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Insights into transgenerational epigenetics from studies of ciliates

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Abstract

Epigenetics, a term with many meanings, is broadly defined as the study of dynamic states of the genome. Ciliates, a clade of unicellular eukaryotes, can teach us about the intersection of epigenetics and evolution due to the advantages of working with cultivable ciliate lineages plus their tendency to express extreme phenotypes such as heritable doublet morphology. Moreover, ciliates provide a powerful model for studying epigenetics given the presence of dimorphic nuclei – a somatic macronucleus and germline micronucleus – within each cell. Here, we exemplify the power of studying ciliates to learn about epigenetic phenomena. We highlight “classical” examples from morphology and physiology including cortical inheritance, mating type, and serotype. In addition, we detail molecular studies including DNA elimination; alternative processing and unscrambling; and copy number determination in model lineages. Based on the implications of such studies, we explore epigenetics as a possible functional mechanism for rapid speciation in ciliates.

Keywords

Ciliates; Cortical inheritance; DNA processing; Mating type; Seotype; Transgenerational epigenetics

Introduction

Epigenetics is a term with a broad array of definitions. The term “epigenetics” was first used by developmental biologist C.H. Waddington to describe how phenotypes arise from a genotype during development (Haig, 2007; Waddington, 1942). Today, the textbook definition of epigenetics regards changes in gene expression that do not rely on alteration of the nucleotide sequence (Burggren and Crews, 2014). Alterations of chromatin, including DNA methylation and histone modification, demonstrate the etymology of the word

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‘epigenetics,’ which describes acting ‘above the genome’ (Haig, 2007). We prefer a broader definition similar to that put forth by Denise Barlow, one of the scientists involved in the discovery of genomic imprinting, who argued, “epigenetics has always been all the weird and wonderful things that can’t be explained by genetics” (McVittie, 2006). Under this umbrella, epigenetics includes phenomena whose molecular details are not yet fully understood. Thus is the case for many of the “classical” examples of epigenetics described below. As such, this broader definition of epigenetics also includes transgenerational inheritance, epigenetic phenomena that are heritable across generations (e.g. following conjugation in ciliates), which are the focus of this manuscript.

Ciliates as models for epigenetic studies

Due to their elaborate ciliature and beauty, ciliates have been subject to study since the invention of the microscope, generating the rich data synthesized in this manuscript. Ciliates, a monophyletic clade of unicellular eukaryotes, are characterized by the presence of cilia in at least one stage of their life cycle and the presence of dimorphic nuclei, with both a somatic macronucleus and germline micronucleus within every cell (e.g. Chalker et al., 2013; Katz, 2001; Yao et al., 2002). This combination of features contributed to the discovery of many epigenetic phenomena in ciliates.

Appreciating transgenerational epigenetics’s strong impact on ciliates and their genomes requires an understanding of ciliate reproduction. During asexual reproduction, division occurs in the transcriptionally-silent micronucleus by mitosis and in the transcriptionally-active somatic macronucleus by amitosis. Sexual reproduction begins with conjugation (i.e. the fusion of two cells of different mating types) and meiosis of the micronucleus in each member of the conjugating pair (Figure 1A). Eventually, one haploid micronucleus from each cell is transferred to the other cell; the haploid nuclei then fuse within each cell to form a zygotic nucleus, which then undergoes mitosis (Chalker et al., 2013; McGrath et al., 2006; Orias, 1998). One of the two mitotic products develops into the micronucleus, presumably by heterochromatin formation that leads to a quiescent nucleus, and the other differentiates into the new macronucleus (Chalker et al., 2013; Katz, 2001; Yao et al., 2002). The DNA content and organization of the developing macronucleus are the same as the micronucleus at the beginning of macronuclear differentiation (Chalker et al., 2013). The developing macronucleus and the parental macronucleus share a cytoplasm, which allows for extensive epigenetic communication between nuclei (Chalker et al., 2013).

In ciliates, inheritance following sexual reproduction is guided by a genome scanning process instead of pure Mendelian inheritance. If Mendelian inheritance were the rule in ciliate sexual reproduction, the parental macronucleus would have no effect on the developing macronucleus. However, this is not the case. While the old macronucleus degrades and contributes no DNA to the new macronucleus, it does influence the development of the new macronucleus through epigenetic processes like RNA-guided genome scanning (Baranasic et al., 2014; Chalker et al., 2013; Chalker and Yao, 2011; Meyer and Duhaucourt, 1996; Nowacki and Landweber, 2009). It is possible, however, that only a portion of the genome is scanned (Hamilton et al., 2016; Schoeberl et al., 2012).

Though the molecular mechanisms underlying epigenetic processes vary, RNA-mediated processes are emerging as a common theme across eukaryotic lineages, including ciliate lineages (Fedoroff, 2012; Maurer-Alcala and Katz, 2015; Yao et al., 2002). RNA-mediated processes (referred to here as genome scanning) involve epigenetic changes to genomes regulated by single-stranded RNAs that silence transposable elements (Chalker et al., 2013; Coyne et al., 2012; Fedoroff, 2012), generate heterochromatin (Chalker and Yao, 2011), and eliminate DNA (Fang et al., 2012; Maurer-Alcala and Katz, 2015; Swart et al., 2014).

Here we highlight the roles that ciliates have played in our understanding of epigenetics over the past 50 years. We organize the manuscript around two major categories of epigenetics in ciliates: 1) “classical” examples of morphological and physiological phenomena, and 2) molecular processes (Table 1). We place ciliate epigenetic phenomena in the context of other eukaryotes to suggest that ciliates have elaborated on an ancient toolbox evolved for silencing transposons. We end by arguing that epigenetic processes in ciliates allow for increased phenotypic variation and genetic diversity.

Part I: Classical examples from morphology and physiology

Classical examples of epigenetic phenomena in ciliates include cortical inheritance, and determination of mating types and serotypes. We define these phenotypic changes as examples based on morphology and physiology in that their discovery relied on light microscopy and behavioral observations.

Cortical inheritance

The inheritance of acquired changes in morphology, also known as cortical inheritance, is an epigenetic phenomenon first observed in ciliates over fifty years ago (Beisson, 2008; Beisson and Sonneborn, 1965). One of the most well studied example of cortical inheritance is doublet morphology, which refers to the acquisition and maintenance of a second, mirrored set of ciliature such as the presence of two mouths (Figure 2A, B; Bell et al., 2008; Christopher and Sundermann, 1995; Frankel and Nelsen, 1986; Landman, 1993). Doublets are produced for a variety reasons and are an example of a phenotypic change that may not require changes in the nucleotide sequences. Though *janus* mutant doublets may be induced genetically in *Tetrahymena thermophila* (Christopher and Sundermann, 1995; Frankel and Nelsen, 1986). Doublet morphology follows Lamarck’s ideas on the inheritance of acquired characteristics. That is, when morphological changes occur, either through errors in cell division or by experimental perturbation, they are transmitted to progeny across asexual and sexual cycles (Landman, 1993). Despite the compelling data on cortical inheritance, Larmarck’s views on inheritance have been largely rejected.

An example of a naturally occurring doublet phenotype is found in the ciliate *Oxytricha fallax* (Class Spirotrichea). If a wild-type *Oxytricha fallax* fails to divide, a ‘doublet’ can form such that the ciliate has two mouths and two mirrored sets of cirri (i.e. bundles of cilia) that are maintained through cell divisions (Landman, 1993). Similarly, *Pleurotricha lanceolata* (Spirotrichea) can form mirror-image symmetrical doublets when subjected to a combination of heat shock and surgery (Landman, 1993). Following encystment, the doublet pattern emerged unchanged and went on to reproduce doublets clonally (Grimes, 1973).

Understanding the functional implications of inheritance of doublet morphology awaits additional studies, but the inheritance of acquired changes to body plans challenges the textbook views on morphological evolution.

Mating types

Non-Mendelian inheritance of mating types is another classic example of epigenetics in ciliates. Genetically determined mating types are the basis for reproductive compatibility in many eukaryotes. For example, the molecular basis for mating types has been determined in lineages such as fungi (e.g. Haber, 2012; Klar et al., 1998) and mammals (e.g. Emmons and Lipton, 2003). In ciliates, mating types can be determined in at least three ways – synclonal, karyonidal and cytoplasmic – where the last two are epigenetically regulated (Hall and Katz, 2011; Phadke and Zufall, 2009). While synclonal inheritance follows Mendelian genetics, karyonidal and cytoplasmic inheritance are epigenetically regulated (Hall and Katz, 2011; Phadke and Zufall, 2009). Karyonidal inheritance occurs when the mating type is determined either stochastically during macronuclear development or predictably by environmental stimuli such as temperature and light exposure (Figure 2C; Cervantes et al., 2013; Hall and Katz, 2011). Cytoplasmic inheritance occurs when the mating type of the offspring reflects the parent's phenotype, in a manner analogous to maternal inheritance in animals (Figure 2D; Hall and Katz, 2011; Phadke and Zufall, 2009; Sonneborn, 1977).

An example of cytoplasmic inheritance found in *Paramecium tetraurelia* (Class Oligohymenophorea) involves mating types described as odd (O) and even (E) (Chalker et al., 2013; Nowacki et al., 2011; Sonneborn, 1977). The mating type is determined by cytoplasmic inheritance during the development of the new macronucleus. The O parent will produce O offspring and the E parent will produce E offspring in a manner that is independent of the alleles carried by each cell (Figure 2D; Chalker et al., 2013; Nowacki et al., 2011; Sonneborn, 1977).

Literature repeatedly demonstrates that Mendelian rules are insufficient to explain the inheritance and evolution of mating types in ciliates. Phadke and Zufall (2009) discuss the rapid diversification of mating types within ciliate species, with a particular emphasis on *Tetrahymena*, *Paramecium* and *Euplotes* species. They suggest that the mode of inheritance (e.g. synclonal, cytoplasmic or karyonidal), the number of mating types (2–12 in different species), and molecular determinants, such as pheromones that are secreted or cell-bound, are all rapidly evolving across the ciliate phylogeny (Phadke and Zufall, 2009). The factors (e.g. genetic, epigenetic, and selective) that drive the elevated rates of evolution, however, remain unclear (Phadke and Zufall, 2009).

Serotypes

Serotypes, determined by the kinds of surface proteins expressed and transported to the cell surface, are also epigenetically regulated in ciliates (Baranasic et al., 2014; Lepere et al., 2008; Simon and Schmidt, 2007). The phenotypic variation created by the various surface antigens enables recognition between cells, may protect ciliates from predators, or could be a defense mechanism against biotic and abiotic factors (Preer, 1986; Simon and Kusch, 2013; Sonneborn, 1970). Over the last 70 years, studies have shown that the inheritance of

surface antigen genes follows Mendelian rules; however, the expression of the antigens is epigenetically regulated (Baranasic et al., 2014; Beale, 1957; Lepere et al., 2008; Sonneborn, 1943).

Microscopy studies revealed that surface antigen expression is not inherited in a Mendelian fashion in *Paramecium* (Beale, 1957). Antibodies were developed against the surface antigens and revealed that the expression of these genes was not inherited by Mendelian genetics but rather by cytoplasmic states (Beale, 1957). Beale (1957) showed that in *Paramecium* the production of surface antigens is determined by the environment, and previous history of the cytoplasmic state. The main conclusion from this study is that cells can produce a vast array of diverse antigens from a set of genes without changing the genotype (Beale, 1957). Numerous hypotheses have been put forth to explain *Paramecium*'s antigen formation (Beale, 1957); however, it was not until recent molecular studies that the details of serotype expression have been demonstrated to involve epigenetic inheritance (see below).

Part II: Molecular studies

While the epigenetic inheritance of morphological and physiological features has been observed in ciliates for decades, our understanding of mechanistic details has emerged more recently through molecular studies. We exemplify this progress through discussions of three epigenetic processes: DNA elimination, genome unscrambling/alternative processing, and copy number determination in extensively fragmenting ciliates. We describe how genome scanning is applicable to all these processes due to their use of small RNAs to direct genomic changes.

Germline soma distinction/DNA elimination

Elimination of particular germline sequences in macronuclear development is the most well understood epigenetic phenomenon in ciliates. DNA elimination in ciliates involves the removal of specific sequences from the developing macronucleus, and retention of sequences present in the old macronucleus (Jonsson et al., 2009; Schoeberl et al., 2012; Swart and Nowacki, 2015). The removed sequences, which can be repetitive elements or unique copy sequences referred to as internally eliminated sequences (IESs), are scattered throughout the micronuclear genome and may interrupt coding regions (Swart and Nowacki, 2015). Mechanisms of elimination have been studied in the classes Oligohymenophorea (*Paramecium* and *Tetrahymena*) and Spirotrichea (*Oxytricha* and *Stylonychia*). Though different in detail, both clades have provided evidence for a scanning mechanism in which elimination or retention of sequences in the developing macronucleus is directed by identity to small RNAs (Fang et al., 2012; Jonsson et al., 2009; Schoeberl et al., 2012; Swart et al., 2014).

In a model proposed for Oligohymenophorea, the process of DNA elimination starts after division of the zygotic nucleus when the entire genome of the new micronucleus is transcribed (Figure 3A; Chalker and Yao, 2011; Swart et al., 2014). Recently, in *Tetrahymena thermophila*, micronuclear limited sequences show evidence of disproportionate representation among transcripts (Schoeberl et al., 2012). At this point,

both the developing macronucleus and new micronucleus have the same genome and DNA content, so these transcripts of the micronucleus correspond to sequences in the developing macronucleus. The double-stranded RNAs (dsRNAs) are processed into small RNAs called scan RNAs (scnRNAs; Heyse et al., 2010; Swart et al., 2014). Then the scnRNAs are transported by a Piwi family protein into the old macronucleus, where they target homologous sequences (Malone and Hannon, 2009; Swart et al., 2014). The scnRNAs with homology to parental macronuclear sequences are degraded and the remaining scnRNAs are sent back into the developing macronucleus (Jonsson et al., 2009; Malone and Hannon, 2009; Swart et al., 2014). Here, the scnRNAs bind to homologous sequences not found in the parental macronucleus and these sequences are targeted for elimination in the developing macronucleus (Jonsson et al., 2009; Malone and Hannon, 2009; Swart et al., 2014). Evidence for this model was first observed in *Paramecium* by injecting the parental macronucleus with plasmids containing IESs (Duharcourt et al., 1995; Yao et al., 2002). About one third of these injected IESs were retained in the new macronucleus and could be passed down to future generations (Duharcourt et al., 1995; Swart et al., 2014). These studies demonstrate the importance influence of the parental macronucleus in recognizing macronuclear DNA to be retained in the next generation.

Small RNA genome scanning in Spirotrichea differs from the mechanism in Oligohymenophorea in that scnRNAs expressed in early development in Spirotrichea (referred to here as piwi-interacting RNAs or piRNAs) mark sequences for retention instead of for deletion (Chen et al., 2014; Fang et al., 2012). In *Oxytricha*, IESs were stably retained when injected with piRNAs homologous to these regions (Fang et al., 2012). Sequencing of piRNAs has revealed an overwhelming majority map to macronuclear destined sequences (MDS), with just 0.11% corresponding to micronuclear limited sequences (Chen et al., 2014). Fang et al. (2012) also reported that, unlike scnRNAs in Oligohymenophorea, where many scnRNAs derive from both the micronucleus and the parental macronucleus; piRNAs in *Oxytricha* derive only from the old macronucleus.

Differences in the function of piRNAs and scnRNAs in Spirotrichea and Oligohymenophorea respectively may be related to the extent of elimination in spirotrichs. In the model genera of Spirotrichea, *Euplotes*, *Stylonychia* and *Oxytricha*, around 85–98% of the sequences present in the germline are eliminated from the developing macronucleus (Fang et al., 2012; Nowacki et al., 2011; Swart and Nowacki, 2015). This value dwarfs the ~30% eliminated in *Paramecium* and in *Tetrahymena* (Arnaiz et al., 2012; Hamilton et al., 2016; Swart and Nowacki, 2015). Since the majority of germline sequences are eliminated in the Spirotrichea and the minority in Oligohymenophorea, the method of elimination for each class involves transfer of the fewest possible scnRNAs into the developing macronucleus (Fang et al., 2012).

Alternative processing and unscrambling

In a few ciliate classes, the pieces of each macronuclear gene (i.e. macronuclear destined sequences or MDSs) are not only interrupted by IESs in the micronucleus, but may also be misordered and some of the pieces may even be inverted (Chen et al., 2014; Jonsson et al., 2009; Nowacki et al., 2011). Genes assembled from misordered MDSs are referred to as

scrambled, and the process of their reordering is called unscrambling (Jonsson et al., 2009; Katz and Kovner, 2010). If the same MDSs are used in the assembly of multiple macronuclear loci, the genes are described as alternatively processed (Chen et al., 2014; Jonsson et al., 2009; Nowacki et al., 2011). Gene scrambling has been reported only in ciliates with gene-sized macronuclear chromosomes, a feature present in three ciliate classes, Armophorea, Phyllopharyngia, and Spirotrichea, with possibly at least two independent origins for this process (Katz, 2001; Katz and Kovner, 2010). These ciliates share features that may support unscrambling, such as the presence of giant polytene chromosomes during macronuclear development (Ammermann, 1987; Chen et al., 2014; Katz and Kovner, 2010; Raikov, 1982).

Molecular mechanisms for unscrambling have been studied in *Stylonychia* and *Oxytricha* (Spirotrichea), where approximately 30% of macronuclear genes are scrambled in the micronucleus genomes (Jonsson et al., 2009). Long-template RNAs derived from the parental macronucleus appear to guide unscrambling (Jonsson et al., 2009; Nowacki et al., 2011; Swart and Nowacki, 2015). These long-template RNAs are theorized to serve as a scaffold for the reorganization of sequences in the developing macronucleus (Nowacki et al., 2011). The new macronuclei of cells injected with artificial RNA templates with altered orders of MDSs inherit the artificial template sequence (Jonsson et al., 2009; Nowacki et al., 2011; Nowacki et al., 2008). Long-template RNAs may also serve as a proofreading mechanism for piRNA elimination (Jonsson et al., 2009). Unscrambling occurs after initial excision, and studies of intermediates (i.e. sequences in the partially developed macronuclear genome) reveal that prior to unscrambling most elimination is imprecise, which would render the new macronucleus dysfunctional (Jonsson et al., 2009; Nowacki et al., 2011). By the time unscrambling occurs, intermediates demonstrate precise elimination, indicating a proofreading mechanism must be present (Jonsson et al., 2009).

Alternative processing is a variation on gene unscrambling that may generate protein diversity (Figure 3B; Katz and Kovner, 2010). Similar to V(D)J recombination in the human immune system, alternative processing involves the reordering of sequences in a multiplicity of arrangements to create diverse gene family members. For example, the ciliate *Chilodonella uncinata* (Phyllopharyngia) generates multiple macronuclear β -tubulin paralogs from an alternatively processed shared micronuclear region (Gao et al., 2015; Katz and Kovner, 2010). In *Oxytricha*, over 1000 MDSs are reused to generate alternatively processed genes, and one MDS can contribute to at most five gene family members (Chen et al., 2014). The ability to recycle MDSs may lead to greater diversity within protein families, as ciliates with extensively processed genomes have higher rates of protein evolution compared to non-scrambling ciliates (Gao et al., 2015; Zufall et al., 2006).

Copy number determination

Copy number of chromosomes in the macronucleus is another epigenetically controlled phenomenon in ciliates with extensively processed, gene-sized macronuclear chromosomes (Bellec and Katz, 2012; Xu et al., 2012). Ciliates with multigene macronuclear chromosomes, such as *Paramecium* and *Tetrahymena*, have roughly even copy numbers of all chromosomes in their macronuclei (Xu et al., 2012). For example, each chromosome in

Paramecium tetraurelia is amplified to ~800 copies (Duret et al., 2008). In contrast, copy numbers in *Oxytricha* and *Chilodonella* range from a couple hundred up to a million for each of 15,000–20,000 different nanochromosomes (Huang and Katz, 2014; Jonsson et al., 2009).

Like unscrambling, copy number determination is thought to rely on long template RNAs from the old macronucleus (Heyse et al., 2010; Nowacki et al., 2011; Xu et al., 2012). In *Stylonychia*, the developing macronucleus begins with roughly the same copy number for each gene, like the zygotic nucleus from which it came (Heyse et al., 2010). Experiments using RNA interference (RNAi) against highly amplified genes demonstrated a decrease in copy number when the corresponding template was degraded (Heyse et al., 2010). Conversely, injection of template RNAs results in an increase in DNA copy number (Heyse et al., 2010; Nowacki et al., 2010). These alterations in *Stylonychia* were stably inherited through vegetative growth; similar results have been obtained for *Oxytricha* (Heyse et al., 2010; Nowacki et al., 2011).

Part III: Implications - The transposon link

A model proposed for the origin of epigenetic mechanisms in ciliates is that they are derived from transposon silencing machinery (Klobutcher and Herrick, 1997). The mechanisms underlying genome scanning in ciliates and epigenetic processes in other eukaryotic lineages share similarities with transposon excision machinery (Arnaiz et al., 2012; Chalker et al., 2013; Coyne et al., 2012; Maurer-Alcala and Katz, 2015; Schoeberl and Mochizuki, 2011; Singh et al., 2014; Swart et al., 2014). For instance, in *Euplotes* and *Paramecium* species, the TA dinucleotides flanking IESs bear resemblance to the termini of Tc1/mariner transposons (Arnaiz et al., 2012; Chalker et al., 2013; Coyne et al., 2012). *Tetrahymena* species use epigenetically regulated heterochromatin formation to remove IESs, similar to other eukaryotes in which small RNA-directed heterochromatin formation is used to control transposons (Chalker and Yao, 2011; Nowacki et al., 2011). Furthermore, in many ciliate lineages, key enzymes involved in the epigenetic removal of IESs are derived from transposases (Chalker et al., 2013; Coyne et al., 2012; Klobutcher and Herrick, 1997; Swart et al., 2014).

Genome scanning has been linked to at least some of the classic examples of epigenetic phenomena in ciliates; for example, both *Paramecium tetraurelia* and *Paramecium septaurelia* use genome scanning in mating type determination (one of our classical examples; Singh et al., 2014). Additionally, *Tetrahymena thermophila* uses programmed DNA rearrangements in mating type determination through karyonidal inheritance (Cervantes et al., 2013). Both scnRNAs and small interfering RNAs (siRNAs) have been implicated as possible epigenetic mechanisms for the inheritance of surface antigens (Baranasic et al., 2014; Lepere et al., 2008). Hence, the epigenetic processes described here likely reflect modification of genome defense machinery that originally evolved to silence invading transposable elements.

Implications - Macroevolution

Epigenetic phenomena may influence macroevolution in ciliates by contributing to rates of speciation. For examples, changes in gene copy number or locations of micronuclear-limited sequences in isolated populations may create barriers to genetic exchange, leading to rapid speciation events (Gao et al., 2015; Hall and Katz, 2011). Similarly, differential loss of duplicated macronuclear destined sequences, which can be corrected through gene unscrambling and alternative processing, may inhibit production of viable offspring between members of previously isolated populations (Gao et al., 2015). This rapid speciation as the result of epigenetic processes may contribute to the complex patterns of morphological and molecular evolution such as cryptic species, which are common in ciliates (Foissner et al., 2008; Hall and Katz, 2011; Lahr et al., 2014; Przybos and Tarcz, 2016; Sonneborn, 1975).

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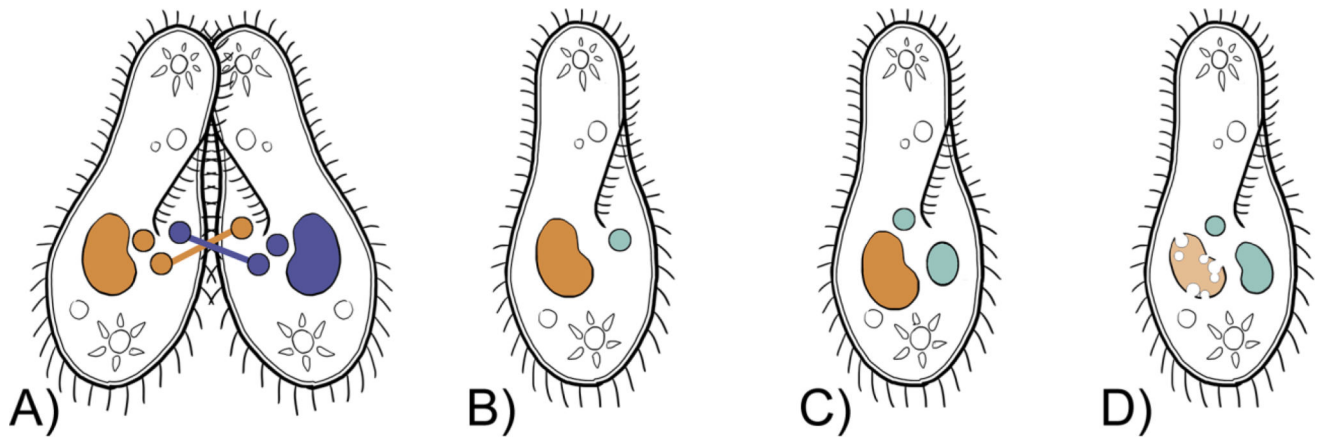


Fig. 1. Simplified version of nuclear morphology following conjugation/sexual reproduction in ciliates, with the small round circles representing micronuclei: A) one haploid micronucleus is exchanged between conjugating cells; B) a zygotic nucleus is formed in the presence of the parental macronucleus; C–D) the zygotic nucleus undergoes division and daughter nuclei differentiate into a new micronucleus and macronucleus; D) the parental macronucleus degrades and is replaced by the new macronucleus.

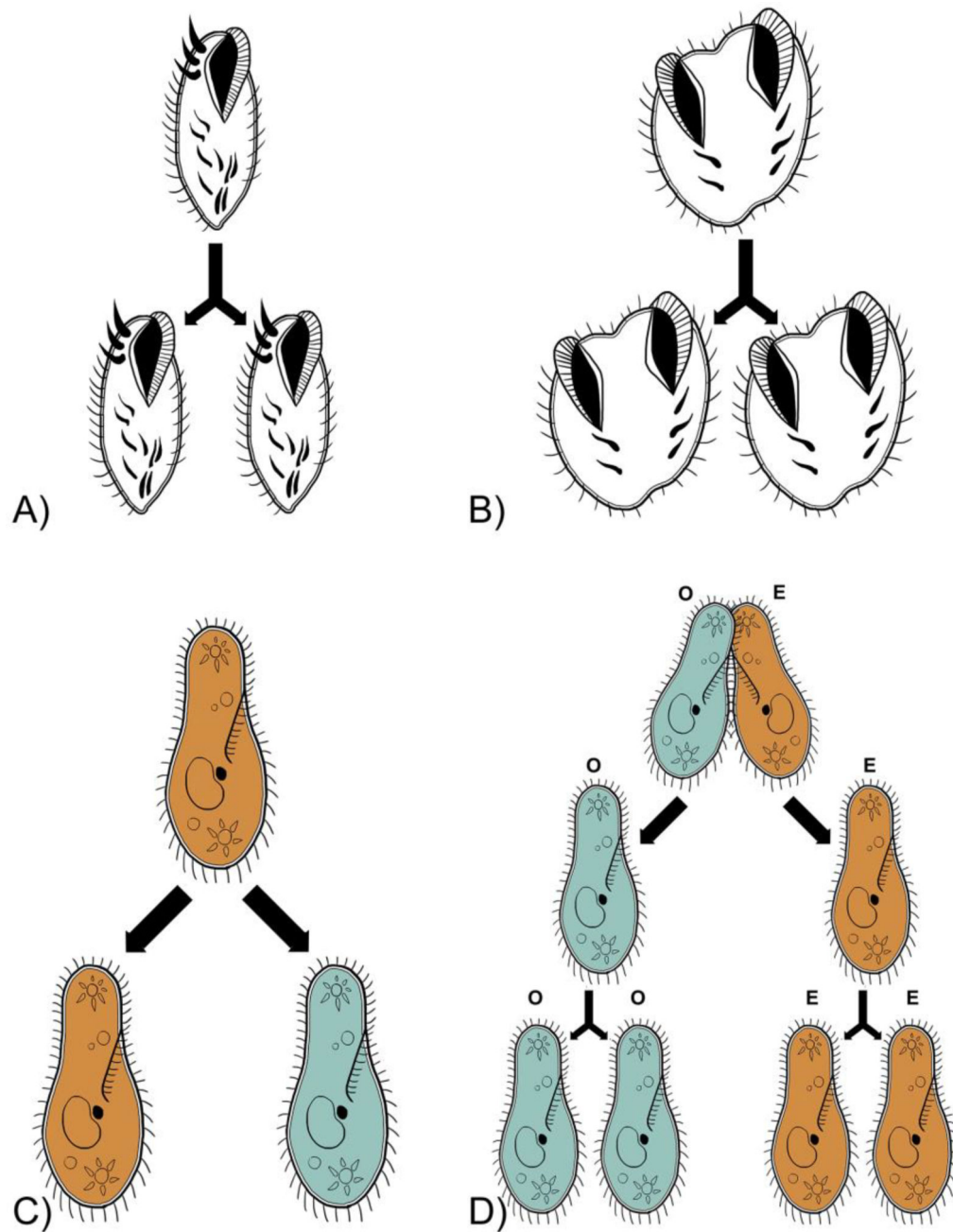


Fig. 2.

Two “classical” examples of epigenetic phenomena in ciliates. Cortical inheritance: following asexual reproduction, the wild type singlet produces progeny that are also singlets (A) while mutant doublets produce doublet offspring (B). Non-Mendelian examples of mating type determination: in karyonidal inheritance, mating type varies either stochastically or is determined by changing environmental factors (e.g. temperature, time of day; C); in cytoplasmic inheritance mating types (O and E) are maternally inherited following conjugation (D; adapted from Chalker et al., 2013; Meyer and Garnier, 2002).

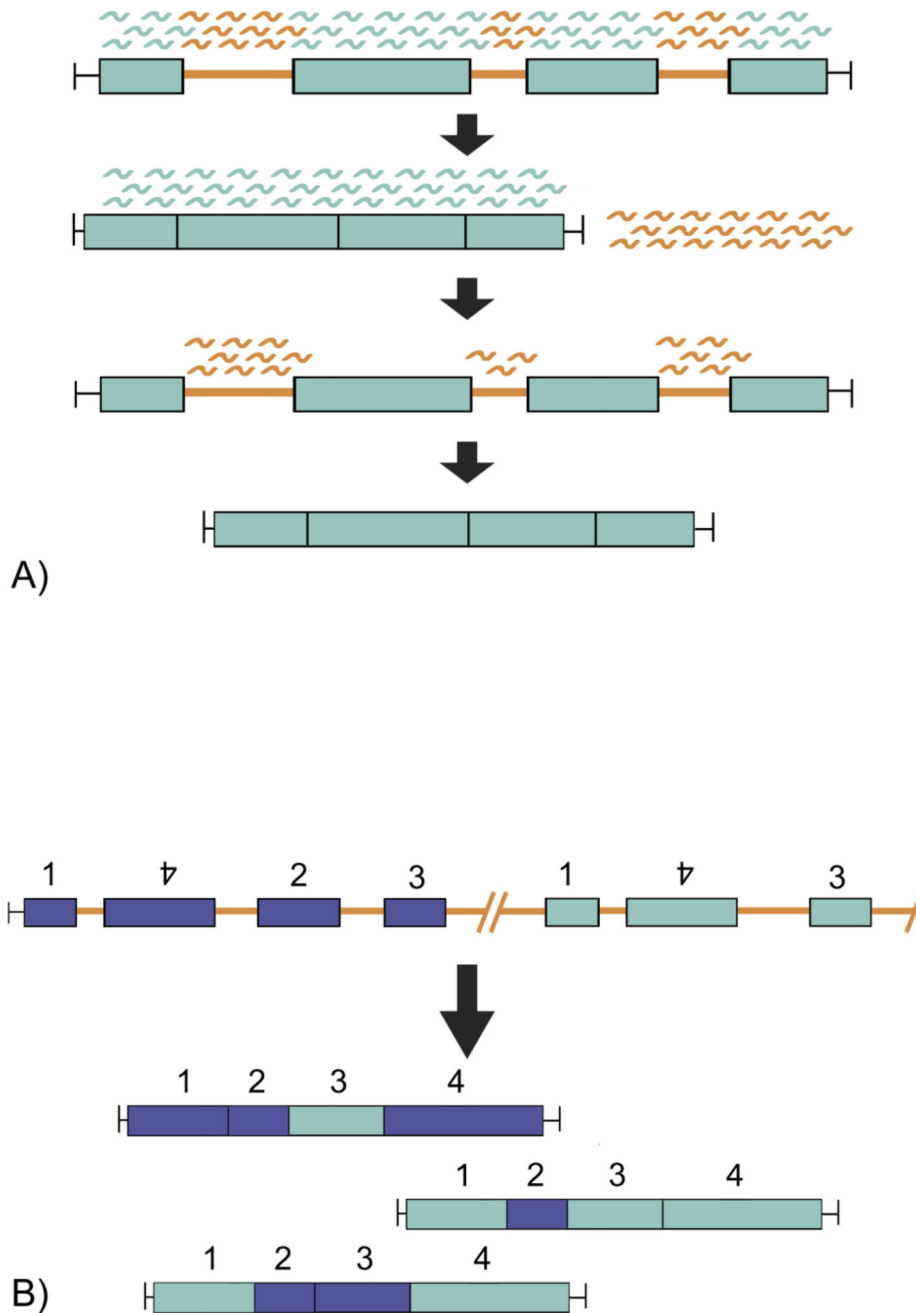


Fig. 3. Two examples of molecular studies of epigenetic phenomena in ciliates. A) DNA elimination: in Oligohymenophorea, the entire micronuclear genome is transcribed into small scnRNAs. These transcripts are transported to the old macronucleus where they bind to regions of homology (MDSs indicated in light blue) and are degraded. The scnRNAs that did not bind to any region of the old macronucleus are transported into the new macronucleus and mark homologous regions (orange) for deletion. B) Alternative processing: MDSs from divergent paralogs are combined in varying orders to create gene variants. In the micronucleus (top) the MDSs labeled 1–4 are in a scrambled order. MDSs

are reassembled and may be used in multiple macronuclear genes in alternative processing, which allows for the creation of several genes from two paralogs.

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Table 1

Summary of examples of epigenetics in ciliates

Classical Examples	Pattern	Processes	References
Cortical inheritance	Doublet morphology	Unknown	Beisson and Sonneborn (1965); Christopher and Sundermann (1995); Grimes (1973); Landman (1993)
Mating types	Cytoplasmic (O & E) & karyondial inheritance	Genome rearrangement	Cervantes et al. (2013); Hall and Katz (2011); Phadke and Zufall (2009); Sonneborn (1977)
Serotypes	Surface antigen A	Genome scanning	Baranasic et al. (2014); Beale (1957); Simon and Schmidt (2007); Sonneborn (1943)
Molecular Examples			
DNA elimination	Removal of micronuclear-limited sequences during development	Genome scanning	Duharcourt et al. (1995); Fang et al. (2012); Hamilton et al. (2016); Jonsson et al. (2009); Swart and Nowacki (2015)
Alternative processing	Only found in ciliates with extensively processed macronuclear genomes	Genome scanning	Gao et al. (2015); Katz and Kovner (2010); Nowacki et al. (2011); Riley and Katz (2001)
Copy number determination	Inheritance of somatic copy number of nanochromosomes	Genome scanning	Bellec and Katz (2012); Heyse et al. (2010); Huang and Katz (2014); Xu et al. (2012)