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Epigenetic influences of mobile genetic elements on ciliate genome architecture and evolution

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Abstract

Mobile genetic elements (MGEs) are transient genetic material that can move either within a single organism's genome or between either individuals or species. While historically considered 'junk' DNA (i.e. deleterious or at best neutral), more recent studies reveal the adaptive advantages MGEs provide in lineages across the tree of life. Ciliates, a group of single-celled microbial eukaryotes characterized by nuclear dimorphism, exemplify how epigenetic influences from a variety of MGEs shape genome architecture and patterns of molecular evolution. Ciliate nuclear dimorphism may have evolved as a response to transposon invasion and ciliates have since co-opted transposable elements to carry out programmed DNA deletion. Another example of the effect of MGEs is in providing mechanisms for lateral gene transfer from bacteria, which introduces genetic diversity and, in several cases, drives ecological specialization in ciliates. As a third example, the integration of viral DNA, likely through transduction, provides new raw genetic material and can change the way host cells defend themselves against other viral pathogens. We argue that the acquisition of MGEs through non-Mendelian patterns of inheritance, coupled with their effects on ciliate genome architecture and expression and persistence throughout evolutionary history, exemplify how the transmission of mobile elements should be considered a mechanism of transgenerational epigenetic inheritance.

Keywords

Transposable elements; lateral gene transfer; virus; transgenerational epigenetic inheritance

INTRODUCTION

Mobile genetic elements (MGEs) are sequences of nucleic acids that can move either within a genome or between the genomes of different individuals or species (e. g. Frost et al. 2005; Kazazian 2004). While originally considered "junk" DNA (Federoff 2012; Kidwell and Lisch 2001), more recent studies have illuminated the important roles MGEs play in structuring eukaryotic genome architecture, diversity, content, and evolution (e. g. Barry 2018; Bourque 2018; Federoff 2012; Kazazian 2004). Mobile genetic elements are argued to have facilitated the evolution of many distinctive traits across eukaryotic lineages, including the immune system of jawed vertebrates, placenta formation in mammals, and

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light-sensing abilities in plants (Feschotte and Pritham 2007; Wells and Feschotte 2020). They have also been responsible for large-scale chromosomal rearrangements and rapid changes in genome size in both plants and animals (e.g. Feschotte and Pritham 2007). In primate genomes, MGEs cause genomic rearrangements, serve as a source of regulatory sequences by providing binding sites for transcription factors, and facilitate epigenetic toolkit development (Lee et al. 2015). Some argue that MGEs may have helped shape genetic material into the earliest genomes (Collens and Katz 2021; Kazazian 2004).

Several hypotheses surround the role of MGEs in shaping the genomes of ciliates, the focus of this article, with most insight emerging from intense study on model ciliate genera such as *Paramecium, Tetrahymena* and *Oxytricha* (Allen and Nowacki 2020; Arnaiz et al. 2012; Bracht et al. 2013; Chen et al. 2014; Chen and Landweber 2016; Guérin et al. 2017; Hamilton et al. 2016; Vogt et al. 2013). Ciliates are a diverse group of unicellular eukaryotes distinguished by both the presence of cilia during at least one life cycle stage and nuclear dimorphism, which is the separation of germline and somatic DNA into distinct nuclei within a single cell (e. g. Jahn and Klobutcher 2002; Katz 2001; McGrath et al. 2007; Prescott 1994). The somatic macronucleus (MAC) is transcriptionally active while the germline micronucleus (MIC) is quiescent throughout most of the ciliate's life cycle (e. g. Duharcourt et al. 2009; Katz 2001; Maurer-Alcalá et al. 2018; Prescott 1994; Prescott 2000; Raikov 1982;). Ciliates are considered to be eukaryotic model organisms (i.e. self-splicing introns and telomeres were both first discovered in ciliates) (Blackburn and Gall 1978; Cech 1985; Hedberg and Johanson 2013; Klobutcher et al. 1981) and they are found in almost every ecosystem, thriving in freshwater, brackish, marine, and terrestrial habitats (Finlay et al. 1998).

While all share dimorphic nuclei, ciliates exhibit a tremendous diversity of nuclear number, ploidy, and genome architecture (McGrath et al. 2007, Maurer-Alcalá et al. 2018; Wang et al. 2017; Yan et al. 2017). Among ciliate species, micronuclear and macronuclear numbers may range from 1 to 20 and 1 to 100, respectively, and ploidy levels can vary from roughly diploid to highly polyploid (McGrath et al. 2007, Parfrey et al. 2010; Raikov 1994; Xu et al. 2012). Some ciliates exhibit long, multi-genic chromosomes, while others possess highly fragmented, gene-sized nanochromosomes that are generated from a transient stage with giant polytene chromosomes (Ammerman 1987; Maurer-Alcalá and Nowacki 2019; Spear and Lauth 1976). Given their plethora of genome structures and their ~1 billion year history on the eukaryotic tree of life (Parfrey et al. 2011; Wright and Lynn 1997), studies in ciliates will help us better define the role of MGEs in shaping genome architecture and evolution in eukaryotes.

Here we review how three different types of MGEs have shaped ciliate genomes: (1) transposable elements (TEs), (2) lateral gene transfer (LGT), and (3) viruses. Transposable elements are DNA sequences that can invade and propagate within genomes and are present in nearly all eukaryotes (e. g. Feschotte and Pritham 2007; Gilbert et al. 2021; Wells and Feschotte 2020). LGT is defined as the movement of genes between distantly related organisms (e. g. Andersson 2005; Cote-L'Heureux et al, submitted; Dunning Hotopp et al. 2007; Sibbald et al. 2020; Soucy et al. 2015; Zhaxybayeva and Doolittle 2011) while transfer of viral sequences between individuals and across lineages is well documented (e.g.

Filée 2009; Filée 2014; Liu et al. 2011; Malik et al. 2017; Schoenfeld et al. 2013). These three types of MGEs are of particular interest as recent evidence suggests they are driving forces in ciliate genome evolution.

While the roles of transposable elements in ciliates are relatively well-characterized, fewer studies focus on genetic material derived from LGT and viral genomes. Transposable elements excise micronuclear-specific sequences during macronuclear development (Chalker and Yao 2011) and are implicated in the origin of nuclear dualism (Bracht et al. 2013; Klobutcher and Herrick 1997; Maurer-Alcalá and Nowacki 2019). Lateral gene transfer, while traditionally observed in prokaryotes, may occur with relative frequency in ciliates and help them enhance their fitness in a given ecological niche (Dobri et al. 2014; Ricard et al. 2006; Xiong et al. 2015). Finally, there is growing recognition of viral interactions with ciliates *via* endosymbionts (Malik et al. 2017), and viral integration and defense-related functions in ciliate genomes (Berjón-Otero, Koslová, and Fischer 2019; Koonin and Krupovic 2017). Below, we summarize information from each type of MGE in separate sections, and then speculate on the epigenetic implications of these mobile elements.

TRANSPOSABLE ELEMENTS ARE ESSENTIAL COMPONENTS OF CILIATE EPIGENETIC TOOLKITS

Ciliate transposon domestication exemplifies the essential role TEs play in ciliate reproduction and nuclear development. Studies in the genera *Paramecium, Tetrahymena,* and *Oxytricha* reveal that large portions of the micronuclear genome, including transposable elements and other repetitive sequences, are selectively excised during macronuclear development (Chalker and Yao 2011). Although TEs are usually considered ‘parasitic’ DNA as their invasion is believed to be detrimental to a host, ciliates rely on tranposase-related proteins to carry out some of this sequence removal (Allen and Nowacki 2017; Bracht et al. 2013; Chalker and Yao 2011; Vogt and Mochizuki 2013; Vogt et al. 2013). RNAi-mediated silencing of transposases in *Paramecium, Tetrahymena,* and *Oxytricha* resulted in inhibition of genome rearrangement and transposon excision (Baudry et al. 2009; Cheng et al. 2010; Nowacki et al. 2009a).

The *Paramecium, Tetrahymena,* and *Oxytricha* micronuclear genomes encode diverse TEs, some of which have been domesticated to carry out DNA excision (Fig. 1; Arnaiz et al., 2012; Chen et al., 2014; Chen and Landweber, 2016; Guérin et al., 2017; Hamilton et al., 2016). The *Paramecium* and *Tetrahymena* MAC genomes encode domesticated PiggyBac-family transposases, and their MIC genomes encode diverse TEs and TE remnants that are typically excised during macronuclear development. For example, the *Paramecium* MIC contains sequences of the L1 subfamily of LINE (long interspersed nuclear element) transposons, as well as multiple DNA transposon subfamilies (Fig. 1; Arnaiz et al. 2012; Guérin et al. 2017). *Tetrahymena*’s micronuclear genome contains LINE and DNA transposon subfamilies, including L1, CRE, Maverick, Helitron, Tc1-Mariner, and PiggyBac (Fig. 1; Hamilton et al. 2016). Meanwhile, the *Oxytricha* MIC genome encodes DNA transposons from the Helitron, Mutator, and Tc1-Mariner subfamilies, the latter of which

are expressed from the MIC to eliminate all germline-limited sequences (Fig. 1; Allen and Nowacki 2017; Chen et al. 2014; Chen and Landweber 2016).

Research on the role of transposon-derived proteins in ciliate DNA excision has shown that ciliates domesticate transposons and repurpose them for new functions in their genomes (Chalker and Yao 2011; Vogt et al. 2013). For example, most internally eliminated sequence (IES) removal in *Tetrahymena* and *Paramecium* is carried out by transposases TPB2 and PiggyMac, respectively (Table 1; Baudry et al. 2009; Bracht et al. 2013; Cheng et al. 2010; Sellis et al. 2021). While these genes are derived from PiggyBac family transposons, they lack terminal inverted repeats and, unlike most transposable elements, are retained in the host MAC genome, indicating that they likely have been domesticated to the benefit of ciliate hosts (Baudry et al. 2009; Vogt et al. 2013). Interestingly, the sequencing of the *Paramecium* germline genome revealed TEs and other non-protein-coding sequences that were not present in DNA extracted from PiggyMac-silenced cells, indicating that the *Paramecium* genome may contain undiscovered excisases (Allen and Nowacki 2020; Guérin et al. 2017). Other *Paramecium* IESs contain inactivated transposons, which are theorized to have co-evolved with *Paramecium* such that they effectively eliminate themselves during MAC development without the use of scnRNA identification pathways (Allen and Nowacki 2017). Transposon domestication may fulfill important functions during nuclear development or simply render transposons nonfunctional. Both cases reflect how transposable elements have elicited genetic responses from ciliates and shaped the structure of their hosts' genomes.

Transposable elements do not always need to be domesticated to influence the ciliate genome, as some ciliates recruit undomesticated transposons to facilitate genome rearrangement (Vogt et al. 2013). For example, *Oxytricha* uses many different TBE (telomere-bearing element) family transposases—which compose almost 20% of its micronuclear genome—to excise germline-restricted DNA (Table 1; Figure 1; Allen and Nowacki 2017; Jangam et al. 2017; Nowacki et al. 2009). Unlike domesticated PiggyBac-family transposases in *Paramecium* and *Tetrahymena*, which are encoded by the macronuclear genome, the TBE transposases that trigger IES excision in *Oxytricha* are expressed from the micronuclear genome, demonstrating that they have not been fully domesticated by their ciliate host (Allen and Nowacki 2017; Vogt et al. 2013; Chen and Landweber 2016; Rzezsutek et al. 2020). This example also counters the narrative that TEs are typically detrimental or "junk" DNA, since *Oxytricha* acquires a large quantity of TBE transposase protein, which is necessary for successful establishment of germline-soma distinction (Cosby et al. 2019; Nowacki et al. 2009a; Vogt et al. 2013; Yerlichi and Landweber 2014). Thus, in this case the transcriptional activity of transposable elements is a vital source of proteins for the developing cell (Table 1; Allen and Nowacki 2017). More broadly, the genetic diversity transposons provide when they invade a cell supplies ciliates an opportunity to develop tools for evolutionary diversification (Bourque et al. 2018; Feschotte and Pritham 2007; Wells and Feschotte 2020).

Some authors argue that nuclear dualism evolved in ciliates as a response to transposable element invasion (Table 1; Allen and Nowacki 2020; Bracht et al. 2013; Klobutcher and Herrick 1997; Maurer-Alcalá and Nowacki 2019). Accommodating TEs while maintaining

some control over genome instability confers huge fitness benefits to the host cell because it equips them with a more dynamic genome (Maurer-Alcalá and Nowacki 2019). However, TEs also can cause catastrophic effects on the ciliate genome, as they are extensive sources of mutations and polymorphisms (Bourque et al. 2018). Thus, nuclear dimorphism and genome rearrangements may have evolved as a mechanism to avoid the harmful effects of transposon invasion, allowing the ciliates to gain stability at the cost of having a more complicated genetic system (Bracht et al. 2013). This example encapsulates that, although transposable elements are sometimes termed “parasitic” or “junk” DNA, they have made significant contributions to shaping ciliate genome architecture and evolution.

LATERAL GENE TRANSFER PLAYS AN IMPORTANT ROLE IN DRIVING CILIATE ADAPTATION AND ECOLOGICAL SPECIALIZATION

Lateral gene transfer (LGT) is the movement of genetic material between organisms by means other than vertical inheritance (Douglass and Langille 2019; Sibbald et al. 2020; Soucy et al. 2015; Zhaxybayeva and Doolittle 2011) and is argued to confer new functions or fitness advantages to ciliates (Dobri et al. 2014; Emameh et al. 2018; Mukhai and Endoh 2004; Newbold et al. 2005; Ricard et al. 2006; Xiong et al. 2015). While traditionally observed in prokaryotes, LGT from bacteria may be frequent in ciliates given that eukaryotes often acquire genes from organisms they take in as food, and many ciliate species are phagotrophic (Doolittle 1998; Keeling and Palmer 2008; Mukhai and Endoh 2004). Studies of LGT in ciliates indicate that bacteria supply ciliates with novel genes, which can provide adaptive advantages and in some cases facilitate ecological specialization (Newbold et al. 2005; Ricard et al. 2006; Xiong et al. 2015). For instance, the *Euplotes raikovi* genome contains a gene involved in protein repair that was likely transferred from an α-proteobacteria (Table 1; Dobri et al. 2014). Several other studies focus on how LGT has enhanced the genome of the model ciliate *Tetrahymena* (Emameh et al. 2018; Mukhai and Endoh 2004). Homology assessment and phylogenetic analyses demonstrate that both the citrate synthase gene (Table 1; Mukhai and Endoh 2004) and β-carbonic anhydrase (Emameh et al. 2018) were acquired through LGT, with the latter possibly involved in virulence and/or homeostasis.

Some ciliates, including species from the orders Entodiniomorphia and Vestibuliferida, have acquired genes *via* LGT that allow them to become ecologically specialized, with the most well-documented examples coming from studies on rumen-dwelling species (Table 1; Miltko et al. 2012; Newbold et al. 2005; Qi et al. 2015; Ricard et al. 2006; Wang et al. 2019; Williams et al. 2020). For instance, *Entodinium caudatum* possesses a glutamate dehydrogenase enzyme used to assimilate ammonia that displays significant sequence similarity to Bacteroidetes-type bacteria, which are abundant in rumen environments (Newbold et al. 2005). A larger-scale study by Ricard and colleagues (2006) on ciliates living in sheep rumens identified 148 ciliate genes primarily involved in carbohydrate metabolism that cluster with bacterial and archaeal genes in phylogenetic analyses and are not present in closely related ciliates. These genes may enable the ciliates to thrive in their anaerobic, carbohydrate-rich environment. Further studies on rumen ciliates detected the production of chitin and pectin-degrading enzymes, some of which bear similarity to those

found in fungi or bacteria and may aid ciliate digestion within the food vacuoles (Miltko et al. 2012; Williams et al. 2020). Lateral gene transfer in ciliates and other microeukaryotes is argued to be widespread (Gabaldón 2020; Grant and Katz 2014; Leger et al. 2018; Ricard et al. 2006), perhaps even comparable to levels found in prokaryotes (Ricard et al. 2006), giving us cause to consider LGT as a possible evolutionary force in ciliate ecological specialization.

LGT may also drive specialization in parasitic ciliates. The facultative fish parasite *Pseudocohnilembus persalinus* hosts 54 putative bacterial-derived genes (Table 1; Xiong et al. 2015). These genes closely resemble those in Proteobacteria, Bacteroidetes, and Firmicutes. Interestingly, they display eukaryote-like structures, indicating that they are integrated into the ciliate genome and not bacterial contaminants. An estimated 20% of these genes may play a role in the ciliate's virulence, contributing to traits like cell adhesion and hemolysis (Xiong et al. 2015). This study reinforces that LGT is present across ciliates with different habitats and life cycles, and shapes ciliate genomes by supplying evolutionary advantages.

LGT-driven specialization is recorded in other diverse protist taxa (Andersson 2009; Cote-L'Heureux et al., submitted; Harding et al. 2017; Keeling and Palmer 2008; Schönknecht et al. 2013; Sibbald et al. 2020; Stairs et al. 2018), supporting our conclusions from ciliate studies that LGT has a significant hand in shaping a species' evolutionary trajectory. There are several reasons why the prevalence of LGT in ciliates may be underestimated, namely the limited molecular data from non-model lineages necessary to assess the true scope of LGT's influence in shaping ciliate genomes. Promising methods for future studies seeking to identify bacterial-derived genes in ciliates include exploiting extensively-fragmented genomes (McGrath and Katz 2007), tracking specific proteins' evolution (Frickey and Kannenberg 2009), and looking for transfer via genomic islands (Emameh et al. 2018).

TRANSFER OF VIRAL DNA INTO THE CILIATE GENOME AND ITS POTENTIAL PROTECTIVE EFFECTS

Similar to LGT, we argue that integration of viral DNA has as yet underappreciated effects in shaping ciliate genomes. Based on the limited data from ciliates coupled with observations from other eukaryotes, we hypothesize that components of viral genomes are widespread in ciliate genomes and may aid host defense. Ciliates frequently interact with viruses in ways that have influenced their genome structure and expression. For instance, ciliates encounter viruses when feeding (Kucera 1992), and *Tetrahymena thermophila* is capable of inactivating certain viruses in the water as it feeds (Pinheiro et al. 2007). Ciliates' ubiquitous contact with viruses may also exert selective pressure to undergo codon reassignments as an antiviral defense strategy (Table 1; Shackleton and Holmes 2008). Indeed, ciliates have experienced numerous independent codon reassignments compared to other eukaryotic lineages (Lozupone et al. 2001). These interactions generate opportunities for viral elements to integrate into ciliate genomes and further shape their evolutionary trajectories.

Endosymbionts may mediate interactions between ciliates and viruses, though the genomic effects of such exchanges may go unnoticed as they do not display the classic phenotypes of viral infection (Malik et al. 2017). Two systems in *Paramecium* exemplify this facilitative role for ciliate symbionts. Paramecia which express the 'killer trait' release toxic particles that kill paramecia lacking this trait upon ingestion (e.g. Pond et al. 1989; Preer and Preer 1967; Schrallhammer and Shweickert 2009; Sonneborn 1938). This capability is conferred by *Caedibacter* endosymbionts, specifically those possessing an organelle called an R-body that is thought to release the toxin (Grosser et al. 2018; Pond et al. 1989; Preer and Jurand 1968; Preer and Preer 1967; Schrallhammer et al. 2012). Observations of phage-like particles near R-bodies, and more recent genomic and transcriptomic sequencing of a *Caedibacter* species, indicate that genes encoding the R-body may be derived from bacteriophages (Pond et al. 1989; Pirritano et al. 2020). In this case, the bacterium serves as a necessary intermediate for viral-derived elements to express a trait that enhances ciliate fitness. Another well-studied system is the association between *Paramecium bursaria* and the large double-stranded DNA virus that infects its green algal symbiont *Chlorella*. Viral particles have been identified on the surface of ciliate cells, in the cytoplasm of the symbiont, and in the digestive vacuoles of the ciliate (Kodama et al. 2017; Milrot et al. 2016; Yashchenko et al. 2008); however, viral integration into the ciliate genome has not been demonstrated (Yashchenko et al. 2012). A more general approach shows that there is almost certainly yet undetected genetic transfer from viruses to eukaryotes mediated by bacterial endosymbionts. Malik and colleagues (2017) characterized the proteomes of 1,223 bacterioviruses, 62 archeoviruses, and 2,155 eukaryoviruses and traced their spread into 1,620 cellular proteomes to estimate the direction of gene transfer. They found a large number of protein fold families shared between bacterioviruses and eukaryotic host proteomes, indicating that genes likely originated in viral lineages and were later transferred to eukaryotes (Malik et al. 2017; Mughal et al. 2020).

The interrelated group of MGEs including Polintons, Polinton-like viruses (PLV), and virophages are ubiquitous across the eukaryotic tree of life and are found in ciliates such as *Tetrahymena thermophila* (Table 1; Kapitonov and Jurka 2006). They are particularly relevant when discussing viral element transfer, as they are known to integrate into host genomes and provide antiviral defense mechanisms. Polintons are a group of large, self-synthesizing transposons that are widespread in eukaryotes, and their size and enzyme similarity with NCLDVs (nucleo-cytoplasmic large DNA viruses) suggest that they evolved from viruses (Fischer and Suttle 2012; Koonin and Krupovic 2017). Computational analysis of protein sequences reveals that Polintons can lead dual lifestyles as either transposons or virions (Krupovic and Koonin 2015). Polinton-like viruses (PLV) share size and sequence similarities with Polintons, yet most lack a retrovirus-type integrase that Polintons possess (Yutin et al. 2015). These viruses have integrated into multiple unicellular eukaryotic genomes. Tlr1 elements (a family of about 30 putative mobile genetic elements) in the *Tetrahymena thermophila* germline genome, which are eliminated during macronuclear development, are a group of PLV, as determined by a helicase and other protein homology (Koonin and Krupovic 2017; Wuitschick et al. 2002). Ciliates may also acquire viral DNA and novel defense mechanisms *via* virophages, which are small, double-stranded DNA viruses that require the presence of another virus to successfully infect a cell (Desnues et

al. 2012). Virophage integration has been documented in numerous microbial eukaryotes including *Tetrahymena,* and transcriptomic data show that virophage-like elements can be expressed in their host (Berjón-Otero et al. 2019; Fischer and Suttle 2011). Interestingly, virophages may also act as "shuttles" to promote gene transfer from their associated giant virus to the host (Filée 2014).

Virophages can play key roles in host defense against their associated giant viruses (Table 1; Berjón-Otero et al. 2019; Blanc et al. 2015; Fischer 2015; Fischer and Hackl 2016; Hackl et al. 2021; Koonin and Krupovic 2016; Mougari et al. 2019; Mougari et al. 2020). While to the best of our knowledge, virophage-mediated defense has not yet been observed in ciliates, it is reasonable to speculate that it occurs. Regardless, virophages and their close evolutionary relatives are proven to integrate into ciliate genomes and survive in multiple forms (Berjón-Otero et al. 2019; Kapitonov and Jurka 2006; Koonin and Krupovic 2017; Wuitschick et al. 2002). When a virophage integrates into its host's genome, it may be activated during subsequent giant virus infections since the virus and virophage promoters are nearly identical (Koonin and Krupovic 2017). Its activation confers a protective effect over the host cell and/or neighboring cells. The mechanism by which this altruistic defense occurs is not fully elucidated, but it is presumed that the virophage suppresses giant virus replication in the host's cytoplasmic virus factory (Koonin and Krupovic 2016).

Our hypotheses that viral integration influences ciliate genome architecture and immune responses are supported by observations in other protists (Blanc et al. 2015; Boratto et al. 2015; Fischer 2015; Fischer and Hackl 2016; Frada et al. 2008; Hackl et al. 2021; Mougari et al. 2019; Mougari et al. 2020; Oliveira et al. 2019). For instance, giant viruses that infect amoebae can exert control over their host's life cycle stage (Boratto et al. 2015; Frada et al. 2008; Oliveira et al. 2019). Virophage-mediated defense has been observed in amoebae (Mougari et al. 2019; Mougari et al. 2020) as well as several species in the SAR (Stramenopiles, Alveolates, and Rhizaria) supergroup, of which ciliates are also members (Blanc et al. 2015; Fischer 2015; Fischer and Hackl 2016; Hackl et al. 2021). Given the diversity of these eukaryotic lineages, virophage influence on ciliate genomes is likely underestimated.

Ciliate-virus interactions, sometimes mediated by bacterial symbionts, likely have under-appreciated effects in shaping ciliate genome content, and may contribute to ciliate immune responses. Viruses may have also played a fundamental role in shaping ciliate, and all eukaryotic, genome organization. Witzany (2016) notes functional similarities between ciliate nuclei and double-stranded DNA viruses, raising the possibility that the dimorphic nuclei of ciliates are derived from viruses that reached a persistent lifestyle. Latent viruses may be associated with sexual reproduction, just as the transcriptionally silent MIC can carry out the transfer of genetic material across generations. DNA virus activation is characterized by DNA amplification and subsequent degradation of non-amplified sequences, which bears some similarity to the transcriptional activation and replication of DNA within the MAC during its development (Witzany 2016). On a broader scale, Collens and Katz (2021) argue that germline-soma distinctions in eukaryotes originated as a response to genetic conflict with MGEs such as viruses, drawing on the widespread occurrence and epigenetic regulation of MGEs as evidence.

Here, we use a broad definition of epigenetics that includes changes in gene expression (i.e. due to chromatin modifications, including through small RNAs) as well as other genotypic changes that have non-Mendelian patterns of inheritance (e.g. LGTs and viruses).

IMPLICATIONS FOR CILIATE EPIGENETICS

The data summarized here indicate that mobile genetic elements frequently integrate into ciliate genomes where they influence genome architecture and evolution. We believe that the transfer and persistence of MGEs leads to transgenerational epigenetic inheritance of their effects on ciliate genomes. We define transgenerational epigenetic inheritance to include non-Mendelian patterns of inheritance of changes in gene expression that are transferred across multiple generations of offspring (e. g. Hanson and Skinner 2016; Neeb and Nowacki 2018). Mechanistically, these changes have previously been linked to the activity of small RNAs, prions, DNA methylation, and histone modifications (Casier et al. 2019; Duempelmann et al. 2020; Liberman et al. 2019).The underlying causes of these inherited changes include transposon invasion, and it is estimated that many are linked to environmental factors like stress (Casier et al. 2019).

Several instances of transgenerational epigenetic inheritance are already described in ciliates. Classical studies identified inheritance of acquired morphological characteristics, mating type determination by environmental conditions or parental phenotype, and serotype expression determination by cytoplasmic states (Beisson and Sonneborn 1965; Pilling et al. 2017; Sonneborn 1943; Sonneborn 1977). Genome scanning, by which scRNAs compare sequences between the MIC and maternal MAC to identify germline-specific DNA destined to be excised, provides an opportunity for changes inflicted upon the maternal MAC to be passed on to the next generation (Allen and Nowacki 2017; Neeb and Nowacki 2018). Other molecular mechanisms such as alternative processing of germline genes in the generation of ciliate gene families are also non-Mendelian in their inheritance (Katz and Kovner 2010).

We argue that the persistent presence of mobile genetic elements such as TEs, lateral gene transfer, and viruses should also be considered instances of transgenerational epigenetic inheritance. These MGEs are foreign material that has been integrated into ciliates' genomes non-Mendelian inheritance. The examples we have discussed illustrate how MGEs have influenced ciliate genome architecture and expression. Importantly, these MGEs have also persisted in the ciliate genome throughout long spans of evolutionary history. In sum, MGEs' impact and persistence in ciliate genomes qualify them as molecular vehicles underlying transgenerational epigenetic inheritance. As such, we should shift the narrative surrounding MGEs and view them not as DNA that is simply deleterious or perhaps neutral, but integral epigenetic components that shape ciliate evolution.

SYNTHESIS

Ciliates, like all eukaryotes, possess chimeric genomes composed of vertically inherited genes as well as various mobile genetic elements. Here we show that transposable elements, lateral gene transfer, and viral elements are substantial forces in shaping ciliate genome architecture and evolution. This conclusion has twofold importance: for one, it leads to

reconsideration as to how we view mobile genetic elements, which are often described as "junk" DNA. While MGEs indeed have detrimental representatives, they are more often neutral or beneficial enough to persist through millions of years of evolutionary history (Arkhipova 2018). Second, the persistence of MGEs in ciliate genomes qualify them as transgenerational epigenetic events because of their non-Mendelian patterns of inheritance. Beyond their demonstrated effects in ciliates, it is likely the MGEs substantially alter the architecture and evolution of genomes in lineages across the eukaryotic tree of life.

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Abbreviations

TE	Transposable element
MGE	mobile genetic element
LGT	lateral gene transfer
MAC	macronucleus
MIC	micronucleus
PLV	Polinton-like viruses
SAR	Stramenopiles, Alveolates, and Rhizaria

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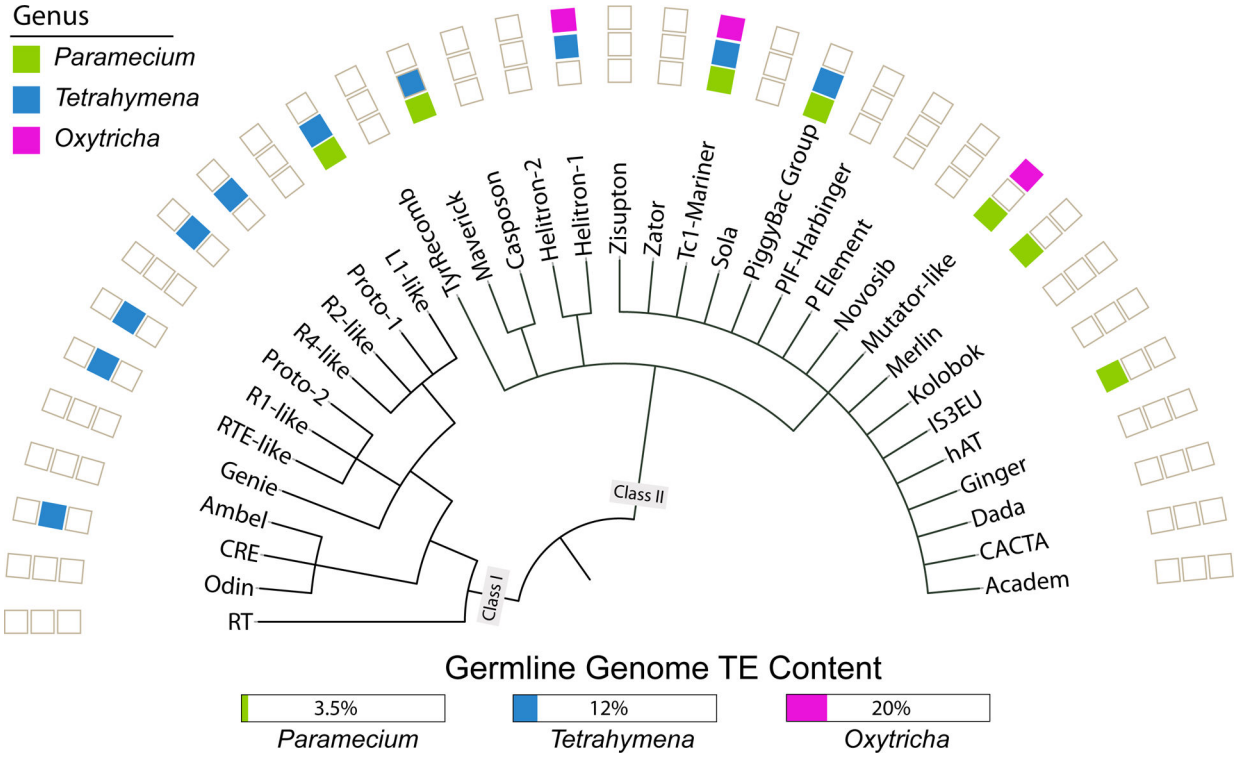

Fig. 1.

Germline genome TE content in three model ciliate genera: *Paramecium* (green), *Tetrahymena* (blue) and *Oxytricha* (pink). **Bottom:** the estimated proportion of the micronuclear genome that is composed of TE or 'TE remnant' sequences (Arnaiz et al., 2012; Chen et al., 2014; Chen and Landweber, 2016; Guérin et al., 2017; Hamilton et al., 2016). **Top:** Classification of selected transposable elements (Curcio and Derbyshire, 2003; Kapitonov and Jurka, 2008; Piégu et al., 2015; Smit et al., 2019; Storer et al., 2021; Wicker et al., 2007).

Table 1.

Evidence for the roles of MGEs (TEs, LGT, and viral elements) in influencing ciliate genome architecture and evolution.

MGE	Evidence in ciliate genomes	References
Transposable elements (TEs)	*Paramecium* and *Tetrahymena* co-opt PiggyBac family transposase for IES excision	Allen and Nowacki, 2017; Allen and Nowacki, 2020; Baudry et al., 2009; Bracht et al., 2013; Chalker & Yao, 2011; Cheng et al., 2010; Sellis et al., 2021; Vogt et al., 2013
	TBE elements in *Oxytricha* carry out IES excision	Allen and Nowacki, 2017; Chen and Landweber, 2016; Jangam et al., 2017; Nowacki et al., 2009a; Rzezsutek et al., 2020; Vogt et al., 2013
	TEs supply proteins and novel genetic material to facilitate evolutionary diversification	Allen and Nowacki, 2017; Bourque et al., 2018; Cosby et al., 2019; Feschotte and Pritham, 2007; Wells and Feschotte, 2020
	TE invasion may have driven the evolution of ciliate nuclear dimorphism	Allen and Nowacki, 2020; Bracht et al., 2013; Klobutcher and Herrick, 1997; Maurer-Alcalá and Nowacki, 2019
Lateral gene transfer (LGT)	*Euplotes raikovi* possesses protein repair gene likely acquired from alphaproteobacteria	Dobri et al., 2014
	Tetrahymena thermophila may have acquired citrate synthase and β-carbonic anhydrase genes from bacteria	Emameh et al., 2018; Mukhai and Endoh, 2004
	Rumen-dwelling ciliates have acquired many bacterial genes related to carbohydrate and anaerobic metabolism that help them thrive in their environments	Newbold et al., 2005; Qi et al. 2015; Ricard et al., 2006; Wang et al., 2019; Williams et al., 2020
	Pseudocohnilembus persalinus hosts 54 genes likely derived from bacteria, 20% of which may contribute to the ciliate's virulence	Xiong et al., 2015
Viral elements	Viruses may exert selective pressure on ciliates to undergo codon reassignments	Lozupone et al., 2001; Shackleton and Holmes, 2008
	Tetrahymena thermophila genome contains Polintons, which can live in both transposon and virion stages, and Tlr elements, which are derived from Polinton-like viruses	Kapitonov and Jurka, 2006; Koonin and Krupovic, 2017; Krupovic and Koonin, 2015; Wuitschick et al., 2002
	Virophages, which can participate in host defense, have been identified in *Tetrahymena*	Berjón-Otero, Koslová, and Fischer, 2019; Koonin and Krupovic, 2017; Krupovic and Koonin, 2015