RNA-mediated trans-generational inheritance in ciliates

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Nuclear dimorphism in ciliates



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.



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.



The scan RNA model for programmed genome rearrangements.



Developing MAC

Developing MAC

New class of small RNAs in *Paramecium*?









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





Sandoval, Swart et al. 2014, Dev Cell.

How to transcribe ultrashort DNA segments?

Cell

Circular Concatemers of Ultra-Short DNA Segments Produce Regulatory RNAs

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Article

Sarah Allen



Allen et al. 2017, Cell.

Are iesRNAs formed from excised IESs?



The concatemer model

• Most IESs are too short to form circles individually



Some iesRNAs map to IES-IES junctions

26-29nt iesRNA		25nt scnRNA	
D* 1. scaffold51_1_with_IES_233_250924_250984_IES	TATTGTAGATTGCTGTCTGTAG	C+ 1. scaffold51_11_with_IES_307_670718_670776_IES	
	TAGATTAACTGTATTGTAGATTGCTGTCT TAATTAACAATATTGTAGATTGCTGTCT TAATTAACAATATTGTAGATTGCTGTCT TTTTTCTGTATATTGTAGATTGCTGTCTG TACTCTAAATTATTGTAGATTGCTGTCTG TAGATACTGTATTGTAGATTGCTGTC TTGAGTCGATATTGTAGATTGCTGTC TACCCTAGAATATTGTAGATTGCTGTCT TACCTCTCTGTATTGTAGATTGCTGTCT TAACCCCTGTATTGTAGATTGCTGTCT TAACCCCTGTATTGTAGATTGCTGTCT TAAAGCTATATTGTAGATTGCTGTCT	FWD 2. HWI-ST1206:91:D16U1ACXX:7:1101:20498:21532 FWD 3. HWI-ST1206:91:D16U1ACXX:7:1101:9553:24206 FWD 4. HWI-ST1206:91:D16U1ACXX:7:1101:14793:64477 FWD 5. HWI-ST1206:91:D16U1ACXX:7:1102:5280:8360 FWD 6. HWI-ST1206:91:D16U1ACXX:7:1102:5280:8360 FWD 7. HWI-ST1206:91:D16U1ACXX:7:1102:5689:72317 FWD 8. HWI-ST1206:91:D16U1ACXX:7:1102:26589:72317 FWD 9. HWI-ST1206:91:D16U1ACXX:7:1102:20519:93913 FWD 9. HWI-ST1206:91:D16U1ACXX:7:1103:16240:20126 FWD 9. HWI-ST1206:91:D16U1ACXX:7:1103:16240:20126 FWD 1. HWI-ST1206:91:D16U1ACXX:7:1103:16240:20126 FWD 11. HWI-ST1206:91:D16U1ACXX:7:1104:7266:53388 FWD 13. HWI-ST1206:91:D16U1ACXX:7:1104:7266:53388 FWD 13. HWI-ST1206:91:D16U1ACXX:7:1104:7266:5338	TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT
FWD 19. HWI-ST1206:91:D16U1ACXX:7:2306:2448:85265 FWD 20. HWI-ST1206:91:D16U1ACXX:7:2306:8355:71357 FWD 21. HWI-ST1206:91:D16U1ACXX:7:2302:16709:27747 FWD 22. HWI-ST1206:91:D16U1ACXX:7:211:18895:23978 FWD 23. HWI-ST1206:91:D16U1ACXX:7:1306:13854:78124 FWD 24. HWI-ST1206:91:D16U1ACXX:7:1306:13854:78124	TCGAGAATATATTG TAGATTG CTG TCT TATCGAGAATATATTG TAGATTG CTG TCTG TTTTGAATG TATTG TAGATTG CTG TCTG TTCT TCCAATATTG TAGATTG CTG TCTG TAAGAATGATATTG TAGATTG CTG TCTG TACCAGAATATTG TAGATTG CTG TCTG TATCCAGAATATTG TAGATTG CTG TCTG TTTCG TAATATTG TAGATTG CTG TCTG TTTTACTG TATTTTAGTAGATTG CTG TCTG AATCCAGAATATTG TAGATTG CTG TCTG TTTCCAGAATATTG TAGATTG CTG TCTG	FWD 14. HWI-S11206:91:D16U1ACXX:7:1104:15317/79440 FWD 15. HWI-S1206:91:D16U1ACXX:7:1105:15449:33553 FWD 16. HWI-ST1206:91:D16U1ACXX:7:1105:15449:33553 FWD 17. HWI-ST1206:91:D16U1ACXX:7:1106:12502:10415 FWD 18. HWI-ST1206:91:D16U1ACXX:7:1106:12502:10415 FWD 19. HWI-ST1206:91:D16U1ACXX:7:1106:12823274 FWD 20. HWI-ST1206:91:D16U1ACXX:7:1106:14265:86968 FWD 21. HWI-ST1206:91:D16U1ACXX:7:1106:14265:86968 FWD 22. HWI-ST1206:91:D16U1ACXX:7:1107:14006:46106 FWD 22. HWI-ST1206:91:D16U1ACXX:7:1107:1407:8832 FWD 24. HWI-ST1206:91:D16U1ACXX:7:1107:1407:417:48832	TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT
FWD 25. HWH-S11206.91:D16U1ACXX:71215.15220.9376 FWD 26. HWH-S11206.91:D16U1ACXX:71210:3944:57946 FWD 27. HWH-ST1206:91:D16U1ACXX:71210:2695:55480 FWD 28. HWH-ST1206:91:D16U1ACXX:71112:2639:66640 FWD 29. HWH-ST1206:91:D16U1ACXX:71112:16038:31249 FWD 30. HWH-ST1206:91:D16U1ACXX:71107:13639:59982 FWD 31. HWH-ST1206:91:D16U1ACXX:71107:13639:59111 FWD 32. HWH-ST1206:91:D16U1ACXX:71101:5145:79794 FWD 33. HWH-ST1206:91:D16U1ACXX:71101:5145:79794 FWD 34. HWH-ST1206:91:D16U1ACXX:71101:5145:79794 FWD 34. HWH-ST1206:91:D16U1ACXX:71111:5145:79794 FWD 34. HWH-ST1206:91:D16U1ACXX:71111:5145:79794 FWD 35. HWH-ST1206:91:D16U1ACXX:71111:5145:79794 FWD 35. HWH-ST1206:91:D16U1ACXX:7111312:9117:13163	TATCTG TTGTATTG TAGATTG CTG TCTG AAATGATTATATTG TAGATTG CTG TCTG TATCGAGAATATTG TAGATTG CTG TCTG TTG TAATTG TATTG TAGATTG CTG TCTG TTC CGAG TATTTG TAGATTG CTG TCTG TAG TATCATATTG TAGATTG CTG TCT TCTAACATATTG TAGATTG CTG TCT TTG G TG TATATTG TAGATTG CTG TCT TTAACTGATATTG TAGATTG CTG TCTG TAACTGATATTG TAGATTG CTG TCTG	FWD 25. HWI-ST1206:91:D16U1ACXX:7:1107:20691:81039 FWD 26. HWI-ST1206:91:D16U1ACXX:7:1108:5064:44039 FWD 27. HWI-ST1206:91:D16U1ACXX:7:1108:5064:44039 FWD 28. HWI-ST1206:91:D16U1ACXX:7:1108:10540:63719 FWD 29. HWI-ST1206:91:D16U1ACXX:7:1108:16781:69701 FWD 30. HWI-ST1206:91:D16U1ACXX:7:1108:16781:69701 FWD 31. HWI-ST1206:91:D16U1ACXX:7:1108:16781:69701 FWD 31. HWI-ST1206:91:D16U1ACXX:7:1108:1655:88272 FWD 33. HWI-ST1206:91:D16U1ACXX:7:1109:1953:44261 FWD 33. HWI-ST1206:91:D16U1ACXX:7:1109:2734:44261 FWD 34. HWI-ST1206:91:D16U1ACXX:7:1109:17266:98986	TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT GGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT
FWD 36. HWI-ST1206:91:D16U1ACXX:7:1106:15243:49633 FWD 37. HWI-ST1206:91:D16U1ACXX:7:1111:7844:65864 FWD 38. HWI-ST1206:91:D16U1ACXX:7:214:9860:72910 FWD 39. HWI-ST1206:91:D16U1ACXX:7:1201:11867:18849 FWD 40. HWI-ST1206:91:D16U1ACXX:7:1206:6573:36438 FWD 41. HWI-ST1206:91:D16U1ACXX:7:1301:19284:63392 FWD 42. HWI-ST1206:91:D16U1ACXX:7:1103:6803:21587 FWD 43. HWI-ST1206:91:D16U1ACXX:7:1103:2904:38472 FWD 44. HWI-ST1206:91:D16U1ACXX:7:1203:4433:89999 FWD 45. HWI-ST1206:91:D16U1ACXX:7:211:11400:66488	TTTGAATGTATTGTAGATTGCTGTCTG TTTAGTTATATTGTAGATTGCTGTCTG TTGTCTAATATTGTAGATTGCTGTCTG TTTCTCATTATTGTAGATTGCTGTCTG TATTATTGTATTG	FWD 35. HWI-S11206:91:D10U1ACXX:7:1110:0435:73347 FWD 36. HWI-ST1206:91:D16U1ACXX:7:1110:14029:90730 FWD 37. HWI-ST1206:91:D16U1ACXX:7:1111:3339:26097 FWD 38. HWI-ST1206:91:D16U1ACXX:7:1111:3339:26097 FWD 39. HWI-ST1206:91:D16U1ACXX:7:1111:18084:47223 FWD 40. HWI-ST1206:91:D16U1ACXX:7:1111:18084:47223 FWD 40. HWI-ST1206:91:D16U1ACXX:7:1111:18084:47223 FWD 41. HWI-ST1206:91:D16U1ACXX:7:111:18084:4723 FWD 42. HWI-ST1206:91:D16U1ACXX:7:111:18084:4723 FWD 44. HWI-ST1206:91:D16U1ACXX:7:2312:2037:19597 FWD 43. HWI-ST1206:91:D16U1ACXX:7:2312:2037:19597 FWD 44. HWI-ST1206:91:D16U1ACXX:7:2312:8490:83746 FWD 44. HWI-ST1206:91:D16U1ACXX:7:2312:8490:83746	TGAATGAGAAATAATATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT
Fub 46. HWI-ST1206:91:D16UACXX:7:1110:7213:95982 Fub 46. HWI-ST1206:91:D16UIACXX:7:1209:16072:82169 Fub 48. HWI-ST1206:91:D16UIACXX:7:1209:16072:82169 Fub 48. HWI-ST1206:91:D16UIACXX:7:2202:18403:24824 Fub 50. HWI-ST1206:91:D16UIACXX:7:2207:9080:30453 Fub 51. HWI-ST1206:91:D16UIACXX:7:2209:11888:60336 Fub 52. HWI-ST1206:91:D16UIACXX:7:1209:11888:60336 Fub 52. HWI-ST1206:91:D16UIACXX:7:1209:11888:60336 Fub 52. HWI-ST1206:91:D16UIACXX:7:1203:1867:7410:3817 Fub 54. HWI-ST1206:91:D16UIACXX:7:2211:9513:35268 Fub 55. HWI-ST1206:91:D16UIACXX:7:1203:16660:9667	TTCATTTATATTG TAGATTG CTG TCTG TAGATTTATATTG TAGATTG CTG TCTG TTG TTCTGTATTG TAGATTG CTG TCTG TTG TTCTGTATTG TAGATTG CTG TCTG TTTAAG TGTATTG TAGATTG CTG TCTG TTTAACTTTATTG TAGATTG CTG TCTG TCTCATTGTATTG TAGATTG CTG TCTG TCTCATTGTATTG TAGATTG CTG TCTG TTCTG TG TATTG TAGATTG CTG TCTG TATTAGAATATTG TAGATTG CTG TCTG	FWD 45. HWI-ST1206:91:D16U1ACXX:7:2313:20016:74107 FWD 46. HWI-ST1206:91:D16U1ACXX:7:2313:5070:74981 FWD 47. HWI-ST1206:91:D16U1ACXX:7:2314:8206:8069 FWD 48. HWI-ST1206:91:D16U1ACXX:7:2314:8206:8069 FWD 49. HWI-ST1206:91:D16U1ACXX:7:2314:17296:94820 FWD 50. HWI-ST1206:91:D16U1ACXX:7:2314:17296:94820 FWD 50. HWI-ST1206:91:D16U1ACXX:7:1112:14662:48145 FWD 51. HWI-ST1206:91:D16U1ACXX:7:1112:5078:76593 FWD 52. HWI-ST1206:91:D16U1ACXX:7:1113:4503:11193 FWD 53. HWI-ST1206:91:D16U1ACXX:7:1113:8611:27034 FWD 54. HWI-ST1206:91:D16U1ACXX:7:1113:8502:28902 FWD 554. HWI-ST1206:91:D16U1ACXX:7:113:3532:28902 FWD 554. HWI-ST1206:91:D16U1ACXX:7:113:3561:40311	TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT TGAATGAGAAATAATATTGTAGAAT

Experimental approach: 'cryptic' IES excision



- 'consensus' 5'TATAG
- Located in unknown genes

Is cryptic IES excision dependent on iesRNAs? Dcl5 silencing



Dependence of iesRNA production on 5' overhangs

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



Sequencing identifies RNA corresponding to concatemers of IESs



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*



Dicer-like enzymes with sequence cleavage preferences







D



Silver staining









size nt





Dicer-like enzymes with sequence cleavage preferences





Hoehener et al. 2018, Cell.



Dcl5 size distribution with IES concatemer





Dcl5 size distribution with IES concatemer



Dcl5 size distribution with IES concatemer Longer



Hoehener et al. 2018, Cell.

Summary of Dcl cleavage preferences





Hoehener et al. 2018, Cell.

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