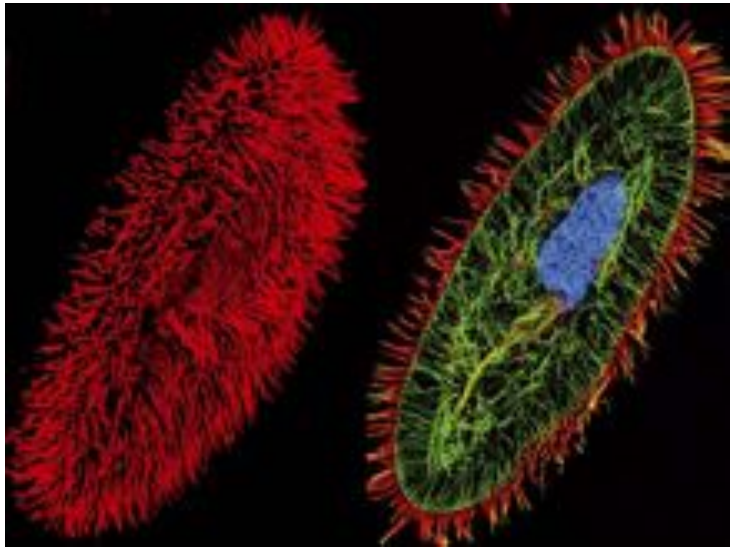
RNA-mediated genome remodeling in ciliates

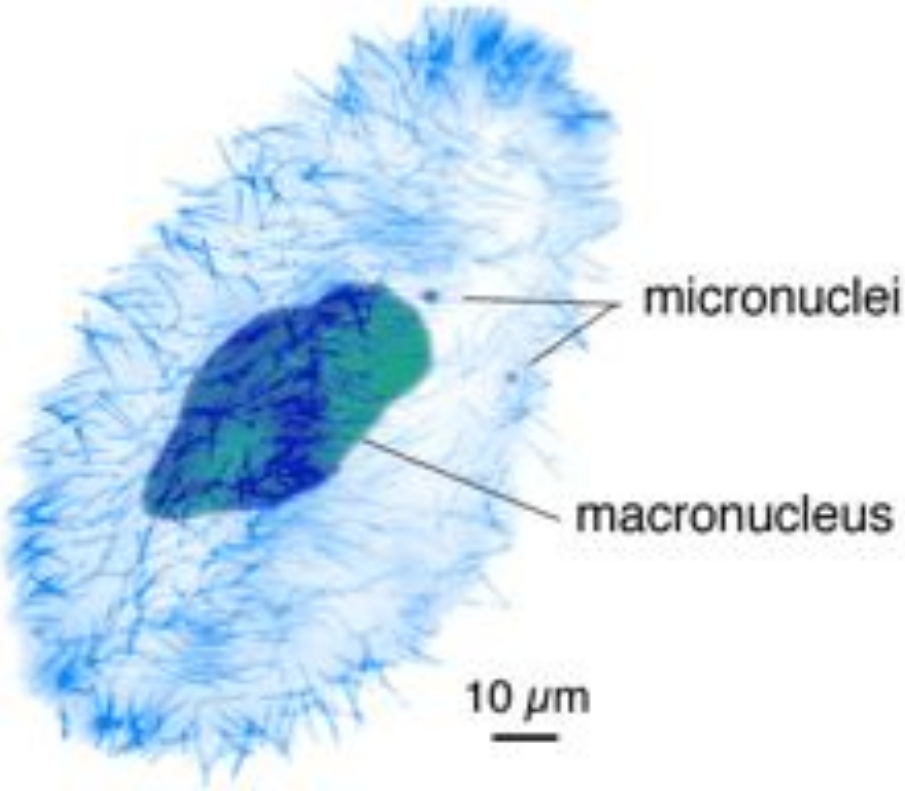
Mariusz Nowacki



University of Bern
Institute of Cell Biology
Switzerland

UNIVERSITÄT BERN

Nuclear dimorphism in ciliates



***Paramecium* cell**

MICRONUCLEUS:

- germline
- transcriptionally **silent**
- diploid
- contains transposons and other germline-limited DNA

MACRONUCLEUS:

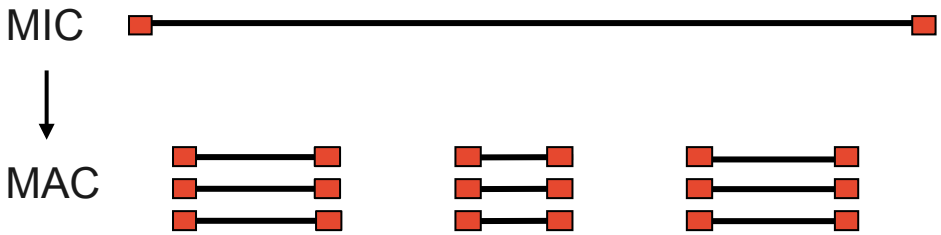
- somatic
- transcriptionally **active**
- highly polyploid
- short chromosomes
- replaced at each sexual process

Macronuclear development

A. Genome amplification

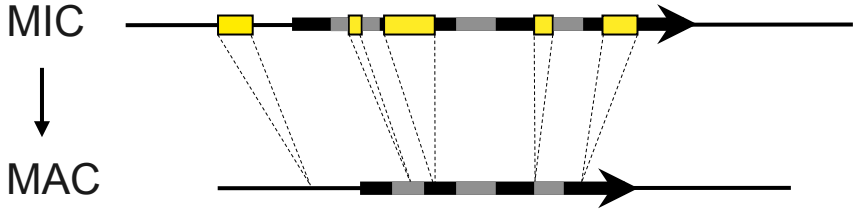
MIC 2n → MAC **up to 10'000n !!!**

B. Genome reduction
up to 98% !!!

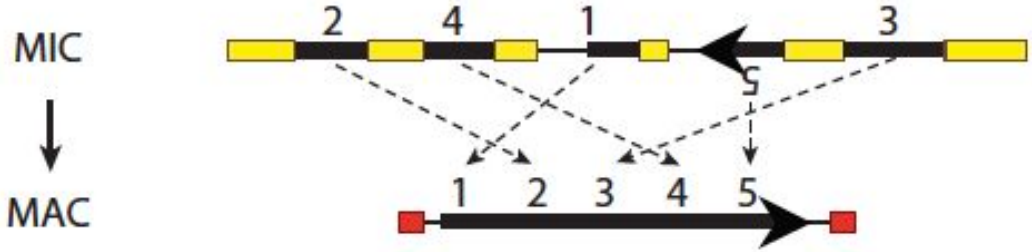


C. Chromosome fragmentation
Down to a 1 gene per chr. !!!

D. Precise excision of short Internal Eliminated Sequences (IES)



E. DNA unscrambling

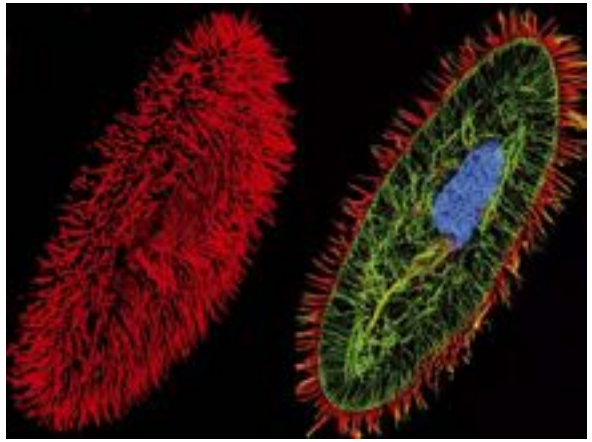


Two distinct types of genome rearrangement in ciliates

Oligohymenophorea

Paramecium, Tetrahymena

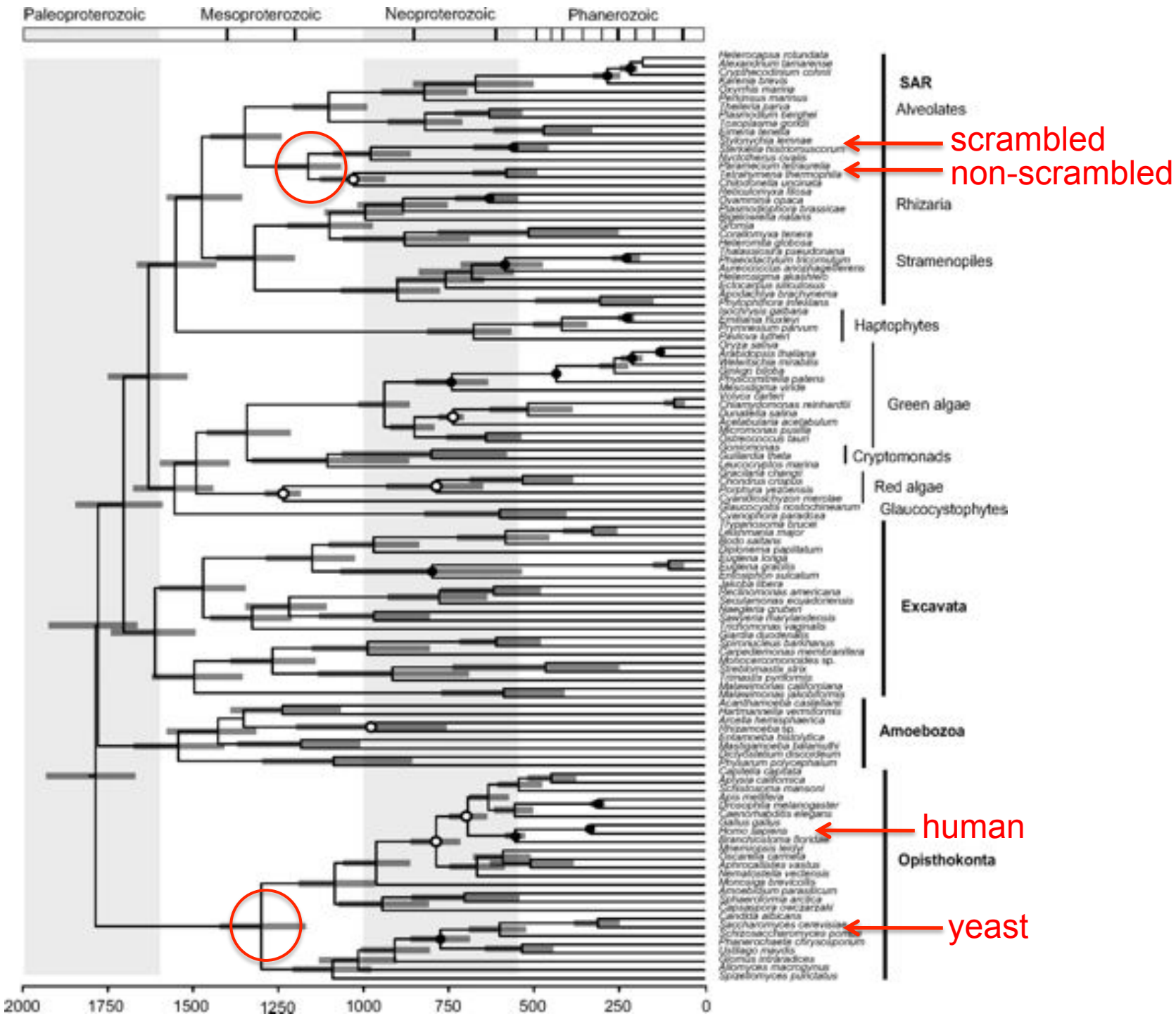
MAC development:
- Long chromosomes
- DNA excision and repair



Stichotrichs

Oxytricha, Stylonychia

MAC development:
- Short chromosomes (nanochromosomes)
- DNA excision and repair
- DNA unscrambling


Paleoproterozoic
Mesoproterozoic
Neoproterozoic
Phanerozoic
SAR
Alveolates
Rhizaria
Stramenopiles
Haptophytes
Green algae
Cryptomonads
Red algae
Glaucocystophytes
Excavata
Amoebozoa
Opisthokonta
scrambled
non-scrambled
human
yeast
2000
1750
1500
1250
1000
750
500
250
0

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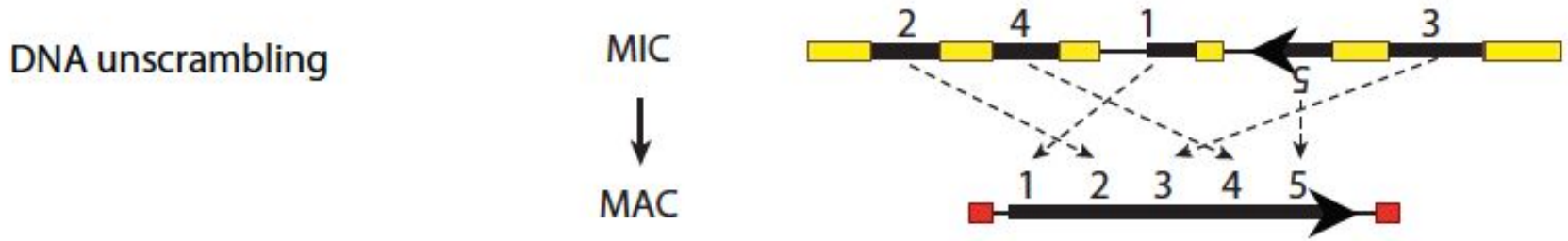
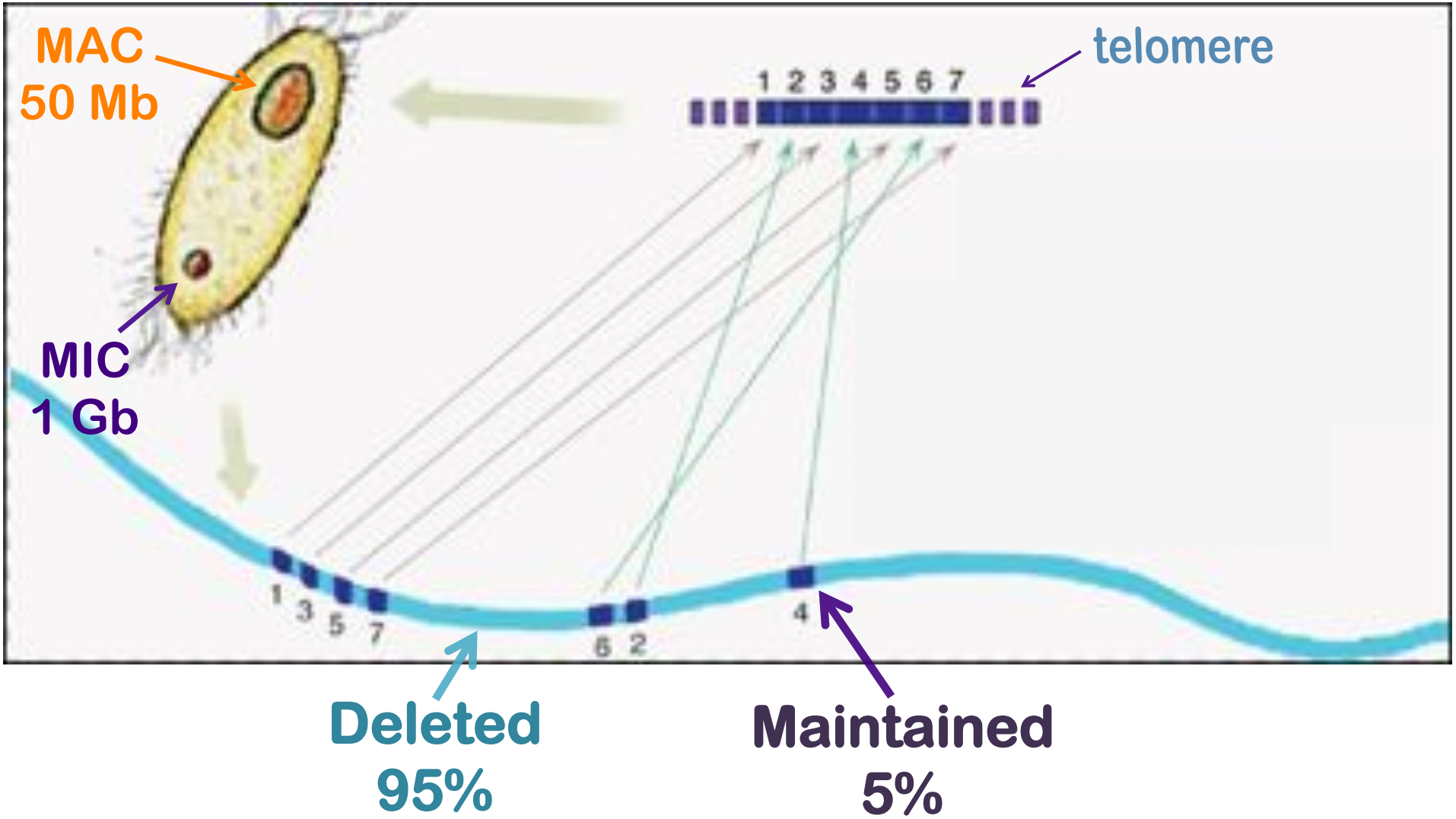
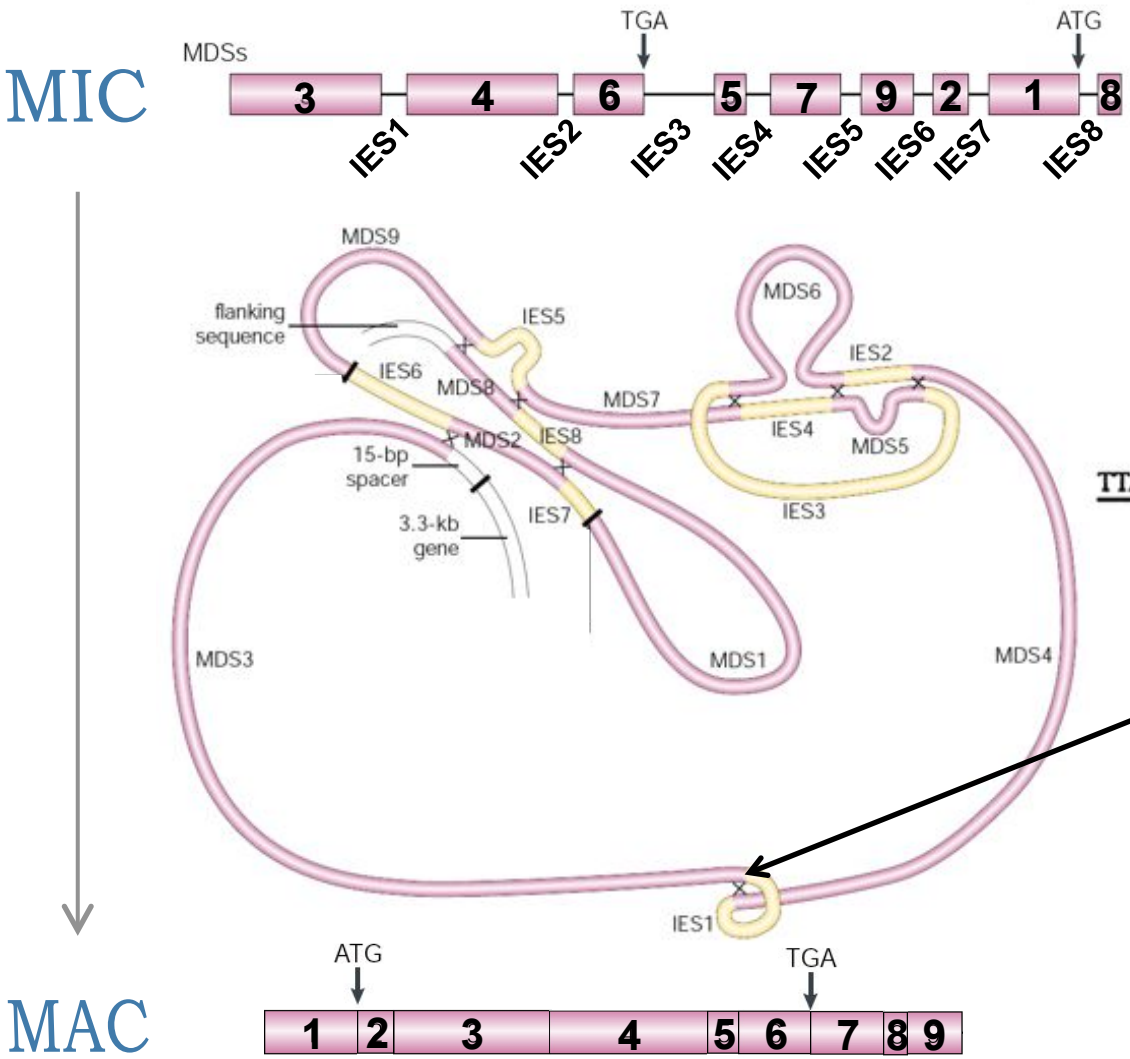
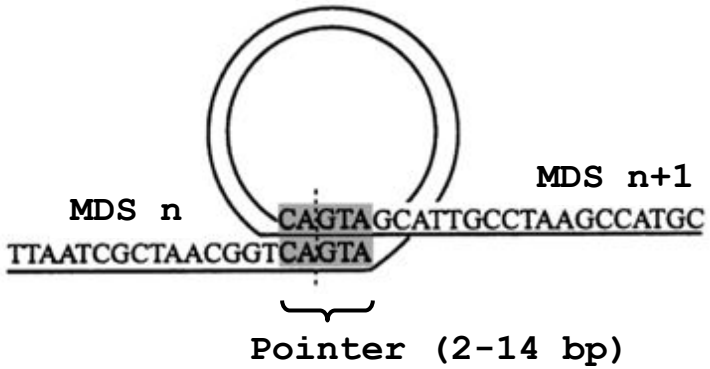


Gene Unscrambling in *Oxytricha*



Detangling of scrambled actinI gene



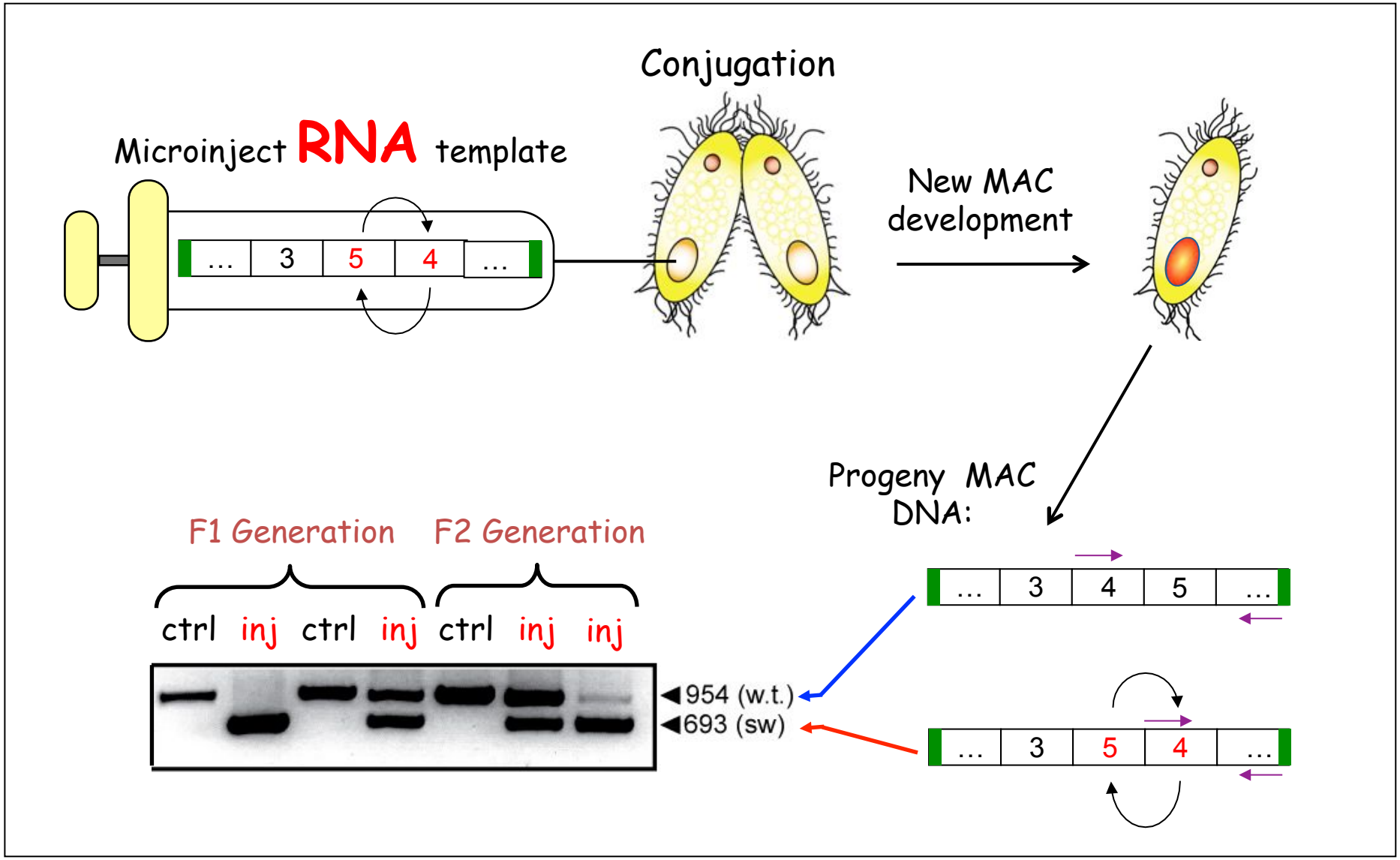


D.M.Prescott, 2000

Pointers are **too short** to guide unambiguously their own alignment

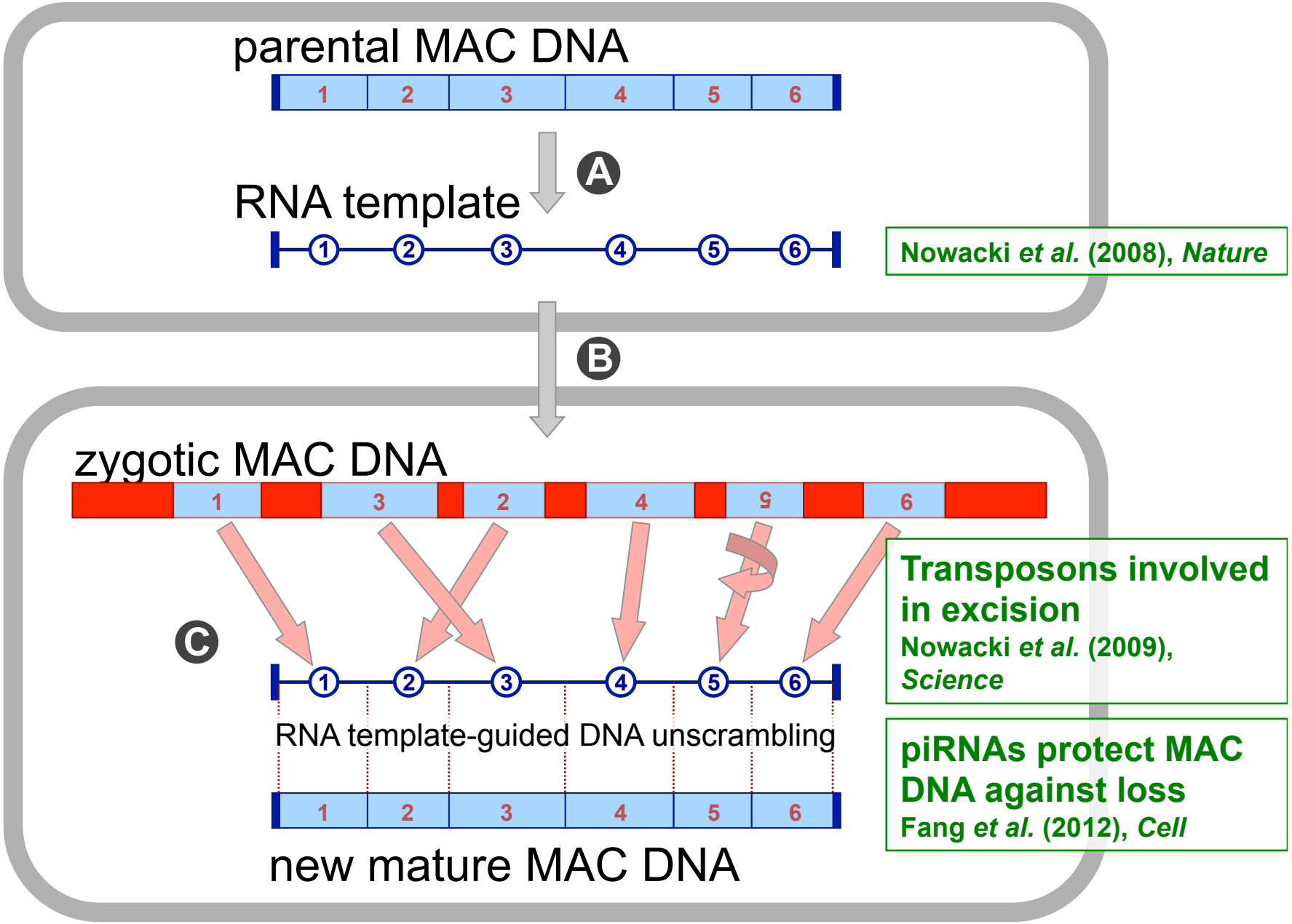
Not enough information to assure correct unscrambling

Microinjection of alternative RNA template



Nowacki M., *et al.* (2008), *Nature,* 451(7175)

RNA template model in *Oxytricha*



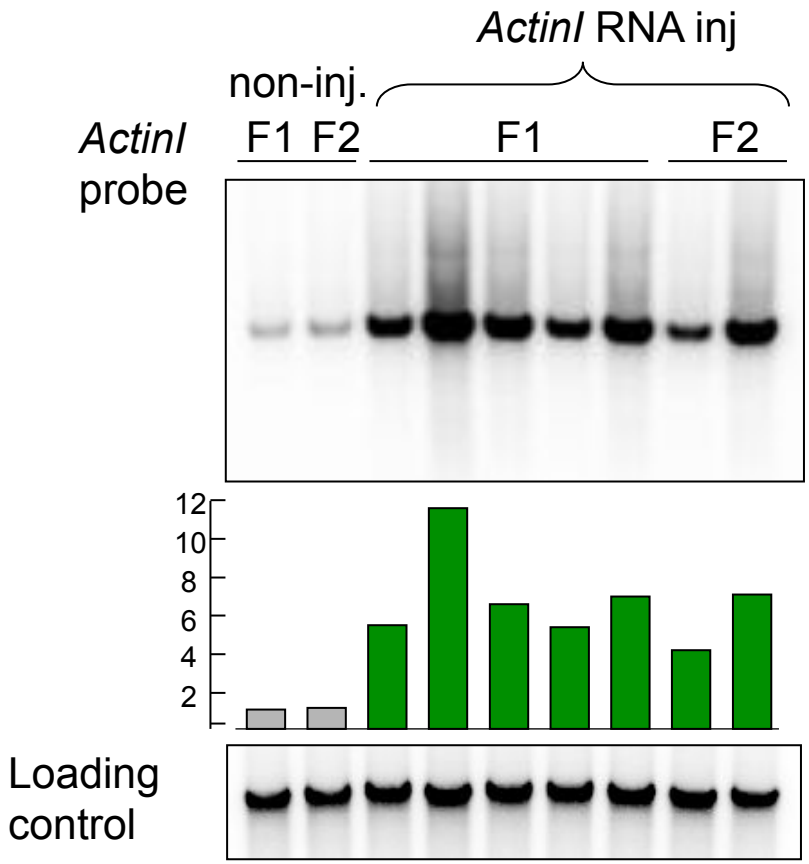
Nowacki *et al.* (2008), *Nature*

Transposons involved in excision
Nowacki *et al.* (2009), *Science*

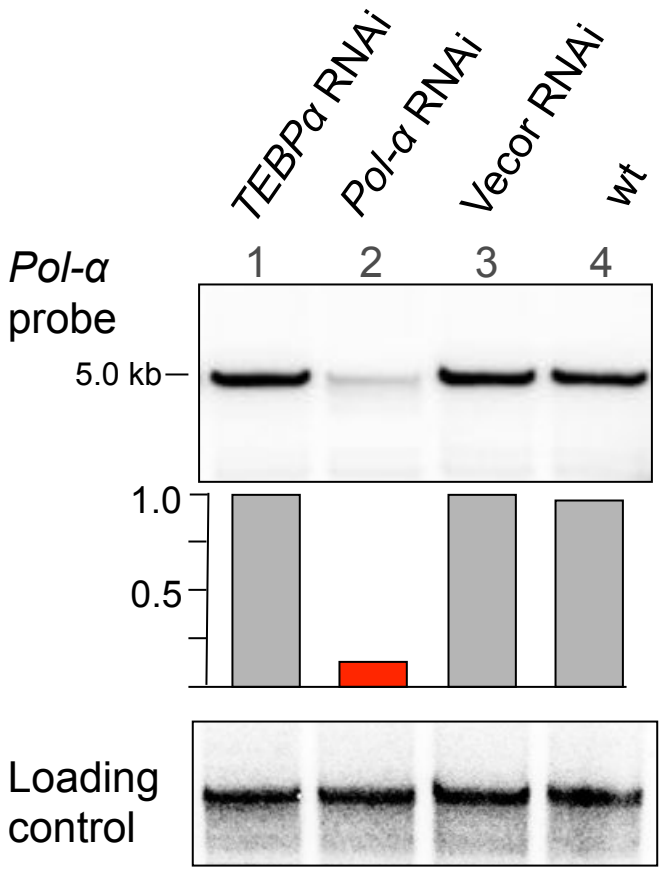
piRNAs protect MAC DNA against loss
Fang *et al.* (2012), *Cell*

Epigenetic inheritance of gene copy number in *Oxytricha*

RNA injection:



RNAi:



Nowacki M., *et al.* (2010), *PNAS*

Conclusion

In Oxytricha, matrnal RNA play an essential role during development by:

- **Providing templates for DNA unscrambling**

- **Regulating chromosome amount in the new macronucleus**

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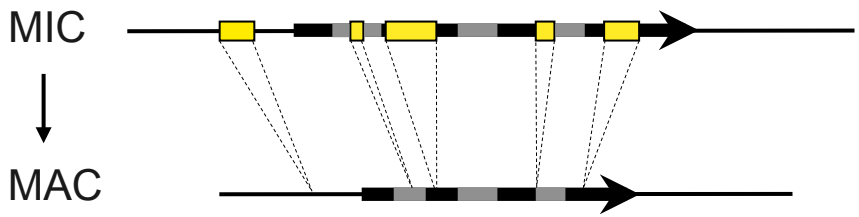
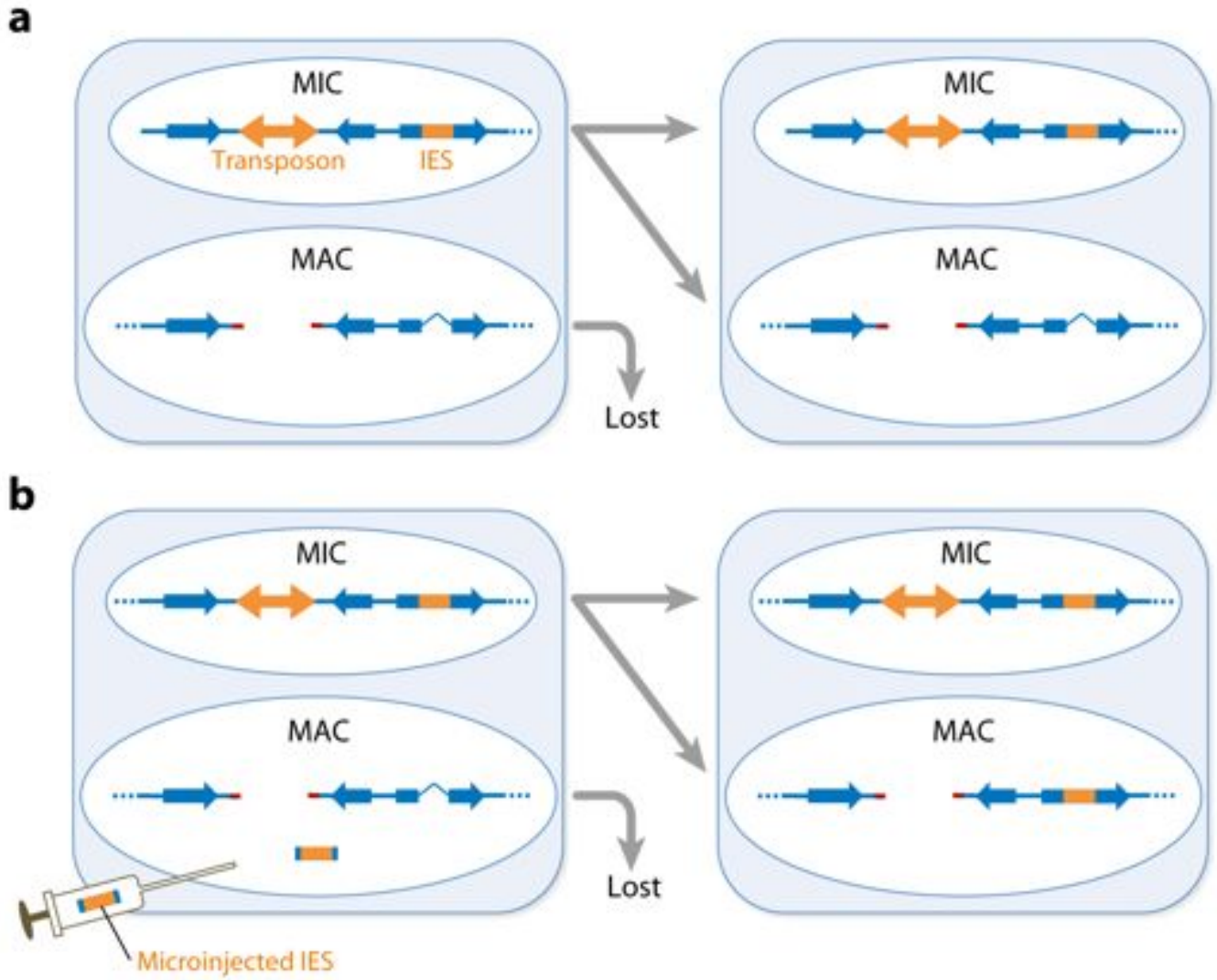
Precise excision of short Internal Eliminated Sequences (IES)

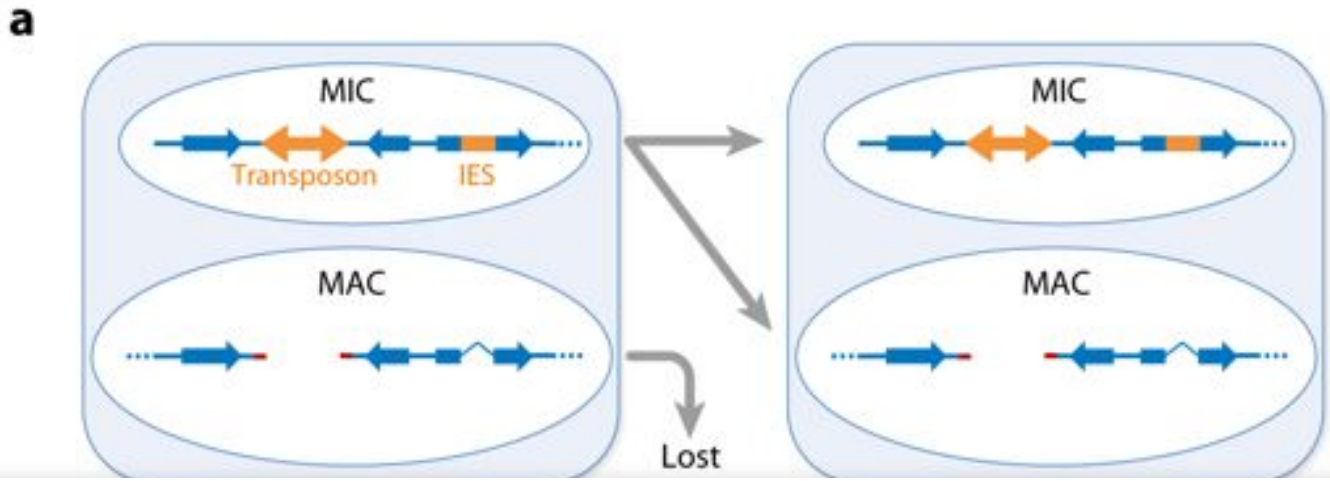


Epigenetic inhibition of internal eliminated sequence (IES) excision.

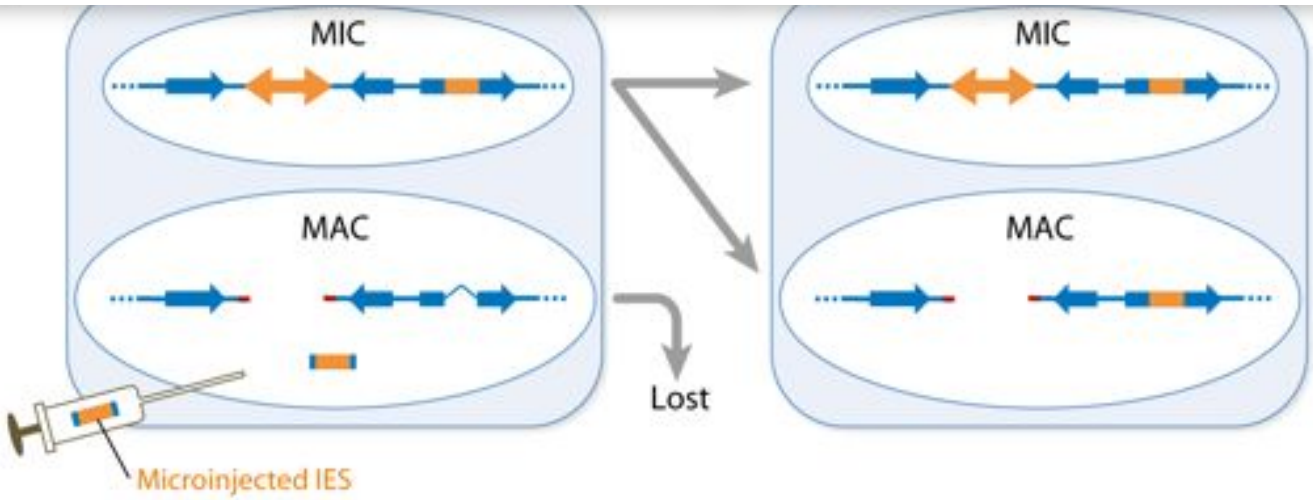


Nowacki M, et al. 2011.
Annu. Rev. Genomics Hum. Genet. 12:367–89

Epigenetic inhibition of internal eliminated sequence (IES) excision.



Works for nearly half of IESs (maternally controlled IESs)

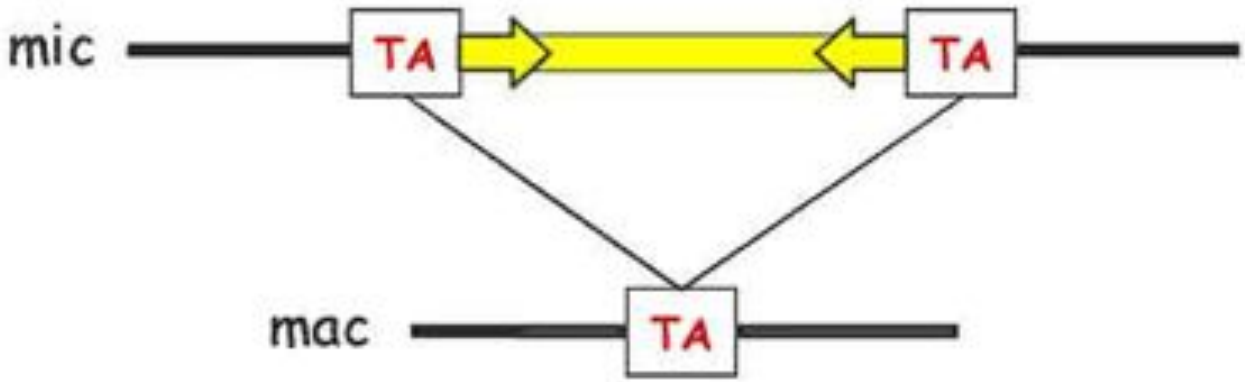


Maternally controlled IESs require Dcl2/3-produced scnRNAs for excision

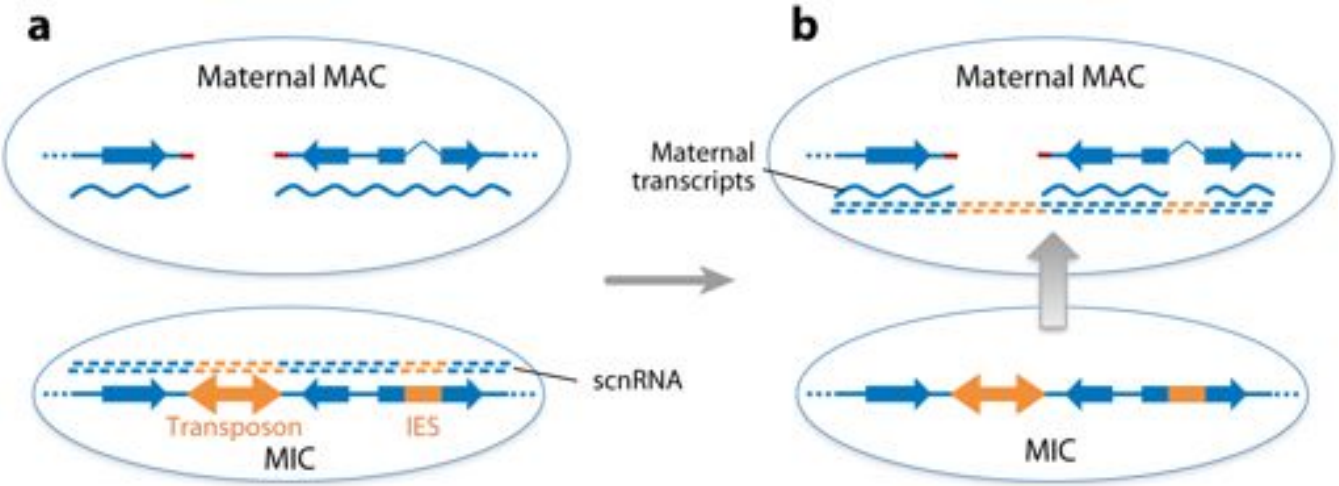
Non-Maternally controlled IESs do not need scnRNAs for their excision

Paramecium Internal Eliminated Sequences (IES)

- ≈ 45 000 per haploid genome
- present in genic and intergenic regions
- Short (28 bp length mode), single-copy, non-coding sequences
- 5´-TAYAGYNR-3´ consensus sequence extending into IES resembles that of Tc1/Mariner transposons



The scan RNA model for programmed genome rearrangements.



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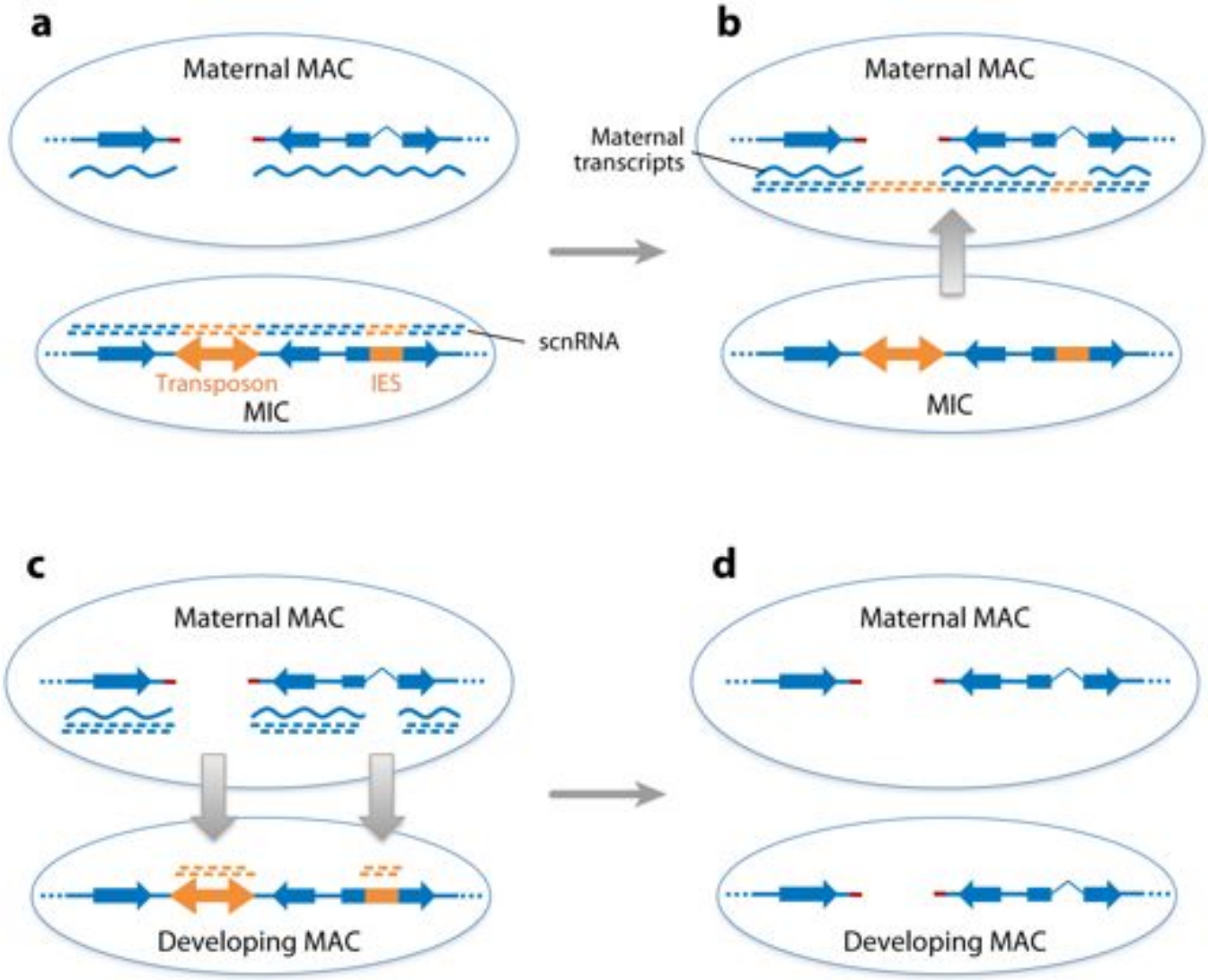
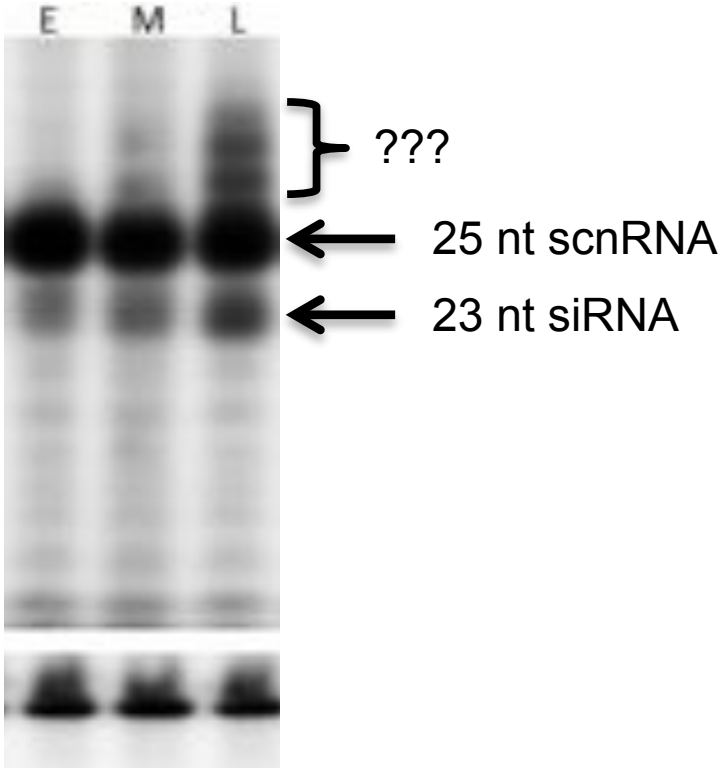
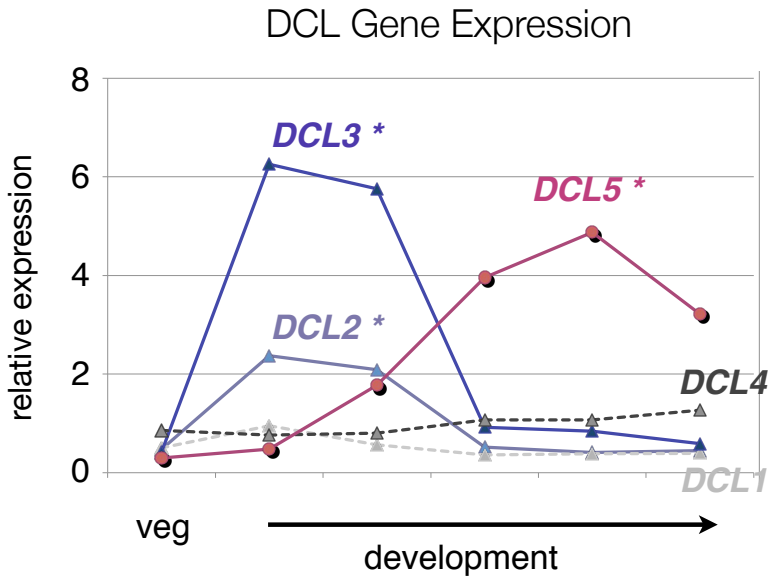
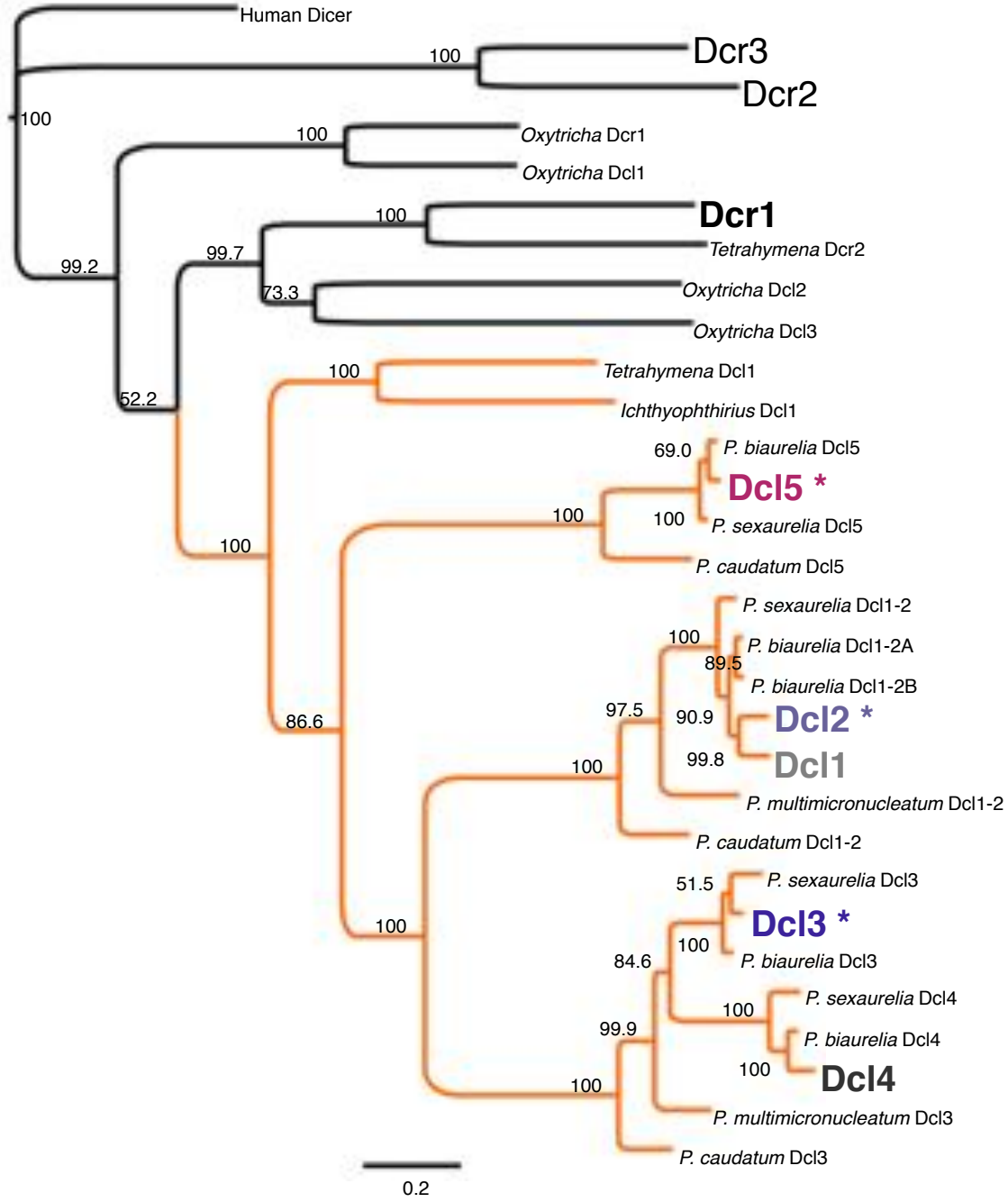


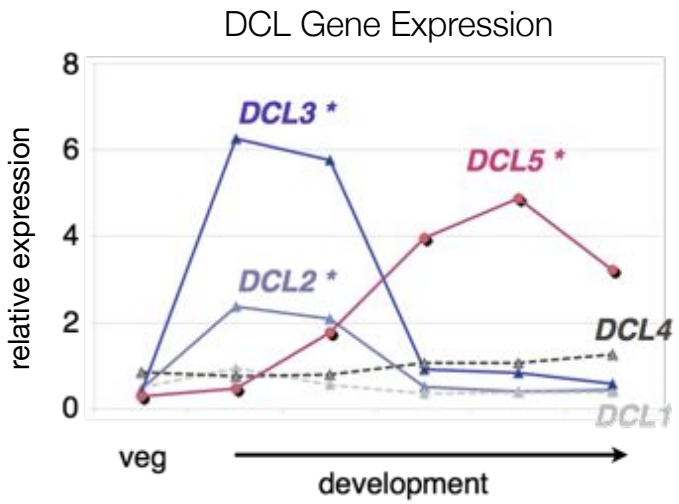
Nowacki M, et al. 2011.
Annu. Rev. Genomics Hum. Genet. 12:367–89

New class of small RNAs in *Paramecium*?









Dcl5

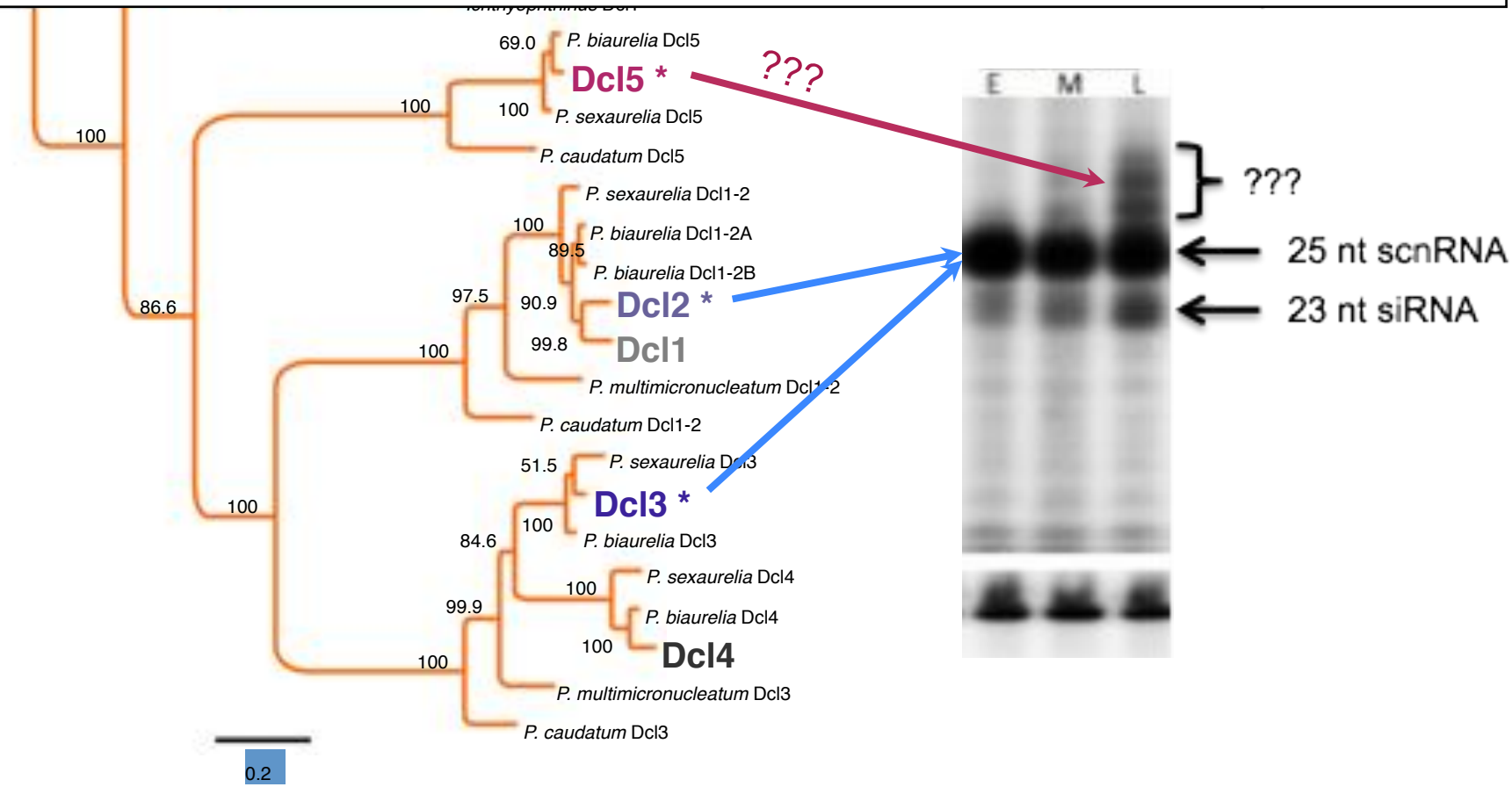
Dcl1/2

Dcl3/4

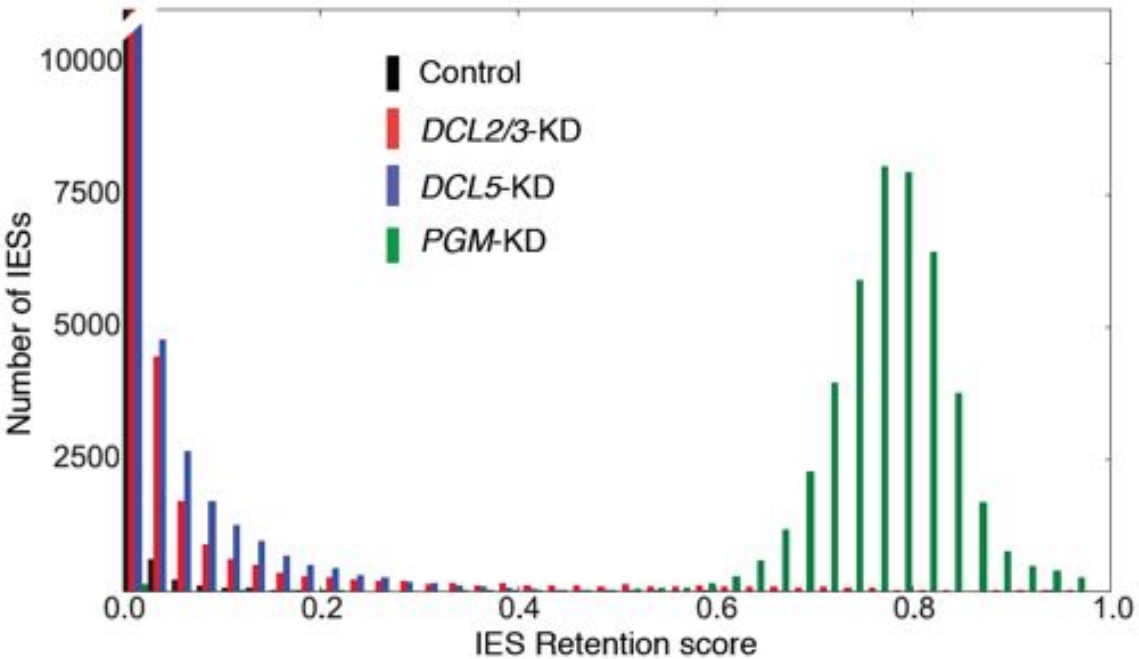
Objective:

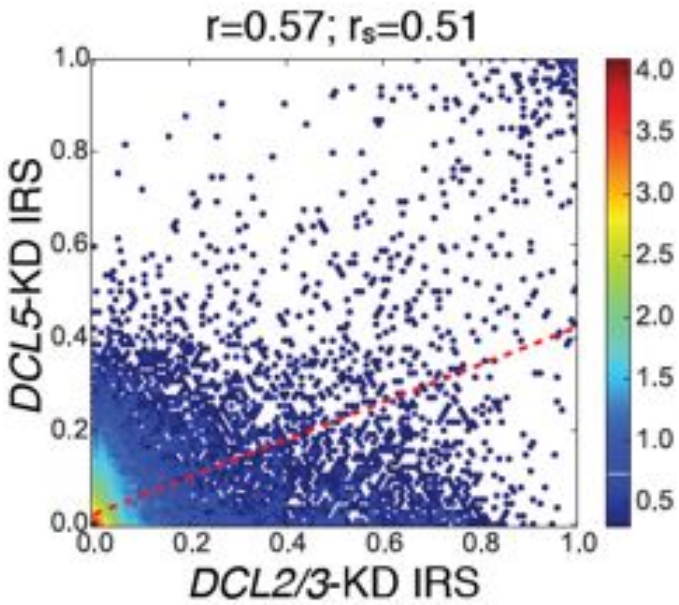
Characterize products of different Dcl proteins

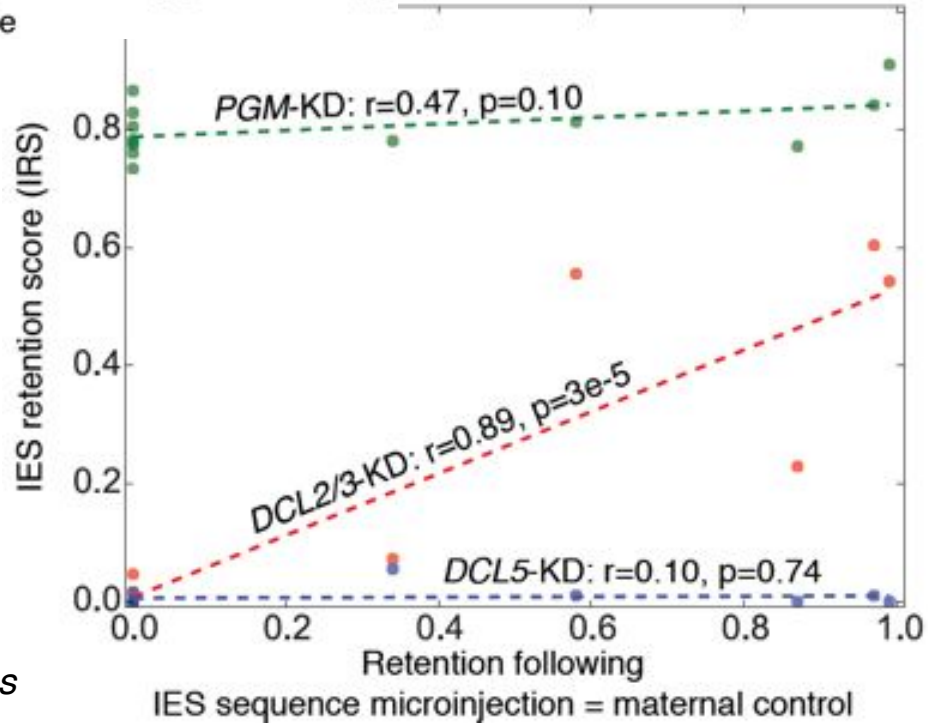
- **Do Dcl2 and Dcl3 produce the same sRNA molecules?**
- **Is Dcl5 responsible for the late 26-29nt sRNAs?**
- **Possible role for the 26-29nt sRNAs?**



Dcl2/3 and Dcl5 are required for IES excision

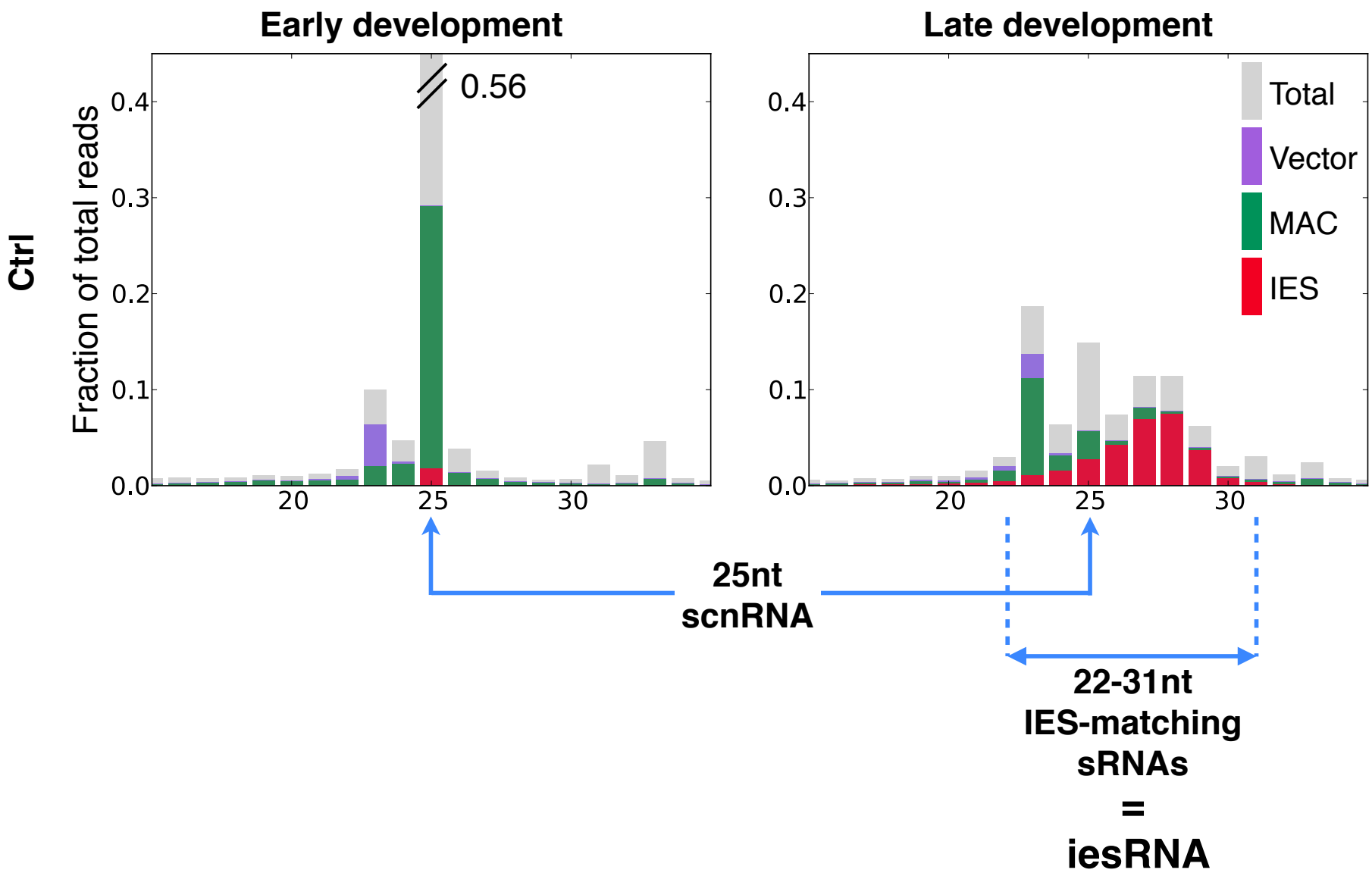


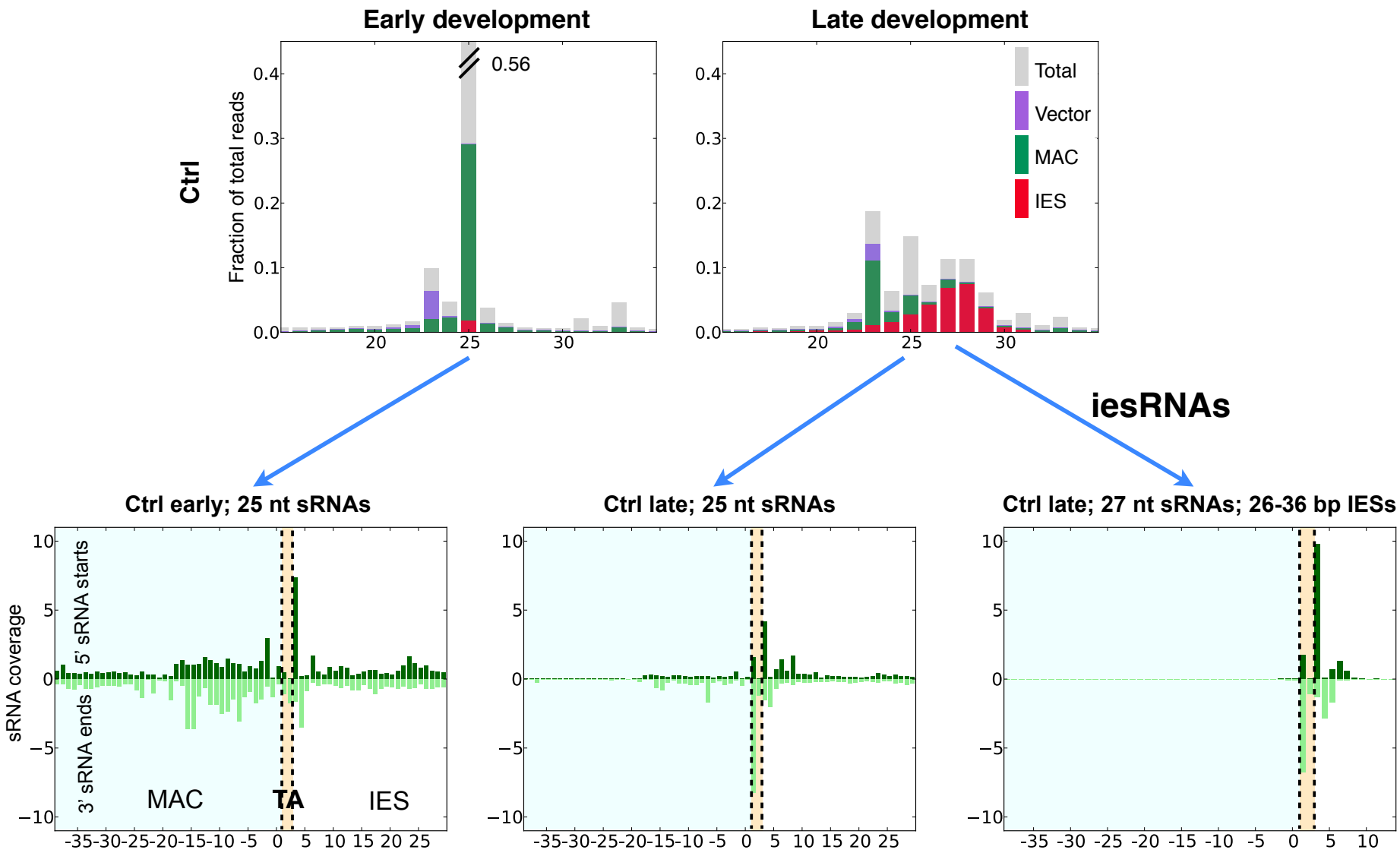




Swart et al. 2014 *Nucleic Acids Res, in press*

sRNA size distributions and source





- **scnRNAs overlap MAC/IES junctions and concentrate at IES ends**
- **<u>iesRNAs map exclusively to IESs</u> and concentrate at IES ends**

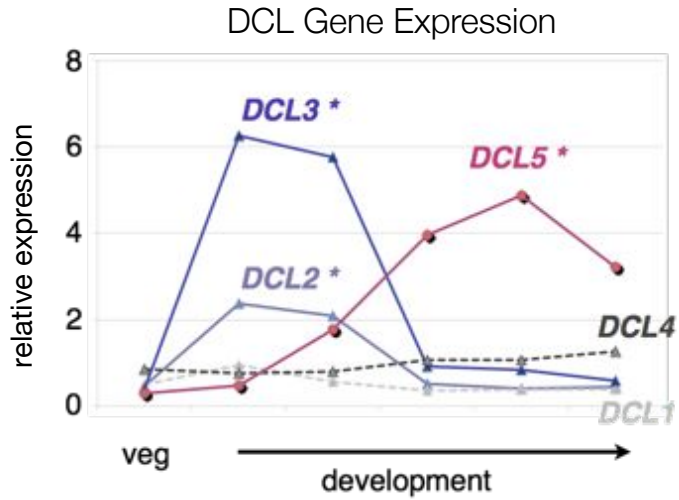
iesRNAs sometimes map across IES/IES junctions

Conclusion:

iesRNAs are produced from excised IESs

Part 2:

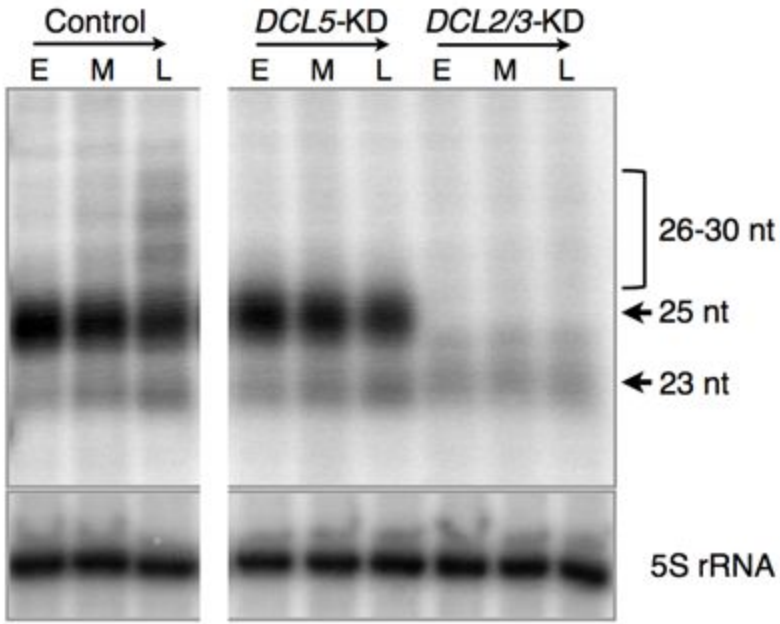
Analysis of Dcl proteins cleavage products

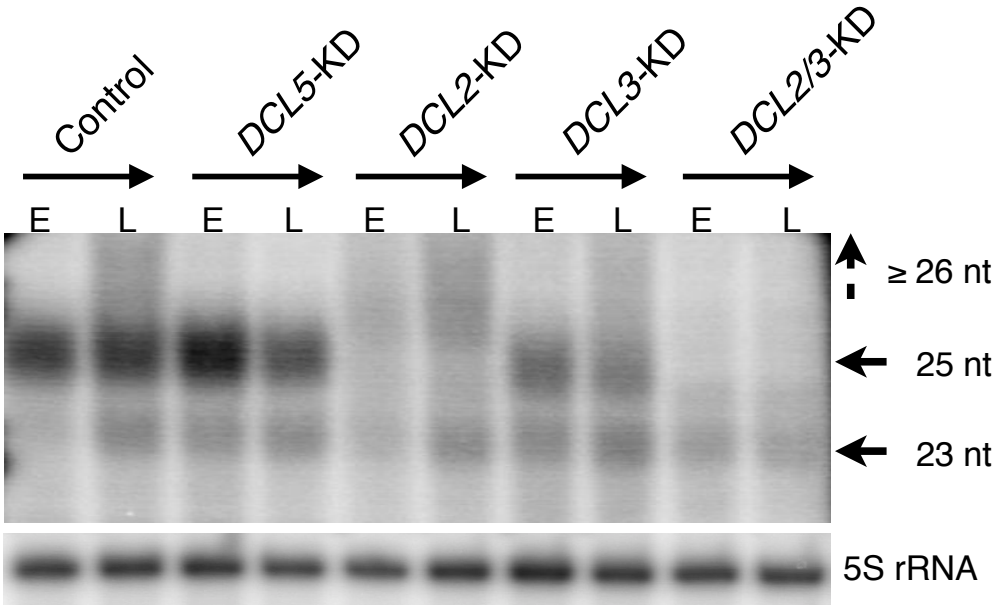


Silencing of Dcl2, Dcl3 and Dcl5 followed by sRNA sequencing and analysis

Sandoval, Swart et al. 2014, *Dev Cell*.

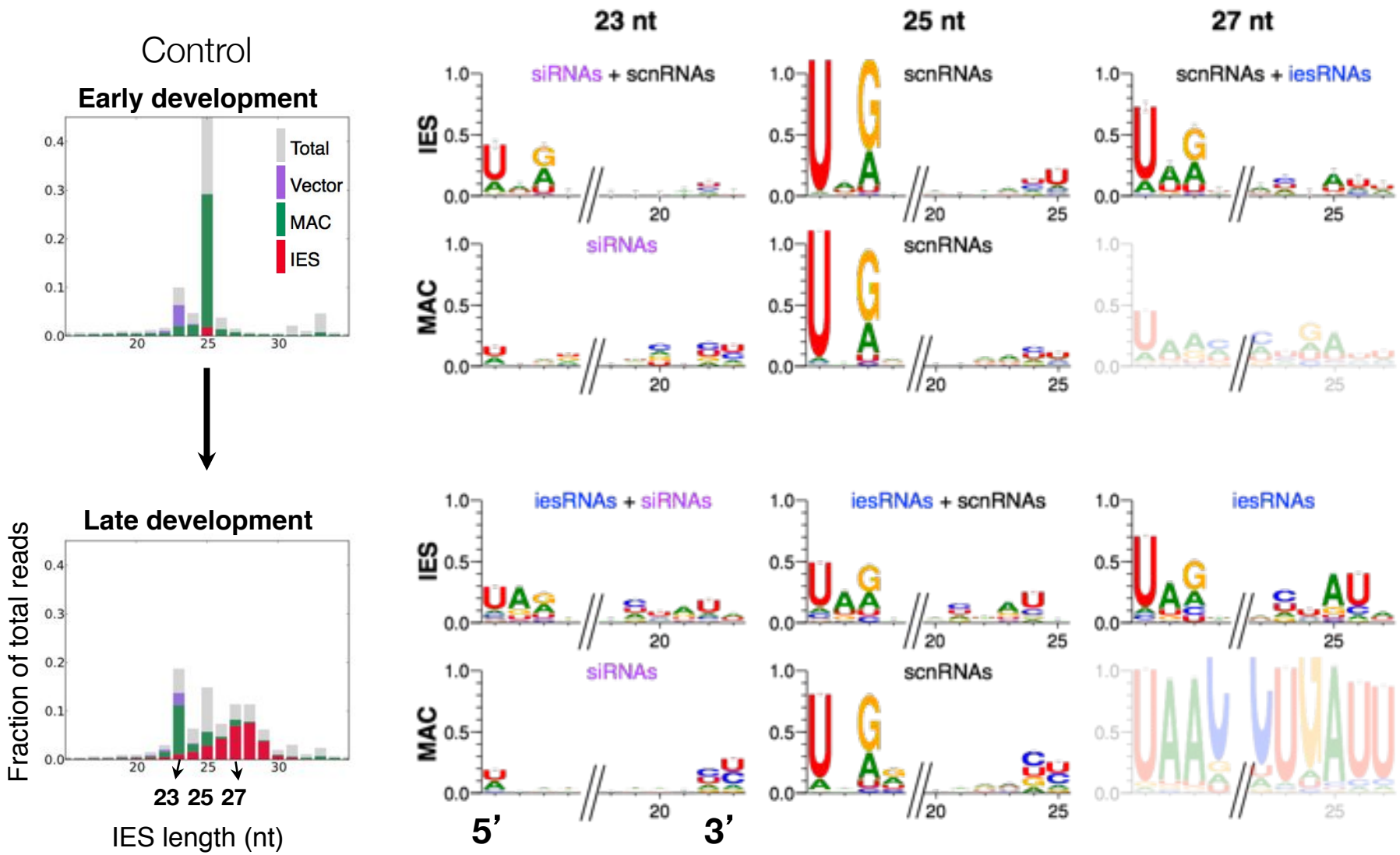
Knockdown of Dcls causes dramatic changes in developmental sRNAs





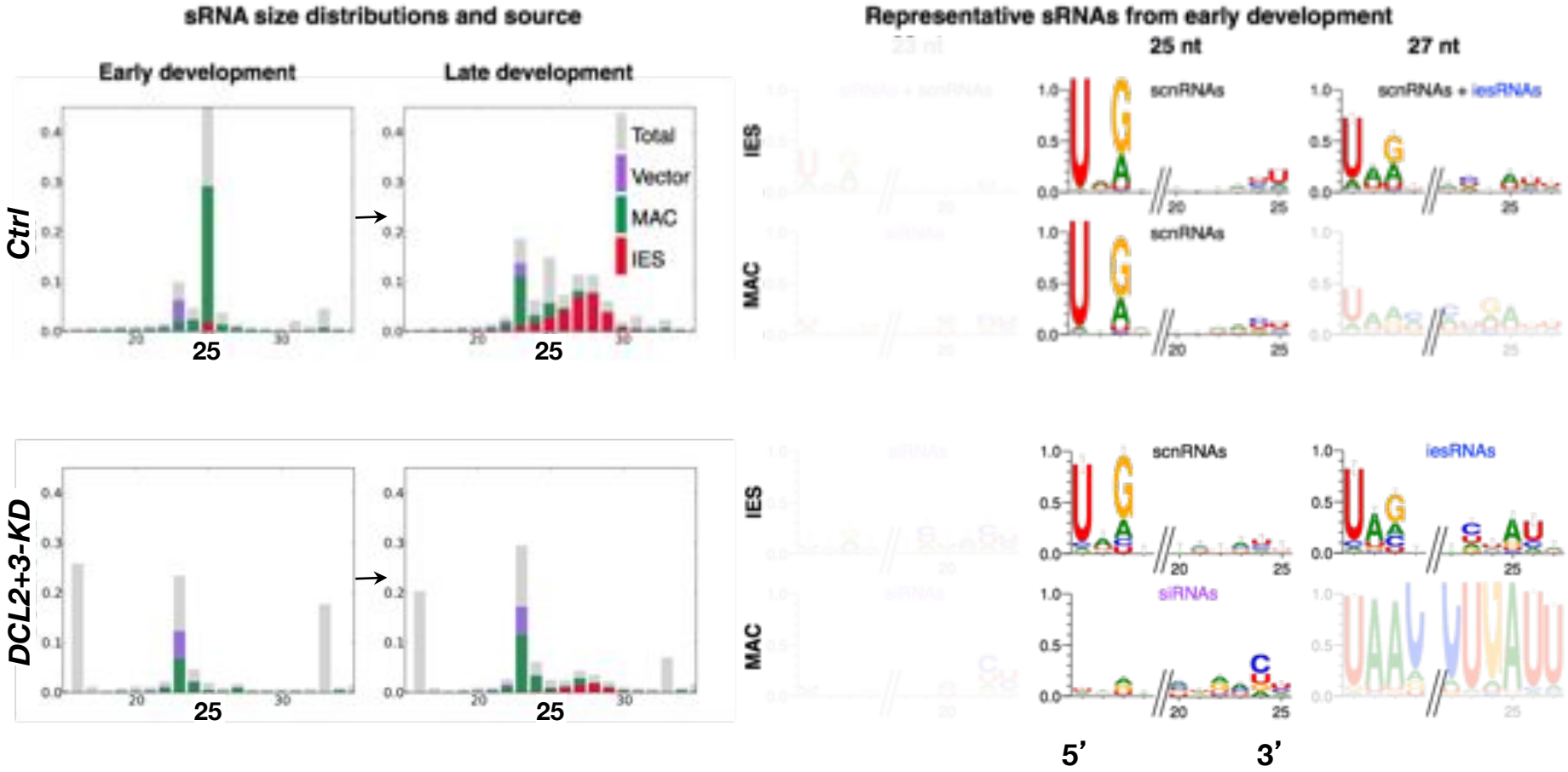
Sandoval, Swart et al. 2014, *Dev Cell*.

iesRNAs are a new class of developmental sRNAs



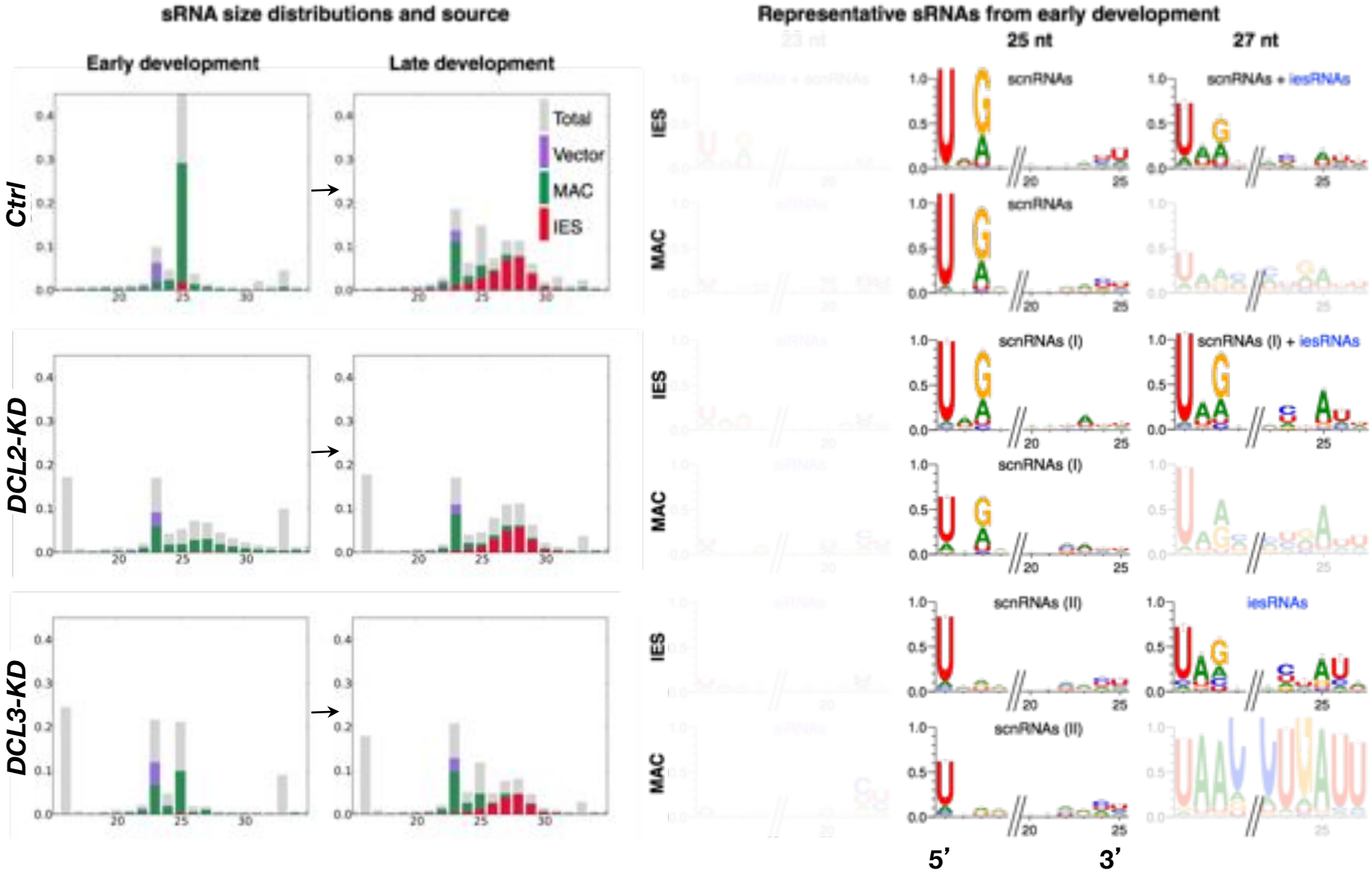
Sandoval, Swart et al. 2014, *Dev Cell*.

A combined *DCL2* and *DCL3* knockdown severely depletes scnRNAs

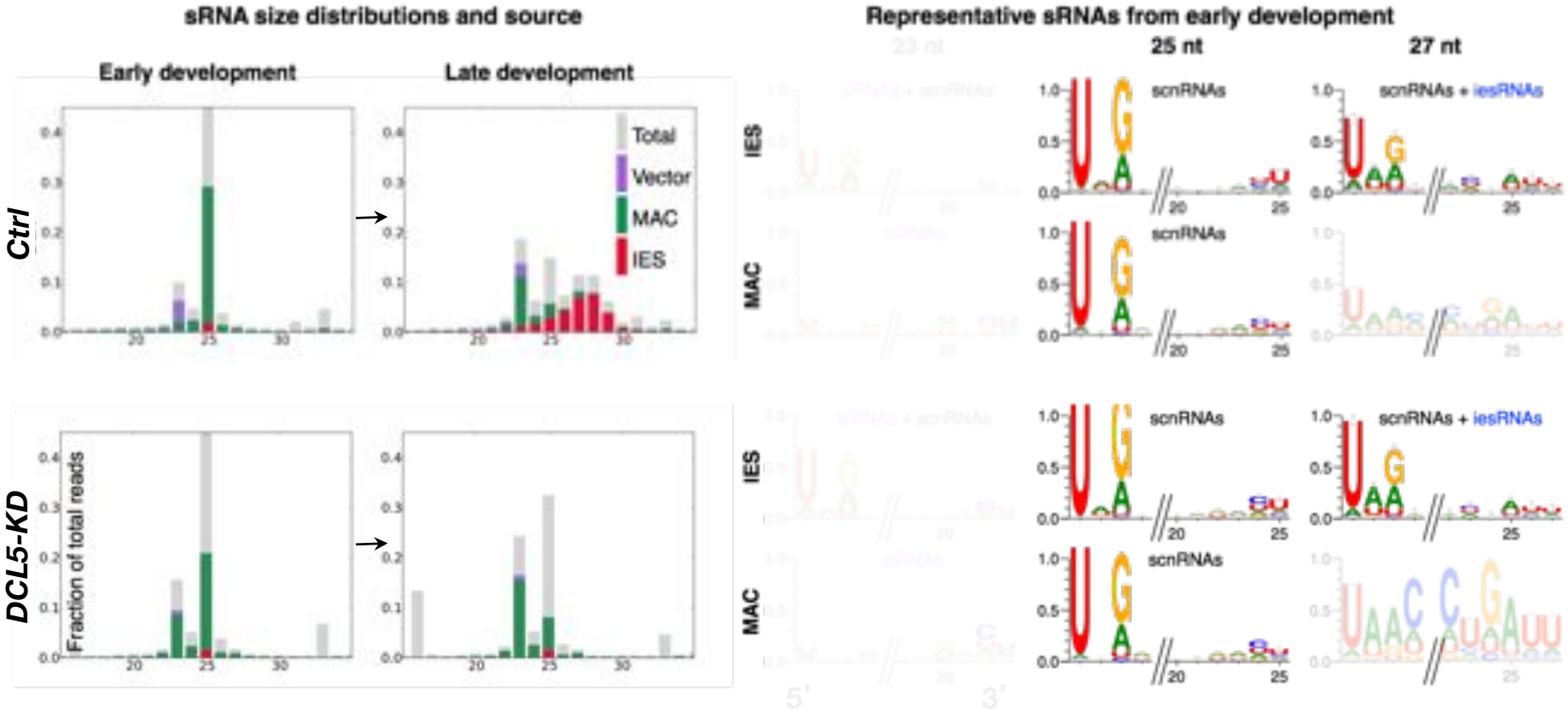


Sandoval, Swart et al. 2014, *Dev Cell*.

Independent *DCL2* and *DCL3* knockdowns reveal different sRNA size and length preferences



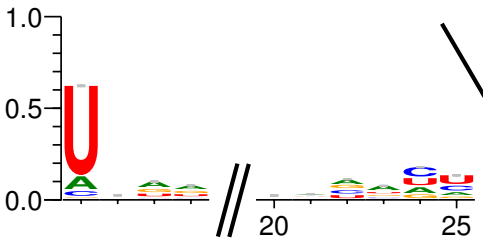
Knockdown of *DCL5* severely depletes iesRNAs



Sandoval, Swart et al. 2014, *Dev Cell*.

Dcl cleavage preferences

Dcl2

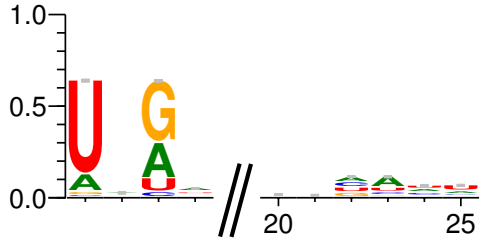


size 25 nt

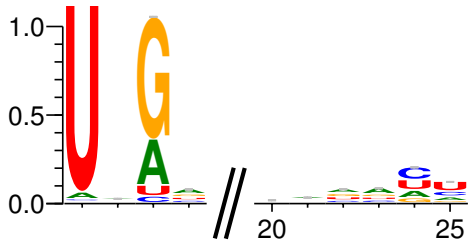
responsible for the size

sequence preference

Dcl3



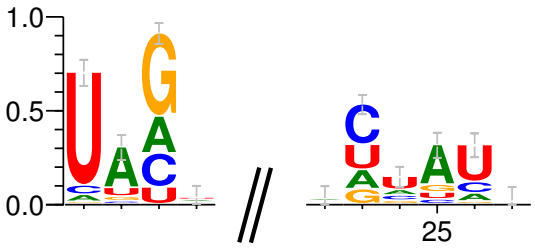
size 22-32 nt



size 25 nt
scnRNA

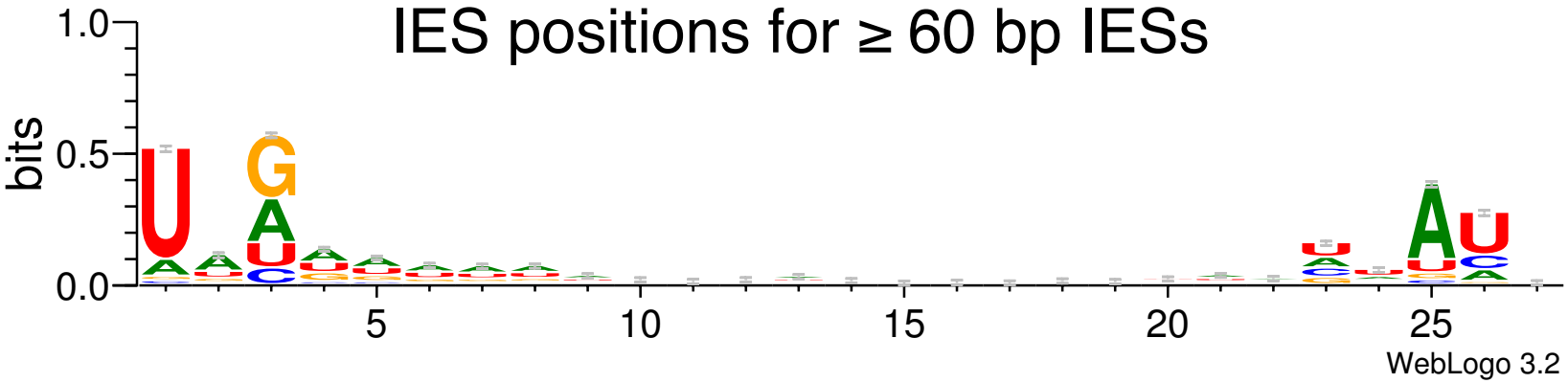
Dcl cleavage preferences

Dcl5



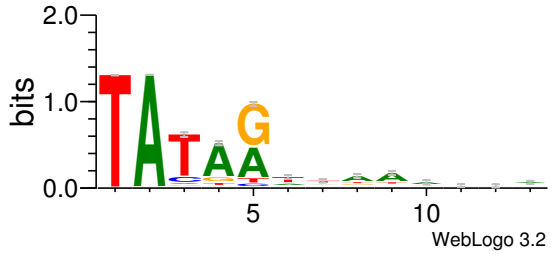
size 22-31 nt

Internal, non-terminal
IES positions for ≥ 60 bp IESs

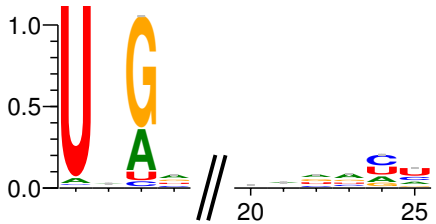


Paramecium sRNAs concentrate at IES boundaries

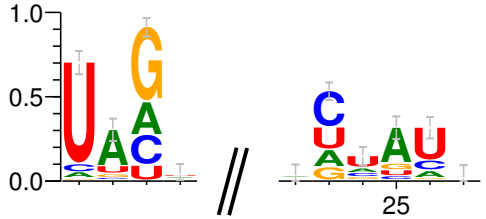
IES end



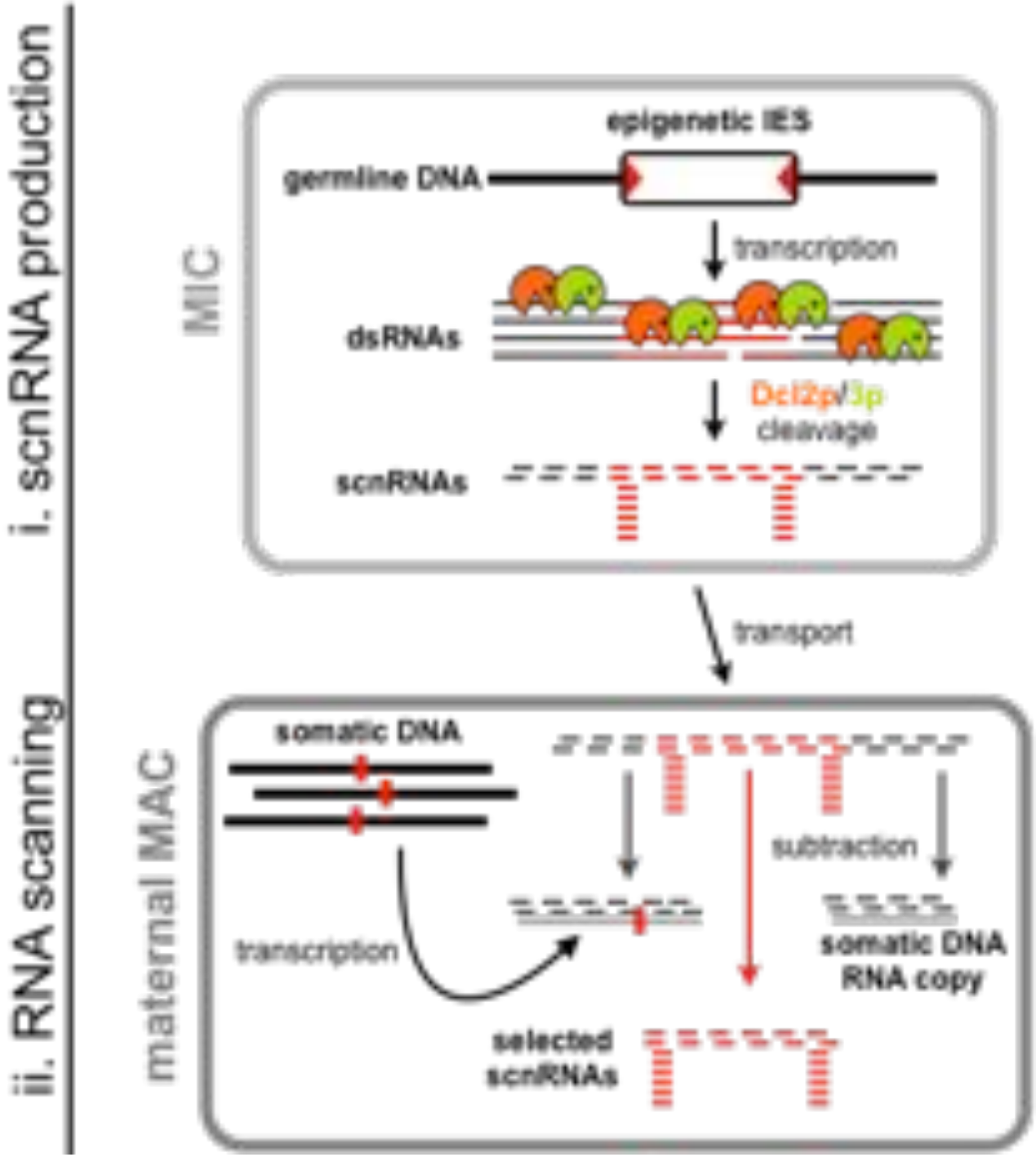
scnRNA

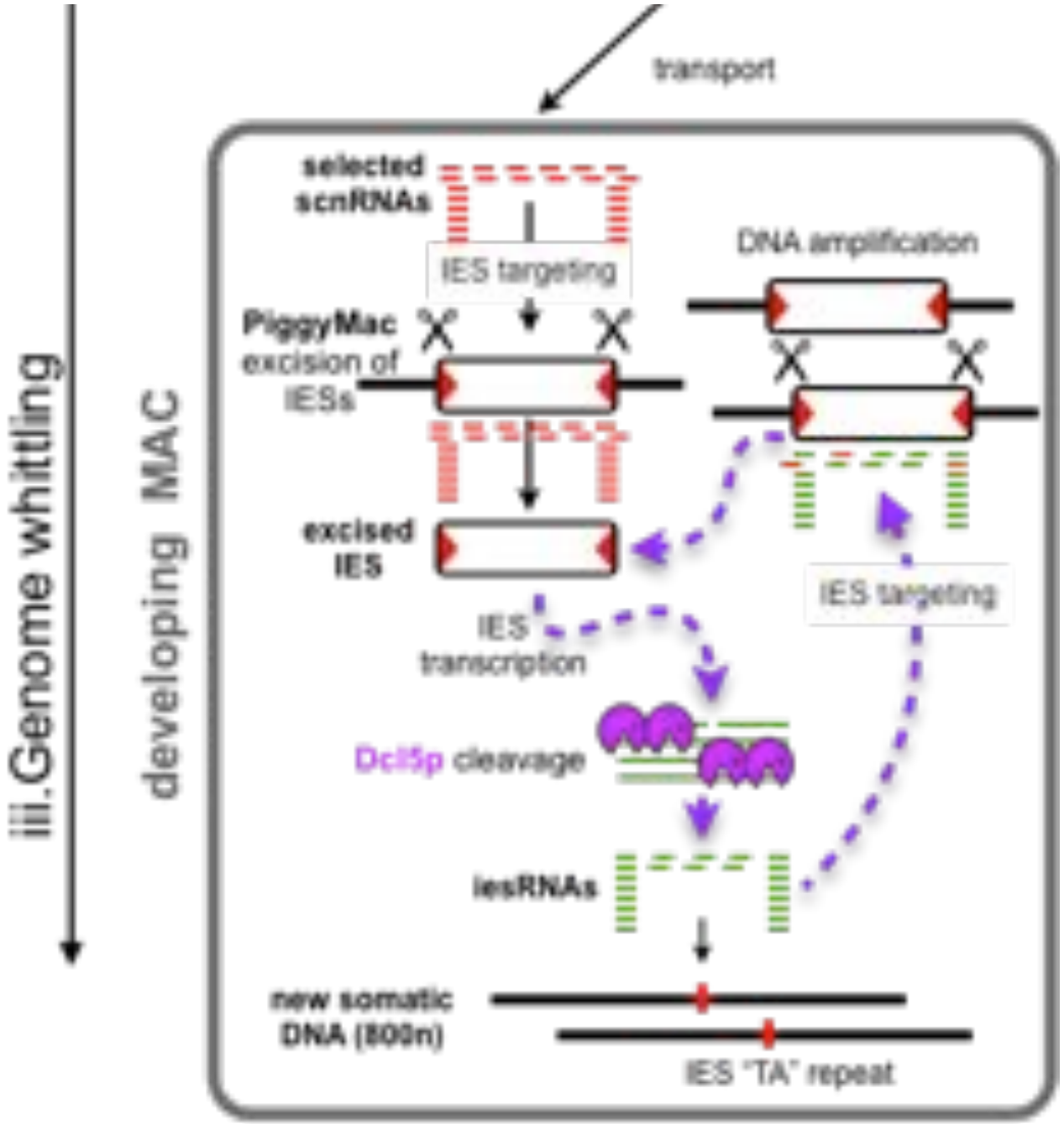


iesRNA



iesRNAs locate 10 fold more frequently at the terminal TNG than at internal TNGs, suggesting that iesRNAs production is biased towards the ends of IESs


i. scnRNA production
ii. RNA scanning
MIC
epigenetic IES
germline DNA
transcription
dsRNAs
Dcl2p/3p
cleavage
scnRNAs
transport
maternal MAC
somatic DNA
subtraction
transcription
somatic DNA
RNA copy
selected
scnRNAs



Sandoval, Swart et al. 2014, *Dev Cell*.

Acknowledgments

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Pamela Sandoval
Estienne Swart
Patricia Romero
Miroslav Arambasic
Michael Ignarski
Dominique Furrer
Aditi Singh
Tina Hoehener
Matthias Kraft
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Hans-Joachim Lipps, Uni-Witten-Herdecke, Germany

Funding:

UNIVERSITÄT BERN

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SCHWEIZERISCHER NATIONALFONDS
FONDO NAZIONALE SVIZZERO
SWISS NATIONAL SCIENCE FOUNDATION

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