Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 3888–3892, April 1995
Genetics

Scrambling of the actin I gene in two *Oxytricha* species

(hypotrichous ciliates/micronuclear DNA/macronuclear-destined sequences/internal eliminated sequences)

MICHELLE DUBOIS AND DAVID M. PRESCOTT

Department of Molecular, Cellular and Development Biology, University of Colorado, Boulder, CO 80309-0347

Contributed by David M. Prescott, January 11, 1995

ABSTRACT **The DNA in a germ-line nucleus (a micronucleus) undergoes extensive processing when it develops into a somatic nucleus (a macronucleus) after cell mating in hypotrichous ciliates. Processing includes destruction of a large amount of spacer DNA between genes and excision of gene-sized molecules from chromosomes. Before processing, micronuclear genes are interrupted by numerous noncoding segments called internal eliminated sequences (IESs). The IESs are excised and destroyed, and the retained macronuclear-destined sequences (MDSs) are spliced. MDSs in some micronuclear genes are not in proper order and must be reordered during processing to create functional gene-sized molecules for the macronucleus. Here we report that the micronuclear actin I gene in *Oxytricha trifallax WR* consists of 10 MDSs and 9 IESs compared to the previously reported 9 MDSs and 8 IESs in the micronuclear actin I gene of *Oxytricha nova*. The MDSs in the actin I gene are scrambled in a similar pattern in the two species, but the positions of MDS–IES junctions are shifted by up to 14 bp for scrambled and 138 bp for the nonscrambled MDSs. The shifts in MDS–IES junctions create differences in the repeat sequences that are believed to guide MDS splicing. Also, the sizes and sequences of IESs in the micronuclear actin I genes are different in the two *Oxytricha* species. These observations give insight about the possible origins of IES insertion and MDS scrambling in evolution and show the extraordinary malleability of the germ-line DNA in hypotrichs.**

Ciliated protozoa of the order Hypotrichida possess a micronucleus (germ-line nucleus) and a macronucleus (somatic nucleus). In hypotrichs, micronuclear DNA is of high molecular weight (>1000 kb) and is not transcribed during vegetative proliferation. Macronuclear DNA molecules are gene-sized (average 2200 bp in *Oxytricha nova*) and serve as templates for all nuclear transcription during vegetative proliferation (1).

When two cells mate, they exchange haploid micronuclei through a transient cytoplasmic bridge. Within each cell, an exchanged haploid micronucleus fuses with a resident haploid micronucleus, then the cells separate, and the new diploid micronucleus in each cell divides by mitosis without cytokinesis. One of the daughter micronuclei develops into a new macronucleus, and one remains a micronucleus. Unused haploid micronuclei and old macronuclei are destroyed.

During the 3 days of development of a micronucleus into a macronucleus, the germ-line DNA is extensively processed to yield somatic DNA. This includes excision of noncoding DNA segments called internal eliminated sequences (IESs) from micronuclear genes (2–4). Also, spacer DNA between genes is destroyed, releasing gene-sized molecules to which telomeres are added (5, 6). During these events in *O. nova*, ≈95% of DNA sequence complexity is eliminated. Finally, the gene-sized DNA molecules are amplified to ≈1000 copies each to yield a mature macronucleus. Macronuclear gene-sized molecules consist of a coding region flanked by two nontranslated regions (a 5′ DNA leader and a 3′ DNA trailer) and short telomeres at each end (1).

IESs are interspersed between macronuclear-destined sequences (MDSs) in a micronuclear gene. During development, the IESs are removed from a gene, its MDSs are spliced, and the spliced MDSs are excised as a macronuclear gene-sized molecule. In some micronuclear genes in *O. nova*, the MDSs are in a scrambled order and must be reordered to produce a functional macronuclear gene. The micronuclear and macronuclear versions of seven genes from *Oxytricha* have been sequenced. Three of these are scrambled in the micronucleus (refs. 7 and 8 and D. C. Hoffman and D.M.P., unpublished data), and four are not (2, 9–11). The actin I gene in the micronucleus of *O. nova* consists of nine MDSs in the scrambled order, MDS3-4-6-5-7-9-2-1-8, with IESs between MDSs, and one inverted MDS (MDS2). The orthodox order of MDSs is defined by their arrangement in the macronuclear gene. Pairs of repeats are present at all MDS–IES junctions (MIJs) and are believed to guide splicing at the time of removal of the IES. In the spliced macronuclear gene, one copy of the repeat is retained.

To learn more about the evolution and significance of IESs and MDS scrambling, we have compared the structure of the micronuclear actin I gene in *O. nova* with the actin I gene in *Oxytricha trifallax WR.**

MATERIALS AND METHODS

Cell culture, micronuclear and macronuclear isolations, and DNA preparation have been described (12). The species *O. trifallax WR* was isolated in 1991 from a mountain lake in Colorado. It is very similar in morphology, behavior, and DNA sequences (unpublished data) to *O. trifallax H.*

PCRs. The PCR mixture was 10 mM KCl/10 mM $(NH_4)_2SO_4$/20 mM Tris·HCl, pH 8.8 (25°C)/6 mM $MgSO_4$/0.1% Triton X-100 containing all four dNTPs (each at 200 μM) and 7.5 pmol of each primer (DNA International, Lake Oswego, OR). Sequences and locations of primers are shown in Fig. 1. Either macronuclear DNA (5 ng) or micronuclear DNA (75 ng) was used as template. After a hot start at 88°C for 5 min, 0.6 unit of Vent (exo-) DNA polymerase (New England Biolabs) was added to each 50-μl reaction mixture. PCRs were carried out in a Hybaid thermal reactor (National Labnet, Woodbridge, NJ) for 35–40 cycles of these steps: 92°C for 60 sec, 48–55°C for 45 sec, and 72°C for 45–90 sec.

Purification and Cloning of PCR Products and Plasmid DNA Isolation. After electrophoresis in 0.8–2.5% agarose gels (FMC BioProducts), PCR products were transferred from the agarose onto diethylaminoethyl (NA45) membranes (Schleicher & Schuell), eluted, and precipitated, as described by the manufacturer. PCR products were then ligated into the *Sma* I

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MDS, macronuclear-destined sequence; IES, internal eliminated sequence; MIJ, MDS–IES junction.

*The sequences reported in this paper have been deposited in the GenBank data base [accession nos. U18940 (macronuclear gene) and U19288 (micronuclear gene)].

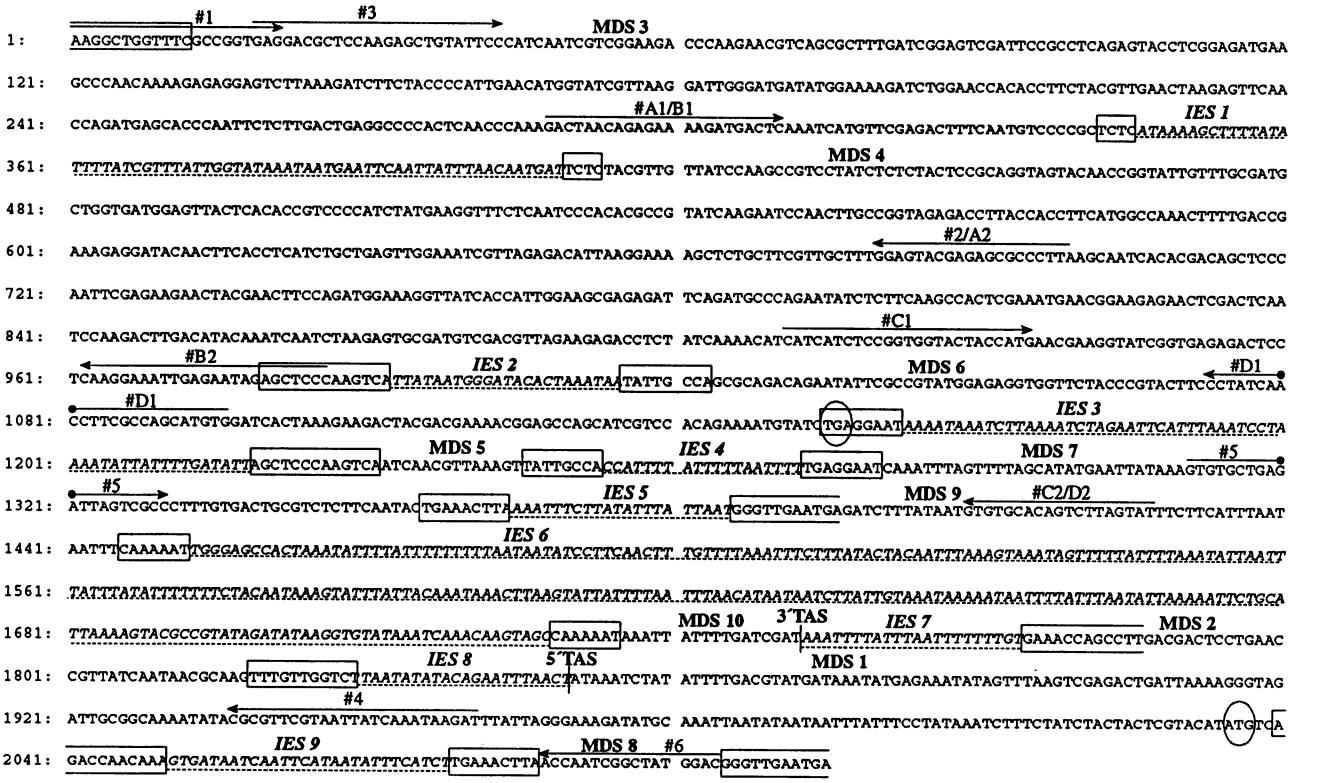

FIG. 1. Nucleotide sequence of the *O. trifallax WR* micronuclear actin I gene. MDSs and IESs are labeled in boldface type. IESs are in italic type and underlined with a dashed line. Repeat sequences are boxed. Terminal boxes with open ends represent repeats whose lengths have not been determined (discussed in text). The 5′ TAS and 3′ TAS are telomere addition sites, the 5′ and 3′ ends of the macronuclear gene (before telomere addition). The start and stop codons are circled. Primer sequences are indicated beneath the arrows; the 3′ end of each sequence is beneath the arrowhead. Primers 1–6 were used to clone sections of the gene; primers A1, A2, B1, etc. were used in subsequent experiments (Fig. 2).

site of pGEM7Zf$^+$ (Promega) and transformed into *Escherichia coli* (DH5α) by using standard methods (13). Plasmid DNAs from transformants were initially isolated with an alkaline lysis procedure (13). Additional plasmid DNA was isolated for sequencing with the RPM kit (Bio 101).

DNA Sequencing and Analysis. DNA sequencing was performed on plasmid DNA templates with protocols from the manufacturer of Sequenase T7 DNA polymerase (Amersham). Sequences were compiled and analyzed with the MACVECTOR and ASSEMBLYLIGN programs (International Biotechnologies).

RESULTS

Sequence of the Actin I Gene in *O. trifallax WR*. The 1502-bp macronuclear actin I gene from *O. trifallax WR* was sequenced from a clone obtained from a screen of a λgt10 phage library of macronuclear DNA. The nucleotide sequence of the coding region of *O. trifallax WR* is 85.4% identical to *O. nova*; the predicted amino acid sequences are 92.2% identical (346 of 375 residues).

The micronuclear sequence of the actin I gene of *O. trifallax WR* was generated from the cloning and sequencing of overlapping PCR products. PCRs used native micronuclear DNA as the template, and primers were designed from the macronuclear sequence. PCRs with primer pairs 1/2, 3/4, and 5/6 (Fig. 1) produced fragments that were cloned into a plasmid and sequenced. At least two plasmid clones of each PCR product were sequenced to reduce the likelihood of sequence errors due to nucleotide changes introduced during PCR. However, some nucleotide differences were still present between the macronuclear and the micronuclear sequence and may reflect the presence of two copies of the actin I gene in micronuclear DNA, as is the case for the actin I gene in *O. nova* (12). The differences do not affect the comparisons of the gene structure between micronuclear and macronuclear DNAs of *O. trifallax WR* or between *O. trifallax WR* and *O. nova*. In particular, none of the nucleotide changes occurs within the repeat sequences at the MIJs.

Comparison of the *O. trifallax WR* Micronuclear and Macronuclear Genes. There are 10 MDSs and 9 IESs in the micronuclear sequence. The MDSs are not in the orthodox macronuclear order but are scrambled in the order MDS3-4-6-5-7-9-10-2-1-8. MDS2 is inverted in the micronuclear version; the coding sequence is on the opposite DNA strand.

At each MIJ is a repeat, one copy of which is retained in the macronuclear sequence after development. For example, one copy in a pair of direct repeats is at the 3′ end of MDS6, and the other copy is at the 5′ end of MDS7. For MDSs that form part of the coding region, one copy of the repeat becomes part of the coding sequence. Repeats are boxed in Fig. 1. MDS2 is inverted, and the repeats for MDS1–MDS2 and for MDS2–MDS3 are likewise inverted in the micronuclear version (refer to Fig. 1). The direct repeats between the nonscrambled MDSs (MDS3–MDS4 and MDS9–MDS10) are 4 bp and 7 bp, respectively. Repeats between scrambled MDSs range from 8 to 13 bp.

Because the micronuclear sequence of the actin I gene of *O. trifallax WR* was generated from PCR products, the lengths of the repeats at the 5′ and 3′ ends of the gene are not known. Repeats are predicted at these locations from the micronuclear actin I sequence of *O. nova*, and PCR experiments indicated the presence of these repeats in *O. trifallax WR* (data not shown). In Fig. 1, the repeat sequences at the 5′ and 3′ ends are depicted as the same lengths as the corresponding repeats of *O. nova* (discussed later).

Micronuclear DNA from *O. trifallax WR* Contains Only Scrambled Copies of the Actin I Gene. Because of scrambling

and the presence of IESs, the sizes of the PCR products generated with a particular primer pair differ from micronuclear and macronuclear DNA templates. PCRs were performed using four primer pairs that were predicted to yield larger PCR products from a scrambled actin I gene than from a nonscrambled version (Fig. 2*A*). When micronuclear DNA was used as template, only PCR products predicted from a scrambled actin I gene were detected; when macronuclear DNA was used as template, only PCR products predicted from a nonscrambled actin I gene were detected (Fig. 2*B*).

Similar Scrambling Patterns of *O. nova* and of *O. trifallax WR*. The order of the MDSs is the same in the two species, with the exception of the presence of MDS10 and an adjacent IES in *O. trifallax WR* (Fig. 3*A*). MDS2 is inverted in both sequences. However, the MIJs are shifted by up to 14 bp for scrambled MDSs and 138 bp for the only two nonscrambled MDSs common to both species (MDS3–MDS4) (Fig. 3*B*). As shown in Fig. 3*B*, the MIJ shifts create different lengths of corresponding MDSs for each species. The sizes of MIJ shifts are reported in terms of the *O. trifallax WR* sequence. For example, a −2-bp MIJ shift means that the MIJ in the *O. trifallax WR* gene is 2 bp upstream of the corresponding MIJ in the *O. nova* gene. MIJ shifts can be precisely determined for only the MDSs that include the coding region. In the 3′ DNA trailer regions, the MDSs of the two species are almost completely different sequences, which makes it impossible to compare them accurately. The lengths of these three MIJ shifts (between MDS6-7-8-9) are, therefore, estimates. Because the MIJs are shifted, repeat sequences are different, as described in Fig. 4.

The repeat lengths for all corresponding pairs of MDSs between the two species are within 2 bp of each other (Fig. 3*A*). For example, the repeat for the MDS4–MDS5 junction is 13 bp in both species, but for the MDS6–MDS7 junction, the repeat is 10 bp in *O. nova* and 8 bp in *O. trifallax WR*. The lengths of the two repeats at the 5′ and 3′ ends of the genes cannot be compared in the two species because the *O. trifallax WR* sequence is PCR-generated, as described above. The repeats between MDS3–MDS4 in both species are 4 bp; such a shorter length repeat is typical of nonscrambled MDSs. Corresponding repeats between scrambled MDSs in both species are generally longer.

Corresponding IESs in the actin I gene (e.g., the IES between MDS4–MDS6) in the two species differ in both size and sequence. The IESs in *O. nova* range from 11 to 110 bp and in *O. trifallax WR* range from 19 to 276 bp. When IESs are ordered according to size, the orders are different between the two species. The sequences of AT-rich 5′ DNA leaders and 3′ DNA trailers are likewise different in the two species.

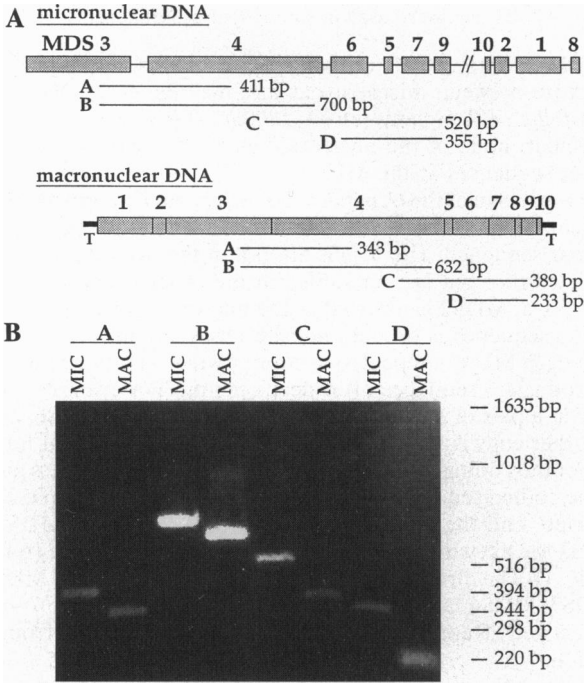

FIG. 2. Only scrambled micronuclear copies and nonscrambled macronuclear copies of the actin I gene are present in native *O. trifallax WR* DNAs. Four PCR primer pairs (A, B, C, and D) were chosen that would generate larger products from a micronuclear DNA template than a macronuclear DNA template. Primer sequences are shown in Fig. 1 as A1, A2, B1, B2, etc. MDSs are labeled shaded boxes; IESs are unlabeled lines. (*A*) Predicted lengths of PCR products are shown for each primer pair, using either micronuclear DNA or macronuclear DNA, as indicated, as template for PCR. (*B*) PCR products from micronuclear DNA (MIC) and macronuclear DNA (MAC) electrophoresed on an ethidium bromide-stained 2% agarose gel.

DISCUSSION

MDS Scrambling of the Actin I Gene in the Two Ciliate Species. The sequences of the macronuclear and micronuclear actin I genes in *O. nova* and *O. trifallax WR* allow the first direct comparison of micronuclear versions of a particular gene between two ciliate species. The MDSs of the micronuclear actin I gene of *O. nova* are in an unorthodox scrambled order MDS3-4-6-5-7-9-2-1-8 (12). The MDSs of *O. trifallax WR*, a closely related hypotrich, are in a similar scrambled order, with the exception of the additional nonscrambled MDS10: MDS3-4-6-5-7-9-10-2-1-8. The conservation of the order of scrambled MDSs suggests that the actin I gene became scrambled before *O. nova* and *O. trifallax WR* diverged in evolution. If this is so, then the presence of an additional MDS and large IES and of MIJ shifting in *O. trifallax WR* means that *Oxytricha* is capable of at least some modification of scrambling while still maintaining the same overall scrambled pattern. In evolution, MDS10 and the accompanying IES must have been lost from the scrambled *O. nova* gene or added to the already scrambled *O. trifallax WR* gene. Although the order of MDS scrambling is conserved, the locations of the MIJs are not conserved between *O. nova* and *O. trifallax WR*. The MIJ shift between nonscrambled MDS3–MDS4 is 138 bp, by far the largest MIJ shift; the MIJ shifts between the scrambled MDSs range from 0 to 14 bp (Fig. 3*B*).

An alternative hypothesis to explain the similarity in scrambling patterns is that scrambling took place independently in the micronuclear actin I gene of both species after the two species separated in evolution. In such a case it is necessary to postulate that the pattern of scrambling is guided by some unknown influence of chromatin structure that yields the same overall patterns of scrambling but is imprecise to the extent that an extra MDS and IES are created and MIJs are shifted.

Repeat Sequences. The scrambled micronuclear genes of *O. nova* contain repeats at each MIJ that are believed to play a role in the unscrambling of the gene during macronuclear development (8, 12). Two copies of each repeat are present in the micronuclear gene, and one copy is retained in the macronuclear gene, which supports the hypothesis of homologous recombination between pairs of repeats to explain removal of IESs and unscrambling of MDSs. Because the sequences of repeat pairs are different within a species as well as between species, the processing mechanism does not recognize a particular sequence.

Repeats appear to fall into two classes. Repeats that presumably serve for splicing nonscrambled MDSs (MDS3–4 for the actin I gene in both species and MDS9–10 in *O. trifallax WR*) range in length from 4 to 7 bp. Repeats that are present between scrambled MDSs in the actin I gene of *O. trifallax WR* range in length from 8 to 13 bp. Similarly, repeats for two nonscrambled sets of MDSs in the micronuclear gene encoding

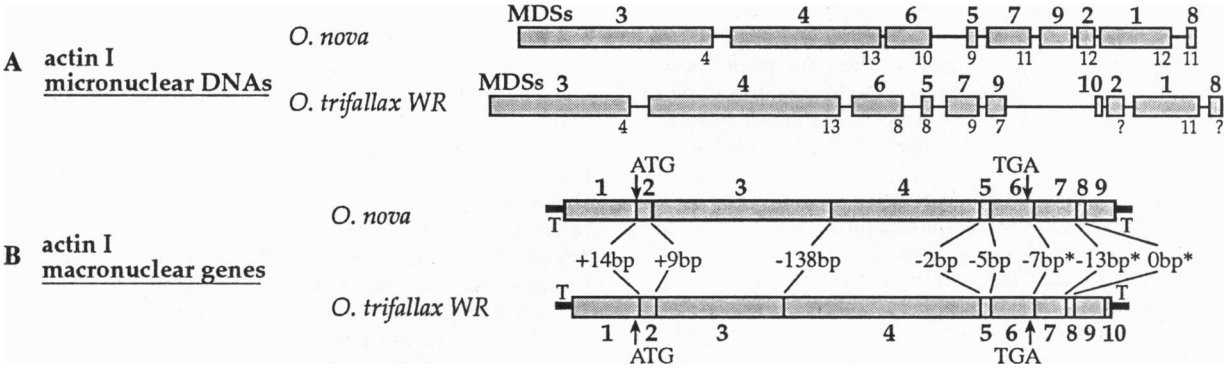

FIG. 3. Comparison of the MDS–IES organizations of the actin I genes of *O. nova* and *O. trifallax WR*. MDSs are depicted as boxes labeled in the boldface type and IESs are depicted as unlabeled lines. (*A*) The micronuclear organizations of the genes. Smaller numbers below each structure represent lengths of repeats present at each MIJ in bp. Repeat lengths are only shown at the 3′ end of the numerically first MDS; another copy of the repeat is present at the 5′ end of the next successive MDS. A question mark indicates that the length of the repeat has not been determined (discussed in text). (*B*) Macronuclear organizations of the genes. Lengths of MIJ shifts are shown between the two gene structures. Negative numbers indicate that the MIJ in *O. trifallax WR* is upstream of the MIJ in *O. nova*. Positive numbers indicate that the MIJ in *O. trifallax WR* is downstream of the MIJ in *O. nova*. Locations of start (ATG) and stop (TGA) codons are shown by arrows. The three MIJ shifts indicated by asterisks are located in the 3′ trailer and are, therefore, approximated (discussed in text). The last MIJ shift (between MDS8 and MDS9) is also approximated because the length of the repeat in *O. trifallax WR* is not known and was estimated to be the same as *O. nova*. T, telomere.

the α-telomere binding protein in *O. nova* are 3 and 5 bp and range from 6 to 19 bp for the 11 sets of scrambled MDSs (8). In the micronuclear gene encoding the α subunit of DNA polymerase in *O. nova*, the repeats for three sets of nonscrambled MDSs are 4 bp and range from 6 to 16 bp for the 41 scrambled sets of MDSs (D. C. Hoffman and D.M.P., unpublished data). The longer repeats represent more sequence information for the recombination model of unscrambling than do shorter repeats.

Absence of a Nonscrambled Micronuclear Version of the Actin I Gene. When using *O. trifallax WR* micronuclear DNA as the template for PCR, single PCR products of lengths predicted by the micronuclear sequence were generated from multiple primer pairs, and no PCR products with lengths predicted by the macronuclear sequence were detected (Fig. 2). In addition, only the predicted PCR products were generated from each of the two species (data not shown) from regions of different size within the micronuclear actin I genes

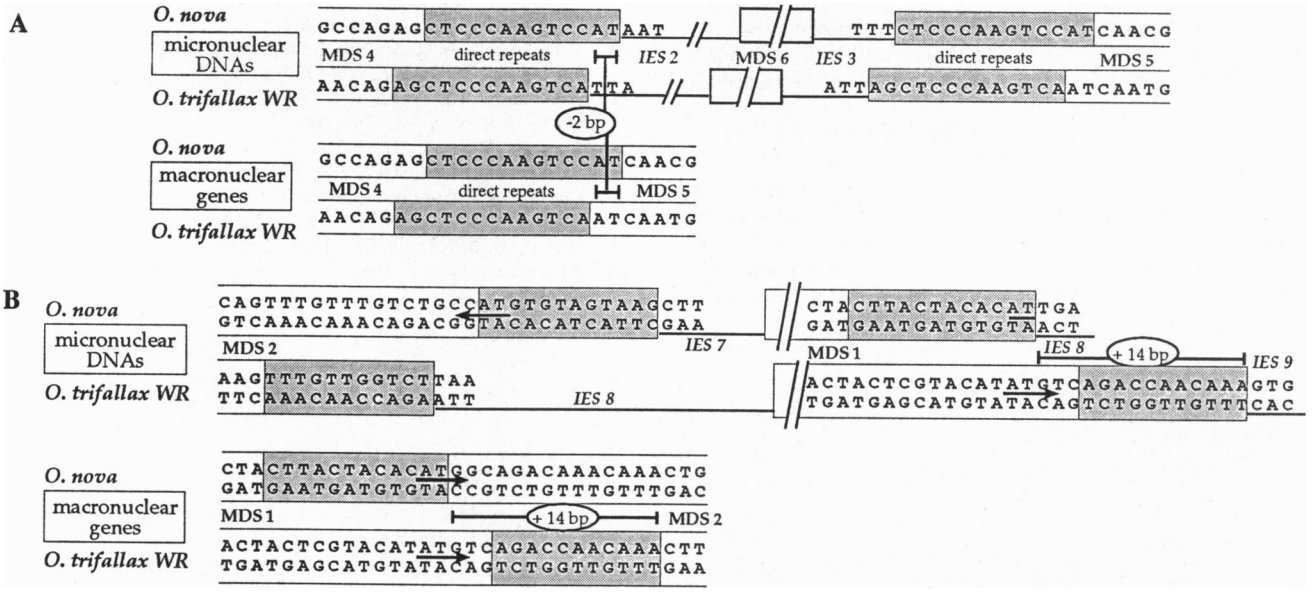

FIG. 4. Illustration of MIJ shifts. MDSs are boxed. Repeat sequences are in shaded boxes. Distances of MIJ shifts are in ovals. (*A*) Sequences involved in the splicing of MDS4–MDS5. (*Upper*) Aligned micronuclear sequences of *O. nova* and *O. trifallax WR*. The repeats of the two species are different sequences, but they are overlapping direct repeats (with 1 nt difference—C vs. A). The MIJ shift of −2 bp results in the shift of the 3′ 2 bp of MDS4 in *O. nova* to the 3′ end after the repeat located at the 5′ end of MDS5 in *O. trifallax WR*. The 5′ 2 bp of the *O. trifallax WR* repeat are not part of the repeat in *O. nova* but are situated immediately 5′ to the repeat in *O. nova*. The repeats for these two MDSs are 13 bp in both species, the longest pairs of repeats present in the micronuclear actin I genes. (*Lower*) Aligned macronuclear sequences after the IESs are excised and MDS4 is spliced to MDS5. One copy of the repeat remains in the macronuclear genes. (*B*) Sequences involved in the splicing of MDS1–MDS2. The ATG start codons are indicated by arrows that point in the direction of transcription. MDS2 is inverted in micronuclear DNA, which creates inverted repeats. (*Upper*) Aligned micronuclear sequences of *O. nova* and *O. trifallax WR*. One copy of a repeat is located at the 3′ end of MDS1. The other copy of the repeat is positioned 5′ of the coding sequence in MDS2 in the direction that transcription proceeds in the macronucleus; because MDS2 is inverted, this repeat is therefore at the 3′ end of the scrambled micronuclear MDS2. This repeat divides the ATG start codon in *O. nova*, but because the MIJ shift is +14 bp (downstream) in *O. trifallax WR*, the ATG is entirely included in MDS1 in *O. trifallax WR*. (*Lower*) Spliced macronuclear sequences of MDS1–MDS2, which lack IESs. MDS2 has been inverted so that all of the coding sequence is in the correct orientation. Only one copy of each repeat remains in the macronuclear genes.

(for example, the 138-bp MIJ shift between MDS3–MDS4, and the addition of the 276-bp IES in *O. trifallax WR*). This is another indication that (*i*) the genes in the two species have differences in some MDS and IES sizes and (*ii*) the only copies of the actin I gene in the micronucleus of *O. trifallax WR* are highly similar in length and organization to the sequenced version.

Species Comparison of IESs. We have compared the sizes and sequences of IESs in corresponding positions in the actin I gene of two ciliate species. The IESs are different in length and very different in sequence, although all IESs are AT-rich. Whether IESs have a function in micronuclear genes is not known; however, any function they may have must be independent of sequence and size because neither trait is conserved between these species. The lack of conservation of size and sequence of IESs between species indicates a rapid evolution and fluidity of IESs.

The positions of IESs within genes are also variable. There is no evidence that IESs actually "move" but there is evidence that they are located in somewhat different positions, while maintaining a similar positional pattern within the same gene in two closely related species. Their positions are not related to intron placement in actin genes of other eukaryotes (14). It is not known whether there are constraints that regulate IES positions within a micronuclear gene.

Origin and Evolution of IESs and Scrambling. IESs might arise from tranpositions or duplications of AT-rich regions (spacer DNA) in the micronuclear genome, they may be generated *de novo* and inserted, or they may be degenerate transposons (15). For nonscrambled MDSs, IES insertion requires a segment of MDS adjacent to the IES insertion to be duplicated at the opposite end of the IES to create the pair of repeats that flanks each IES. When MDSs become scrambled, flanking repeat sequences must move with their MDS. Conceivably, scrambling could occur by formation of repeats without IES formation, but no such cases are known. Also, all repeat sequences initially might be longer and remain longer for scrambled MDSs but shorten over time for nonscrambled MDSs.

Three events have occurred in the modification of a scrambled micronuclear gene that was originally functional. IESs were inserted, pairs of repeat sequences were formed, and MDSs were scrambled. IES insertion and repeat pair formation were probably simultaneous because repeat pairs are presumbly essential for IES excision during macronuclear development. The factors that determine IES insertion, IES location, and repeat formation are totally unknown. However, once IESs are in place in a scrambled or nonscrambled gene, a micronuclear gene may undergo additional evolution with changes in IES length and/or sequence and with the insertion and/or removal of MDSs and IESs.

IES insertion, repeat pair formation, and scrambling might have taken place in a single concerted process. Alternatively, IES insertion and repeat pair formation might have preceded scrambling. Then MDSs could be scrambled and inverted in any pattern as long as each MDS moved with its two flanking terminal repeats; the repeats are presumably used to guide unscrambling. Once a scrambling configuration was established in the ancestor of the two species, the recombination machinery might have interacted with sets of aligned repeats and shifted the MIJs without unscrambling the gene in the germ-line genome.

We thank John Doedens and members of the laboratory for their helpful comments and Gayle Prescott for assistance with the manuscript. This work was supported by Grant GM19199 from the National Institute of General Medical Sciences.

1. Prescott, D. M. (1994) *Microbiol. Rev.* **58,** 233–267.
2. Klobutcher, L. A., Jahn, C. L. & Prescott, D. M. (1984) *Cell* **36,** 1045–1055.
3. Jahn, C. L., Krikau, M. F. & Shyman, S. (1989) *Cell* **59,** 1009–1018.
4. Tausta, S. L. & Klobutcher, L. A. (1989) *Cell* **59,** 1019–1026.
5. Boswell, R. E., Klobutcher, L. A. & Prescott, D. M. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 3255–3259.
6. Roth, M. & Prescott, D. M. (1985) *Cell* **41,** 411–417.
7. Greslin, A. F., Prescott, D. M., Oka, Y., Loukin, S. H. & Chappell, J. C. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 6264–6268.
8. Mitcham, J. L., Lynn, A. J. & Prescott, D. M. (1992) *Genes Dev.* **6,** 788–800.
9. Herrick, G., Cartinhour, S. W., Williams, K. R. & Kotter, K. P. (1987) *J. Protozool.* **34,** 429–434.
10. Ribas-Aparicio, R. M., Sparkowski, J. J., Proulx, A. E., Mitchell, J. D. & Klobutcher, L. A. (1987) *Genes Dev.* **1,** 323–336.
11. Mitcham, J. L., Prescott, D. M. & Miller, M. K. (1994) *J. Eukaryot. Microbiol.* **41,** 478–480.
12. Prescott, D. M. & Greslin, A. F. (1992) *Dev. Genet.* **13,** 66–74.
13. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
14. Weber, K. & Kabsch, W. (1994) *EMBO J.* **13,** 1280–1286.
15. Klobutcher, L. A. & Jahn, C. L. (1991) *Curr. Opin. Genet. Dev.* **1,** 397–403.