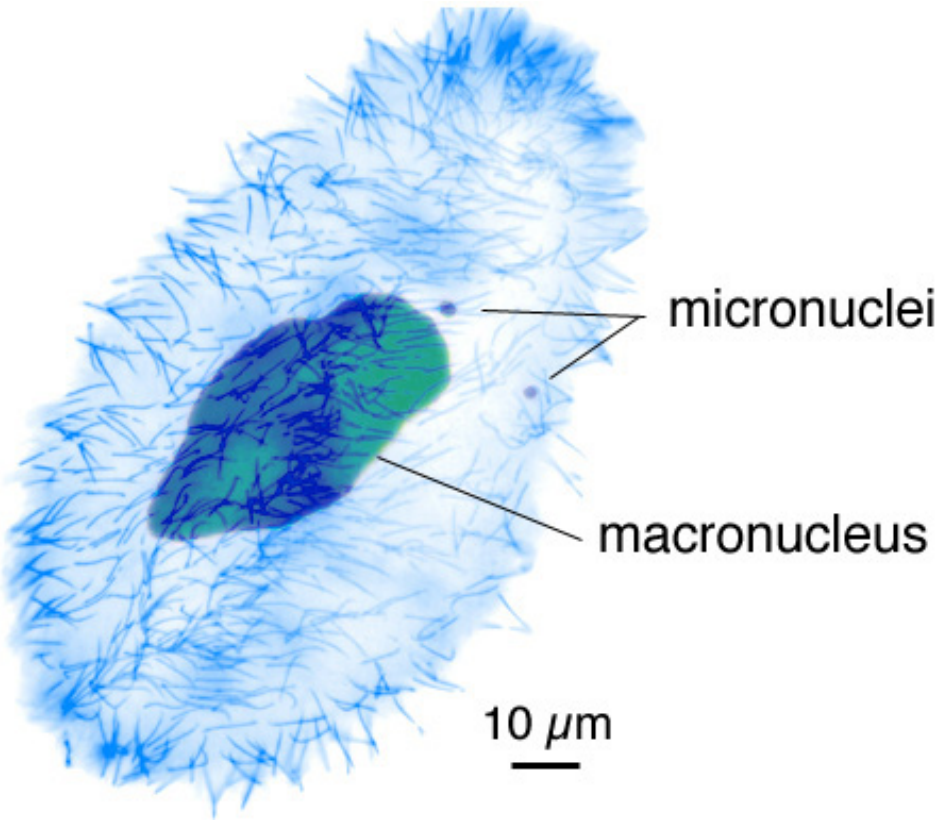
RNA-mediated trans-generational inheritance in ciliates

Mariusz Nowacki

University of Bern
Institute of Cell Biology
Switzerland

Nuclear dimorphism in ciliates



***Paramecium* cell**

MICRONUCLEUS:

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

MACRONUCLEUS:

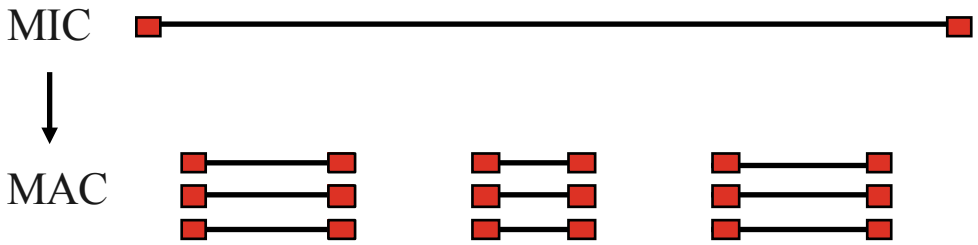
- somatic
- transcriptionally **active**
- highly polyploid
- short chromosomes
- replaced at each sexual process

Macronuclear development

A. Genome amplification

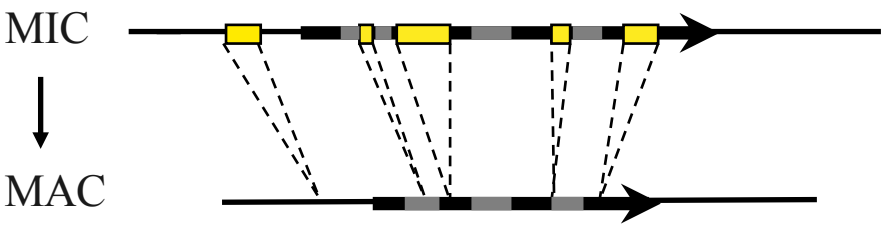
MIC 2n → MAC **800n**

B. Genome reduction by **20%**

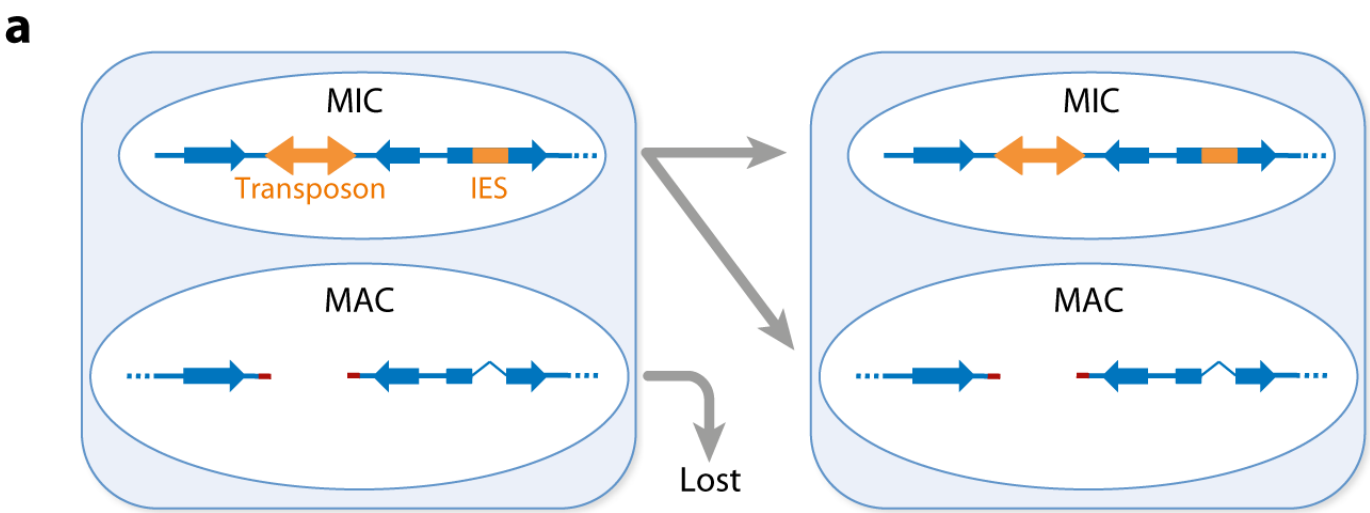


C. Chromosome fragmentation

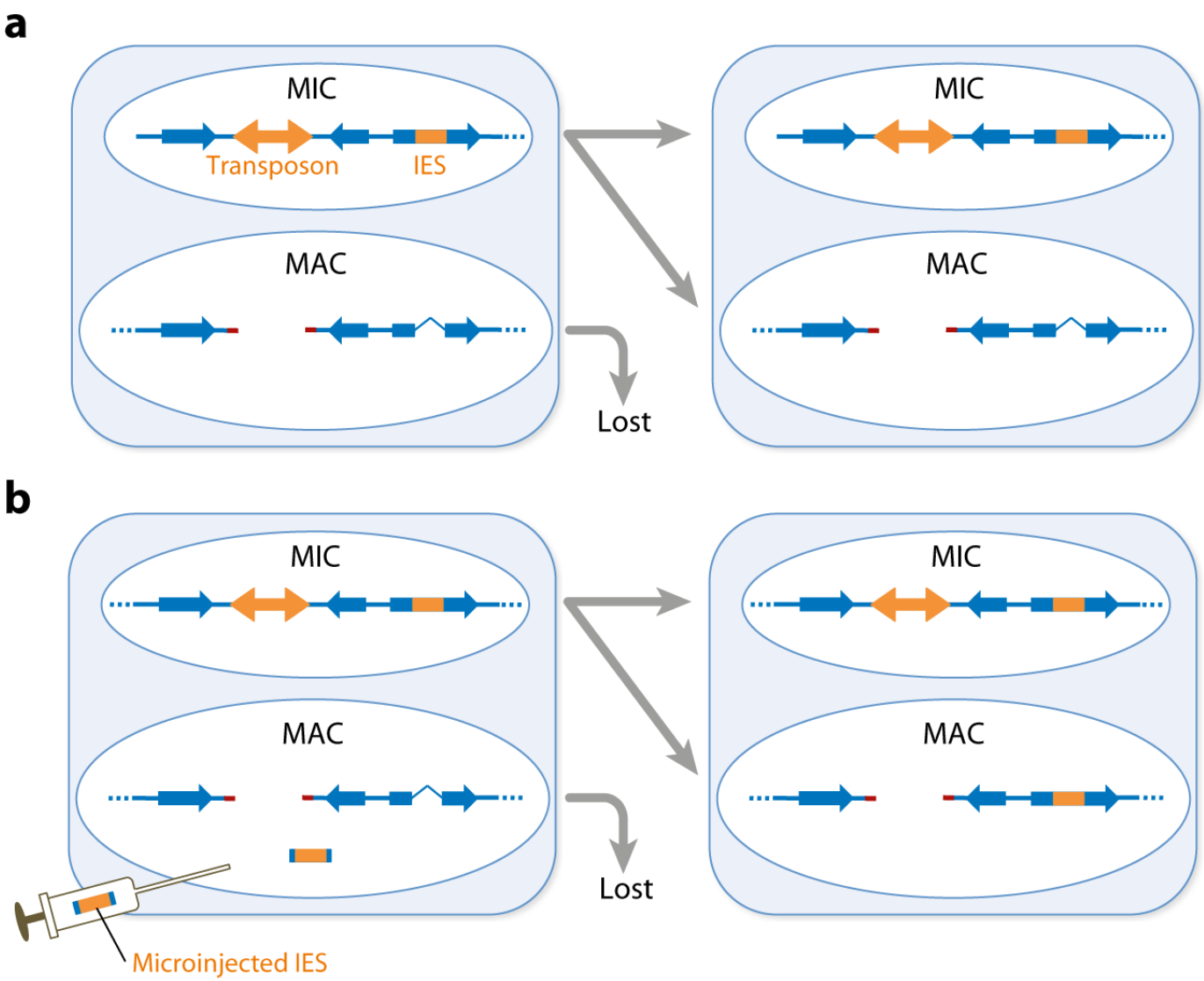
D. Precise excision of short Internal Eliminated Sequences (IES)



Epigenetic inhibition of DNA excision.



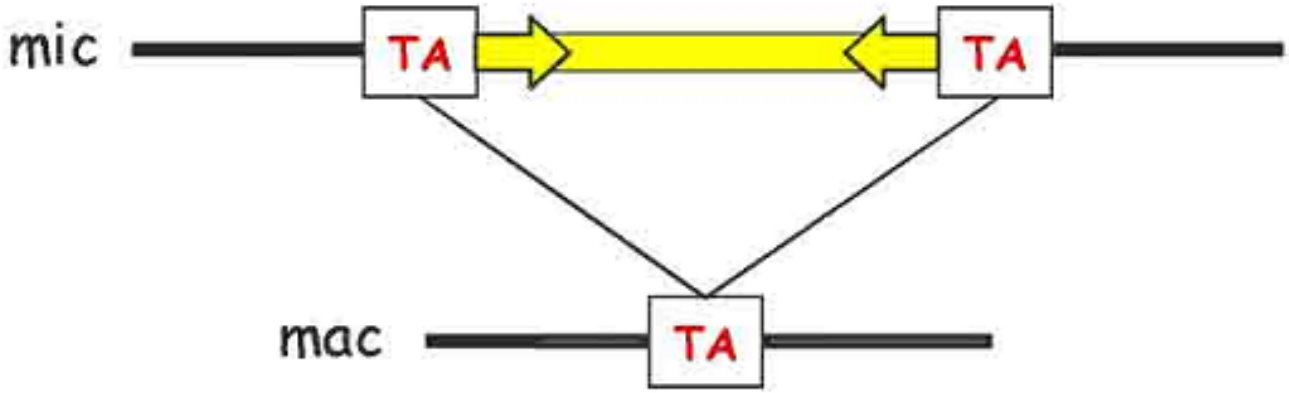
Epigenetic inhibition of DNA excision.



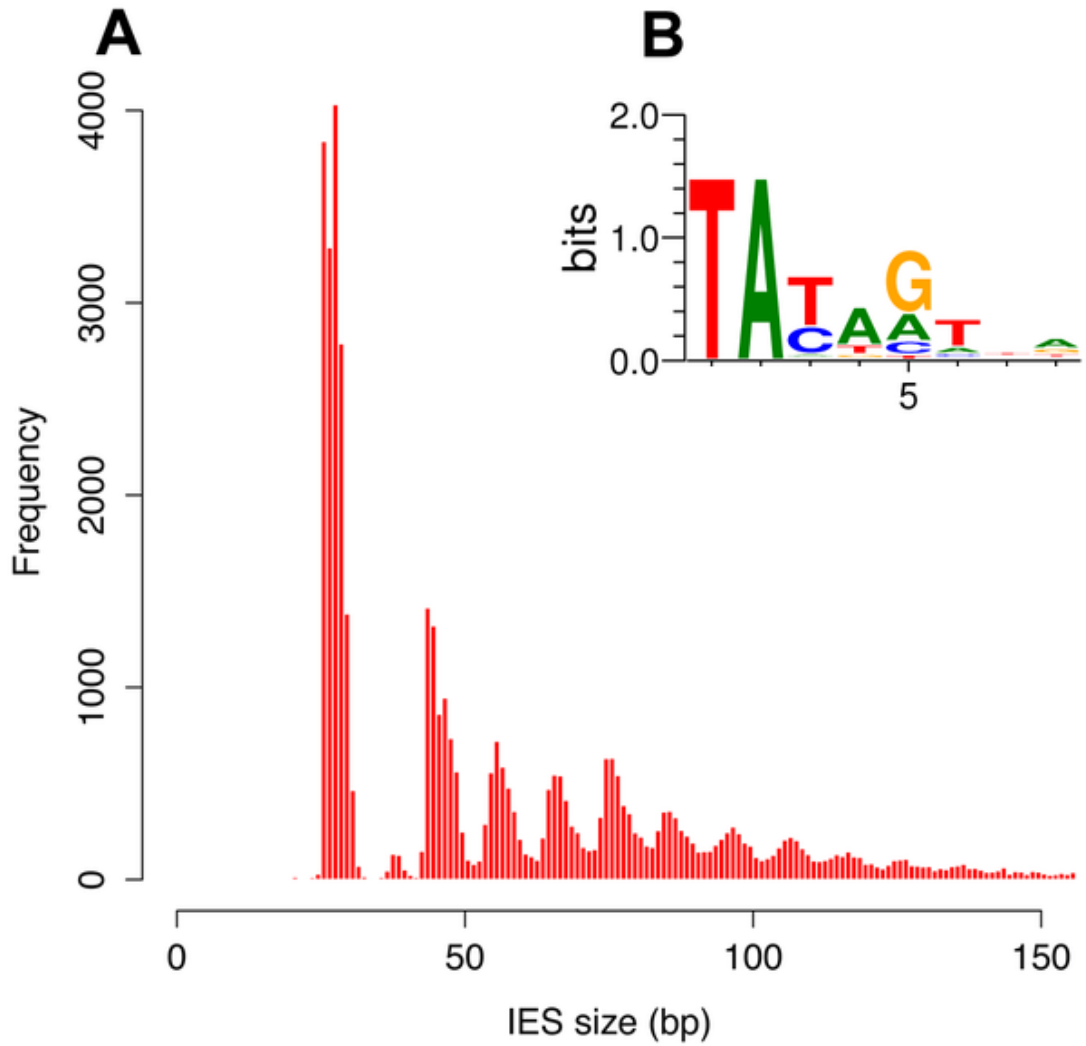
Nowacki M, et al. 2011.
Annu. Rev. Genomics Hum. Genet. 12:367–89

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

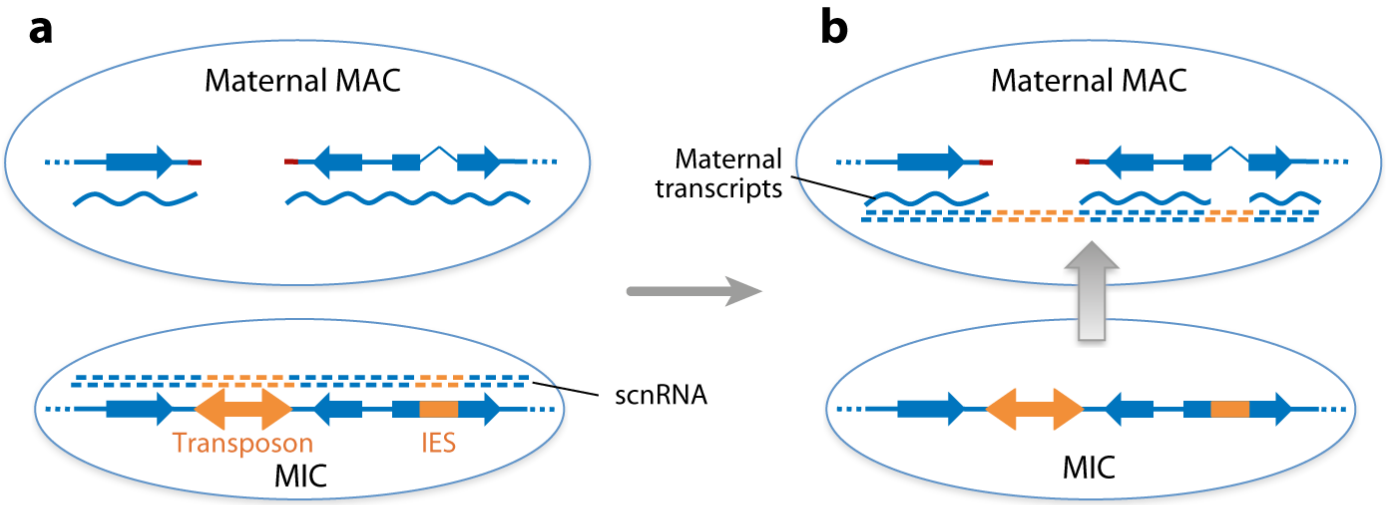


Paramecium Internal Eliminated Sequences (IES)



Arnaiz O, Mathy N, Baudry C, Malinsky S, Aury JM, et al. (2012) PLOS Genetics 8(10): e1002984.

The scan RNA model for programmed genome rearrangements.



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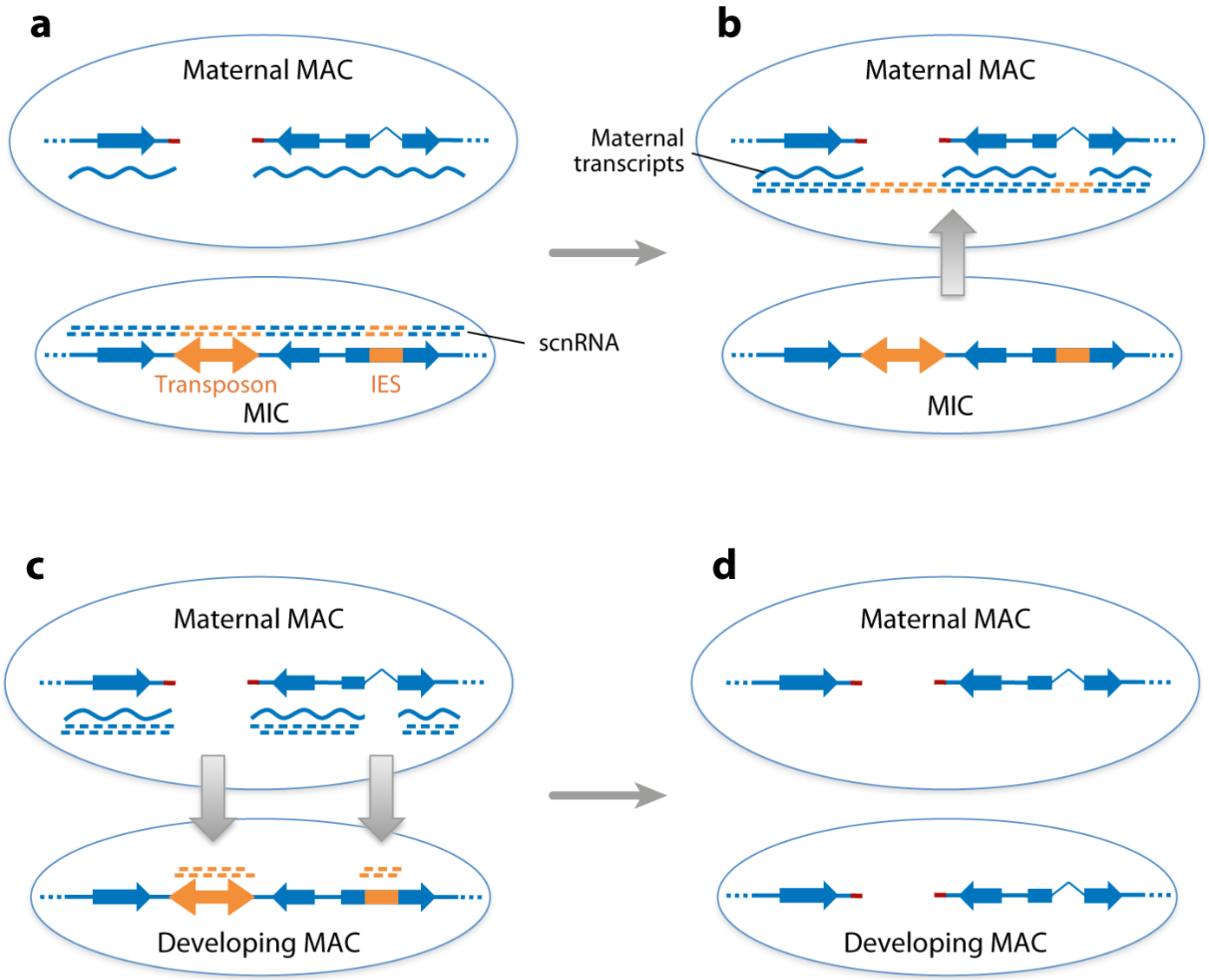
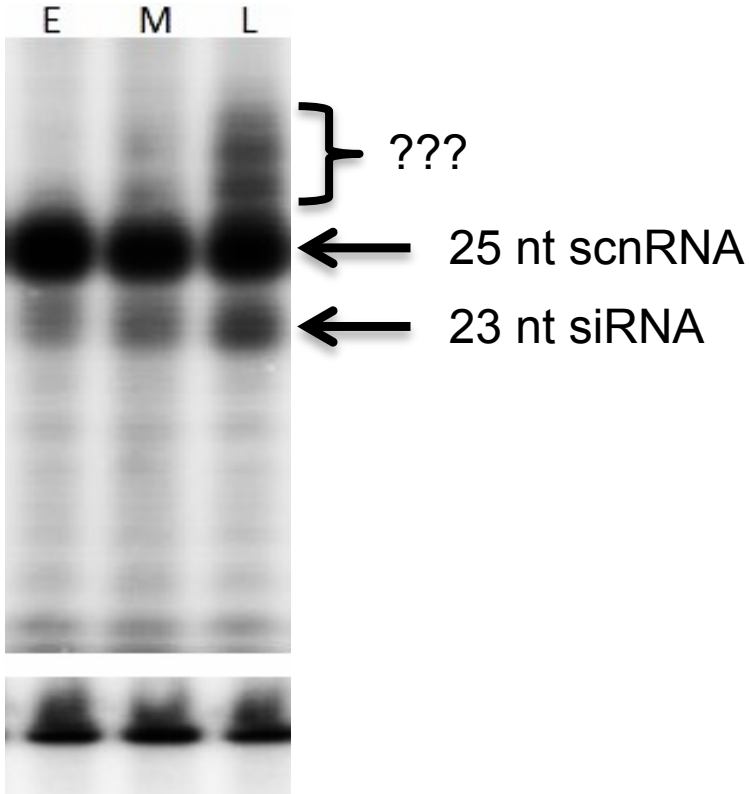
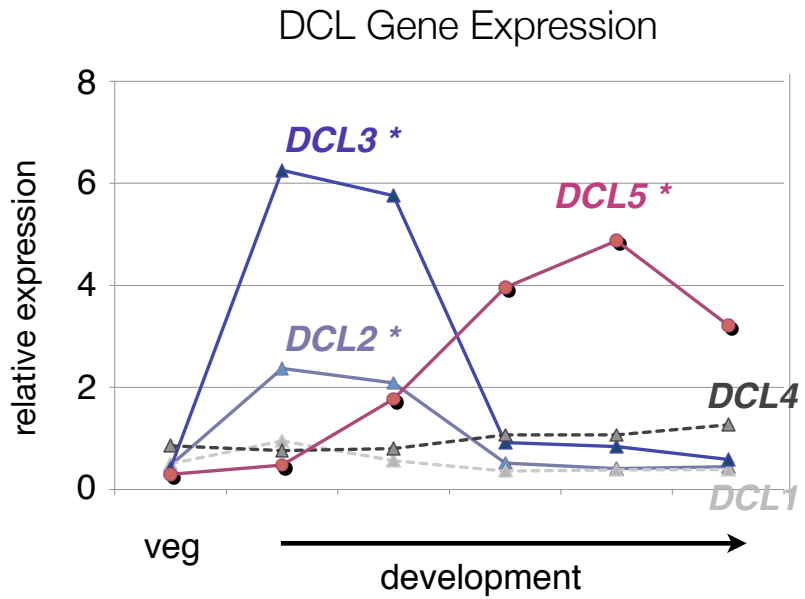
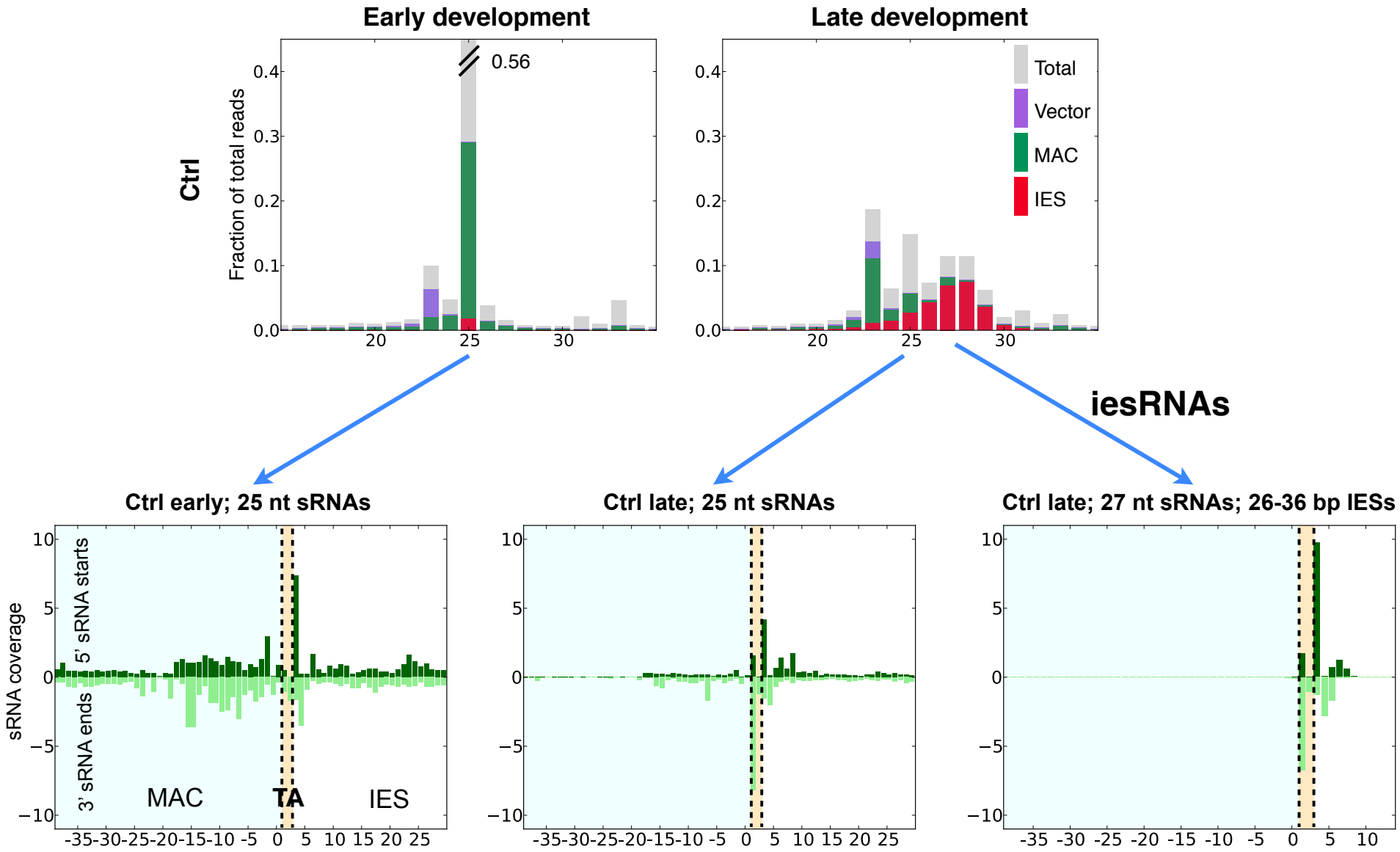


New class of small RNAs in *Paramecium*?





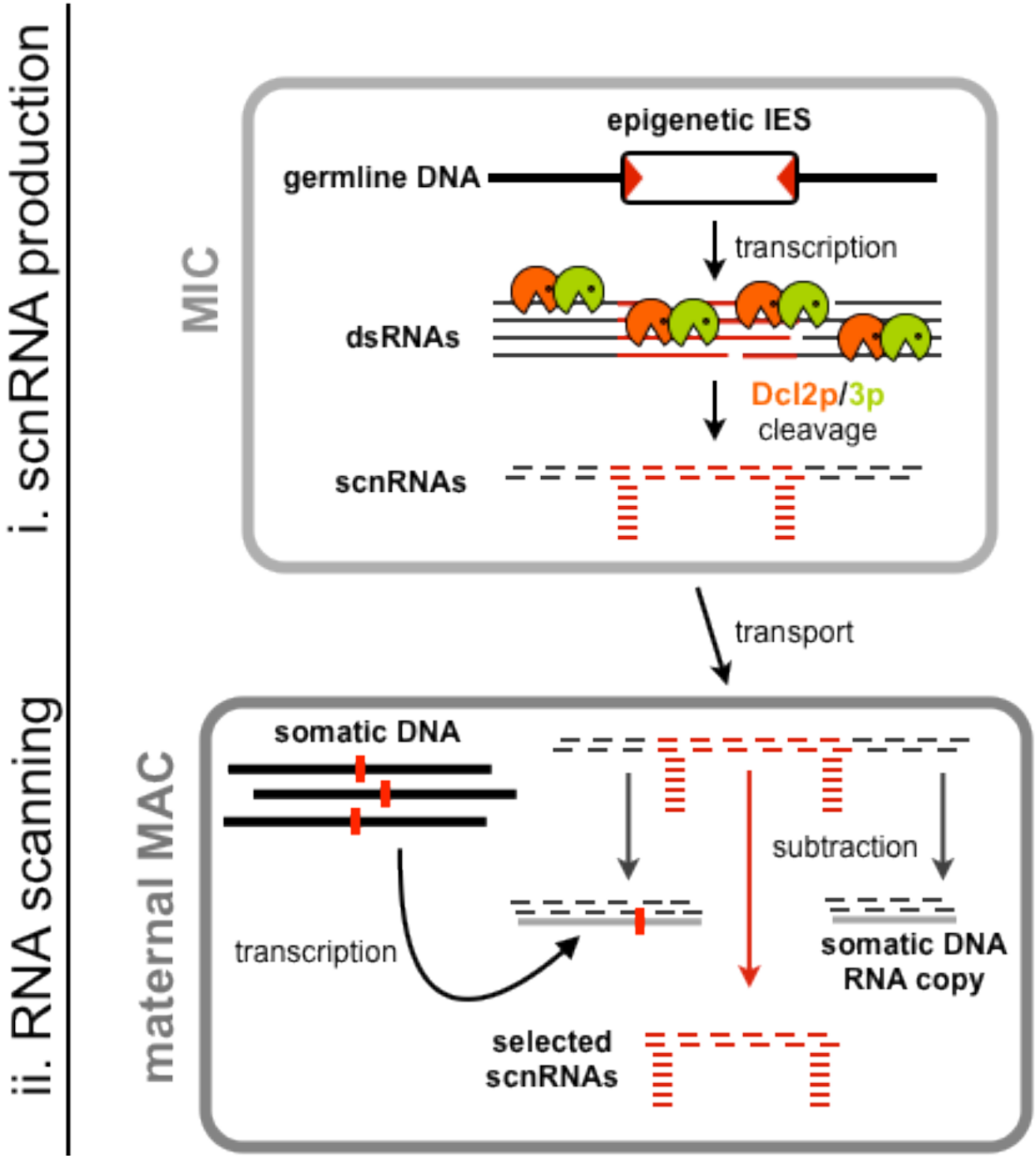
Sandoval, Swart et al. 2014, *Dev Cell*.

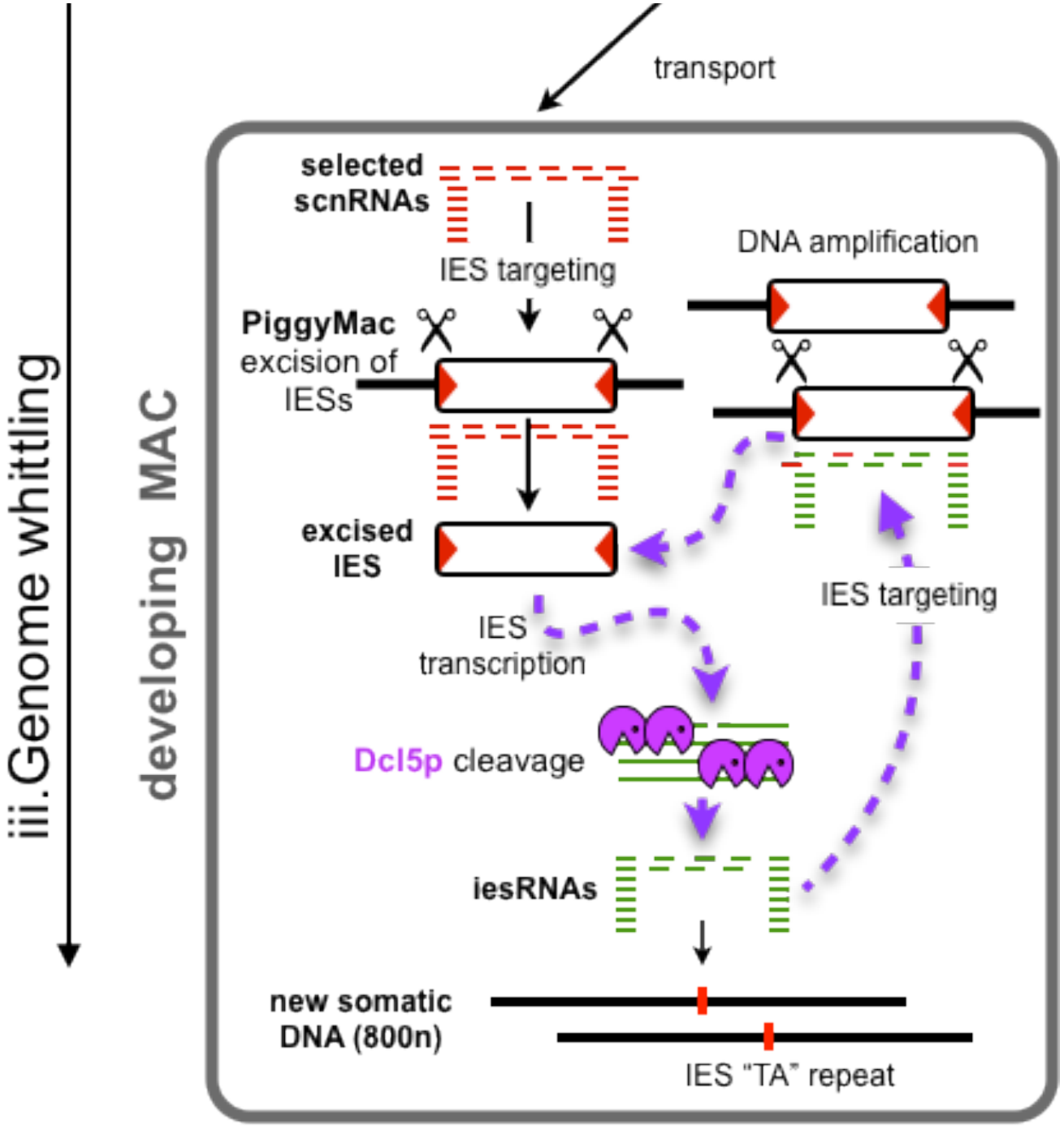


- scnRNAs overlap MAC/IES junctions and concentrate at IES ends

- <u>iesRNAs map exclusively to IESs</u> and concentrate at IES ends

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


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*.

How to transcribe ultrashort DNA segments?

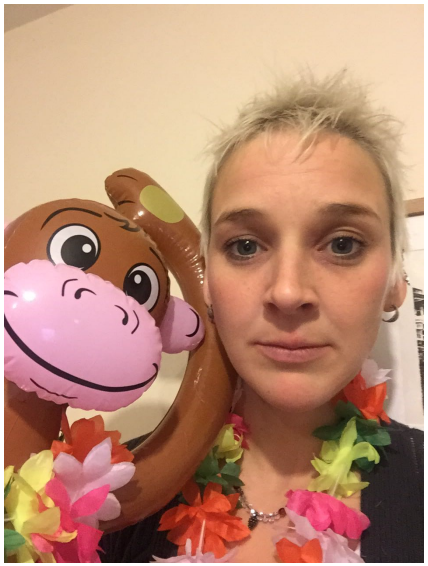
Cell

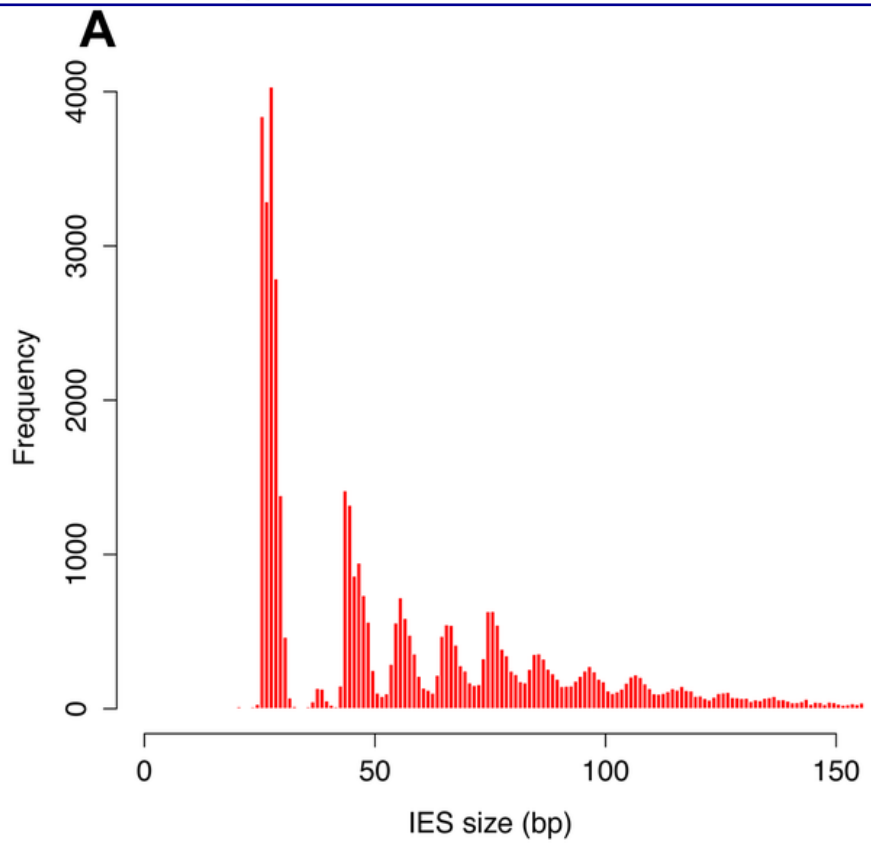
Article

Circular Concatemers of Ultra-Short DNA Segments Produce Regulatory RNAs

Sarah E. Allen,[1] Iris Hug,[1] Sylwia Pabian,[1] Iwona Rzeszutek,[1] Cristina Hoehener,[1] and Mariusz Nowacki[1,2,*]
[1]Institute of Cell Biology, University of Bern, Baltzerstrasse 4, 3012 Bern, Switzerland
[2]Lead Contact
*Correspondence: mariusz.nowacki@izb.unibe.ch
http://dx.doi.org/10.1016/j.cell.2017.02.020

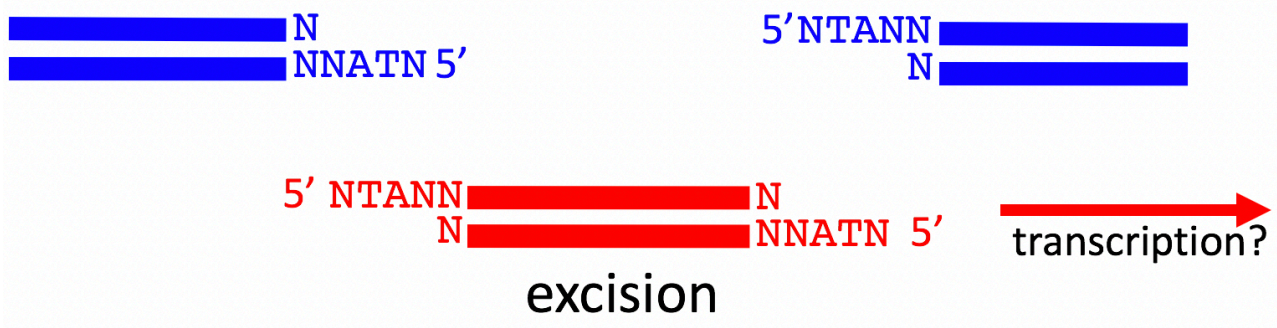
Sarah Allen





Allen et al. 2017, *Cell*.

Are iesRNAs formed from excised IESs?



Assembly of RNA polymerase complex on a 27 bp excised IES poses problems

Allen et al. 2017, *Cell*.

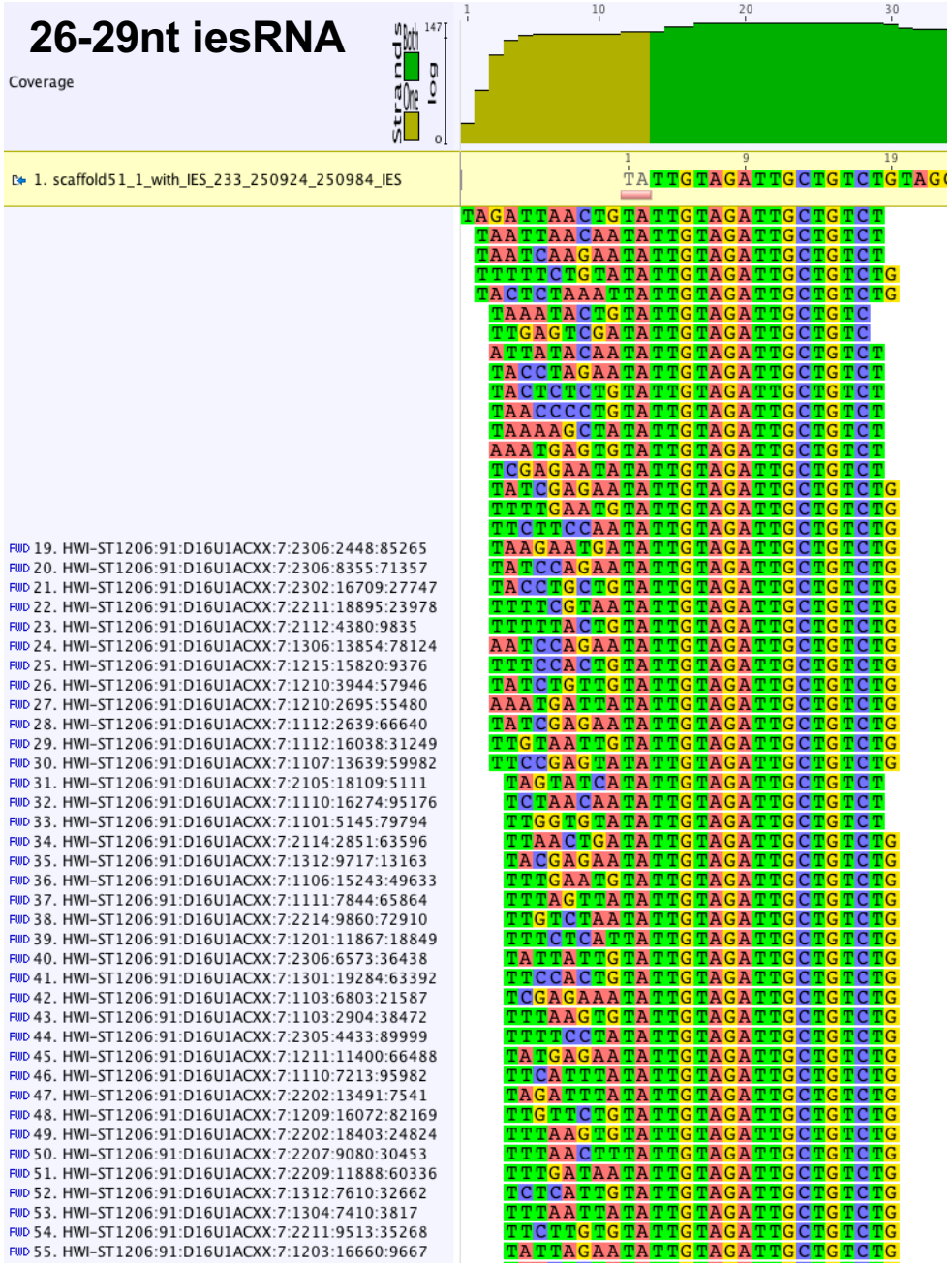
The concatemer model

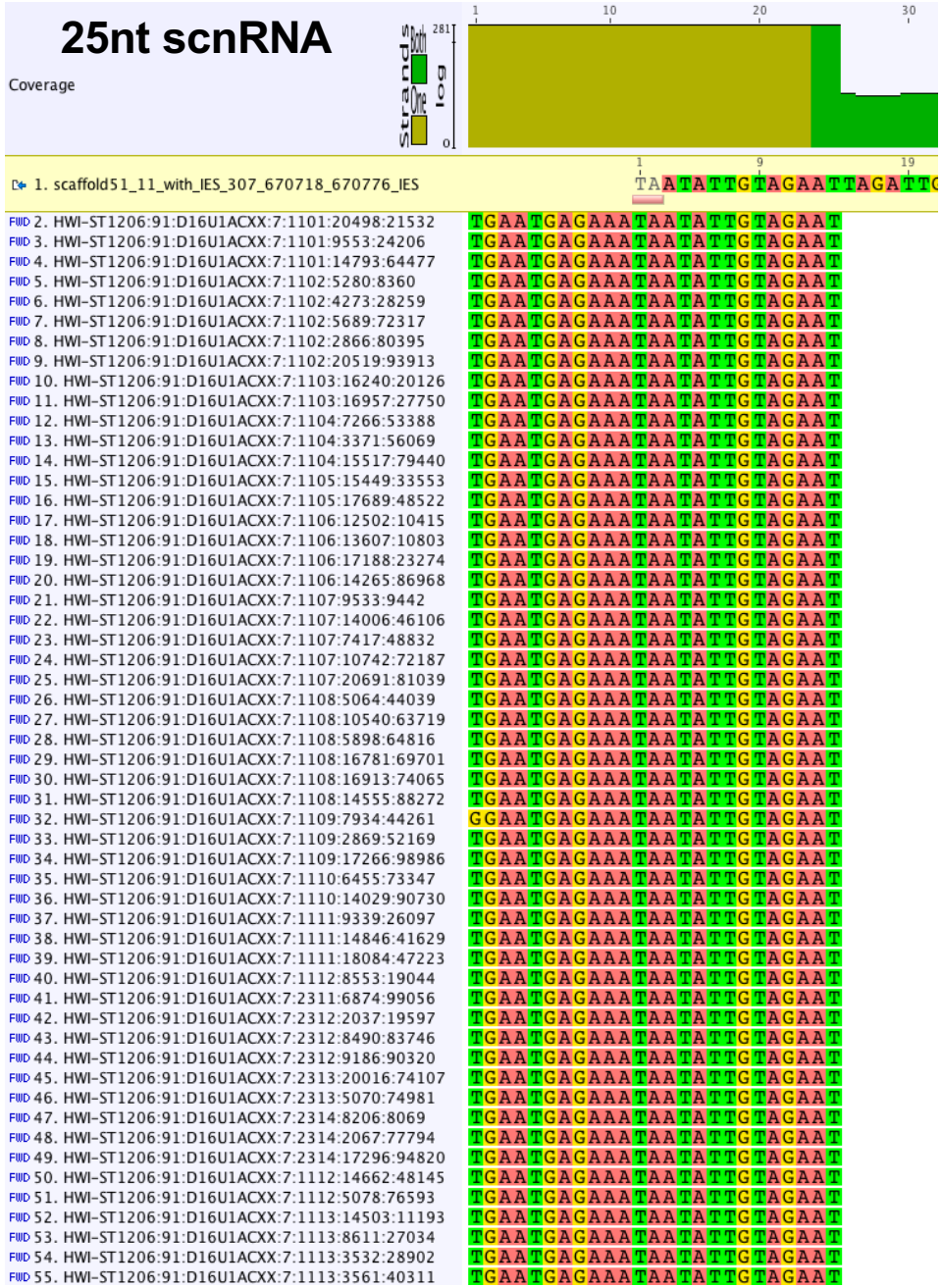
- Most IESs are too short to form circles individually

Long IES (>200 bp)

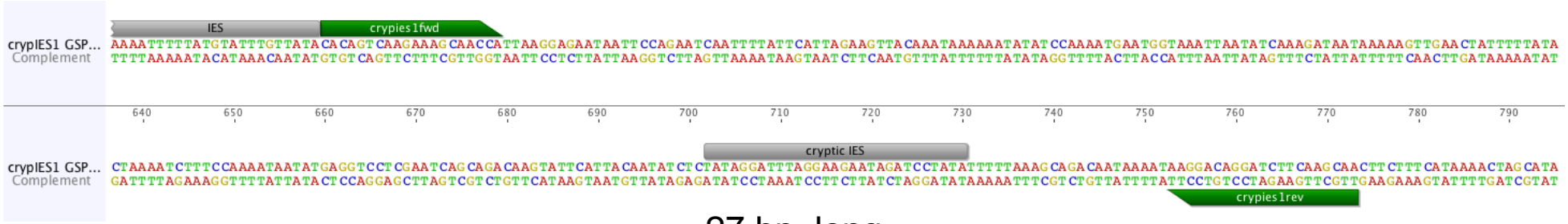
Allen et al. 2017, *Cell*.

Some iesRNAs map to IES-IES junctions





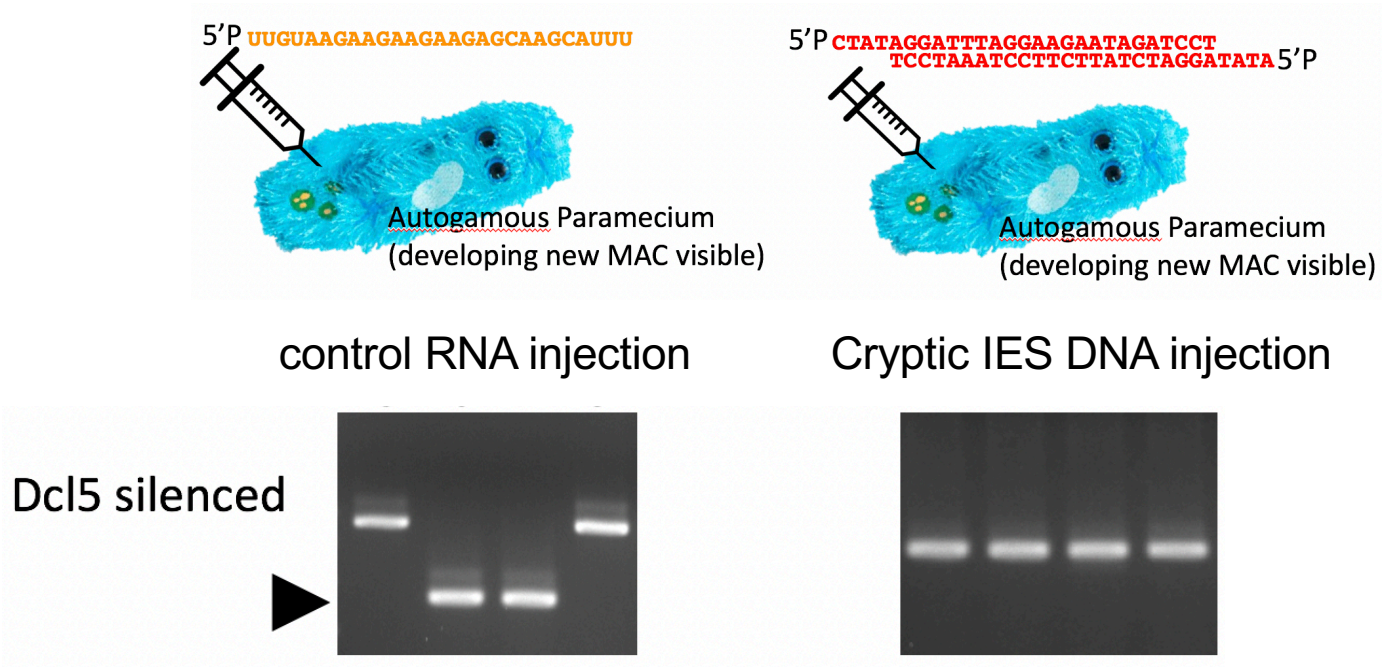
Experimental approach: ‘cryptic’ IES excision



- 27 bp long
- ‘consensus’ 5’TATAG
- Located in unknown genes

Allen et al. 2017, *Cell*.

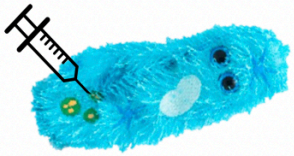
Is cryptic IES excision dependent on iesRNAs? Dcl5 silencing



Allen et al. 2017, *Cell*.

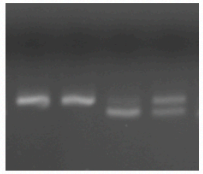
Dependence of iesRNA production on 5’ overhangs

Injection of cryptic IESs with 3’ overhangs does not induce excision

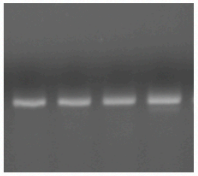


3’ overhangs

ctrl (cryp4)

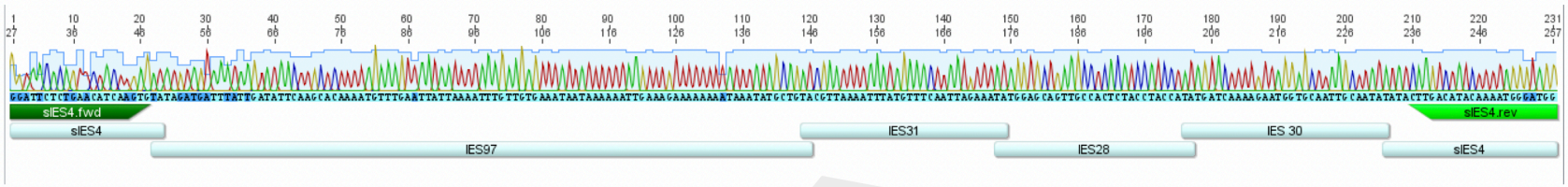


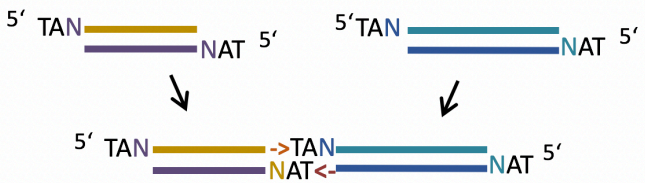
cryp2 3’ overhangs

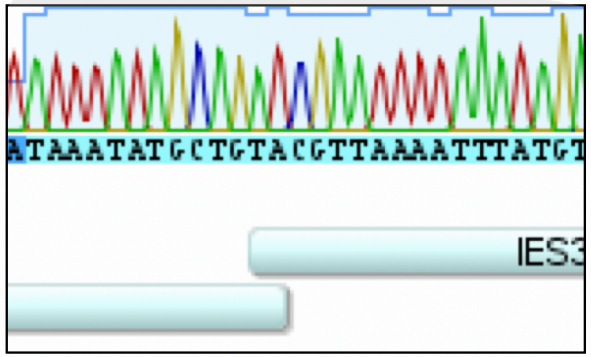


Allen et al. 2017, *Cell*.

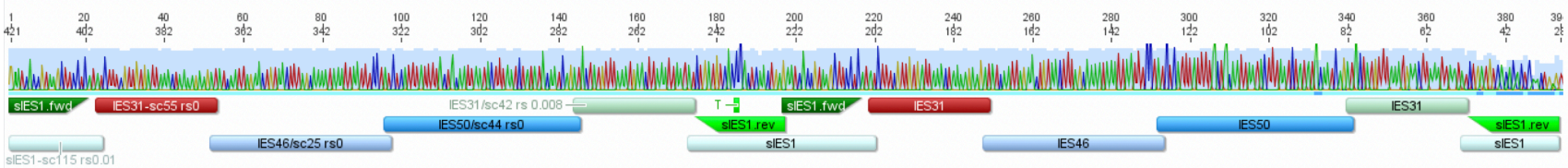
Sequencing identifies RNA corresponding to concatemers of IESs

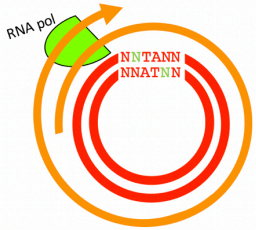






Concatemer repeat

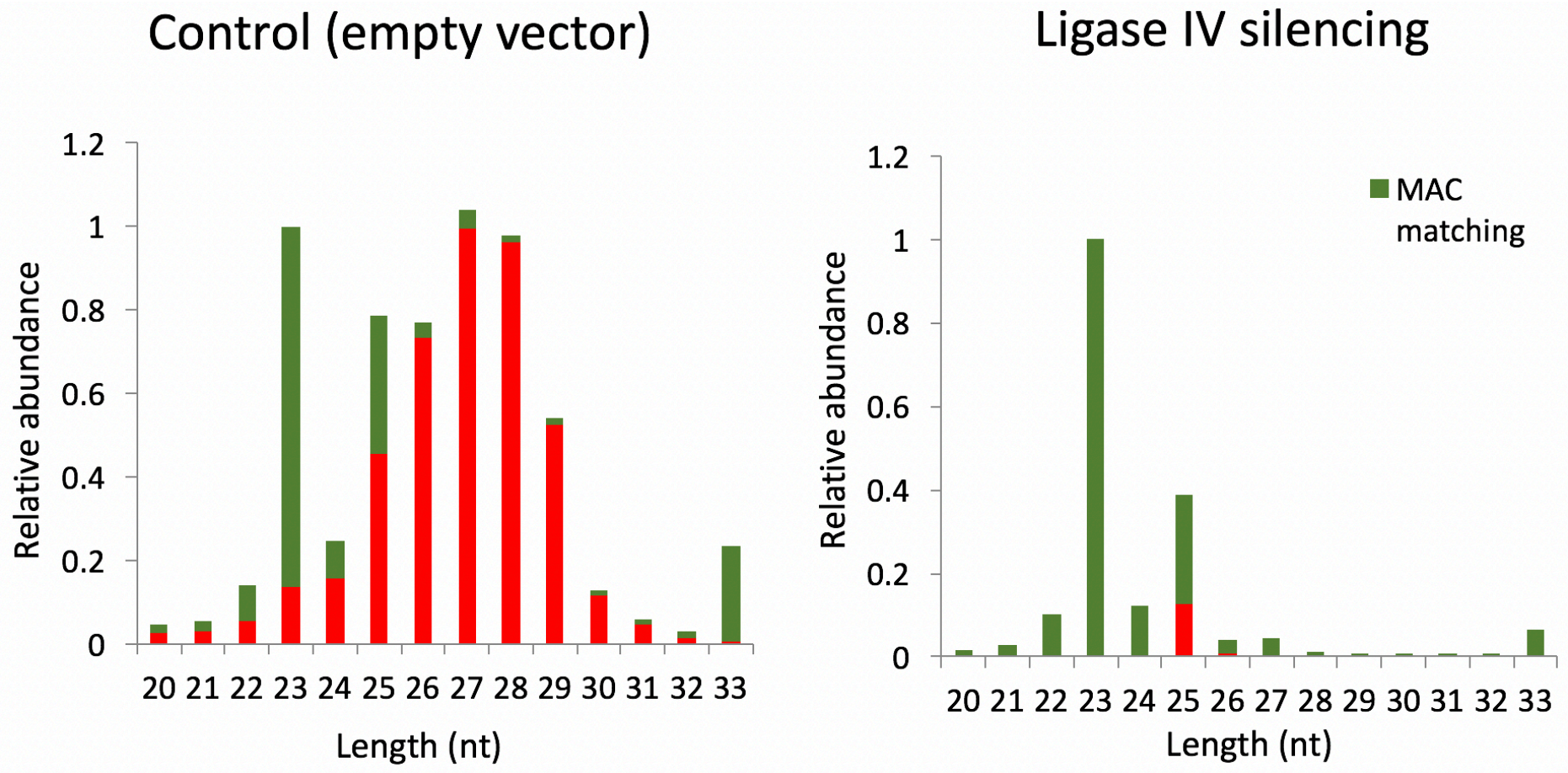




Allen et al. 2017, *Cell*.

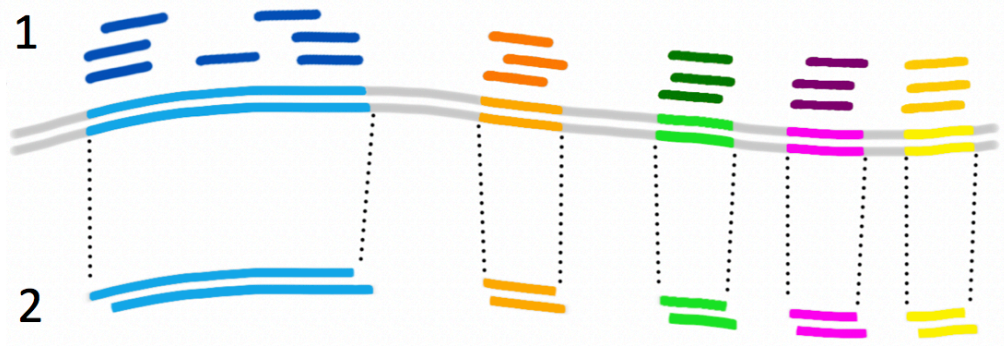
How are putative concatemers formed? Ligase IV?

Silencing of Ligase IV by RNAi feeding



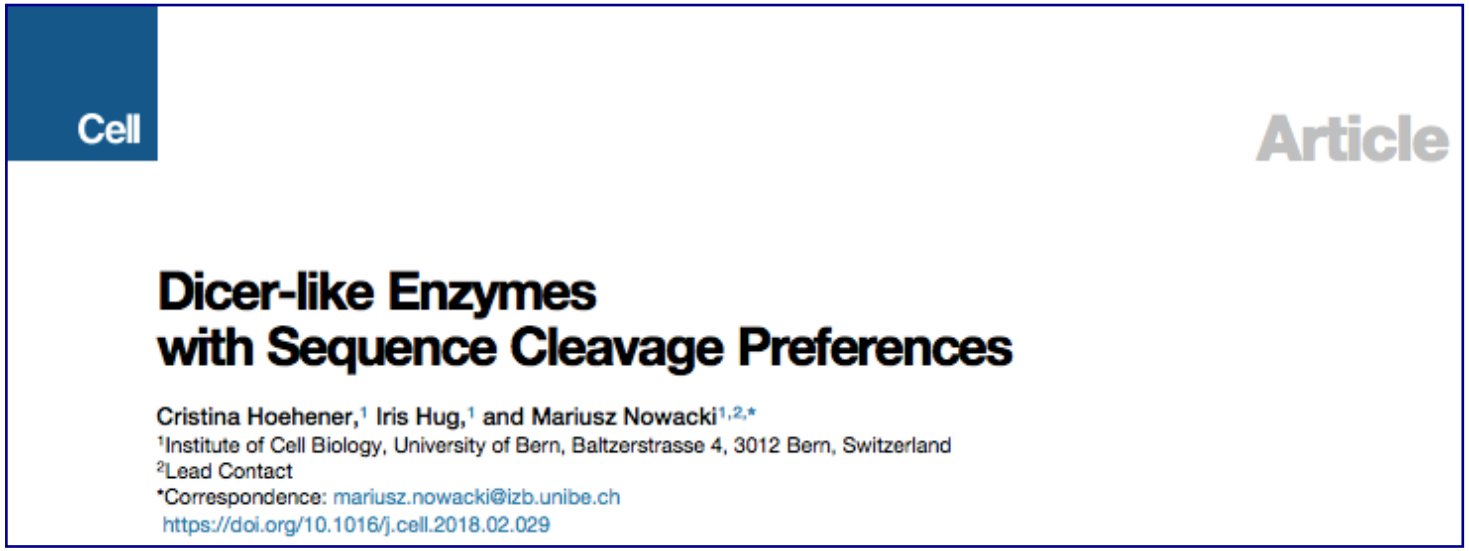
Allen et al. 2017, *Cell*.

A model for small RNA production from short DNA fragments in *Paramecium*



Allen et al. 2017, *Cell*.

Dicer-like enzymes with sequence cleavage preferences


Cell

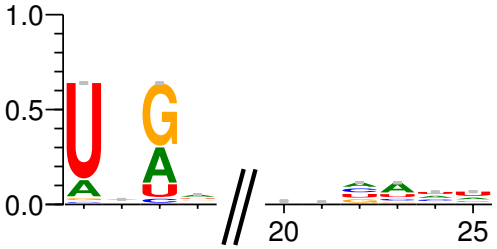
Article

Dicer-like Enzymes
with Sequence Cleavage Preferences

Cristina Hoehener,[1] Iris Hug,[1] and Mariusz Nowacki[1,2,*]
[1]Institute of Cell Biology, University of Bern, Baltzerstrasse 4, 3012 Bern, Switzerland
[2]Lead Contact
*Correspondence: mariusz.nowacki@izb.unibe.ch
https://doi.org/10.1016/j.cell.2018.02.029

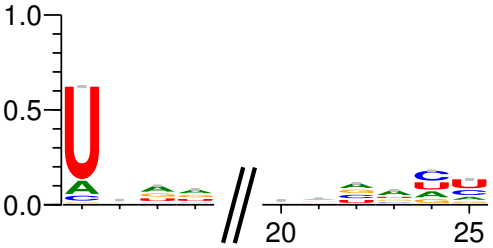
Tina Höhener

Dcl3



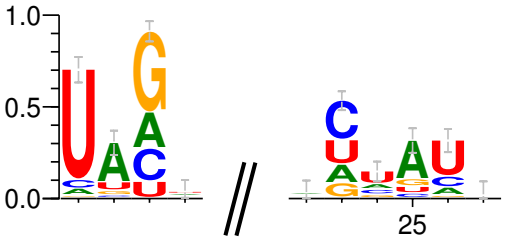
size 22-32 nt

Dcl2

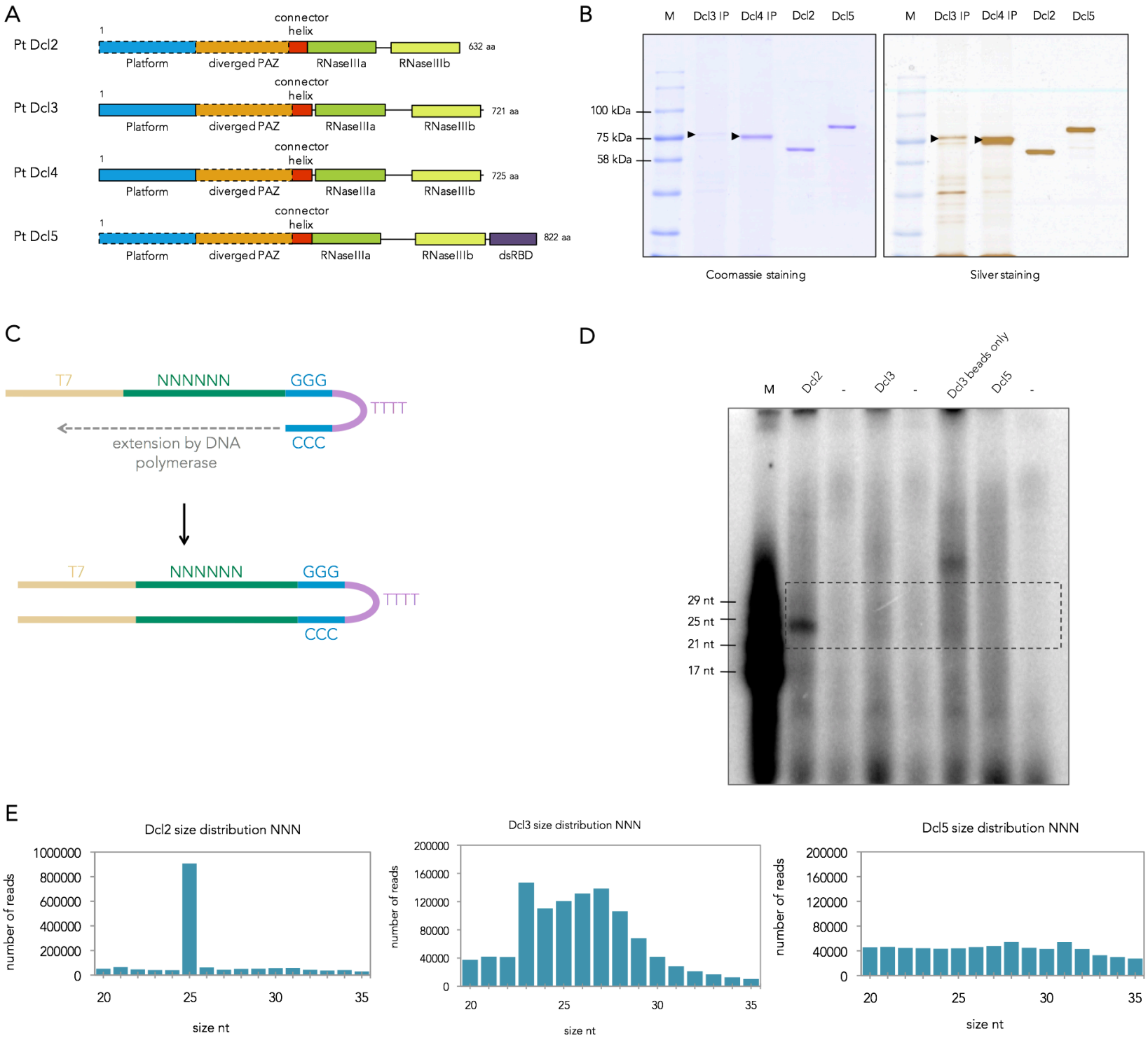


size 25 nt

Dcl5



size 22-31 nt

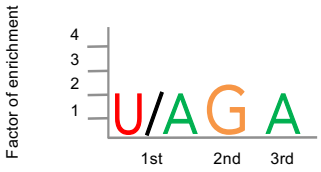

A
Pt Dcl2
Pt Dcl3
Pt Dcl4
Pt Dcl5
connector helix
Platform
diverged PAZ
RNaseIIIa
RNaseIIIb
dsRBD
632 aa
721 aa
725 aa
822 aa
B
M
Dcl3 IP
Dcl4 IP
Dcl2
Dcl5
100 kDa
75 kDa
58 kDa
Coomassie staining
Silver staining
C
T7
NNNNNN
GGG
TTTT
CCC
extension by DNA polymerase
D
M
Dcl2
Dcl3
Dcl3 beads only
Dcl5
29 nt
25 nt
21 nt
17 nt
E
Dcl2 size distribution NNN
Dcl3 size distribution NNN
Dcl5 size distribution NNN
number of reads
size nt

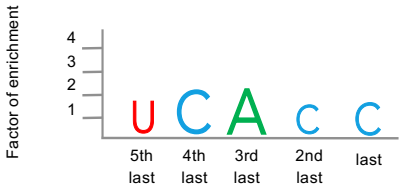
Dicer-like enzymes with sequence cleavage preferences

5' end

3' end

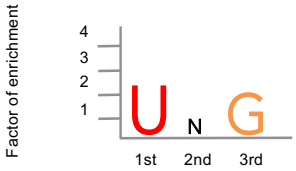
Dcl2





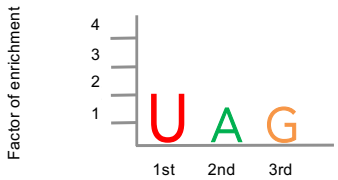
5′-UNNNNNNNNNNNNNNNNNNNUCUNN -3′
3′-NNANNNNNNNNNNNNNNNNNAGA- 5′

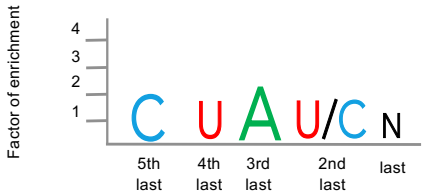
Dcl3



5′-UNGNNNNNNNNNNNNNNNNNCNANN -3′
3′-NNANCNNNNNNNNNNNNNNNNGNU- 5′

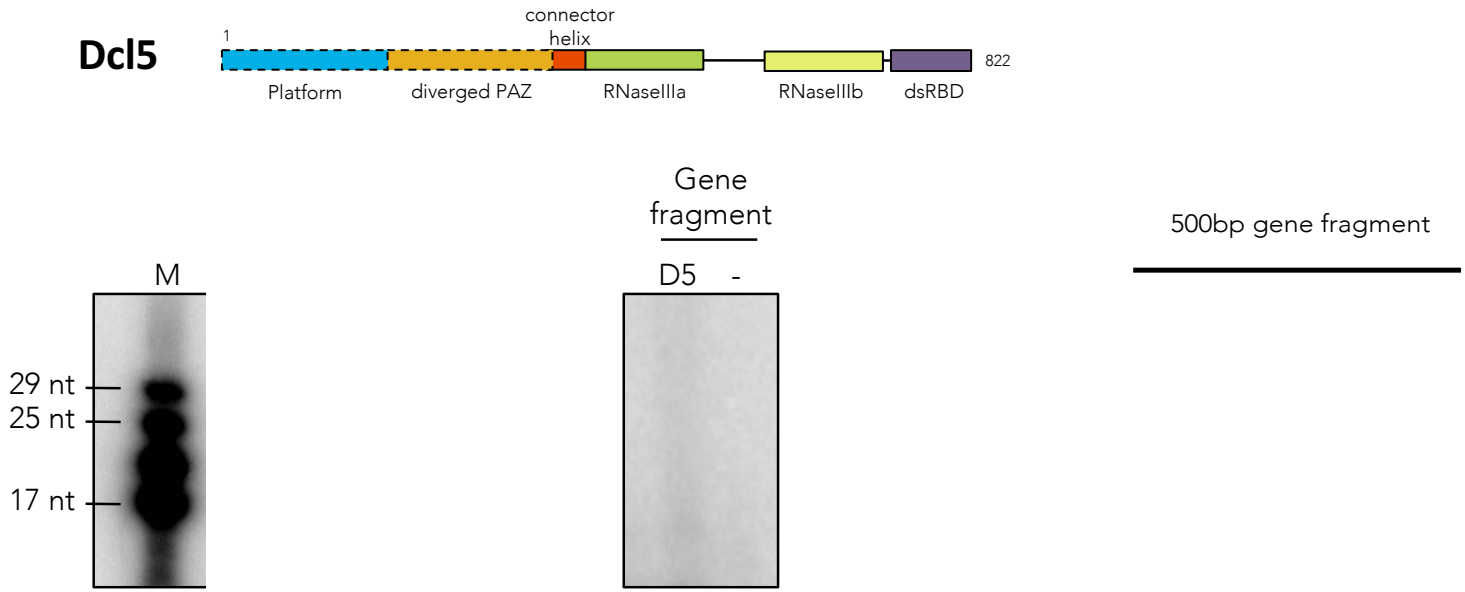
Dcl5



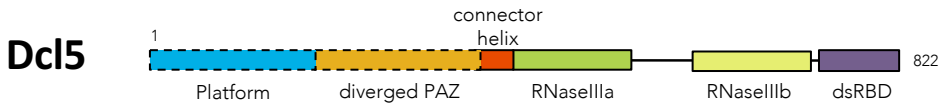


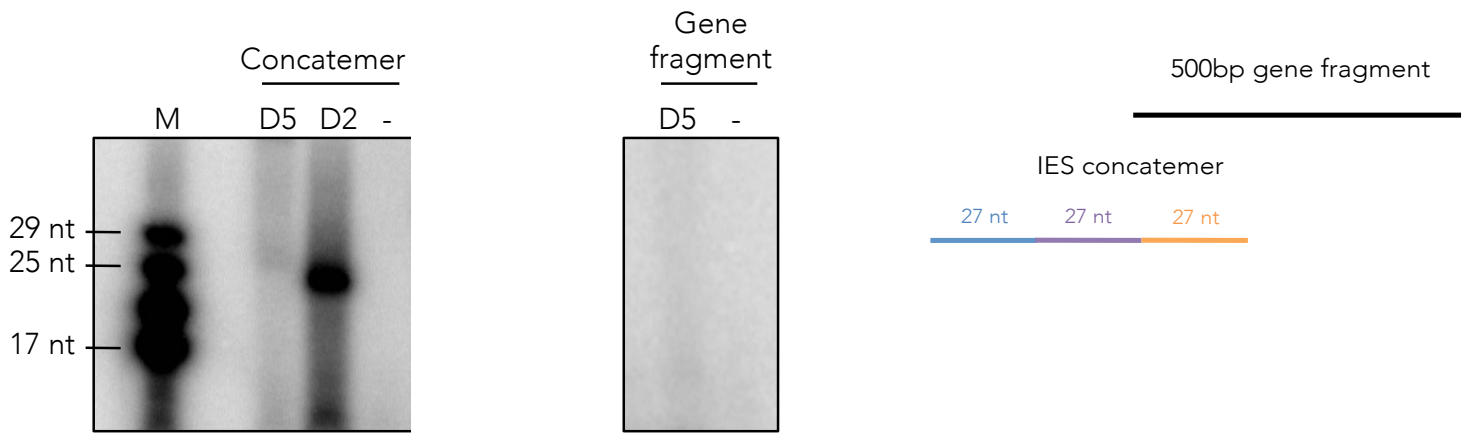
5′-UAGNNNNNNNNNNNNNNNNNCUAUN -3′
3′-NUAUCNNNNNNNNNNNNNNNNNGAU- 5′

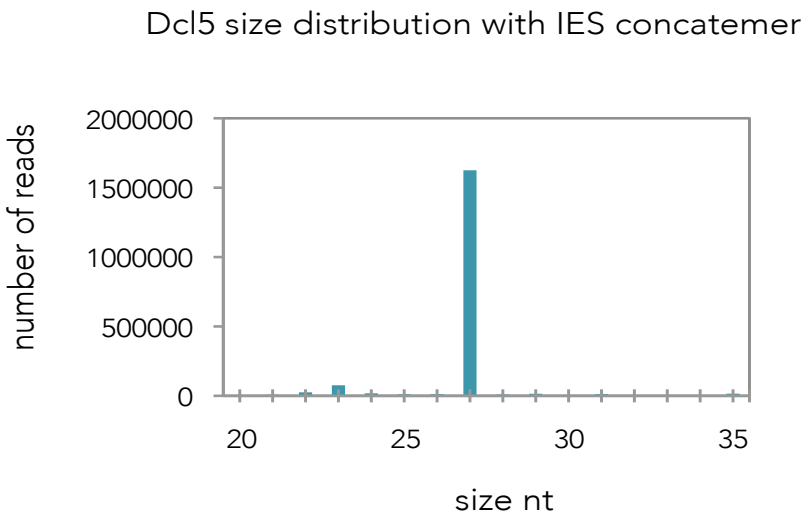
Hoehener et al. 2018, *Cell*.



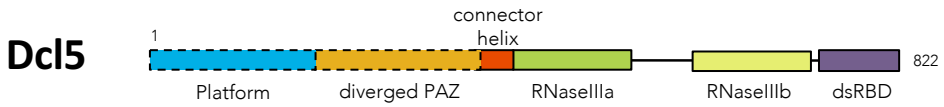
Hoehener et al. 2018, *Cell*.

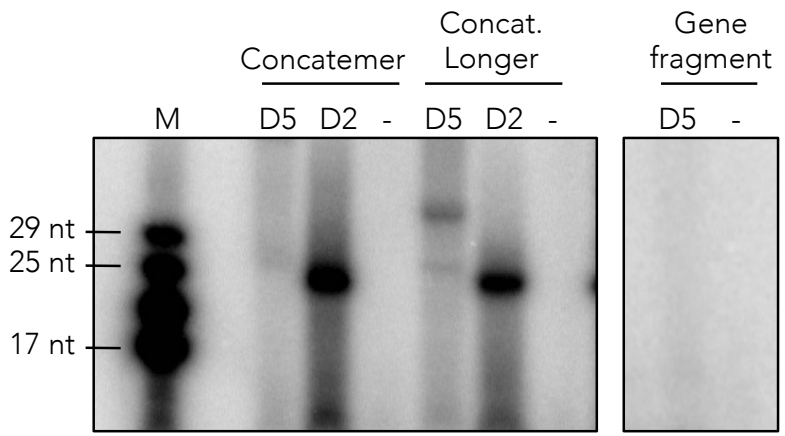


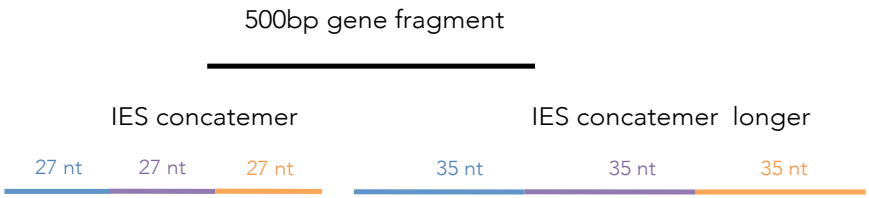


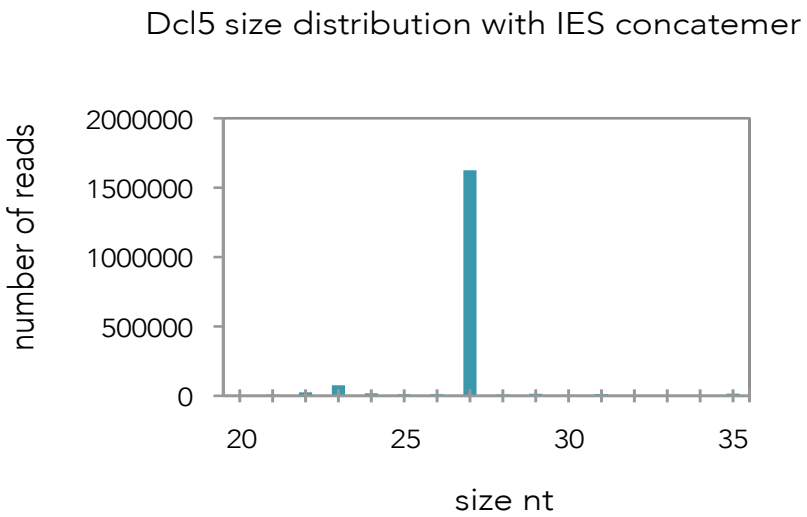


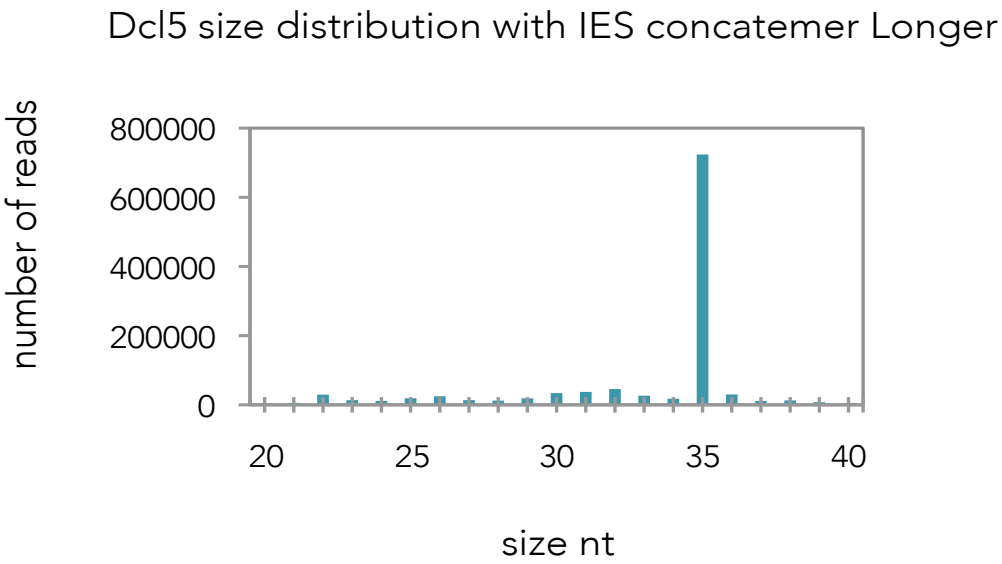
Hoehener et al. 2018, *Cell*.





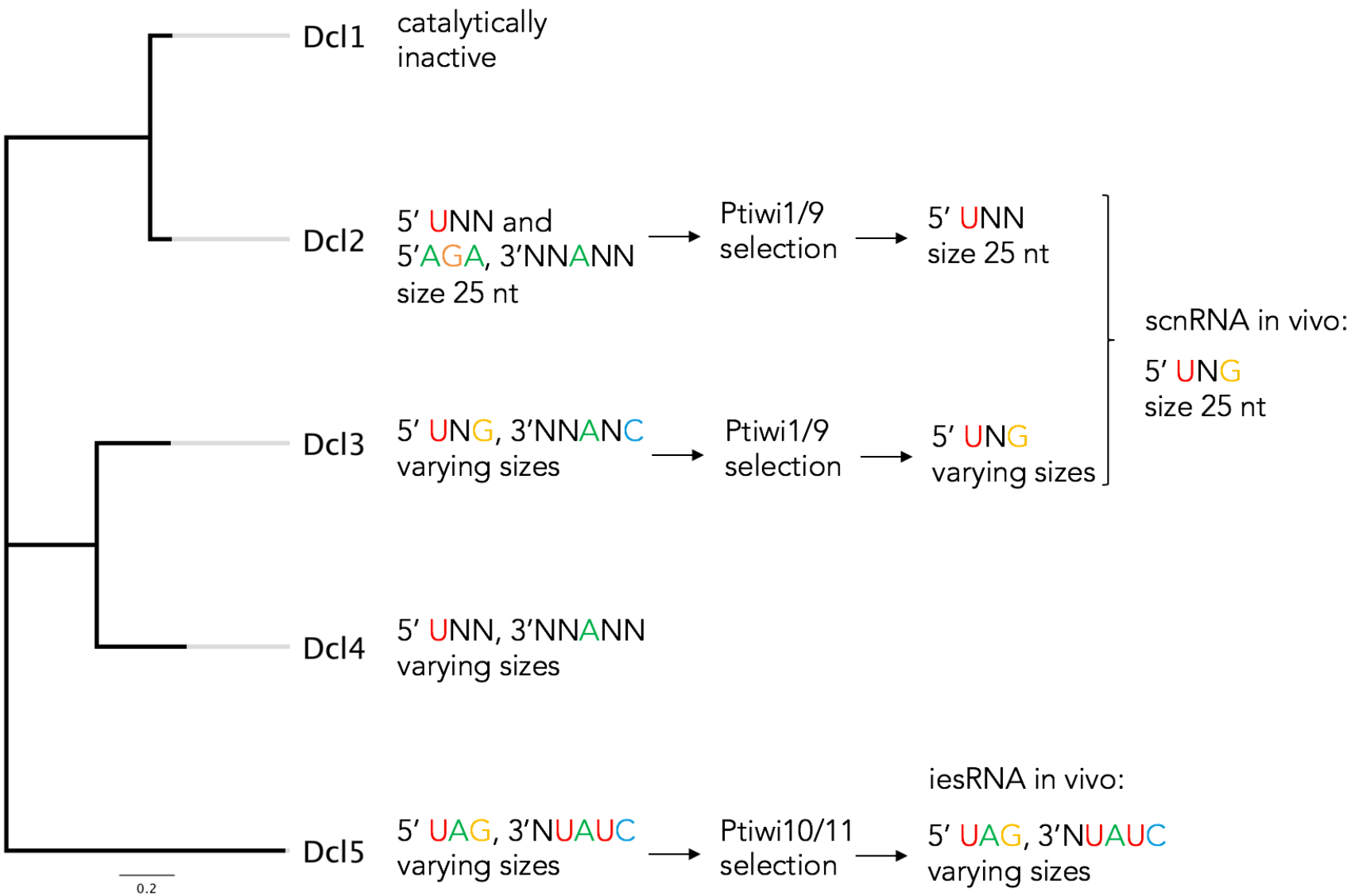


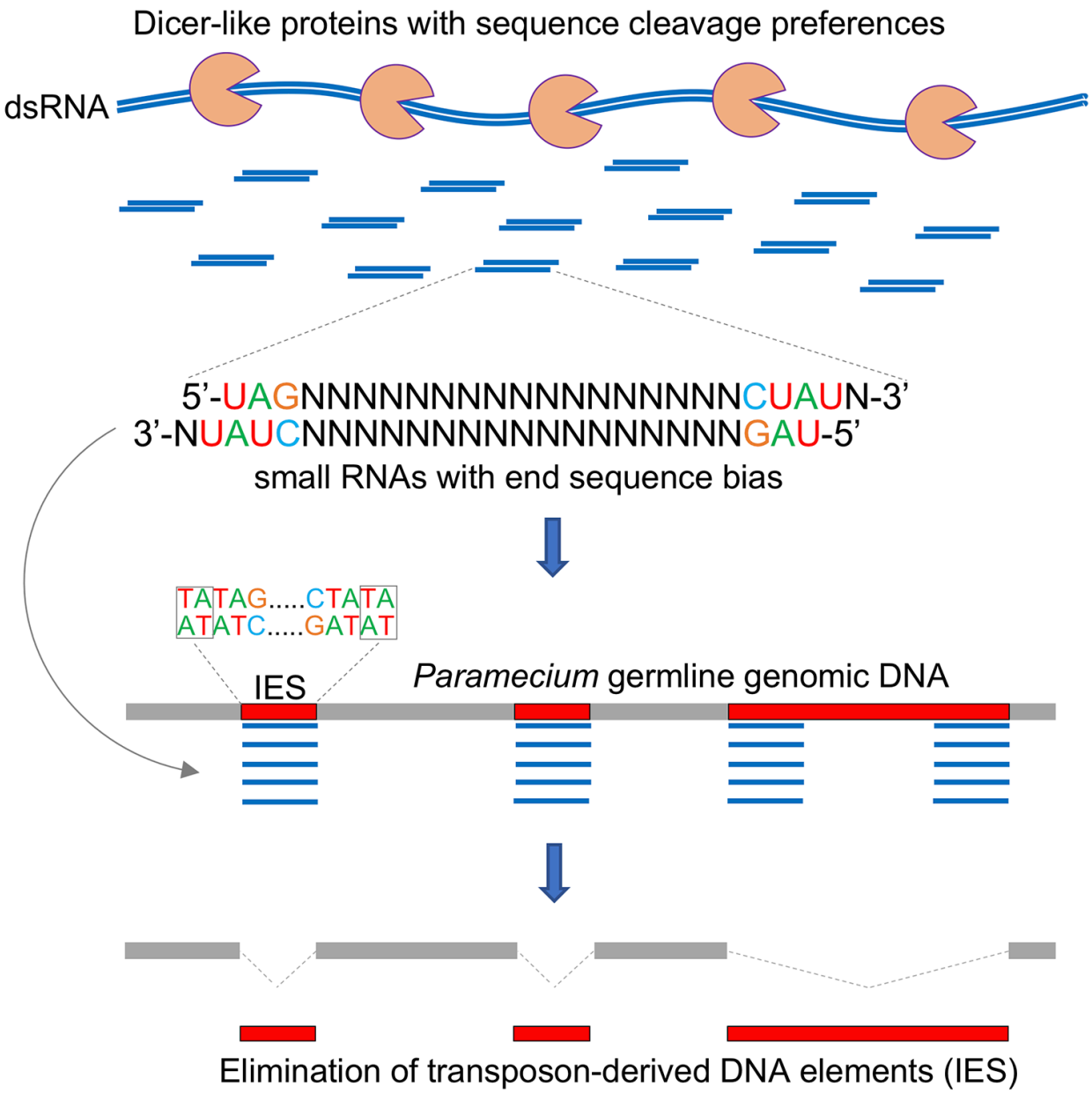




Hoehener et al. 2018, *Cell*.

Summary of Dcl cleavage preferences





Hoehener et al. 2018, *Cell*.

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