GENOME DOWNSIZING DURING CILIATE DEVELOPMENT: Nuclear Division of Labor through Chromosome Restructuring

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ABSTRACT
The ciliated protozoa divide the labor of germline and somatic genetic functions between two distinct nuclei. The development of the somatic (macro-) nucleus from the germinal (micro-) nucleus occurs during sexual reproduction and involves large-scale, genetic reorganization including site-specific chromosome breakage and DNA deletion. This intriguing process has been extensively studied in *Tetrahymena thermophila*. Characterization of *cis*-acting sequences, putative protein factors, and possible reaction intermediates has begun to shed light on the underlying mechanisms of genome rearrangement. This article summarizes the current understanding of this phenomenon and discusses its origin and biological function. We postulate that ciliate nuclear restructuring serves to segregate the two essential functions of chromosomes: the transmission and expression of genetic information.

INTRODUCTION
Large-scale DNA rearrangement, or chromatin diminution, was first described by Boveri in 1887 in the Ascarid worm (13). It involves the elimination of a vast amount of chromatin from all somatic progenitor cells and fragmentation of their chromosomes following a precise developmental program. This type of rearrangement has since been observed in some ciliated protozoa (82), arthropods (11) and vertebrates (64, 73, 74). The global nature of this process clearly
distinguishes it from other types of DNA rearrangement and poses very interesting biological questions: How is the precision of this massive rearrangement achieved; how are the events coordinated; are they related to other types of DNA rearrangement; and how do they affect gene expression and the dynamics of chromosomes? Its occurrence challenges our fundamental understanding of chromosome structure and genome organization.

The relative simplicity of ciliates offers an excellent setting for analysis of this phenomenon. Recent studies have revealed intriguing processes that make up this drastic genomic change, including internal DNA elimination and chromosome breakage, as well as gene dimerization, amplification, and shuffling (82, 102, 103). In this article, we summarize our current understanding, using *Tetrahymena thermophila* as a primary example. We focus on two major events—DNA deletion and chromosome breakage—that occur in all ciliates studied, and that appear to be the most important processes. Molecular studies have allowed in-depth understanding of *cis*-acting sequences, and have provided models for their regulatory mechanisms. These studies have also revealed intermediates that could help elucidate the biochemical steps. Studies on *trans*-acting factors, although just begun, have identified an interesting candidate linking DNA deletion to heterochromatin formation. In addition, several studies have implicated these processes in the determination of unorthodox genetic traits in Tetrahymena and Paramecium, thereby revealing an interesting new aspect of heredity. A detailed and intriguing picture is emerging of this extensive reorganization of the ciliate somatic genome. Finally, we speculate on the roles of these events in somatic nuclear differentiation, in particular, the possibility that germline-specific sequences have crucial roles in the organization and transmission of mitotic and/or meiotic chromosomes.

**Nuclear Dualism**

All ciliates display nuclear dualism (83), a special feature particularly relevant to DNA rearrangement. *Tetrahymena* represents a typical case. Each cell contains two drastically different nuclei, a micronucleus and a macronucleus, that separately carry out the germinal and the somatic functions of the genome. *Tetrahymena* cells grow and divide indefinitely by binary fission, during which time the micronucleus divides mitotically but the macronucleus divides by amitosis, that is, without notable chromosome condensation and spindle formation. The macronucleus is active in transcription, whereas the micronucleus is transcriptionally inert (52). The germline function of the micronucleus is revealed during sexual conjugation (76). It undergoes meiosis and mitosis to produce two gametic nuclei in each partner. Reciprocal exchange and nuclear fusion then occur to produce diploid zygotic nuclei, which divide to produce progenitors for both new micro- and macronuclei. The old macronuclei degenerate
during this process. Formation of the new macronucleus takes only a few hours in Tetrahymena, and it is during this time that all DNA rearrangement processes occur. The macro- and micronucleus thus represent one of the simplest forms of soma and germline differentiation. Ciliates differ from metazoa mainly in that both nuclei reside in the same cell throughout the life cycle. This unique lifestyle is perhaps the main reason why nuclear differentiation has reached such an extreme. The macronucleus is solely responsible for gene expression and the micronucleus for faithful gene transmission (through mitosis and meiosis). Thus the basic functions of chromosomes are carried out separately in these two nuclei in ciliates, a situation not found in other dividing cells.

CHROMOSOME BREAKAGE

Breakage in Tetrahymena

By pulse field gel electrophoresis, it is estimated that breakage of the five micronuclear pairs of chromosomes in *T. thermophila* occurs at 50 to 200 reproducible sites to give rise to macronuclear chromosomes with an average size of about 600 kilobases (kb) (2, 21). The first breakage sites studied were those flanking the ribosomal RNA gene, or rDNA. The rDNA is present in a single copy in the micronuclear genome, but it is excised during macronuclear development (101). Flanking micronuclear DNA at both ends contains a conserved 15-base pair (bp) sequence within a short stretch that is eliminated from the macronucleus (56, 110). This sequence, now known as Cbs (for chromosome breakage sequence), is present in multiple copies in the micronuclear genome, but it is apparently absent from the macronucleus (109). The sequence organization of one micronuclear breakage site is presented in Figure 1. Five Cbs-containing micronuclear clones were each found to correspond to a site of breakage. Conversely, three randomly selected breakage sites were each associated with a copy of Cbs (109, 112). These correlations provide strong evidence that Cbs is present at most, if not all, sites of breakage in *T. thermophila* and that it is likely to be a cis-acting determinant for breakage. Proof of this hypothesis has come through the use of an rDNA-based transformation system.

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  mie: AAGAAAATAAATTCATTCAATTTATAAAGAAGGTTGGTTTAAGAAAATAAAAATTATTATTTTTTTTTATTATTT
  mac: AAGAAAATAAATGGGGTT(n)AACCCCTATTATTT
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*Figure 1.* DNA sequence structure of a chromosome breakage junction in *T. thermophila*. The top line shows micronuclear sequence. Cbs is boxed; sequences retained in the macronucleus are underlined. The bottom line shows terminal sequences, of the two macronuclear chromosomes derived from breakage, with telomeric repeats in parentheses (109).
Deletion of Cbs from either end of the rDNA eliminates breakage at that site. Conversely, insertion of Cbs at novel sites generates corresponding new sites of breakage (104). Thus, Cbs is the necessary and sufficient signal for breakage in *T. thermophila*.

Further analysis has revealed a tight coupling between Cbs-induced breakage and telomere addition. Tetrahymena telomerase apparently will not act on restriction enzyme-generated breaks in vivo (30). In vitro, it shows a requirement for primers of particular sequence composition (38, 40). As long as Cbs is present, no such limitations are observed in vivo. Addition of telomeric repeats always begins within a distance of 4 to 22 bp from the end of Cbs (30, 109, 112). This distance is independent of the orientation of Cbs and the nature of the flanking DNA sequence. Usually, addition of telomeric G4 T2 repeats initiates at or before a T or G nucleotide on the 3′ end of the broken duplex. However, if a stretch devoid of T and G is inserted, the normal distance relationship is maintained, and addition occurs following an A or C, even if short sequences designed to favor telomere addition are placed immediately adjacent to Cbs (30). This suggests that the initial breakage event occurs not within Cbs itself, followed by exonucleolytic digestion from that point, but at a short distance from both of its ends. The microheterogeneity of telomere addition sites could result from imprecise breakage or from limited exonucleolytic degradation, perhaps carried out by telomerase itself (20).

Current data favor a model in which a complex of *trans*-acting factors cooperate to carry out breakage and end healing (see Figure 2). Designation of a site for breakage occurs by the binding of a *trans*-acting Cbs recognition factor. Cutting activity may reside within this factor or within an accessory factor recruited to both sides of Cbs. The final component is telomerase, which we predict interacts directly with the breakage complex. Assembly of the entire complex may be required for breakage to proceed; this would ensure that every broken end is efficiently healed. After breakage, the recognition complex could dissociate and act catalytically at other Cbs sites. Telomerase would remain associated with the ends and, perhaps following limited digestion, cap them with telomeric repeats. Further elucidation of the breakage mechanism will require the analysis of its *trans*-acting factors, which may be identified by biochemical and/or genetic means. Their comparison with other DNA rearrangement factors will be very interesting with regard to the evolutionary origins of chromosome breakage.

**Breakage in Other Ciliates**

Chromosome breakage in the Paramecium genus occurs to a similar extent as in Tetrahymena, giving rise to macronuclear chromosomes with an average size of 300 kb (22, 80). However, the process is considerably less precise. Telomere addition occurs at various locations within 200–800-bp regions (10, 16, 31).
Furthermore, several such regions may be found at a particular chromosome end, separated by 2 to 13 kb. At least two chromosomal locations, alternative events may lead to either telomerization or internal deletion, which suggests a possible mechanistic link between the two processes (3, 16). As yet, no conserved sequence has been identified near the sites of telomere addition in Paramecium that may be a signal analogous to Cbs (10, 31), but recognition of a weakly conserved sequence may be hampered by the heterogeneity of ends. Perhaps cis-acting signals will be discovered in the as yet largely unexplored micronuclear-limited regions flanking telomere addition sites. Nevertheless, such a signal would clearly act by a mechanism distinct from Cbs. Initial breakage near the signal followed by extensive degradation could explain the distribution of telomere addition sites, but this seems inconsistent with the ability of Paramecium telomerase to cap injected linear DNA molecules with no detectable shortening (35). Alternatively, cis- and trans-acting signals could
activate a large region for potential breakage events. The particular site chosen could depend on local DNA sequence and/or chromatin context. A similar model has been proposed for the nematode *Ascaris suum* (72). Several reports show a novel and unusual influence of the old macronucleus on selection of telomere addition sites in the newly developing one, as described further below (29, 68). Clearly, more work is needed to understand this phenomenon and the mechanism of DNA fragmentation in Paramecium.

Macronuclear development in the hypotrichous ciliates, such as Stylonychia, Oxytricha, and Euplotes, takes several days, beginning with endoreplication to give rise to polytene chromosomes (61). Fragmentation of the chromosomes into thousands of pieces then occurs, accompanied by a drastic decrease in nuclear DNA content. Further fragmentation of the remaining DNA, which includes clusters of macronuclear-retained sequences, gives rise to the mature macronuclear minichromosomes, each containing only a single gene with minimal flanking DNA.

In two Oxytricha species, alternative use of some fragmentation sites has been observed (45, 59). There is also a small degree of heterogeneity in telomere addition site selection (8, 45). No conserved DNA sequence has been recognized at sites of breakage, although, as in Paramecium, weakly conserved signals may be obscured by the heterogeneity of ends. In contrast, *Euplotes crassus* carries out telomere addition at unique nucleotide locations (8). It is the only ciliate known to display such precision. Interestingly, two macronuclear gene precursors overlap by 6 bp in the micronucleus, suggesting that fragmentation may occur by a staggered break followed by fill-in of the recessed ends. A similar staggered break may occur in *O. nova*, where two macronuclear genes appear to overlap in the micronuclear genome by 5 bp (59).

Most macronuclear gene precursors in *E. crassus* do not overlap, but are separated by short spacers. A 14-bp consensus \(5'\cdot A/T A/T A/T TCAA Y A/T YYTA T-3'\) has been recognized either 10 bp into the macronuclear DNA end or, in inverted orientation, 4 bp into the flanking, eliminated region (8). The 6-bp difference between these distances is consistent with the proposed 6-bp staggered cut noted above; fill-in of the staggered cut on the macronuclear-retained molecule would add 6 bp to the distance between the consensus and the site of telomere addition. Although weakly conserved, this sequence is a strong, but still unproven, candidate for a signal directing the breakage event and/or its precise location. Besides bearing no sequence similarity to Cbs, being much more weakly conserved and not being present at all breakage sites, the proposed signal differs in other respects from Cbs. It is often retained in the macronucleus, unlike Cbs, which is always eliminated. It also appears to act in a unidirectional, orientation-dependent manner, unlike Cbs. Interestingly, the most well-conserved core of this consensus \(5'\cdot TCA-3'\) is also found near the ends of Tec elements,
a family of micronuclear-specific transposons of *E. crassus*, described further below. Its distance from the transposon target site duplication is equal to the distance, noted above, between the sequence and telomere addition sites. This suggests a possible link between chromosome breakage and the elimination of these transposons from the macronucleus (8, 51).

**Summary**

As yet, there is little evidence for mechanistic similarity in chromosome fragmentation among the three ciliate groups studied in detail. Breakage in *Tetrahymena*, with its invariant use of a well-defined *cis*-acting signal, is the most easily reconciled with cases of sequence-specific DNA cutting in other organisms, although it has unique features. The initial breakage event in *Euplotes*, probably a precise, staggered, double-strand break, would be similar to those occurring in many DNA rearrangement events. However, the loose sequence requirements pose a challenge as to how breakage sites are defined. This is true to an even greater extent in *Oxytricha* and especially in *Paramecium*. The complication of old macronuclear influence on telomere site selection in *Paramecium* makes it even harder to present a unified picture of chromosome breakage in ciliates at this time. However, our knowledge is at an early stage. In particular, direct analysis of *cis*-acting sequences in species other than *T. thermophila*, the study of *trans*-acting factors, and possibly the analysis of breakage events in hypotrichs at the borders of macronuclear gene clusters may establish some link between these different phylogenetic groups.

**INTERNAL DNA DELETION**

*Deletion in Tetrahymena*

In addition to chromosome fragmentation, reorganization of the macronuclear genome involves extensive removal of micronuclear-limited DNA. Renaturation kinetics of *Tetrahymena* DNA show that ~15% of the micronuclear genome is eliminated from the macronucleus (106). By comparison of corresponding micronuclear and macronuclear clones (15, 105), it is estimated that internal deletion removes specific segments from over 6000 chromosomal sites. Deletion elements of *Tetrahymena* range in size from a few hundred bp to greater than 13 kb and vary greatly in sequence (82). The complete or partial sequence of five deletion elements shows that all are AT-rich (103). In the micronucleus, these elements are flanked by direct repeats of 4–8 bp of varied sequences. None has yet been found to interrupt gene coding regions, although one has been found within an intron (41).

Much of our knowledge of the deletion process has resulted from characterization of a 9.3-kb region of the micronuclear genome (105), diagrammed
in Figure 3, containing three deletion elements referred to as R, M, and L for right, middle, and left. In the macronucleus, site-specific deletion removes 1.1 kb from the R region, 0.9 kb or 0.6 kb from the M region, and >2 kb from the L region (the left boundary of L is unknown). The M element has been most well-characterized and serves as our primary example to illustrate the essential features of deletion. Alternative deletion of the M element results in two equally likely outcomes: removal of either a 0.6-kb or a 0.9-kb segment that differ only in their left boundaries, M1 or M2 (see Figure 3) (4, 6). Other such cases of alternative rearrangement have been described (47, 97, 99).

The transformation system described above has been used to analyze deletion of M-element DNA (36, 37). Deletion of vector-borne elements occurs accurately when introduced into conjugating cells, and analysis of in vitro mutagenized M-element DNA has identified important cis-regulatory sequences. Inspection of the M-element sequence revealed an unusual polypurine tract, 5′-AAAAAGGGGG-3′ (A5G5), located ∼45 bp from each of the alternative left deletion boundaries, M1 and M2 (see Figure 4). A nine of ten bp complementary sequence, 5′-CCCCCTATTATT-3′ (C5T5), was found ∼45 bp from the right deletion boundary, M3. The importance of this sequence was tested in the transformation assay. Substitutions in the center of the A5G5 next to M1 abolished the use of this junction (37). Insertion of A5G5 between M2 and M3 created novel deletion boundaries 43–47 bp from the end of the insertion (36). Thus the A5G5 sequence is necessary and sufficient to specify the boundary of deletion. It also appears to specify the deletion junction from a specific distance. When the spacing between the M3 direct repeat and the rightward C5T5 tract is altered, selection of the deletion boundary is still specified 42–53 bp from C5T5 (36). Furthermore, the specific terminal repeats, a hallmark of deletion elements, have no essential role in regulating deletion.
Figure 4  The M element region and cis regulatory sequences. Macronuclear-destined and eliminated sequences are shown as wide boxes and solid or shaded bars, respectively. The two alternative left boundaries, M1 and M2, and the right boundaries are indicated. The $A_5G_5$ and $C_5T_5$ flanking regulatory sequences are indicated by the triangles. Internal promoting sequences are shown as ovals. Short, direct repeats found at each boundary are indicated by arrowheads.

The $A_5G_5$ flanking regulatory sequence is the only defined sequence controlling deletion; however, it is not sufficient to promote rearrangement of the M element. Removal of the entire 0.6-kb eliminated region or substitution with macronuclear DNA or E. coli phage DNA abolishes deletion activity. Preliminary evidence indicates that the M element contains multiple, nonoverlapping internal promoting sequences that stimulate deletion additively (see Figure 4) and function independently of distance or orientation relative to the deletion boundaries (M-C Yao, R Callahan, R Godiska & C-H Yao, unpublished information).

Thus the M element is controlled by two classes of cis regulatory sequence—one in the flanking DNA and another inside the eliminated region. The nature of these sequences distinguishes the M element from transposons, which are of consistent structure and carry entirely internal regulatory sequences. Does this view of M element deletion apply generally to other Tetrahymena deletion elements? Extensive mutagenesis of the DNA flanking the R element has shown that specific sequences within this region determine the boundary of deletion in a distance- and orientation-dependent fashion analogous to $A_5G_5$ (D Chalker, A Wilson, A La Terza, C Kroenke & M-C Yao, unpublished information). Furthermore, other deletion elements also contain internal sequences able to stimulate excision. Chimeric elements created by replacement of the essential 0.6-kb region of the M element with micronuclear-limited DNA of other deletion elements rearrange efficiently. Taken together, these data indicate that flanking regulatory and internal promoting sequences exist in many, if not all, elements.

The eliminated elements are generally lost rapidly from developing macronuclei, but a low abundance of long-lived circular forms has been detected by PCR (87, 108). The major products detected contain one copy of the terminal repeat
sequence that borders the eliminated region in micronuclear DNA, indicating perfect excision of the elements. The persistence and extremely low abundance of these circular molecules suggest that they are aberrant by-products of the normal mechanism. Potential cleavage intermediates have been identified by a ligation-mediated PCR assay (88), suggesting the following model for deletion.

A staggered double-strand break exposes a 3′ adenosine at one junction of the macronuclear-retained DNA. This attacks the same strand at the opposite boundary in a direct transesterification reaction. The deletion element is cleaved from the remaining strand and the resulting gap is repaired. The direct transesterification mechanism is similar to known transposon excision reactions. Although the structure and regulatory sequences of the Tetrahymena deletion elements are quite varied, if this model is correct, the actual excision may share some features with other mobile elements.

The only characterized protein putatively involved in DNA deletion is Pdd1p, formerly named p65 (67), an abundant protein preferentially localized to developing macronuclei. By fluorescence in situ hybridization and immunocytochemistry, Pdd1p has been colocalized with micronuclear specific sequences during the time at which DNA deletion occurs. Late in macronuclear differentiation, Pdd1p and associated DNA are found in electron-dense structures distributed around the nuclear periphery (M Madireddi, R Coyne, J Smothers, K Mickey, M-C Yao, & CD Allis, in preparation). Pdd1p contains two chromodomains, a sequence motif that characterizes a family of proteins including Drosophila HP1 and Polycomb (63, 78). Members of this family associate with heterochromatin and/or mediate transcriptional repression. Pdd1p may promote a specialized DNA conformation required for DNA deletion. The implications of the heterochromatin/DNA deletion relationship are discussed later.

Based on the identification of cis regulatory sequences and the putative involvement of Pdd1p, we propose the model for excision of deletion elements shown in Figure 5. Initially, trans-acting factors recognize the internal promoting sequences of a given element thereby marking it for deletion. Factors that recognize the flanking regulatory sequences (e.g. A5 G 5 ) along with Pdd1p then associate with the element. The boundaries of the macronuclear-destined sequences are brought into proximity by alteration of the chromatin structure mediated by Pdd1p. The flanking regulatory factors specify the excision boundaries. They themselves may perform the actual excision or recruit deletion enzymes to the junctions. Macronuclear sequences are joined and the eliminated sequence is targeted for degradation.

**DNA Deletion in Other Ciliates**

DNA deletion occurs universally among ciliates, but to widely differing extents. The extreme occurs in the hypotrichs in which all repetitive DNA and as much as
95% of unique sequences are removed from the macronucleus (82). The size and structure of the deletion elements are quite heterogeneous within and among ciliate species. The structure and excision products of some elements have been extensively characterized; however, technical limitations have prevented examination of deletion requirements in other ciliates as described above for Tetrahymena. The deletion elements of other ciliates fall into two major classes: transposon-like elements and internal eliminated sequences (IES).

Transposon-like elements include TBE (telomere-bearing elements) of Oxytricha (43) and Tec elements of Euplotes (7, 49, 95). The Tel1 element of Tetrahymena may also be a member of this group (17). The exclusively micronuclear TBE and Tec elements are found in thousands to tens of thousands of copies, respectively. TBEs are 4.1 kb long, have 78-bp terminal inverted repeats.
bounded by 17 bp of telomeric sequence, and are flanked by a 3-bp target-site
duplication 5′–ANT-3′ (100). Tec elements are 5.3 kb in length, terminate with
∼ 700-bp inverted repeats, and are flanked by the target duplication 5′-TA-3′
(7, 49, 50). Each has the coding capacity for a putative transposase that may
mediate mobility in the micronucleus and/or excision from the macronucleus.
They are members of the Tc1/mariner transposon family, several of which read-
ily undergo somatic excision (24). The consistent size and end structure as well
as the coding potential of these elements are quite distinct from the deletion
elements of Tetrahymena described above.

IES elements are AT-rich, micronuclear-limited sequences of heterogeneous
size (from tens to hundreds of base pairs) and sequence. They often interrupt
gene coding sequences, and therefore their precise excision is essential. Flank-
ing direct repeats (5′-TA-3′, for Paramecium and Euplotes IESs, and 2–6-bp
variable sequences in Oxytricha) probably guide this precision. The small size
(as short as 14 bp) and diverse sequence of IESs present a conundrum as to how
these sequences are recognized and excised. Paramecium and Euplotes IESs
contain similar consensus end sequences and identical flanking repeats (5′-TA-
3′) as Tec elements, suggesting that excision mechanisms of these elements are
related (58).

Excision products of TBE, Tec, and larger IES elements are abundant circular
molecules (49, 95). TBEs circles contain one copy of the 3-bp direct repeat
connecting the ends of the element (100). In contrast, circular forms of excised
Tec and IES elements in Euplotes contain both copies of the TA direct repeat,
separated by 10 bp derived from flanking sequence that is partially heteroduplex
(51, 62). This suggests a common origin for some transposon-like elements and
IESs. Alternatively, convergence may account for this similarity.

Gene shuffling (81, 82) is a remarkable phenomenon found in _O. nova_ in
which micronuclear regions that consist of a set of macronuclear-destined
sequences in scrambled order are reorganized by IES excision to produce a
functional gene in the macronucleus (39, 70). Junctions between scrambled
segments and IESs contain direct repeats matching the segments to which they
will be joined rather than the adjacent micronuclear segments. These repeats
are 6 to 19 bp, much longer than the 2–6-bp repeats flanking typical IESs.

Survey of the eliminated DNA of ciliates has identified a diversity of deletion
elements, and the relationships among them remain uncertain. Some IESs and
transposon-like elements have similar terminal sequences and excision prod-
ucts, but these features are not shared by Tetrahymena deletion elements. The
short sequence repeats flanking the ends of all known elements are not es-
sential for deletion to occur in Tetrahymena, although they may help guide
its precision. Characterized excised forms are exclusively circular, although
these forms appear to be deletion by-products in Tetrahymena. Also, most
hypotrich IES elements are too small to form circles, suggesting that circularization is not a general feature of deletion. Clearly some IESs are too small to contain the internal promoting sequences defined in Tetrahymena, and the fact that they interrupt coding DNA places restrictions on flanking regulatory sequences. However, only a small part of the genomes of these organisms has been studied. It is possible that deletion elements may be discovered in the hypotrichs similar to those found in Tetrahymena, and vice versa.

**Epigenetic Control of DNA Rearrangement**

Non-Mendelian inheritance in ciliates has been an enigma ever since the realization that the primary determinant of mating type during new macronuclear development in Paramecium is specified by the old macronucleus (89). With the discovery of genome rearrangement, macronuclear-controlled alternative rearrangement has been suspected to underlie the phenomenon, and recent studies of Paramecium and Tetrahymena have provided supporting evidence. The first such evidence came from studies of a non-Mendelian mutant of *P. tetraurelia*, d48, that, despite containing a wild-type micronuclear A surface antigen gene, fails to carry out proper chromosome fragmentation, resulting in deletion of the gene from new macronuclei (29). The hereditary determinant is the absence of the A gene from the old macronucleus, and normal fragmentation is restored by injection of cloned A gene fragments (55, 111). Similarly, in *P. primaurelia*, high-copy-number plasmids containing the G surface antigen gene induce alternative fragmentation patterns on the micronuclear G gene in the subsequent macronuclear development (68). Similar non-Mendelian determinants affect the processing of an IES of the G gene (26). Failure to remove the IES can be induced in new macronuclei by the presence of high-copy-number, IES-containing plasmids in the old macronucleus.

Evidence for macronuclear control of DNA rearrangement also exists in Tetrahymena. When cells carrying high copy numbers of the normally micronuclear-limited M or R deletion element in the macronucleus are mated, they fail to excise the corresponding element in the new macronucleus (16a). Even macronuclear development occurring in wild-type cells is inhibited when the mating partner contains deletion elements in its macronucleus, showing that inhibition is mediated through diffusible cytoplasmic factors.

The mechanism(s) of epigenetic inheritance is yet to be determined. In Tetrahymena, sequestration of specific, deletion-promoting factors by the deletion elements is the simplest interpretation of the results. An alternative hypothesis has been favored for IES retention in the Paramecium G gene in which a nucleic acid factor specific for the retained sequence inhibits deletion in the developing nucleus by homologous pairing to the target (26). Both views account for the necessary specificity and diffusible nature of the deletion block.
Much further work will be required for a clear understanding of this unusual phenomenon.

**BIOLOGICAL SIGNIFICANCE**

In this section, we consider the following questions: What evolutionary forces may have driven the establishment of genome rearrangement in ciliates? Once the mechanisms were in place, how might they have been used for other purposes? What can we learn from ciliates about genome activities in general?

Several proposals have been made for evolutionary advantages resulting from chromosome fragmentation and internal deletion. These include opportunities for the control of gene expression by differential amplification and alternative rearrangement as well as the diversification of somatic genome complements resulting from “phenotypic assortment”—the random homozygosing of macronuclear alleles that occurs during vegetative growth due to amitosis (14). Several cases of differential gene amplification in ciliates are known (9, 44, 90); the rDNA is the best studied example (101) and so far the only one associated with a gene product of known function. Alternative rearrangement, both in chromosome breakage (16, 45, 59) and internal deletion (4, 47, 97, 99), is also well characterized. The control of mating type (69, 75) and the expression of surface antigen genes (25, 69) in Paramecium and Tetrahymena are candidates for this type of control, but further study will be required to confirm this. Phenotypic assortment has so far been observed only in Tetrahymena and has not yet been shown to offer a selective advantage. These topics have been more extensively reviewed elsewhere (82, 92, 102). For the rest of this review, we focus on another proposal related to the distinctive activities of the germline vs somatic genomes, respectively, the transmission and expression of the genetic material (103).

**A Proposed Rationale for DNA Elimination**

One advantage offered by DNA elimination is the opportunity to “streamline” the somatic genome, thus saving the energy (and space in the nucleus) that might have been devoted to maintaining DNA sequences not essential for vegetative function. This consideration gains weight when the germline genome has become increasingly occupied by repetitive, transposon-like and satellite sequences, as with many hypotrichous ciliates. As noted above, it is possible that elimination of Tec and TBE elements is, or was at an earlier time, carried out by transposon-encoded factors. Similarities between transposon and IES terminal DNA sequences (58, 96) and excision products (51, 62) suggest that some of these factors may be shared, either through convergent evolution or because IESs are degenerate remnants of ancient transposons (42, 60). However,
much of the DNA eliminated in hypotrichs as well as Tetrahymena is apparently unrelated to transposons. It may eventually become apparent that elimination of these sequences is derived from transposon-related processes, but whatever the origin of the deletion machinery, natural selection may have favored those organisms that have used it to rid the somatic genome of other unwanted sequences besides transposons.

Ciliate macronuclei are unique among dividing eukaryotic nuclei in never carrying out chromosome condensation or mitosis (83); meiosis is also restricted to the germline. Some of the chromosome mechanics necessary for these activities require localized DNA sequences that are not only dispensable in the ciliate macronucleus, but may even interfere with its normal functioning (103). We consider four types (not all mutually exclusive) of such sequences: centromeres, telomeres, matrix attachment regions (or other sequences required for mitotic chromosome condensation), and heterochromatin.

Unfortunately, ciliate centromeres are completely uncharacterized, but they are almost surely absent from the minimal-length chromosomes of hypotrichs. Centromeres are often associated with heterochromatin, which is dealt with below. Telomeres have been shown to enhance chromosome stability (86) and plasmid segregation efficiency (28, 66) in budding yeast. There is also evidence for their involvement in premeiotic chromosome movements in fission yeast (18). It is interesting, then, that micronuclear and macronuclear telomeres of ciliates are strikingly different. In Oxytricha, micronuclear telomeres are much longer and more heterogeneous in length (23). Micronuclear telomeres of *T. thermophila* are eliminated from the macronucleus (57). Their sequence organization is different from that of macronuclear telomeres in several respects. These differences suggest a possible germline-restricted function, perhaps related to mitosis or meiosis, as suggested by the yeast studies described above. Examination of micronuclear telomeres from other ciliates would prove quite interesting. Another future direction would be the identification of proteins that interact specifically with each class of telomere and their analysis by reverse genetics.

Another germline-restricted activity in ciliates is chromosome condensation. Analysis of condensation in other systems has revealed the requirement for two types of protein, members of the SMC family (46, 79) and topoisomerase II. Topo II is contained in the so-called chromosome scaffold (12, 27, 34) and is thought to bind to specific DNA regions known as SARs or MARs (for scaffold or matrix attachment regions) (33). So far, specific DNA sequences have not been identified that mediate interaction with SMC proteins, but their existence seems likely, especially in the case of the *C. elegans* DPY27 protein, which binds only to the X chromosome (19). Thus, both classes of protein known to be required for chromosome condensation appear to interact with dispersed
DNA elements. A highly speculative proposal would be that such elements are among the eliminated DNA sequences in ciliates and that their removal from macronuclear chromosomes frees them from the cycles of condensation and decondensation carried out in the micronucleus. Clear advantages to the organism would result from such a release, including the ability to carry out transcription throughout the cell cycle. This may be of special importance to the ciliates, due to their large cellular volume and high rate of division. The organization of macronuclear chromosomes might also be greatly simplified, because there is no need for the sequences thought to insulate genes from special chromatin effects seen in other eukaryotes (54, 84). One test of this hypothesis would be by analysis of the germline activities (if any) of deletion elements. We would predict that they play a role in chromosome condensation, perhaps mediated by topo II and/or members of the SMC family.

Another form of chromatin compaction common to eukaryotes is heterochromatin formation. Originally defined cytologically by a condensed appearance throughout the cell cycle, the term heterochromatin is now more generally applied to chromosome regions displaying characteristics such as unusual chromatin structure, transcriptional repression, and late replication (65). Assignment of functions to heterochromatin has been controversial, but recent studies support roles at least in centromere function, sister chromatid adhesion, and meiotic achiasmate chromosome segregation (48, 65). As described above, several members of the chromodomain family interact with heterochromatin (63, 78). Disruption of genes encoding these proteins can lead to defects in the condensation and/or segregation of chromosomes (1, 53).

Interestingly, in higher organisms that undergo chromatin diminution, nearly all heterochromatin is eliminated from the somatic nuclei (11, 64, 71). In ciliates, classically defined heterochromatin has not been observed. The so-called “heterochromatin blocks” in hypotrich polytene chromosomes are probably over- rather than underreplicated, as expected for true heterochromatin (91). Recent evidence, however, shows that micronuclear-limited regions of the E. crassus genome are underreplicated during the polytene stage (32). Examination of T. thermophila macronuclear anlagen during the period of DNA elimination reveals dense, heterochromatin-like bodies containing eliminated DNA and the chromodomain protein Pdd1p (67; M Madireddi, R Coyne, J Smothers, K Mickey, M-C Yao & CD Allis, in preparation). This protein is currently the best link between DNA elimination and heterochromatin formation in ciliates.

Heterochromatic regions may thus represent another chromosomal substructure that is actively eliminated from the macronuclear genome. Its removal from the somatic nuclei of ciliates and certain higher organisms shows that it
has no essential somatic function. Removal of heterochromatin, as with the (perhaps overlapping) germline-specific functional elements described above, might produce efficient, “streamlined” somatic chromosomes, more able to carry out their essential role, transcription, without the complicating structural requirements for condensation and segregation imposed on most eukaryotic chromosomes. The study of ciliates may provide insight into how eukaryotic chromosomes balance the sometimes conflicting demands on chromosomes for both efficient expression and faithful transmission of the genetic material.

A Proposed Rationale for Chromosome Breakage

Whether or not elimination of specific DNA sequences is responsible, macronuclear chromosomes do not condense prior to karyokinesis. Failure to do so in other eukaryotes leads to defects in chromosome segregation, followed by cell death (53, 85, 93, 94). How do ciliates avoid such a fate, even with their less sophisticated amitotic nuclear division? Chromosome fragmentation may be the solution they have found. Clearly, hypotrichs, with their tiny chromosomes, are spared the entanglements and random breakage that could easily result from segregation of full-length uncondensed chromosomes, especially in a highly polyploid nucleus. The situation is less clear with Tetrahymena and Paramecium, whose chromosomes are similar in length to those of S. cerevisiae. Little is known about the amitotic mechanism, but it is likely that chromosome fragmentation may be required for the process, or at least may facilitate it.

Macronuclei of the most primitive ciliates, the karyorelictids, cannot, in fact, divide at all and must be regenerated from the micronucleus at each vegetative fission (83). Molecular data on this group are entirely lacking, but it has been suggested that a key event in the evolution of advanced ciliates from such an ancestral state was acquiring the ability for the macronucleus to divide, partly due to controlled chromosome breakage (42, 77). One feasible test of the connection between fragmentation and macronuclear division capability would be to prevent chromosome breakage by mutating an essential trans-acting factor and observing the effects on macronuclear development and fission.

Conclusion

We propose that the remarkable phenomenon of genome rearrangement in ciliates may be related to their unusual property of nuclear dualism and its attendant segregation of the functions required of chromosomes: the transmission and expression of genetic information. Knowledge gained from other systems on the mechanisms behind these functions has provided clues toward examining this hypothesis in ciliates. Conversely, exploration of the unusual properties of ciliates may provide understanding into chromosome function in general.
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