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Analysis of the micronuclear B type surface protein gene in *Paramecium tetraurelia*

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ABSTRACT

The micronuclear DNA of *Paramecium* contains sequences that are precisely excised during the formation of the macronuclear (somatic) genome. In this paper we show that four eliminated sequences ranging in size from 28 to 416 base pairs, are present in or near the micronuclear copy of the B surface protein gene. Each excised sequence is bounded by the dinucleotide 5′-TdA-3′. Comparison of the micronuclear B gene with the previously determined micronuclear sequence of the A surface protein gene shows that although the positions of at least three of the eliminated sequences are conserved in both genes, the sequences are highly divergent. Transformation of vegetative macronuclei with fragments of the micronuclear B gene results in replication and maintenance of the DNA, but the micronuclear specific sequences are not removed. Previous studies have shown that the correct incorporation of the B gene into the new macronucleus requires copies of the macronuclear B gene in the old macronucleus. Using macronuclear transformation, we show that the micronuclear B gene can substitute for the macronuclear B gene with regard to its role in DNA processing. This suggests that the macronuclear DNA is not acting as a guide for the excision of the micronuclear specific sequences.

INTRODUCTION

Developmentally controlled DNA rearrangements occur within many eukaryotic organisms. Examples include mating type determination in yeast, the formation of antibody genes in mammals and chromosome loss in the somatic cells of Ascaris. Ciliated protozoans present a unique opportunity for the study of DNA rearrangements. Each cell contains a polygenomic, transcriptionally active macronucleus and a diploid, non-transcribed micronucleus. Both are created during sexual reproduction from a single diploid fertilization nucleus. The formation of a new macronuclear genome involves chromosomal fragmentation as well as the elimination of micronuclear specific DNA through splicing events (reviewed in 1, 2 and 3). These internal eliminated sequences (IESs) are located both within and outside coding regions, and consist of relatively short (14 bp to about 2 kb) sequences. Estimates from work in *Euplotes crassus* suggest that tens of thousands of IESs are eliminated during the formation of its macronuclear genome (4). Few common sequence features of IESs have been identified. All have direct repeats of 2–10 bp at the border of the eliminated sequence, one copy of which is retained in the macronucleus. In some organisms such as *Euplotes crassus*, the direct repeats always include the dinucleotide 5′-TdA-3′. The related *Oxytricha nova* also has direct repeats but they do not necessarily include the sequence 5′-TdA-3′ (3). *Tetrahymena* is the only organism in which the sequence requirements for IES removal have been experimentally investigated. Instead of a sequence within the IES, a polypurine tract was identified about 45 bp outside of the IES that is necessary and sufficient to allow DNA splicing *in vivo* (5).

The recent development of a reliable purification scheme for *Paramecium* micronuclear DNA has allowed the cloning and sequencing of the micronuclear copy of the A surface protein gene (6,7). The A gene is one member of a family of surface protein genes in *Paramecium* (reviewed in 8,9). Analysis of the micronuclear A gene shows that it contains seven IESs within the coding region of the gene, and one IES at position −10 relative to the start of translation. Each IES begins and ends with the dinucleotide 5′-TdA-3′, one copy of which is retained in the macronuclear DNA. Some have longer inverted repeat sequences that include 5′-TdA-3′ (itself an inverted and direct repeat). The micronuclear structure of *Paramecium* surface protein genes is of particular interest because of the remarkable regulatory system that controls the processing of these genes into the new macronucleus.

Each *Paramecium* cell contains two diploid micronuclei and a transcriptionally active macronucleus that has roughly 1000 copies of each gene (10). In addition to its vegetative functions the macronucleus acts in a very specific manner to control DNA processing during sexual reproduction. A cell line that contains wild type micronuclei but has macronuclear deletions of both the A and B genes will not incorporate either gene into the new macronucleus which is formed during sexual reproduction (11). If the macronuclear A gene is transformed into the old macronucleus, the micronuclear A gene is correctly processed into the new macronucleus. Interestingly, this rescue is gene

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specific so that transformation with the A gene does not rescue the B gene, and transformation with the B gene rescues B but not A (11). Experiments have demonstrated that correct processing is not the result of a protein product made from the A gene, but there is no adequate molecular explanation for this communication between the old macronucleus and developing macronucleus (12,13). Until recently all studies have focused on the macronuclear requirements for DNA processing. Clearly, knowledge of the structure of the micronuclear precursors will be required to understand the molecular details which control processing of surface protein genes. In this paper we show that the micronuclear B gene contains sequences which are precisely eliminated during macronuclear development. Comparison of the micronuclear A and B genes shows that IESs contain few conserved nucleotide sequences. In addition, when the micronuclear DNA is transformed into a vegetative macronucleus, the IESs are not removed and they do not prevent replication of the cloned DNA in the macronucleus. Finally, we show that injection of the micronuclear clone of the B gene into the macronucleus can provide the information necessary to process correctly the B gene during macronuclear development. This suggests that the macronuclear DNA does not act as a guide for the removal of IESs.

MATERIALS AND METHODS

Cell lines and cultivation

Paramecium tetraurelia stock 51 is homozygous for both the A and B genes. d12.141 is a homozygous A− and B− mutant which has typical, Mendelian genetics (14). It was originally derived from the Mendelian A− mutant d12, which contains both macronuclear and micronuclear deletions of the A gene (15,16). The M1 cell line was created by replacing the micronuclei of d12.141 with wild-type, stock 51 micronuclei (11)

All cells were cultured in 0.25% wheat grass medium buffered with 0.45 g/liter Na_2HPO_4 (pH is approximately 7.0). Medium was inoculated with a non-pathogenic strain of *Klebsiella pneumoniae* one or two days prior to being used.

Isolation of DNA

Large scale DNA isolations were performed as follows: Packed cells (0.1−0.2 ml) were resuspended in 0.7 ml of culture fluid and then quickly squirted into 2.1 ml of lysing solution (10 mM Tris−HCl, pH 9.5, 50 mM sodium EDTA, 1% SDS,) at 65°C. After 10 min, 7 ml of saturated CsCl was added and the solution was centrifuged in a vTi65.1 rotor at 55 000 r.p.m. for approximately 20 h. DNA containing fractions were collected and dialyzed overnight against TE (10 mM Tris−HCl, 0.5 mM EDTA, pH 8.0). Small scale DNA isolations were performed as follows: 100 ml cultures of each cell line were pelleted, resuspended in 0.4 ml of culture fluid and quickly squirted into 0.8 ml of lysing solution at 65°C. After 10 min, lysates were extracted with phenol:chloroform and the DNA was precipitated in two volumes of ethanol. Pellets were resuspended in 0.5 ml TE, treated with ribonuclease A for 20 min at room temperature (2.5 μl of a 1 mg/ml ribonuclease A solution) followed by a 1 h treatment of SDS and proteinase K at room temperature (1 μl of 5% SDS and 12.5 μl of a 2 mg/ml proteinase K solution). The DNA solution was then extracted twice with phenol:chloroform before a final ethanol precipitation.

Library screening and DNA sequence determination

The micronuclear library was generously provided by John Preer, Louise Preer and Joy Steele, at Indiana University (7). The library was screened using standard methods (17). A *Hin*dIII fragment from the 5′ end of the gene (subclone pSB2.1H) was used as a probe and duplicate filters were probed with a *Pst*I−*Eco*R1 fragment from the 3′ end of the gene (pSB1.2PE). For DNA sequencing, restriction fragments of the phage clone were subcloned into pUC119. The resulting plasmids were transformed into either *E.coli* strain JM101 to produce DNA for single-stranded sequencing, or DH5α to produce DNA for double-stranded sequencing using standard chain termination protocols (17). Sequencing reactions were performed using the Sequenase version 2.0 DNA sequencing kit (US Biochemicals, Cleveland, OH). DNA sequence was determined from both strands of all IES regions. The sequence of one strand of DNA was determined

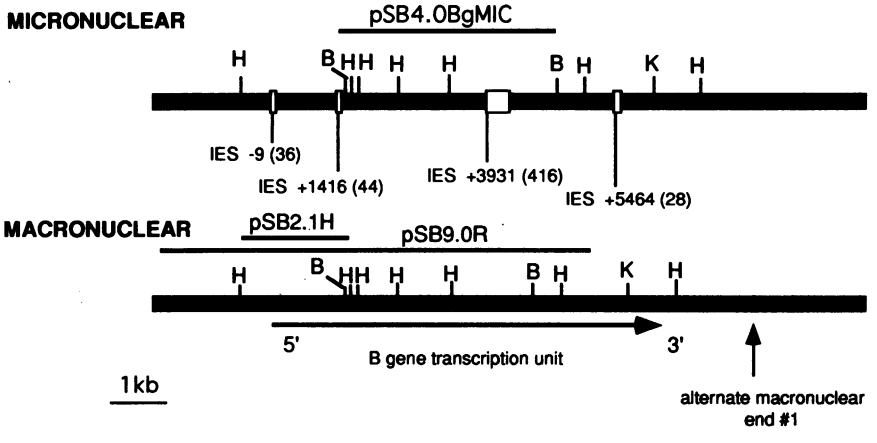

Figure 1. Maps of the micronuclear and macronuclear B surface protein gene. Comparison of the micronuclear and macronuclear B gene DNA sequences show that four internal eliminated sequences are located within or near the B gene. The location of the IESs are shown with white boxes. IES numbers refer to the macronuclear DNA sequence relative to translation start. The numbers in parenthesis indicate the size of the IES in base pairs. The thin lines labeled pSB4.0BgMIC, pSB2.1H and pSB9.0R indicate subclones used in this study. H, *Hin*dIII; B, *Bgl*II; K, *Kpn*I. The DNA sequence was determined for both clones from the *Hin*dIII site upstream of the gene to the *Hin*dIII site downstream of the 3′ end.

for regions of the micronuclear gene which corresponded to the previously determined sequence of the macronuclear B gene. The authenticity of the lambda clone was confirmed by polymerase chain reaction amplification of a segment of the micronuclear genome using one primer within an IES and one in the adjacent macronuclear destined DNA. The amplified fragment gave the expected products after restriction digestion (data not shown). Sequence analysis was performed using the University of Wisconsin GCG sequence analysis software package version 6.2 Copyright (c) 1989 John Devereaux (18). The nucleotide sequence data of the 51B micronuclear gene can be found in the EMBL, GenBank, and DDBJ Nucleotide Sequence Databases under the accession number U07603.

Southern blots

Southern blot analysis was performed according to Sambrook *et al.* (17). Filters were washed in 10×Denhardt's solution, 0.1% SDS, 0.2 M phosphate buffer and 5×SET (1×SET = 0.15 M NaCl, 30 mM Tris, 2 mM EDTA) at 65°C for one hour. The filters were then incubated with hybridization solution (1×Denhardt's, 20mM phosphate buffer, 5×SET and 0.25% SDS) for one hour at 65°C before adding labelled probe. After an overnight incubation, filters were washed 3 times for 30 min each in 0.2×SET, 0.1% SDS, 0.1% sodium pyrophosphate and 25 mM phosphate buffer at a high stringency temperature (71–74°C).

Transformation by microinjection

Microinjections were performed as previously described (19). Prior to microinjection with DNA, cell lines that had undergone micronuclear transplantation were brought through autogamy using the daily isolation method (20) and allowed to grow for at least 2 fissions. Newly formed macronuclei were injected with either a mixture of pSB4.0BgMIC and pSA10XS, or a mixture of λSB-MIC and pSA10XS. pSB4.0BgMIC is a pUC119 plasmid subclone of the micronuclear B gene and contains a 416 base pair IES. This is the largest IES in the B gene. pSA10XS is a plasmid containing the entire coding region of the A gene as well as 1.5 kb of upstream and approximately 300 base pairs of downstream noncoding sequence (21) and λSB-MIC1 is a full length phage clone of the micronuclear B gene. Plasmid DNA was dissolved at a final concentration of 1–2 mg/ml in 114 mM KCl, 20 mM NaCl, 3 mM NaH_2PO_4, pH 7.4 as described by Tondravi and Yao (22). In order to reduce the viscosity of the phage DNA, the micronuclear B gene phage clone was digested with *Sma*I prior to microinjection. *Sma*I cleaves both left and right λ arms but leaves the *Paramecium* insert DNA intact. Phage DNA was injected at a concentration of 1.5 mg/ml. Between 3 and 6 pl of solution was injected into each cell using a glass microneedle 1–2 μm in diameter at the tip. Following microinjection, each cell was placed in 0.75 ml of fresh medium and cultured at 27°C.

Scoring for rescue of cell lines

Each injected cell was cultured for 3 days at 27°C at which time wells had usually reached a density of 500–1000 cells. 50 μl of culture medium was removed from each well (approximately 50 cells) and scored for surface protein A and protein B expression. Cells were considered transformed if A or B surface protein expression was found, even if less than 100% of the cell line showed expression. Uninjected control cells were also routinely checked for A and B expression but no expression of either surface protein was ever found. Selected transformed cell lines were then taken through autogamy to induce the formation of new macronuclei. Following autogamy, 100 ml of each cell line was cultured for DNA isolation. Genomic DNA was digested with either *Hin*dIII or *Hin*dIII and *Pst*I, blotted to nitrocellulose and probed with either a mixture of nick translated pSA1.4H and pSB2.1H, or nick translated pSB4.0BgMIC.

RESULTS

Isolation and DNA sequence of the micronuclear B gene

A micronuclear phage library that was previously used to isolate the micronuclear copy of the A surface protein (7) was screened with a nick translated probe made from the plasmid pSB2.1H, a subclone containing the 5′ end of the macronuclear B gene (see Figure 1). A duplicate set of filters was screened with a probe

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                                                           36
B(-9)     TAGAATGTAAACCTAAAGTT ATAGTATAATCCCATCATATCTCTAATTTCAACTAT AATACTTTTAATGAATAAATAA
A(-10)    TTCTTAGCAAATTTAATTTT ATAGTTAAGTAATCAATGTCC.......TTAACTAT AATACTTTAATGAATTAGAAAT
                                                           29

                                                                                  44
B(1416) TCGTGAATGCTCCATCAACT ATAGTAATTTTATAATAATATTTATATAATAATTATATAGG............TAT AATACTACTAATGATTTATG
A(1416) GTTTAAATGCTCCATCAACT ACAGTTATTATTTAATATTTTCCAAATTAATTATCC///TCTCTCTCTTCGACTAT AACACCACACACGACCTTTG
                                                                                  74

                                                                                  416
B(3931) CAACAATTGCTAATTGTTAT ACAGCTTAATTAAGAAACATTTTCAACAAT///TTATAAATTAAATTAATCAGCTGT AGATCAACTGCTGGATATTG
A(4578) ATACAAAATGCCAAGCCTAT ACAGCTAACGTAAGAAATTTTATTGTAAAT///AAAAAATTTCATTTCCTAGACTGT AACACTAAATGTACTTCAAT
                                                                                  882

                                                  28
B(5464)  GAACTAACTAAGCATATTGT ATTAATAAATTTATTTTATTTCTTCCAT ACTGTTAAGACTTGTAATAC
A(6435)  TTACTTCCCCTGCATATTGT ATCGATAATATTGTTATTAATATATTAT ACAATAAAGACTTGTAGTAC
                                                  28
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Figure 2. Comparison of B and A gene internal eliminated sequences. The sequence of each B gene IES is presented above the most likely related A gene IES. The A gene sequence is from previously published work by Steele, *et al.* (7). The numbers in parentheses indicate the position of the IES with regard to translation start of the macronuclear sequence of the A and B genes. Bold letters are internal eliminated DNA sequence, dots represent spaces introduced to align the junctions of the macronuclear and eliminated DNA, and slashes indicate sequence not shown. The total size of each IES is indicated by the number at the right hand side of the IES.

made from the plasmid pSB1.2PE, a subclone derived from the 3′ region of the macronuclear B gene. In order to maximize the chance of obtaining a clone which spanned the area corresponding to the full length coding region of the macronuclear B gene, only plaques which hybridized to both clones were chosen for further purification. One clone, λSB-MIC1, which contained a 14 kb *Paramecium* DNA insert was chosen for further analysis.

The sequence of the micronuclear B gene was determined within the area corresponding to the entire coding region of the macronuclear gene as well as 563 nucleotides of the 5′ flanking DNA. Figure 1 shows that the micronuclear B gene contains 4 IESs, which range in size from 28 base pairs to 416 base pairs. Except for the presence of the IESs, the micronuclear B gene is 100% identical to the macronuclear B gene. The corresponding region of the A gene contains 8 IESs (7). Comparison of IES locations between these two related genes (70% nucleotide identity within the coding region) shows that at least three IES sites are conserved. One of these is located at the start site of transcription which is −10 for the A gene, and −9 for B (numbers refer to the macronuclear sequence with translation start designated as +1). The other conserved IES locations are in the 5′ end (+1416 for both the A and B genes), and 3′ end (+6435 for the A gene, +5464 for B) of each gene. The conserved positions of these IESs relative to the homologous coding regions of the A and B genes was demonstrated by computer generated alignment (BESTFIT) of the A and B gene macronuclear DNA sequences (data not shown). Despite homologous positions in both genes, the eliminated regions have highly divergent sequences (Figure 2). The only recognizable similarity is in the −9 IES. The −10 IES in the A gene has a 10 nucleotide inverted repeat and the corresponding −9 IES in the B gene has an inverted repeat that is 6 nucleotides long. The IESs in the 3′ end of the B gene have the same size in both genes (28 bp), yet there is no clear identity between the two IESs. The macronuclear sequence near the B gene IES +3931 could not be accurately aligned with the A gene because the nucleotide sequence identity is much lower in this region of the gene (14). Nevertheless, it is likely that the B gene +3931 IES and the +4578 A gene IES are evolutionarily related. They are in the same general area of their respective genes, and they have identical sequences just inside the junction of the macronuclear DNA. Six nucleotides are identical at the 5′ junction and 4 nucleotides at the 3′ junction (Figure 2). As was previously shown for the A gene, all the IESs in the B gene begin and end with the dinucleotide 5′-TdA-3′ and have a higher A+T content than coding regions. A seven nucleotide inverted repeat can be identified in the largest B gene IES sequence. Most but not all of the IESs in the A gene contain inverted repeats (7). No additional conserved sequence features could be identified inside the IESs nor within 50 bp of the bordering macronuclear destined sequences.

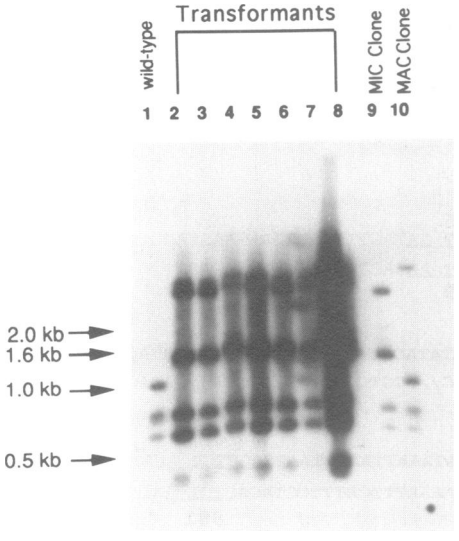

Figure 3. The micronuclear B gene is not processed in vegetatively growing cells. Genomic DNA was obtained from pre-autogamous d12.141 cells that were transformed with pSB4.0BgMIC, a subclone that contains the largest IES in the micronuclear B gene (see Figure 1). Genomic DNA was digested with *Hin*dIII, and Southern analysis was performed as described in the text. Lane 1, wild-type cells; lanes 2−8, transformed cell lines; lane 9, the B gene micronuclear subclone pSB4.0BgMIC, lane 10, the B gene macronuclear subclone pSB9.0R.

The micronuclear B gene is not processed in vegetatively growing cells

To determine if an IES could be removed in the macronucleus of a vegetatively growing cell, we injected a fragment of the micronuclear B gene, pSB4.0BgMIC, into the macronucleus of d12.141, a Mendelian A-, B- mutant. pSB4.0BgMIC is a subclone from the central portion of the micronuclear B gene which contains a 416 base pair IES. The full length A gene clone, pSA10XS, was co-injected along with the micronuclear B gene plasmid so that transformants could be easily selected by serotype A expression. DNA from 7 transformants was obtained and digested with both *Pst*I and *Hin*dIII. This double digest was chosen because it produces a markedly different restriction pattern in the micronuclear and macronuclear B gene clones: a 1.5 kb restriction fragment in the micronuclear B gene which contains the +3931 IES, and a 1.1 kb restriction fragment in the macronuclear B gene without the IES. The Southern blot was probed with nick-translated pSB4.0BgMIC and washed under conditions stringent enough to prevent cross hybridization with related genomic sequences (see Materials and Methods). Figure 3 shows that the 1.5 kb *Pst*I−*Hin*d III fragment of the micronuclear B gene is present in all 7 transformants. This indicates that most of the IESs are not removed and that the presence of an IES does not inhibit replication and maintenance of macronuclear DNA. This experiment cannot rule out that some small amount of DNA is correctly processed.

The micronuclear B gene permanently rescues the B mutation in M1 cells

In an earlier study, a cell line was created that contains a wild type micronucleus but has macronuclear deletions of the A and B surface protein genes. This cell line, called M1, is unable to incorporate either gene into the new macronucleus which is formed during sexual reproduction (11). Interestingly, transformation of the M1 cell line with the macronuclear B gene is sufficient to allow the correct processing of the B gene into the next macronucleus, but does not affect the processing of the A gene (11). In order to determine if the cloned micronuclear B gene could also rescue the B mutation in M1 cells, we microinjected M1 cells with the entire micronuclear B gene phage clone (λSB1MIC). The full length macronuclear A gene plasmid, pSA10XS, was co-injected along with the micronuclear clone so that transformants could be selected on the basis of A gene

expression. DNA was obtained from transformants both before and after autogamy, digested with *Hin*dIII, blotted to nitrocellulose and simultaneously probed with nick translated pSB2.1H and pSA1.4H. Following overnight hybridization, filters were stringently washed so that the two probes would not cross-hybridize. All nine transformants obtained contained both the A and B genes before autogamy. Eight transformants were permanently rescued for both the A and B genes following autogamy, and one transformant was not rescued for either the A or B gene. Figure 4 shows representative transformed cell lines after autogamy.

Although the Southern hybridization in Figure 4 shows that the B gene is incorporated into the new macronucleus, we investigated the possibility that the resulting macronuclear B gene still contained IESs. To determine if the DNA in the macronucleus of the rescued cell lines was wild-type, or contained IES sequences after autogamy, DNA from each transformant was digested with *Pst*I and *Hin*dIII. A Southern blot of the digested DNA was probed with nick-translated pSB4.0BgMIC. All of the cell lines that were rescued with the micronuclear B gene contained a 1.1 kb *Pst*I−*Hin*dIII fragment, indicating that after autogamy the B gene in the macronucleus does not contain IES sequences and has the normal macronuclear B gene pattern (data not shown).

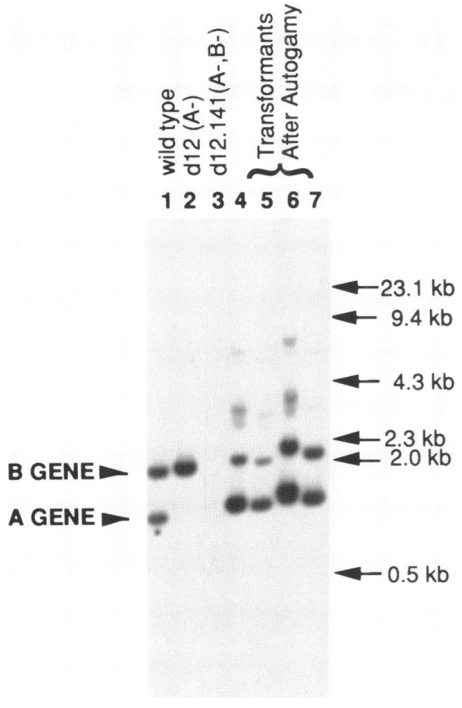

Figure 4. Rescue of the M1 cell line after macronuclear transformation with the micronuclear copy of the B gene. The M1 cell line was co-injected with the macronuclear copy of the A gene (pSA10XS) and the micronuclear B gene (λSB-MIC1). Autogamy was induced in the transformed cell lines and total genomic DNA isolated from post-autogamous cells. DNA was digested with *Hin*dIII, electrophoresed on an agarose gel and blotted to nitrocellulose. The filter was probed with a 2.1 kb *Hin*dIII fragment of the B gene (pSB2.1H) and a 1.4 kb *Hin*dIII fragment of the A gene (pSA1.4H). The respective A and B gene bands are indicated by arrows. The bands above the A and B gene signal are sequences which cross hybridize with the B gene. The slight difference in mobility of the bands in lane 6 is the result of overloading the gel (data not shown). A total of eight cell lines were rescued from nine transformants (data not shown).

DISCUSSION

IES structure

The gene encoding surface protein B contains 4 micronuclear specific sequences which are deleted during the processing of micronuclear DNA into macronuclear DNA. Interestingly, at least three and possibly all four of the IESs in the B gene are related to IESs in the A surface protein gene. Since there is limited sequence similarity between IESs, this relationship is most easily recognized by comparing the sequence of the macronuclear DNA adjacent to the eliminated regions. The presence of the IESs in the same locations in both genes suggests that they were present in a common ancestral gene prior to the duplication and divergence of A and B. It is clear that the sequence of IESs diverges more quickly than the coding regions in the 5′ and 3′ regions of surface protein genes. The only feature that shows some conservation is the inverted repeat at the border of the IES. It is impossible to tell from the current data whether the additional IESs found in the A gene are the result of insertions after the genes diverged or the elimination of IESs from the B gene. The lack of sequence conservation within IESs is consistent with the idea that the signals for removal of an IES lie outside the eliminated sequence. Work in *Tetrahymena* has shown that a polypurine sequence located about 45 bp outside the IES is sufficient to control its elimination (5). We could not identify any conserved primary sequence within 100 bp flanking *Paramecium* eliminated sequences, but this search would be complicated if there are multiple signals. In *Tetrahymena* the polypurine sequence is not found outside of all IESs, suggesting that different classes of signals may be used.

Although a B gene plasmid containing an IES could be replicated in the macronucleus of vegetatively growing cells, the IES was not removed. This suggests that IES removal is regulated in a developmental fashion. The fact that the cell is able to replicate DNA plasmids which contain an IES in the macronucleus is not surprising, since even DNA derived entirely from a procaryotic source can be replicated by the cell (23). It appears that the *Paramecium* vegetative macronucleus does not have a mechanism for recognizing and destroying DNA which is normally micronuclear specific. These results are consistent with previous work in *Tetrahymena* in which a micronuclear-specific sequence was experimentally introduced into the macronucleus and was replicated and maintained as part of a vector that contained ribosomal DNA (24).

Micronuclear DNA and the control of surface protein gene processing

The best studied example of macronuclear DNA processing in *Paramecium* is the A surface protein gene. A mutant cell line, called d48, has a wild type micronucleus but contains a macronuclear deletion of the A gene. Despite this normal micronucleus, the A gene is not correctly incorporated into the new macronucleus and a telomere is formed near the 5′ end of the A gene rather than 8−15 kb downstream of the gene (25). Microinjection of the macronuclear A gene or fragments of the A gene into the d48 macronucleus permanently rescues the mutation after the next round of sexual reproduction. Recently, we have shown that a mutant cell line called M1, which has

macronuclear deletions of the A and B genes but has a normal micronucleus can be rescued after transformation with the macronuclear B gene (11). Thus, a similar mechanism controls both genes. Studies of the A gene have shown that processing is gene specific (related sequences don't rescue), dependent on the length of DNA (bigger is better), but not dependent on protein products from the A gene (12,13). Models that propose base pairing interactions between the nucleic acid in the old macronucleus and the developing macronucleus are attractive but there is no direct experimental support for this idea. One simple model is that the sequence of macronuclear DNA (in the form of DNA or RNA) acts as a guide for the removal of IESs in the developing macronucleus. This model predicts that a micronuclear clone should not rescue the mutant M1 cell line. Since the micronuclear B gene was sufficient to allow correct formation of the macronuclear B gene (including correct IES removal) it is unlikely that IES removal is guided by nucleic acid from the old macronucleus. This experiment does not demonstrate that IES removal is unrelated to the DNA processing defect in d48 and the M1 cell lines. A large number of variable DNA rearrangements are known to occur at macronuclear telomere addition sites (26), and recent work on the formation of the macronuclear end near the G surface protein gene in *P.primaurelia* suggests that the removal of IESs and formation of macronuclear telomeres are part of a single processing pathway (27).

Although the number of cells tested was small, the cloned micronuclear B gene rescued the B mutation in the M1 cell line as efficiently as the macronuclear A gene rescued the A mutation (about 90% of transformed lines). This is interesting because internal deletions of the macronuclear A gene substantially drop the efficiency of rescue (from about 90% to 20%). Large insertions into macronuclear DNA have not been investigated. Since the IESs would cause frameshifts in the B gene, rescue by the micronuclear clone is also consistent with previous work showing that a protein product is not required from the DNA to allow macronuclear DNA processing.

Characterization of the micronuclear A and B genes is an important step in understanding the defective processing of DNA in the d48 and M1 strains. The timing of IES removal can now be examined in wild type cells and compared with the mutant d48 and M1 cell lines. Investigations of the DNA rearrangements and micronuclear sequences that occur at the sites of wildtype macronuclear telomere formation will be useful to understand the defects in the mutant strains.

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