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Evidence for Transcriptional Self-Regulation of Variable Surface Antigens in *Paramecium tetraurelia*

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Variable surface antigens are commonly found on free-living and parasitic protozoa, yet the regulation of antigen expression and switching is not fully understood in any system. A cell line of *Paramecium tetraurelia* stock 51 can express at least 11 different antigens yet only one type is found on the surface at any one time. Previous studies have shown that mutually exclusive expression of *Paramecium* surface antigens can be overcome if two antigen genes contain the same 5′ coding region. In this article we utilize a gene chimera containing portions of A51 and B51 to analyze the effect of a frameshift mutation on transcription and steady-state mRNA levels. We show that a frameshift mutation near the 3′ end prevents expression of the protein on the cell surface and reduces the rate of transcription of the corresponding gene. The difference in transcription is not the result of differences in plasmid copy number. We propose that expression of the antigen on the cell surface is part of a self-regulatory pathway that influences transcription of the corresponding gene. A model incorporating the previous and current data is presented.

Transcription Variable surface antigen Paramecium GPI-anchored protein

VARIABLE surface antigens are a common feature of both parasitic and free-living protozoa. Examples include trypanosome variant surface glycoproteins (21) as well as the variable surface antigens of *Tetrahymena* (34,35) and *Paramecium* (4,25). *Paramecium tetraurelia* stock 51 has a repertoire of at least 11 different surface antigens, yet only one type is found on the surface at any one time. Although environmental conditions such as temperature, pH, and salinity can cause surface antigen switching, it is clear that they do not control mutual exclusion. Early genetic experiments demonstrated that isogenic cell lines under identical conditions can show stable expression of different surface antigens [reviewed in (4)].

Each antigen is encoded by a separate unlinked gene and is named according to the corresponding antisera that kills cells expressing that antigen (A51, B51, C51, etc.). Complete sequences of the *Paramecium tetraurelia*, A51, C51 (20), B51 (31), and D51 (30) and *Paramecium primaurelia*, G156, G168 (22,23) genes have been reported.

The surface antigens themselves are high molecular mass, 250–310 kDa, cysteine-rich glycoproteins that are attached to the cell surface via a glycosylinositol phospholipid anchor [(3), J. Van Houten, personal communication]. These abundant proteins constitute 50% of the ciliary membrane protein and 3.5% of the total cellular protein (24). Although the function of surface antigens is not known, their abundance and the fact that no paramecia have been found without a surface antigen imply that they have an important function, perhaps serving as a protective coat.

Studies have shown that mutually exclusive expression is controlled primarily at the level of transcription (16,19), and based on its α-amanitin sensitivity, transcription is most likely mediated by an RNA polymerase II-like enzyme (8). In at least one case there is also evidence for posttranscriptional reg-

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ulation, which suggests that some examples of mutual exclusion may involve multiple regulatory mechanisms (8). Promoter deletion studies have shown that the 5′ upstream region of surface antigen genes is required for transcription, but substitution experiments showed that this region is not sufficient to control mutually exclusive expression (16,19). Remarkably, the 5′ coding region of surface antigen genes contains a major regulatory element for mutually exclusive transcription. A chimeric gene that contains the first 885 base pairs of the B51 coding region attached to the remainder of the coding sequence from A51 is coexpressed with B51 (15). The precise location of the *cis* regulatory elements within the coding region remains unknown.

Several models of surface antigen gene expression have been postulated, and some of these speculate that the surface antigen itself is involved in the regulation of mutual exclusion (1,2,5,6). Self-regulation is an attractive idea because it helps explain the relatively stable expression of a single antigenic type in the face of many alternative antigens. A genetic analysis of allelic exclusion between G156 and G168 alleles in *P. primaurelia* provided some support for this hypothesis. Observations of the homozygous strains as well as a strain isogenic for 168 but containing the G156 gene showed that the expression patterns tend to follow the antigen gene itself rather than regulatory genes in the host background. This and other data led Capdeville (2) to conclude that surface antigen expression is self-regulated.

In this article, we use chimeric genes developed in our laboratory to investigate whether the presence of a specific antigen on the cell surface can influence the transcription of its corresponding gene. We demonstrate that transcription of a chimeric surface protein is downregulated when a frameshift mutation is placed near the 3′ end of the gene. Other experiments show that substitution of the 3′ coding region between A51 and B51 genes does not alter mutually exclusive transcription. We present a model to explain these results in the context of previous studies of *Paramecium* variable surface antigen expression.

MATERIALS AND METHODS

Cell Lines, Media, Growth Conditions, and Immobilization Assays

P. tetraurelia stock 51 is homozygous for the A51 and B51 surface antigen genes. Cell line d12.141 was originally derived from stock 51 and contains both macronuclear and micronuclear deletions of the A51 gene as well as a complete macronuclear deletion of the B51 gene (28,31). All cells were cultured in a 0.25% wheat grass medium buffered with sodium phosphate and supplemented with 1 μg/ml stigmasterol. The medium was inoculated with a nonpathogenic strain of *Klebsiella pneumoniae* 1–2 days prior to use. Stock 51 or transformed d12.141 cell lines were maintained at 27°C. Serotype identification was carried out using the immobilization assay described by Sonneborn (33). Each injected cell was cultured for 3 days or until a density of 500–1000 cells/ml was achieved. Fifty microliters of the culture was removed, and 50 μl of antisera [anti-A51 or anti-B51 diluted 1:100 in Dryl's solution (0.1 M sodium phosphate monobasic, 0.1 M sodium phosphate dibasic, 0.1 M sodium citrate, 0.1 M calcium chloride)] was added. The fraction of cells immobilized by the antisera after 15 min indicates the fraction expressing the corresponding surface antigen.

Microinjection

Microinjections were performed as previously described (9). DNAs were dissolved at a final concentration of about 2 μg/μl in 1× TE, pH 7.4, or in a microinjection buffer [114 mM KCl, 20 mM NaCl, 3 mM NaH_2PO_4 (pH 7.4)]. Between 3 and 6 pl of this solution was injected into each cell using a glass microneedle 1–2 μm in diameter at the tip. A higher rate of transformation was obtained when microinjecting cells that were cultured for 3–4 days (at 27°C) after autogamy.

Total DNA and RNA Isolations, RNA Blotting, and Hybridization

RNA was isolated using spin columns (Rneasy Kit, Qiagen, Valencia, CA) and DNA isolations were carried out as previously described (26,32). Filters containing total genomic DNA for hybridization were prepared as follows. Approximately 5 μg of each genomic DNA sample was denatured in 0.3 M NaOH, heated to 65°C for 30 min, neutralized with 7.5 M NH_4OAc, and applied to Schleicher & Schuell maximum strength Nytran with a vacuum slot blotter. The filters were UV fixed with 1200 μJ light (UVP, CL-1000 Ultraviolet Crosslinker). RNA slot blots were prepared as described in Sambrook et al. (29). Hybridization and washes were performed exactly as described in Leeck and Forney (15). Copy number determination required stripping and reprobing filters three successive times, first with the 1.4-kb A51 fragment, then a 2.0-kb B51 fragment, and finally with a 2.2-kb α-tubulin gene. Hybridization signals were counted using a Packard InstantImager (Packard Instrument Co. Inc.) and copy number was calculated by dividing the signal from transformants by the signal from wild-type cells. The α-tubulin probe was

used to correct for small errors in loading. For RNA slot blots, the rDNA probe was used to correct for loading errors. To determine the amount of RNA, we measured the counts (Packard Bell InstantImager) from the RNA slot blots and divided the counts of the transformed lines by the counts of wild-type A51 or B51. Therefore, the amount shown in Fig. 2 is the average value (four individual transformed lines from each co-microinjection) relative to the wild-type A51 or B51 RNA level. Stripping of the filters was done as recommended for nylon membranes (29) or by boiling in 0.1% SDS for 2–5 min.

In Vitro Nuclear Run-on Transcription Assays

Transcriptional activity was examined using the run-on transcription assay previously described except 100 µl cytoskeletal frameworks were used instead of 50 µl (8). Micro Bio-spin 6 chromatography columns were used to remove unincorporated radioactive nucleotides (Bio-Rad). Filters containing 0.5 µg DNA for hybridization to labeled RNAs were prepared the same way as the genomic DNA, described above. Approximately 10^7 total counts of labeled RNA were incubated with the membranes.

Radiolabeled DNA Probes

DNAs that were used to make probes were either gel-purified fragments or PCR products that were purified using Qiagen spin columns. The primers used to amplify specific regions of A51, B51, and α-tubulin included: AAGCTTATTTAACTGGAA (+1235, A51) and GCATAGATCTATAGTCTGTACAATTAGCTG (+2620, A51), GCTTAGTTAGTTCCTACAG (+5440, B51), and AATGCTTCTGGAATACTGG (+3400, B51), and ATTAGAATCAGAGAGGGC and GGA GATCATACAAACAGC (for α-tubulin). The templates for A51 and B51 were lambda SA1 and lambda SB2, respectively (7,31). α-Tubulin was amplified from pTc2 (provided by John Preer, Bloomington, IN). [α-^{32}P]dCTP radiolabeled DNA probes were prepared by nick-translation or by random primer labeling kit (Amersham Inc.). Typical specific activity was 2×10^7 cpm/µg.

Plasmid Construction

pSB (previously known as pSB11.6BX) is the full-length cloned version of the B51 gene (31). PSB/A is a chimeric gene that was made previously, originally known as pSB(−1649–+885)A (15). To make the pSB/A(fs) (pSB/A with the frameshift mutation) DNA construct, primers GTAC**GAATTC**TCTGAT CCAAAAGTTTG (+7026, *Eco*RI site in boldface) and universal primer and template pSA-K were used to generate the PCR product. The PCR product was digested with *Eco*RI and *Bam*HI, then gel purified (UltraClean 15 DNA purification kit, Mo Bio Laboratories, CA). It was cloned into *Eco*RI/*Bam*HI digested pSB/A. The frameshift was created by deleting a single nucleotide at position +7032. pSA-K=pSA is a derivative of the A51 gene clone (36). The pSA/B-Cterm construct was made using primers AGTC**G GATCC**TGAAACTATCTGAC (+8135, *Bam*HI site in boldface) and ACTGATGGTGGATGTGTT (+5994) and template pSB. The PCR product was purified, digested with *Bam*HI and *Eco*RI, and subcloned into *Bam*HI/*Eco*RI digested pSA.

RESULTS

A Surface Antigen Gene Frameshift Mutation Downregulates its Own Transcription

Previous regulatory models of *Paramecium* variable surface antigen expression proposed roles for the surface antigens in the control of their own expression, but there is little direct molecular data to support these theories. The major feature of surface antigen expression that complicates the experimental analysis is the mutually exclusive nature of expression (each cell is limited to a single type of antigen on its surface at any one time). We have partially overcome this problem by constructing genes containing roughly the first 900 bp of coding sequence from one surface antigen and the remainder of the coding sequence from another antigen. These chimeric genes allow coexpression with the wild-type gene that contains the corresponding 5′ coding region. The chimeric gene pSB/A (Fig. 1A) was made by replacing the 51A sequence between −1652 and +885 with the equivalent region of the B51 gene. Microinjecting pSB/A with pSB resulted in coexpression of A51 (actually pSB/A) and B51 surface antigens (15). This coexpression system allows us to measure transcriptional activity and steady-state RNA levels when one surface antigen is not expressed on the cell surface.

Paramecia generally transcribe and express a single antigen on the cell surface at any one time, yet pSB/A and pSB have the same 5′ coding region and both are transcribed and expressed on the cell surface. We prevented expression of a functional protein from pSB/A by deleting one nucleotide at position +7032, creating a frameshift and a subsequent stop codon at position +7301. The resulting plasmid, called pSB/A(fs) (pSB/A frameshift), does not express protein on the cell surface when transformed into cells (data not shown). To analyze the effect of the frameshift on transcription, two sets of coinjec-

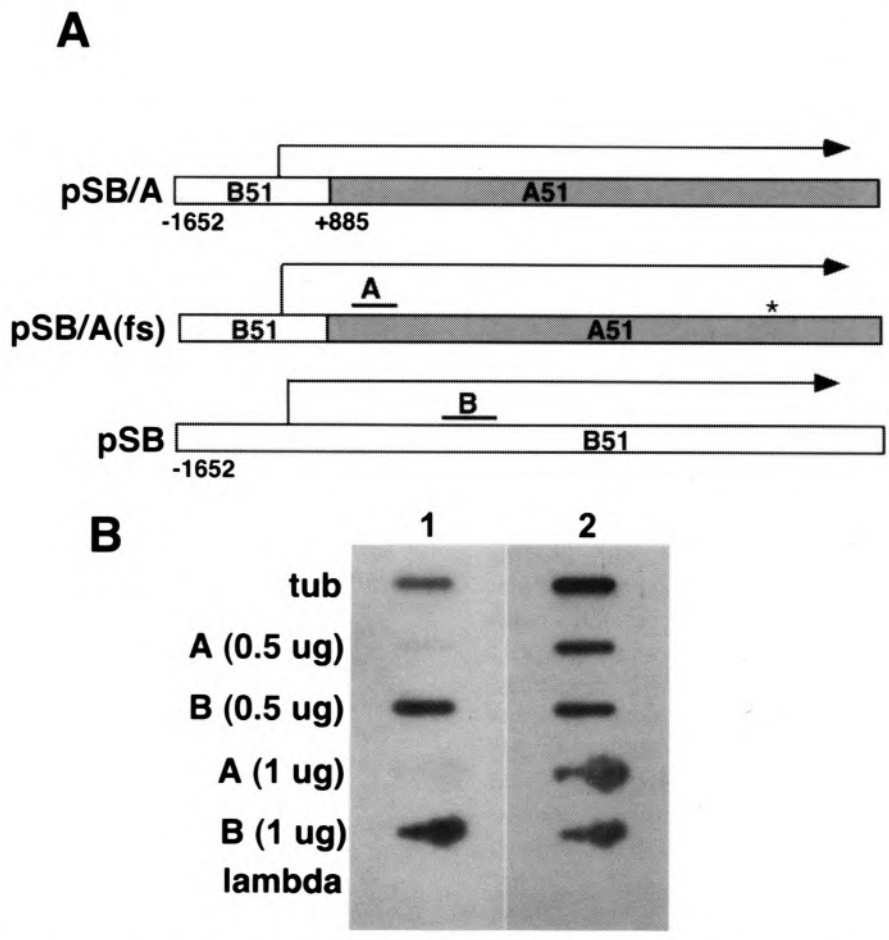

FIG. 1. Surface antigen expression regulates transcription. (A) DNA constructs of pSB/A and pSB were microinjected into an A-B- cell line (d12.141). The asterisk (*) indicates the location of the nucleotide deleted to create a frameshift. The bar lines labeled A or B on top indicate DNA fragments that were attached to the Nytran membrane for hybridization. A is a 1.4-kb fragment of the A51 gene. B is a 2-kb fragment of the B51 gene. (B) Nuclear run-on transcription assays. Blot 1 was hybridized with the ^{32}P-labeled RNA from pSB/A(fs) + pSB co-microinjected cells. Blot 2 was hybridized with ^{32}P-labeled RNA from pSB/A + pSB co-microinjected cells. Tub = *Paramecium* tubulin gene. Lambda is phage lambda DNA, which is used as a negative control for the hybridization reaction. The numbers in parentheses indicate the amount of the fragment attached to the membrane. Different amounts were used to be sure that the DNA on the membrane is not the limiting factor for the signal.

tion were performed: 1) pSB/A + pSB and 2) pSB/A(fs) + pSB. In all experiments the constructs were transformed into a cell line that has deletions of the endogenous A51 and B51 genes. Nuclei were isolated from transformants and run-on transcription analysis was performed. The radioactive RNA probes from the run-on reaction of pSB/A(fs) + pSB transformed lines and pSB/A + pSB transformed lines were hybridized separately to filters that contained the α-tubulin gene, 1.4-kb *Hin*dIII fragment of A51 (labeled A), 2.0-kb *Hin*dIII fragment of B51 (labeled B), and lambda DNA. Run-on analysis of four different pSB/A(fs) + pSB transformed cell lines was performed. Each cell line displayed a five- to sixfold lower transcription rate of pSB/A(fs) compared with the wild-type pSB construct (Fig. 1B, 1). In contrast, analysis of two transformants coinjected with pSB/A + pSB showed that the transcription rate for pSB/A and pSB was essentially the same (Fig. 1B, 2). The data suggest that the presence of the antigen on the cell surface alters the transcription of the gene.

Lower Transcription Rate Is Not Due to Copy Number Differences

Although the macronuclear copy number of plasmids in transformed cell lines can vary considerably (100-fold), the copy number ratio between two coinjected DNAs is generally maintained throughout the vegetative life of a clone (13). To eliminate the possibility that the differences in the transcription rate between pSB/A(fs) and pSB are due to copy

number differences, genomic DNA was isolated and Southern blots were performed to determine the copy number. The copy numbers are presented as multiples of the wild-type macronuclear copy number (Table 1). As indicated in the table, the copy number of the chimeric gene [pSB/A or pSB/A(fs)] is greater than pSB for all the transformants analyzed. Therefore, the reduced transcription of pSB/A(fs) cannot be accounted for by copy number differences.

Steady-State RNA Levels Correspond to Transcriptional Activity

The presence of a frameshift mutation and resulting stop codon could affect the stability of RNA. To examine this possibility, total RNA was isolated and RNA slot blots were prepared. The RNA slot blots were probed with either the 2.0-kb *Hin*dIII fragment of B51, 1.4-kb *Hin*dIII fragment of A51, or the rDNA gene. The rDNA gene was used as an internal control to correct for loading errors. For calculation purposes, the pSB/A(fs) transcript (A51 probe) and B51 RNA levels are shown relative to the wild-type A51 or B51 RNA level, respectively. The average value of four cell lines was used to make the graphs in Fig. 2. Figure 2A indicates that there is about sixfold less of the chimeric transcript from the frameshift construct [pSB/A(fs)] compared with pSB/A when they are co-injected with pSB. Because transcription is decreased by approximately sixfold, the sixfold lower pSB/A(fs) transcript level can be accounted for solely by the reduced transcription rate. The data also suggest that nonsense-mediated decay does not contribute significantly to the decreased steady-state RNA levels. As expected, the level of B51 RNA in both sets of transformants [pSB/A(fs) + pSB and pSB/A + pSB] is essentially the same because in both cases the B51 surface antigen is expressed (Fig. 2B).

Examination of the graph in Fig. 2A shows that in both cotransformation experiments the transcript from the chimeric gene is less abundant than the normal wild-type A51 RNA level. The reason for this difference is not known, but it does not alter the conclusion that relative to the normal pSB/A transcript the frameshift transcript has a decreased abundance. These data suggest that the difference in transcriptional activity accounts for the lower levels of surface antigen transcript and that surface antigen expression does not alter mRNA stability.

TABLE 1
COPY NUMBER OF INJECTED PLASMIDS AND PERCENTAGE OF CELLS EXPRESSING A51 AND B51

	Copy Number		Serotype (%)	
Cell Line	pSB/A	pSB	A51	B51
pSB/A + pSB				
1	82	74	100	100
2*	100	63	95	98
pSB/A(fs) + pSB				
1*	4.6	4.2	0	100
2	33	28	0	100
3	2.1	1.7	0	100
4	15.1	14.9	0	95

Autoradiogram quantitation was done using an Imager. pSB/A is a chimeric gene illustrated in Fig. 1. pSB is the B51 gene plasmid. Expression was determined by immobilization assays prior to total DNA, total RNA, and nuclei isolation. α-Tubulin was used to correct the loading errors. pSB/A(fs) was created by deleting a nucleotide at the 3′ end of pSB/A. Copy numbers are presented as multiples of the wild-type macronuclear copy number.

*Molecular data from these cell lines are also shown in other figures.

Substitution of the C-Terminal Region of Surface Antigen Genes Does Not Alter Mutual Exclusion or mRNA Stability

Although previous studies have shown that the 5′ coding region contains an important controlling element for mutual exclusion, the role of the 3′ coding region was not addressed (15). Because this region is altered in our frameshift construct, we examined whether substitution of the 3′ coding region affects mutually exclusive transcription. The chimeric gene pSA/B-Cterm was constructed by substituting the A51 gene sequence, from +7026 to +8554 (+8154 is translation stop), with the B51 gene sequence, from +6054 to +8138 (7188 is translation stop) (Fig. 3A). Cells expressing the chimeric antigen react only with A51 antiserum and not B51 antiserum (data not shown). Thus, when pSA/B-Cterm is coinjected with pSB we can distinguish which surface antigen is expressed on the cell surface. Unlike the pSB/A chimeric gene (Fig. 1A), pSA/B-Cterm did not confer coexpression when it was co-injected with pSB. Transformed cells expressed only the pSA/B-Cterm chimeric protein on the cell surface as assayed with antisera (27°C favors A51 expression). Northern blots (Fig. 3B) confirmed that no pSB mRNA is present when the chimeric gene is transcribed. In contrast, co-injection of pSA/B-Cterm with pSA results in transcription of both genes (Fig. 3B, probe 1 and probe 3). The data clearly demonstrate that transcription of pSA/B-Cterm is regulated the same as the A51 gene and mutual exclusion is not altered. Comparison of RNA levels between wild-type cells and cell lines cotransformed with pSA/B-Cterm + pSB show that the level of RNA is similar in both cell

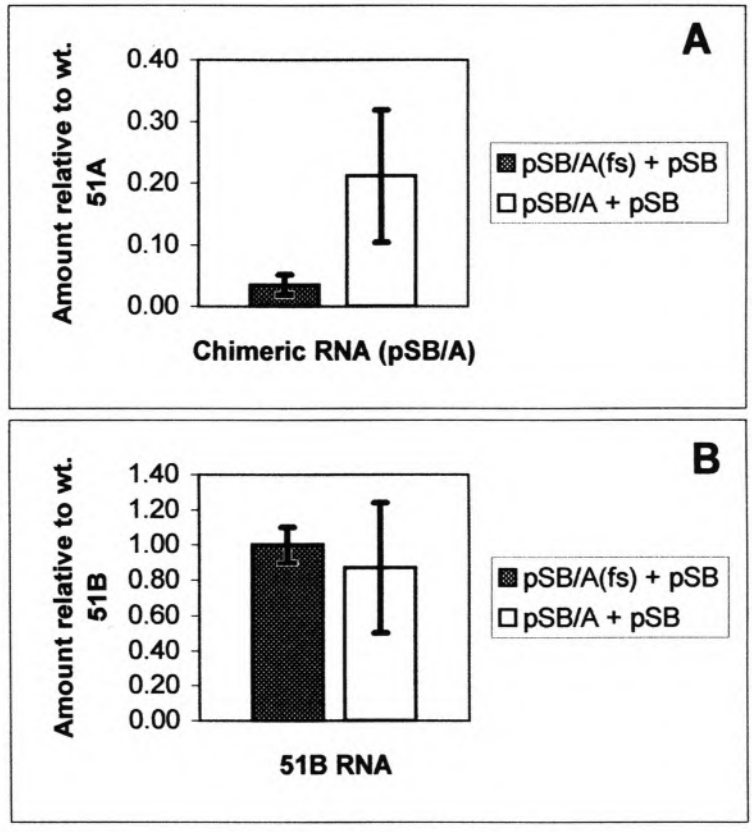

FIG. 2. Stability of steady-state mRNA levels. The mRNA level shown was the average of four individual transformed cell lines and is presented as the amount relative to the wild-type A51 or B51. A RNA slot blot was hybridized with the 1.4-kb *Hin*dIII fragment of the A51 gene (A). The blot was stripped and reprobed with a 2-kb *Hin*dIII fragment of the B51 gene (B). rDNA probe was used to normalize small loading errors.

lines (Fig. 3B, probe 1 and probe 3), which suggests that the A51 RNA stability is unaffected by the B51 sequence substitution.

DISCUSSION

The unusual characteristics of *Paramecium* variable surface antigen expression create a complex system for experimental analysis. Although the regulation of surface antigen expression is controlled primarily at the level of transcription, our previous work has defined components that are required for general transcription and others that control mutual exclusion. The 5′ upstream sequences are required for general transcription as demonstrated by upstream deletions of the A51 gene (16,19). Nevertheless, plasmid constructions that attach the B51 5′ upstream sequence onto the A51 coding region do not affect mutual exclusion. Despite having the same upstream sequences as the B51 gene, the resulting chimeric gene is cotranscribed with A51, not with B51. In this example, it is clear that the upstream sequences are required for transcription but do not distinguish between different surface antigens. In contrast, substitution of the first 885 nucleotides of the coding region of A51 with B51 sequences changes the pattern of transcription so the chimeric gene is simultaneously transcribed with B51 (15).

The experiments in this article show that a frameshift mutation in pSB/A [creating pSB/A(fs)] results in a substantially reduced rate of transcription. This difference in transcriptional activity cannot be accounted for by differences in copy number because the ratio between pSB/A(fs) and pSB is similar in different transformants. In fact, each cell line has slightly more copies of pSB/(fs) than pSB (Table 1). Run-on assays performed on four independent transformants were consistent with roughly a sixfold decrease in transcriptional activity. A corresponding sixfold decrease in steady-state RNA levels was also

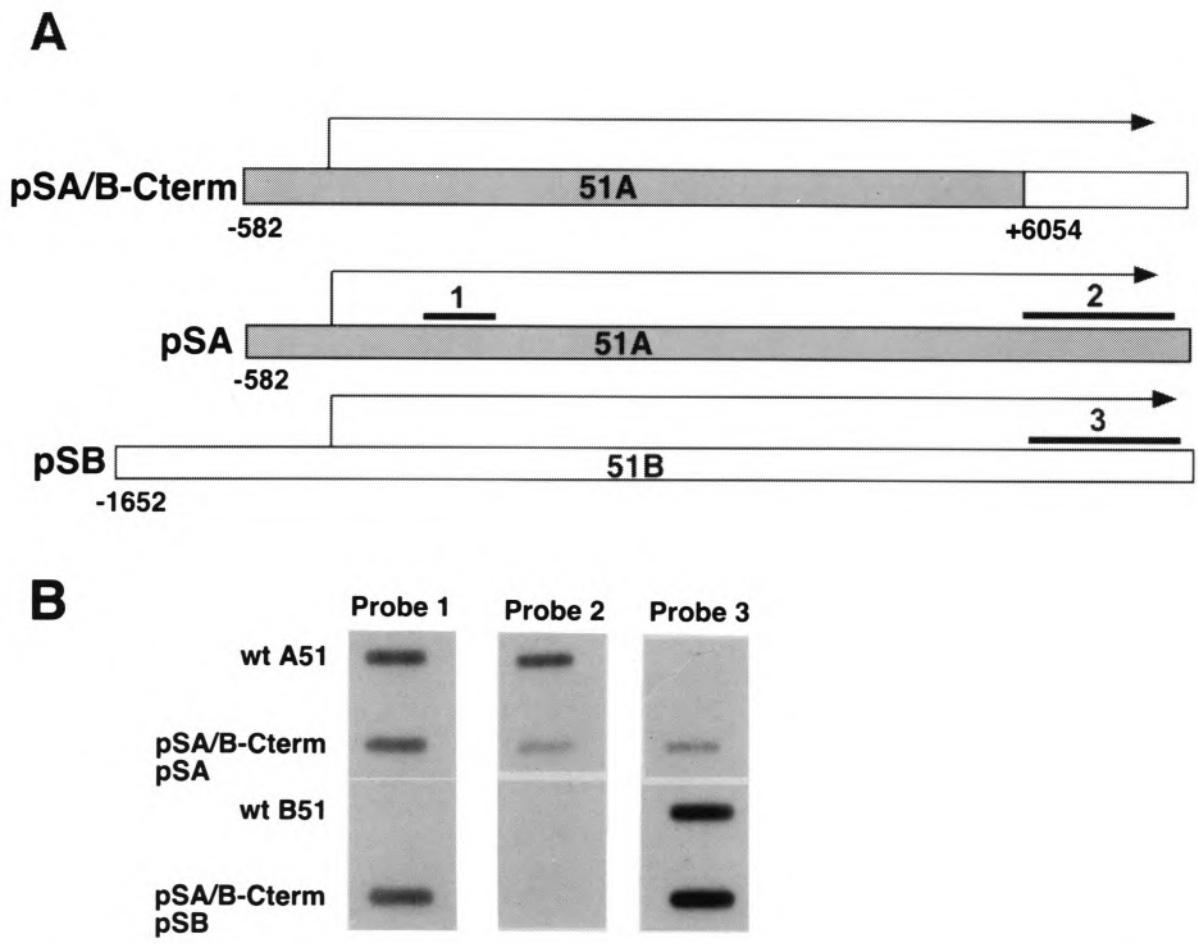

FIG. 3. Analysis of the 3′ coding region. (A) The pSA/B-Cterm was co-injected with either pSA or pSB into the A-B- cell line (d12.141). Solid lines above the constructs represent the fragments used as probes for the Northern blots. (B) Northern blots. The three filters were prepared with identical samples. Each was hybridized with the probe indicated. The transformed lines of pSA/B-Cterm + pSA or pSA/B-Cterm + pSB injection expressed A51 surface antigen at the time of isolation.

observed, making it unlikely that nonsense-mediated decay [reviewed in (12)] is occurring in this system. The differences in transcription rates lead us to conclude that a portion of the variable surface antigen transcriptional pathway is self-regulatory. Because the sequences upstream of nucleotide position +885 are identical in both the chimeric gene [pSB/A(fs)] and pSB, we must presume that sequences downstream of +885 also influence transcription. Additional experiments in this article show that substitution of the 3′ coding region (+6054) and downstream sequences between A51 and B51 failed to alter transcription; therefore, the middle of the coding region must be considered a target for factors that alter transcription. We summarize the previous and current data into a model shown in Fig. 4. This model features repressors that act upon the coding region (between +885 and +6054) to inhibit transcription and activators upstream of +885. Competition between repressors and activators controls mutual exclusive transcription. Expression of a specific antigen on the cell surface is able to signal the release of repressors for the corresponding gene. The frameshift in pSB/A(fs) prevents expression on the surface, and therefore lowers transcription activity of the gene due to continued repression. Although direct evidence for this hypothesis is not available, it is noteworthy that we have been unsuccessful in expressing chimeric surface antigens that contain large (>1 kb) substitutions within the central region of the gene (Y. You and J. Forney, unpublished). It is not yet known whether this is a result of defects in transcription. Interestingly, no introns have been detected in *Paramecium* surface antigen coding regions even though they

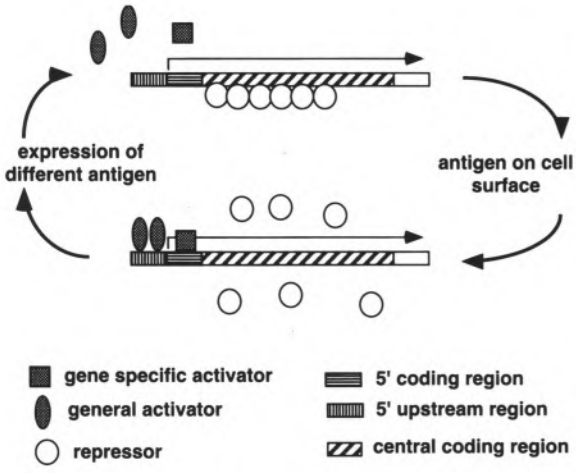

FIG. 4. General model for variable surface antigen expression. Previous work has shown that the 5′ upstream region is required for transcription and the 5′ coding region controls mutual exclusion. Experiments in this article implicate the central coding region as a site for repressor binding. Release of repressors is mediated by a signal originating from the antigen itself. Expression of a particular antigen is dependent on competition between activators and repressors. The *trans*-acting factors have not been identified and are shown for the purpose of clarifying the model.

are commonly found in other *Paramecium* genes. This fact is also consistent with the possibility that the coding region acts as a *cis* element in transcriptional control.

The signal that causes reduced transcription of the frameshift construct [pSB/A(fs)] is unknown but of great interest. In theory, one could propose an interaction between the surface antigen itself and a gene-specific repressor resulting in reduced repression and stable transcription of the corresponding surface antigen. Although attractive, this direct interaction hypothesis is complicated by the fact that variable surface proteins are presumably translated directly into the endoplasmic recticulum and processed to the membrane. The opportunities for direct interaction between the mature GPI-anchored surface protein and a repressor located in the nucleus seem limited. Alternatively, the GPI-anchored surface protein may communicate to a repressor via a signal transduction pathway. There is considerable evidence supporting a role for GPI-anchored proteins in signaling even though the precise mechanism is unknown (11,17,18,27). In one case it was demonstrated that binding of monoclonal antibodies to GPI-anchored antigens on T-lymphocytes resulted in activation of interleukin-2 synthesis (10). The possibility of a signal transduction pathway controlling surface antigen expression in *Paramecium* remains speculative and tests will require additional information about the mature protein and its processing. Our current efforts include affinity tagging the protein for further investigations.

Ours is not the first model to invoke variable surface antigens as positive or negative regulators of their own expression [reviewed in (6)]. Kimball proposed a model in which the *Paramecium* surface antigens are positive self-regulators, such that expression of one surface antigen causes continued expression until other factors alter the system (14). The hypothesis accounts for the stable expression of a surface antigen in the face of multiple alternatives. One of the few pieces of experimental data supporting a self-regulation theory came from genetic analyses of allelic exclusion (2). Although many surface antigen alleles are coexpressed, others such as G156 and G168 of *Paramecium primaurelia* show exclusion. After backcrossing the G156 allele into a strain isogenic with 168, the pattern of surface antigen expression was examined for various homozygous and heterozygous strains. Based on the results, Capdeville (2) concluded that the expression characteristics generally follow the antigen gene itself rather than the host background and proposed that the surface antigen itself positively maintains its own expression.

Addressing the possibility that surface antigens play a role in their own expression has been difficult. Failure to express one antigen naturally leads to expression of another. It was only our ability to coexpress two antigens (B51 and the chimera) that allowed us to obtain quantitative data on transcription as a function of antigen expressed on the surface. It is important to note that our experiments do not directly evaluate the contribution of the N-terminal region of the antigen on transcription, because it is supplied by the normal B51. Indeed, the polypeptide encoded by this region could be a major factor regulating expression because substitution of this portion of the gene alters mutual exclusion. Evaluation of potential *trans*-acting components from this portion of the gene will require alternative approaches because *cis*-acting components are required for our cotranscription experiments.

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