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

Robert S. Coyne, Douglas L. Chalker, and Meng-Chao Yao

Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, Washington 98104

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

557

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 life style 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

mic: AAGAAAATAAT TTTCAAATTTAATA `AAAGAGGTTGGTTTA` AAAAAATAAAAATTTTTAGTTTTTTT ATTTATTT

mac: AAGAAAATAAT (GGGGTT)n   n(AACCCC) ATTTATTT

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).

(98, 107). 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 G_4T_2 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).
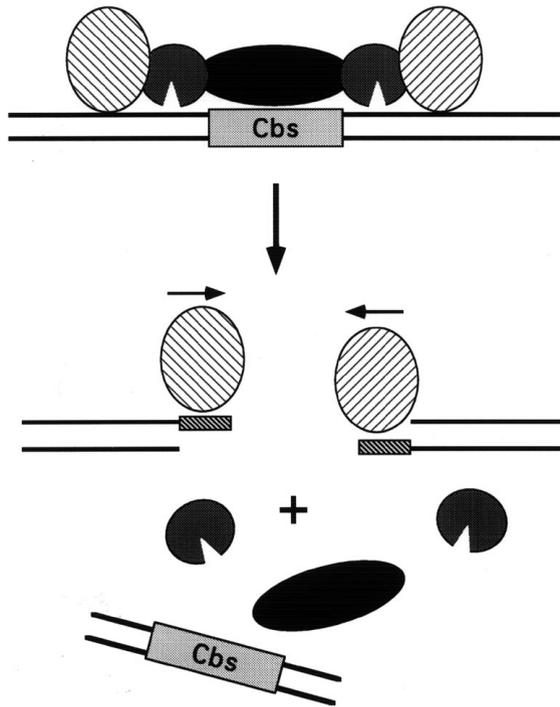
Figure 2 Model for chromosome breakage in *T. thermophila*. Three types of *trans*-acting factor are postulated. A Cbs-recognition factor is represented by the black oval, a nuclease activity by the shaded "Pac-Men," and telomerase by the striped ovals. After breakage, telomeric repeats (striped boxes) are added to the 3' broken strands by telomerase, as the other components dissociate.

Furthermore, several such regions may be found at a particular chromosome end, separated by 2 to 13 kb. At 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 ($5'$-$^A/_T$$^A/_T$$^A/_T$TCAAY$^A/_T$YYTAT-$3'$) 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 ($5'$-$^A/_T$TCAA-$3'$) 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
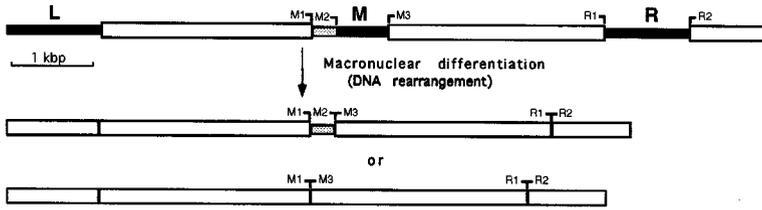
Figure 3 Cloned micronuclear DNA containing one partial and two complete deletion elements, designated L, M, and R. The open boxes represent macronuclear-destined sequences and the solid or shaded bars represent eliminated sequences. Rearrangement produces two variant macronuclear chromosomes by alternative deletion of the M element. The boundaries of the M and R elements are designated M1, M2, and M3, and R1 and R2, respectively.

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′ (A_5G_5), located $\sim$ 45 bp from each of the alternative left deletion boundaries, M1 and M2 (see Figure 4). A nine of ten bp complementary sequence, 5′-CCCCCTTATTT-3′ (C_5T_5), was found $\sim$ 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 A_5G_5 next to M1 abolished the use of this junction (37). Insertion of A_5G_5 between M2 and M3 created novel deletion boundaries 43–47 bp from the end of the insertion (36). Thus the A_5G_5 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 C_5T_5 tract is altered, selection of the deletion boundary is still specified 42–53 bp from C_5T_5 (36). Furthermore, the specific terminal repeats, a hallmark of deletion elements, have no essential role in regulating deletion.
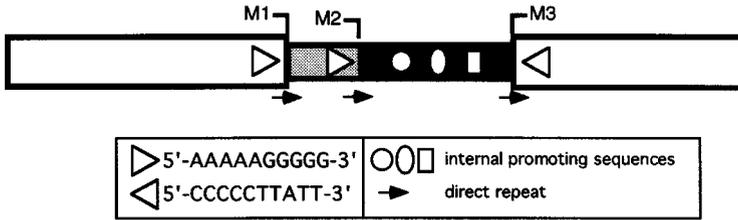
Figure 4   The M element region and *cis* regulatory sequences. Macronuclear-destined and eliminated sequences are shown as wide boxes and solid or shaded bars, respectively. The two alternative left boundaries, M1 and M2, and the right boundaries are indicated. The A_5G_5 and C_5T_5 flanking regulatory 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. A_5G_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
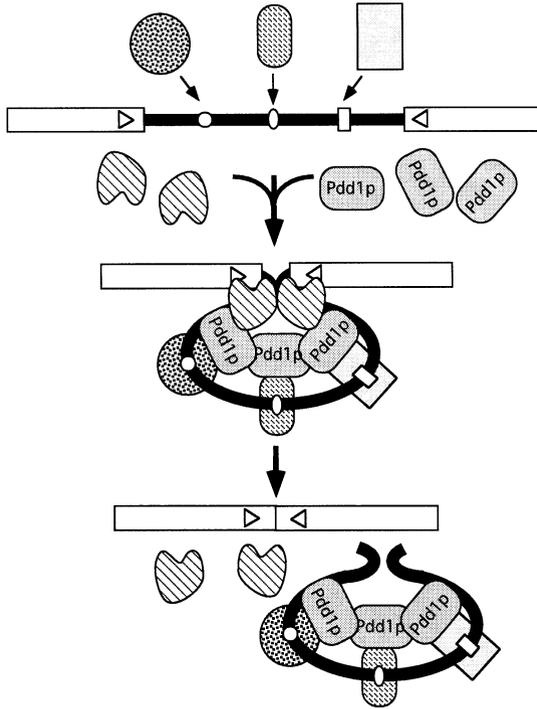
Figure 5 Model of internal deletion. Open boxes are macronuclear-destined sequences; the wide solid line is the eliminated DNA. The triangles represent flanking regulatory sequences. The small circle, oval, and square indicate internal promoting sequences of different character that give the element a unique identity. The putative *trans*-acting factors that recognize *cis* regulatory sequences or mediate protein/protein interaction (as proposed for Pdd1p) are illustrated.

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 readily 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. Flanking 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 tranposon-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 products, but these features are not shared by Tetrahymena deletion elements. The short sequence repeats flanking the ends of all known elements are not essential 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 *s*caffold or *m*atrix *a*ttachment *r*egions) (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.

Visit the *Annual Reviews home page* at
http://www.annurev.org.

Literature Cited

1. Allshire RC, Nimmo ER, Ekwall K, Javerzat J-P, Cranston G. 1995. Mutations derepressing silent centromeric domains in fission yeast disrupt chromosome segregation. *Genes Dev.* 9:218–33

2. Altschuler MI, Yao MC. 1985. Macronuclear DNA of *Tetrahymena thermophila* exists as defined subchromosomal-sized molecules. *Nucleic Acids Res.* 13(16):5817–31

3. Amar L. 1994. Chromosome end formation and internal sequence elimination as alternative genomic rearrangements in the ciliate *Paramecium*. *J. Mol. Biol.* 236:421–26

4. Austerberry CF, Allis CD, Yao MC. 1984. Specific DNA rearrangements in synchronously developing nuclei of *Tetrahymena*. *Proc. Natl. Acad. Sci. USA* 81(23):7383–87

5. Austerberry CF, Snyder RO, Yao MC. 1989. Sequence microheterogeneity is generated at junctions of programmed DNA deletions in *Tetrahymena thermophila*. *Nucleic Acids Res.* 17(18):7263–72

6. Austerberry CF, Yao MC. 1988. Sequence structures of two developmentally regulated, alternative DNA deletion junctions in *Tetrahymena thermophila*. *Mol. Cell Biol.* 8(9):3947–50

7. Baird SE, Fino GM, Tausta SL, Klobutcher LA. 1989. Micronuclear genome organization in *Euplotes crassus*: A transposonlike element is removed during macronuclear development. *Mol. Cell Biol.* 9(9):3793–807

8. Baird SE, Klobutcher LA. 1989. Characterization of chromosome fragmentation in two protozoans and identification of a candidate fragmentation sequence in *Euplotes crassus*. *Genes Dev.* 3:585–97

9. Baird SE, Klobutcher LA. 1991. Differential DNA amplification and copy number control in the hypotrichous ciliate *Euplotes crassus*. *J. Protozool.* 38(2):136–40

10. Baroin A, Prat A, Caron F. 1987. Telomeric site position heterogeneity in macronuclear DNA of *Paramecium primaurelia*. *Nucleic Acids Res.* 15:1717–28

11. Beerman S. 1977. The diminution of heterochromatic chromosomal segments in *Cyclops* (Crustacea, Copepoda). *Chromosoma* 60:297–344

12. Berrios M, Osheroff N, Fisher P. 1985. In situ localization of DNA topoisomerase II, a major polypeptide component of the *Drosophila* nuclear matrix fraction. *Proc. Natl. Acad. Sci. USA* 82:4142–46

13. Boveri T. 1887. Über Differenzierung der Zellkerne während der Furchung des Eies von *Ascaris megalocephala*. *Anat. Anz.* 2:688–93

14. Bruns PJ. 1986. Genetic organization of *Tetrahymena*. See Ref. 32a, pp. 27–44

15. Callahan RC, Shalke G, Gorovsky MA. 1984. Developmental rearrangements associated with a single type of expressed alpha-tubulin gene in *Tetrahymena*. *Cell* 36:441–45

16. Caron F. 1992. A high degree of macronuclear chromosome polymorphism is generated by variable DNA rearrangements in *Paramecium primaurelia* during macronuclear differentiation. *J. Mol. Biol.* 225(3):661–78

16a. Chalker DL, Yao M-C. 1996. Non-Mendelian, heritable blocks to DNA rearrangement are induced by loading the somatic nucleus of *Tetrahymena thermophila* with germline-limited DNA. *Mol. Cell. Biol.* 16:3658–67

17. Cherry JM, Blackburn EH. 1985. The in-

ternally located telomeric sequences in the germline chromosomes of *Tetrahymena* are at the conserved ends of transposon-like elements. *Cell* 43:747–58

18. Chikashige Y, Ding DQ, Funabiki H, Haraguchi T, Mashiko S, et al. 1994. Telomere-led premeiotic chromosome movement in fission yeast. *Science* 264:270–73

19. Chuang P-C, Albertson DG, Meyer BJ. 1994. DPY27: a chromosome condensation protein homolog that regulates *C. elegans* dosage compensation through association with the X chromosome. *Cell* 79:459–74

20. Collins K, Greider CW. 1993. *Tetrahymena* telomerase catalyzes nucleolytic cleavage and nonprocessive elongation. *Genes Dev.* 7:1364–76

21. Conover RK, Brunk CF. 1986. Macronuclear DNA molecules of *Tetrahymena thermophila*. *Mol. Cell Biol.* 6(3):900–5

22. Cummings DJ. 1975. Studies on macronuclear DNA from *Paramecium aurelia*. *Chromosoma* 53:191–208

23. Dawson D, Herrick G. 1984. Telomeric properties of C4A4-homologous sequences in micronuclear DNA of *Oxytricha fallax*. *Cell* 36:171–77

24. Doak TG, Doerder FP, Jahn CL, Herrick G. 1994. A proposed superfamily of transposase genes: transposon-like elements in ciliated protozoa and a common D35E motif. *Proc. Natl. Acad. Sci. USA* 91(3):942–46

25. Doerder FP, Berkowitz MS. 1987. Nucleo-cytoplasmic interaction during macronuclear differentiation in ciliate protists: genetic basis for cytoplasmic control of *SerH* expression during macronuclear development in *Tetrahymena thermophila*. *Genetics* 117:13–23

26. Duharcourt S, Butler A, Meyer E. 1995. Epigentic self-regulation of developmental excision of an internal eliminated sequence in *Paramecium tetraurelia*. *Genes Dev.* 9:2065–77

27. Earnshaw WC, Halligan B, Cooke CA, Heck M, Liu LF. 1985. Topoisomerase II is a structural component of mitotic chromosome scaffolds. *J. Cell Biol.* 100:1706–15

28. Enomoto S, Longtine MS, Berman J. 1994. Enhancement of telomere-plasmid segregation by the X-telomere associated sequence in *Saccharomyces cerevisiae* involves *SIR2*, *SIR3*, *SIR4* and *ABF1*. *Genetics* 136(3):757–67

29. Epstein LM, Forney JD. 1984. Mendelian and non-Mendelian mutations affecting surface antigen expression in *Paramecium tetraurelia*. *Mol. Cell Biol.* 4:1583–90

30. Fan Q, Yao M-C. 1996. New telomere formation coupled with site-specific chromosome breakage in *Tetrahymena thermophila*. *Mol. Cell Biol.* 16(3):1267–74

31. Forney JD, Blackburn EH. 1988. Developmentally controlled telomere addition in wild type and mutant paramecia. *Mol. Cell Biol.* 8:251–58

32. Frels JS, Tebeau CM, Doktor SZ, Jahn CL. 1996. Differential replication and DNA elimination in the polytene chromosomes of *Euplotes crassus*. *Mol. Biol. Cell.* In press

32a. Gall JG, ed. 1986. *The Molecular Biology of Ciliated Protozoa*. Orlando, FL: Academic

33. Gasser SM, Laemmli UK. 1987. A glimpse of chromosomal order. *Trends Genet.* 3:16–21

34. Gasser SM, Laroche T, Falquet J, Boy de la Tour E, Laemmli UK. 1986. Metaphase chromosome structure-involvement of topoisomerase II. *J. Mol. Biol.* 188:613–29

35. Gilley D, Preer JJ, Aufderheide KJ, Polisky B. 1988. Autonomous replication and addition of telomerelike sequences to DNA microinjected into *Paramecium tetraurelia* macronuclei. *Mol. Cell Biol.* 8(11):4765–72

36. Godiska R, James C, Yao MC. 1993. A distant 10-bp sequence specifies the boundaries of a programmed DNA deletion in *Tetrahymena*. *Genes Dev.* 7(12A):2357–65

37. Godiska R, Yao MC. 1990. A programmed site-specific DNA rearrangement in *Tetrahymena thermophila* reguires flanking polypurine tracts. *Cell* 61:1237–46

38. Greider CW, Blackburn EH. 1987. The telomere terminal transferase of *Tetrahymena* is a ribonucleoprotein enzyme with two kinds of primer specificity. *Cell* 51(6):887–98

39. Greslin AF, Prescott DM, Oka Y, Loukin SH, Chappell JC. 1989. Reordering of nine exons is necessary to form a functional actin gene in *Oxytricha nova*. *Proc. Natl. Acad. Sci. USA* 86(16):6264–68

40. Harrington LA, Greider CW. 1991. Telomerase primer specificity and chromosome healing. *Nature* 353(6343):451–54

41. Heinonen TY, Pearlman RE. 1994. A germ line-specific sequence element in an intron in *Tetrahymena thermophila*. *J Biol Chem* 269(26):17428–33

42. Herrick G. 1994. Germline-soma relationships in ciliated protozoa: the inception and evolution of nuclear dimorphism in one-celled animals. *Sem. Dev. Biol.* 5:3–12

43. Herrick G, Cartinhour S, Dawson D, Ang D, Sheets R, Lee A, Williams K. 1985. Mobile elements bounded by C4A4 telomeric repeats in *Oxytricha fallax. Cell* 43:759–68

44. Herrick G, Cartinhour SW, Williams KR, Kotter KP. 1987. Multiple sequence versions of the *Oxytricha fallax* 81-MAC alternate processing family. *J. Protozool.* 34(4):429–34

45. Herrick G, Hunter D, Williams K, Kotter K. 1987. Alternative processing during development of a macronuclear chromosome family in *Oxytricha fallax. Genes Dev.* 1(10):1047–58

46. Hirano T, Mitchison TJ, Swedlow JR. 1995. The SMC family: from chromosome condensation to dosage compensation. *Curr. Opin. Cell. Biol.* 7:329–36

47. Howard EA, Blackburn EH. 1985. Reproducible and variable genomic rearrangements occur in the developing somatic nucleus of the ciliate *Tetrahymena thermophila. Mol. Cell Biol.* 5(8):2039–50

48. Irick H. 1994. A new function for heterochromatin. *Chromosoma* 103:1–3

49. Jahn CL, Krikau MF, Shyman S. 1989. Developmentally coordinated en masse excision of a highly repetitive element in *Euplotes crassus. Cell* 59:1009–18

50. Jahn CL, Nilles LA, Krikau MF. 1988. Organization of the *Euplotes crassus* micronuclear genome. *J. Protozool.* 35(4):590–601

51. Jaraczewski JW, Jahn CL. 1993. Elimination of Tec elements involves a novel excision process. *Genes Dev.* 7(1):95–105

52. Karrer KM. 1986. The nuclear DNA of holotrichous ciliates. See Ref. 32a, pp. 85–110

53. Kellum R, Alberts B. 1995. Heterochromatin protein 1 is required for correct chromosome segregation in *Drosophila* embryos. *J. Cell Sci.* 108:1419–31

54. Kellum R, Schedl P. 1991. A position-effect assay for boundaries of higher order chromosomal domains. *Cell* 64:941–50

55. Kim CS, Preer JR, Polisky B. 1994. Identification of DNA segments capable of rescuing a non-Mendelian mutant in *Paramecium. Genetics* 136:1325–28

56. King BO, Yao MC. 1982. Tandemly repeated hexanucleotide at *Tetrahymena* rDNA free end is generated from a single copy during development. *Cell* 31(1):177–82

57. Kirk KE, Blackburn EH. 1995. An unusual sequence arrangement in the telomeres of the germ-line micronucleus in *Tetrahymena thermophila. Genes Dev.* 9:59–71

58. Klobutcher LA, Herrick G. 1995. Consensus inverted terminal repeat sequence of *Paramecium* IESs: resemblance to termini of Tc1-related and Euplotes Tec transposons. *Nucleic Acids Res.* 23(11):2006–13

59. Klobutcher LA, Huff ME, Gonye GE. 1988. Alternative use of chromosome fragmentation sites in the ciliated protozoan *Oxytricha nova. Nucleic Acids Res.* 16(1):251–64

60. Klobutcher LA, Jahn CL. 1991. Developmentally controlled genomic rearrangements in ciliated protozoa. *Curr. Opin. Genet. Dev.* 1(3):397–403

61. Klobutcher LA, Prescott DM. 1986. The special case of the hypotrichs. See Ref. 32a, pp. 111–54

62. Klobutcher LA, Turner LR, LaPlante J. 1993. Circular forms of developmentally excised DNA in *Euplotes crassus* have a heteroduplex junction. *Genes Dev.* 7(1):84–94

63. Koonin EV, Zhou S, Lucchesi JC. 1995. The chromo superfamily: new members, duplication of the chromodomain and possible role in delivering transcription regulators to chromatin. *Nucleic Acids Res.* 23:4229–33

64. Kubota S, Kuro-O M, Mizuno S, Kohno S. 1993. Germ line-restricted, highly repeated DNA sequences and their chromosomal localization in a Japanese hagfish (*Eptatretus okinoseanus*). *Chromosoma* 102:163–73

65. Lohe AR, Hilliker AJ. 1995. Return of the H-word (heterochromatin). *Curr. Opin. Genet. Dev.* 5:746–55

66. Longtine MS, Enomoto S, Finstad SL, Berman J. 1992. Yeast telomere repeat sequence (TRS) improves circular plasmid segregation, and TRS plasmid segregation involves the *RAP1* gene product. *Mol. Cell Biol.* 12:1997–2009

67. Madireddi MT, Davis M, Allis D. 1994. Identification of a novel polypepetide involved in the formation of DNA-containing vesicles during macronuclear development in *Tetrahymena. Dev. Biol.* 165:418–31

68. Meyer E. 1992. Induction of specific macronuclear developmental mutations by microinjection of a cloned telomeric gene in *Paramecium primaurelia. Genes Dev.* 6(2):211–22

69. Meyer E, Keller A-M. 1996. A Mendelian mutation affecting mating-type determination also affects developmental genomic rearrangements in *Paramecium tetraurelia*. *Genetics* 143:191–202

70. Mitcham JL, Lynn AJ, Prescott DM. 1992. Analysis of a scrambled gene: the gene encoding alpha-telomere-binding protein in *Oxytricha nova*. *Genes Dev.* 6(5):788–800

71. Muller F, Bernard V, Tobler H. 1996. Chromatin diminution in nematodes. *BioEssays* 18:133–38

72. Muller F, Chantal W, Spicher A, Tobler H. 1991. New telomere formation after developmentally regulated chromosomal breakage during the process of chromatin diminution in *Ascaris lumbricoides*. *Cell* 67:815–22

73. Nakai Y, Kubota S, Goto Y, Ishibashi T, Davison W, Kohno S. 1995. Chromosome elimination in three Baltic, south Pacific and north-east Pacific hagfish species. *Chrom. Res.* 3:321–30

74. Nakai Y, Kubota S, Kohno S. 1991. Chromatin diminution and chromosome elimination in four Japanese hagfish species. *Cytogen. Cell Genet.* 56:196–98

75. Orias E. 1981. Probable somatic DNA rearrangements in mating type determination in *Tetrahymena thermophila*: a review and a model. *Dev. Genet.* 2:185–202

76. Orias E. 1986. Ciliate conjugation. See Ref. 32a, pp. 45–84

77. Orias E. 1991. Evolution of amitosis of the ciliate macronucleus: gain of the capacity to divide. *J. Protozool.* 38:217–21

78. Paro R, Hogness DS. 1991. The Polycomb protein shares a homologous domain with a heterochromatin-associated protein of *Drosophila*. *Proc. Natl. Acad. Sci. USA* 88:263–67

79. Peterson CL. 1994. The SMC family: novel motor proteins for chromosome condensation? *Cell* 79:389–92

80. Preer JR Jr, Preer LB. 1979. The size of macronuclear DNA and its relationship to models for maintaining genic balance. *J. Protozool.* 26:14–18

81. Prescott DM. 1993. Restructuring of DNA sequences in the germline genome of *Oxytricha*. *Curr. Opin. Genet. Dev.* 3(5):726–29

82. Prescott DM. 1994. The DNA of ciliated protozoa. *Microbiol. Rev.* 58(2):233–67

83. Raikov IB. 1982. *The Protozoan Nucleus*. Wien/New York: Springer-Verlag, (Alfert M, Beearmann W, Goldstein L, Porter KR, Sitte P, eds. Cell Biol. Monogr. Vol. 9)

84. Roseman RR, Geyer PK. 1993. The *su(Hw)* protein insulates expression of the *Drosophila melanogaster* white gene from chromosomal position-effects. *EMBO J.* 12:435–42

85. Saka Y, Sutani T, Yamashita Y, Saitoh S, Takeuchi M, et al. 1994. Fission yeast *cut3* and *cut14*, members of a ubiquitous protein family, are required for chromosome condensation and segregation in mitosis. *EMBO J.* 13:4938–52

86. Sandell LL, Zakian VA. 1993. Loss of a yeast telomere: arrest, recovery, and chromosome loss. *Cell* 75(4):729–39

87. Saveliev SV, Cox MM. 1994. The fate of deleted DNA produced during programmed genomic deletion events in *Tetrahymena thermophila*. *Nucleic Acids Res.* 22(25):5695–701

88. Saveliev SV, Cox MM. 1995. Transient DNA breaks associated with programmed genomic deletion events in conjugating cells of *Tetrahymena thermophila*. *Genes Dev.* 9(2):248–55

89. Sonneborn TM. 1977. Genetics of cellular differentiation: stable nuclear differentiation in eukaryotic unicells. *Annu. Rev. Genet.* 11:349–67

90. Steinbruck G. 1983. Over-amplification of genes in macronuclei of hypotrichous ciliates. *Chromosoma* 88:156–63

91. Steinbruck G. 1986. Molecular reorganization during nuclear differentiation in ciliates. *Results Probl. Cell Differ.* 13(105):105–74

92. Steinbruck G. 1986. Molecular reorganization during nuclear differentiation in ciliates.In *Germ Line-Soma Differentiation*, ed. W Hennig, pp. 105–74. Berlin: Springer-Verlag

93. Strunnikov AV, Hogan E, Koshland D. 1995. *SMC2*, a *Saccharomyces cerevisiae* gene essential for chromosome segregation and condensation, defines a subgroup within the SMC family. *Genes Dev.* 9:587–99

94. Strunnikov AV, Larionov VL, Koshland D. 1993. *SMC1*: an essential yeast gene encoding a putative head-rod-tail protein is required for nuclear division and defines a new ubiquitous protein family. *J. Cell Biol.* 123:1635–48

95. Tausta SL, Klobutcher LA. 1989. Detection of circular forms of eliminated DNA during macronuclear development in *E. crassus*. *Cell* 59(6):1019–26

96. Tausta SL, Turner LR, Buckley LK, Klobutcher LA. 1991. High fidelity developmental excision of Tec1 transposons and internal eliminated sequences in

Euplotes crassus. Nucleic Acids Res. 19(12):3229–36

97. Tondravi MM. 1988. DNA rearrangements associated with the H3 surface antigen gene of *Tetrahymena thermophila* that occur during macronuclear development. *Curr. Genet.* 14(6):617–26

98. Tondravi MM, Yao MC. 1986. Transformation of *Tetrahymena thermophila* by microinjection of ribosomal RNA genes. *Proc. Natl. Acad. Sci. USA* 83(12):4369–73

99. White TC, Allen SL. 1986. Alternative processing of sequences during macronuclear development in *Tetrahymena thermophila. J. Protozool.* 33:30–38

100. Williams K, Doak TG, Herrick G. 1993. Developmental precise excision of *Oxytricha trifallax* telomere-bearing elements and formation of circles closed by a copy of the flanking target duplication. *EMBO J.* 12(12):4593–601

101. Yao M-C. 1986. Amplification of ribosomal RNA genes. See Ref. 32a, pp. 179–201

102. Yao M-C. 1989. Site-specific chromosome breakage and DNA deletion in ciliates. In *Mobile DNA*, ed. DE Berg, MM Howe, pp. 715–34. Washington, DC: Am. Soc. Microbiol.

103. Yao M-C. 1996. Programmed DNA deletions in *Tetrahymena*: mechanisms and implications. *Trends Genet.* 12:26–30

104. Yao M-C, Yao C-H, Monks B. 1990. The controlling sequence for site-specific chromosome breakage in *Tetrahymena. Cell* 63:763–72

105. Yao MC, Choi J, Yokoyama S, Austerberry CF, Yao CH. 1984. DNA elimination in *Tetrahymena*: a developmental process involving extensive breakage and rejoining of DNA at defined sites. *Cell* 36(2):433–40

106. Yao MC, Gorovsky MA. 1974. Comparison of the sequences of macro- and micronuclear DNA of *Tetrahymena pyriformis. Chromosoma* 48(1):1–18

107. Yao MC, Yao CH. 1989. Accurate processing and amplification of cloned germ line copies of ribosomal DNA injected into developing nuclei of *Tetrahymena thermophila. Mol. Cell Biol.* 9(3):1092–99

108. Yao MC, Yao CH. 1994. Detection of circular excised DNA deletion elements in *Tetrahymena thermophila* during development. *Nucleic Acids Res.* 22(25):5702–8

109. Yao MC, Zheng K, Yao CH. 1987. A conserved nucleotide sequence at the sites of developmentally regulated chromosomal breakage in *Tetrahymena. Cell* 48(5):779–88

110. Yao MC, Zhu SG, Yao CH. 1985. Gene amplification in *Tetrahymena thermophila*: formation of extrachromosomal palindromic genes coding for rRNA. *Mol. Cell Biol.* 5(6):1260–67

111. You Y, Scott J, Forney J. 1994. The role of macronuclear DNA sequences in the permanent rescue of a non-Mendelian mutation in *Paramecium tetraurelia. Genetics* 136:1319–24

112. Yu GL, Blackburn EH. 1991. Developmentally programmed healing of chromosomes by telomerase in *Tetrahymena. Cell* 67(4):823–32