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**Title**

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**Permalink**

<https://escholarship.org/uc/item/0w96771d>

**Journal**

Developmental Cell, 28(2)

**ISSN**

1534-5807

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**Publication Date**

2014

**DOI**

10.1016/j.devcel.2014.01.009

Peer reviewed

Published in final edited form as:

*Dev Cell*. 2014 January 27; 28(2): 111–112. doi:10.1016/j.devcel.2014.01.009.

## An Expanding World of Small RNAs

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### Abstract

In ciliate protozoans, small RNAs (sRNAs) are integral to guiding large-scale genomic rearrangements after mating. Sandoval et al. (2014) report in this issue of *Developmental Cell* the discovery of a class of *Parameciums* RNAs, produced by a unique Dicer-like enzyme, that likely provides late stage quality control in this process.

In recent years, evidence has emerged to illustrate the incredibly diverse functionality of small RNAs (sRNAs) in many different aspects of gene regulation. These 20–30 nucleotide (nt) RNAs can silence the expression of genes by interactions with mRNAs (microRNAs and silencing RNAs) and are important for gene regulation through chromatin modification (piRNAs and siRNAs) (Wilson and Doudna, 2013, Luteijn and Ketting, 2013). In the ciliated protozoans, sRNAs have been shown to act to epigenetically program large-scale genomic rearrangements and aid in the retention and elimination of specific DNA sequences.

The mechanism of biogenesis of many of the different classes of small RNAs are varied and many are still being worked out, but they are often processed from a double-stranded region of RNA that is generated either by fold-back secondary structure, by RNA-dependent RNA polymerases or by annealing of complementary RNAs (Wilson and Doudna, 2013, Luteijn and Ketting, 2013). Generation of many classes of small RNAs from double-stranded regions involves the function of a double-stranded RNA endonuclease of the Dicerfamily, which produces cleavage product RNAs with characteristic 2 nt 3' overhangs.

Nuclear dimorphism and complex genome rearrangements are two defining characteristics of molecular biology in ciliated protozoans. Ciliates contain two separate caches of genetic information: a transcriptionally silent germline micronucleus (MIC) and a somatic macronucleus (MAC) containing hundreds to thousands of amplified DNA molecules from which genes are transcribed during vegetative growth. When a mating event occurs, micronuclei undergo meiosis and haploid micronuclei are exchanged. A new diploid micronucleus is formed and after a mitotic division, one of the daughter micronuclei forms a new macronucleus. Macronuclear development involves the elimination of from 5% to 95% of the micronuclear DNA (depending on the ciliate species), the fragmentation of the chromosomes, the addition of telomeres, the removal of many thousands of internally eliminated sequences (IESs) and the ligation of flanking macronuclear destined sequences

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(MDSs), and the amplification of macronuclear DNA to the appropriate high copy number (Chalker and Yao, 2011).

It has been shown that epigenetic information from the parental macronucleus guides the elimination and retention of sequences in the developing macronucleus. Small RNAs are the mechanism by which that epigenetic information is transferred from parent to daughter. In *Tetrahymena* and *Paramecium*, scnRNAs produced in the micronucleus are filtered against the parental macronucleus, and those that do not match the parental macronucleus then go on to direct chromatin modification and DNA elimination in the developing macronucleus (Duharcourt et al., 1995, Chalker and Yao, 1996, Mochizuki et al., 2002). In the *Stichotrichous* ciliates, in which up to 95% of micronuclear sequences are eliminated, a somewhat opposite approach has been proposed to occur. 27ntmacRNAs produced in the parental macronucleus early in mating (Zahler et al., 2012, Fang et al., 2012) function to mark MDS sequences in the developing macronucleus for retention (Fang et al., 2012).

Unlike typical eukaryotic genomes that generally code for one or two Dicer/Dicer-like proteins, ciliates often have many more homologs. *Paramecium tetraurelia* for example has 8 different Dicers; three from the Dicer class and five from the Dicer-like class, which contains only RNase III domain pairs and lacks the N-terminal helicase domain of dicers (Sandoval et al., 2014). Previous work with Dicer-like proteins lacking the N-terminal helicase domains of traditional Dicers suggests that these domains are not required for catalytic activity and may not be necessary for sRNA biogenesis (MacRae, et al., 2006). These expansions of Dicer and Dicer-like genes in *Paramecium* appear to arise from gene duplication during evolution. While some are ubiquitously expressed and seem to function in RNAi mechanisms, specific expression of particular Dicer/Dicer-like proteins at distinct stages of macronuclear development has been observed, and may provide clues to their potential distinct functions in sRNA biogenesis and function during the large-scale genomic rearrangements.

Gene knockdown along with high-throughput deep sequencing of small RNAs has now allowed Sandoval et al. (2014) to discover a novel class of sRNAs in *Paramecium* whose production requires the Dicer-like protein *DCL5*. Reporting in this issue of *Developmental Cell*, Sandoval et al. (2014) took advantage of the use of siRNAs to silence expression of three mating-specific *DCL* proteins to show distinct functions in the generation of different classes of mating-specific small RNAs. *DCL2* and *DCL3* are expressed early in macronuclear development and cooperate in the production of the 25nt scnRNAs. *DCL5* is expressed later in macronuclear development and they demonstrate that it is required for the production of a novel class of 26–30nt long sRNAs that is produced late in macronuclear development. These *DCL5*-dependent sRNAs are derived from internally eliminated sequences (IESs) and they have been named iesRNAs. Based on the observation that iesRNAs do not contain sequences from the flanking IES/MDS junctions (these junctions can be detected in the scnRNA class), this new class of sRNA is produced from IESs after their excision from the micronuclear chromosomes. Given that the chromosomes are amplified at this late stage in macronuclear development, the authors suggest a role for iesRNAs in genome quality control, helping to ensure the full removal of all IESs matching these sequences from the amplified chromosomes late in the process of macronuclear development.

This paper from Sandoval et al. (2014) shows the beauty of combining reverse genetics with high-throughput sequencing to distinguish and identify a new class of sRNAs. The discovery of iesRNAs raises additional questions about the mechanism of small RNA biogenesis and the control of DNA elimination. How are eliminated IES DNA sequences specifically transcribed to provide precursors for *DCL5*? Are transcripts made in both directions from

these excised DNA fragments or is an RNA-dependent RNA polymerase responsible for making the opposite strand? It is clear from this new study that the small *Paramecia* have a more complex and dynamic system of small RNA regulation of DNA elimination than was previously thought. This study is a reminder that even in a simple organism, the extent and complexity of small RNA function is not simple at all.

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