J. Phycol. **29,** 209–216 (1993)

TWO DISTINCT SMALL-SUBUNIT RIBOSOMAL RNA GENES IN THE NORTH AMERICAN TOXIC DINOFLAGELLATE *ALEXANDRIUM FUNDYENSE* (DINOPHYCEAE)[1]

Christopher A. Scholin,[2] *Donald M. Anderson*

Biology Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

and

Mitchell L. Sogin

Marine Biological Laboratory, MBL St., Woods Hole, Massachusetts 02543

ABSTRACT

Two distinct small-subunit ribosomal RNA genes, termed the A gene and B gene, were detected in a clonal isolate of the toxic dinoflagellate, Alexandrium fundyense *Balech. The two sequences, which occur in roughly a 1:1 ratio in polymerase chain reaction-amplified material, differ at approximately 40 positions scattered throughout the length of the molecule. Transcripts of the B sequence were not detected in total RNA extracts from nutrient-replete and ammonium-starved (sexually induced) cultures or nutrient-replete log-phase cultures harvested at 2-h intervals over a complete circadian cycle. Many of the position changes in the B gene deviate from universally and eukaryotic-conserved small-subunit rRNA sequences. In contrast, the A gene is expressed under all culture conditions tested and does not violate any conserved sequence positions. Thus, the B sequence is not represented by stable transcripts and is likely a pseudogene. The B gene may*

[1] Received 9 March 1992. Accepted 14 December 1992.

[2] Current address and address for reprint requests: Monterey Bay Aquarium Research Institute, 160 Central Avenue, Pacific Grove, California 93950.

serve as a useful marker for fine-scale population and taxonomic analyses of some Alexandrium *species.*

Key index words: Alexandrium fundyense; *polymerase chain reaction (PCR); pseudogene; Pyrrophyta; red tide; small-subunit rRNA*

Small-subunit ribosomal RNA (16S-like rRNA) sequences are widely accepted for evaluating the evolutionary histories of organisms (Olsen et al. 1986, Sogin et al. 1986a, b, Field et al. 1988). These molecules have also been used as species- and even strain-specific markers and consequently appear to have potential in addressing both population ecology and fine-scale taxonomic questions (e.g. Gobel et al. 1987, McCutchan et al. 1988, Stahl et al. 1988, Amann et al. 1990, Distel et al. 1991). While assessing the utility of nuclear 16S-like rRNA gene sequences (16S-like rDNA) to delineate populations of closely related toxic dinoflagellates, we discovered significant sequence variation between 16S-like rDNAs from a clonal *Alexandrium fundyense* Balech culture. This variation may provide a useful tool for discriminating between closely related species or strains of *Alexandrium.*

Toxic dinoflagellates of the genus *Alexandrium* are responsible for paralytic shellfish poisoning (red tides) along the northeastern coasts of the United States and Canada and other temperate coastal waters throughout the world. These organisms pose an important problem in population biology and taxonomy as well as a serious economic and public health concern (Anderson 1989). An alarming trend in recent years has been the apparent natural and human-assisted dispersal of toxic *Alexandrium* to regions of the world previously free of their presence (Anderson 1989, Hallegraeff and Bolch 1991, 1992). However, techniques to unambiguously distinguish between populations of these organisms are presently insufficient to test dispersal theories.

Alexandrium species are also the subject of an ongoing taxonomic controversy, and only recently has an international agreement been reached on the appropriate genus designation (Steidinger 1990, Steidinger and Moestrup 1990). Although the confusion over genus names appears to be over, concerns about species assignments continues (Taylor 1985). For example, in a recent revision of *Alexandrium* taxonomy, the closely related toxic species *A. tamarense* (Lebour) Balech, *A. catenella* (Whedon et Kofoid) Balech, and *A. fundyense* were distinguished on the basis of fine-scale morphological features (Balech 1985, Balech and Tangen 1985). Other authorities, however, believe these organisms (commonly referred to as the tamarensis group or tamarensis/catenella complex) represent a single species complex comprised of numerous biochemically distinct varieties (Taylor 1985, Cembella and Taylor 1986, Cembella et al. 1987).

The disagreement over fine-scale taxonomic indicators inspired a search for additional biochemical and genetic markers that would be useful in clarifying *Alexandrium* systematics. Detailed toxin composition and enzyme electrophoretic studies, in conjunction with traditional, morphologically based taxonomic analyses, have all been applied to assess the genetic similarity of *Alexandrium* isolates (Maranda et al. 1985, Cembella and Taylor 1986, Cembella et al. 1987, Hayhome et al. 1989). Collectively, these markers represent complex character states that depend on the coordinated expression of multiple genes; equitable comparisons of such characters require fastidious culturing, harvesting, preparative, and analytical procedures. Despite such efforts, population and taxonomic boundaries within and between *Alexandrium* species have remained coarse. Thus, at present, genetic markers specific for many strains of *Alexandrium* are lacking, and there is disagreement over the relative importance of morphologically based taxonomic criteria. This, in turn, has complicated efforts to understand the population dynamics and potential dispersal of these toxic organisms.

In an attempt to identify molecular markers for species or strains of *Alexandrium,* we undertook the sequence analysis of nuclear 16S-like rDNA. This analysis does not depend on the physiological state of the organism nor the concomitant expression of other genes and therefore has many advantages over morphological and biochemical studies conducted previously. Surprisingly, 16S-like rDNAs from a clonal, toxic, eastern North American *A. fundyense* (strain GtCA29, formerly *Protogonyaulax tamarensis* Taylor; Hayhome et al. 1989) amplified using the polymerase chain reaction (PCR; Saiki et al. 1988) contained two distinct sequences. In contrast, the 16S-like rDNA sequence recently reported for a nontoxic, western European *A. tamarense* (Destombe et al. 1992) includes only a single class of genes. Here, we describe the characterization of the two classes of genes in *A. fundyense,* attempts to determine whether or not both are expressed, their relationship to other known, functional 16S-like rRNAs, and the implications of this finding with regard to the use of rDNA sequences as genetic and taxonomic markers for *Alexandrium* species. The use of these genes in biogeographic studies will be presented elsewhere (Scholin and Anderson 1992, Scholin and Anderson, unpubl.).

MATERIALS AND METHODS

A culture of *Alexandrium fundyense* strain GtCA29, established from a cyst isolated from Gulf of Maine sediments ~32 km east of Portsmouth, New Hampshire, was rendered clonal by isolation of a single swimming cell. This culture was maintained as asexually reproducing or "sexually induced" in f/2 or ammonia-encystment medium, respectively, as described by Anderson et al. (1984).

RNA isolation. All stock solutions for RNA isolation were prepared with DEPC-treated (Sigma) ddH_2O using baked glassware and disposable, presterilized glass or plasticware. Where appropriate, solutions were filtered and autoclaved.

Approximately 2 L of a mid-late log culture (2–5000 cells·

mL^{-1}) was concentrated on a 20-μm Nitex mesh, backwashed with sterile sea water into a disposable 50-mL centrifuge tube, and briefly spun to pellet the cells. The supernatant was removed by aspiration. The cells were resuspended in ~10 mL of sterile sea water, transferred into a disposable 15-mL centrifuge tube, and pelleted again. Supernatant was removed as before, and the tube was immediately immersed in liquid nitrogen where it was stored until further processing.

The frozen cell pellet was allowed to thaw at room temperature briefly, resuspended in 5.5–6.0 mL of freshly prepared guanidine isothiocyanate lysis buffer (5 M guanidine isothiocyanate, 25 mM NaCit, pH 7.0, 25 mM EDTA, 25 mM EGTA, 0.5% sarkosyl, 2.0% mercaptoethanol), placed in a nitrogen bomb (Parr Instrument Co.) that was then pressurized to ~2000 psi for ~5 min, and released to atmospheric pressure. The resulting lysate was collected into a fresh 15-mL disposable centrifuge tube and extracted three times with phenol : chloroform (1:1; phenol equilibrated with 10 mM Tris pH 8.0 and 0.1% mercaptoethanol) and once with chloroform. Following the final extraction, the aqueous phase was transferred to baked, Corex centrifuge tubes, and the nucleic acids were precipitated by the addition of 2.5 volumes of 100% EtOH, 1/20 volume of 4 M NH_4OAc (pH 5.0) and chilling at −70° C for >1 h. Precipitates were collected by centrifugation at ~4° C for 20 min at 10,000 rpm in a Beckman model J2-2l centrifuge fitted with a JA-20 rotor. The supernatants were discarded; the pellets were briefly drained and then resuspended in 2–4 mL of DEPC-treated ddH_2O. Total RNA was precipitated by adding $LiCl_2$ to a final concentration of 2 M (Ausubel et al. 1987) and leaving the samples on ice overnight. The precipitated RNA was collected as earlier. The pellets were carefully rinsed with chilled 2 M $LiCl_2$, resuspended in a total volume of ~2 mL of DEPC-treated ddH_2O, and precipitated once more using $LiCl_2$ as above. Precipitates were collected again by centrifugation, and the RNA pellet was resuspended in 1mL of DEPC-treated ddH_2O. An aliquot of this was used for quantification (absorbance at 260 nm), and the remainder precipitated immediately (Ausubel et al. 1987). Precipitated RNA samples were stored at −70° C until needed for sequencing. For sequencing, an aliquot of the RNA precipitate was transferred to a microcentrifuge tube, collected by centrifugation, and resuspended in DEPC-treated ddH_2O to a final concentration of ~1 $mg \cdot mL^{-1}$.

DNA extraction. Approximately 50 mL of a mid-log culture (~2000–3000 cells·mL^{-1}) was briefly centrifuged to pellet the cells. The supernatant was discarded. The cell pellet was resuspended in 2.0 mL of STE (10 mM NaCl, 10 mM Tris HCL, pH 7.5, 1 mM EDTA pH 8.0) and disrupted in a French pressure cell. SDS was added to a final concentration of 1%, and the resulting solution was extracted twice with phenol equilibrated with STE, once with STE-saturated phenol : chloroform : isoamyl alcohol (PCI; 24:24:1) and once with chloroform : isoamyl alcohol (CI; 24:1). DNA was precipitated by the addition of 2 volumes of EtOH and 1/10 volume of 3 M NaOAc, followed by incubation at −20° C for >2 h. The precipitate was collected by centrifugation, rinsed with 80% EtOH, spun again, and resuspended in 200 μL of LT (10 mM Tris HCL, pH 7.5, 10 mM NaCL, 0.5 mM EDTA, pH 8.0). The concentration of the DNA was determined by diluting an aliquot of the resuspended material and reading its absorbence at 260 nm (Ausubel et al. 1987).

PCR amplification of 16S-like rDNA. Universal eukaryotic primers containing polylinker restriction sites (Medlin et al. 1988) were used to amplify full-length 16S-like rDNAs (Sogin 1990) with 30 cycles of a Perkin-Elmer Cetus DNA Thermal Cycler set as follows: 2-min denaturation at 94° C, 2-min ramp to 37° C, 2-min primer annealing at 37° C, 3-min ramp to 72° C; and 6-min extension at 72° C. Three replicate 100 μL amplification reactions were conducted in parallel using 1, 10, and 100 ng, respectively, of *A. fundyense* genomic DNA. PCR products were subjected to agarose gel electrophoresis; amplifications using 10 ng and 100 ng of genomic DNA yielded the best product. Products from each replicate amplification were purified by extracting once with an equal volume of STE PCI and once with CI. Afterward, they were concentrated by EtOH precipitation and resuspended in 10 μL LT. The concentration of 16S-like rDNAs in each replicate was determined by diluting an aliquot of the material and reading its absorbance at 260 nm.

Cloning of 16S-like rDNA. Purified, concentrated products from two PCR amplification reactions were mixed, digested with *Bam*HI and *Sal*I (New England Biolabs), and ligated into *Bam*HI/*Sal*I-cut replicative forms of M13 mp18 and M13 mp19 bacteriophage (Messing 1983) as described by Medlin et al. (1988). Individual clones were subsequently grown and screened by agarose gel electrophoresis for the presence of a correctly sized insert; 22 mp18 (coding strand) and 24 mp19 (noncoding strand) positive (insert-containing) clones were identified.

Preparation of M13 DNA for sequencing. The population of PCR products was sampled by infecting *E. coli* (JM109) with a mixture of all positive mp18 or mp19 phage. Single stranded, "pooled" mp18 and mp19 templates (i.e. mixtures of all mp18 or mp19 recombinants, respectively) were isolated as described by Messing (1983). For analysis of individual clones, templates were prepared separately from four clones in mp18 and four clones in mp19.

Sequencing of 16S-like rDNA. All sequencing reactions were carried out using modified T7 polymerase (Pharmacia or USB sequenase V 2.0) with deoxyadenosine triphosphate [α^{35}S] label (Amersham) and dideoxy chain termination (Sanger and Coulson 1975). Coding and noncoding strands of the amplified, cloned 16S-like rDNA products were sequenced in their entirety using a series of primers complementary to conserved sites in the molecule (Elwood et al. 1985).

Sequencing of 16S-like rRNA. Primers complementary to conserved regions at *Dictyostelium discoideum* nucleotide positions 1139–1125 and 906–892 (Sogin and Gunderson 1987) were end-labeled with adenosine triphosphate [γ^{35}S] (Amersham; Ausubel et al. 1987) and used to sequence (Lenaers et al. 1991) a portion of the 16S-like rRNA that encompasses multiple nucleotide differences in the A and B genes.

RESULTS

PCR amplification of 16S-like rDNAs from *A. fundyense* (GtCA29) yielded a product of approximately 1800 nucleotides. Individual 16S-like rDNA clones were combined, and the resultant "pool" was sequenced, permitting assessment of genomic heterogeneity and potential errors introduced during early rounds of the PCR reaction (Medlin et al. 1988). This analysis revealed both sequence ambiguities and length heterogeneities in the cloned 16S-like rDNA PCR products. The length differences obscured the pooled clone sequencing ladders, making it impossible to resolve portions of the sequence. Therefore, eight individual 16S-like rDNA clones (four each of the coding and noncoding strands) were sequenced separately to characterize regions of heterogeneity. Sequences obtained from pooled and individual clones revealed the existence of at least 40 differences in the cloned 16S-like rDNAs. Two distinct classes of genes were identified and termed the A gene (1802 base pairs) and B gene (1800 base pairs). The sequences differ by 13 transitions, 24 transversions, 2 single base-pair deletions and 1 single base-pair insertion; 32 of these differences were unambiguously identified by the analysis of individual 16S-like rDNA clones (positions 172–1300). The remaining eight heterogeneities occurred in regions that were not sequenced with in-

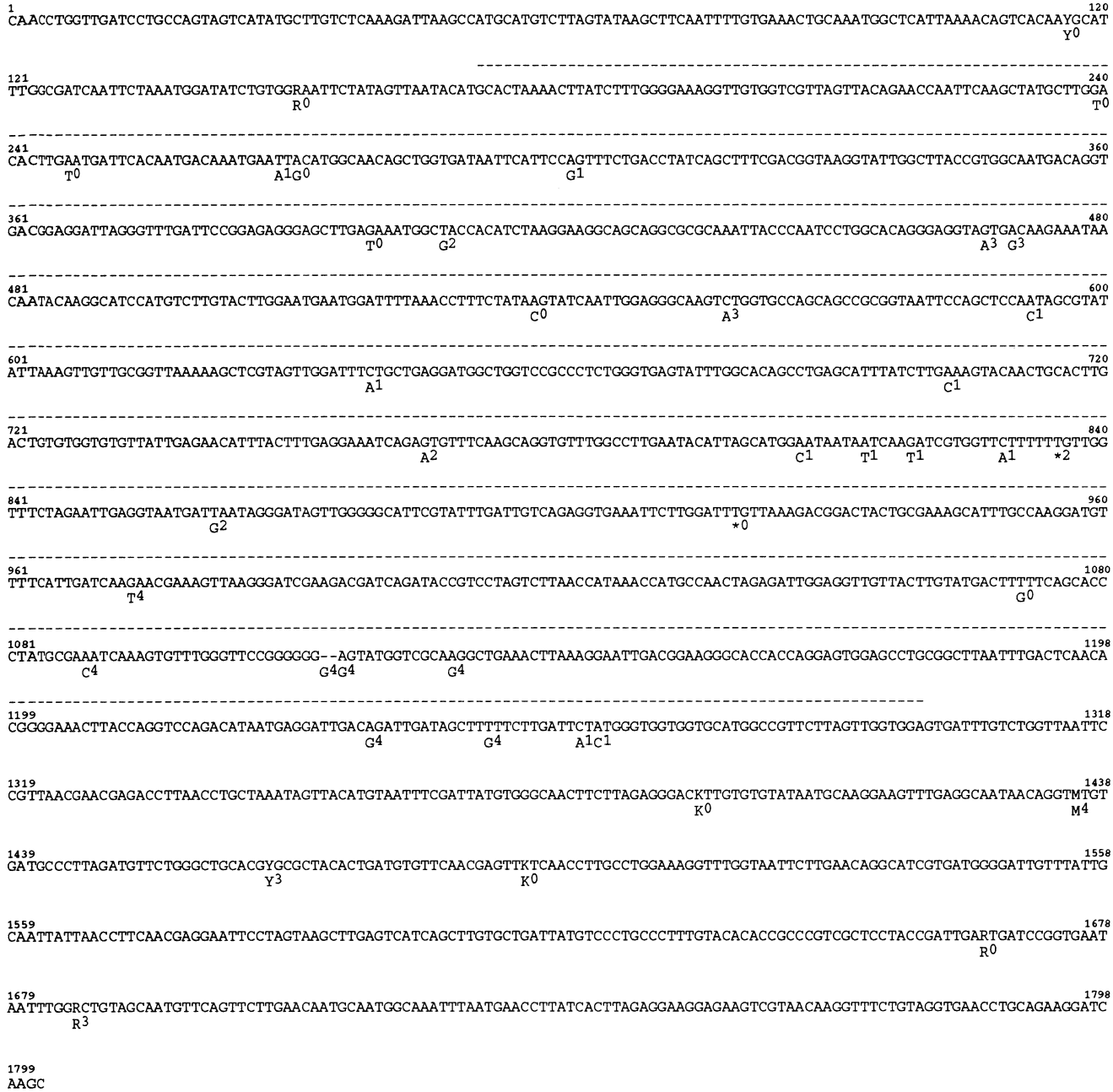

FIG. 1. Nucleotide sequences of the A and B 16S-like rDNAs from *A. fundyense* (strain GtCA29). The A sequence is presented in its entirety; substitutions in the B sequence are indicated below each line, with superscripts to indicate whether that position in the sequence is evolutionarily variable (0) or conserved among dinoflagellates (1); dinoflagellates, apicomplexans, and ciliates (2); eukaryotes (3); or eukaryotes and prokaryotes (universally conserved; 4). *Denotes single-base deletions in the B gene. Two alignment gaps (—) in the A gene are necessary to accommodate the B gene's single-base insertion (between A gene nucleotides 1114 and 1115) and corresponding superscript. Dashed line above the sequences indicate the portion of the molecules sequenced with individual 16S-like rDNA clones. Ambiguities identified outside this region are designated as follows: R = G or A; Y = C or T; K = G or T; M = C or A (IUPAC ambiguity codes).

dividual clones (Fig. 1). Of the eight individual clones sequenced, five are A genes and three are B genes.

The A gene and B gene exist as a "family" of sequences in the PCR products, each with its own "intrafamily" variations. However, sequences within either the A gene family or B gene family are nearly identical (>99% similar). For simplicity, the terms A gene (or A sequence) and B gene (or B sequence) are used throughout the remainder of this article as a designation of the A gene family and B gene family, respectively. A gene clones included molecules that differ at six positions (four transitions, two transversions); B gene clones included molecules that also differ at six positions (four tran-

sitions, two transversions). The substitutions within the A and B genes are not reported in Figure 1 because they are represented by only a single 16S-like rDNA clone and are not corroborated by other sequence data (cf. Scholin 1992).

To determine whether or not both genes encode stable transcripts, total RNA was isolated from mid-log cultures that were grown under both nutrient-replete (asexually reproducing) and ammonium-starved (sexually induced; Anderson et al. 1984) conditions. RNA was also extracted from a nutrient-replete, log-phase culture at 2-h intervals over a complete circadian cycle. Reverse transcriptase (RTase) sequencing of a portion of the 16S-like rRNAs known to contain multiple differences between the two genes revealed that transcripts of the A gene were clearly present in all samples. In contrast, there is no evidence for the presence of B gene transcripts (Fig. 2).

Figure 2 illustrates the identification of 16S-like rRNAs by RTase sequencing. If transcripts of the A and B genes were both present in cellular RNA, a G/U ambiguity would appear at position 974 and the single-base deletion at position 920 would cause a single-base shift for some fraction of the sequencing ladder above the position where it occurs in the autoradiograph. By both criteria, there is no evidence for B gene transcripts; overexposing the autoradiograph also failed to reveal any trace amounts of B gene 16S-like rRNAs (data not shown).

Further analysis of the A and B sequences was undertaken by comparing them to 131 eukaryotic and 13 prokaryotic 16S-like rRNAs (Neefs et al. 1990). Of the 32 differences that were identified by sequencing individual A and B gene clones, the majority were at positions that are not variable in functional 16S-like rRNAs: seven occurred at universally conserved sites, three occurred at positions conserved among all eukaryotes, four occurred at sites conserved among dinoflagellates, apicomplexans, and ciliates, 11 occurred at nucleotides conserved among several dinoflagellates (*Amphidinium caterae* Hulburt, *Crypthecodinium cohnii* (Seligo) Chatton, *Prorocentrum micans* Ehrenberg), and seven occurred at evolutionarily variable sites. Discrepancies between the A and B genes that violate evolutionarily conserved sequence positions map exclusively to the B gene (Fig. 1) (two of the individually sequenced A gene clones each contain one transition at different positions in the molecule, and both of these substitutions *do* deviate from universally conserved positions. Because these substitutions are represented by single clones and not substantiated by other sequence data, we suspect they are PCR or cloning artifacts [cf. Scholin 1992]).

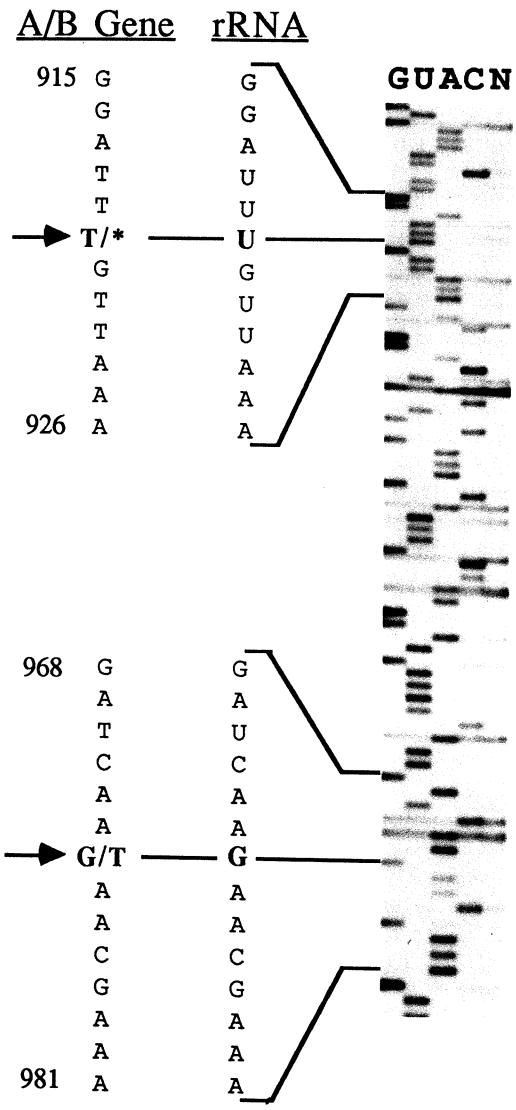

FIG. 2. Sequencing gel of 16S-like rRNAs from total RNA extracts of *A. fundyense* (GtCA29), with a comparison to known A and B 16S-like rDNA gene sequences. Numbers indicate nucleotide positions in the A gene (Fig. 1). The complement of specific nucleotide termination reactions are indicated above each lane. N represents no ddNTP addition. Arrows indicate differences between the A and B genes and the identity of that position in the expressed 16S-like rRNAs. *Indicates a single-base deletion within the B gene. The single-base deletion (position 920) occurs at an evolutionarily variable position. The G/T transversion (position 974) occurs at a universally conserved position (Fig. 1).

DISCUSSION

Two distinct 16S-like rRNA genes, named the A gene and B gene (Fig. 1), were identified by sequencing PCR-amplified 16S-like rDNAs from a clonal culture of *A. fundyense.* These two sequences are ~97% identical. In addition, the A and B genes were estimated to be present in an approximately 1:1 ratio in the PCR-amplified, cloned SsrDNAs.

We initially considered the possibility that *A. fundyense* (GtCA29) was not a true clone but in fact was a mixture of two distinct *Alexandrium* isolates. If this were the case, the culture would contain both A and B 16S-like rDNAs transcripts. However, it contained only A gene transcripts (Fig. 2). Thus, it was highly unlikely that the two, distinct 16S-like rDNAs

cloned from *A. fundyense* originated from a mixed culture. Two lines of reasoning then led us to speculate that the B gene might be expressed under more specialized circumstances. First, the apicomplexan *Plasmodium berghei* is known to carry two 16S-like rRNA genes that are differentially expressed over the course of its life cycle (Gunderson et al. 1987), and dinoflagellate 16S-like rRNAs do share a unique, common evolutionary history with apicomplexans (Gajadhar et al. 1991). We suspected that an analogous switch in gene expression might occur in *A. fundyense* as it progressed through a developmental cycle, perhaps associated with the induction of sexuality. Second, because dinoflagellates are known to exhibit strong circadian rhythms in total RNA synthesis and translational regulation of a gene involved with luminescence (Waltz et al. 1983, Morse et al. 1989), we reasoned that differential expression of the A and B genes might occur during the light and dark phases of growth. However, B gene transcripts do not appear in total cellular RNA in response to sexual induction (nitrogen starvation; Anderson et al. 1984) or over the course of a circadian cycle (data not shown). Consequently, it appears that the B gene is either transcriptionally inactive and/or encodes an unstable product.

The probable nature of the B gene became apparent when it was compared to other 16S-like rRNA sequences. Nucleotide substitutions in the B gene, but not the A gene, violate many highly conserved sequences (Fig. 1) (two of the individually sequenced A gene clones each contain one transition at different positions in the molecule, and both of thee substitutions *do* deviate from universally conserved positions. Because these substitutions are represented by single clones and not substantiated by other sequence data, we suspect they are PCR or cloning artifacts [cf. Scholin 1992]). The B gene's deviations from evolutionarily conserved motifs are especially significant because these sequence elements are considered essential to the basic core structure of a functional ribosome (Raue et al. 1988). The fact that the B sequence varies from these highly conserved elements and that B gene transcripts are apparently rare or absent in RNA extracts leads us to conclude that the B sequence is a pseudogene.

Two lines of evidence suggest that the A and B genes are present in approximately equal proportions in the PCR products. First, sequence ambiguities (i.e. two different nucleotide terminations at the same position in the sequencing ladder) observed in the pooled clone sequences always appeared with nearly equal band intensities, indicating that templates harboring alternative nucleotides are equally abundant. Those ambiguities characterized by sequencing single 16S-like rDNA clones revealed that one of two nucleotide alternatives was indeed contiguous with either the A or B sequence. Second, of the eight individual clones sequenced, five are A genes and three are B genes, suggesting that the proportions of cloned A and B sequences are roughly equivalent.

The B sequence cannot result from PCR artifact for several reasons. First, if errors were being introduced randomly, both genes would be expected to show multiple deviations from evolutionarily conserved positions, yet the B gene alone displays this type of variance. Second, both sequences have been reproducibly amplified and detected by either restriction fragment polymorphism analysis (RFLP; Scholin and Anderson 1992, Scholin and Anderson, unpubl.) or direct sequencing of the PCR products (Scholin 1992). Thus, the A and B genes must be present in the extracted DNA from *A. fundyense.* Furthermore, they consistently appear in a near 1:1 ratio (Scholin and Anderson 1992, Scholin and Anderson, unpubl.), as predicted from sequencing pooled and individual 16S-like rDNA clones.

If the ratio of A and B genes in PCR products reflects their abundance in the extracted DNA, then the two genes may be present in near equal numbers within the *A. fundyense* genome. This, however, raises an interesting question: Why are so many copies of an apparent pseudogene being maintained? One possibility is that the B gene is perpetuated simply as a result of its linkage to other, functional rRNA genes. Analyzing individual A and B gene rDNA cistrons along with their respective promoter regions would be useful in addressing this possibility. Viral or other insertional elements in close proximity to the B sequence should not be discounted as players in the B gene's transcriptional inactivity and/or maintenance (Jakubczak et al. 1992).

Despite the fact that we cannot fully explain the origin and apparent abundance of the B gene within *A. fundyense,* it nonetheless holds promise as a biogeographic and taxonomic marker for this group of organisms. If the B gene is a pseudogene and is no longer under selective pressure, then it is likely to be evolving rapidly. These features should make the B gene a very sensitive marker for identifying and distinguishing between groups of morphologically similar, but geographically distinct, strains or species of *Alexandrium.* This supposition is supported by comparing the A and B genes to the 16S-like rDNA sequence recently reported for a nontoxic, western European *A. tamarense* (Destombe et al. 1992). The *A. tamarense* 16S-like rDNA is approximately 98% identical to the *A. fundyense* A gene and lacks "B-like" homology. In addition, we have independently examined PCR-amplified 16S-like rDNAs from the western European *A. tamarense* using an RFLP-based assay and find no evidence for presence of the B gene (Scholin and Anderson 1992, Scholin and Anderson, unpubl.). Therefore, both actively expressed 16S-like rRNA genes and the B gene appear to be informative biogeographic and taxonomic characters of *Alexandrium* species.

The finding of two small-subunit rRNA genes in *A. fundyense* underscores the risk associated with us-

ing a single clone of a multigene family as *the* representative sequence of an organism's genotype. Multiple clones must be pooled prior to sequencing, or individually sequenced, in order to assess the homogeneity of cloned products and reduce the possibility of obtaining artifactual data (Sogin 1990). In this regard, the extensive 16S-like rRNA data base is a substantial resource that should be used in conjunction with sequencing methods that utilize either PCR-amplified 16S-like rDNAs or their corresponding RNA transcripts.

We thank D. M. Kulis for his help in culturing *A. fundyense* and E. Ariztia-Carmona and C. Bibeau for their assistance in cloning and sequencing of the rDNAs. This work was supported by grants from the National Science Foundation No. OCE 89-11226 (D.M.A.), National Institute of Health No. GM32964 (M.L.S.), Woods Hole Oceanographic Institution Ocean Ventures Fund (C.A.S.), and the Vettesen Foundation. Contribution No. 7992 from the Woods Hole Oceanographic Institution.

Amann, R. I., Krumholz, L. & Stahl, D. A. 1990. Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. *J. Bacteriol.* 172:762–70.

Anderson, D. M. 1989. Toxic algal blooms and red tides: a global perspective. *In* Okaichi, T., Anderson, D. M. & Nemoto, T. [Eds.] *Red Tides: Biology, Environmental Science and Toxicology.* Elsevier, New York, pp. 11–20.

Anderson, D. M., Kulis, D. M. & Binder, B. J. 1984. Sexuality and cyst formation in the dinoflagellate *Gonyaulax tamarensis*: cyst yield in batch cultures. *J. Phycol.* 20:418–25.

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Siedman, J. G., Smith, J. A. & Struhl, K. 1987. *Current Protocols in Molecular Biology,* Vols. 1 and 2. Wiley Interscience, New York.

Balech, E. 1985. The genus *Alexandrium* or *Gonyaulax* of the *tamarensis* group. *In* Anderson, D. M., White, A. W. & Baden, D. G. [Eds.] *Toxic Dinoflagellates* Elsevier, New York, pp. 33–8.

Balech, E. & Tangen, K. 1985. Morphology and taxonomy of toxic species in the *tamarensis* group (Dinophyceae): *Alexandrium excavatum* (Braarud) comb. nov. and *Alexandrium ostenfeldii* (Paulsen) comb. nov. *Sarsia* 70:333–43.

Cembella, A. D., Sullivan, J. J., Boyer, G., Taylor, F. J. R. & Andersen, R. J. 1987. Variation in paralytic shellfish toxin composition within the *Protogonyaulax tamarensis/catenella* species complex: red tide dinoflagellates. *Biochem. Syst. Ecol.* 15:171–86.

Cembella, A. D. & Taylor, F. J. R. 1986. Electrophoretic variability within the *Protogonyaulax tamarensis/catenella* species complex: pyridine linked dehydrogenases. *Biochem. Syst. Ecol.* 14:311–23.

Destombe, C., Cembella, A. D., Murphy, C. A. & Ragan, M. A. 1992. Nucleotide sequence of the 18S ribosomal RNA genes from the marine dinoflagellate *Alexandrium tamarense* (Gonyaulacales, Dinophyta). *Phycologia* 31:121–4.

Distel, D. L., DeLong, E. F. & Waterbury, J. B. 1991. Phylogenetic characterization and in situ localization of the bacterial symbiont of shipworms (Teredinidae: Bivalvia) by using 16S rRNA sequence analysis and oligodeoxynucleotide probe hybridization. *Appl. Environ. Microbiol.* 57:2376–82.

Elwood, H. J., Olsen, G. J. & Sogin, M. L. 1985. The small subunit ribosomal RNA gene sequences from the hypotrichous ciliates *Oxytricha nova* and *Strylonychia pustulata. J. Mol. Biol. Evol.* 2:399–410.

Field, K. G., Olsen, G. J., Lane, D. J., Giovannoni, S. J., Ghiselin, M. T., Raff, E. C., Pace, N. R. & Raff, R. A. 1988. Molecular phylogeny of the animal kingdom. *Science (Wash. D.C.)* 239: 748–53.

Gajadhar, A. A., Marquart, W. C., Hall, R., Gunderson, J., Ariztia-Carmona, E. V. & Sogin, M. L. 1991. Ribosomal RNA sequences of *Sarcocystis muris, Theileria annulata,* and *Cryptthecodinium cohnii* reveal evolutionary relationships among apicomplexans, dinoflagellates, and ciliates. *Mol. Biochem. Parisitol.* 45:147–54.

Gobel, U. B., Geiser, A. & Stanbridge, E. J. 1987. Oligonucleotide probes complementary to variable regions of ribosomal RNA discriminate between *Mycoplasma* species. *J. Gen. Microbiol.* 133:1969–74.

Gunderson, J. H., Sogin, M. L., Wollet, G., Hollingdale, M., de la Cruz, V. F. & McCutchan, T. F. 1987. Structurally distinct, stage-specific ribosomes occur in *Plasmodium. Science (Wash. D.C.)* 238:933–7.

Hallegraeff, G. & Bolch, C. J. 1991. Transport of toxic dinoflagellate cysts via ship's ballast water. *Mar. Poll. Bull.* 22:27–30.

——— 1992. Transport of toxic dinoflagellate cysts via ship's ballast water: implications for plankton biogeography and aquaculture. *J. Plankton Res.* 14:1067–84.

Hayhome, B. A., Anderson, D. M., Kulis, D. M. & Whitten, D. J. 1989. Variation among congeneric dinoflagellates from the northeastern United States and Canada. I. Enzyme electrophoresis. *Mar. Biol. (Berl.)* 101:427–35.

Jakubczak, J. L., Zenni, M. K., Woodruff, R. C. & Eickbush, T. H. 1992. Turnover of R1 (type 1) and R2 (type 2) retrotransposable elements in the ribosomal DNA of *Drosophila melanogaster. Genetics* 131:129–42.

Lenaers, G., Scholin, C. A., Bhaud, Y., Saint-Hilaire, D. & Herzog, M. 1991. A molecular phylogeny of dinoflagellate protists (Pyrrhophyta) inferred from the sequence of the 24S rRNA divergent domains D1 and D8. *J. Mol. Evol.* 32:53–63.

Maranda, L., Anderson, D. M. & Shimizu, Y. 1985. Comparison of toxicity between populations of *Gonyaulax tamarensis* of eastern North American waters. *Estuarine Coastal Shelf Sci.* 21:401–10.

McCutchan, T. F., de la Cruz, V. F., Lal, A. A., Gunderson, J. H., Elwood, H. J. & Sogin, M. L. 1988. Primary sequences of two small subunit ribosomal RNA genes from *Plasmodium falciparum. Mol. Biochem. Parasitol.* 28:63–8.

Medlin, L., Elwood, H. J., Stickel, S. & Sogin, M. L. 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene* 71:491–9.

Messing, J. 1983. New M13 vectors for cloning. *Meth. Enzymol.* 101:20–78.

Morse, D., Milos, P. M., Roux, E. & Hastings, J. W. 1989. Circadian regulation of bioluminescence in *Gonyaulax* involves translational control. *Proc. Nat. Acad. Sci. U.S.A.* 86:172–6.

Neefs, J-M., Van de Peer, Y., Hendriks, L. & De Wachter, R. 1990. Compilation of small-subunit ribosomal RNA sequences. *Nucl. Acid. Res. (Suppl.)* 18:2237–317.

Olsen, G. J., Lane, D. J., Giovannoni, S. J., Pace, N. R. & Stahl, D. A. 1986. Microbial evolution: a ribosomal RNA approach. *Annu. Rev. Microbiol.* 40:337–65.

Raue, H. A., Klootwijk, J. & Musters, W. 1988. Evolutionary conservation of structure and function of high molecular weight ribosomal RNA. *Proc. Biophys. Molec. Biol.* 51:77–129.

Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science (Wash. D.C.)* 239:487–91.

Sanger, F. & Coulson, A. R. 1975. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *J. Mol. Biol.* 94:441–8.

Scholin, C. A. 1992. Analysis of toxic and non-toxic Alexandrium (Dinophyceae) species using ribosomal RNA gene sequences. Ph.D. Thesis, Massachusetts Institute of Technology/Woods Hole Oceanographic Institution, 251 pp.

Scholin, C. A. & Anderson, D. M. 1992. Population analysis of toxic and nontoxic *Alexandrium* species using ribosomal RNA signature sequences. *In* Smayda, T. J. & Shimizu, Y. [Eds.]

Fifth International Conference on Toxic Marine Phytoplankton. Elsevier, New York (in press).

Sogin, M. L. 1990. Amplification of ribosomal RNA genes for molecular evolution studies. *In* Innis, M. A., Gelfand, D. H., Sninsky, J. J. & White, T. J. [Eds.] *PCR Protocols: A Guide to Methods and Applications.* Academic Press, New York, pp. 307–14.

Sogin, M. L., Elwood, H. J. & Gunderson, J. H. 1986a. Evolutionary diversity of eukaryotic small-subunit rRNA genes. *Proc. Nat. Acad. Sci. U.S.A.* 83:1383–7.

Sogin, M. L. & Gunderson, J. H. 1987. Structural diversity of eukaryotic small subunit ribosomal RNAs: evolutionary implications. *Endocytobiology III. Ann. N.Y. Acad. Sci.* 503:125–39.

Sogin, M. L., Ingold, A., Karlok, M., Nielsen, H. & Engberg, J. 1986b. Phylogenetic evidence for the acquisition of ribosomal RNA introns subsequent to the divergence of some of the major *Tetrahymena* groups. *EMBO J.* 5:3625–30.

Stahl, D. A., Flesher, B., Mansfield, H. R. & Montgomery, L. 1988. Use of phylogenetically based hybridization probes for studies of ruminal microbial ecology. *Appl. Environ. Microbiol.* 54:1079–84.

Steidinger, K. A. 1990. Species of the *tamarensis/catenella* group of *Gonyaulax* and the fucoxanthin derivative-containing gymnodiniods. *In* Graneli, E., Sundstrom, B., Edler, L. & Anderson, D. M. [Eds.] *Toxic Marine Phytoplankton.* Elsevier, New York, pp. 11–6.

Steidinger, K. A. & Moestrup, Ø. 1990. The taxonomy of *Gonyaulax, Pyrodinium, Alexandrium, Gessnerium, Protogonyaulax* and *Goniodoma. In* Graneli, E., Sundstrom, B., Edler, L. & Anderson, D. M. [Eds.] *Toxic Marine Phytoplankton.* Elsevier, New York, pp. 522–3.

Taylor, F. J. R. 1985. The taxonomy and relationships of red tide dinoflagellates. *In* Anderson, D. M., White, A. W. & Baden, D. G. [Eds.] *Toxic Dinoflagellates.* Elsevier, New York, pp. 11–26.

Waltz, B., Waltz, A. & Sweeny, B. M. 1983. A circadian rhythm in RNA in the dinoflagellate, *Gonyaulax polyedra. J. Comp. Physiol.* 151:207–13.