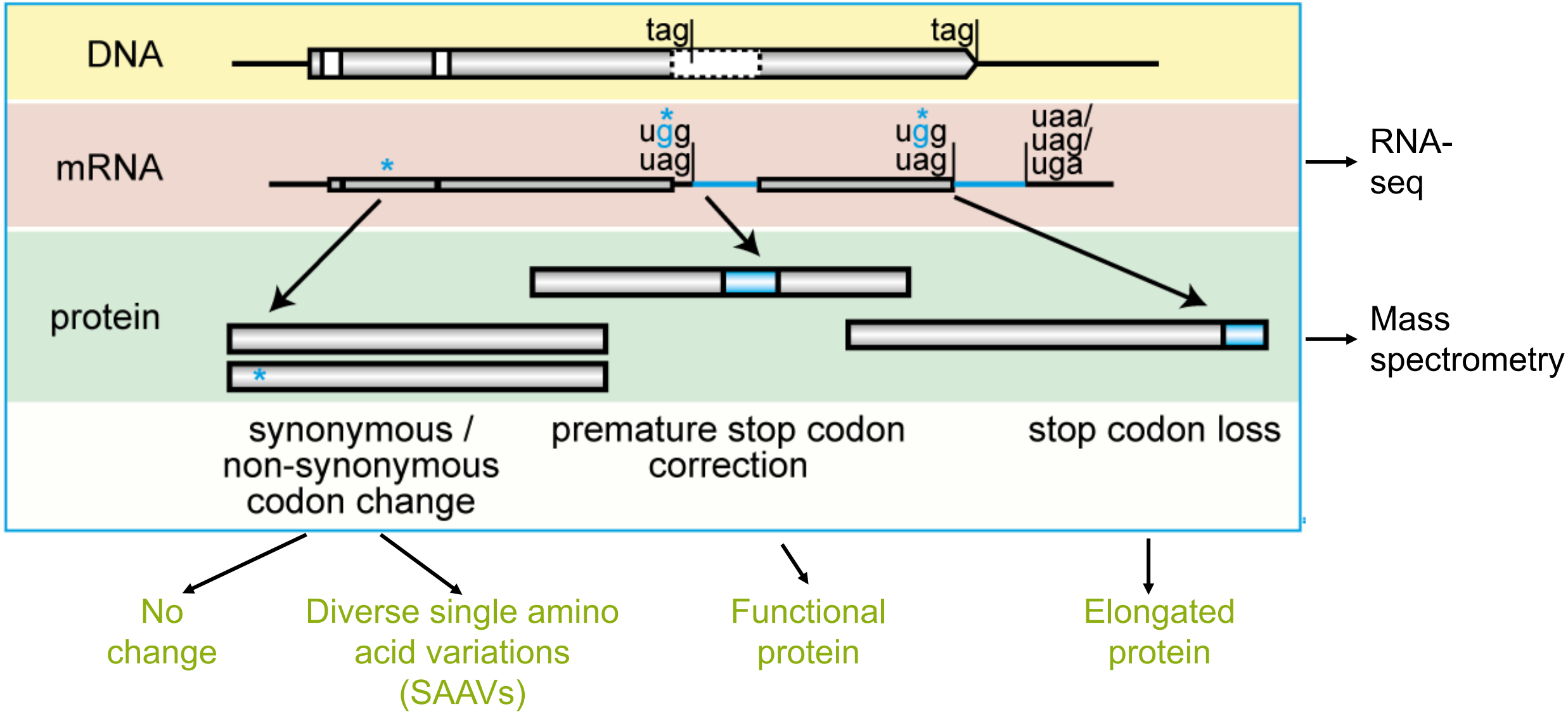
A-to-I RNA Editing: Deamination of Adenosine



- Deamination of adenosine to inosine
- Inosine pairs with cytidine:
  - ➢ **Effectively A-to-G exchange**
    - Changes in RNA structure
    - Changes in protein sequence

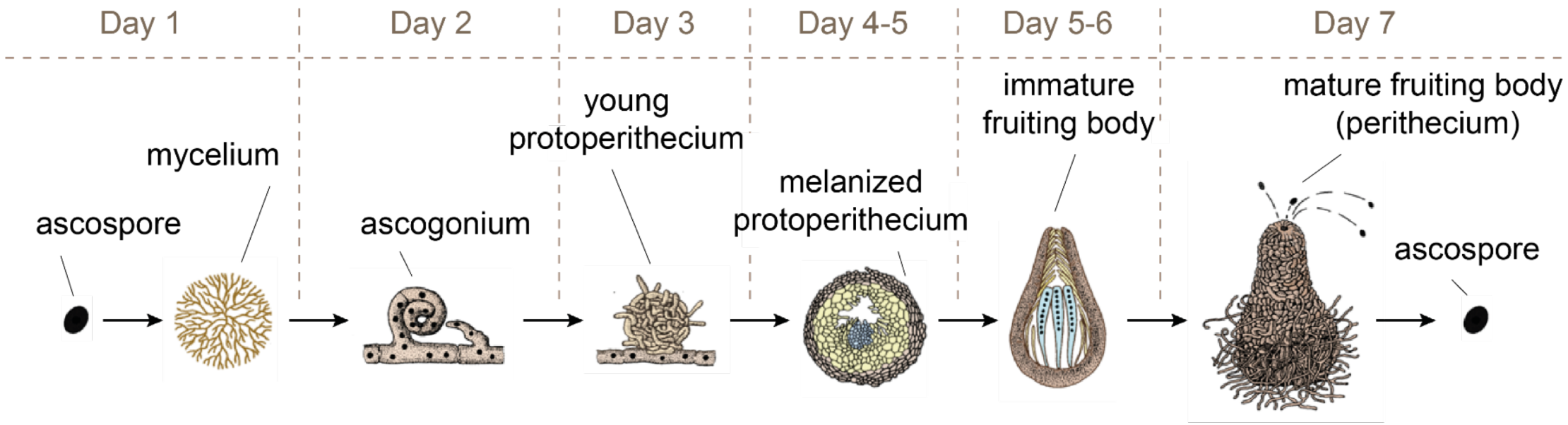
Nishikura 2016, Rosenthal 2015

Effect of A-to-I Editing on mRNA and Proteins



Modified from Teichert (2018)

A-to-I Editing in *Sordaria macrospora*



RNA-based (collaboration with M. Nowrousian, Ruhr University Bochum)

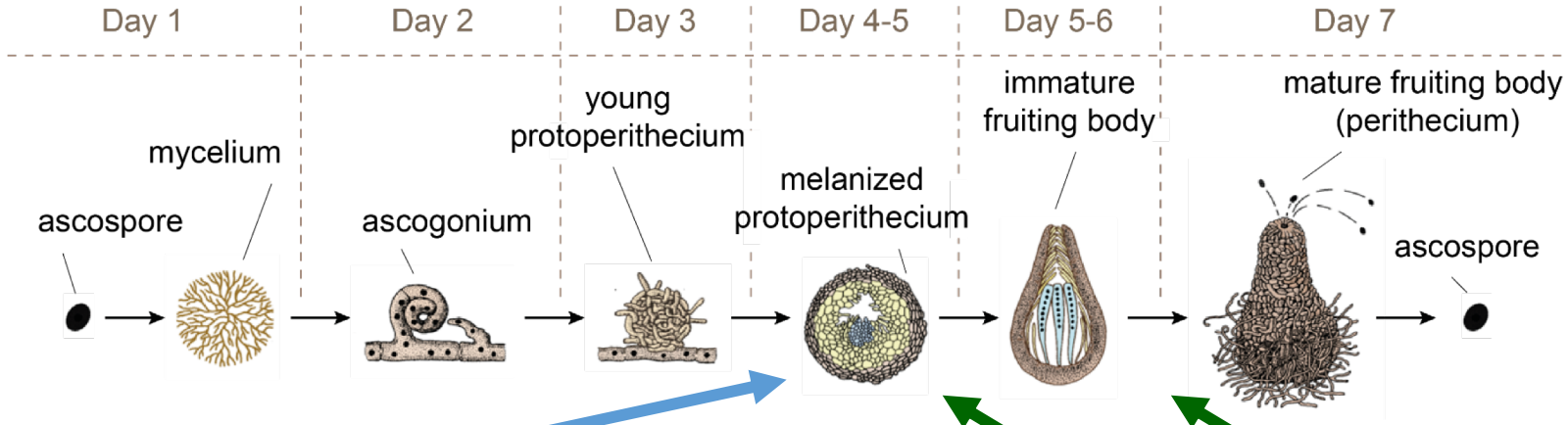
- RNA-seq

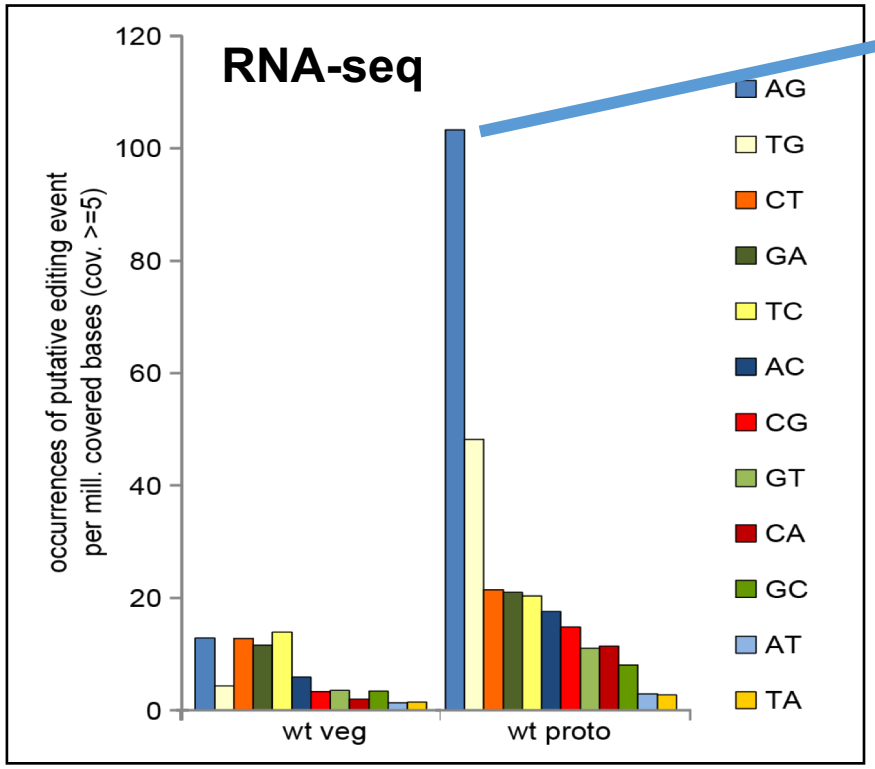
Protein-based (collaboration with A. Sickmann, B. Blank-Landeshammer, ISAS e.V. Dortmund)

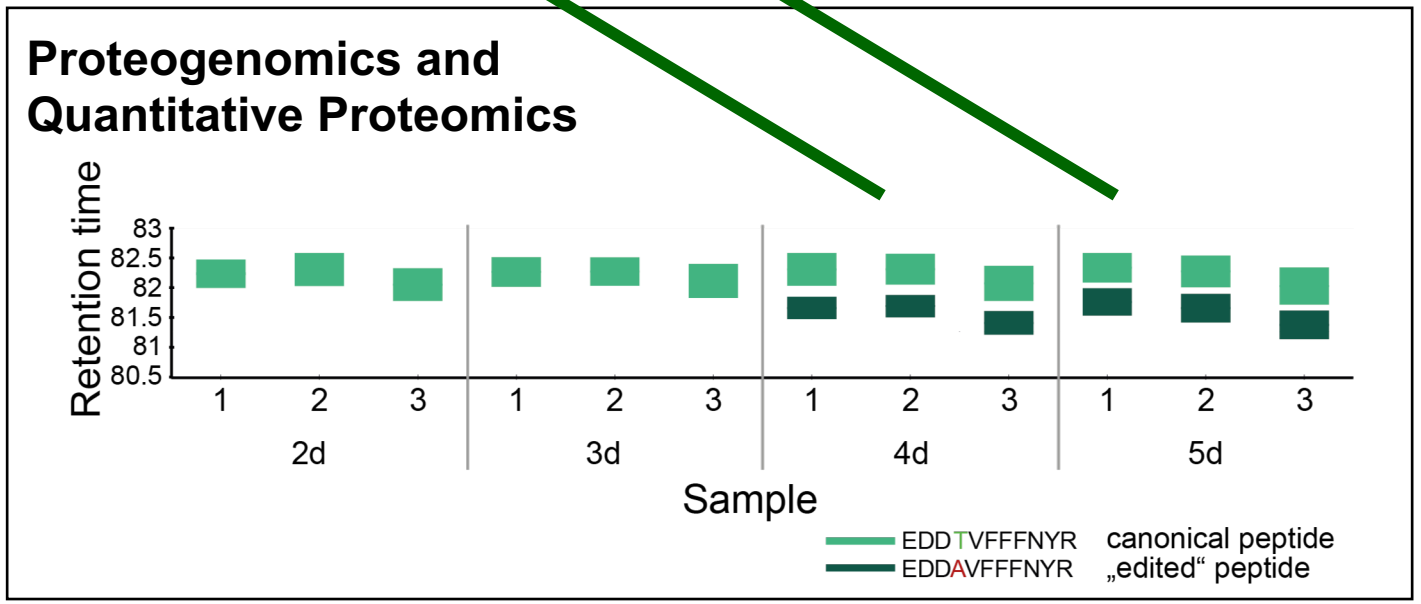
- Proteomics
- Proteogenomics

Modified from Teichert et al. (2014)

A-to-I Editing in *Sordaria macrospora*



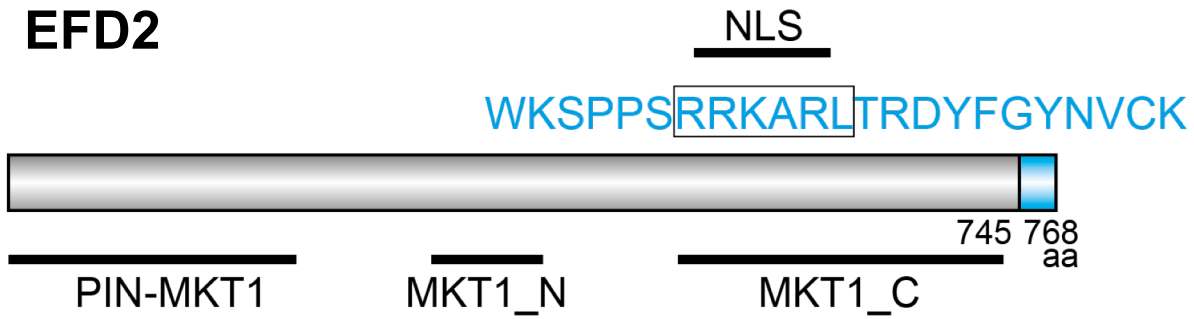
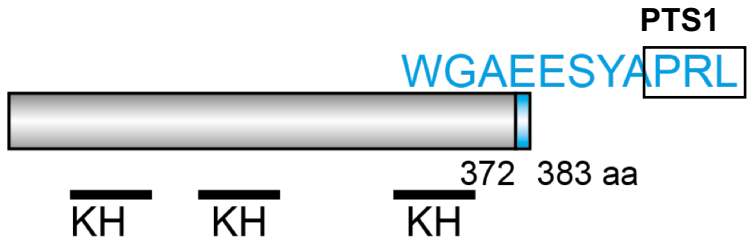




→ **Protein level evidence for 35 stop loss events and 113 SAAVs**

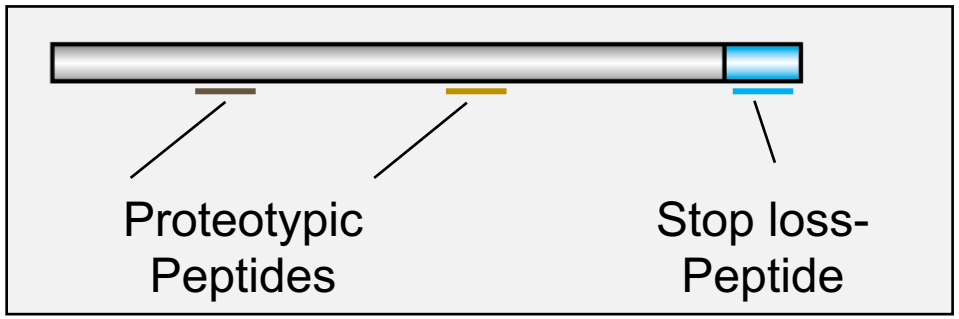
Blank-Landeshammer et al. (2019), Teichert et al. (2014), Teichert et al. (2017)

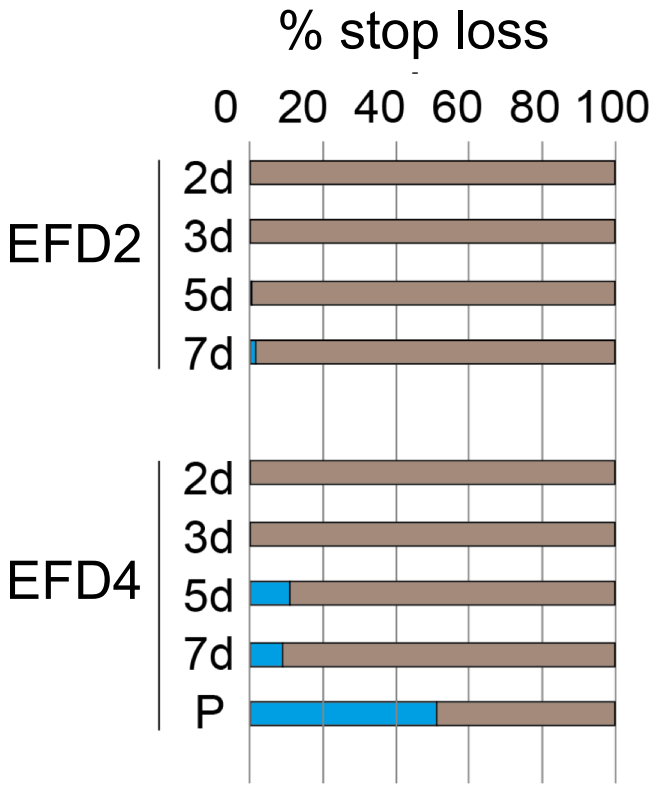
EFD Proteins Gain Signals and Domains

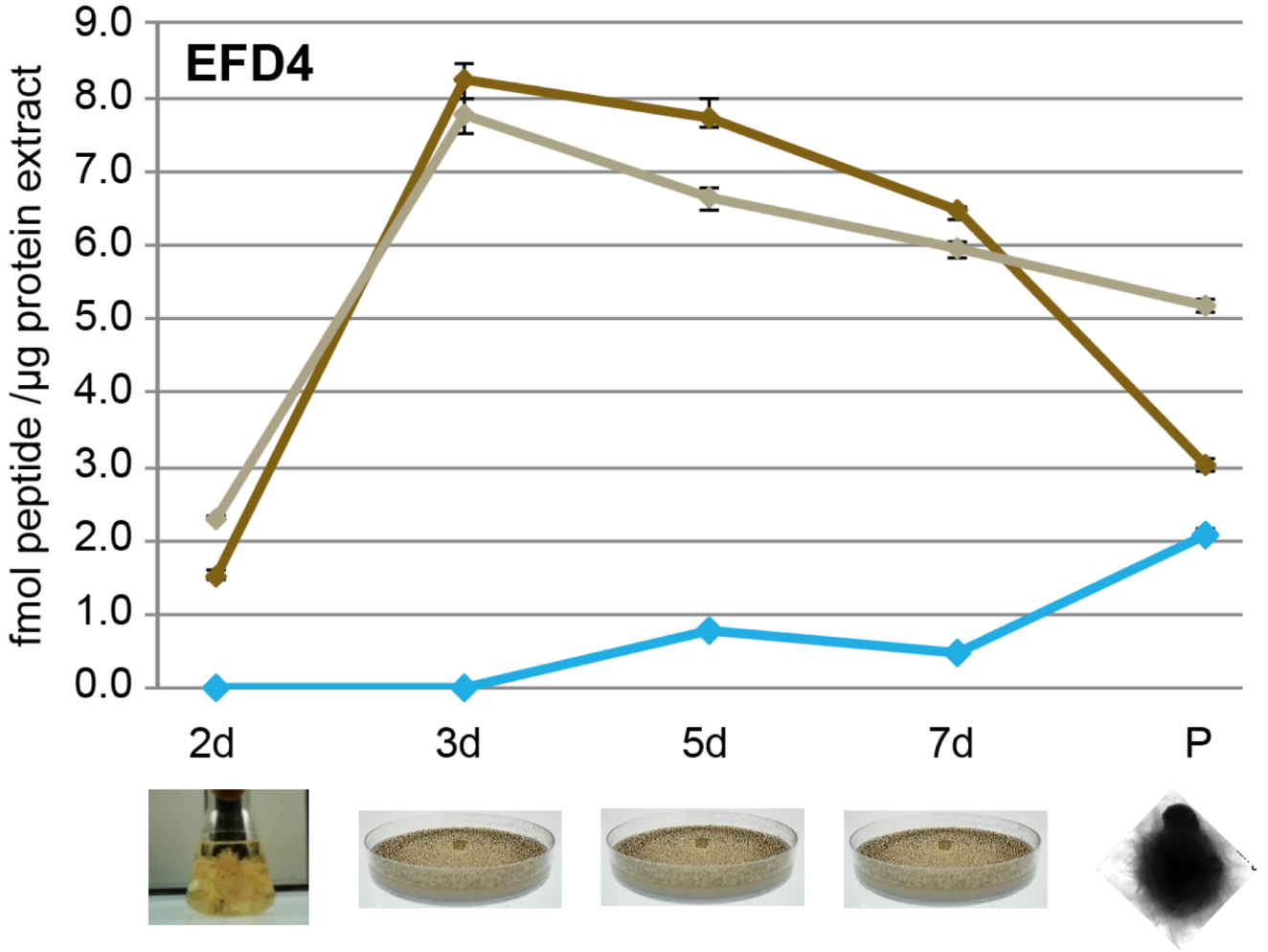
	(predicted) function	Δ phenotype
EFD2 	RNA-binding protein	Aberrant ascospore morphology and number
EFD4 	RNA-binding protein	Aberrant ascus and ascospore morphology, fewer ascospores

NLS, nuclear localization signal; PTS1, peroxisomal targeting signal; KH, MKT, RNA-binding domains

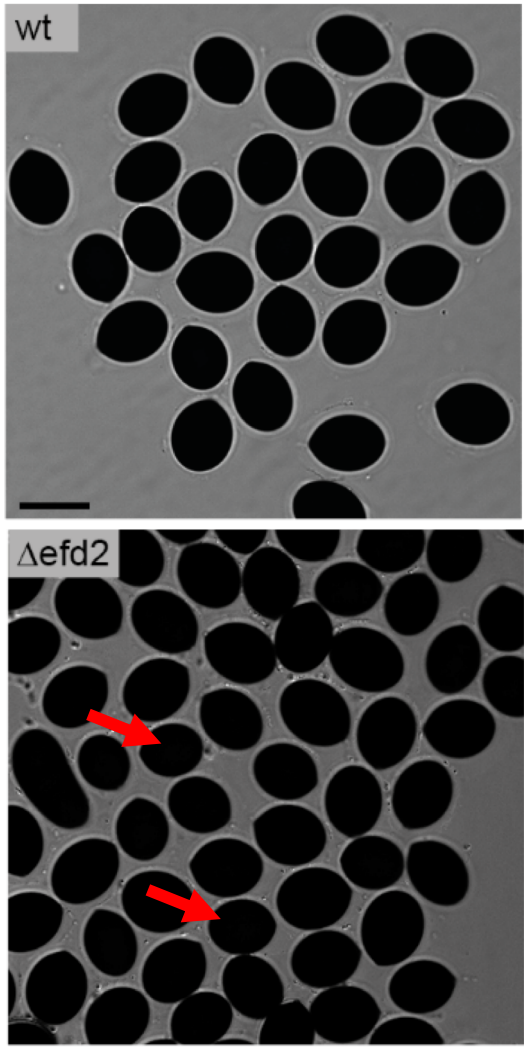
Quantification of Stop loss Editing in EFD2 and EFD4



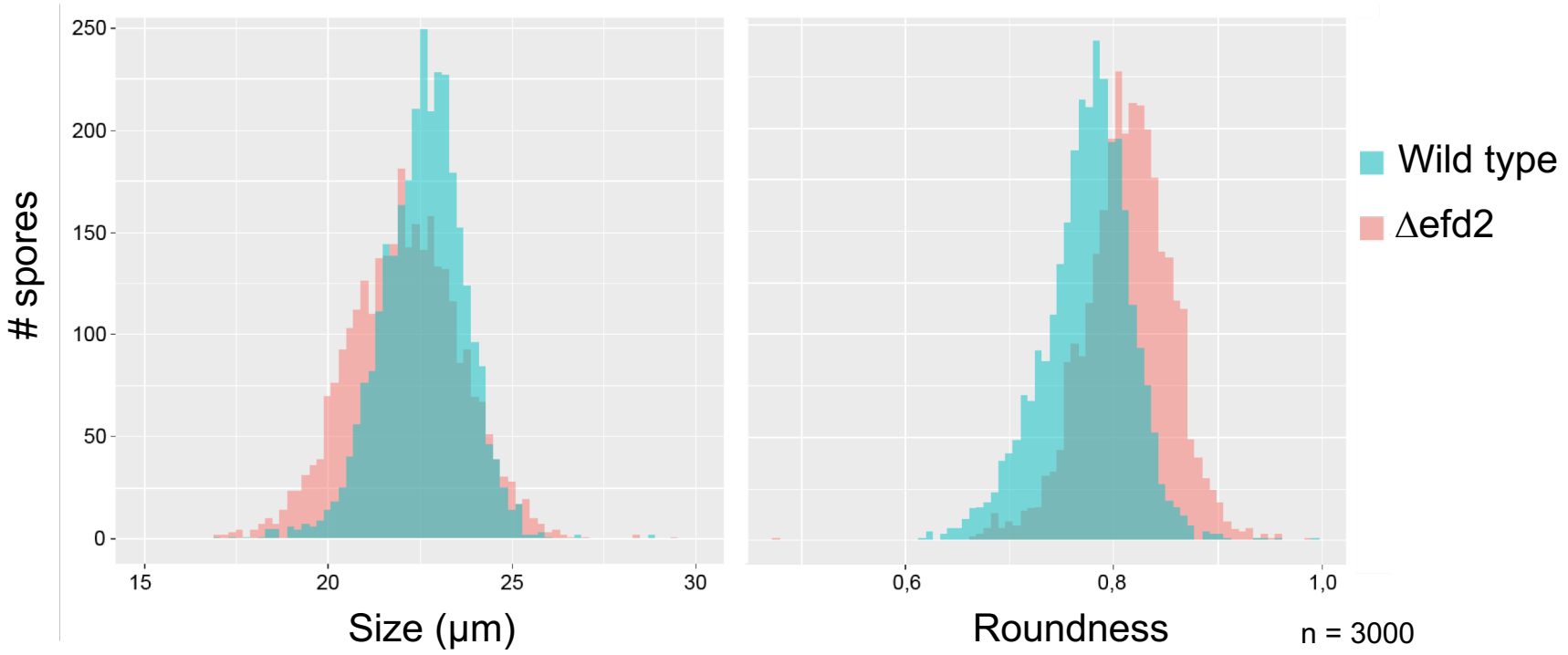




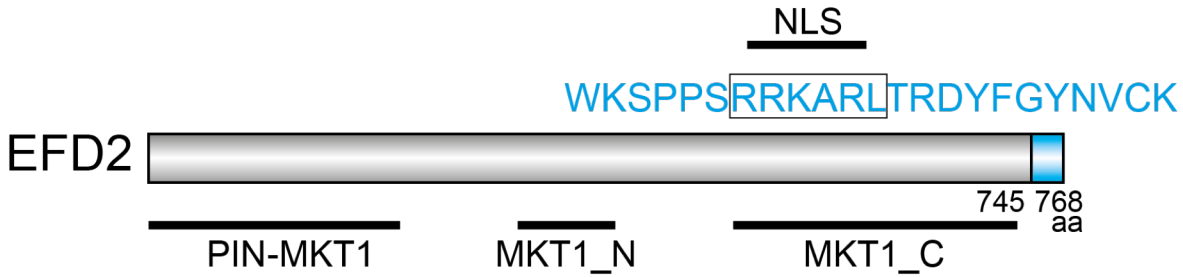
Lack of *efd2* leads to smaller and rounder ascospores



bar, 20 µm

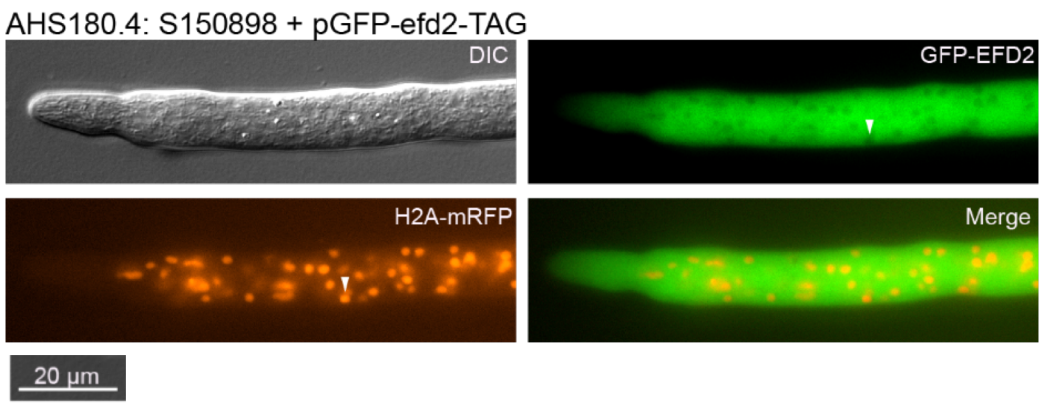


EFD2 localizes to the nucleus in sexual spores



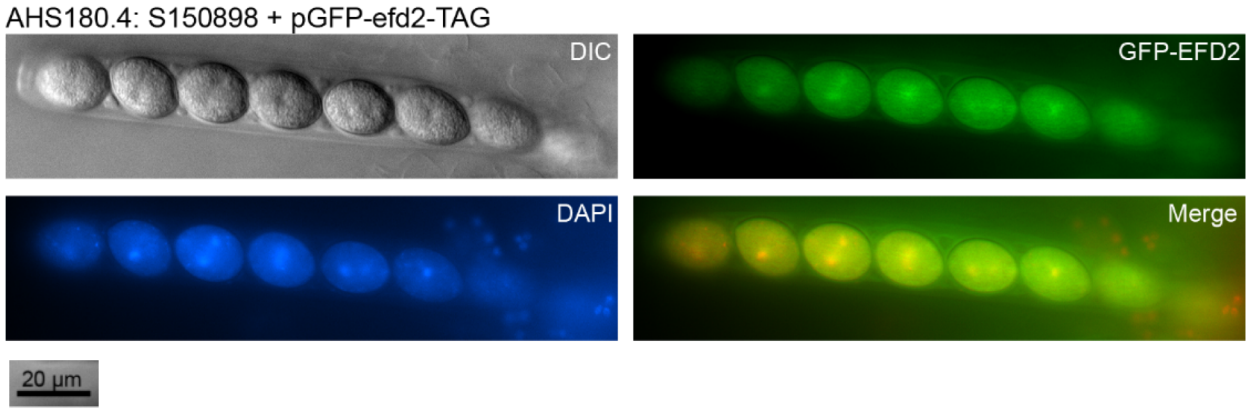
2 days, vegetative growth, no editing

Short protein



6 days, sexual development, editing

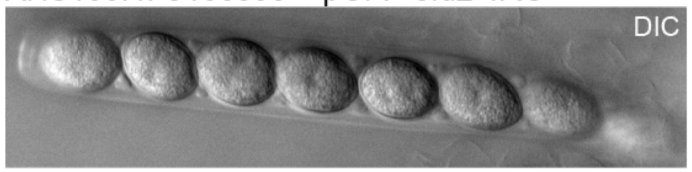
Long protein

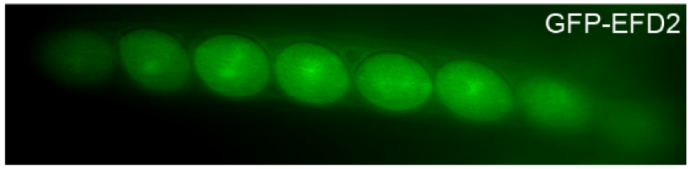


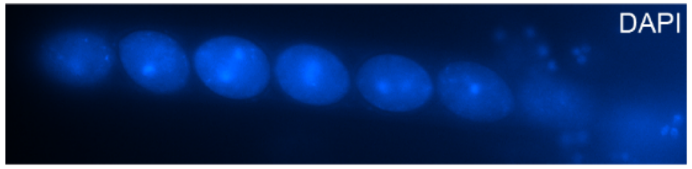
Is RNA editing required for EFD2 nuclear localization?

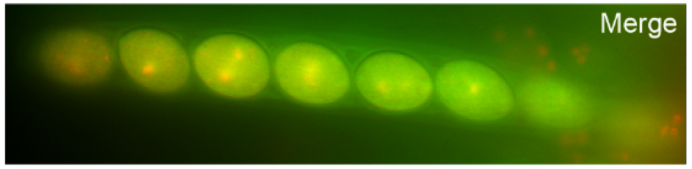
„tag“
Native allele

AHS180.4: S150898 + pGFP-efd2-TAG



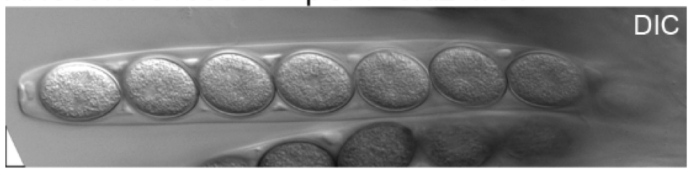


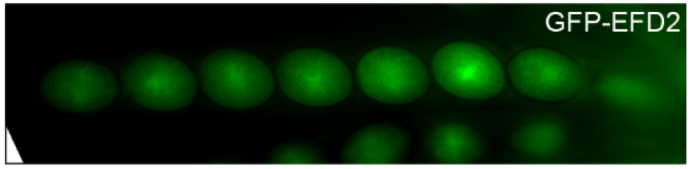


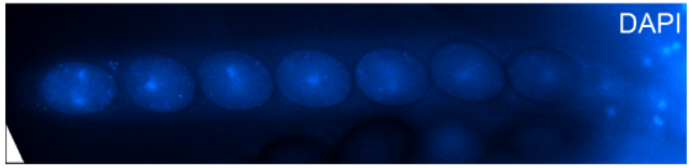


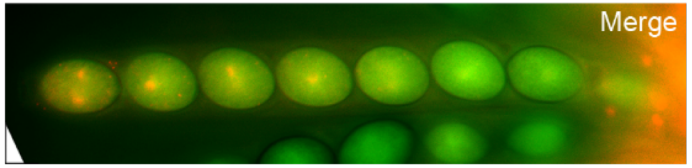
„taa“
Non-editable allele

AHS80.6: S150898 + pGFP-efd2-TAA



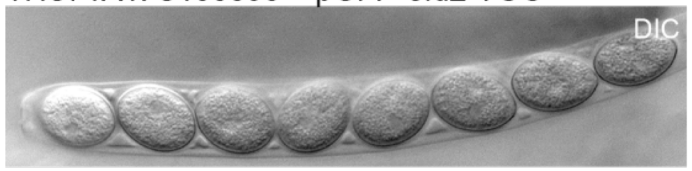


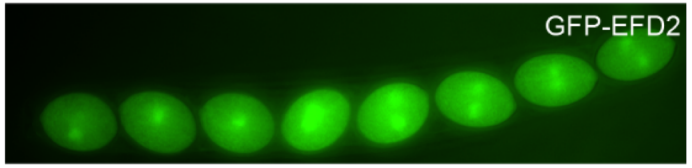


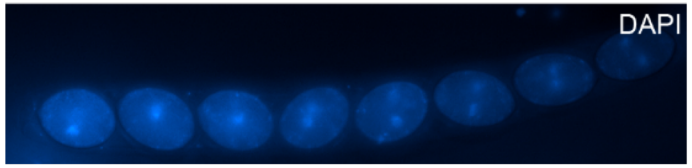


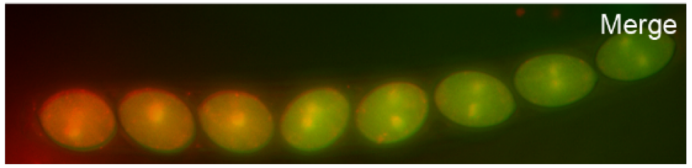
„tgg“
Always edited allele

THS71A1: S150898 + pGFP-efd2-TGG

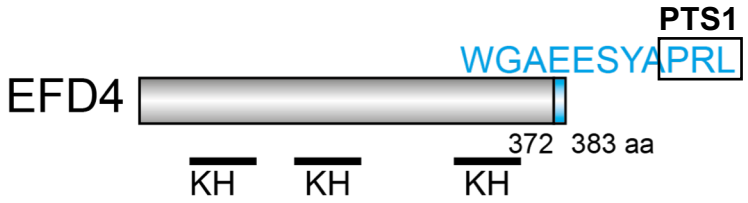




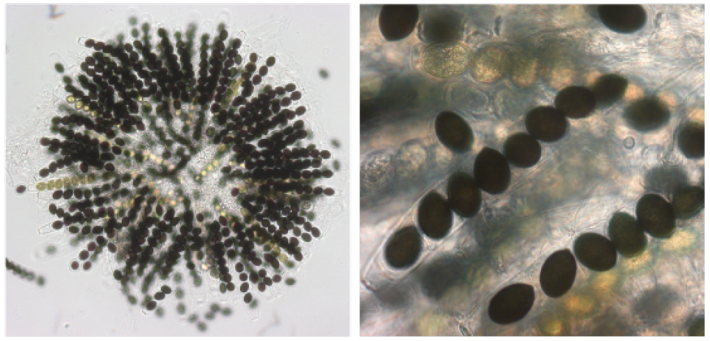




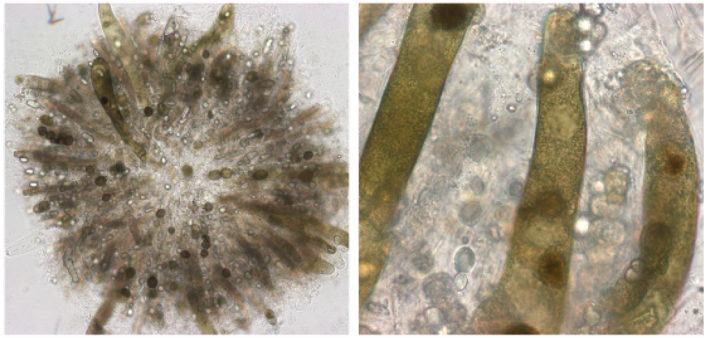
Both EFD4 isoforms are required for ascospore formation



Wild type

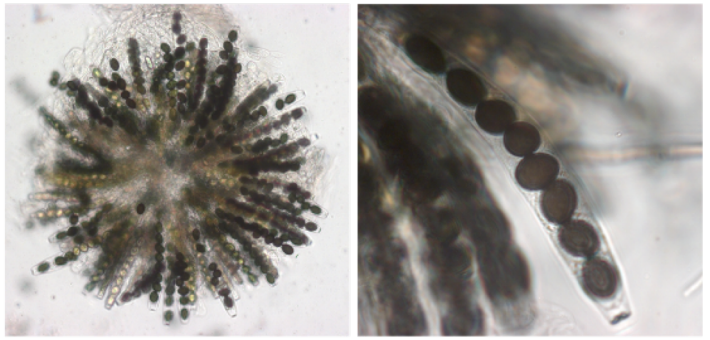


Δefd4

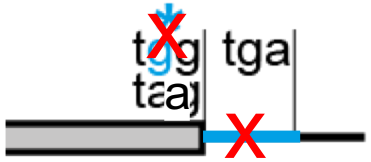


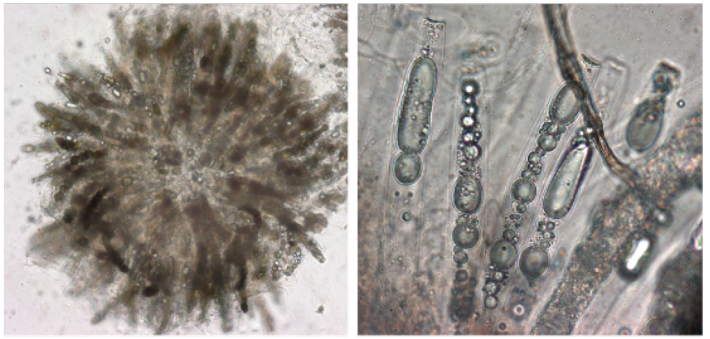
→ Target different alleles to the native locus

„tag“
Native allele

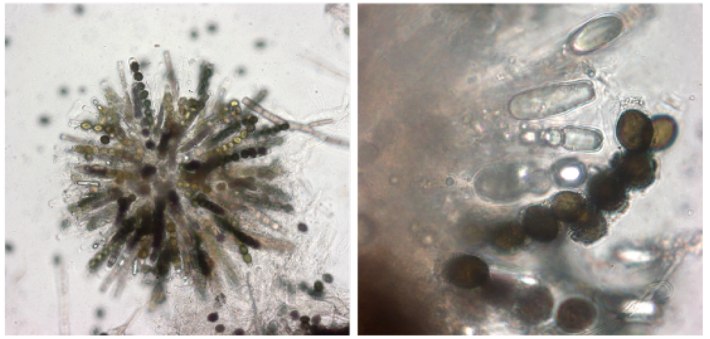


„taa“
Non-editable allele





„tgg“
Always edited allele



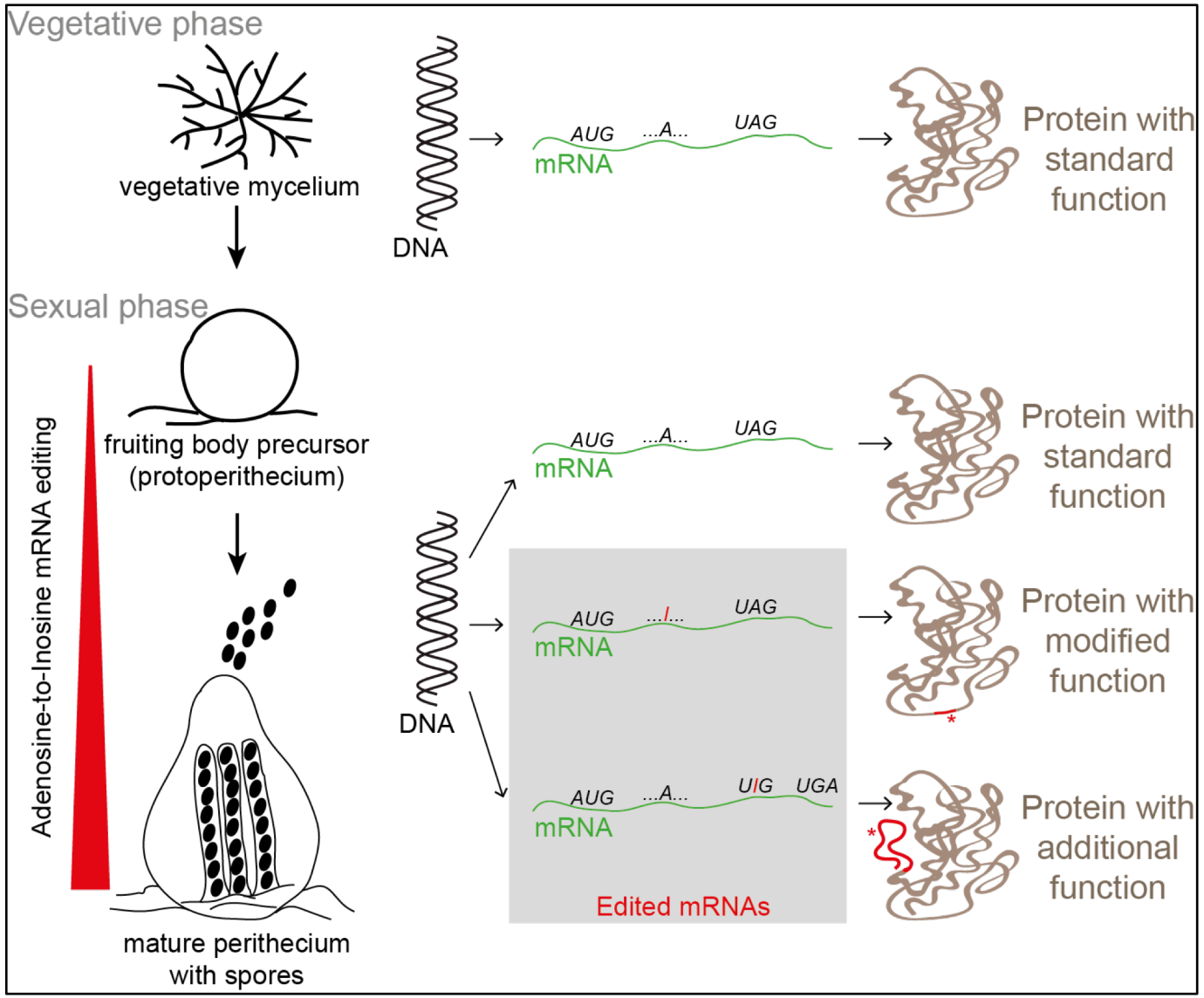
A-to-I editing of nuclear protein-coding transcripts

WHO?

- Filamentous ascomycetes
- Perithecia + apothecia producers
- Homothallic + heterothallic species

WHEN?

- Starts before meiosis
- Levels increase during ascus formation in fruiting bodies



WHY?

- Ascospore formation
- Generative tissue formation
- Provision of RNAs/proteins for germination

How?

- ?

Modified from Teichert (2020)

Thanks!

RNA Editing Group
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Stefanie Pöggeler

CICESE, Mexiko
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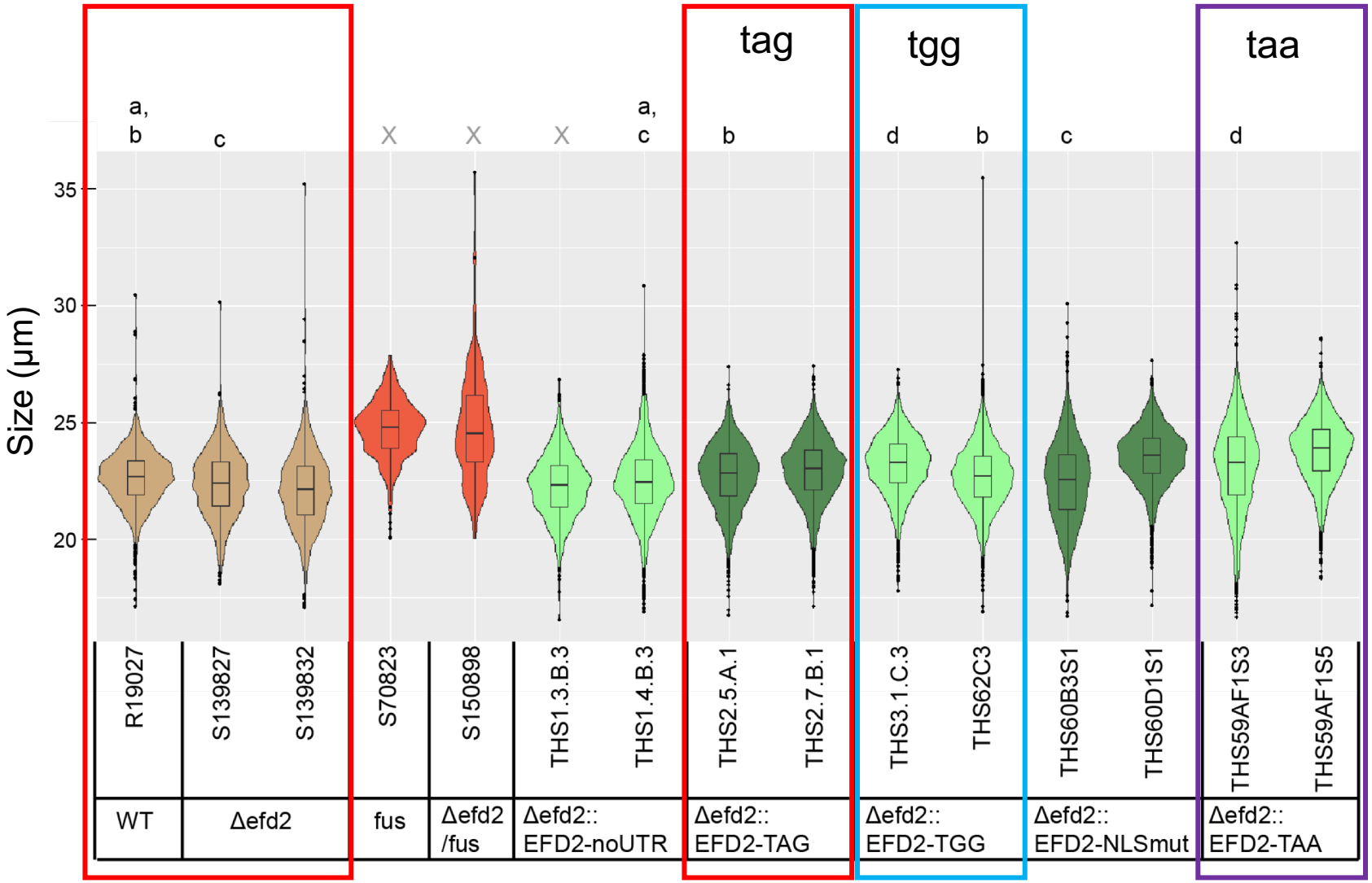
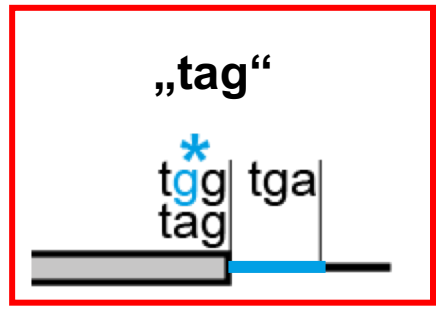
UC Berkeley
Louise Glass

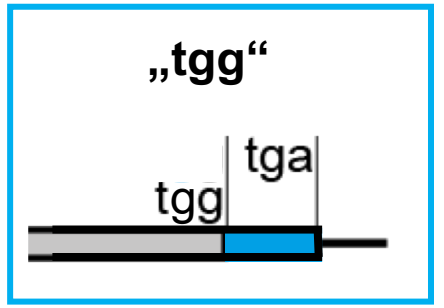
Deutsche Forschungsgemeinschaft
DFG

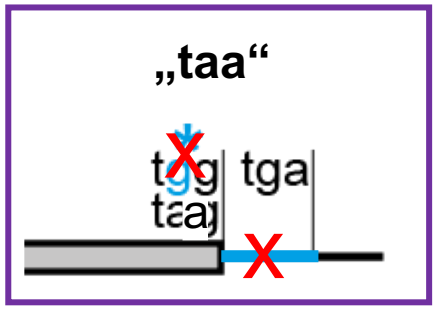
RUB
Protein
Research
Department

Function and localization of *efd2* alleles

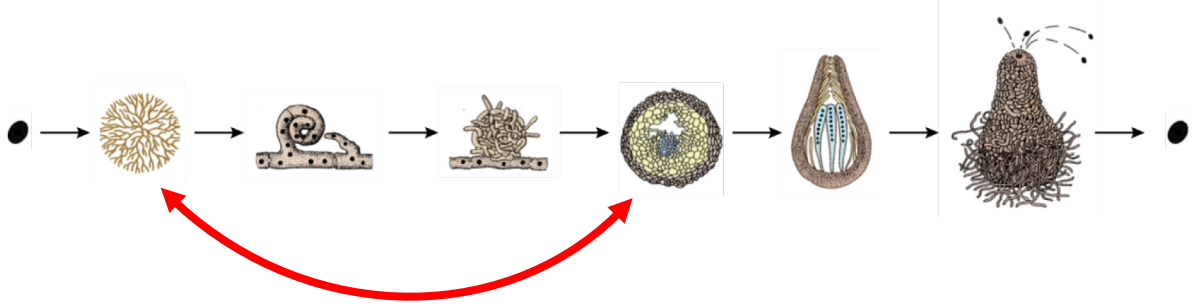




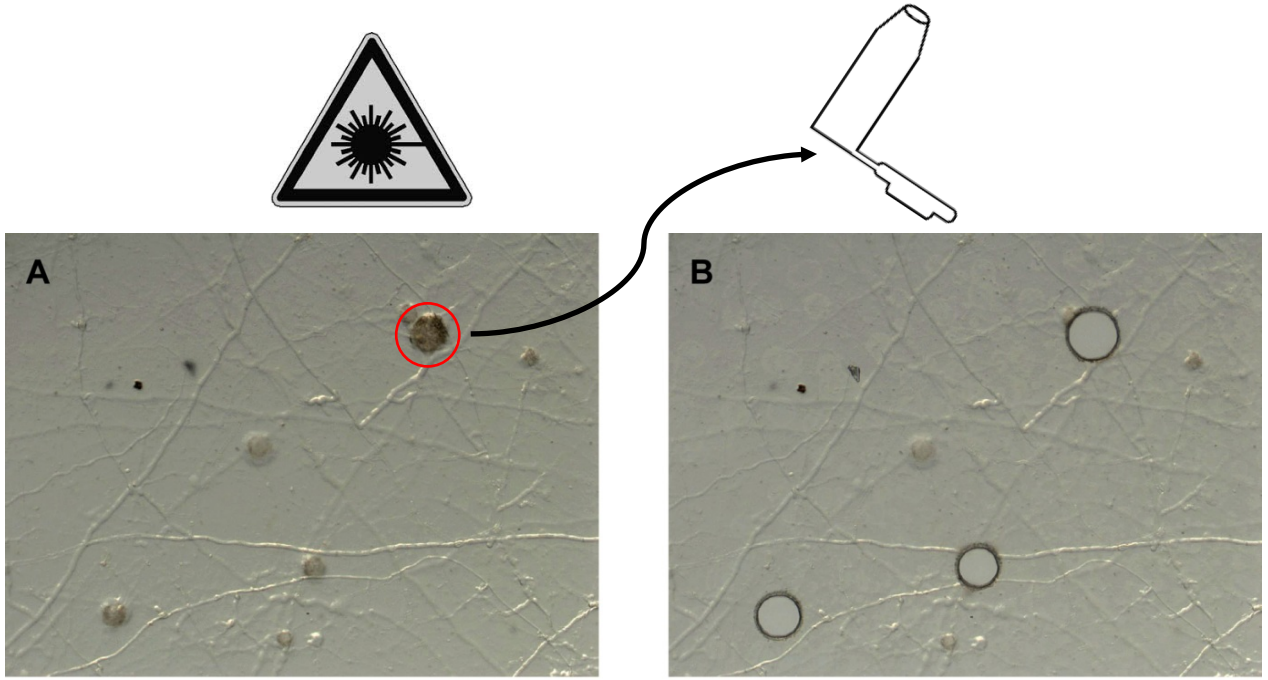


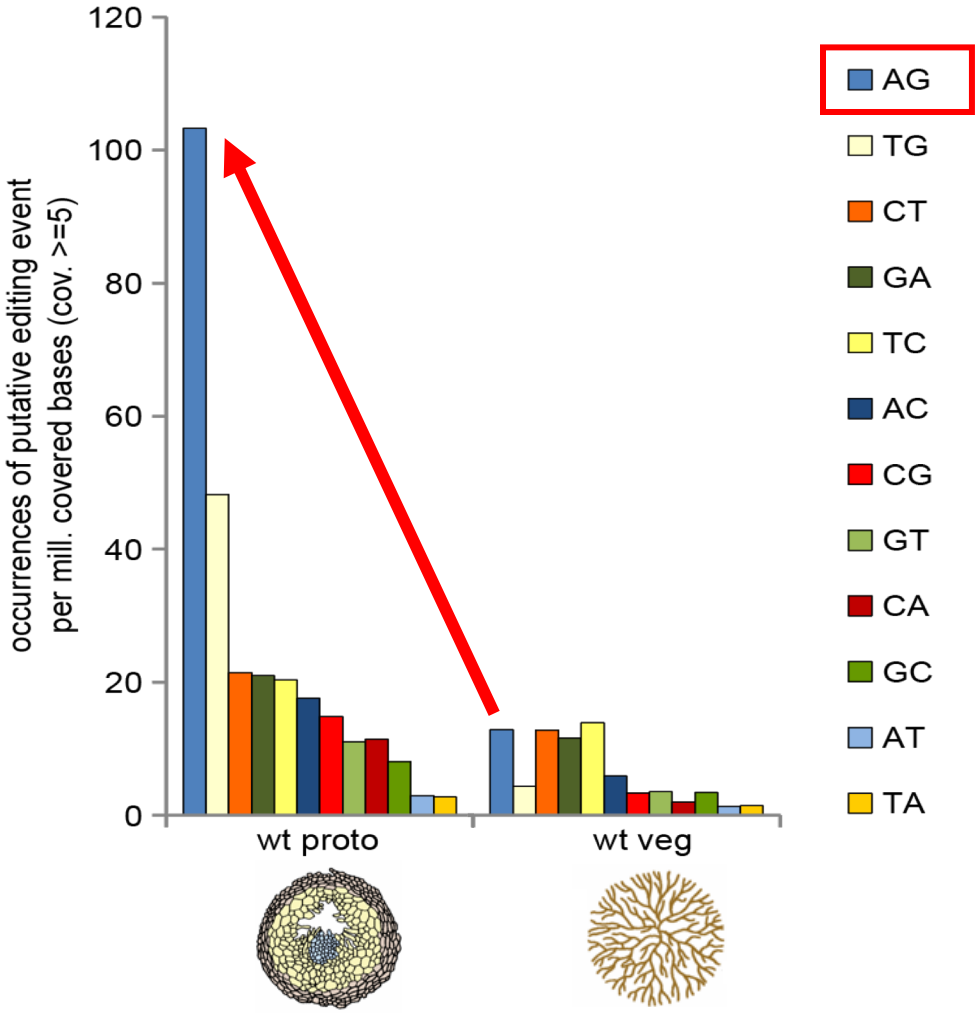


Identification of editing sites by RNA-seq



Isolation of protoperithecia by microdissection





Identification of ***efd*** genes
(**e**dited in **f**ruiting-body **d**evelopment)

Identification of editing sites by proteomics

Problem

Proteomics depends on translation of annotated genes

NINDSETR

NINDSETR EPSEWGAEESYAPR

Solution

Artificial databases, based on RNA-seq data

- *S. macrospora* RNA-seq
  (Teichert et al. 2012, Dirschnabel et al. 2014)
- *N. crassa* RNA-seq
  (Liu et al. 2017)

Protein database

>EFD4
MSASQDSIPANGGSIEENLDNLNINDSETRLGPDGEPAPRTDEEY
SQTTLTLRAIVSSKEAGVIIGKGGQNVANLRDETGVKAGVSKVVQG
VHDRVLTITGGCDAVSKAYAAVARSLLEGAPSVGMGGVISANGTH
PLKLLISHNQMGTVIGRQGLKIKHIQDVSGVRMVAQKEMLPQSTER
VVEVQGTPEGIERAVWEICKCLVDDWQRGTGTVLYNPVVRGPGA
PVSGGERNYPQERSYGSSRVTRTGNGADFSSNSGGRAYNRRSD
SDAASRGPPTHDENGEEIQTQNISIPADMVGCIIGRAGSKISEIRKQ
SGARISIAKGPHDESGERMFTIMGSAKANETALYLLYENLEAEKTR
RSQQALEPSE*

>EFD4_ed
MSASQDSIPANGGSIEENLDNLNINDSETRLGPDGEPAPRTDEEY
SQTTLTLRAIVSSKEAGVIIGKGGQNVANLRDETGVKAGVSKVVQG
VHDRVLTITGGCDAVSKAYAAVARSLLEGAPSVGMGGVISANGTH
PLKLLISHNQMGTVIGRQGLKIKHIQDVSGVRMVAQKEMLPQSTER
VVEVQGTPEGIERAVWEICKCLVDDWQRGTGTVLYNPVVRGPGA
PVSGGERNYPQERSYGSSRVTRTGNGADFSSNSGGRAYNRRSD
SDAASRGPPTHDENGEEIQTQNISIPADMVGCIIGRAGSKISEIRKQ
SGARISIAKGPHDESGERMFTIMGSAKANETALYLLYENLEAEKTR
RSQQALEPSEWGAEESYAPRL*

Identification of editing sites by proteogenomics

„Standard“ proteogenomics

- **Matching** to 6-frame genome translation

Peptides from within C-terminal elongations

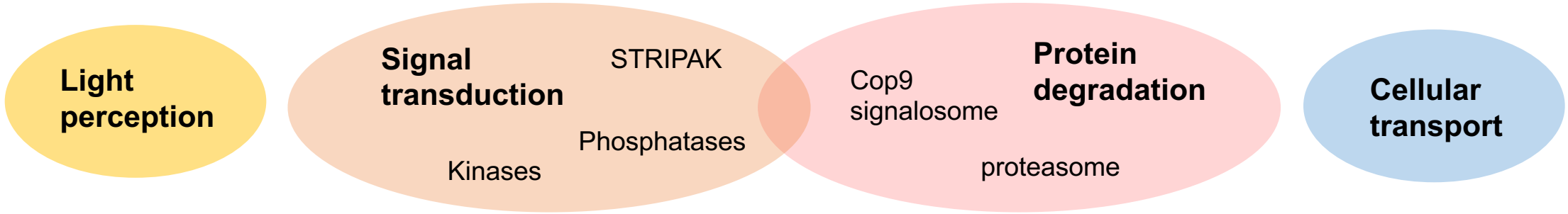
Novel proteogenomics approach

- *De novo* peptide sequencing
- **Alignment** to 6-frame genome translation

Peptides harboring single amino acid variations (SAAVs)

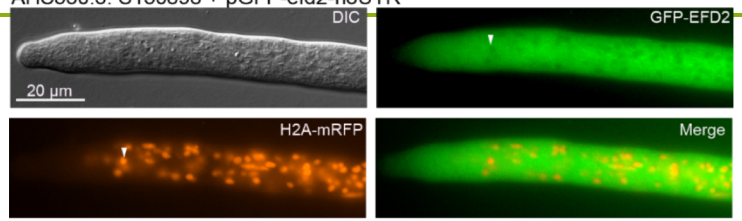
Peptides overlapping Stop to W exchanges

→ Protein level evidence for >30 stop loss events and >100 SAAVs

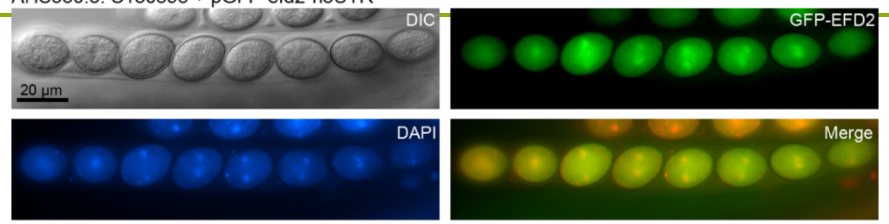


Blank-Landeshammer et al. (2019) mBio, accepted for publication

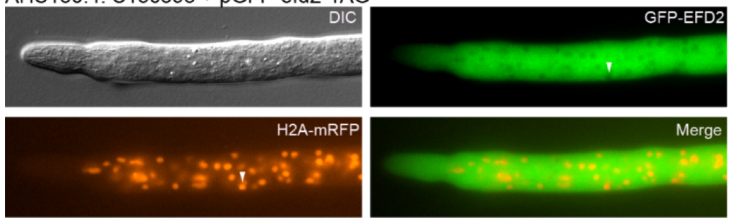
AHS530.3: S150898 + pGFP-efd2-noUTR



AHS530.3: S150898 + pGFP-efd2-noUTR



AHS180.4: S150898 + pGFP-efd2-TAG



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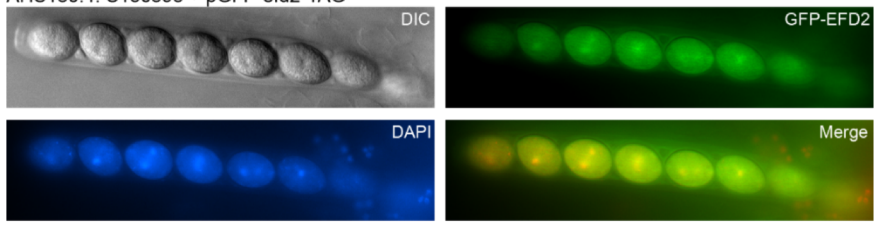
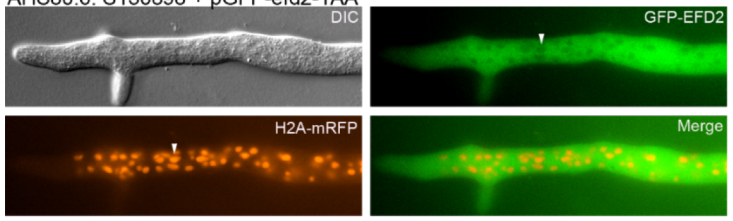
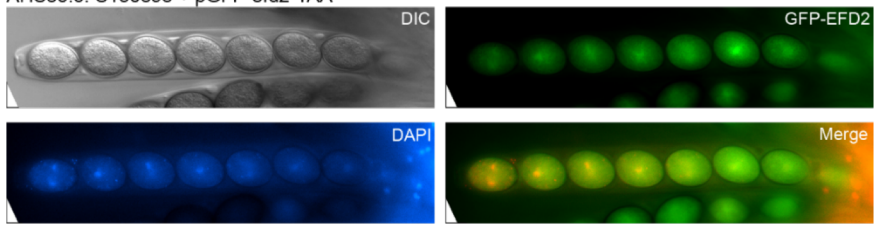


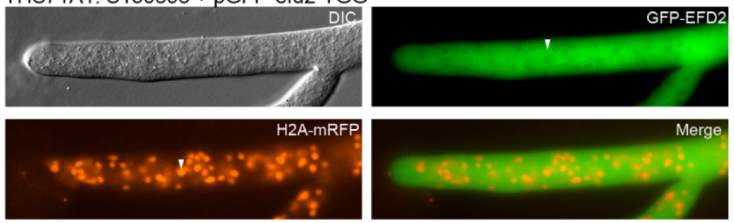
AHS80.6: S150898 + pGFP-efd2-TAA



AHS80.6: S150898 + pGFP-efd2-TAA



THS71A1: S150898 + pGFP-efd2-TGG



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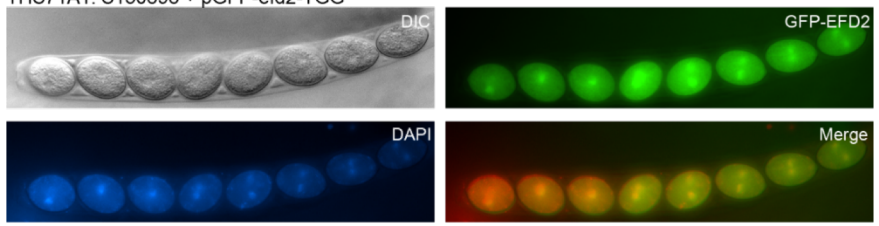
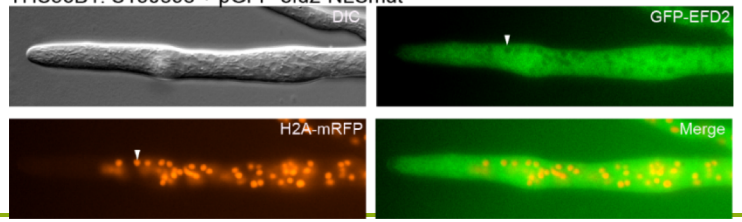
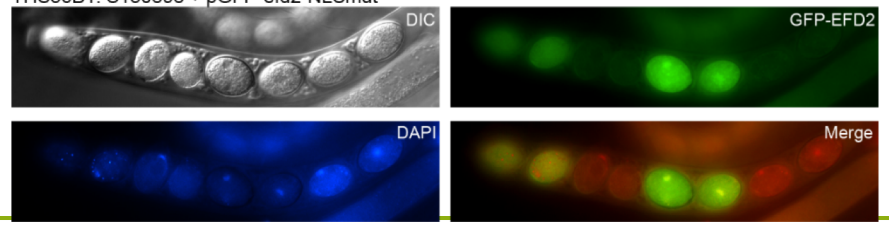


THS56B1: S150898 + pGFP-efd2-NLSmut



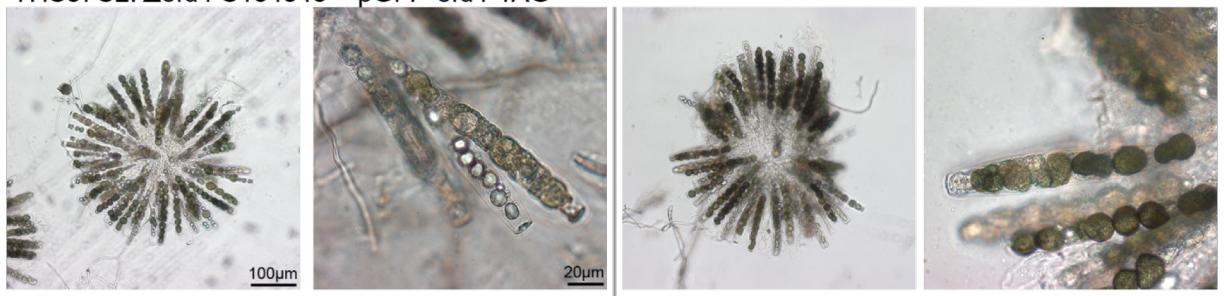
THS56B1: S150898 + pGFP-efd2-NLSmut



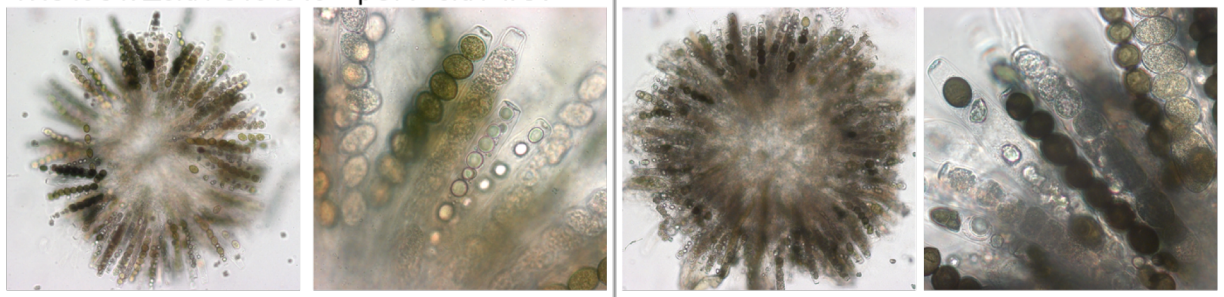
7 Tage

10 Tage

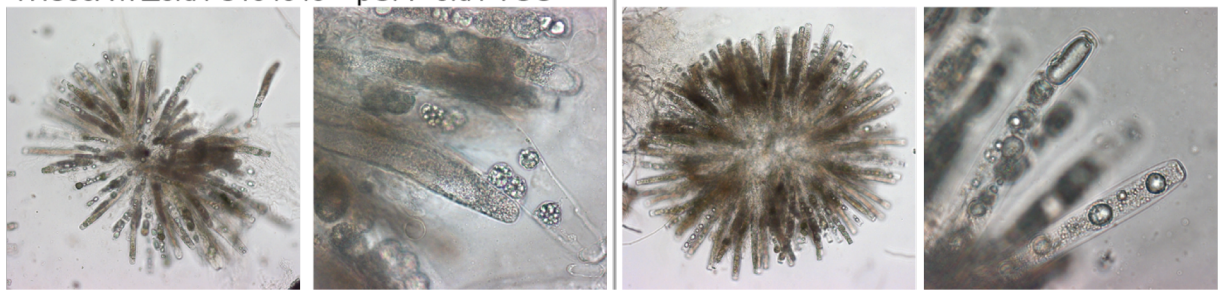
THS57C2: Δefd4 S154548 + pGFP-efd4-TAG



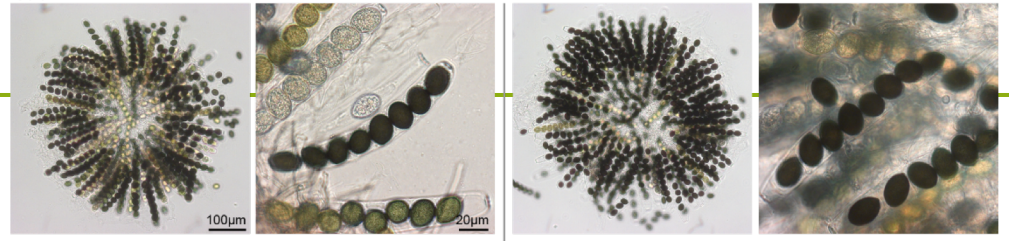
THS49C1: Δefd4 S154548 + pGFP-efd4-TAA



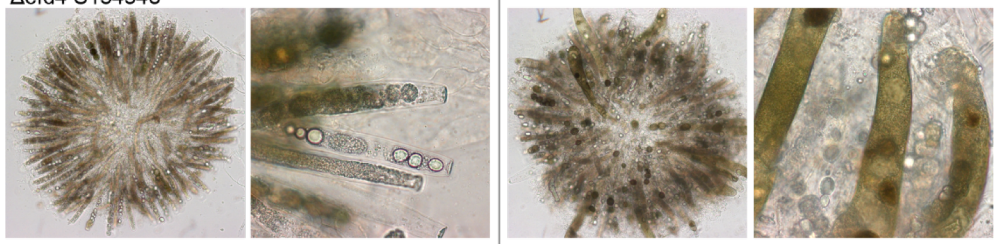
THS58A1: Δefd4 S154548 + pGFP-efd4-TGG



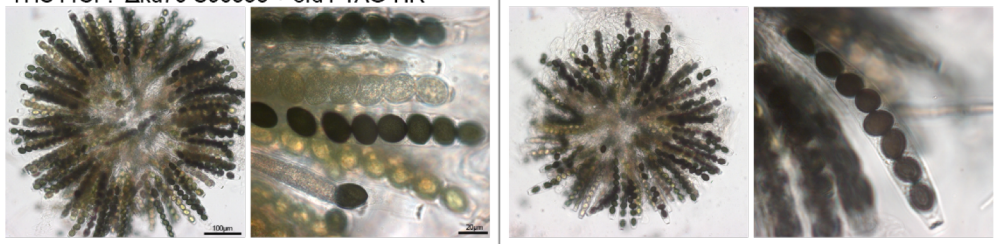
WT



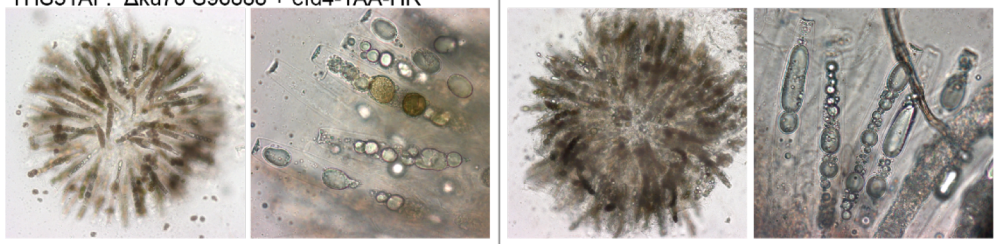
Δefd4 S154548



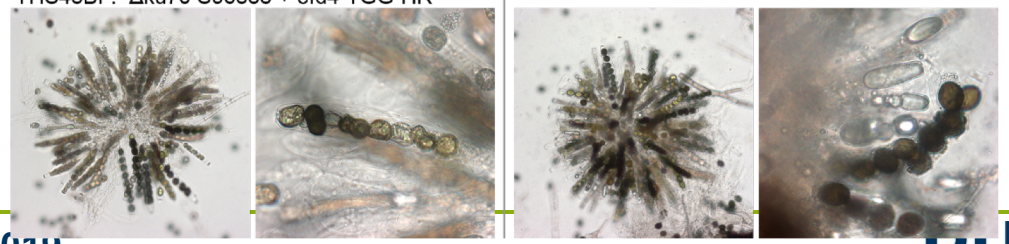
THS44CF: Δku70 S96888 + efd4-TAG-HR



THS31AF: Δku70 S96888 + efd4-TAA-HR



THS45BF: Δku70 S96888 + efd4-TGG-HR



Conserved stop-loss EFD proteins

Protein	Function of predicted protein	# gained aa	Loc. change	Domain change
EFD2	**RNA-binding protein**	**23**	**X; NLS**	
EFD4	RNA-binding protein	11	X; PTS1	
EFD5	Protein phosphatase 2C homolog	29	X	X; DUF
EFD6	Serine/threonine-protein kinase	76	X	
EFD7	Sorting nexin	89	X	
EFD8	N-alpha-acetyltransferase	143	X	
EFD9	Myoadenylate deaminase	72	X	
EFD11	40S ribosomal protein	44	X	
EFD12	Putative GTPase-activating protein	9		
EFD15	Uracil catabolism protein	15	X	
EFD16	Serine/threonine-protein phosphatase 2A activator	34	X	
EFD17	Translation initiation factor eIF-2B subunit gamma	63	X	
EFD18	Ubiquitin-conjugating enzyme E2	33	X	
EFD19	UPF0160 protein C27H6.8	16		
EFD20	White collar 1	131	X	X; HDAC
EFD21	NEDD8-conjugating enzyme UBC12	21		
EFD22	Cullin-associated NEDD8-dissociated protein 1, C-terminal part	20		
EFD25	Importin alpha re-exporter	18	X	
EFD26	Glycogen debranching enzyme	46	X	
EFD27	Heat shock protein	47	X	
EFD28	Fatty acid synthase subunit alpha	15	X; NLS	X; PPT
EFD29	Sphingolipid C9-methyltransferase	27	X	
EFD30	Casein kinase II subunit alpha	20		
EFD31	ATP-dependent RNA helicase	39	X	X; DEAD box
EFD32	26S proteasome regulatory subunit	19		
EFD33	Heat shock protein hsp98	19		
EFD34	Eukaryotic translation initiation factor 3 subunit E	82	X	
EFD35	Catalase-peroxidase	101	X	

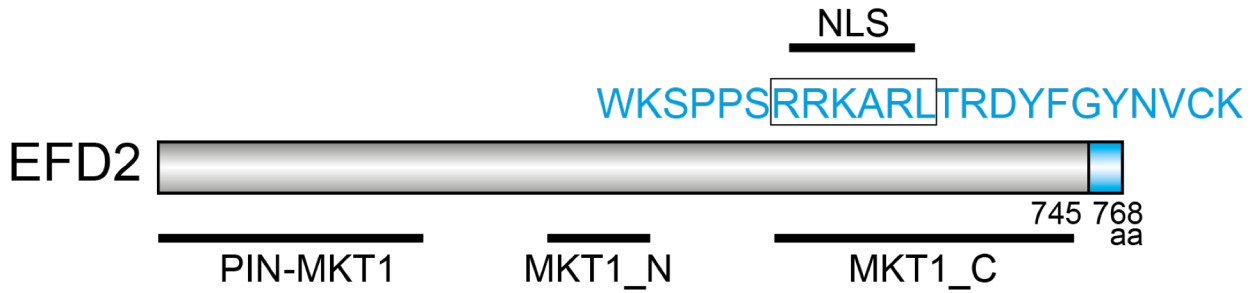
Localization:
PSORT

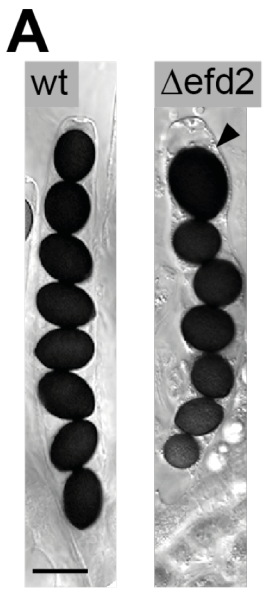
Targeting signals:
ELM

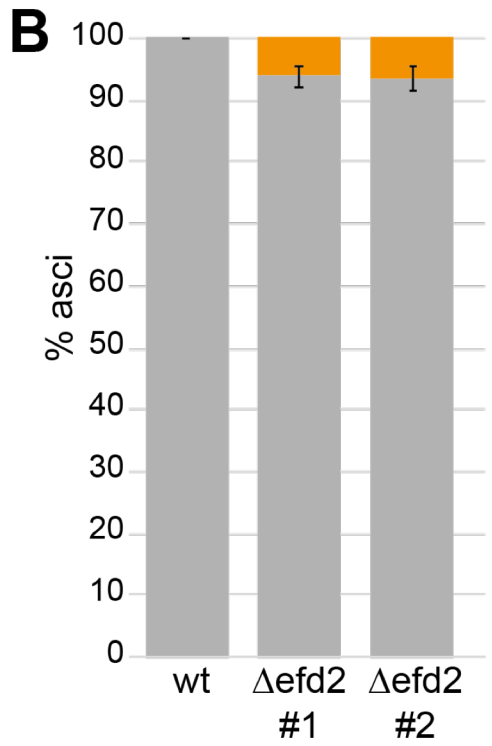
Domains:
BLAST CDS

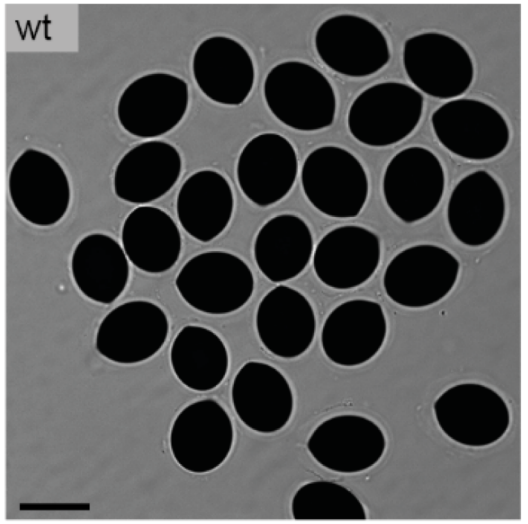
RUB

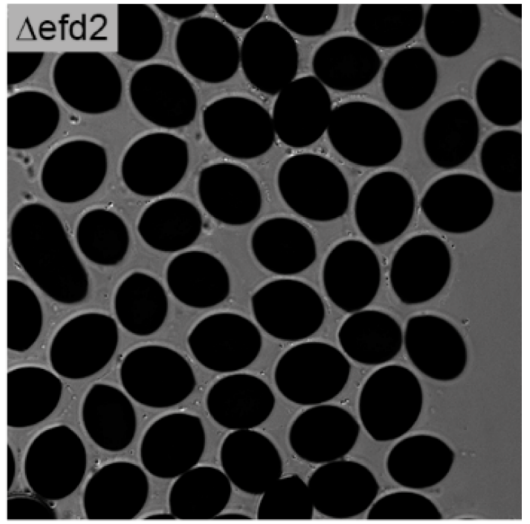
The *efd* genes function in ascospore formation







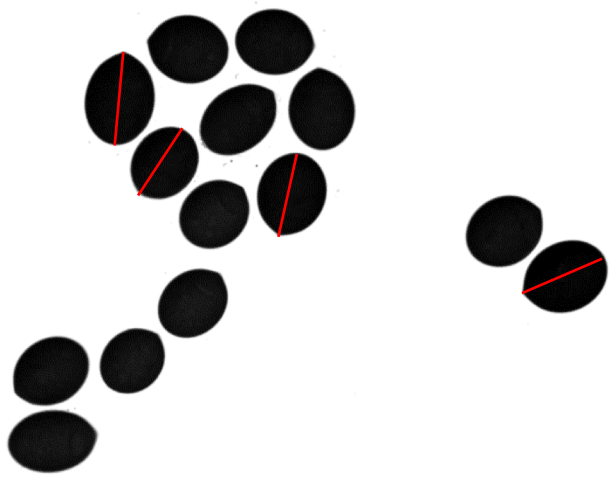


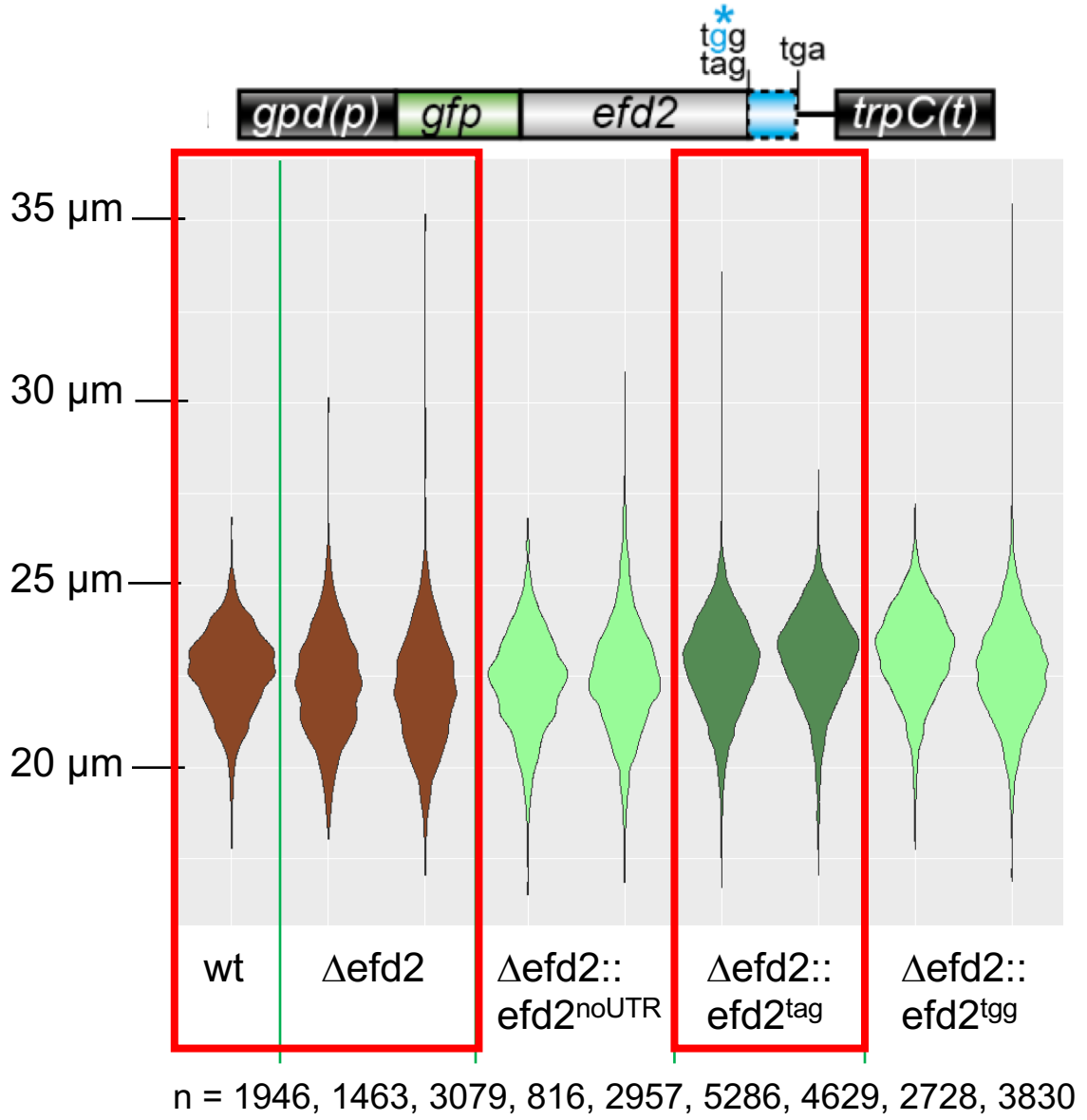


NLS, nuclear localization signal; PIN-MKT1, MKT1, RNA-binding domains; bar, 20 µm

Quantification of ascospore size

- 6 day cultures
- Spores on Petri dish lids
- Fiji: Feret`s diameter
- R studio





Questions – Conclusions - Outlook

1. **Which sites become edited? Which sites are of biological significance?**
   - Sites with higher conservation?
   - Protein evidence
   - ➢ RNA-seq data of perithecia (M. Nowrousian, R. Lütkenhaus)
   - ➢ Proteome data of distinct tissue and cell types (S. Pöggeler)

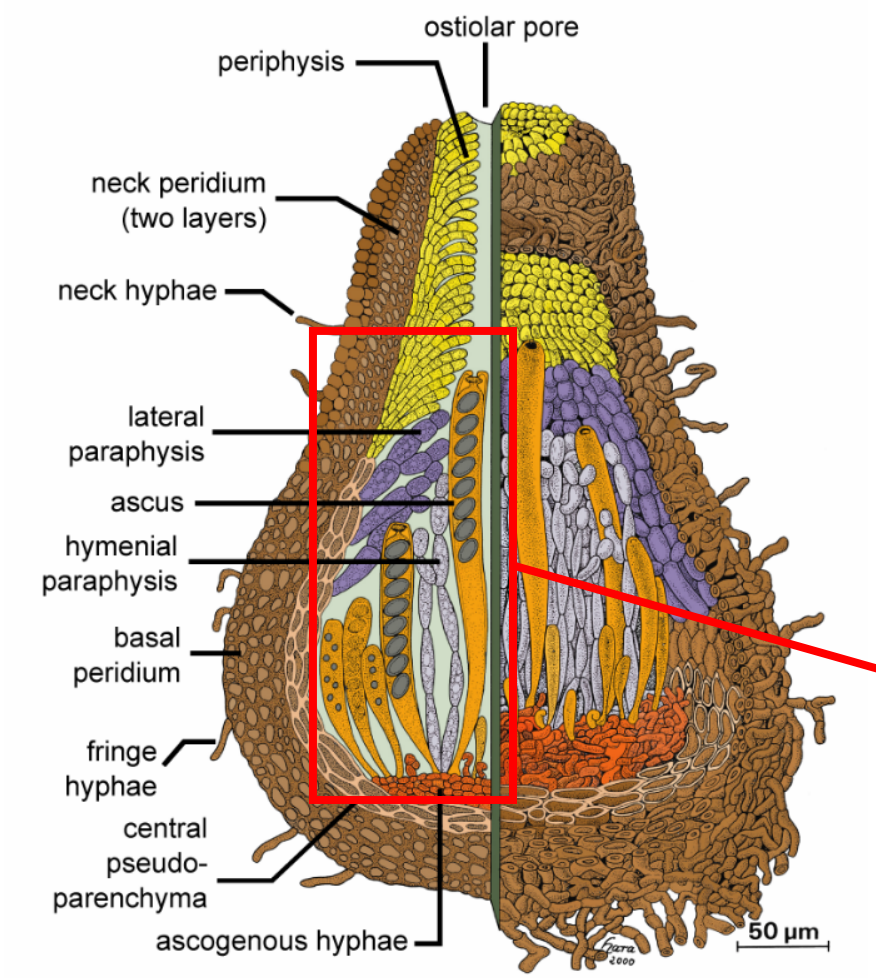
2. **What is the consequence of editing for protein function?**
   - Stop loss: Gain of localization signals, domains, small motifs
   - ➢ SAAVs: Fine tuning of protein function?

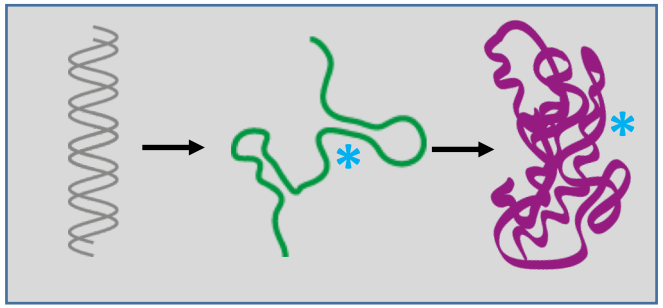
3. **What is the fungal editing mechanism?**
   - ➢ Reporter strain that gains antibiotic resistance through editing
   - ➢ Forward genetic mutagenesis approach

4. **Why have certain fungi evolved editing?**

Working Hypothesis

RNA editing is required for adaptation of protein functions to generate offspring

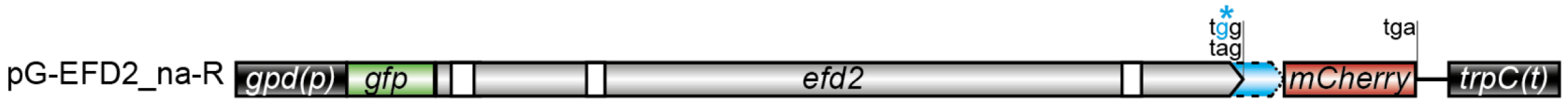


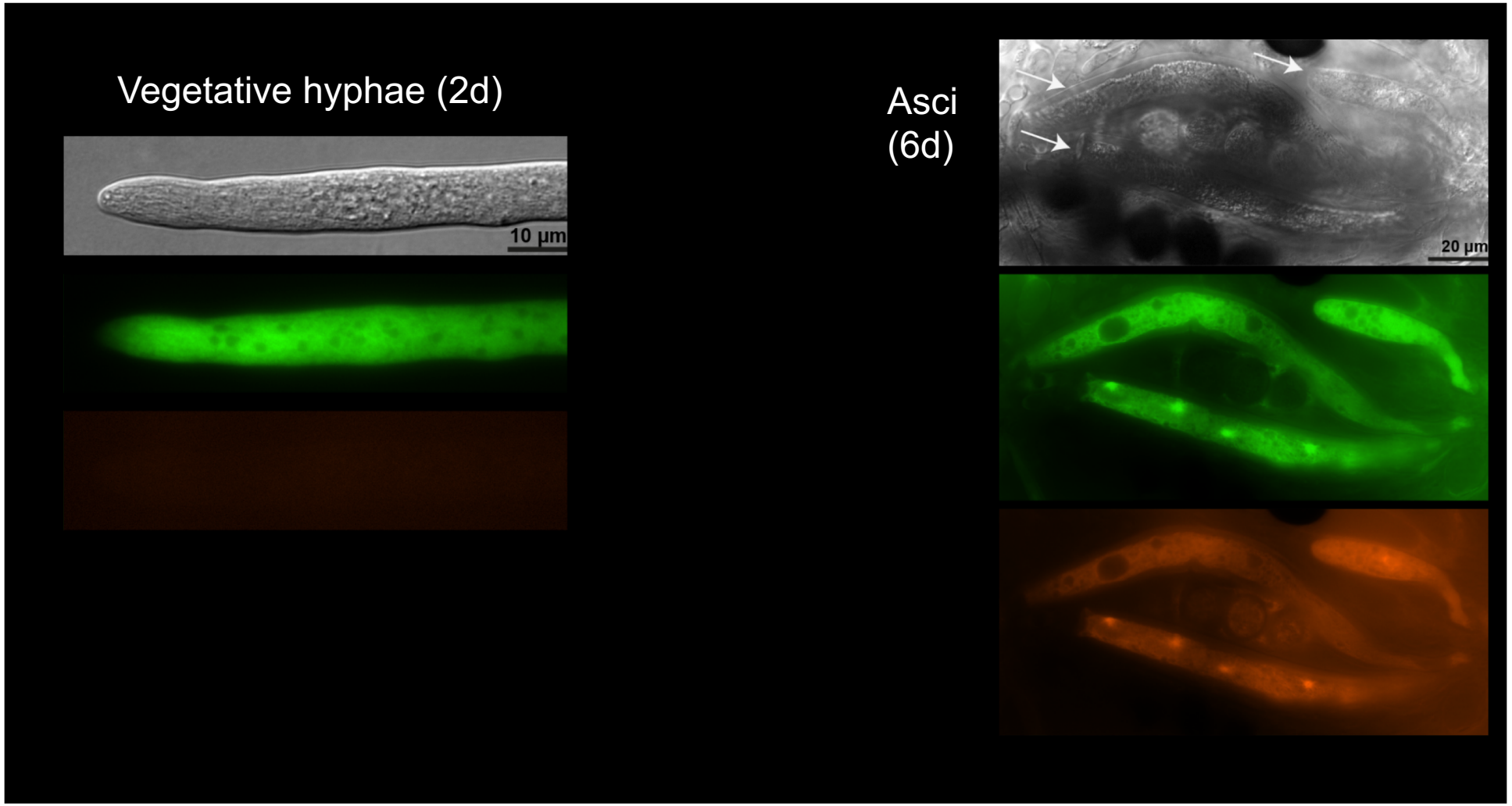


EFD2
EFD4
EFD5
EFD6
EFD16

EFD6
?
Dikaryon
Karyogamy
Meiosis
MI
MII
Mitosis
Generation of
spores
?
?

Visualization of editing *in vivo*

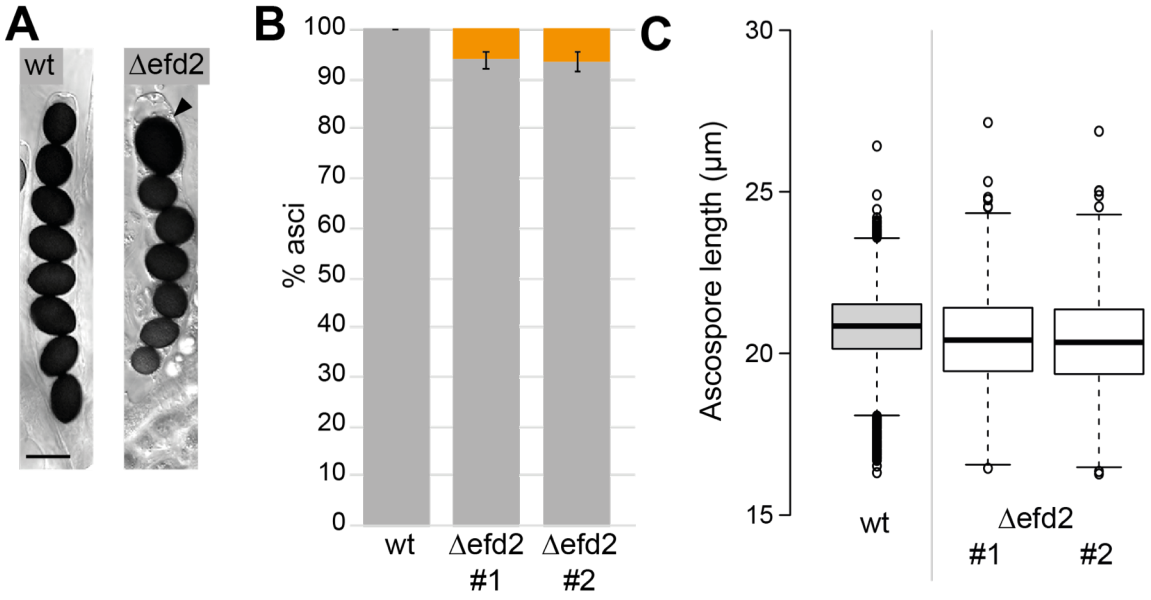




EFD Proteins Function in Ascospore Generation

Δefd2

- Asci with 7 spores
- Variable spore size



3-4 biological replicates with $n \geq 160$ each

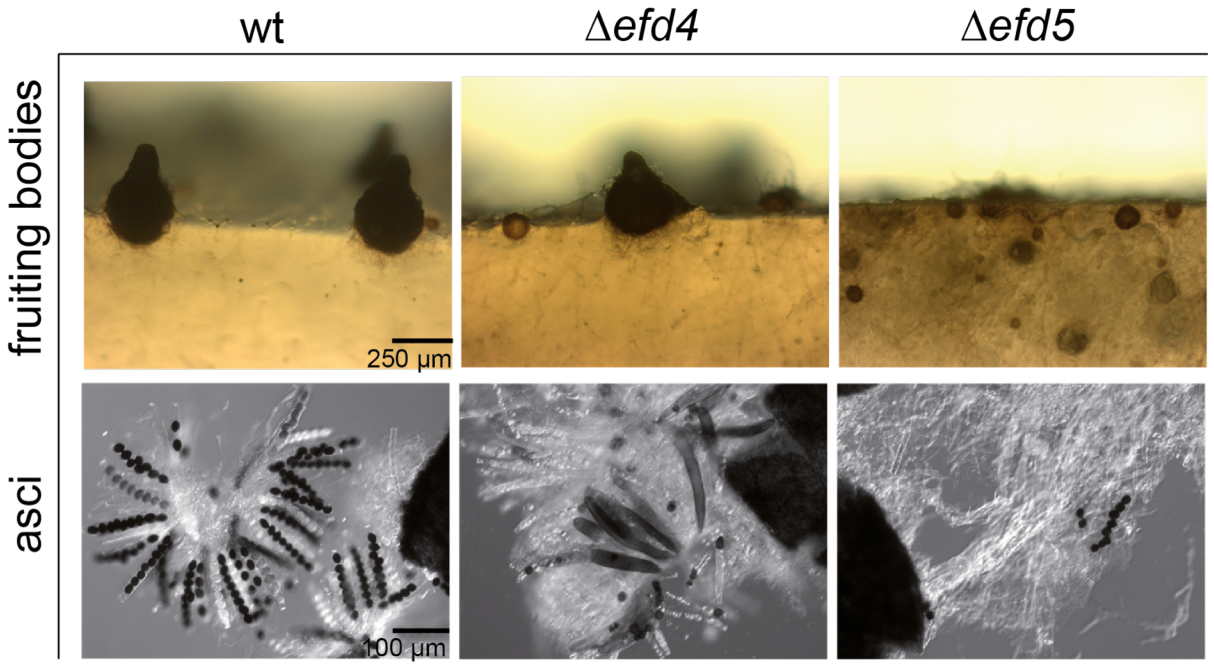
n = 8950, 1777, 3845

Δefd4

- Few spores
- Black asci

Δefd5

- Few perithecia
- Few spores



Working Hypothesis

DNA

RNA processing
RNA stability
RNA structure

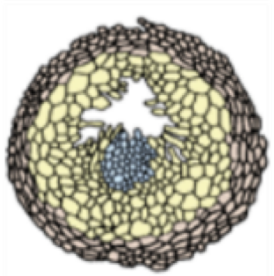
Protein localization – **EFD2**
Protein activity
Protein charge
Protein-protein interaction
Protein function – **EFD5**

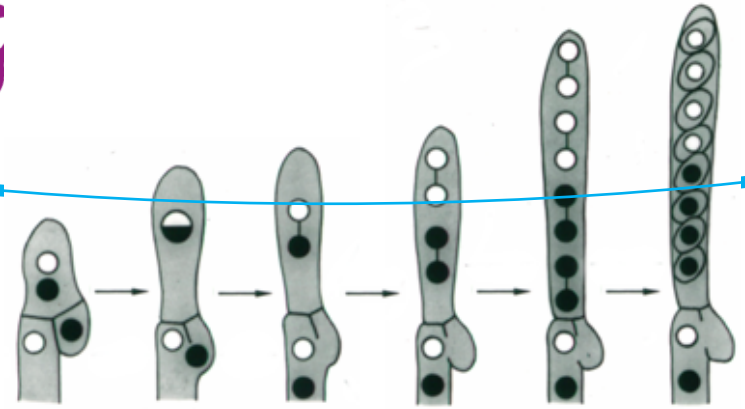
mRNA binding / translation control – **EFD2**, **EFD4**

Transport modification (nucleus, peroxisomes, …) – **EFD25**

Signal transduction – **EFD5**

Generation of asci and ascospores





RNA editing – when you cannot trust the DNA

The central dogma

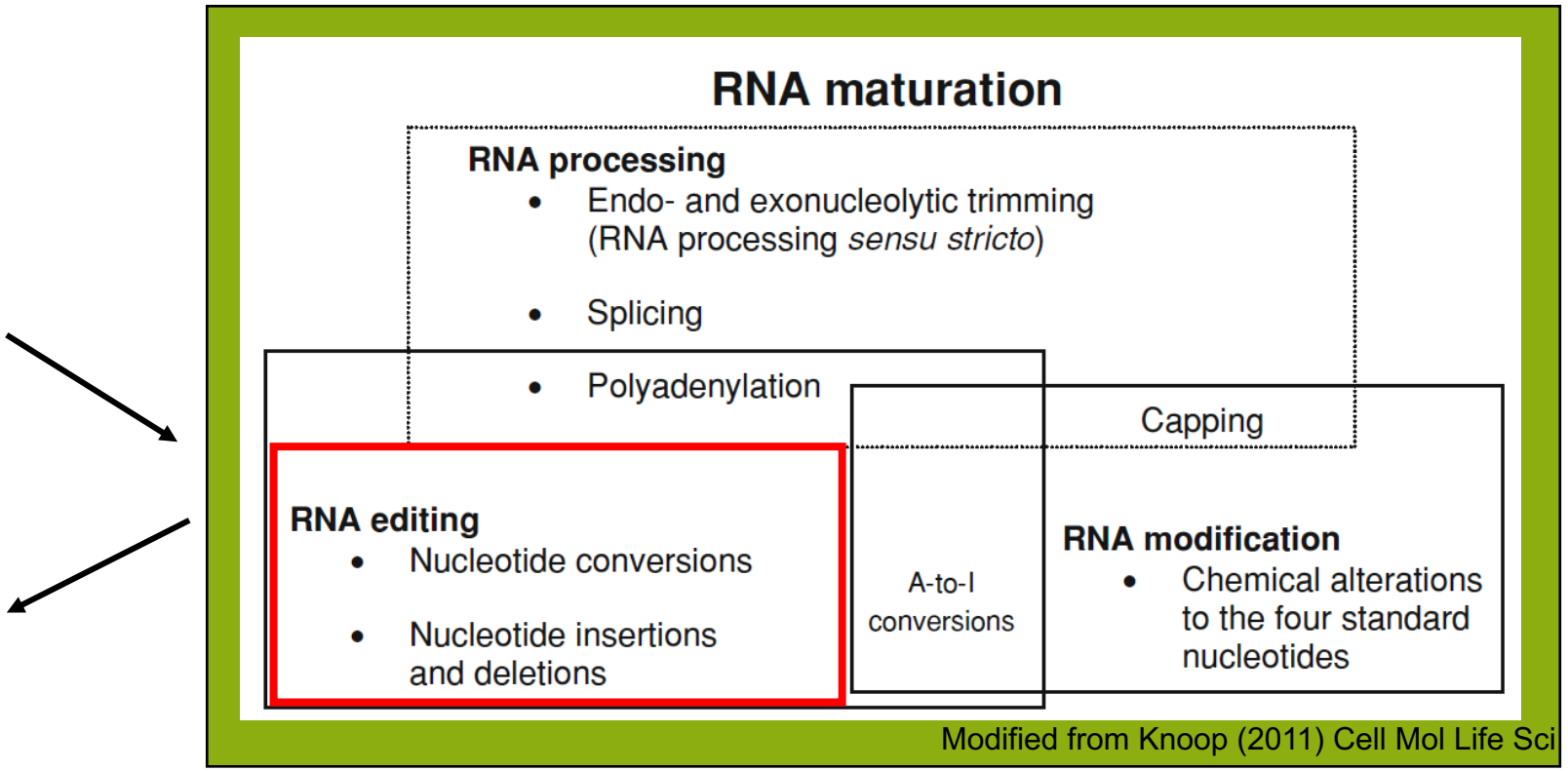
DNA

↓

mRNA

↓

protein



- Adenosine (A) to Inosine (I)
- Cytidine (C) to Uridine (U)
- U indels