nen, A. Narvanen, V. M. Wasenius, P. Partanen, I. Virtanen, *FEBS Lett.* **224**, 156 (1987)], and gelsolin (*17*). Polyclonal antibodies were used to detect profilin [J. H. Hartwig, K. A. Chambers, K. L. Hopcia, D. J. Kwiatkowski, *J. Cell Biol.* **109**, 1571 (1989)], and fodrin [J. Morrow, Yale University; A. S. Harris, L. A. Green, K. J. Ainger, J. S. Morrow, *Biochim. Biophys. Acta* **830**, 147 (1985)].

8. We obtained RNA from cells by the guanidinium isothiocyanate–cesium chloride method [J. M. Chirgwin, A. E. Przybyla, R. J. MacDonald, W. J. Rutter, *Biochemistry* **18**, 5294 (1979)] and quantitated it by measuring absorbance at 260 nm. Ribonuclease protection analysis of ABP message RNA was performed as described (*17*) with a 2.1-kb ABP cDNA (7) random oligonucleotide–labeled probe [A. P. Feinberg and B. Vogelstein, *Anal. Biochem.* **132**, 6 (1983)]. Southern blot analysis was done by standard methods using the 2.1-kb ABP cDNA to probe the membrane.
9. For videomicroscopy, cells were plated on a 25-mm glass cover slip and placed in a Leiden incubation chamber (Motion Analysis, Greenville, NY) at 37°C in an atmosphere of 5% CO_2. Differential interference contrast (DIC) microscopy was performed on a Zeiss Axiovert 405M inverted microscope with either a 40×, 0.9 numerical aperture (N.A.) Planneofluor objective or a 63×, 1.4 N.A. Planapochromatic objective. For time-lapse recordings, images were transmitted to a Hamamatsu 2400 videocamera and recorded on a Panasonic 6750AG video recorder.
10. Overlapping ABP cDNA clones were assembled into one complete construct in Bluescript SK+ (Stratagene) by standard methods and ligated to LK444, which contains the human β-actin promoter [P. Gunning, J. Leavitt, G. Muscat, S. Y. Ng, L. Kedes, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4831 (1987)]. The complete LKABP plasmid was transfected into the cells by calcium phosphate coprecipitation. After two days, the culture medium was replaced with medium containing G418 (1000 μg/ml) (Gibco-BRL, Gaithersburg, MD). Resistant colonies of cells were serially diluted into multiwell plates and cultured separately.
11. Medium in which cells from lines M1 to M6 were grown had both chemokinetic and chemotactic activity. The greatest stimulation of motility occurred with medium conditioned by M4 cells, so that medium was used in all subsequent assays. For motility assays, 5×10^4 cells were placed in each well of a 48-well, two-compartment chamber (Nucleopore, Pleasanton, CA) separated by an 8-μm pore size polycarbonate filter. To create a gradient of chemoattractant, conditioned medium was added to one of the chambers. After incubation (2 hours at 37°C), the filter was removed, stained, and examined with a 10× objective, and the number of cells that had migrated through the membrane was counted for each well.
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13. The elastic modulus of a pellet of cells depends on the elasticity of the individual cell cortices in the same manner as measurements of continuous networks of micron size gels depend on the viscoelasticity of the individual microgels [H.-F. Eicke, C. Quellet, G. Xu, G. Riess, in *Physical Networks: Polymers and Gels*, W. Burchard and S. Ross-Murphy, Eds. (Elsevier, London, 1990), pp. 169–184] and can be measured in a torsion pendulum. Packed cells (400 μl) were applied to the bottom plate of a torsion pendulum [P. A. Janmey, *J. Biochem. Biophys. Methods* **22**, 41 (1991)]. Oscillatory measurements were begun one min later and continued for an hour. A constant shear stress was then applied for 10 min, during which time the displacement of the sample, quantitated as shear strain, was measured. The stress was then removed and the recovery of the sample toward its original undeformed state was measured. Oscillatory measurements were repeated after this experiment, and for all samples the value of G' measured was unchanged from that before imposition of the steady stress, confirming that the slow deformation did not alter the structure of the sample. The optical density and cell number were measured after all experiments, confirming that the samples contained approximately equal amounts of cell mass and that the cells remained rounded and intact during the measurements.
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18. We thank K. Weimer, M. DeSisto, and J. Lamb for technical help. Supported by NIH grants AI 08051 (C.C.C.), HL 19429 (T.P.S.), AR 38910 (P.A.J.), DK 38452 (J.H.H.), and HL 02158 (J.B.G.), and grants from the Whittaker Foundation and the Council for Tobacco Research. T.P.S. is an American Cancer Society Research Professor and D.J.K. is an Established Investigator of the American Heart Association.

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Cyclic 2′,3′-Phosphates and Nontemplated Nucleotides at the 3′ End of Spliceosomal U6 Small Nuclear RNA's

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Spliceosomal U6 small nuclear RNA (U6 RNA) in species as diverse as man, frog, fruitfly, and soybean have at their 3′ ends a cyclic 2′,3′-phosphate (>p) apparently derived from uridylic acid residues that were added post-transcriptionally. The 3′ ends of U6 RNA's from various sources may be processed in different ways, or to different extents, depending on the organism or stage of development. The presence of a >p terminus on U6 RNA may influence the activity of U6 RNA either directly during splicing or indirectly by ensuring that the RNA has a defined length or proper conformation (or both).

U6 RNA, ONE OF FIVE SMALL NUclear RNA's (snRNA's) that function in pre-mRNA splicing (*1*), has several unusual characteristics that distinguish it from other snRNA's. It is synthesized by RNA polymerase III, rather than II, it lacks the Sm antigen binding site present in other spliceosomal snRNA's, and it has a methyl triphosphate at its 5′ end rather than a hypermethylated cap (*2, 3*). Mutational probing of U6 RNA has revealed that the 3′ terminal domain, extending from the region of interaction between U4 and U6 (*4*) to the 3′ end, is important for function (*5*). Alterations of the phylogenetically conserved length of this 3′ terminal domain decrease the activity of yeast U6 RNA in an in vitro splicing system (*6*). Also, base-pairing of sequences in this region with U2 RNA is essential for splicing in vertebrates (7), but not in yeast (*8*).

The major 3′ end sequence of mammalian U6 RNA is reported to be (5′) G-U-U-C-C-A-U-A-U-U-U-U-(U)$_{OH}$ (3′) (*9*). Like other RNA pol III transcripts that end with three to six U's (*10*), newly synthesized U6 RNA binds to the nuclear antigen La and is precipitated by antibodies to La (anti-La) (*11–13*). However, the major, mature form of U6 RNA (either free or complexed with U4 snRNA) is not precipitable by anti-La and it differs at its 3′ end in a hitherto undetermined way from the newly made U6 RNA (*11*). The sequence at the 3′ end of the RNA was deduced by labeling with pCp (*9*) or from U6 gene sequences (*14*), and therefore post-transcriptional alterations would have gone undetected. We show that the major 3′ end of U6 RNA contains a cyclic 2′,3′-phosphate (>p) end group, as opposed to the *cis* 2′,3′-diol end group of the anti-La precipitable U6 RNA's; this explains the lack of binding of mature U6 RNA to La antigen and reveals a novel feature of RNA metabolism.

In mammalian cells, the size of the steady-state population of U6 RNA's is heterogeneous, and multiple forms can be detected by RNA blot analysis (*15*), the most abundant form having the fastest mobility (Fig. 1A, lane 1). Some of this heterogeneity could be attributed to maturation of U6 RNA because the gel mobilities of newly made ^{32}P-labeled U6 RNA's increased with time (lanes 2 and 3) (*16*). Although this trend would be consistent with a shortening of the RNA during maturation, direct nucleotide sequence analyses (Fig. 1B) indicated that the mature U6 RNA (lane 3) was actually longer than the newly transcribed U6 RNA (lane 2).

RNase T1 fingerprint analyses (*17, 18*) of U6 RNA's made in vivo showed that the 3′ ends of U6 RNA's changed with time (Fig. 1B); this alteration might account for the inability of mature U6 RNA to bind La

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antigen (*11, 13*). The U6 RNA's isolated from short-term labeled cells (5 hours) produced a mixture of 3′ end oligonucleotides, including the previously identified 3′ oligonucleotide of the major, mature form of U6 RNA (No. 19) and a group of heterogeneous 3′ oligonucleotides. These metabolically unstable minor 3′ oligonucleotides contained 3′-OH groups and differed from one another by having progressively more UMP residues, as indicated by their fingerprint mobilities (Fig. 1B) and sequence analyses (*19*). Among pulse-labeled U6 RNA's (Fig. 1A, lane 2), slower migrating RNA's have longer forms of the minor 3′ end oligonucleotides (*19*).

In fingerprints of long-term labeled (mature) U6 RNA (Fig. 1B, 20 hours) the major 3′ end oligonucleotide (No. 19) predominated, and the heterogeneity of the minor 3′ end oligonucleotides was less pronounced. As already observed (*11, 13*), oligonucleotide No. 19 was absent from U6 RNA's isolated by precipitation with anti-La. Despite its apparent large size, this major 3′ end oligonucleotide predominated in the rapidly migrating, apparently smaller mature forms of U6 RNA (Fig. 1A, lane 3), an indication that the 3′ end of mature U6 RNA was modified. Coincident with an increased yield of oligonucleotide No. 19 was the appearance of a related oligonucleotide denoted by a star (see below).

In vitro transcription of a cloned mouse U6 gene in S100 extracts of HeLa cells (*12*) produced primary transcripts shorter than the endogenous U6 RNA's (Fig. 1A, lane 6). Thus, the 3′ end heterogeneity of cellular U6 RNA's might result from post-transcriptional elongation of primary transcripts (*16*) by addition of UMP residues (*12, 20*). Ribonuclease (RNase) T1 fingerprinting (Fig. 1B, in vitro) showed that the U6 transcripts made in extracts lacked the major 3′ end oligonucleotide (No. 19), but contained a collection of shorter 3′ end oligonucleotides. Likewise, the mature 3′ end is not present in U6 RNA's synthesized in vivo after injection of mouse (or frog) U6 genes into *Xenopus laevis* oocytes (*13*). As noted by others (*12, 20*), endogenous U6 RNA's in the S100 extracts became end-labeled during incubation with labeled UTP (Fig. 1A, lanes 4 to 6) but not GTP (*19*).

To identify the modification at the 3′ end of mature U6 RNA, we redigested the major 3′ oligonucleotide (Fig. 1B, No. 19) with RNase T2 or nuclease P1, which produce 3′- or 5′-nucleoside monophosphates, respectively, and analyzed the products by two-dimensional chromatography with either acidic (Fig. 2, A and B) or neutral solvents (C and D) in the second dimension (*21*). An unexpected product was observed when the nuclease P1 digestion products were chromatographed in the neutral solvent (arrowhead in Fig. 2D); in the acidic solvent (about 2 N HCl), this product migrated like pUp (Fig. 2B). Because the most likely candidate for an acid-labile 3′ end group is a cyclic 2′,3′-phosphate (U>p), we propose that the nuclease P1 product is pU>p. RNase T2 digestion (Fig. 2, A and C) would convert this modified end group to 3′-Up, enzymatically.

As a direct test for the presence of U>p at the 3′ end of U6 RNA, long-term labeled U6 RNA was treated either with acid (to open a cyclic phosphate), acid plus phosphatase (to remove a newly susceptible phosphate), or phosphatase alone (which would not remove a cyclic phosphate) (*22*). RNase T1 fingerprints of such treated U6 RNA's demonstrated that only the 3′ end oligonucleotide was affected (Fig. 3). Treatment with acid to open >p converted the major 3′ oligonucleotide No. 19 (Fig. 3A) to a more slowly migrating form (star in Fig. 3B). A

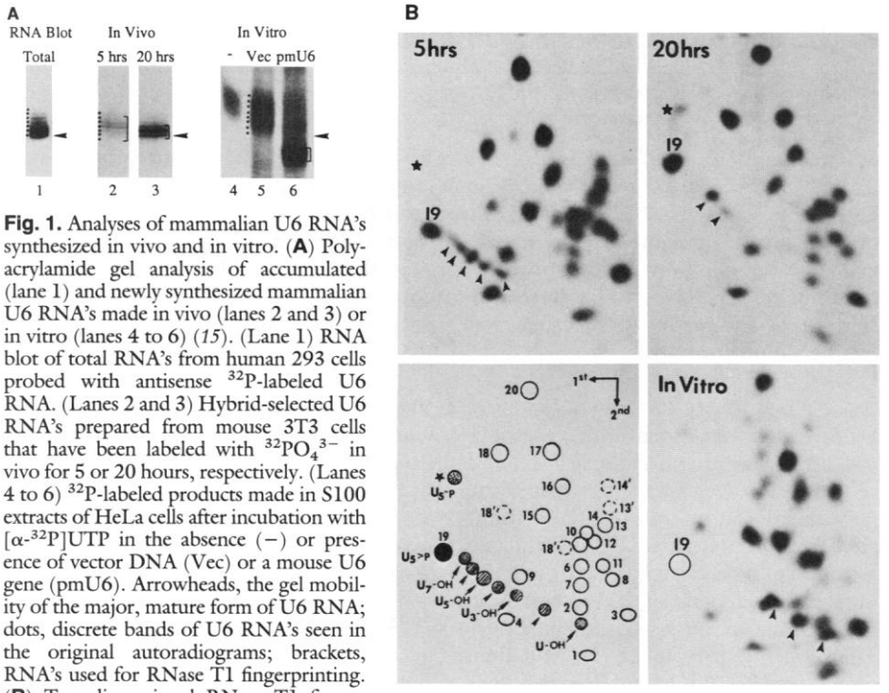

Fig. 1. Analyses of mammalian U6 RNA's synthesized in vivo and in vitro. (**A**) Polyacrylamide gel analysis of accumulated (lane 1) and newly synthesized mammalian U6 RNA's made in vivo (lanes 2 and 3) or in vitro (lanes 4 to 6) (*15*). (Lane 1) RNA blot of total RNA's from human 293 cells probed with antisense ^{32}P-labeled U6 RNA. (Lanes 2 and 3) Hybrid-selected U6 RNA's prepared from mouse 3T3 cells that have been labeled with $^{32}PO_4^{3-}$ in vivo for 5 or 20 hours, respectively. (Lanes 4 to 6) ^{32}P-labeled products made in S100 extracts of HeLa cells after incubation with [α-^{32}P]UTP in the absence (−) or presence of vector DNA (Vec) or a mouse U6 gene (pmU6). Arrowheads, the gel mobility of the major, mature form of U6 RNA; dots, discrete bands of U6 RNA's seen in the original autoradiograms; brackets, RNA's used for RNase T1 fingerprinting. (**B**) Two-dimensional RNase T1 fingerprints of ^{32}P-labeled U6 RNA's (*17, 18*) made in vivo (5 and 20 hours) or in vitro, as marked in (A). RNase T1 oligonucleotides are numbered according to (*9*); oligonucleotides 13′, 14′, and 18′ are the result of incomplete modification of U6 RNA's that were immature (5 hours) and made in vitro (*12*). The termini of the major 3′ oligonucleotide (No. 19, solid black), a related product (*, dotted), or minor 3′ end oligonucleotides (arrowheads, crosshatched) are shown. Some oligonucleotides are not labeled in U6 RNA made in vitro with [α-^{32}P]UTP.

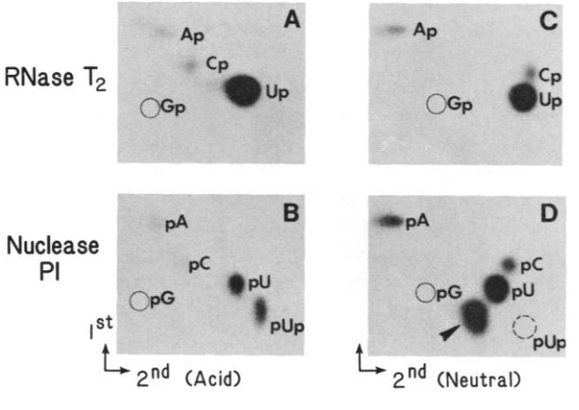

Fig. 2. Analysis of the 3′ end oligonucleotide of mature U6 RNA. RNase T2 (**A** and **C**) and nuclease P1 (**B** and **D**) digests of the major 3′ end oligonucleotide (U-U-C-C-A-UA-U-U-U-U-U>p) were analyzed by two-dimensional thin-layer chromatography with acidic (A and B) or neutral (C and D) second-dimension solvents (*21*). Positions of pG and Gp (circles), pUp (broken circle) and pU>p (arrowhead) are marked. The ratio of ^{32}P in pUp (or pU>p) to pU was about 2:3 (B and D); this differs from the expected value of 2:7 for a uniformly labeled oligonucleotide No. 19, indicating that the 3′ terminal UMP residue (having higher specific activity than the internal residues) may be subject to considerable turnover.

small amount of this slowly migrating product, which ends with a 3′- (or 2′-) monophosphate (*19*), was also evident in the fingerprints shown in Fig. 1B (*23*). Treatment with acid plus phosphatase converted oligonucleotide No. 19 to one that comigrated with one of the minor 3′-OH ends (Fig. 3C); the mobility of this product shows that it has a series of five UMP residues at its 3′ end (*19*), indicating that the mature 3′ terminus is . . A-U-U-U-U-U>p (*24*). When U6 RNA was treated with phosphatase alone, it had the same RNase T1 fingerprint as untreated U6 RNA (*19*), a result consistent with the 3′ end group being a phosphatase-resistant >p. Oxidation of a mixture of the multiple forms of U6 RNA by $NaIO_4$ changed only the minor 3′ end oligonucleotides (Fig. 3D), confirming that they had 2′,3′-OH end groups.

Modifications at the 3′ ends of U6 RNA's from other species were assayed (RNA blotting) by examining the effects of various treatments (*22*) on RNA gel mobility (*15*). The gel mobility of U6 RNA was reduced by removal of a 3′ phosphate (by phosphatase), but increased by removal of a nucleoside (by oxidation plus β elimination) (*19*). For example, the mobility of soybean (*Glycine max.*) U6 RNA was not affected by oxidation–β elimination or phosphatase treatment alone (Fig. 4A, lanes 1 to 3), but it changed when the RNA was first treated with acid (lanes 4 to 6); hence, essentially all soybean U6 RNA had >p at its 3′ end. Likewise, most *Saccharomyces cerevisiae* U6 RNA's also have a blocked 3′ end, being resistant to oxidation plus β elimination (Fig. 4B, lanes 1 and 2). Because the blocking group was removed by phosphatase treatment without prior exposure to acid (lanes 3 to 5), most U6 RNA's of *S. cerevisiae* contained 3′- (or 2′-) monophosphate at their 3′ ends (*25*). In contrast, U6 RNA of *Trypanosoma brucei rhodesiense* had solely 2′,3′-hydroxyl ends, being sensitive to oxidation–β elimination even in the absence of phosphatase treatment (Fig. 4C, lanes 1 to 3).

Table 1. Structures at the 3′ ends of accumulated U6 snRNA's. The 3′ ends of U6 RNA's were deduced from RNA blot analysis of total RNA's (*34*) as illustrated in Fig. 4.

Organism	Cell type	3′ ends (ratio)
Man	293 cells	>p, −OH (9:1)
Mouse	3T3 cells	>p, −OH (9:1)
X. laevis	Oocyte	>p, −OH (9:1)
	Tadpole	−p, −OH (1:1)
	A6 cells	−p, −OH (1:1)
Soybean		>p
D. melanogaster	Embryos (10–12 hours)	>p, −p, −OH (1:1:1)
	KcO cells	>p, −p (2:1)
C. elegans		−Blocked, −OH (20:1)
S. cerevisiae		−p, (−OH)
S. pombe		−p, −OH (1:1)
T. brucei rhodesiense		−OH

Similar assays on RNA's from several other organisms revealed U6 RNA's that consisted of mixtures of forms with different 3′ end groups (Table 1). Both *Drosophila melanogaster* and *Xenopus laevis* U6 RNA's gave complex patterns of 3′ ends that changed with development (*19*); it remains to be determined whether similar developmental changes occur in mammals. The U6 RNA's of *Caenorhabditis elegans* had unidentified blocking groups on their 3′ ends that were resistant to phosphatase digestion even after acid treatment (*19*).

The two modifications of U6 RNA 3′ ends described above, elongation by UMP addition and formation of the 3′ >p, ensure that the 3′ terminal domain has a discrete length. Primary U6 transcripts (*26*), being made by RNA polymerase III, often have fewer than five UMP residues at their 3′ ends (*27*); these molecules can be sufficiently elongated post-transcriptionally (*12, 13, 16, 20*, our data).

The UMP-addition activity might also regenerate the 3′ ends of mature U6 RNA molecules that have somehow been shortened (legend to Fig. 3) in much the same way that tRNA nucleotidyl transferase regenerates the 3′ ends of tRNA's (*28*). The formation of >p on the fifth UMP then fixes the precise length of the RNA in a way that could inhibit subsequent shortening or elongation. Moreover, the presence of >p (or 2′- or 3′-p) at the 3′ end of U6 RNA reduces its affinity for the abundant nuclear antigen La (*13*); binding of La would probably interfere with U6 RNA function during splicing, although formation of the terminal >p is not required for association of U6 RNA with U4 snRNP's (small nuclear ribonucleoproteins) (*13, 19*).

The enzymes responsible for modification of the 3′ ends of U6 RNA remain unknown. HeLa cells contain a terminal uridylyltransferase (TUTase) that is a required host factor for poliovirus RNA replication, but which has no known cellular substrate (*29*). Both HeLa cells and *X. laevis* oocyte nuclei contain an RNA 3′ terminal phosphate cyclase that catalyzes conversion of a 3′ terminal phosphate to the cyclic 2′,3′-phosphodiester in an adenosine triphosphate–dependent reaction (*30*). It is unknown if any of these enzymes is responsible for 3′ end modification of U6 RNA.

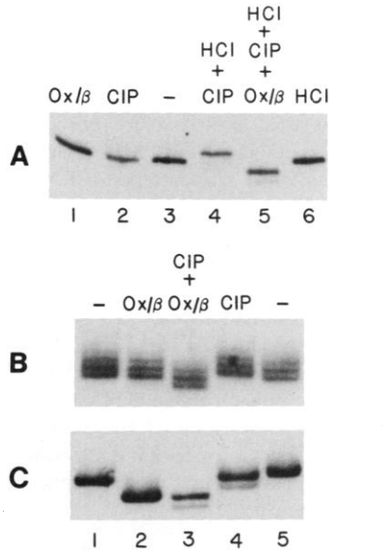

Fig. 4. Analyses of 3′ termini of U6 RNA's of various species. RNA blot analyses (*15*) of soybean, *Glycine max.* (**A**), yeast, *S. cerevisiae* (**B**) and trypanosome, *T. brucei rhodesiense* (**C**) U6 RNA's that were treated with acid (HCl), calf intestinal phosphatase (CIP), or sodium periodate plus lysine (Ox/β), either alone or sequentially, starting with the reagent listed on top. Changes in U6 RNA gel mobilities reflect alterations at the 3′ end of the RNA's.

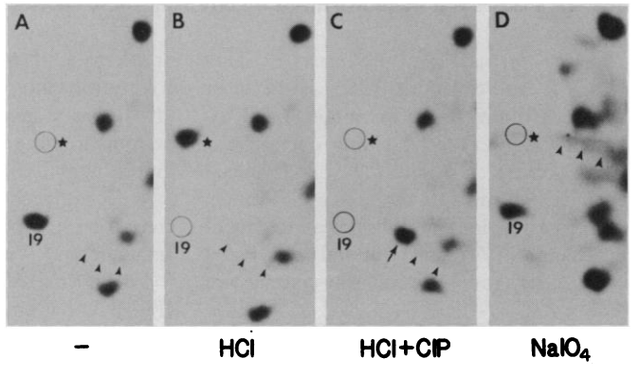

Fig. 3. Identification of the 3′ end modification of mature U6 RNA. RNase T1 fingerprints (A to D) of mouse U6 RNA's that were untreated (**A**) or treated with either acid (HCl) (**B**), acid plus calf intestinal phosphatase (HCl+CIP) (**C**), or sodium periodate (**D**). For clarity, only the parts of the fingerprints containing the 3′ end oligonucleotides are shown. The precise length of the 3′ terminal oligouridylate region in the major 3′ end oligonucleotide in (D) was determined by comparison with synthetic U6 RNA's of defined length (*19*).

Similar modifications of RNA's have been detected in other systems. For example, the 3′ ends of guide RNA's that participate in RNA editing of mitochondrial transcripts in kinetoplastid protozoa are post-transcriptionally elongated by UMP addition (*31*). The two other known types of RNA's with cyclic phosphates at their 3′ ends are both metabolically active, being intermediates in RNA cleavage and ligation events: the 5′ splicing intermediates of intron-containing tRNA's of mammalian cells (*32*) and the cleavage products of hammerhead and hairpin ribozymes (*33*). In a similar fashion, the cyclic phosphate of U6 RNA may participate directly in one of the steps of pre-mRNA splicing by forming a transient, covalent linkage with another spliceosomal component.

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15. Preparation of RNA's by the urea-lysis method, hybrid-selection of U6 RNA's to filter-bound pmU6 (−315 to +256) DNA [G. Das, D. Henning, D. Wright, R. Reddy, *EMBO J.* 7, 503 (1988)] and RNA blot analysis were as described (*17*). Electrophoresis was for 3.5 to 4 hours at 40 V/cm in fully denaturing (0.4 mm thick and 40 cm long) 8% (29:1) polyacrylamide gels containing 7 M urea, 0.5× TEB (45 mM tris-borate, 1.15 mM EDTA, pH 8.3). Antisense U6 RNA probes were prepared by transcription of pmU6(−150 to +5) DNA with T7 RNA polymerase (Promega) in the presence of 50 μM [α-^{32}P]UTP (NEN, Dupont) (30 to 50 μCi/nmol). In vitro transcription of pmU6(−315 to +286) DNA in S100 extracts of HeLa cell was according to T. H. Steinberg, D. E. Mathews, R. D. Durbin, R. R. Burgess [*J. Biol. Chem.* **265**, 499 (1990)].
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21. RNase T2 or nuclease P1 digests were separated by two-dimensional chromatography on cellulose thin-layer plates with isobutyric acid, water, and ammonium hydroxide (66:33:1) in the first dimension (bottom to top) [M. Silberklang, A. M. Gillum, U. T. RajBhandary, *Methods Enzymol.* **59**, 58 (1979)] and either isopropanol, water, and concentrated HCl (70:15:15) [S. Nishimura, *Prog. Nucleic Acids. Res. Mol. Biol.* **12**, 49 (1972)] or isopropanol, saturated ammonium sulfate and 1 M sodium acetate, pH 7.0 (2:80:18) in the second dimension [M. M. Konarska, R. A. Padgett, P. A. Sharp, *Cell* **38**, 731 (1984)].
22. RNA's were incubated with 10 mM HCl (acid treatment) or calf intestinal phosphatase (CIP) (phosphatase treatment) [A. C. Forster, C. Davies, C. J. Hutchins, R. H. Symons, *Methods Enzymol.* **181**, 583 (1990)] or subjected to oxidation plus β elimination [H. C. Neu and L. A. Heppel, *J. Biol. Chem.* **239**, 2927 (1964)].
23. Presumably a small fraction of U>p was hydrolyzed during RNase T1 fingerprinting, accounting for the observed "streak" between oligonucleotides No. 19 and the asterisk in Fig. 1B.
24. Blocking of U6 RNA 3′ ends by >p (or 3′-p) could account, at least in part, for the low efficiency of 3′ end-labeling by pCp and RNA ligase [R. Reed, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 8031 (1990)]. Moreover, 3′ end-labeled molecules would represent only the subset of U6 RNA's lacking 3′ end modification.
25. Because of potentially overlapping mobilities of multiple bands, some yeast U6 RNA's may have undetectable phosphatase-resistant 3′ ends (either −OH or >p).
26. At least one mouse U6 RNA gene encodes only four uridylic acid residues (Y. Ohshima, N. Okada, T. Tani, Y. Itoh, M. Itoh, *Nucleic Acids Res.* **9**, 5145 (1981)].
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Sibling Species in *Montastraea annularis*, Coral Bleaching, and the Coral Climate Record

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Measures of growth and skeletal isotopic ratios in the Caribbean coral *Montastraea annularis* are fundamental to many studies of paleoceanography, environmental degradation, and global climate change. This taxon is shown to consist of at least three sibling species in shallow waters. The two most commonly studied of these show highly significant differences in growth rate and oxygen isotopic ratios, parameters routinely used to estimate past climatic conditions; unusual coloration in the third may have confused research on coral bleaching. Interpretation or comparison of past and current studies can be jeopardized by ignoring these species boundaries.

MONTASTRAEA ANNULARIS (ELLIS and Solander, 1786) is the most abundant, wide-ranging, and intensively studied reef-building coral of the tropical western Atlantic (*1, 2*). Skeletal characters in this species (*3, 4*) are routinely used to assess local and global environmental change (*5–7*), but temporal and spatial comparisons involving multiple colonies are problematic if the enormous variation in colony morphology shown by this species (*1, 8*) has a genetic basis. No systematic examination of genetic influences on colony morphology has been attempted, however.

We recognized three often sympatric, discrete morphotypes of *M. annularis* on Panamanian and Venezuelan reefs using the following criteria in the field: Morphotype 1 (Fig. 1A) has small polyps, and large colonies form groups of columns that widen distally. Living tissue is found mainly on tops of columns, and margins are senescent. Morphotype 2 (Fig. 1B) also has small

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