

Identification of fertility restorers for S male-sterile maize: beyond PPRs

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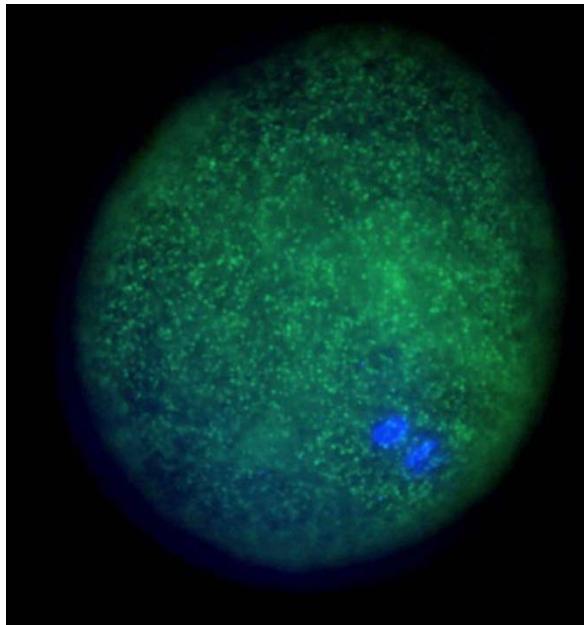
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Mitochondrial function - a genetic collaboration



Bi-nucleate maize pollen
nuclei-DAPI
mitochondria-GFP
(Karen Chamusco)

Plant mitochondrial genomes

- 31-36 protein-coding genes
- 17-25 tRNA genes
- 3 rRNA genes

Plant nuclear genomes

- ~2,500 protein-coding genes predict mitochondrial targeting (Emanuelsson et al., J Mol Biol 300:1005)

• ~ 6 nuclear-encoded tRNAs imported into plant mitochondria

Mitochondrial function - a genetic challenge

Primary metabolism

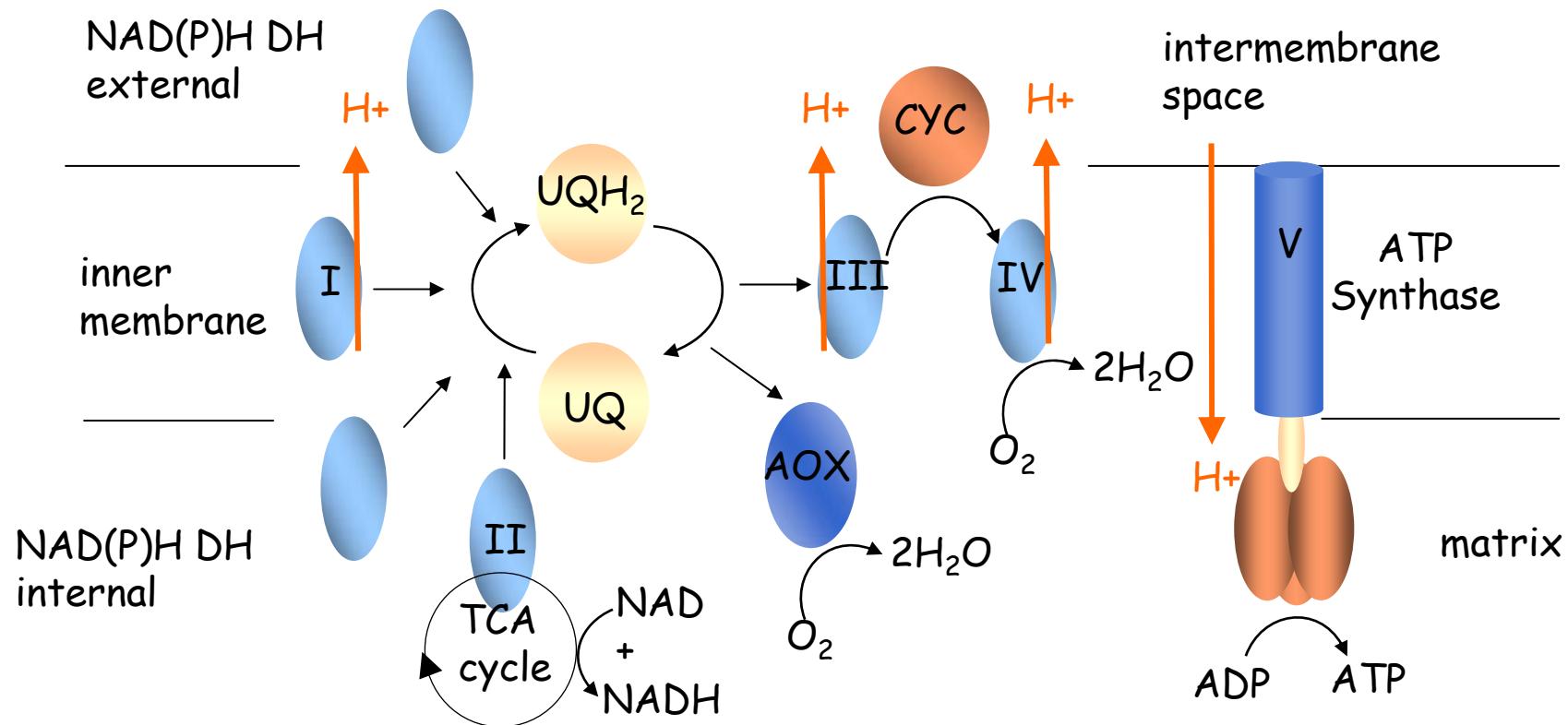
- TCA cycle
- Respiratory electron transfer
- ATP synthesis

Anabolic reactions

- Fe-S centers

Cellular signaling

- Calcium, ROS & PCD

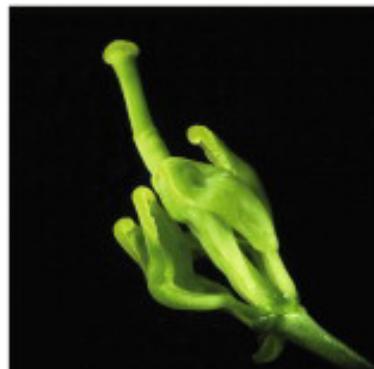


(Modified from Rasmusson et al., Ann Rev Plant Biol 55:23)

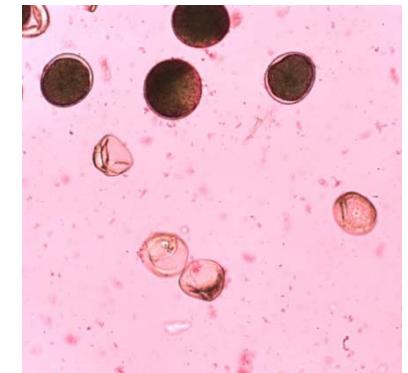
Cytoplasmic male sterility - a partial solution

- Mitochondrial gain-of-function mutations
- Diversity of male-sterility phenotypes
- Viability and female fertility usually unaffected
- Expression governed by nuclear restorer-of-fertility loci

Changes in floral organ identity:
stamen to carpel stamen to petal



Failed
pollen development
Anther abortion or function

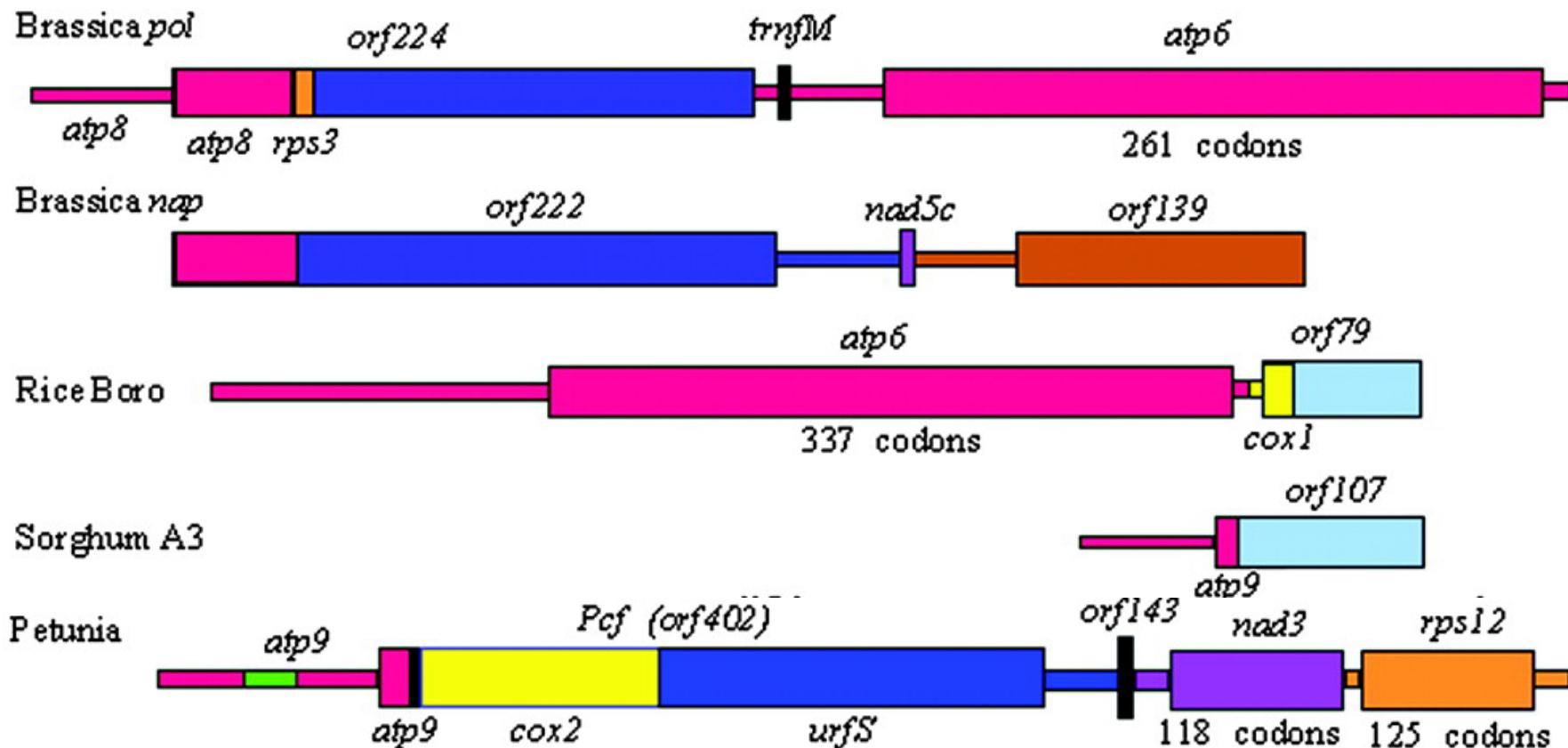


(Carlsson et al.,
Mitochondrion 8:74)

(Linke & Börner,
Mitochondrion 5:387) KC Chamusco

Diversity of CMS genes

- Chimeras of mitochondrial and unknown sequences
- Some examples of common ancestry
- Expressed courtesy of recruited mit promoters



(Modified from Hanson & Bentolila Plant Cell 16:S154)

Fertility restorer genes

Most are pentatricopeptide repeat (PPR) protein coding genes

PPR proteins

- Encoded by a highly expanded plant gene family
- Most are plastid or mitochondria-targeted
- Site-specific RNA binding proteins comprised of degenerate ~35 aa repeats
- Mediate organelle gene expression

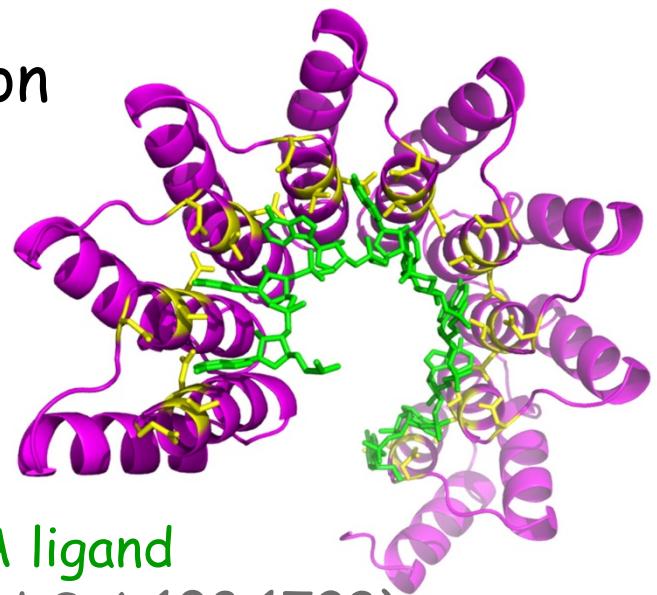
Transcription

RNA processing

Intron splicing

RNA editing

Translation



(Modeled PPR motifs & RNA ligand
from Fujii et al. Proc Natl Acad Sci U S A 108:1723)

Fertility restorer & Rf-PPR-like (RFL) genes

Restorers

- Specialized class of PPR protein coding genes
- Adaptive evolution for silencing specific CMS gene targets

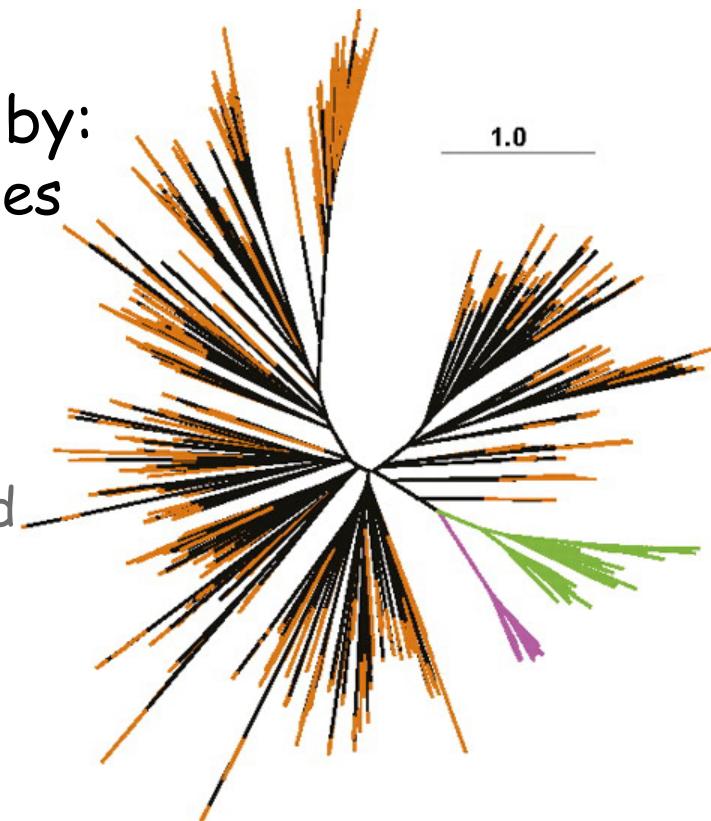
Restorers and RFLs

- Distinguished from other PPRs by:
 - Genomic clusters of related genes
 - Rapid evolution
 - Diversifying selection

(Phylogeny of 1,085 PPR proteins predicted to be targeted to mitochondria including

212 RFL proteins

from Fujii et al. Proc Natl Acad Sci USA
108:1723)



Fertility restorer genes

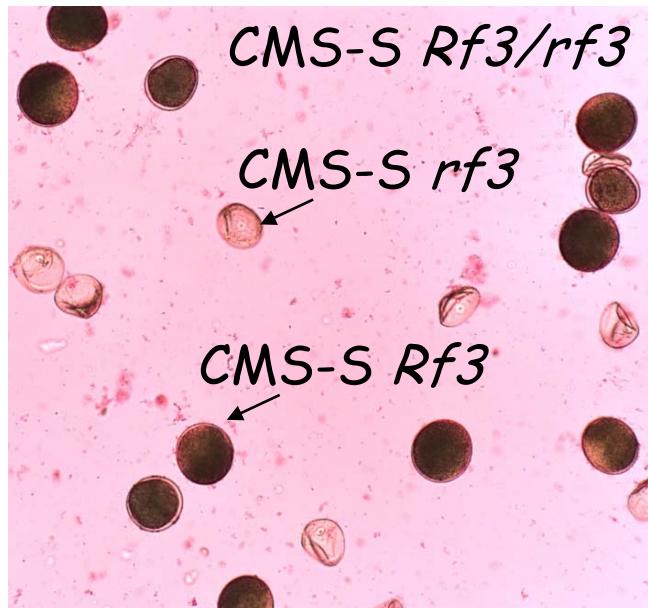
Non-PPR restorers

- *Rf4* of CMS-T maize - mitochondrial aldehyde dehydrogenase (Cui et al. Science 272:1334)
- *Rf2* for Lead-rice CMS - mitochondrial glycine-rich protein (Itabashi et al. Plant J 65:359)
- *Rf17* for Chinese wild rice CMS - 178 aa of unknown function (Fujii & Toriyama Proc Natl Acad Sci USA 106:9513)
 - Loss-of-function allele is restorer

Insights & limitations for mitochondrial genetics

Fertility restoration in CMS-S maize - a novel paradigm

A gametophytic system of CMS and fertility restoration
(Buchert, Proc Natl Acad Sci USA 47:1436)



Karen Chamusco

- Pollen collapse
 - bi-cellular stage
 - apoptotic-like programmed cell death
- Rescued by *restorer-of-fertility* alleles
- In S-cytoplasm, only the restoring allele transmits through the male
- In S-cytoplasm restoring & non-restoring alleles transmit through the female

Native fertility restoration systems of CMS-S maize

Rf3, Rf9

Likely result from adaptive evolution



- Homozygous viable
- *Rf3* restoring allele is dominant (i.e. gain of function)
2N pollen (Kamps et al. *Genetics* 142:1001)
Sporophytic transcript effects
(Wen et al. *Curr Genet* 35:521)
- Found in Mexican landraces & teosinte
(Gabay-Laughnan et al. *Genetics* 166: 959)
- *Rf3* maps to a cluster of *PPR* genes
(Xu et al. *Plant Mol Biol Repr* 27:511)

Restorer-of-fertility mutations in CMS-S maize



Susan Gabay-Laughnan

$rfI^*00-9050$
| |
| |
year row

new mutant
(locus unknown)

Restoring alleles recovered via mutation

- Homozygous lethal rfI^*
- Homozygous viable rfv^*

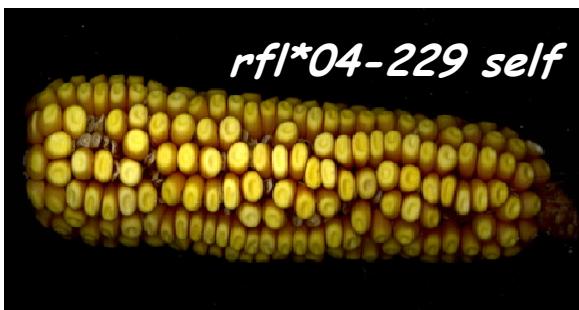
Spontaneous mutation

- 41 non-allelic rfI^*
- 6 non-allelic rfv^*

24 B73 UniformMu families

- 7 non-allelic rfI^*
- 3 rfv^* (allelism TBD)

rf1 mutations rescue CMS-S pollen but cause seed-lethal phenotypes



Recessive, loss-of-function mutations

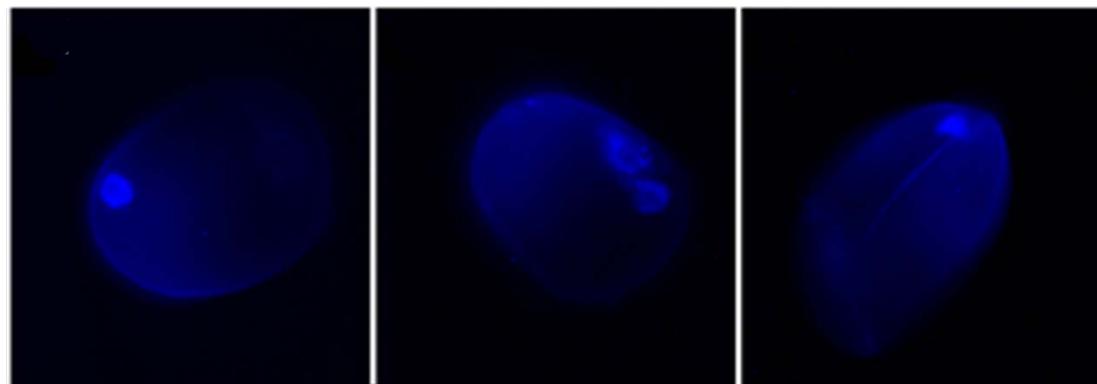
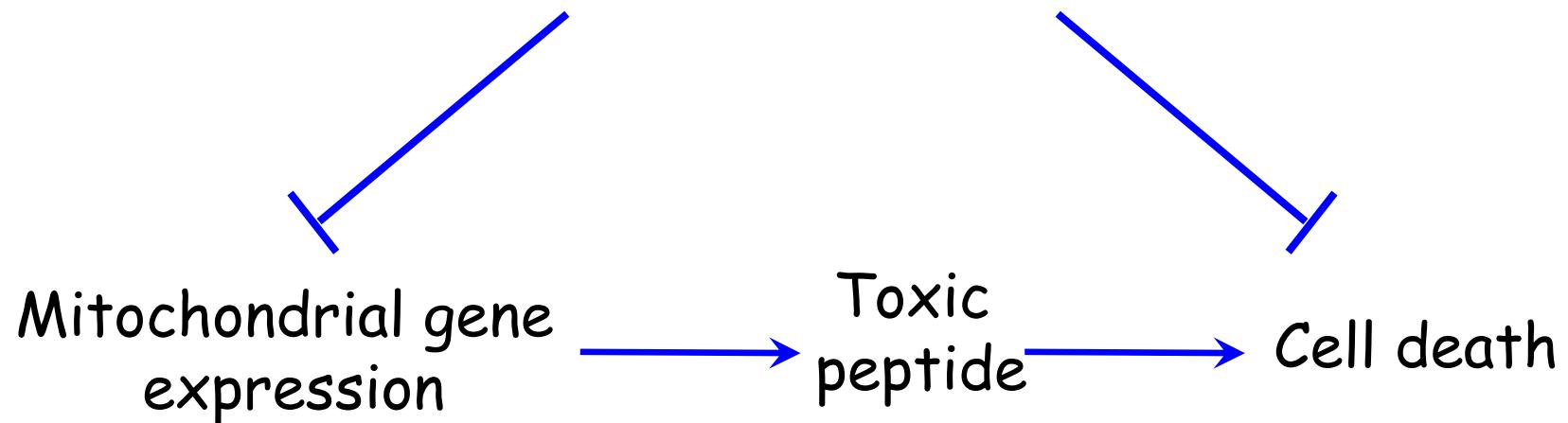
- Homozygous seed-lethal phenotype
- 2N pollen studies (*rf1/1*)
(Wen, Ruesch et al. *Genetics* 165:771)

Disrupt expression of CMS-S in pollen

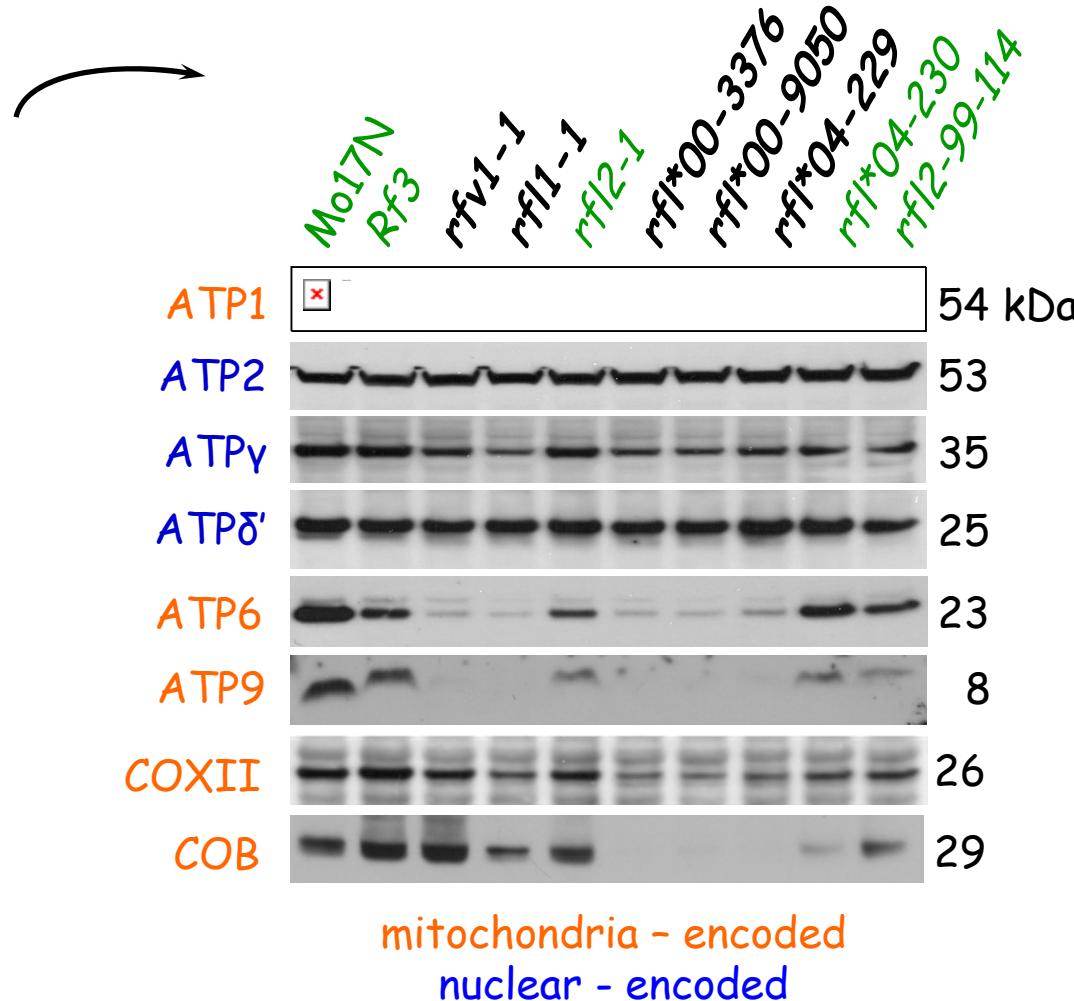
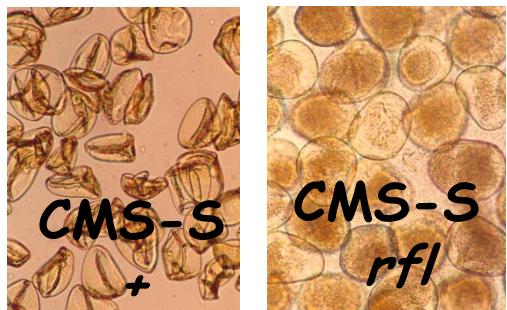
Disrupt mitochondrial functions
essential to seed (but not pollen)
development

Convenient tests of allelism

restorer-of-fertility mutations

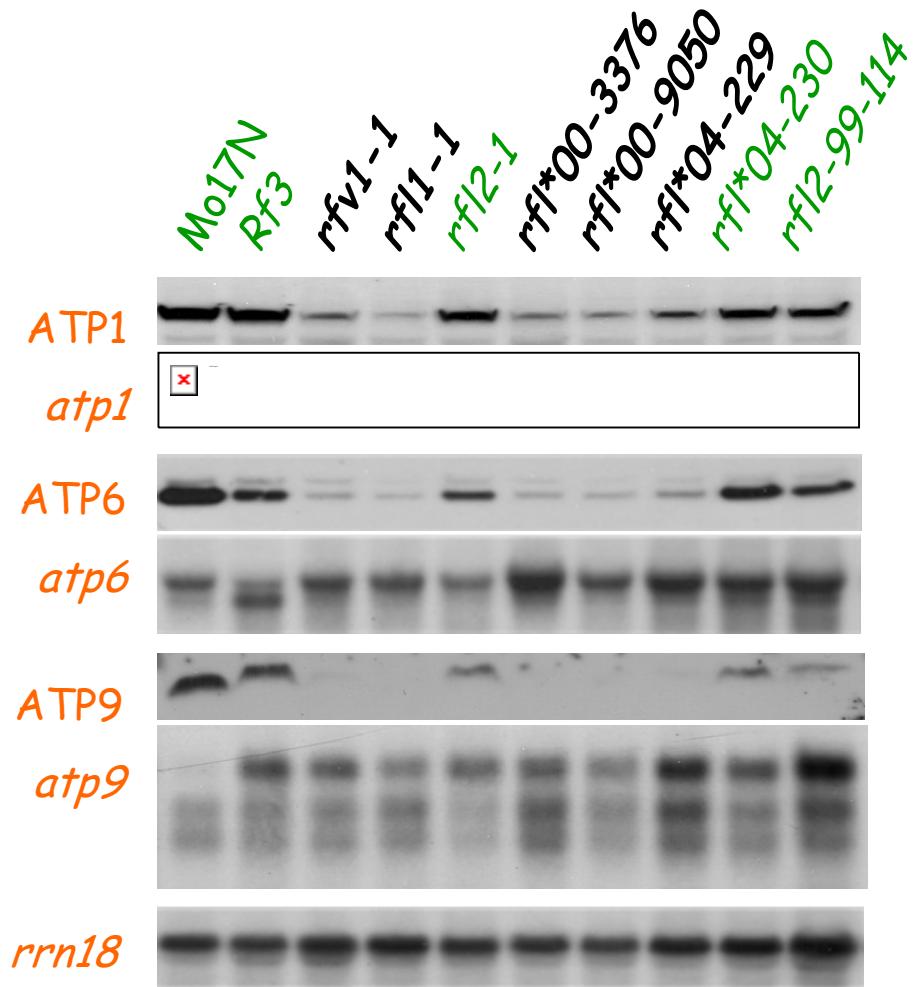


Loss of mitochondrial gene products in restored CMS-S pollen



Liming Zhao, Victor Ortega, Ashley Andersen

Post-transcriptional loss of mitochondrial gene products in restored CMS-S pollen



Liming Zhao

Wild-type transcript editing in CMS-S *rfl*04-229* pollen for:

atp1

atp6

atp9

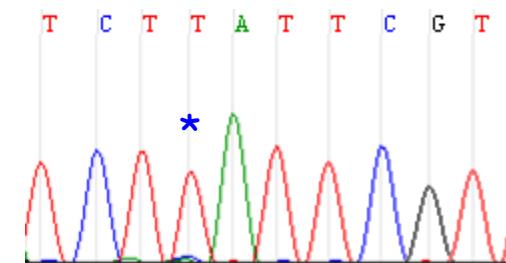
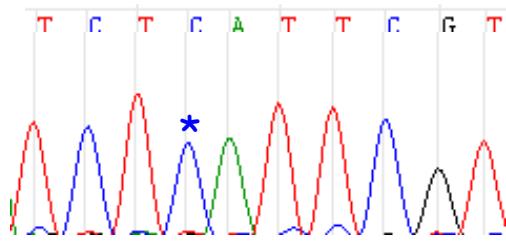
cob

atp9
genomic

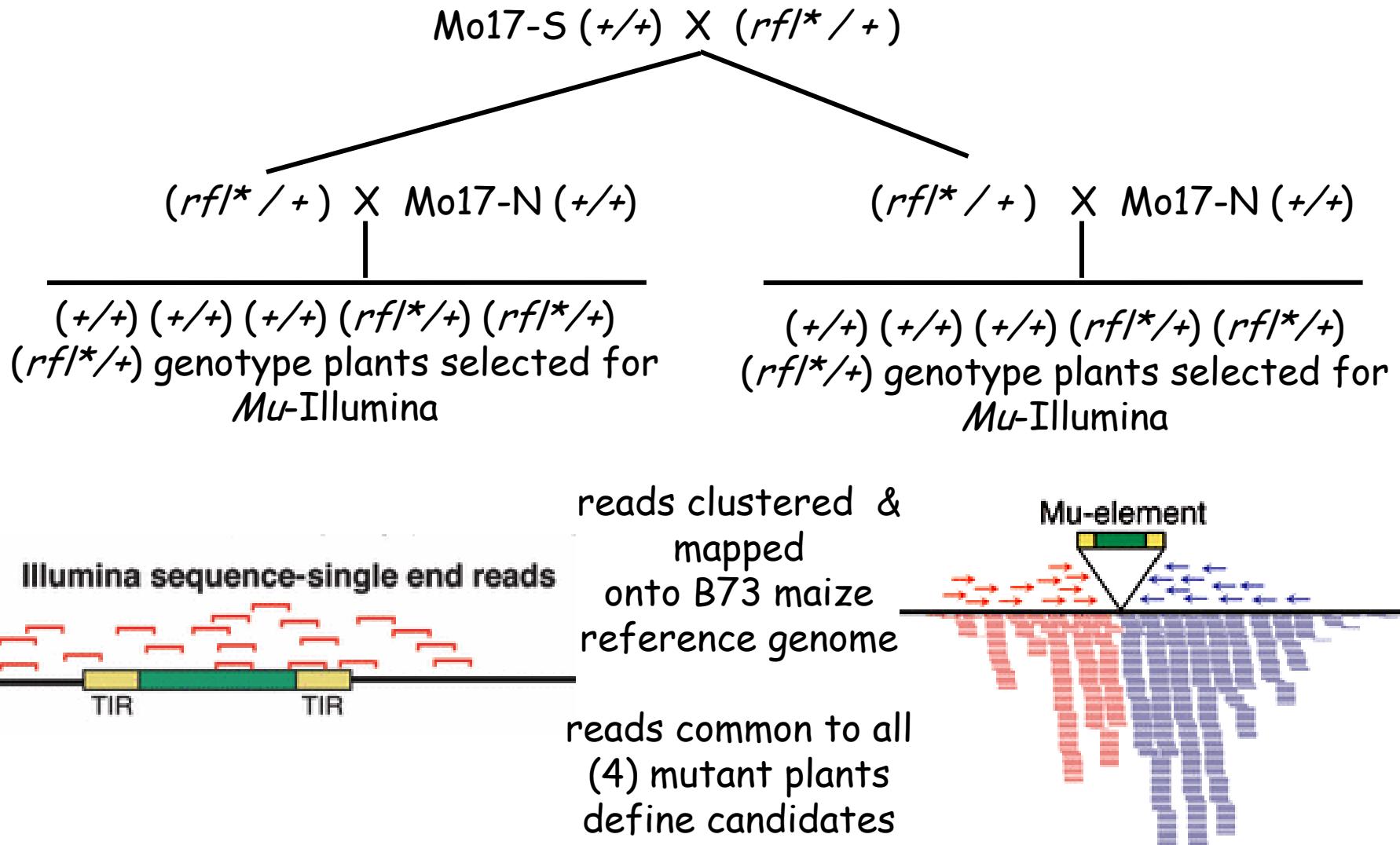
N +
pollen *atp9*
cDNA

S *rfl*04-229*
pollen *atp9*
cDNA

TCA>TTA
S71L



Mu-Illumina for identification of restorer loci



(Modified from Williams-Carrier et al. The Plant J. 63:167)

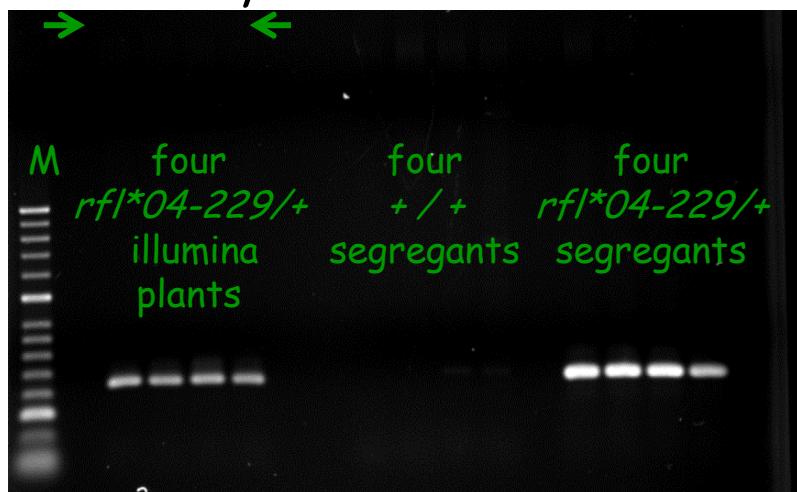
Mu-Illumina identifies a single *rfl*04-229* candidate

Mitochondrial 60S ribosomal protein L6

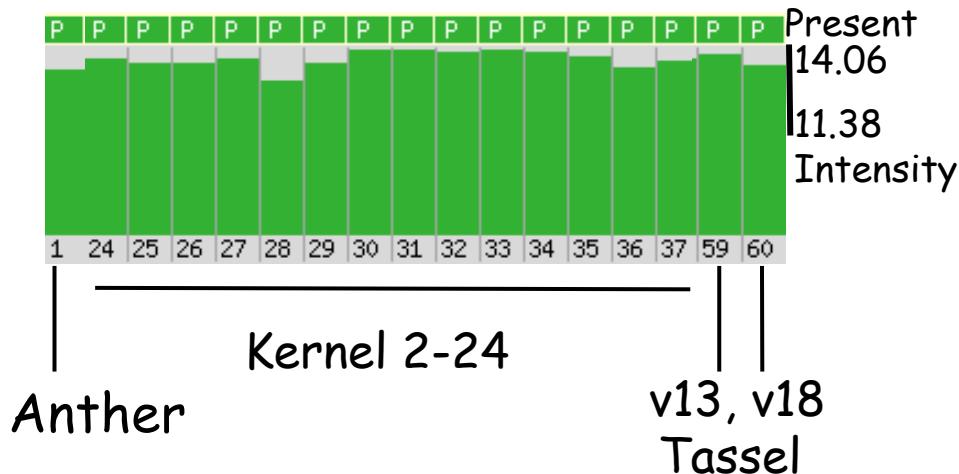
Chromosome 4: 154,418,377-154,420,600



MuTIR-rp16 PCR



rp16 transcript expression across tissues



Identification of fertility restorers for S male-sterile maize: ? beyond ribosomal proteins

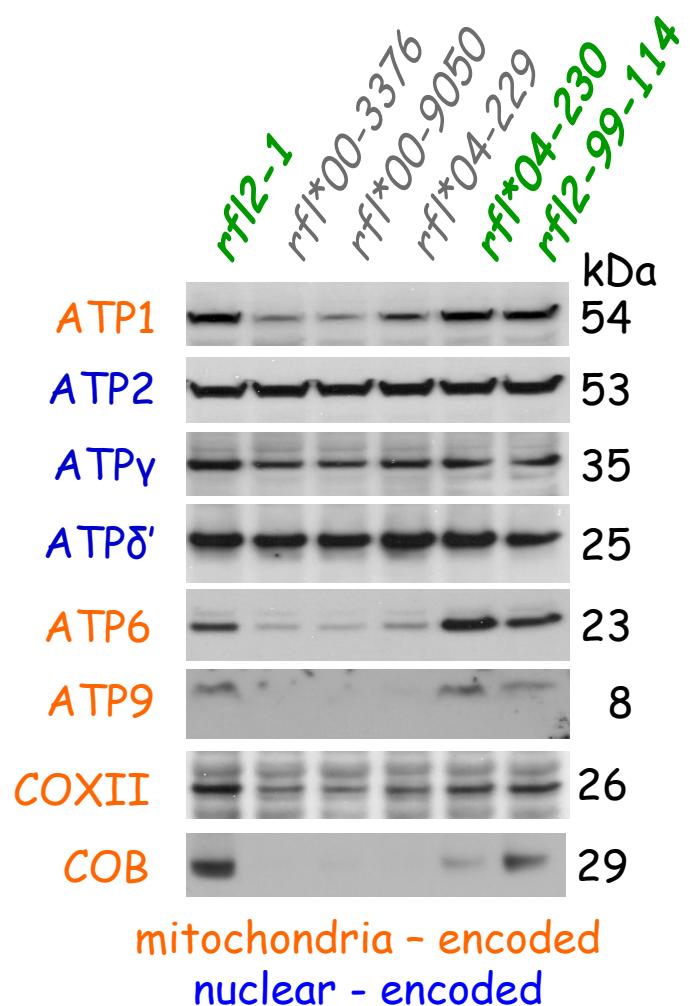
*rf1** alleles without global mitochondrial protein loss

- *rf1 *04-230*
- *rf12*

many additional *rf1** mutants
*rfv** mutants

Likely candidates:

- *ppr* genes with day jobs
- PPR-interacting partners
- Plant cell death effectors



S male-sterile maize: a unique tool for forward genetic analysis of plant mitochondrial function

Native restorers

- Adaptive evolution
- Homozygous viable
- Gain of function for silencing CMS



Restorers by mutation

- Homozygous lethal
- Loss of function for mitochondrial gene expression &/or signaling

Unique biology & genetics of maize male gametophyte

- CMS-S "reporter gene"
- Genetic instability and mutant detection in haploid cells
- Metabolic plasticity with respect to mitochondrial function
- Mu-Illumina and UniformMu insertion resources

Collaborators

Pollen collapse and fertility restoration in S male-sterile maize

Mutagenesis

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McCarty, Mark
Settles, Karen Koch,
Brandon Futch &
John Baier
Horticultural
Sciences Department
University of Florida

Tagging

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Carrier & Alice Barkan
Institute of Molecular
Biology
University of Oregon

Sanger sequencing

University of Florida ICBR
DNA Sequencing Core
Laboratory

Antibodies

Bethyl Laboratories
Tom Elthon, Maureen
Hanson, Hirokazu
Handa, Dan
Kliebenstein, Sam
Levings, Tom Mason,
Jean-Michel
Grienenerger, Pat
Schnable

Acknowledgements

Chase lab

<u>Postdocs</u>	&	<u>Bioscientists</u>
Terry Kamps		Karen Chamusco
Lanying Wen		Sarah Gullett
Liming Zhao		Victor Ortega

Graduate Students

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Victoria Read
Yong Tan

Funding - University of FL

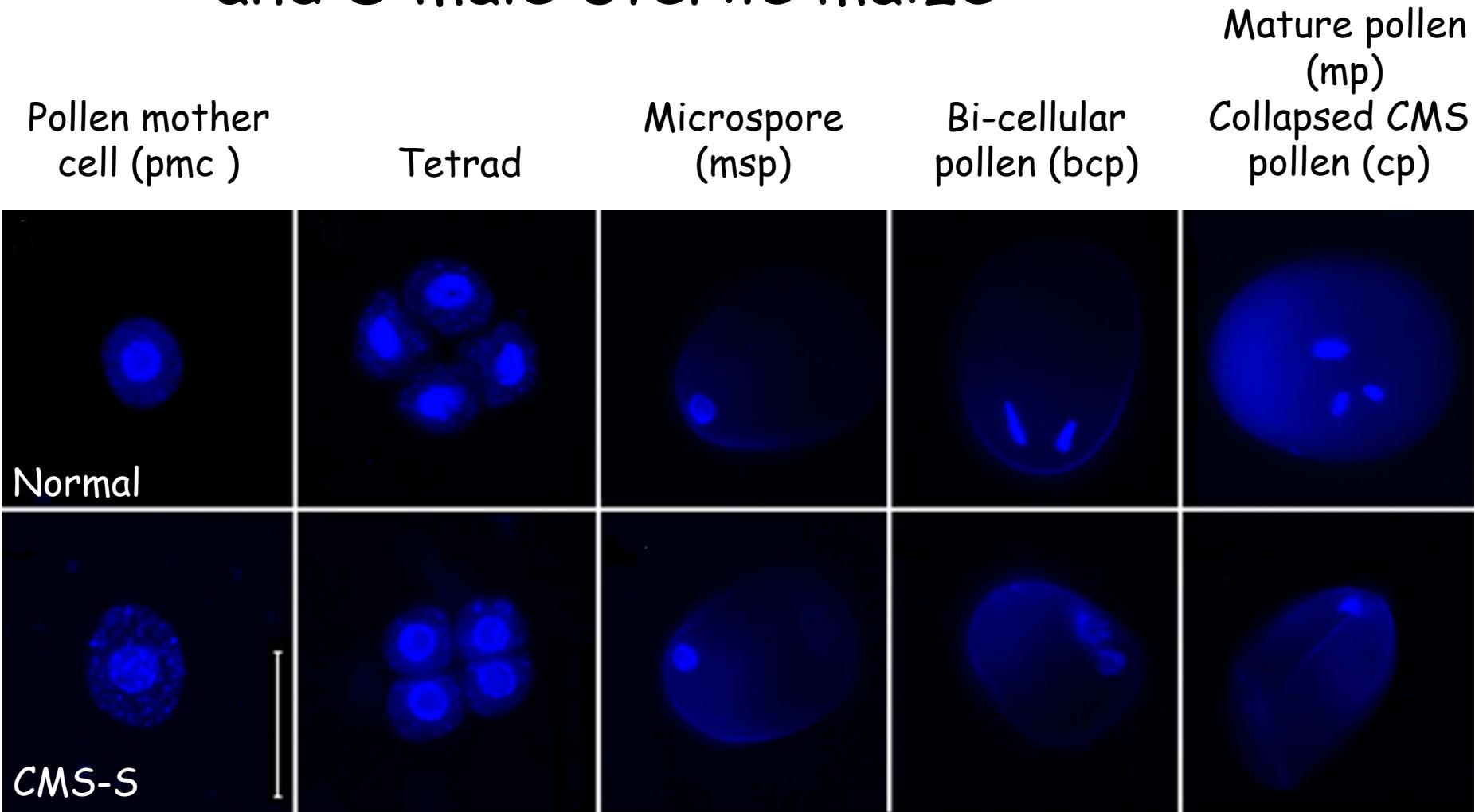
National Research Initiative of the
USDA CSREES, 2001-0534-10888,
2005-35301-1570
National Science Foundation IOS-
0816782
UF/IFAS Horticultural Sciences Dept.
UFL University Scholars Program
UFL HHMI Science for Life Program

Funding - University of OR

National Science Foundation grant IOS-
0922560

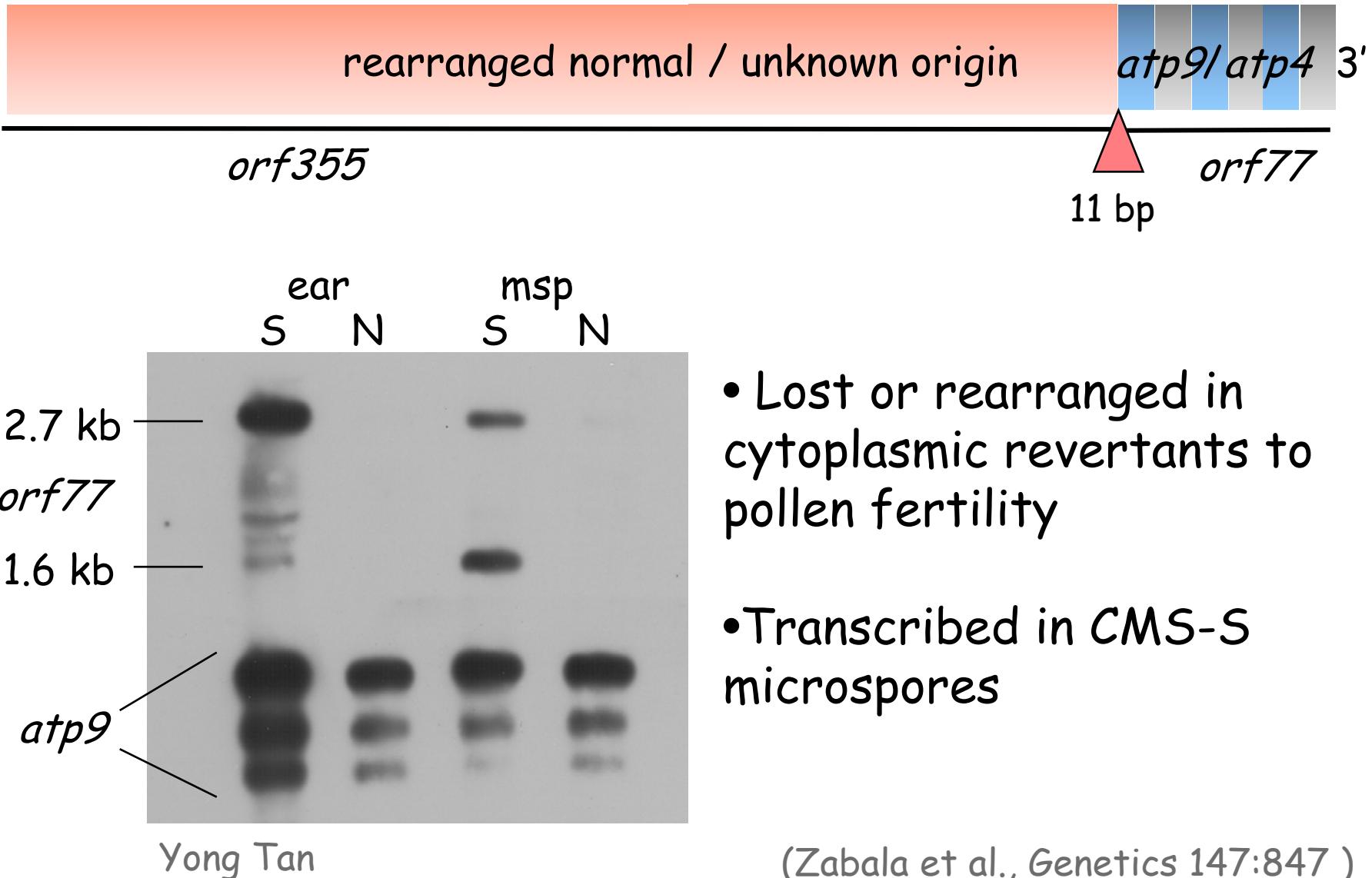


Pollen development in male-fertile and S male-sterile maize

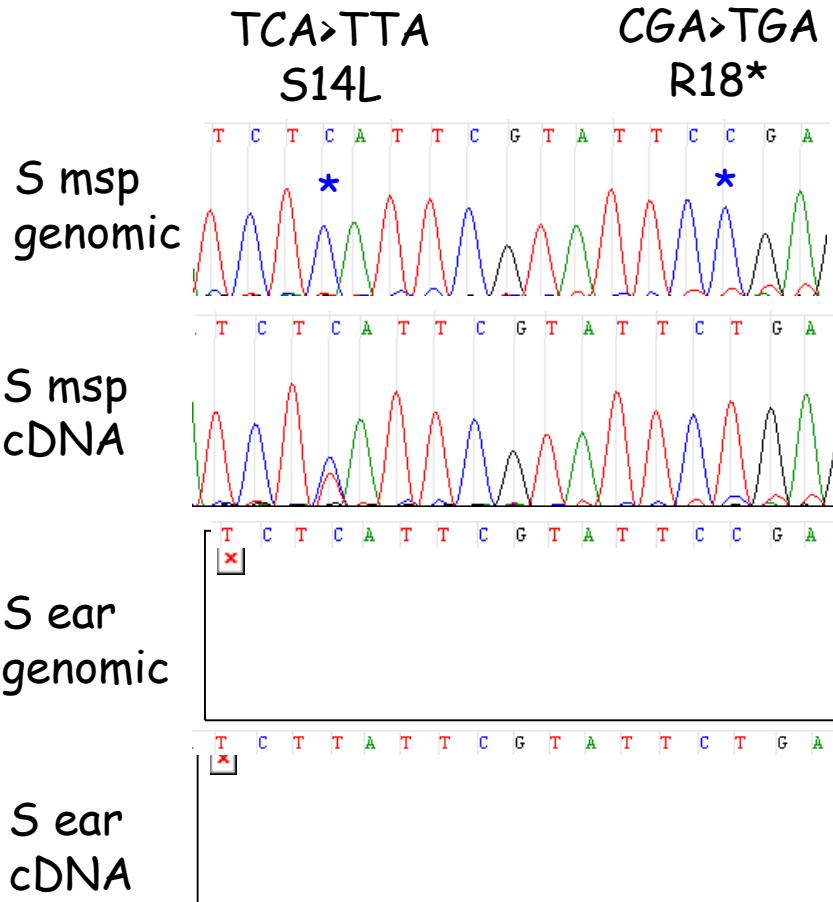


Karen Chamusco

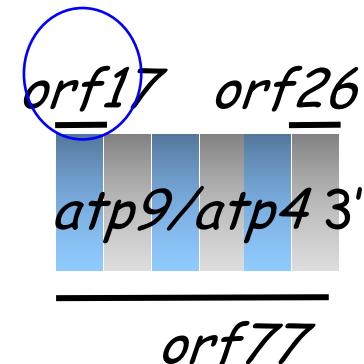
Multiple candidate orfs for CMS-S maize



Mitochondrial C-to-U RNA editing events create additional candidate CMS orfs



Yong Tan

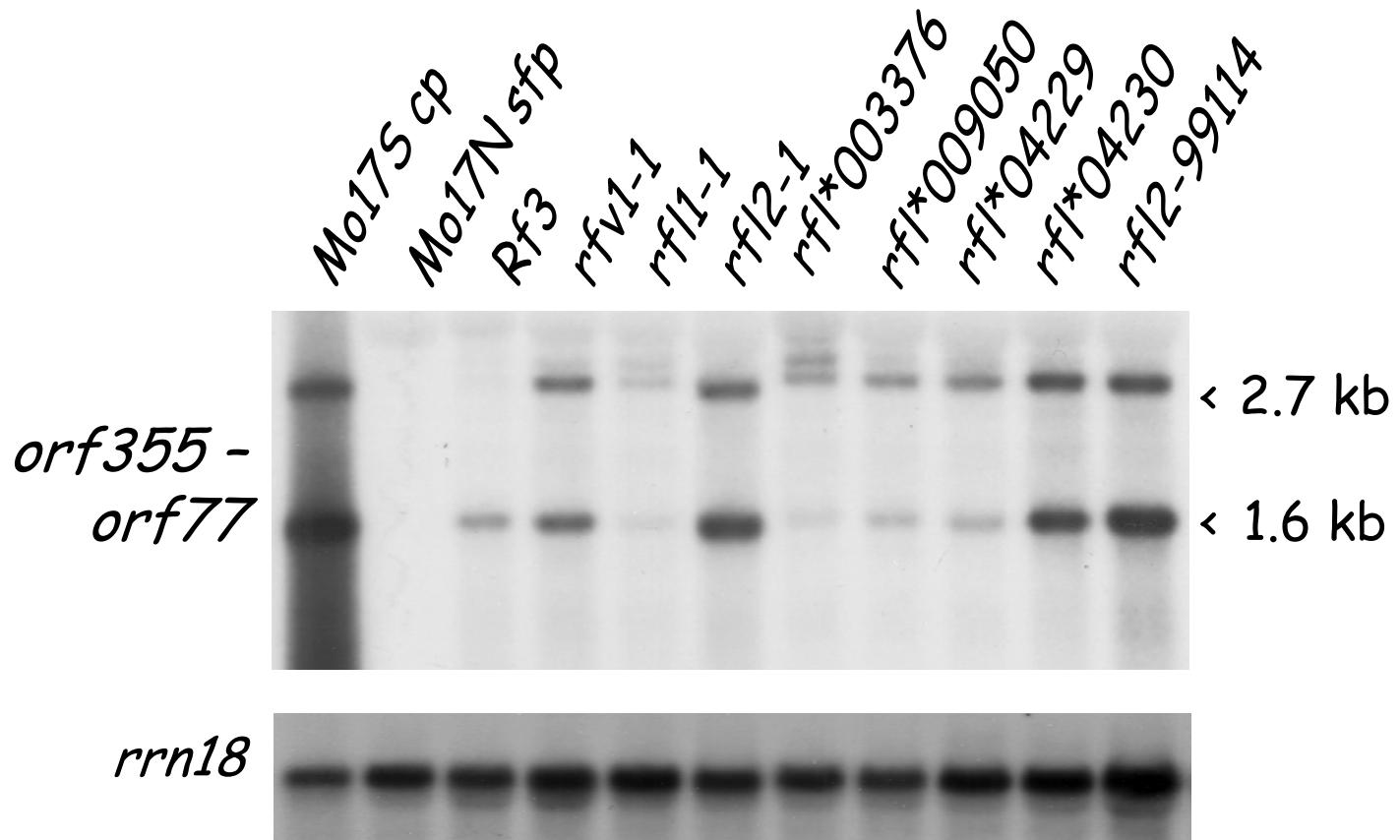


Candidate CMS orfs

orf	Predicted Protein	
	MW (kDa)	pI
<i>orf355</i>	39.9	5.1
<i>orf77</i>	9.1	9.5
<i>orf77 S14L</i>	9.1	9.5
<i>orf17</i>	2.0	3.1
<i>orf17 S14L</i>	2.0	3.1
<i>orf26</i>	2.9	10.6

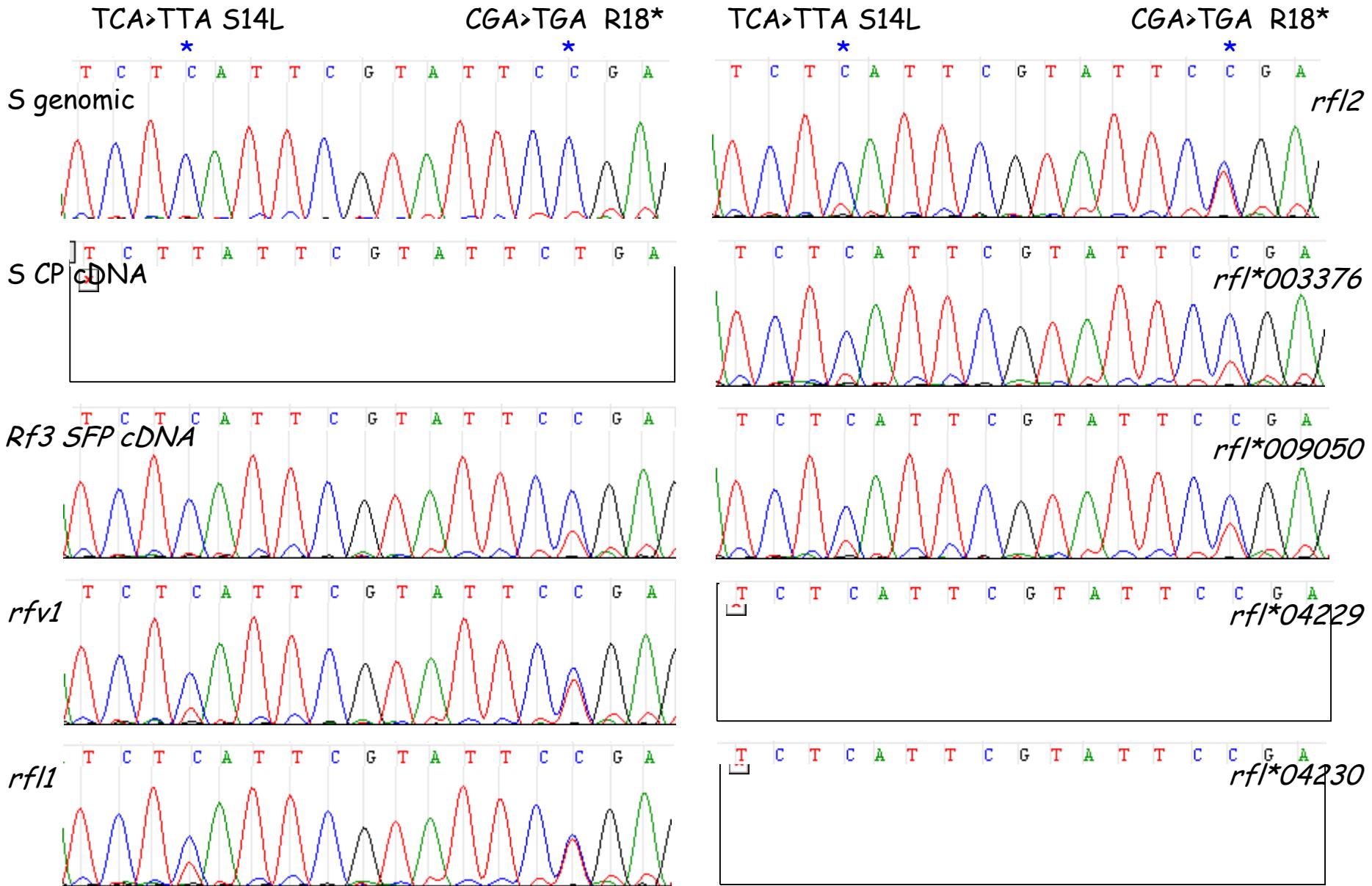
(Gallagher et al., Curr Genet 42:179)

Restorer-associated changes in *orf355*- *orf77* transcript accumulation

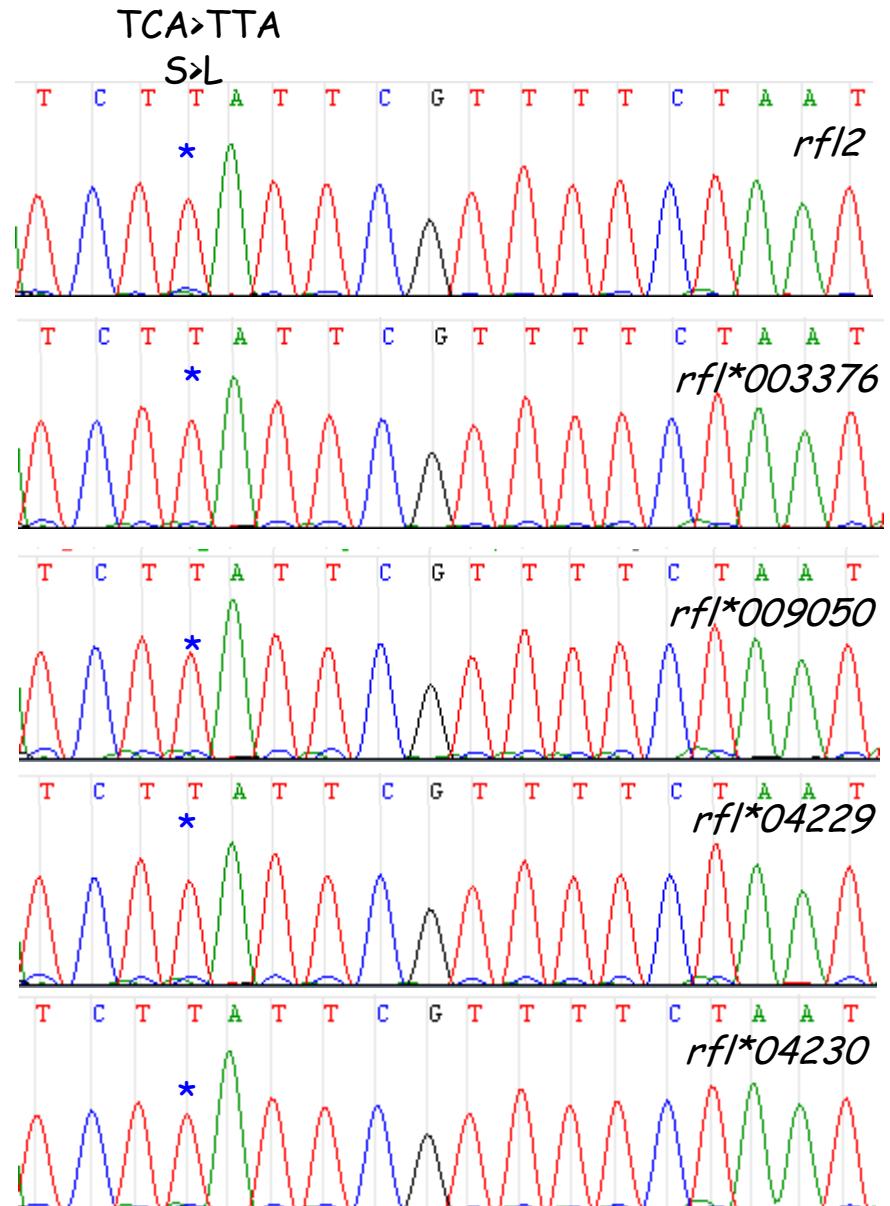
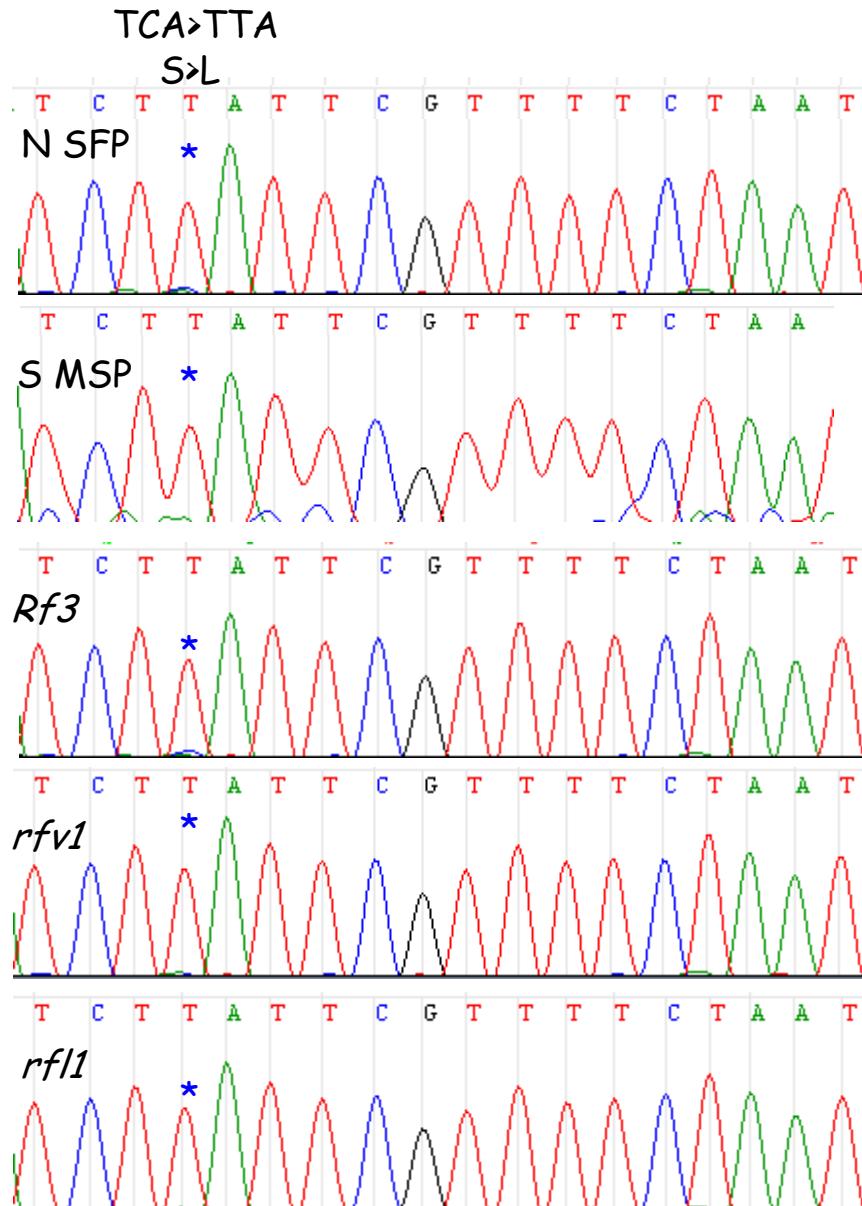


Liming Zhao

Restoration - associated changes in *orf77* transcript editing



Wild-type *atp9* transcript editing in restored CMS-S pollen



Identification of fertility restorers for S male-sterile maize: ? beyond ribosomal proteins

*rfl*04-230* candidates

