Published in final edited form as:

Res Microbiol. 2011 ; 162(6): 578–586. doi:10.1016/j.resmic.2011.05.001.

***Tetrahymena thermophila*, a unicellular eukaryote with separate germline and somatic genomes**

Eduardo Orias^{*}, Marcella D. Cervantes, and Eileen P. Hamilton

Department of Molecular, Cellular and Developmental Biology, University of California at Santa Barbara, Santa Barbara, CA 93106, USA

Abstract

Tetrahymena thermophila is a ciliate -- a unicellular eukaryote. Remarkably, every cell maintains differentiated germline and somatic genomes: one silent, the other expressed. Moreover, the two genomes undergo diverse processes, some as extreme as life and death, *simultaneously in the same cytoplasm*. Conserved eukaryotic mechanisms have been modified in ciliates to selectively deal with the two genomes. We describe research in several areas of *Tetrahymena* biology, including meiosis, amitosis, genetic assortment, selective nuclear pore transport, somatic RNAi-guided heterochromatin formation, DNA excision and programmed nuclear death by autophagy, which has enriched and broadened knowledge of those mechanisms.

Keywords

Aneuploidy; Apoptosis; Argonaute superfamily; Cell cycle; Chiasmata; Chromosome; Conjugation; Copy number control; Crescent; Double-strand break; Epigenetics; Genome scan; Haplotype; Importin; Macronucleus; Micronucleus; Non-coding transcript; Nuclear pore complex; Nucleoporin; Phenotypic assortment; Recombination; Somatic polyploidy; Transposon

1. Introduction

Tetrahymena thermophila is a free-living unicell that belongs to the ciliated Protozoa, a major, ecologically successful monophyletic group of unicellular eukaryotes. Their closest known relatives, Dinoflagellates and Apicomplexans, which include Plasmodium and other obligate parasites, are also unicellular. Species of the genus *Tetrahymena* live in freshwater environments distributed throughout the world. A remarkable, virtually unique feature of the ciliates is that they maintain stably differentiated germline and somatic nuclear genomes *within a single cell*. The germline genome is housed in the micronucleus (MIC) while the somatic genome is housed in the macronucleus (MAC). The MIC genome, which is transcriptionally silent in vegetative cells, is the store of genetic information for the sexual progeny. The MAC genome is the primary source of gene transcripts in the cell; its “working” gene products (proteins and non-protein coding RNAs) maintain the functions required for the life of the organism and thus the MAC genome directly determines the phenotype of cell.

© 2011 Elsevier Masson SAS. All rights reserved.

^{*}Correspondence and reprints: Eduardo Orias: Eduardo.Orias@lifesci.ucsb.edu; Marcella D. Cervantes: Marcella.Cervantes@lifesci.ucsb.edu; Eileen P. Hamilton: Eileen.Hamilton@lifesci.ucsb.edu .

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

As will be described in this review, the two genomes are genetically and developmentally related but exhibit dramatic differences in structure and function. These include DNA content, histones and their modifications, timing of cell cycle events and mode of genome distribution at cell division. During asexual multiplication, these differences are faithfully inherited potentially indefinitely, as *Tetrahymena* cells are somatically immortal. Throughout their life cycle, germline and somatic genomes undergo diverse processes, with radically different outcomes, *at the same time and in the same cytoplasm*. Twice during conjugation, the non-reproductive, sexual stage of the cell cycle, entire genome copies are efficiently and precisely aligned and compared in a matter of hours for different purposes: during meiosis to undergo evolutionarily conserved homologous recombination and later to identify and eliminate germline-specific transposon and other DNA sequences in the newly differentiated somatic genome via an RNAi-guided mechanism.

Much is now understood about mechanisms that establish and maintain *Tetrahymena* genome differences, in part because of the multidimensional experimental advantages of this unicellular organism (genetic, biochemical, molecular, genomic). In this review, we cover special properties of *Tetrahymena*'s genomes and the fascinating genetic, cellular and developmental mechanisms, mostly using conserved eukaryotic biology, that shape them.

2. Germline and somatic genomes during the life cycle

As is typical of ciliates, the life cycle of *T. thermophila* has two major components: asexual reproduction by binary fission and conjugation, the non-reproductive sexual stage (Fig. 1). During asexual reproduction, the MIC genome divides mitotically, while the MAC divides amitotically. During conjugation, the parental MIC in each conjugant undergoes meiosis, haploid gamete nuclei are formed and reciprocal fertilization gives rise to a diploid zygotic nucleus. Progeny MICs and MACs differentiate from mitotic copies of the zygote nucleus. The parental MAC genome contributes no DNA to the sexual progeny and is destroyed through autophagy-mediated programmed nuclear death. We return to these events in more detail later in this review.

2.1. The vegetative micronuclear genome

The diploid MIC contains 5 pairs of metacentric chromosomes (Ray, 1956). Early analysis of MIC DNA suggested that the MIC genome had about 15% greater DNA sequence complexity than the MAC genome, accounted for primarily by moderately repetitive sequences (Yao and Gorovsky, 1974). If this estimate is correct, then the MIC genome size should be at least 120 Mb based on the known MAC genome size (below). A preliminary MIC sequencing project was recently carried out (K. Collins, personal communication) and a new project designed to generate a high coverage sequence and assembly of the MIC genome is underway (R. S. Coyne, personal communication).

The size difference between the two genomes is mainly due to “internally eliminated sequences” (IESs), which are deleted in a site-specific manner over the course of a few hours during MAC differentiation. There are an estimated 6,000 distinct IESs in the MIC genome. IESs include transposable elements (Tel element, Cherry and Blackburn, 1985; the Maverick family DNA Tlr transposon, Pritham et al., 2007; Wuitschick et al., 2002) and non-LTR retrotransposons related to LINE elements in mammalian genomes, Fillingham et al., 2004) and additional repeated or unique MIC sequences. A striking aspect of IES removal is that IES identity (that is, what constitutes IES DNA and what does not) is not permanently encoded in the germline DNA. Instead, that determination is made de novo at each conjugation episode by an RNAi-mediated genome-wide comparison between the parental MAC genome, which lacks IES DNA, and the MIC genome, which has all the IES DNA. This remarkable mechanism is described in detail in a subsequent section.

2.2. The vegetative macronuclear genome

The macronuclear genome has been sequenced (Eisen et al., 2006; Coyne et al., 2008). This sequence draft is of high quality, facilitated by the paucity but not complete absence of a repeated sequence in the MAC and the use of a whole-genome homozygous cell line as MAC DNA source. The MAC genome contains ~104 Mb of DNA and is thus comparable in size to the genome of the nematode *Caenorhabditis elegans*. It contains ~25,000 known or predicted protein-coding genes, a number comparable to that of the human genome. There is general agreement that the lineages that gave rise to ciliates and Metazoa diverged at or very near the base of the eukaryotic phylogenetic tree (e.g., Parfrey et al., 2010). Yet, *T. thermophila* and humans share more orthologs with one another than are shared between humans and their more closely related microbial eukaryote model organism, the yeast *S. cerevisiae* (Eisen et al., 2006). *Tetrahymena* genomic resources, including genome sequence, transcriptome expression profiles, subcellular organelle proteomes and genetic maps, are listed and referenced as Supplementary Material.

The *T. thermophila* MAC genome consists of approximately 180 chromosomes (Hamilton, Dear and Orias, in preparation). These are generated during MAC differentiation by site-specific breakage at highly conserved, germline-encoded chromosome breakage sequences (Cbs) (Fan and Yao, 2000; Hamilton et al., 2006) and concerted telomere addition to the newly formed ends (Fan and Yao, 1996). The MAC chromosome which encodes the 35S ribosomal RNA precursor (the rDNA) is unique in that it is maintained at ~9,000 copies per MAC (Gall, 1974). The remaining chromosomes are often referred to as the “bulk chromosomes”. Based on genetic data from a handful of loci, they were inferred to be maintained at an average of ~45 G1 copies per cell (Doerder et al., 1992). The MAC genome sequencing project confirmed the uniformity of bulk chromosome average copy number (Eisen et al., 2006).

Developmentally programmed chromosome fragmentation is shared by all ciliates thus far investigated. It is moderate in *Tetrahymena*, resulting in the order of hundreds of genes per MAC chromosome, unlike cases of extreme fragmentation in other ciliate clades, where most MAC chromosomes contain a single gene (Prescott, 1994; Riley and Katz, 2001). The evolutionary pressures that drove chromosome fragmentation are not clear. Chromosome fragmentation may have co-evolved with amitotic MAC division (see below) as one way to insure integrity of distribution of rather extended DNA molecules by making them smaller. A better understood consequence of chromosome fragmentation in *T. thermophila* is discussed in the section on amitotic division.

3. Multiple modes of genome distribution at nuclear division

Three distinct modes of genome distribution at nuclear division have been documented in *Tetrahymena*: MIC mitosis, MIC meiosis and MAC amitosis. Nuclear divisions are classically defined as being closed or open based on the retention or breakdown of the nuclear envelope during nuclear division. By this criterion, all nuclear divisions in *Tetrahymena* are closed. Recently, the central channel “FG-repeat” nucleoporin Nup98 has been found to play an important role as a nuclear pore “gatekeeper” (reviewed in Iwamoto et al., 2010; see also section 4 in this review). Its behavior is considered to provide a more discriminating indicator of whether mitosis is open or closed (reviewed by De Souza and Osmani, 2007). Recent investigations suggest that *T. thermophila* MIC-specific Nup98 proteins remain associated with the nuclear pore complex during mitosis (Iwamoto et al., 2009) and that nuclear division may indeed be fully closed. During asexual multiplication in *T. thermophila*, progress of the MIC and MAC through the cell cycle is strikingly asynchronous. The MIC has an abbreviated G1 phase: its S phase begins minutes after the completion of mitosis. MIC mitosis occurs during MAC G2, while MIC S-phase occurs

during MAC amitosis (Woodard et al., 1972; Doerder and Debault, 1975). Maintaining nuclear envelope closure may play a critical role in facilitating the temporal dissociation of mitotic and amitotic cell cycle events.

3.1. MIC meiosis: Stretching the MIC genome beyond the length of the cell

In *Tetrahymena*, meiosis occurs in the absence of cell division, a property shared with ciliates in general. It also exhibits three other unusual properties. First, unlike many other eukaryotes, meiosis does not involve a cytologically observable synaptonemal complex nor does the genome encode any recognizable homologs relevant to this structure (Wolfe et al., 1976; Mochizuki et al., 2008). Nonetheless, conserved homologous meiotic recombination does occur and allows the construction of conventional genetic linkage maps of the *Tetrahymena* MIC genome (Brickner et al., 1996).

Second, during prophase of meiosis I, the MIC elongates ~50-fold, becoming about twice the length of the cell that houses it. The resulting linear structure is called the “crescent” because it is usually crescent or U-shaped (Ray, 1956). All centromeres cluster at one end, while all MIC telomeres are clustered at the other, more tapered end (Loidl and Scherthan, 2004; Cervantes et al., 2006; Cui and Gorovsky, 2006). The *Tetrahymena* crescent is reminiscent of the meiotic bouquet arrangement, which is conserved among eukaryotes (see Davis and Smith, 2006). Homologous meiotic recombination occurs in the crescent, as Dmc1, which in *Tetrahymena* is the recombination protein required for interhomolog meiotic recombination, prominently localizes to this structure (Howard-Till et al., 2011). Double-strand breaks (DSBs) -- but not crossovers -- and the operation of the ATM/ATR DNA damage signaling pathway are normally required for crescent elongation. DSBs are catalyzed by a conserved eukaryotic nuclease Spo11p homolog. The conserved Hop2p homolog promotes DSB resolution in a way that favors interhomolog cross-overs, cytologically visualized as chiasmata (Mochizuki et al., 2008; Loidl and Mochizuki, 2009).

A major challenge of meiosis in *Tetrahymena* is the alignment of the two copies of each parental MIC genome (size >120 Mb) with one another, with high precision, in a few hours. This task is complicated by the presence of many transposon copies and other repeated sequences in the MIC genome. The crescent may facilitate this alignment in several ways: by stretching out the DNA, by physically constraining the two ends (centromere and telomere) of homologous arms, and by limiting the homology search to essentially one-dimensional space.

Third, *Tetrahymena* exhibits an estimated one to four chiasmata per bivalent (Loidl and Scherthan, 2004). Chiasmata are generally required for the orderly separation of meiotic bivalents, thus avoiding aneuploidy. If chiasmata occurred at totally random genome locations in *Tetrahymena*, the Poisson distribution of this small number of chiasmata would predict the relatively frequent occurrence of chiasmata-less chromosomes with consequent abnormal segregation of those chromosomes. As in other eukaryotes (e.g. in *Saccharomyces cerevisiae*, Sun et al., 1989, and *Schizosaccharomyces pombe*; Cervantes et al., 2000), chiasmata in *Tetrahymena* may be targeted to specific locations (Loidl and Scherthan, 2004).

3.2. MAC amitosis: Random distribution of the expressed genome

MAC chromosome copies are randomly distributed during MAC amitosis in the course of asexual propagation (Allen and Nanney, 1958; Orias and Flacks, 1975). Consequently, a cell with a “heterozygous” MAC containing a comparable number of *A* and *a* alleles at a single locus generates descendants whose *A:a* allele ratios can vary widely between ~45 *A*: 0 *a* (pure *A* type) to 0 *A*: ~45 *a* (pure *a* type). Once a cell of pure MAC genotype is generated, it

remains pure during subsequent asexual propagation. The process that generates pure MAC genotypes is called assortment. When all loci are considered, and after enough fissions, every MAC has the potential to become a whole genome “homozygote”, pure for a single, randomly selected whole-genome haplotype (Orias, 2000).

Spontaneous MAC homologous recombination is rare. As a consequence, parental allele combinations of *two* loci that reside on the *same* MAC chromosome have a strong tendency to coassort (assort together) during asexual propagation (Longcor et al., 1996; Wong et al., 2000). Independent assortment generally means that two loci are on different MAC chromosomes – but not always, as rare hotspots of MAC recombination have been detected (Deak and Doerder, 1998; Hamilton and Orias, unpublished observations).

Maintenance of a uniform MAC chromosome copy number (Eisen et al., 2006) in spite of amitotic division requires the existence of a presently unidentified mechanism that independently regulates the copy number of each bulk chromosome (Preer and Preer, 1979). Amitotic distribution also implies that every *Tetrahymena* cell has to live with transient, random short-term variation in MAC DNA content and copy number of individual bulk chromosomes. Indeed, progeny can survive severe aneuploidy in their newly differentiated MACs; they grow slowly at first but later reach normal growth rates (Bruns et al., 1983). Copy number control mechanisms undoubtedly ensure that gene dosage imbalances are transient.

Assortment has additional genetic consequences. *Tetrahymena* is an outbreeder and heterozygosity is common in the natural environment (Nanney, 1980). Considering just a single heterozygous locus, assortment of the ~45 copies can generate phenotypes that essentially vary continuously over almost a two order-of-magnitude dynamic range, in contrast to the three phenotypes possible in diploid nuclei. Another consequence of assortment, coupled with MAC chromosome fragmentation, is that a heterozygous cell that starts with just one DNA polymorphism in every MAC chromosome can, after enough fissions, independently assort up to at least $\sim 2^{180}$ different whole-genome haplotypes, thus enabling its descendants to explore an enormous amount of genetic diversity space. Assortment and coassortment enrich the range of genetic mechanisms available to *Tetrahymena* and provide useful tools for genetic mapping as well as for a myriad of experimental purposes (Turkewitz et al., 2002).

4. Maintaining nuclear differentiation: the critical role of selective transport across the nuclear envelope

Differential MIC and MAC protein composition was described in *Tetrahymena* long ago (Allis et al, 1979) and these functional differences were shown to be maintained by controlling the selectivity of protein transport into each nucleus (White et al., 1989). In eukaryotes, transport of macromolecules > 40 kDa across the nuclear envelope relies on highly conserved “FG-repeat” nucleoporins, which act as “gatekeepers” in the nuclear pore central channel (Iwamoto et al., 2010). Importin β -superfamily proteins are transport receptors or carriers, which “unlock the gate” and transport cargos across the nuclear pore by interacting with phenylalanine residues of the gatekeepers (FG-repeat nucleoporins). Importin α proteins are “adaptors”, which connect cargos to importin β -proteins through specific nuclear localization signals in the cargos (Terry et al., 2007).

Recent studies support the hypothesis that in *Tetrahymena* differential MIC and MAC function is regulated by using MIC- and MAC-specific transport adaptors and nuclear pore “gatekeepers” to restrict nuclear access to proteins specifically targeted to either nucleus. *T. thermophila* importin α -like proteins have high MIC/MAC specificity; among 10 homologs

thus far identified, 9 have MIC-specific localization, while the exception localizes specifically to the MAC (Malone et al., 2008). For the MAC-specific importin α -like protein, either the N-terminus (putative importin β -binding domain) or the C-terminus (putative cargo-binding domain) was sufficient to target the protein to the MAC nuclear envelope in vivo. Reciprocal swapping of the putative importin β -binding domains of two nuclear specific importin α -like proteins yielded chimeric proteins with promiscuous MIC/MAC localization and with defects in transport (Malone et al., 2008).

Among 13 conserved core nucleoporins identified in *Tetrahymena*, all but four localize to both MIC and MAC. The four exceptions are the only homologs of the central channel nucleoporin Nup98 (Iwamoto et al., 2009). Strikingly, the two Nup98 homologs with MAC-specific localization have conserved GLFG repeats in their N-termini, while the two homologs that localize specifically to the MIC instead carry novel NIFN repeats. When N-terminal domains were swapped between MIC- and MAC-specific Nup98 homologs, nuclear localization of the overexpressed chimeric proteins was predominantly according to the C-terminal domains, which contain nucleoporin2 domains (Fig. 2), suggesting that the C-terminal domain is important for selectively targeting the Nup98p gatekeepers to the correct nucleus. Nuclear pores containing the MAC-repeats:MIC-localized Nup98p chimera blocked the passage of MIC- as well as MAC-targeted cargo into the MIC. Likewise, nuclear pores containing the MIC-repeats:MAC-localized Nup98p chimera blocked the passage of MAC- as well as MIC-targeted cargo into the MAC (Fig. 2). In contrast, nuclear pores with either chimeric Nup98 allowed passage of a control protein, histone 2B, normally targeted to both nuclei. Thus, the Nup98 N-terminal repeats domain functions to prevent transport of the nucleus-specific cargo to the wrong nucleus, but it is not sufficient to allow transport of nuclear-specific cargo to the right nucleus. The findings on importin α -like and Nup98 proteins suggest that faithfully targeted nuclear localization and transport require that the N- and C-terminal domains be correctly matched in each protein.

In contrast to the importin α -like proteins, *Tetrahymena* importin β -like proteins are relatively non-nuclear-specific: 6 localize to both MIC and MAC, 2 localize exclusively to the MAC and one is found primarily in the cytoplasm (Malone et al., 2008). Thus, if importin β -like proteins physically participate in *selective nuclear transport* in *Tetrahymena*, they seem to contribute little or no specificity to transport selectivity. Examples are known in other organisms where importin- β can be bypassed in nuclear transport (Terry et al., 2007).

MIC/MAC-specific nuclear transport machinery may be established at the time when newly divided MIC and MAC diploid anlagen are deposited at opposite locations in each conjugant (stage 5, Fig. 1) and may well be the key event that determines the alternative fate of the pluripotent anlagen (Goldfarb and Gorovsky, 2009).

Interestingly, importin- α -like and Nup98p studies have revealed that, among the nuclear-specific transport proteins identified in *Tetrahymena*, the MAC-specific homologs have features and organization that are the most evolutionarily conserved with other eukaryotes, while MIC-specific counterparts are the most divergent. This difference could be correlated with the lack of gene expression of the MIC genome, which is arguably the most striking evolutionary deviation of the ciliate MIC from typical eukaryotic nuclei.

5. Programmed RNAi-guided DNA excision from the somatic genome

Transposons behave as molecular parasites that pose a constant threat to genome stability. Complete transposons encode a transposase that, if expressed, allows them to insert themselves at new locations in the genome, potentially disrupting genes or transcriptional units. Because they can spread so readily, every organism has found a way to live with its

transposons by keeping them in check. Diverse eukaryotes share an ancient and highly conserved mechanism to silence transposons by converting potentially active transposon chromatin to repressive heterochromatin using an RNAi-guided process that targets histone H3 lysine 9 and 27 methylation (Malone and Hannon, 2009). Argonaute proteins, which are a key component of the mechanism of repressive heterochromatin formation, are members of a highly conserved protein superfamily found also in bacteria. Some Argonaute proteins have the capacity to display on their surface a specific short (20-30 nt) single-stranded RNA molecule (the “guide strand”) which allows them to interact by sequence complementarity to specific cognate transcripts and initiate specific heterochromatin formation, a phenomenon known as transcriptional gene silencing (TGS) (Malone and Hannon, 2009). Other Argonaute-small RNA complexes have the capacity to destroy or sequester cognate transcripts, a phenomenon known as post-transcriptional gene silencing (PTGS). Both of these conserved mechanisms function in *Tetrahymena*.

Tetrahymena cells not only use conserved heterochromatin biology to silence transposons in the newly differentiating somatic nucleus, but they take this process to its ultimate conclusion: they excise and destroy the silenced DNA (Fig. 3). Transposon DNA is found in the *Tetrahymena* germline genome, where it normally poses minimal threat because germline genes are not expressed. During the differentiation of a new MAC genome, excision of IES (transposon and other repeated sequence) DNA occurs at an estimated 6,000 distinct sites per haploid genome. IES excision has been observed in every ciliate species that has been investigated. While the main elements of heterochromatin formation are highly conserved among living eukaryotes, the way in which the guide RNAs are generated in *Tetrahymena* is illuminating and impressive in terms of its magnitude and carefully organized timing during a period of just a few hours. Key steps involved in this remarkable process of heterochromatin formation and excision are listed in Table 1, along with the proteins experimentally demonstrated to be required at each step and relevant specific literature references. A more detailed narrative of the current model follows.

Promiscuous (non-coding) transcripts are generated by bidirectional transcription of the entire MIC genome during meiosis. These transcripts putatively reanneal to form double-stranded RNA molecules, which are digested to short (27-30 bp) double-stranded RNA (scan RNA = scnRNA) by a process requiring a specific dicer (one of 3 distinct homologs encoded in the genome). scnRNA molecules are exported to the cytosol, where they form a complex with a specific member of the piwi/argonaute-family (one of 8 genome-encoded paralogs with distinct functions; see Supplementary Material). The scnRNA “passenger” strand is removed, generating “mature” Ago-scnRNA complexes displaying the “guide” strand.

Mature Ago-scnRNA complexes are then selectively transported into the *parental* MAC, a process dependent on the novel protein “Gentleman-in-waiting” (Gwi1p). Because in the absence of Gwi1p, the Ago-scnRNA remains in the cytoplasm, Gwi1p may be a link in the selective targeting of Ago-scnRNA complexes to the nuclear pore complex of the parental macronucleus and not to the other nuclei present in the same cytoplasm. Once in the parental MAC, the Ago-scnRNA complexes perform a “genome scan” by interacting with whole-MAC-genome non-coding transcripts; those that find cognate RNA sequences (MAC-destined sequence) are disabled and ultimately destroyed. The survivors, which are the IES-derived Ago-scnRNA complexes, are transported to the *newly differentiating* MAC anlagen. These IES-derived Ago-scnRNA complexes are the physical basis of the epigenetic IES-identifying information which is transmitted from the old to the new somatic genome across sexual generations.

Once in the MAC anlagen, IES-derived Ago-scnRNA complexes interact with cognate sequences in non-coding transcripts of the entire MAC anlagen genome (which at this stage represents the whole germline genome) and are required for the induction of classical histone modifications (H3K9 and K27 me3), in turn required for heterochromatin formation. The heterochromatin aggregates into discrete intra-anlagen “elimination bodies” and it is then excised (spliced out) by a largely uncharacterized mechanism that requires a transposase in the *Tetrahymena* genome derived from a piggyBac transposon.

6. Programmed nuclear death by autophagy

During conjugation, selected nuclei (three non-participating meiotic products, the parental MAC and a new MIC) are destroyed by programmed nuclear death (PND). The best studied case of PND is the selective resorption of the parental MAC after the new MACs begin to differentiate (Akematsu et al., 2010, Akematsu and Endoh, 2010, and references therein cited). These studies, and earlier ones referenced therein, have shown that PND in *Tetrahymena* shares highly conserved elements with the caspase-independent, apoptosis inducing factor (AIF)-mediated type of programmed cell death (apoptosis) pathway in yeast and multicellular eukaryotes. This pathway is characterized by dependence on two components stored in mitochondria: AIF and endonucleases that carry out the coarse degradation of nuclear DNA into pieces 10's of kb long. Potential molecular marks for the degradation system (negatively charged phospholipids and certain sugars on the outer membrane leaflet) have been detected on the parental MAC surface but are missing in co-existing nuclei in the same cytosol; these marks are analogous to those on the surface of apoptotic cells targeted for phagocytosis in metazoa and are referred to as the “eat-me” signal. Initiation of PND in *Tetrahymena* requires the PI3-kinase signaling pathway, which is necessary for many types of autophagy in yeast and animals. However, much remains unknown about commitment to the death program, how the PND program is aborted if differentiation of the new MAC fails and the primary event that *selectively* dooms the parental MAC to PND.

PND is executed by macroautophagy of the parental MAC and is accompanied by dramatic changes in its nuclear envelope, including the loss of nuclear pores. This process involves two special features. 1) The parental macronuclear envelope is *directly* transformed into an autophagic membrane. This direct transformation differs from autophagy in other eukaryotes, in which isolation of the autophagic compartment is accomplished by *engulfment* by a topologically spherical vesicle. 2) The mechanism of delivery of AIF and endonucleases to the parental MAC is fusion with a more conventional mitochondria-containing autophagic compartment; this process ensures that the lethal, mitochondrial-stored weapons are targeted exclusively to the parental MAC.

The discovery of a conserved AIF-dependent pathway in *Tetrahymena* establishes a robust mechanistic link between PND in this unicell and apoptosis in multicellular organisms. Cell survival is essential; thus in *Tetrahymena*, the AIF pathway clearly functions exclusively as a programmed nuclear death pathway and *not* as an apoptotic program. Conservation of the AIF pathway in eukaryotic groups as distantly related as ciliates, green plants, metazoa/fungi and Dictyostelium suggests that it represents an ancient cell biological mechanism, perhaps already present in the last common eukaryotic ancestor, which evolved or was subsequently recruited as a way to induce the suicide of a unicell by targeting its nuclear DNA. It also suggests that the AIF pathway may well exist undiscovered in other unicellular eukaryotes. Studies on programmed nuclear death in *Tetrahymena* are broadening and enriching knowledge of eukaryotic autophagy and apoptosis, while providing a useful model system for the elucidation of unanswered questions about these processes.

7. Perspectives

Every organism alive today draws on a remarkable set of biological mechanisms inherited from an unbroken line of ancestors who managed to survive over billions of years in a highly complex and changing environment. *Tetrahymena* is one of those model organisms that, because of serendipitous biological properties (Turkewitz et al., 2002) and the ingenuity of human investigators, can relatively easily be experimentally coaxed to yield knowledge about those fundamental biological mechanisms.

By possessing germline/soma differentiation, embodied in two functionally distinct nuclei that coexist in a shared cytosol, the ciliates are a beautiful “experiment of Nature”. This review has largely been focused on research in *Tetrahymena* that promises to continue to illuminate diverse areas of the biology of the *nucleus*, the hallmark of eukaryotes. These processes are important for genome maintenance, such as meiosis, mitosis and amitosis, transcriptional gene silencing, selective transport and programmed death. Particularly valuable for experimental research are the nuclear gymnastics of conjugation required to transition from diploid to haploid and back to diploid genome stages in synchronous progression in less than a day, the need to align and compare relatively large genomes and the prodigious abundance in which macromolecules used stoichiometrically and required in excess (e.g., for heterochromatin formation), must be quickly synthesized.

By all the most powerfully constructed phylogenies, the ciliates are very distantly related to the few, relatively narrow evolutionary groups of eukaryotes (metazoa/fungi and plants) whose study over the years has generated most of our current knowledge of eukaryotic molecular, cellular and developmental biology. Ciliates and other diverse groups of unicellular eukaryotes have a great deal to contribute to the dissection of core, conserved eukaryotic mechanisms. They can also greatly help to illuminate the impressively modern cell biology already possessed by the last common ancestor of the eukaryotes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Ms. Teal R. Hamilton for drawing Fig. 1 and Drs. Tokuko Haraguchi and Kazufumi Mochizuki for permission to reproduce Figs. 2 and 3, respectively. We acknowledge Drs. Takahiko Akematsu, Martin A. Gorovsky, Tokuko Haraguchi, Masaaki Iwamoto, Geoffrey Kapler, Joseph Loidl, Kazufumi Mochizuki, Ronald E. Pearlman and anonymous reviewers for significant improvements of the manuscript. We take responsibility for any remaining errors. We gratefully acknowledge support from grant RR-009231 from the National Center for Research Resources of the National Institutes of Health and grant MCB-1025069 from the National Science Foundation. We regret that for reasons of space and reference limits we could not include and cite other ground-breaking areas of *Tetrahymena* research enabled by the availability of the genome sequence, or cite more than one or two relevant reviews. We apologize to the authors of work not covered or indirectly cited through reviews.

References

- Akematsu T, Endoh H. Role of apoptosis-inducing factor (AIF) in programmed nuclear death during conjugation in *Tetrahymena thermophila*. *BMC Cell Biol.* 2010; 11:13. [PubMed: 20146827]
- Akematsu T, Pearlman RE, Endoh H. Gigantic macroautophagy in programmed nuclear death of *Tetrahymena thermophila*. *Autophagy.* 2010; 6:901–911. [PubMed: 20798592]
- Allen SL, Nanney DL. An analysis of nuclear differentiation in the selfers of *Tetrahymena*. *Amer. Naturalist.* 1958; 92:139–160.IV: Model of subnuclear segregation in the macronucleus of ciliates. *Amer. Naturalist.* 92:161–170. Appendix by Schensted.

- Aronica L, Bednenko J, Noto T, DeSouza LV, Siu KW, Loidl J, Pearlman RE, Gorovsky MA, Mochizuki K. Study of an RNA helicase implicates small RNA-noncoding RNA interactions in programmed DNA elimination in *Tetrahymena*. *Genes Dev.* 2008; 22:2228–2241. [PubMed: 18708581]
- Bednenko J, Noto T, DeSouza LV, Siu KW, Pearlman RE, Mochizuki K, Gorovsky MA. Two GW repeat proteins interact with *Tetrahymena thermophila* argonaute and promote genome rearrangement. *Mol Cell Biol.* 2009; 29:5020–5030. [PubMed: 19596782]
- Bowman GR, Smith DG, Siu KW, Pearlman RE, Turkewitz AP. Genomic and proteomic evidence for a second family of dense core granule cargo proteins in *Tetrahymena thermophila*. *J. Eukaryot Microbiol.* 2005; 52:291–297. [PubMed: 16014006]
- Brickner JH, Lynch TJ, Zeilinger D, Orias E. Identification, mapping and linkage analysis of randomly amplified DNA polymorphisms in *Tetrahymena thermophila*. *Genetics.* 1996; 143:811–821. <http://www.genetics.org/cgi/reprint/143/2/811>. [PubMed: 8725229]
- Bruns PJ, Brussard TB, Merriam EV. Nullisomic *Tetrahymena*. II. a Set of Nullisomics Define the Germinal Chromosomes. *Genetics.* 1983; 104:257–270. [PubMed: 17246135]
- Cervantes MD, Farah JA, Smith GR. Meiotic DNA breaks associated with recombination in *S. pombe*. *Mol Cell.* 2000; 5:883–888. [PubMed: 10882124]
- Cervantes MD, Xi X, Vermaak D, Yao MC, Malik HS. The CNA1 histone of the ciliate *Tetrahymena thermophila* is essential for chromosome segregation in the germline micronucleus. *Mol Biol Cell.* 2006; 17:485–497. [PubMed: 16251352]
- Chalker DL. Dynamic nuclear reorganization during genome remodeling of *Tetrahymena*. *Biochim Biophys Acta.* 2008; 1783:2130–2136. [PubMed: 18706458]
- Cheng CY, Vogt A, Mochizuki K, Yao MC. A domesticated piggyBac transposase plays key roles in heterochromatin dynamics and DNA cleavage during programmed DNA deletion in *Tetrahymena thermophila*. *Mol Biol Cell.* 2010; 21:1753–1762. [PubMed: 20357003]
- Cherry JM, Blackburn EH. The internally located telomeric sequences in the germ-line chromosomes of *Tetrahymena* are at the ends of transposon-like elements. *Cell.* 1985; 43:747–758. [PubMed: 3000613]
- Couvillion MT, Lee SR, Hogstad B, Malone CD, Tonkin LA, Sachidanandam R, Hannon GJ, Collins K. Sequence, biogenesis, and function of diverse small RNA classes bound to the Piwi family proteins of *Tetrahymena thermophila*. *Genes Dev.* 2009; 23:2016–2032. [PubMed: 19656801]
- Coyne RS, Thiagarajan M, Jones KM, Wortman JR, Tallon LJ, Haas BJ, Cassidy-Hanley DM, Wiley EA, Smith JJ, Collins K, Lee SR, Couvillion MT, Liu Y, Garg J, Pearlman RE, Hamilton EP, Orias E, Eisen JA, Methé BA. Refined annotation and assembly of the *Tetrahymena thermophila* genome sequence through EST analysis, comparative genomic hybridization, and targeted gap closure. *BMC Genomics.* 2008; 9:562. [PubMed: 19036158]
- Cui B, Gorovsky MA. Centromeric histone H3 is essential for vegetative cell division and for DNA elimination during conjugation in *Tetrahymena thermophila*. *Mol Cell Biol.* 2006; 26:4499–4510. [PubMed: 16738316]
- Davis L, Smith GR. The meiotic bouquet promotes homolog interactions and restricts ectopic recombination in *Schizosaccharomyces pombe*. *Genetics.* 2006; 174:167–177. [PubMed: 16988108]
- De Souza CP, Osmani SA. Mitosis, not just open or closed. *Eukaryot Cell.* 2007; 6:1521–1527. [PubMed: 17660363]
- Deak JC, Doerder FP. High frequency intragenic recombination during macronuclear development in *Tetrahymena thermophila* restores the wild-type SerH1 gene. *Genetics.* 1998; 148:1109–1115. [PubMed: 9539428]
- Doerder FP, Deak JC, Lief JH. Rate of phenotypic assortment in *Tetrahymena thermophila*. *Developmental Genetics.* 1992; 13:126–132. [PubMed: 1499154]
- Doerder FP, Debault LE. Cytofluorimetric analysis of nuclear DNA during meiosis, fertilization and macronuclear development in the ciliate *Tetrahymena pyriformis*, syn. 1. *J. Cell Sci.* 1975; 17:471–493. [PubMed: 806605]

- Eisen JA, Coyne RS, Wu M, Wu D, Thiagarajan M, Wortman JR, Badger JH, Ren Q, et al. Macronuclear genome sequence of the ciliate *Tetrahymena thermophila*, a model eukaryote. *PLoS Biol.* 2006; 4:e286. [PubMed: 16933976]
- Fan Q, Yao M. New telomere formation coupled with site-specific chromosome breakage in *Tetrahymena thermophila*. *Mol Cell Biol.* 1996; 16:1267–1274. [PubMed: 8622671]
- Fan Q, Yao MC. A long stringent sequence signal for programmed chromosome breakage in *Tetrahymena thermophila*. *Nucleic Acids Res.* 2000; 28:895–900. [PubMed: 10648780]
- Fillingham JS, Thing TA, Vythilingum N, Keuroghlian A, Bruno D, Golding GB, Pearlman RE. A non-long terminal repeat retrotransposon family is restricted to the germ line micronucleus of the ciliated protozoan *Tetrahymena thermophila*. *Eukaryot Cell.* 2004; 3:157–169. [PubMed: 14871946]
- Gall JG. Free ribosomal RNA genes in the macronucleus of *Tetrahymena*. *Proc. Nat. Acad. Sci., USA.* 1974; 71:3078–3081. [PubMed: 4528573]
- Goldfarb DS, Gorovsky MA. Nuclear dimorphism: two peas in a pod. *Curr Biol.* 2009; 19:R449–R452. [PubMed: 19515351]
- Gould SB, Kraft LG, van Dooren GG, Goodman CD, Ford KL, Cassin AM, Bacic A, McFadden GI, Waller RF. Ciliate pellicular proteome identifies novel protein families with characteristic repeat motifs that are common to Alveolates. *Mol Biol Evol.* 2011; 28:1319–31. [PubMed: 21127172]
- Hamilton EP, Williamson S, Dunn S, Merriam V, Lin C, Vong L, Russell-Colantonio J, Orias E. The highly conserved family of *Tetrahymena thermophila* chromosome breakage elements contains an invariant 10-base-pair core. *Eukaryot Cell.* 2006; 5:771–780. [PubMed: 16607024]
- Herrmann L, Erkelenz M, Aldag I, Tiedtke A, Hartmann MW. Biochemical and molecular characterisation of *Tetrahymena thermophila* extracellular cysteine proteases. *BMC Microbiol.* 2006; 6:19. [PubMed: 16507097]
- Howard-Till RA, Lukaszewicz A, Loidl J. The recombinases Rad51 and Dmc1 play distinct roles in DNA break repair and recombination partner choice in the meiosis of *Tetrahymena*. *PLoS Genetics.* 2011 in press.
- Iwamoto M, Asakawa H, Hiraoka Y, Haraguchi T. Nucleoporin Nup98: a gatekeeper in the eukaryotic kingdoms. *Genes Cells.* 2010; 15:661–669. [PubMed: 20545767]
- Iwamoto M, Mori C, Kojidani T, Bunai F, Hori T, Fukagawa T, Hiraoka Y, Haraguchi T. Two distinct repeat sequences of Nup98 nucleoporins characterize dual nuclei in the binucleated ciliate *tetrahymena*. *Curr Biol.* 2009; 19:843–847. [PubMed: 19375312]
- Jacobs ME, DeSouza LV, Samaranayake H, Pearlman RE, Siu KW, Klobutcher LA. The *Tetrahymena thermophila* phagosome proteome. *Eukaryot Cell.* 2006; 5:1990–2000. [PubMed: 17012537]
- Kilburn CL, Pearson CG, Romijn EP, Meehl JB, Giddings TH Jr, Culver BP, Yates JR 3rd, Winey M. New *Tetrahymena* basal body protein components identify basal body domain structure. *J. Cell Biol.* 2007; 178:905–912. [PubMed: 17785518]
- Kurth HM, Mochizuki K. Non-coding RNA: a bridge between small RNA and DNA. *RNA Biol.* 2009; 6:138–140. [PubMed: 19276654]
- Loidl J, Mochizuki K. *Tetrahymena* meiotic nuclear reorganization is induced by a checkpoint kinase-dependent response to DNA damage. *Mol Biol Cell.* 2009; 20:2428–2437. [PubMed: 19297526]
- Loidl J, Scherthan H. Organization and pairing of meiotic chromosomes in the ciliate *Tetrahymena thermophila*. *J. Cell Sci.* 2004; 117:5791–5801. [PubMed: 15522890]
- Longcor MA, Wickert SA, Chau MF, Orias E. Coassortment of genetic loci during macronuclear division in *Tetrahymena thermophila*. *Eur. J. Protistol.* 1996; 32(Suppl. 1):85–89.
- Madinger CL, Collins K, Fields LG, Taron CH, Benner JS. Constitutive secretion in *Tetrahymena thermophila*. *Eukaryot Cell.* 2010; 9:674–681. [PubMed: 20348385]
- Malone CD, Falkowska KA, Li AY, Galanti SE, Kanuru RC, LaMont EG, Mazzarella KC, Micev AJ, Osman MM, Piotrowski NK, Suszko JW, Timm AC, Xu MM, Liu L, Chalker DL. Nucleus-specific importin alpha proteins and nucleoporins regulate protein import and nuclear division in the binucleate *Tetrahymena thermophila*. *Eukaryot Cell.* 2008:1487–1499. [PubMed: 18676955]
- Malone CD, Hannon GJ. Small RNAs as guardians of the genome. *Cell.* 2009; 136:656–668. [PubMed: 19239887]

- Miao W, Xiong J, Bowen J, Wang W, Liu Y, Braguinets O, Grigull J, Pearlman RE, Orias E, Gorovsky MA. Microarray analyses of gene expression during the *Tetrahymena thermophila* life cycle. *PLoS One*. 2009; 4:e4429. [PubMed: 19204800]
- Mochizuki K, Novatchkova M, Loidl J. DNA double-strand breaks, but not crossovers, are required for the reorganization of meiotic nuclei in *Tetrahymena*. *J. Cell Sci*. 2008; 121:2148–2158. [PubMed: 18522989]
- Nanney, DL. Experimental ciliatology: an introduction to genetic and developmental analysis in ciliates. Wiley; New York: 1980.
- Noto T, Kurth HM, Kataoka K, Aronica L, DeSouza LV, Siu KW, Pearlman RE, Gorovsky MA, Mochizuki K. The *Tetrahymena* argonaute-binding protein Giw1p directs a mature argonaute-siRNA complex to the nucleus. *Cell*. 2010; 140:692–703. [PubMed: 20211138]
- Orias E. Toward sequencing the *Tetrahymena* genome: exploiting the gift of nuclear dimorphism. *J. Eukaryot Microbiol*. 2000; 47:328–333. [PubMed: 11140445]
- Orias E, Flacks M. Macronuclear genetics of *Tetrahymena*. I. Random distribution of macronuclear gene copies. *Genetics*. 1975; 79:187–206. [PubMed: 805746]
- Parfrey LW, Grant J, Tekle YI, Lasek-Nesselquist E, Morrison HG, Sogin ML, Patterson DJ, Katz LA. Broadly sampled multigene analyses yield a well-resolved eukaryotic tree of life. *Syst Biol*. 2010; 59:518–533. [PubMed: 20656852]
- Preer JR Jr. Preer LB. The Size of Macronuclear DNA and Its Relationship to Models for Maintaining Genic Balance. *J. Euk. Microbiol*. 1979; 26:14–18.
- Prescott DM. The DNA of ciliated protozoa. *Microbiol Rev*. 1994; 58:233–267. [PubMed: 8078435]
- Pritham EJ, Putliwala T, Feschotte C. Mavericks, a novel class of giant transposable elements widespread in eukaryotes and related to DNA viruses. *Gene*. 2007; 390:3–17. [PubMed: 17034960]
- Ray C Jr. Meiosis and nuclear behavior in *Tetrahymena pyriformis*. *J. Protozool*. 1956; 3:88–96.
- Riley JL, Katz LA. Widespread distribution of extensive chromosomal fragmentation in ciliates. *Mol. Biol. Evol*. 2001; 18:1372–1377. [PubMed: 11420375]
- Smith DG, Gawryluk RM, Spencer DF, Pearlman RE, Siu KW, Gray MW. Exploring the mitochondrial proteome of the ciliate protozoon *Tetrahymena thermophila*: direct analysis by tandem mass spectrometry. *J. Mol Biol*. 2007; 374:837–863. [PubMed: 17959197]
- Smith JC, Northey JG, Garg J, Pearlman RE, Siu KW. Robust method for proteome analysis by MS/MS using an entire translated genome: demonstration on the ciliome of *Tetrahymena thermophila*. *J. Proteome Res*. 2005; 4:909–919. [PubMed: 15952738]
- Stover NA, Krieger CJ, Binkley G, Dong Q, Fisk DG, Nash R, Sethuraman A, Weng S, Cherry JM. *Tetrahymena* Genome Database (TGD): a new genomic resource for *Tetrahymena thermophila* research. *Nucleic Acids Res*. 2006; 34:D500–3. Database issue. [PubMed: 16381920]
- Sun H, Treco D, Schultes NP, Szostak JW. Double-strand breaks at an initiation site for meiotic gene conversion. *Nature*. 1989; 338:87–90. [PubMed: 2645528]
- Terry LJ, Shows EB, Wente SR. Crossing the nuclear envelope: hierarchical regulation of nucleocytoplasmic transport. *Science*. 2007; 318:1412–1416. [PubMed: 18048681]
- Turkewitz AP, Orias E, Kapler G. Functional genomics: the coming of age for *Tetrahymena thermophila*. *Trends Genet*. 2002; 18:35–40. [PubMed: 11750699]
- White EM, Allis CD, Goldfarb DS, Srivastva A, Weir JW, Gorovsky MA. Nuclear specific and temporally restricted localization of proteins in *Tetrahymena* macronuclei and micronuclei. *J. Cell Biol*. 1989; 109:1983–1992. [PubMed: 2553740]
- Wolfe J, Hunter B, Adair WS. A cytological study of micronuclear elongation during conjugation in *Tetrahymena*. *Chromosoma*. 1976; 55:289–308. [PubMed: 824106]
- Wong L, Klionsky L, Wickert S, Merriam V, Orias E, Hamilton EP. Autonomously replicating macronuclear DNA pieces are the physical basis of coassortment groups in *Tetrahymena thermophila*. *Genetics*. 2000; 155:1119–1125. [PubMed: 10880474]
- Woodard J, Kaneshiro E, Gorovsky MA. Cytochemical studies on the problem of macronuclear subnuclei in *tetrahymena*. *Genetics*. 1972; 70:251–260. [PubMed: 17248560]

- Wuitschick JD, Gershan JA, Lochowicz AJ, Li S, Karrer KM. A novel family of mobile genetic elements is limited to the germline genome in *Tetrahymena thermophila*. *Nucleic Acids Res.* 2002; 30:2524–2537. [PubMed: 12034842]
- Yao MC, Gorovsky MA. Comparison of the sequences of macro- and micronuclear DNA of *Tetrahymena pyriformis*. *Chromosoma.* 1974; 48:1–18. [PubMed: 4218159]

Fig. 1. The *Tetrahymena* life cycle

Left: vegetative (asexual) reproduction phase. V0: vegetative cell. V1: cell undergoing binary fission. MIC and MAC divide mitotically and amitotically, respectively. Right: conjugation, the sexual (non-reproductive) stage of the life cycle. The following events occur in synchrony in each conjugant. 1. Starved cells of different mating type meet and form pairs. 2. Meiosis generates four haploid products, three of which are resorbed by programmed nuclear death (PND). 3. The functional meiotic product undergoes mitosis, generating the haploid migratory (anterior) and stationary (posterior) gamete pronuclei. Migratory pronuclei are reciprocally exchanged. 4. Incoming migratory and resident stationary pronuclei fuse, generating the diploid fertilization nucleus. 5. The fertilization nucleus undergoes two rounds of mitosis, generating four diploid anlagen, which will differentiate into new polyploid MACs (anterior two) and new diploid MICs (posterior two). 6. New MACs and MICs take up central position and characteristic arrangement. Parental MAC migrates posteriorly and is resorbed by PND. 7. Exconjugant cells separate. One new MIC is resorbed by PND. Not shown: at the first post-conjugation cell division (directly following stage 7), a new MAC and a mitotic daughter of the surviving new MIC are distributed to each daughter cell, thus restoring the vegetative -- one MIC, one MAC -- nuclear organization (stage V0).

Fig. 2. Chimeric Nup98s inhibits nuclear accumulation of native nucleus-specific proteins (A) GFP-labeled nuclear-targeted proteins (green fluorescence) were co-expressed with mCherry-labeled (red fluorescence) chimeric Nup98 proteins. Rows: MLH (MIC-targeted linker histone H1), H1 (MAC-targeted linker histone H1) or H2B (histone normally targeted to both nuclei) “BigMac” column: cells overexpressing MIC-repeats:MAC-localized chimeric Nup98. “BigMic” column: cells overexpressing MAC-repeats:MIC-localized chimeric Nup98. “Wild type” column: cells expressing GFP fusion constructs only. DNA was stained with DAPI (blue fluorescence). Arrows and arrowheads indicate MIC- and MAC-localizing mCherry-BigMic, respectively. Double arrowheads indicate MAC-localizing mCherry-BigMac. Scale bars represent 20 μ m. (B) Quantification of GFP fluorescence intensity in MICs and MACs. Values are percentages relative to the intensity in wild-type control. Columns represent the average and SD. (C) A model representative of chimeric Nup98 functions. Localization of the GLFG or NIFN repeats of Nup98 to the inappropriate nuclear pore complexes inhibits nuclear accumulation of native nucleus-specific cargo to that nucleus. This figure is reproduced, with permission, from Iwamoto et al., 2009.

Fig. 3. RNAi-guided transposon IES heterochromatin formation and excision

1. Bidirectional MIC genome transcription. 2. Generation of short double-stranded scnRNAs. 3. Ago-scnRNAs complex is formed and Ago-scnRNA passenger strand is removed. 4. Mature Ago-scnRNA complexes are transported to the *parental* MAC. Ema1p RNA helicase is recruited. Genome scanning is performed. Ago-ScnRNA complexes displaying MAC-destined cognate sequences are disabled. 5. Surviving ago-IES-derived scnRNA-complexes are transported to the *newly differentiating* MAC anlagen. Second genome scan occurs. MIC-limited DNA sequence cognate to scnRNA acquires classical heterochromatin marks. Heterochromatin is spliced out and destroyed in "elimination bodies". This figure is reproduced, with permission, from Kurth and Mochizuki, 2009.

Table 1
Programmed RNAi-guided IES heterochromatin formation and excision in the
***Tetrahymena* newly differentiating somatic genome**

Step	Required component
A. scan RNA biogenesis in the meiotic (parental) MAC	
1. Bidirectional MIC genome transcription	RNA polymerase II
2. Generation of short double stranded RNA scnRNAs	Dicer-like protein 1 (Dcl1p)
B. Mature scnRNA-Argonaute protein complex formation in the cytosol	
3. Ago-scnRNAs complex are formed	<i>Tetrahymena</i> piwi/Argonaute protein 1 (Twi1p)
4. Ago-scnRNA passenger strand is removed	DDH-motif-dependent Slicer activity of Twi1p (Noto et al., 2010)
C. Genome scanning in the parental MAC	
5. Mature Ago-scnRNA complexes are transported to the <i>parental</i> MAC	Novel “gentleman-in-waiting” protein 1 (Giw1p) (Noto et al., 2010)
6. Genome scanning is performed. Ago-ScnRNA complexes displaying MAC-destined cognate sequence are disabled.	DEXH-box RNA helicase (Ema1p) (Aronica et al., 2008)
D. IES Heterochromatin formation in the MAC anlagen	
7. Surviving ago-IES-derived scnRNA-complexes are transported to the <i>newly differentiating</i> MAC anlagen.	
8. Second genome scan occurs. DNA sequence cognate to scnRNA acquires classical repressive (H3K9 and K27 me3) heterochromatin marks	<ul style="list-style-type: none"> - RNA helicase (Ema1p) - Histone methyltransferase: Enhancer-of-zeste-like 1 (Ezl1p) - Chromodomain-containing (H3K9- and K27-binding) Programmed DNA Degradation proteins 1 and 2 (Pdd1p and Pdd2p)
E. Excision and destruction of IES heterochromatin on the MAC anlagen	
9. Heterochromatin “elimination bodies” are formed	PHD finger, localized in Anlagen 5 protein (Lia5p) WG-repeat Wag1p and cnjB proteins (Bednenko et al., 2009) Pdd2p Centromere histone 3 (Cna1p) (Cui and Gorovsky, 2006)
10. Heterochromatin is spliced out and destroyed	-“Domesticated” <i>Tetrahymena</i> piggyBac transposase (Tpb2p) (Cheng et al., 2010) - “Localized in Anlagen 5” protein (Lia5p)

The process takes 14-16 h at 30°C in synchronized cultures containing up to billions of conjugating pairs. Failure of a given event results in developmental arrest and lethality. Contributions up until 2008 are reviewed by Chalker (2008). More recent contributions are cited within this table. Currently step E has been demonstrated only in ciliates but may also occur in other eukaryotes where “chromatin diminution” has been reported. Steps A-D are widely conserved among eukaryotes, but steps A and C are greatly exaggerated in ciliates, thus facilitating their study.