10. W. V. Shaw, *Methods Enzymol.* **43**, 737 (1975).
11. R. Jaenisch *et al.*, *Cell* **24**, 519 (1981).
12. E. F. Wagner, L. Covarrubias, T. A. Stewart, B. Mintz, *ibid.* **35**, 647 (1983); J. Löhler, R. Timpl, R. Jaenisch, *ibid.* **38**, 597 (1984).
13. K. A. Mahon, P. A. Overbeek, H. Westphal, in preparation.
14. R. P. Woychik, T. A. Stewart, L. G. Davis, P. D'Eustachio, P. Leder, *Nature (London)* **318**, 36 (1985).
15. P. A. Chatis *et al.*, *J. Virol.* **52**, 248 (1984); L. DesGroseillers and P. Jolicoeur, *ibid.*, p. 448; J. Lenz *et al.*, *Nature (London)* **308**, 467 (1984); B. Davis, E. Linney, H. Fan, *ibid.* **314**, 550 (1985).
16. P. A. Overbeek, A. B. Chepelinsky, J. S. Khillan, J. Piatigorsky, H. Westphal, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7185 (1985).
17. J. S. Khillan, A. Schmidt, P. A. Overbeek, B. de Crombrugghe, H. Westphal, *ibid.*, in press.
18. M. Mercola, J. Goverman, C. Mirell, K. Calame, *Science* **227**, 266 (1985).
19. J. M. Chirgwin, A. E. Przybyla, R. J. MacDonald, W. J. Rutter, *Biochemistry* **18**, 5294 (1979); E. Jonas, T. D. Sargent, I. B. Dawid, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5413 (1985).
20. G. Church and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 1991 (1984).
21. J. W. Gordon, G. A. Scangos, D. J. Plotkin, J. A. Barbosa, F. H. Ruddle, *ibid.* **77**, 7380 (1980).
22. R. L. Brinster *et al.*, *Cell* **27**, 223 (1981).
23. We thank C. Gorman and B. Howard for the pRSV-CAT plasmid and J. Coffin, P. Duesberg, and K. Mahon for helpful suggestions.

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Autolytic Processing of Dimeric Plant Virus Satellite RNA

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Associated with some plant viruses are small satellite RNA's that depend on the plant virus to provide protective coat protein and presumably at least some of the proteins necessary for satellite RNA replication. Multimeric forms of the satellite RNA of tobacco ringspot virus are probable in vivo precursors of the monomeric satellite RNA. Evidence is presented for the in vitro autolytic processing of dimeric and trimeric forms of this satellite RNA. The reaction generates biologically active monomeric satellite RNA, apparently is reversible to form dimeric RNA from monomeric RNA, and does not require an enzyme for its catalysis.

THE KNOWN SMALL SATELLITE RNA's have 400 or fewer nucleotide residues in their simplest form and have been discovered in association with the members of five groups or potential groups of RNA plant viruses (*1, 2*). They replicate detectably only when co-inoculated with the respective supporting virus. Small satellite RNA's become encapsidated in coat protein specified by the supporting virus but, with two exceptions, have no extensive nucleotide sequence homology with the virus RNA (*2*). The satellite RNA of tobacco ringspot virus (STobRV RNA) was the first small satellite RNA to be discovered (*3*). In mixed TobRV plus STobRV RNA infections, more than 90 percent of the encapsidated RNA may be STobRV RNA.

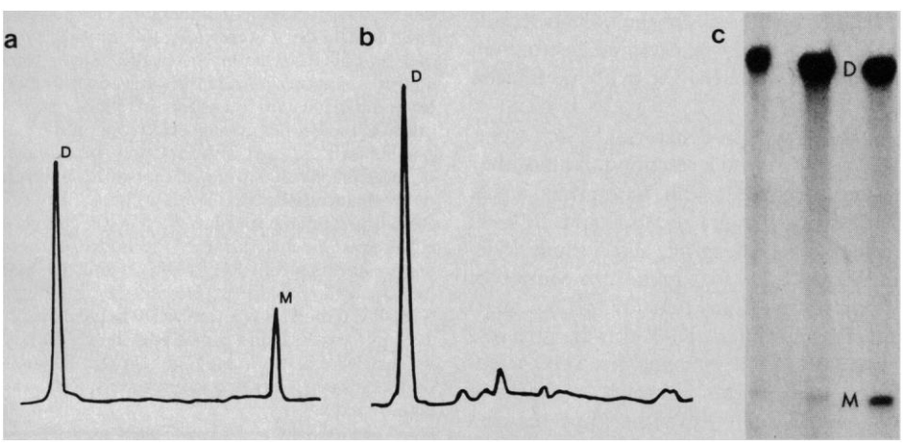

Fig. 1. Formation of RNA with the electrophoretic mobility of monomeric STobRV RNA from the dimeric form. After incubation in buffered solution as indicated below, the samples were heated to 80°C for 30 seconds in 50 percent formamide containing an excess of EDTA over divalent metal ion. Electrophoresis was at 350 V in 10 cm long, 7 percent polyacrylamide gel (a and b) or at 1500 V in 40 cm long, 6.5 percent polyacrylamide gel (c), both containing 7*M* urea. NC-87 strain dimeric STobRV RNA, when incubated in 33 m*M* tris, 5.5 m*M* HCl, 10 mg of SDS per milliliter, and 20 m*M* $MgCl_2$ (*p*H ~7.4) for 15 minutes at 30°C generated a zone with the mobility of the monomeric STobRV RNA from virus particles (a), whereas the control incubation with 2 m*M* EDTA in place of $MgCl_2$ did not (b). Detection was by silver stain (*18*) and optical densitometry. The sample for (c) was dimeric budblight strain STobRV RNA partially 5′ end-labeled (less than 5 percent of the molecules derivatized) by incubation with bacteriophage T4 polynucleotide kinase and [γ-^{32}P]ATP. The dimeric RNA was incubated in 100 m*M* tris, 45 m*M* HCl, 10 mg of lithium dodecyl sulfate (LDS, *p*H 8.0) per milliliter, and either 50 m*M* EDTA on ice or at 25°C (left-hand and central lanes, respectively) or 5 m*M* $MgCl_2$ at 25°C (right-hand lane) for 30 minutes before electrophoresis and detection by autoradiography. D and M indicate the zones for dimeric and monomeric STobRV RNA, respectively.

Unencapsidated, double-stranded STobRV RNA from infected tissue is composed of linear and circular multimeric forms of both polarities (*4, 5*), whereas encapsidated STobRV RNA is linear and of one polarity, designated arbitrarily as (+). The encapsidated (+)RNA is principally in the monomeric form (359 nucleotide residues), with decreasing amounts of the dimer and each of the succeeding, repetitive-sequence, multimeric forms. Observations of multimeric linear and circular forms of satellite RNA's and (the independently replicating) viroid RNA's and of enzymes capable of circularizing RNA (*5–9*) have led to the formulation of replication models that include rolling-circle transcription and the processing of multimeric transcripts to form the monomeric RNA (*4, 8, 9*).

We studied two independent isolates of STobRV RNA, designated budblight and NC-87 (*10*). Virus particles (*11*) from leaves of infected common bean (*Phaseolus vulgaris* cv. Black Valentine) were chromatographed on 2 percent agarose beads (Sepharose CL-2B, virus particle distribution constant ≈0.6) in 10 m*M* NaH_2PO_4, 2 m*M* EDTA, and 0.25*M* NaCl (*p*H ~6.5; column linear flow rate, 0.6 mm/min). RNA was isolated by a two-phase phenol extraction procedure (*11*), and purification of monomeric, dimeric, and trimeric forms of the satellite RNA was by electrophoresis at 30 volt/cm through 0.5 mm thick, 6.5 percent polyacrylamide gels in 7*M* urea, 90 m*M* tris-borate, and 1 m*M* EDTA buffer (*p*H 8.3) (*12, 13*).

Electrophoresis of purified dimeric and trimeric STobRV RNA preparations gave single zones of the expected mobilities, but only if the RNA was analyzed directly after isolation (Fig. 1b). After storage of RNA at

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−20°C as a precipitate under 0.1*M* sodium acetate and 3 m*M* acetic acid in 67 percent ethanol for as little as 1 week, a new zone with the mobility of monomeric STobRV RNA often appeared. In later experiments we found that the chelating resin Chelex 100 (Bio-Rad) greatly reduced the spontaneous production of monomeric RNA during storage. We were unable to mimic the generation of monomer-like RNA by brief treatments of freshly isolated dimeric RNA with low concentrations of ribonucleases T1 or U2 under various conditions. However, dimeric RNA incubated in a solution of magnesium ions at room temperature rapidly generated RNA with the mobility of monomeric STobRV RNA (Fig. 1). The conversion of dimeric RNA to putative monomeric RNA varied from 10 to 65 percent in similar experiments. Trimeric STobRV RNA generated zones with the mobilities of dimeric and monomeric RNA.

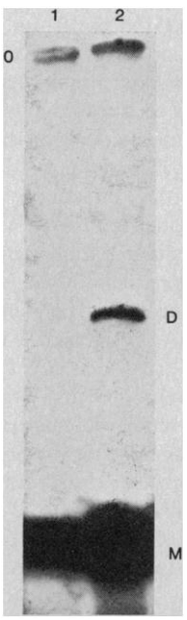

Fig. 2. Formation from the monomeric form of RNA with the electrophoretic mobility of linear dimeric STobRV RNA. Electrophoretically purified monomeric budblight strain STobRV RNA was partially labeled (approximately 5 percent of the molecules) by introducing 5′-phosphoryl groups labeled in ^{32}P in a reaction catalyzed by T4 bacteriophage polynucleotide kinase. The RNA was incubated in 100 m*M* 2-(*N*-morpholino) ethane sulfonic acid (MES), 45 m*M* NaOH, 10 mg of LDS per milliliter (*p*H ~ 6), and either 30 m*M* zinc chloride (lane 2) or 20 m*M* Na_2EDTA (lane 1) for 24 hours at 4°C. The samples were heated in formamide solution and analyzed by electrophoresis through a 5 percent polyacrylamide gel containing 7*M* urea. Detection was by autoradiography with an image-intensifying screen placed just above the zones for monomeric RNA to avoid the heavy exposure that those zones otherwise would cause. The origin (O) and the positions of the zones for dimeric (D) and monomeric (M) RNA are indicated.

The biological activity of the RNA derived from dimeric STobRV RNA provides evidence that it is authentic monomeric STobRV RNA. The derived monomeric RNA had a biological activity greater than or equal to that of the residual dimeric RNA that remained after incubation (Table 1). However, the biological activity of the derived monomeric RNA apparently was less than that of monomeric or dimeric RNA from virus particles, possibly as a result of damage to the former RNA during incubation or additional electrophoretic purification (or both).

Monomeric satellite RNA was purified from plants that had been inoculated with a mixture of the ST strain of TobRV and monomeric RNA derived in vitro from either budblight dimeric or trimeric STobRV RNA. These RNA's and standard STobRV monomeric RNA were subjected to partial digestion with base-specific ribonucleases to compare the sequences of residues 5 through 160, those residues most accessible by this method (*14*). The sequences were the same, which further indicates that the monomeric RNA derived from dimeric and trimeric budblight STobRV RNA gave rise to budblight STobRV RNA, even when the ST strain was the supporting virus.

Glyoxal in 50 percent dimethysulfoxide (*15*), but not 50 percent dimethylsulfoxide alone (Table 2), completely prevented dimer RNA conversion to monomer and did not reveal any other more rapidly migrating species. Thus dimeric RNA did not appear to have hidden breaks in the polynucleotide chain. Sufficiently concentrated ethidium bromide reduced the yield of monomeric RNA (Table 2).

When protein-denaturing detergents were included in the reaction mixtures, they did not affect the yield of monomeric RNA. During an overnight incubation at 37°C in Proteinase K (10 μg/ml) and sodium dodecyl sulfate (SDS, 10 mg/ml), a sample of *Escherichia coli* β-galactosidase (100 μg/ml) added to the dimeric RNA was digested to a level undetectable by a sensitive silver-staining procedure (*16*). However, the yield of monomeric RNA from the same reaction was not reduced. Other experiments showed that the extent of reaction was not altered over an eightfold range of dimeric RNA concentration. These results argue against a protein requirement for the dimer-to-monomer conversion and in favor of its being the result of a unimolecular, presumably autolytic RNA processing reaction.

Partial nucelotide sequence analyses of the 5′ ends of monomeric and dimeric STobRV RNA from virus particles and monomeric RNA derived in vitro from dimeric RNA were carried out as described (*14, 17*). Prior treatment of the RNA's with alkaline phosphatase did not alter the extent of 5′ phosphorylation by adenosine [γ-^{32}P]triphosphate ([γ-^{32}P]ATP) in a reaction catalyzed by bacteriophage T4 polynucleotide kinase (*4*). Partial digestion of each RNA with base-specific nucleases gave parallel "ladders" of zones, indicating that all three have the same 5′ terminal sequence, and digestion with nuclease P1 (*18*) released adenosine-5′-phosphate as the only labeled nucleotide (*19*). Cytidylate was the only 3′ terminal residue detected when monomeric RNA derived from dimeric RNA was incubated in acidic solution, to open 2′,3′-cyclic phosphodiester groups, before phosphatase treatment and end-labeling as described (17).

These results indicate that the dimeric RNA cleaves at a specific phosphodiester bond to generate 2′,3′-cyclic phosphodiester and 5′-hydroxyl groups at the new ends. (However, end-labeling was not quantitative, and it is conceivable, although unlikely, that all the detected terminal sequences of the monomeric RNA produced in vitro were derived from terminal sequences of the original dimeric RNA.) The nucleotide sequence of the junction region of budblight

Table 1. Biological activity of monomeric STobRV RNA derived from dimeric RNA in an autolytic reaction. All inocula contained TobRV, and inoculations were to 12 primary leaves of cowpea seedlings (*Vigna unquiculata* cv. Blackeye 5). Reaction products of monomeric RNA and the residual (uncleaved) dimeric RNA (first two rows of data only) were electrophoretically purified, and all RNA additions to the inocula were adjusted to approximately the same concentration by comparisons of intensities of ethidium bromide–stained zones after gel electrophoresis. The satellite index (*1, 28*) is the fraction (percentage) of local lesions that were of the small, chlorotic type characteristic of TobRV plus STobRV RNA. In experiment 1, NC-87 STobRV dimeric RNA was incubated for 15 minutes at room temperature in 33 m*M* tris-HCl and 20 m*M* $MgCl_2$ (*p*H 8). In experiment 2, budblight STobRV dimeric RNA similarly was incubated in 100 m*M* tris-HCl and 5 m*M* $MgCl_2$ (*p*H 8), and inocula were at 1× (first number) and 5× (second number) relative concentrations. ND, not determined.

Additions to inoculum	Satellite index	
	Experiment 1	Experiment 2
Monomeric STobRV RNA derived from dimeric RNA	30	5, 20
Residual dimeric STobRV RNA	1.7	0, 15
None	0	0
Monomeric STobRV RNA from TobRV capsids	ND	ND, 92
Untreated dimeric STobRV RNA	ND	13, ND

dimeric STobRV RNA (*17*) is 5′ . . . AAAUAUUCUAGCCCGAUACCCUGUCACCGGAUGUGCUUUCCGGUCUGAUG . . . 3′. The underlined adenylate residue in this sequence indicates the first residue in the sequence for monomeric STobRV RNA. The adenylate residue and the preceding cytidylate residue form the junction expected if dimeric RNA is simply two joined monomeric budblight STobRV RNA molecules. They also form the only C-A pair within 50 nucleotide residues of the proposed junction. Thus the autolysis appears to be a phospho-transfer reaction between an adenylate residue and the 2′-hydroxyl group of a cytidylate residue to produce a new 5′-hydroxyl terminal adenosine residue and a new cytosine residue with a 2′,3′-cyclic phosphodiester group, both of which are characteristic of monomeric STobRV RNA (*17*).

The 2′,3′-cyclic phosphodiester end that forms during autolysis of multimeric STobRV RNA is a suitable substrate for an RNA ligase such as that from wheat germ (*7*). This type of enzyme is a candidate for catalyzing circularization of monomeric STobRV RNA (*6, 20*), as would be necessary as a first step in the proposed rolling-circle models (*4, 8, 9*) of RNA replication. Simlarly, autolytic processing itself is consistent with such a model.

Two well-characterized RNA's that facilitate the cleavage of P–O bonds are the M1 RNA of the bacterial RNA–processing enzyme ribonuclease P (*21*) and the self-splicing ribosomal RNA precursor of the protozoan *Tetrahymena* (*22*). The reactions of these RNA's differ from the autolysis of STobRV multimeric RNA's. For example, they require magnesium ions specifically and proceed by a 5′-phosphoryl intermediate rather than the 2′,3′-cyclic phosphodiester group that is the terminus of STobRV RNA. Also, exogenous guanosine participates in the splicing reaction of the ribosomal RNA precursor (*22*). Production of monomeric RNA from dimeric STobRV RNA was promoted by divalent ions not only of magnesium but also of zinc and lead; even the polyamine spermidine was effective (Table 2). Monovalent ammonium, lithium, sodium, and potassium ions did not appreciably stimulate the magnesium ion–promoted reaction (Table 2), nor did guanine, adenine, guanosine, or adenosine, as expected if the phospho-transfer reaction that we indicate above is responsible for chain cleavage. No 2′-, 3′-, or 5′-monophosphate of the four standard ribonucleosides increased the extent of monomeric RNA production or, when labeled with ^{32}P, was incorporated into the products.

Two other RNA's appear to cleave autolytically in a manner similar to that of dimeric STobRV RNA. These are a transcript, synthesized in vitro, of a cloned dimeric copy of potato spindle tuber viroid (PSTV) (*20*) and the p2Sp1 RNA of *E. coli* cells infected with bacteriophage T4 (*23*). The PSTV autolysis reaction occurs under conditions that are similar to those reported here, whereas the most efficient p2Sp1 RNA reaction conditions are quite different (*23*). However, the cleaved bond is Cp-A in p2Sp1 RNA as well as in dimeric STobRV RNA.

Table 2. Effects of various agents and *p*H on the extent of conversion of dimeric STobRV RNA, of the budblight and NC-87 strains, to monomeric RNA. Incubation of dimeric RNA at a concentration of 25 n*M* or less was for 15 minutes at 30°C or for 30 minutes at 22°C. Each autolysis reaction mixture, except those with ethidium bromide (EthBr), contained 10 mg of SDS or LDS per milliliter. Analysis was by electrophoresis and optical densitometry of silver-stained gels (Fig. 1). Values for percentage processing are the areas under the monomer zone tracing divided by the combined areas of the monomer and dimer zone tracings. DMSO, dimethylsulfoxide; MES, *N*-morpholinoethanesulfonic acid.

*p*H	Buffer and other additions	Divalent ion or polyamine	Percentage processing
	NC=87		
7.4	Tris-HCl (33 m*M*) + NaCl (100 m*M*)	$MgCl_2$ (20 m*M*)	14
		EDTA (2 m*M*)	8
	Tris-HCl (33 m*M*)	$PbCl_2$ (5 m*M*)	23
	Tris-HCl (33 m*M*)	$MgCl_2$ (20 m*M*)	21
	+ DMSO (50 percent)	$MgCl_2$ (20 m*M*)	26
	+ DMSO (50 percent)	$ZnCl_2$ (10 m*M*)	26
	Tris-HCl (33 m*M*)	EDTA (2 m*M*)	6
	Budblight		
8	Tris-HCl (200 m*M*)	Spermidine (0.1 m*M*)	31
		Spermidine (1 m*M*)	23
		$MgCl_2$ (5 m*M*)	27
		EDTA (2 m*M*)	7
	Tris-HCl (100 m*M*)	$MgCl_2$ (5 m*M*)	18
	+EthBr (32 μ*M*)	$MgCl_2$ (5 m*M*)	16
	+EthBr (64 μ*M*)	$MgCl_2$ (5 m*M*)	12
	+EthBr (127 μ*M*)	$MgCl_2$ (5 m*M*)	9
	+EthBr (254 μ*M*)	$MgCl_2$ (5 m*M*)	5
	Tris-HCl (100 m*M*)	EDTA (10 m*M*)	4
	NC=87		
4.2	Sodium acetate (200 m*M*)	$MgCl_2$ (10 m*M*)	14
4.8	Sodium acetate (200 m*M*)	$MgCl_2$ (10 m*M*)	11
6.2	Sodium MES (200 m*M*)	$MgCl_2$ (10 m*M*)	9
6.8	Tris-HCl (200 m*M*)	$MgCl_2$ (10 m*M*)	15
8.0	Tris-HCl (200 m*M*)	$MgCl_2$ (10 m*M*)	24

Our data (Table 2) on the *p*H-dependence of autolysis show a minimum in the extent of reaction between *p*H 5 and 7. The *p*H of optimum stability for RNA in solution is approximately 6.5, and at all *p*H values the predominant initial reaction of the RNA is phosphodiester bond cleavage to create new 5′-hydroxyl and 2′,3′-cyclic phosphodiester termini (*24*). Although our data on *p*H-dependence are insufficient to rule out specific ion and several other possible effects, we postulate that the autolysis of dimeric STobRV RNA resembles ordinary RNA phosphodiester bond cleavage in solution but at a bond whose liability is strongly enhanced by a particular, divalent ion–stabilized conformation of the RNA molecule. The data do not favor the specific metal ion catalysis that has been postulated for some systems (*25*) because of the chemically disparate types of metal ions and polyamine (Table 2) that have been effective.

If, as our results indicate, autolysis produces 5′-hydroxyl and 2′,3′-cyclic phosphodiester terminal groups, and if no nucleotide or nucleoside is lost or incorporated in the reaction, there is the potential for a limited reversal of the autolysis reaction aided by the energy maintained in the cyclic phosphodiester. 5′-Phosphorylated monomeric STobRV RNA, when incubated at 4°C with an excess (approximately 1 mg/ml) of unmodified monomeric RNA for periods of hours, generated labeled RNA with the mobility of linear dimeric STobRV RNA from virus particles (*4*), as detected by electrophoresis either in urea-containing gels (Fig. 2) or in nondenaturing gels. EDTA prevented the reaction. The presumed dimeric RNA survived the usual denaturation treatment that precedes gel electrophoresis in 7*M* urea. It also survived derivatization with glyoxal. Comigration of the reaction product with linear dimeric RNA under several conditions indicates that it is neither a circular RNA, which in urea-containing gels migrates more slowly than the corresponding linear form (*5, 26*), nor some other nonlinear form of the RNA. The yields of the dimer, estimated from Ceren-

kov counts of excised zones, averaged 0.5 percent in four experiments, with a range of 0.16 to 1.0 percent.

STobRV RNA does not have detectable messenger activity (*27*). Its nucleotide sequence nevertheless has several functions: it serves as a template for transcription into RNA; it undergoes autolysis; and it is encapsidated. STobRV RNA greatly reduces the yield of TobRV and the severity of symptoms that TobRV alone induces. Because of these multiple functions, probably less than the entire nucleotide sequence of dimeric STobRV RNA is necessary for the autolysis reaction alone.

REFERENCES AND NOTES

1. I. R. Schneider, in *Beltsville Symposia in Agricultural Research*, J. A. Romberger, Ed. (Allenheld, Osmun, Montclair, NJ, 1977), vol. 1, pp. 201–219.
2. A. F. Murant and M. A. Mayo, *Annu. Rev. Phytopathol.* **20**, 49 (1982); J. M. Kaper and M. E. Tousignant, *Endeavor (New Series)* **8**, 194 (1984); R. I. B. Francki, *Annu. Rev. Microbiol.* **39**, 151 (1985).
3. I. R. Schneider, *Science* **166**, 1627 (1969).
4. M. C. Kiefer, S. D. Daubert, I. R. Schneider, G. Bruening, *Virology* **121**, 262 (1982).
5. J. M. Sogo and I. R. Schneider, *ibid.* **117**, 401 (1982); H. J. M. Linthorst and J. M. Kaper, *ibid.* **137**, 206 (1984).
6. P. A. Kiberstis, J. Hazeloff, D. Zimmern, *EMBO J.* **4**, 817 (1985).
7. M. Konarska, W. Filipowicz, H. J. Gross, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1474 (1982).
8. A. D. Branch, H. D. Robertson, E. Dickson, *ibid.* **78**, 6381 (1981); A. D. Branch and H. D. Robertson, *Science* **223**, 450 (1984).
9. R. H. Symons *et al.*, in *Subviral Pathogens of Plants and Animals: Viroids and Prions*, K. Maramorosch and J. J. McKelvey, Jr., Eds. (Academic Press, New York, 1985), pp. 235–263.
10. R. P. Kahn, H. A. Scott, R. L. Monroe, *Phytopathology* **52**, 1211 (1962); I. R. Schneider, R. M. White, G. V. Gooding, *Virology* **50**, 902 (1972).
11. I. R. Schneider, R. Hull, R. Markham, *Virology* **47**, 320 (1972).
12. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning, A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982), pp. 156, 174–177.
13. Zones were located in the electrophoresis gel by autoradiography or by staining with toluidine blue or ethidium bromide. Excised gel was soaked (*14*) overnight in ammonium chloride (0.5*M*), Na_2EDTA (0.5 m*M*), and SDS (0.5 mg/ml). RNA was recovered from the soak solution by precipitation with two volumes of ethanol. Yields were approximately 80 percent for monomeric STobRV RNA and 50 percent for the dimeric form.
14. J. Haseloff and R. H. Symons, *Nucleic Acids Res.* **9**, 2741 (1981).
15. G. K. McMaster and G. G. Carmichael, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4835 (1977).
16. Photochemically activated silver staining detected either RNA or RNA and protein. The procedure was that of C. R. Merril, D. Goldman, S. A. Sedman, and M. H. Ebert [*Science* **211**, 1437 (1981)] except that the rinses that preceded the dichromate oxidation step were either (i) 10 percent methanol for 1 hour or longer, three changes of water over a 15-minute period, 10 percent methanol for 30 and 5 minutes, 40 percent methanol for 20 minutes, and finally water for 10 minutes (for RNA detection) or (ii) 10 percent glutaraldehyde for 30 minutes and three washes with water for 10 minutes each (for detection of both RNA and protein).
17. J. M. Buzayan, W. L. Gerlach, G. Bruening, P. Keese, A. R. Gould, *Virology*, in press.
18. S. D. Daubert and G. Bruening, *Virology* **98**, 246 (1979).
19. S. Nishimura *et al.*, *Biochim. Biophys. Acta* **142**, 133 (1967).
20. H. D. Robertson, D. L. Rosen, A. D. Branch, *Virology* **142**, 441 (1985).
21. C. Guerrier-Takada and S. Altman, *Science* **223**, 285 (1984); M. Baer and S. Altman, *ibid.* **228**, 999 (1985).
22. A. J. Zaug, J. R. Kent, T. R. Cech, *ibid.* **224**, 574 (1984).
23. N. Watson, M. Gurevitz, J. Ford, D. Apirion, *J. Mol. Biol.* **172**, 301 (1984).
24. W. C. Ginoza *et al.*, *Nature (London)* **203**, 606 (1964).
25. K. Haydock and L. C. Allen, *Prog. Clin. Biol. Res.* **172A**, 87 (1985); R. S. Brown *et al.*, *Nature (London)* **303**, 543 (1983).
26. A. D. Branch and H. D. Robertson, personal communication.
27. R. A. Owen and I. R. Schneider, *Virology* **80**, 222 (1977).
28. T. O. Diener, I. R. Schneider, D. R. Smith, *ibid.* **57**, 577 (1974); I. R. Schneider, *ibid.* **45**, 108 (1971).

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HTLV-III *gag* Protein Is Processed in Yeast Cells by the Virus *pol*-Protease

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The *gag-pol* gene of HTLV-III (human T-lymphotropic virus), the virus linked to AIDS (acquired immune deficiency syndrome), was expressed in yeast, and processing of the *gag* precursor into proteins of the same size as those in the virion was observed. Processing of the *gag* gene in yeast cells mimics the process that naturally occurs in mammalian cells during maturation of virions. Therefore it was possible to perform mutational analysis of the virus genome to localize the gene that codes for the protease function to the amino terminal coding region of the *pol* gene. Since this region overlaps the *gag* gene, it is likely that ribosomal frameshifting occurs from *gag* to *pol*. Antibodies in all of the AIDS patients' sera tested recognized the yeast synthesized *gag* proteins, although the sera showed differences in relative reactivity to the individual *gag* proteins and the precursor. This yeast system should be valuable not only for production of viral proteins for diagnostic or vaccine purposes but also for analysis of the genetics and biochemistry of viral gene functions—parameters that are difficult to study otherwise with this virus.

THE RETROVIRUS HTLV-III AND the closely related variants of this virus, LAV and ARV, are the causative agents of the disease acquired immune deficiency syndrome (AIDS) (*1*). Molecular cloning and nucleotide sequence analysis of HTLV-III and its variants have demonstrated that this viral genome exhibits many of the structural features of the avian and mammalian retroviruses. Thus, the viral genome contains the three genes (*gag, pol*, and *env*) characteristic of all retroviruses (*2*). In addition, the HTLV-III genome contains two short open reading frames whose functions are unknown (*2*).

One of the viral genes, *gag*, encodes a precursor which is processed into core proteins during virion maturation. From DNA sequence data and analysis of isolated viral proteins, the HTLV-III *gag* precursor is about 56 kD and is processed into species of approximately 24, 16, and 14 kD (*2*) (Fig. 1A). The protease responsible for this processing is typically encoded by the retroviral genome. It is included in the 3′ end of the *gag* gene in avian retroviruses and in the 5′ end of the *pol* gene in mammalian viruses (*3*). In Moloney murine leukemia virus (MuLV), the protease is a *gag-pol* read through product (*4*) and, for Rous sarcoma virus (RSV), a *gag-pol* fusion protein is produced by a frameshift between overlapping reading frames (*5*). A therapeutic agent that could inhibit this protease might block virus spread. It is, therefore, important to identify the region of the HTLV-III genome that encodes this protease and to develop in vivo and in vitro systems in which the proteolysis of the *gag* gene precursor can be studied. Our results show that the processing reaction is carried out very efficiently in yeast cells and suggest that the yeast system may be used for the development of inhibitors of this process. We have illustrated the utility of the system by map-

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