Published in final edited form as:

Curr Opin Microbiol. 2009 December ; 12(6): 638–643. doi:10.1016/j.mib.2009.09.012.

Epigenetic inheritance in ciliates

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Summary

2009 marks not only the 200th anniversary of Darwin's birth but also publication of the first scientific evolutionary theory, Lamarck's *Philosophie Zoologique*. While Lamarck embraced the notion of the inheritance of acquired characters, he did not invent it [1]. New phenomena discovered recently offer molecular pathways for the transmission of several acquired characters. Ciliates have long provided model systems to study phenomena that bypass traditional modes of inheritance. RNA, normally thought of as a conduit in gene expression, displays a novel mode of action in ciliated protozoa. For example, maternal RNA templates provide both an organizing guide for DNA rearrangements in *Oxytricha* and a template that can transmit spontaneous mutations that may arise during somatic growth to the next generation, providing two such mechanisms of so-called Lamarckian inheritance. This suggests that the somatic ciliate genome is really an "epigenome", formed through templates and signals arising from the previous generation. This review will discuss these new biological roles for RNA, including noncoding "template" RNA molecules. The evolutionary consequences of viable mechanisms in ciliates to transmit acquired characters may create an additional store of heritable variation that contributes to the cosmopolitan success of this diverse lineage of microbial eukaryotes.

Introduction and background

The subject of cytoplasmic inheritance has long been associated with ciliated protozoa because of myriad examples of non-Mendelian inheritance. In 1937 [2], *Paramecium* became one of the first single-celled eukaryotic model systems, with the observation that mating type determination was non-Mendelian. The same genotypes can produce two opposite mating types, and mating type determinants were cytoplasmically transmitted to sexual progeny. Sonneborn introduced the concept of "plasmagenes" [3], which together with nuclear genes were thought to determine all phenotypic characters.

The exceptional variety of ciliate characters under cytoplasmic inheritance raises the question of why so many examples of unconventional transmission appear in these organisms. Because sex in ciliates (Figure 1) is not reproduction, which occurs asexually, ciliate conjugation imparts to a new zygote not only a diploid genome derived from both parents but also the entire protoplasm of one parent. Though multicellular organisms may also transmit large amounts of protoplasm to their progeny, this usually contributes nutrients, rather than fully differentiated functions, although miRNAs and mRNAs are also present [4–7]. In ciliate exconjugants, a fully functional and integrated organelle system is only gradually taken over by a new nuclear

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genome. Given this situation in ciliates, the abundance of non-Mendelian characters that persist after conjugation should be less surprising.

Classic examples include "cortical inheritance" [8]. For instance, after conjugation, *Paramecium* cells sometimes fail to separate and produce abnormal doublets that only occasionally revert back to "singlets". Sonneborn found that the genetic determinants of the doublet character were located not in the nucleus or in the cytoplasm, but in the cell cortex itself, or outer layer. Cortical inheritance also exists in *Oxytricha* [9–11]. Environmentally induced or acquired phenotype changes in organisms can therefore transmit to further generations, even if they are not determined by nucleic acids. This, of course, does not exclude a role in cortical organization of genes, since they encode the proteins that comprise the structures. Genetic mutations do exist that result in altered cortical organization (reviewed in [12]). However, among epigenetic phenomena in ciliates, cortical structural inheritance is the least understood at the molecular level, and begs further investigation.

Homology-dependent epigenetic inheritance in ciliates

Recently there have been expanding cases of non-Mendelian inheritance termed homology-dependent, i.e. sequence-dependent. Like in many other eukaryotes, sequence-dependent silencing of a gene can occur during vegetative growth; in addition, these mechanisms can participate in the programming of genome rearrangements during development. One particular distinguishing feature in ciliates is the decoupling of germline and somatic functions by two distinct nuclei, the diploid micronucleus (MIC) and a DNA-rich macronucleus (MAC). Micronuclear meiosis initiates sexual events and after haploid nuclei exchange, new micro- and macronuclei develop from the zygotic nucleus; the old MAC is eventually lost and replaced by the new one.

Advances in ciliate molecular biology have shown that very similar developmental alternatives can be determined by programmed genome rearrangements. Indeed, the development of a new macronucleus involves extensive rearrangements of the germline genome, including elimination of transposons and other repeated sequences and the precise excision of numerous single-copy Internal Eliminated Sequences (IESs) from coding and non-coding sequences. Genome-wide rearrangements discard nearly all non-genic DNA, resulting in streamlined gene-rich genomes. In *Paramecium*, for instance, ~40,000 genes occupy only 72 Mb [13]. In some cases, for example in the ciliate *Oxytricha*, reordering, or "unscrambling", of the remaining DNA segments (Macronucleus Destined Segments, or MDSs) also occurs by translocation or inversion.

The amazingly high degree of specificity and reproducibility suggests a general mechanism for programming rearrangements. The parental ciliate cell must provide sufficient amount of information in order to produce a fully functional new macronucleus. Details of the mechanism that allows the cell to perfectly recognize hundreds of thousands of DNA sequences for elimination or rearrangement remain largely unknown. However, the discovery of homology-dependent maternal effects that can modify rearrangements patterns has shed some light on this process. In *Paramecium*, both types of developmentally-regulated genome rearrangements—precise excision of IESs and imprecise DNA elimination—can be controlled by homology-dependent maternal effects that score the presence or absence of a gene in the macronucleus [14–18]. Microinjection of a specific DNA sequence into the parental macronucleus can prevent that sequence from being eliminated from the progeny's somatic genome. This appears to be general for a subset (approximately one third) of *Paramecium* IESs [19–20] termed maternally controlled IESs, or mIESs. These observations imply trans-nuclear genome comparison during development.

The earliest model for epigenetic programming of DNA rearrangements, proposed by *Paramecium* researchers, assumed that the whole genome of the parental MAC is transcribed, and that transcripts are exported to the developing MAC, where they regulate rearrangements in a homology-dependent manner. Two possibilities for regulating IES excision were proposed: either the maternal transcripts simply inhibit excision of homologous sequences or they provide homologous templates for repair after constitutive elimination [19]. The latter hypothesis was rejected after demonstrating that mutations engineered in the sequence injected into the old MAC were not copied in the IES maintained in the new MAC; this was recently confirmed by showing that cleavage does not occur in engineered cell lines containing the IES permanently present in the MAC [21]. On the other hand, there has been recent evidence in support of a *protective* role of parental MAC transcripts in *Paramecium*: use of RNAi to degrade specific transcripts results in elimination of homologous DNA sequences in the new MAC for both genes [22] and IESs [23]. However, a template role for maternal transcripts remains an attractive model to explain the specificity of rearrangements in other ciliates, including *Oxytricha*, which is likely over one billion years distant from *Paramecium* and requires more complex DNA rearrangements via *descrambling*, in addition to removal of hundreds of thousands of intervening IESs, to construct functional, transcriptionally-active genes in the macronucleus. In this case, the maternal transcripts would provide templates for cleavage and repair during the DNA descrambling process.

RNA template-guided genome unscrambling

A recent study [24] demonstrates that maternal RNA templates can orchestrate massive genome rearrangements in *Oxytricha*, supporting an epigenetic model for sequence-dependent comparison between germline and somatic genomes (Figure 2). This work revealed the existence of maternal RNA that have the potential to guide DNA assembly. Furthermore, disruption of specific RNA molecules disables rearrangement of the corresponding gene, while injection of artificial templates reprograms the DNA rearrangement pathway. These observations suggest that RNA molecules guide genome rearrangement. The current data also suggest that the entire somatic genome might be equally transcribed during a narrow window of development, and the resulting RNA copy or cache of the genome would then be transported to the developing somatic nucleus of the sexual progeny to guide rearrangement. This implies the existence of a new system of inheritance, independent of the germline genome, where somatic genetic information is bequeathed through a transient RNA genome. This new system of inheritance in *Oxytricha* appears to be different from, though related to, the system described in *Paramecium* and *Tetrahymena*. These two distant ciliate lineages therefore acquired different variations on an apparently RNAi-like mechanism.

The opportunity for RNA-guided DNA repair is most profound in the ciliate *Oxytricha*, because it deletes 95% of its germline genome through global DNA rearrangements. These severely fragment its germline chromosomes and then sort and reorder the hundreds of thousands of MDS pieces remaining. Because the programmed information for reordering comes from transiently-expressed maternal template RNAs, changes that arise in the maternal somatic genome, whether at the level of point mutations or new DNA rearrangement patterns, have an opportunity to be passed on to the next generation, bypassing the usual mode of inheritance via DNA. Furthermore, the occasional transfer of point mutations in these RNA templates to the F1 generation provides a mechanism for stable inheritance of acquired, spontaneous somatic substitutions, without altering the germline genome. This suggests that the somatic macronuclear genome is really an "epigenome", formed through templates and signals arising from the previous generation. This mechanism of inheritance beyond the conventional DNA genome is capable of epigenetically transferring rearrangement information across multiple generations, at least to the F3, hinting at the power of RNA molecules to reshape genomic information. While details of the mechanism are unknown, a recent study in our lab [25]

implicates a role for germline-limited DNA transposons in the DNA rearrangement process, and the epigenetic transfer of specific point substitutions [24] points to an involvement of RNA-directed DNA proofreading.

Small RNA-guided programming of DNA rearrangements

In ciliates, at least two distinct RNAi pathways appear to mediate different types of homology-dependent effects. Post-transcriptional gene silencing (PTGS) was first shown to be induced in *Paramecium* by untranslatable transgenes [26,27], or by feeding cells with *E. coli* producing double-stranded RNA [28]. In both cases, silencing of targeted genes correlates with the accumulation of homologous ~23-nt siRNAs [22,29]. The same siRNAs are also involved in vegetative silencing and the deletions induced when *Paramecium* cells are fed with bacteria producing long dsRNA corresponding to a target gene. In *Tetrahymena*, a class of ~23–24-nt RNAs is produced endogenously from a limited number of loci [30]. Experimentally induced hairpin transcripts are processed into siRNAs and cause the silencing of homologous genes [31]. In both *Tetrahymena* and *Paramecium*, a Dicer-related protein is involved in the biogenesis of siRNAs [32,33]. However, the 23-nt siRNAs cannot explain other epigenetic effects, such as the maternal inheritance of alternative DNA rearrangements that do not depend on experimental silencing.

A second RNAi-like pathway, described in *Tetrahymena thermophila*, is restricted to conjugation. During developmental DNA rearrangements, elimination of micronuclear-specific DNA in this organism appears targeted by histone H3 K9 methylation [34,35]. Recent advances have shown a requirement for meiosis-specific Dicer- and Piwi-like proteins in the accumulation of scnRNAs (scan RNAs), a complex class of ~26–31-nt RNAs that appear in meiotic micronuclei [36–38]. These Dicer- and Piwi-like proteins are also required later for DNA elimination in the developing macronucleus, suggesting that rearrangement patterns in *Tetrahymena* are determined by scnRNA-directed methylation of histone H3 on lysine positions 9 (H3K9) and 27 (H3K27) marking germline-specific sequences in the developing MAC [35,37,39]. Histone methylation-dependent DNA elimination in ciliates suggests analogy to heterochromatin-related transcriptional gene silencing in other eukaryotes. This analogy is important because similar proteins might be involved in both phenomena. The observation that *Tetrahymena* scnRNAs appear to migrate from parental to zygotic macronucleus and become progressively enriched in micronucleus-specific sequences during conjugation [39] suggests that they may mediate the trans-nuclear comparison that is required to mimic the parental genome organization in the zygotic macronucleus. ScnRNAs that are able to find and possibly pair with homologous sequences in the parental macronucleus would be degraded along with destruction of the old macronucleus, whereas germline-specific RNAs would be exported to the newly developing macronucleus to target DNA elimination [40].

Current experimental data point to a combination of the scan RNA and the maternal transcript models: scnRNAs may instead be compared to an RNA copy of the maternal MAC genome, rather than to the MAC DNA itself (Figure 3). The elusive mechanism by which ciliates recognize germline sequences for elimination may thus involve a natural genomic subtraction, computed *in vivo* by RNA-RNA pairing interactions. Both *Tetrahymena* and *Paramecium* researchers have turned to a model proposing scnRNA selection through their interaction with long maternal RNAs [23,41]. The interaction between these two novel types of noncoding RNAs during development is the key to our understanding the large-scale genome reconstruction that is glorified in ciliates.

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Figure 1. *Oxytricha*'s sexual cycle [25]

All vegetative cells (**stages 1 and 10**) contain one macronucleus (MAC) and two micronuclei (MIC). The two MIC are genetically identical, but for simplicity we show only one here. **(2)** When deprived of food, two cells of different mating-types partially fuse, to initiate conjugation. **(3)** Both vegetative micronuclei in each partner enter meiosis I. **(4)** One product from each meiosis I is promoted to meiosis II, and one of the four meiosis II products is promoted to a post-meiotic mitosis. **(5)** The sister products of one mitosis are promoted to develop into gametic nuclei: a posterior stationary, and an anterior migratory nucleus; this happens in both partners, so that both partners emerge **(6)** with identical zygotic genotypes when the exchanged migratory nucleus fuses with the retained stationary nucleus **(7)**, resulting in genetically-identical exconjugant cells. **(8)** The newly-formed zygotic nucleus divides twice:

one daughter nucleus is destroyed, two become the new MICs, and **(9)** one differentiates into the new MAC. (Details of *Oxytricha*'s nuclear events are described in ref. [42]). Inside the circle are representative MIC and MAC versions of the *O. trifallax actin I* gene (MDSs in purple; IESs and flanking DNA in yellow).

Figure 2. Model for RNA-templated genome rearrangements in *Oxytricha* [24]

Bidirectional RNA transcription of all DNA nanochromosomes in the old, maternal macronucleus (MAC) produces an RNA genome cache (A). Transport of these RNA transcripts to the newly developing macronucleus (B), where they may act as scaffolds to guide rearrangements (deletion, permutation and inversion) of corresponding micronuclear (MIC) DNA sequences (C). In this illustration, segments 2 and 3 are switched and segment 5 is inverted (number upside down) relative to the final macronuclear order.

Figure 3. Scan RNA model for programmed DNA deletions in *Paramecium* and *Tetrahymena* [23, 41]

Like in *Oxytricha*, the parental somatic genome (MAC DNA) is entirely transcribed during conjugation (A). The meiotic micronucleus produces short, 25–30-nt dsRNA, so-called scnRNA that derive from the entire germline genome (B), and are subsequently transported to the parental nucleus (C). Pairing between an RNA copy of the parental genome and scnRNA takes place prior to new MAC development, allowing selection of unpaired MIC-specific scnRNA (D). MIC-specific scnRNAs target deletions of homologous DNA sequences in the developing MAC (E), leading to a mature new MAC. Blue boxes represent MAC-destined DNA, which in the MIC are separated from each other by germline-specific sequences (red boxes) like IESs or transposons.