K–EDTA and the DNA purified by phenol-chloroform extraction and ethanol precipitation.
12. S. Mukherjee, H. Erickson, D. Bastia, *Cell* **52**, 375 (1988).
13. M. A. Seyfred, M. P. Kladde, J. Gorski, *Mol. Endocrinol.* **3**, 305 (1989).
14. PCR products obtained from a nuclear ligation assay performed on G1I and GH_3 nuclei were digested with either Hinf I (G1I) or Hae III (GH_3) and then analyzed by Southern (DNA) blotting with a ^{32}P-labeled Hind III–Pst I *PRL* probe. Hinf I digestion of 871- and 1102-bp PCR products obtained from G1I nuclei formed the predicted (based on the known DNA sequence) 494- and 725-bp fragments, respectively. Hae III digestion of the 871- and 973-bp PCR products obtained from GH_3 nuclei resulted in the formation of three predicted fragments at 729, 627, and 244 bp. The formation of the unique Pst I junction (−1953 to −12 bp) by the ligation of the 5′ distal Pst I site of *PRL* at −1953 bp with the proximal promoter Pst I site at −12 bp was confirmed by dideoxynucleotide sequencing of the 871-bp PCR product.
15. To calculate the ligation frequency of the Pst I fragments, we scanned Southern blots of Pst I *PRL*-Tn*5*-BPV DNA fragments by a Hoefer laser densitometer, and the ratios of the 1941-bp and 2172-bp fragments compared to the full-length 12.4-kb Xba I fragment were determined. Bands corresponding to the 702-bp internal standard, the 871-bp P_3-P_4 Pst I DNA fragments, and the 1102-bp P_3-P_5 Pst I DNA fragments were excised from the nylon membrane and counted in a scintillation counter. The ligation frequency was determined by the following equation:

$$\frac{\dfrac{P_3\text{-}P_4 \text{ (or } P_3\text{-}P_5\text{) Pst I PCR fragment (cpm)}}{\text{PCR internal standard (cpm)}}}{\dfrac{\text{1941- (or 2172-) bp Pst I DNA fragment}}{\text{12.4-kb Xba I DNA fragment}}}$$

where the Pst I and Xba I DNA fragments are measured in scanning units.
16. For comparison, see Fig. 4C.
17. A Pst I DNA PCR standard used with *PRL* primers I and II (below) was prepared by digesting a 1941-bp Pst I *PRL* DNA fragment (P_3 to P_4) (Fig. 2) with Hae III (−1748) and then adding Pst I linkers to the ends of the DNA fragment. The 1772-bp Pst I fragment was gel purified, circularized with T4 DNA ligase, and then linearized with Hind III. The Hind III digestion products were amplified by PCR with *PRL* primers I [(−1495) 5′-GGAAGATATAGATAAATAAATCGTT-3′ (−1519)] and II [(−418) 5′-TTTAATTTACCCAATAATCTTGAAT-3′ (−394)], which yielded a 702-bp DNA fragment. We obtained the Pst I DNA PCR standard used with Tn*5* primers III and IV (below) by ligating in vitro an 1154-bp Pst I fragment from P_4 (−10) to P_6 (+1144) (Fig. 4) followed by linearization with Pvu II. The Pvu II digestion products were amplified by PCR with the Tn*5* primers III [(+89) 5′-TCCACCAAGCGGCCGGAGAACCTG-3′ (+65)] and IV [(+830) 5′-CTGAGCGGGACTCTGGGGTTCGAAATGACC-3′ (+859)], which produced a 414-bp DNA fragment. The 414-bp PCR product of Tn*5* primers III and IV and the 702-bp PCR product of *PRL* primers I and II were gel-purified and quantitated by comparison of ethidium bromide intensities on an agarose gel with known quantities of DNA. The 702-bp fragment was also used as an internal standard to monitor amplification efficiency (Fig. 4).
18. K. E. Cullen and M. A. Seyfred, unpublished results.
19. For comparison, see Fig. 4A.
20. J. C. Wang and G. N. Giaever, *Science* **240**, 300 (1988).
21. Estrogen appeared to have little or no effect on the formation of the 1941-bp P_3-P_4 chromatin fragment (Fig. 4). In other experiments, estrogen enhanced the formation of the 1941-bp P_3-P_4 chromatin fragment, which resulted in a corresponding increase in the amount of P_3-P_4 ligation products formed. Upon correction for the efficiency of the PCR amplification and the amount of substrate available for ligation, estrogen increased the ligation frequency two- to threefold compared to the control.
22. E. B. Crenshaw III, K. Kalla, D. M. Simmons, L. W. Swanson, M. G. Rosenfeld, *Genes Dev.* **3**, 959 (1989).
23. G. Felsenfeld and J. D. McGhee, *Cell* **44**, 375 (1986).
24. Pst I ligation fragments were amplified by PCR with primer (0.5 μM each) in 50 mM KCl, 10 mM tris-Cl (pH 8.3), 0.01% gelatin, 1.5 or 2.5 mM $MgCl_2$ (optimized for each primer set), and Taq polymerase (20 U/ml) (Boehringer Mannheim Biochemicals) in 100 μl of reaction volumes. Samples were preheated for 5 min at 95°C, placed on ice for 5 min, and then subjected to cycling conditions of 94°C for 60 s, 55°C for 30 s, and 72°C for 30 s for three cycles followed by 27 cycles of 94°C for 15 s, 55°C for 15 s, and 72°C for 15 s. A 25-μl aliquot of the PCR reaction was analyzed on a 1.2% agarose gel, blotted to nylon, and then hybridized with a specific ^{32}P-labeled DNA probe.
25. The authors would like to thank G. Mosig, W. LeStourgeon, R. Morse, and R. Simpson for critically reading this manuscript. Supported by grants from NIH [DK42731 (M.A.S.) and T32HD07048 (K.E.C.)] and the Vanderbilt University Research Council.

19 February 1993; accepted 13 May 1993

An NAD Derivative Produced During Transfer RNA Splicing: ADP-Ribose 1″-2″ Cyclic Phosphate

Gloria M. Culver, Stephen M. McCraith, Martin Zillmann, Ryszard Kierzek, Neil Michaud, Richard D. LaReau, Douglas H. Turner, Eric M. Phizicky*

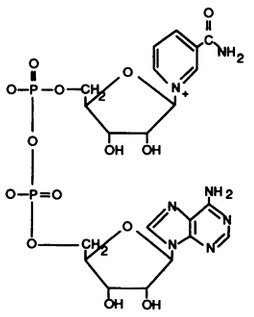
Transfer RNA (tRNA) splicing is essential in *Saccharomyces cerevisiae* as well as in humans, and many of its features are the same in both. In yeast, the final step of this process is removal of the 2′ phosphate generated at the splice junction during ligation. A nicotinamide adenine dinucleotide (NAD)–dependent phosphotransferase catalyzes removal of the 2′ phosphate and produces a small molecule. It is shown here that this small molecule is an NAD derivative: adenosine diphosphate (ADP)–ribose 1″-2″ cyclic phosphate. Evidence is also presented that this molecule is produced in *Xenopus laevis* oocytes as a result of dephosphorylation of ligated tRNA.

Intron-containing tRNA genes are ubiquitous in the nuclei of eukaryotes (*1*). Introns invariably occur one base 3′ of the anticodon, and intron removal is an essential event. Transfer RNA splicing is best understood in *S. cerevisiae*. Splicing there is initiated by an endonuclease that recognizes precursor tRNA (pre-tRNA) structure, measures the length of the anticodon stem to locate the intron, and excises the intron to generate two half-molecules (*2*). These half-molecules are substrates for tRNA ligase, which catalyzes four individual steps to restructure both ends of the half-molecules and then join them. Ligation generates a mature-length tRNA bearing a 2′ phosphate at the splice junction (*3*). An NAD-dependent phosphotransferase that catalyzes removal of the splice junction 2′ phosphate has been identified in yeast (*4*, *5*). This enzyme efficiently removes an internal 2′ phosphate from tRNA or from an oligonucleotide as small as a dimer; however, it will not detectably remove a terminal 5′, 3′, 2′, or 2′-3′ cyclic phosphate from an oligonucleotide (*4*, *6*). Dephosphorylation of ligated tRNA in vitro is accompanied by transfer of the 2′ phosphate to an acceptor molecule, which forms an unusual small molecule that we call the phosphotransfer product. We show here that the phosphotransfer product is an NAD derivative: ADP-ribose 1″-2″ cyclic phosphate (Fig. 1). This phosphotransfer product is also made in *Xenopus* oocytes, which indicates that dephosphorylation of ligated tRNA occurs by the same process in vivo.

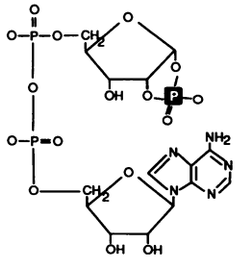
To elucidate the structure of the phosphotransfer product, we treated it with various enzymes. Phosphotransfer product bearing a radiolabeled transferred phosphate can be generated, after endonucleolytic cleavage, ligation, and phosphotransfer (*5*), from a pre-tRNA^{Phe} transcript labeled with [α-^{32}P]adenosine triphosphate (ATP). Surprisingly, the purified phosphotransfer product was resistant to hydrolysis with calf intestinal phosphatase (Fig. 2, lane 2) and bacterial alkaline phosphatase. This phosphatase resistance is highly unusual, as both phosphatases are nonspecific and would be expected to remove any O-linked phosphomonoester (*7*). Although the molecule was pyrophosphatase sensitive (Fig. 2, lane 3), subsequent treatment with

G. M. Culver, S. M. McCraith, R. D. LaReau, E. M. Phizicky, Department of Biochemistry, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Rochester, NY 14642.
M. Zillmann and N. Michaud, Department of Biology, University of Rochester, Rochester, NY 14627.
R. Kierzek, Institute of Bioorganic Chemistry, Polish Academy of Science, 60-704 Poznan, Noskowskiego 12/14, Poland.
D. H. Turner, Department of Chemistry, University of Rochester, Rochester, NY 14627.

*To whom correspondence should be addressed.



NAD
(AppN)



ADP-ribose
1"-2" cyclic phosphate
(Appr> P)

Fig. 1. Structures of NAD and ADP-ribose 1"-2" cyclic phosphate.

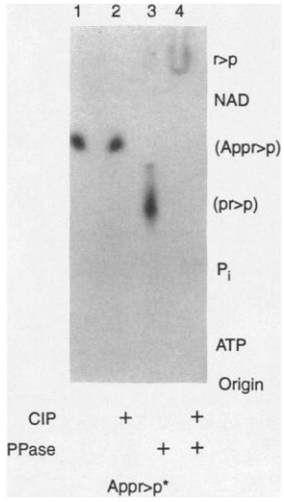


Fig. 2. Analysis of the phosphotransfer product bearing a labeled transferred phosphate. The phosphotransfer product was prepared from ligated tRNA as described (*5*), applied to PEI (polyethylenimine) cellulose thin layer chromatography plates, developed in 1.2 M $NaHCO_2$ (pH 3.5), eluted with 0.5 M NH_4CO_3 (pH 8.0), dried, and analyzed. Phosphatase reactions contained 300 mM NaCl, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, and 2.4 U of calf intestinal phosphatase and were incubated at 37°C for 30 min. Pyrophosphatase reactions contained 10 mM tris-HCl (pH 9.0), 14 mM $MgCl_2$, and 1.0 U of pyrophosphatase from *Crotalus adamanteus* venom (type II; Sigma) and were incubated at 37°C for 60 min. The phosphotransfer product was treated as indicated, applied to PEI plates, and chromatographed in 1.0 M LiCl. Lane 1, no treatment; lane 2, phosphatase treatment; lane 3, pyrophosphatase treatment; and lane 4, sequential treatment with pyrophosphatase and then with phosphatase. CIP, calf intestinal phosphatase; PPase, pyrophosphatase. The asterisk indicates the position of radiolabeled phosphate.

phosphatase did not release labeled inorganic phosphate (P_i) (Fig. 2, lane 4), which indicates that phosphatase resistance is not a result of steric blockage.

The phosphatase resistance of the phosphotransfer product is a result of the cyclization of the transferred phosphate. Sequential treatment of the phosphotransfer product with ribonuclease (RNase) T2, which is capable of resolving both cyclic phosphates and phosphodiesters into phosphomonoesters (*8*) (Fig. 3), and then with phosphatase resulted in the release of P_i. Furthermore, the phosphotransfer product could be regenerated by chemical cyclization of the purified RNase T2 digestion product (*9*). These results indicate that the phosphotransfer product bears a cyclic phosphate.

One possible role of NAD in the phosphotransfer reaction is that it acts as the phosphate acceptor (*5*). This hypothesis is consistent with the observation that the phosphotransfer product, like NAD, is sensitive to treatment with pyrophosphatase (Fig. 2, lane 3). We used [^{32}P-adenylate]NAD to demonstrate directly that NAD acts as the phosphate acceptor. In several chromatographic systems, phosphotransfer product made from [^{32}P-adenylate]NAD and unlabeled 2′ phosphate donor comigrated with the phosphotransfer product bearing a labeled transferred phosphate (*10*). Furthermore, the molecule made from labeled NAD was also phosphatase resistant and pyrophosphatase sensitive. Because pyrophosphatase treatment of this molecule released labeled adenosine monophosphate (AMP) rather than a phosphorylated derivative of AMP, this indicates that the phosphate is transferred to the original nicotinamide mononucleotide (NMN) moiety of NAD.

Two experiments indicate that nicotinamide is released as a consequence of the phosphotransfer reaction. First, purified phosphotransfer product, made from [^{32}P-adenylate]NAD (Fig. 3, lane 1), was treated with RNase T2 to open the cyclic phosphate (Fig. 3, lane 2) and then with phosphatase to remove it (Fig. 3, lane 3). A molecule that comigrated with ADP-ribose, but not with NAD, was generated by this treatment, which indicates that nicotinamide is not present. Second, [^{3}H]NAD, labeled in the nicotinamide ring (*11*), was used to show directly that nicotinamide is released as a consequence of the phosphotransfer reaction. Consistent with these results, a molecule that comigrated with ribose cyclic phosphate (r > p) was generated from phosphotransfer product bearing a labeled transferred phosphate after pyrophosphatase and phosphatase treatment (Fig. 2, lane 4) (*12*, *13*). These experiments indicate that the phosphotransfer product is ADP-ribose with a cyclic phosphate on the terminal ribose. Release of nicotinamide could supply the energy necessary for cyclization.

To determine the cyclic phosphate position, we used purified r > p generated from the phosphotransfer product (Fig. 2, lane 4). Cleavage of this molecule with a cyclic phosphodiesterase isolated from yeast (*14*) or a similar enzyme from wheat germ (*15*) resulted in the production of ribose-1-phosphate, as indicated by comigration with ribose-1-phosphate generated from nucleoside phosphorylase and radiolabeled P_i (*12*). This indicates that the 1" position of ADP-ribose is involved in the cyclic phosphate linkage. Treatment of r > p with acid produced a different molecule that was resistant to periodate treatment (*16*). Because periodate reacts with vicinal hydroxyl groups, ribose-2-phosphate is resistant to periodate, whereas ribose-1-phosphate and ribose-3-phosphate are sensitive to this treatment. Thus, the acid product is ribose-2-phosphate, and the structure of the phosphotransfer product is ADP-ribose 1"-2" cyclic phosphate (Appr > p) (Fig. 1).

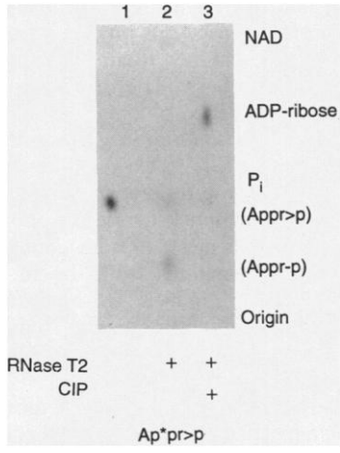


Fig. 3. Analysis of phosphotransfer product made from [^{32}P-adenylate]NAD. Phosphotransfer product was prepared and purified as described (Fig. 2), except a chemically synthesized 2′ phosphate donor (U_p^pU) (*26*) was used with 13.5 μCi of [^{32}P-adenylate]NAD (specific activity: 800 Ci per millimole of NAD). RNase T2 reactions contained 0.1 U of RNase T2 in 50 mM $NH_4CH_3CO_2$ (pH 5.2) and were incubated at 37°C for 60 min. Phosphotransfer product was treated as indicated, applied to PEI plates and developed in 1.2 M $NaHCO_2$ (pH 3.5). Lane 1, no treatment; lane 2, RNase T2 treatment; and lane 3, sequential treatment with RNase T2 and then with phosphatase. Abbreviations are as in Fig. 2.

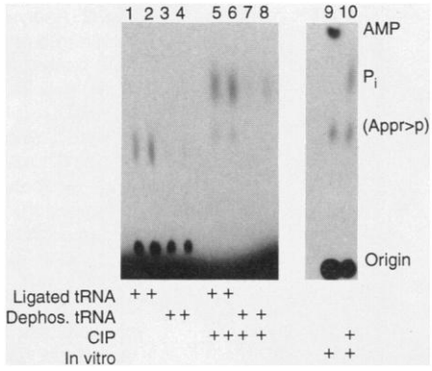


Fig. 4. Fate of the transferred phosphate in microinjected *Xenopus* oocytes. Samples (50 nl) of either ligated tRNA or dephosphorylated ligated tRNA, prepared from [α-^{32}P]ATP–labeled pre-tRNA^{Phe} transcript (2.5×10^{-14} to 5.0×10^{-14} mol; 3140 Ci per millimole of tRNA), were microinjected into *Xenopus* oocytes (*27*). Five samples of each were incubated, frozen in liquid nitrogen, thawed, homogenized in H_2O, phenol extracted, and ethanol precipitated. Supernatants (small molecules) were treated as indicated and chromatographed as described (Fig. 3). Lanes 1 and 2, ligated tRNA in 15-min and 30-min incubations, respectively. Lanes 3 and 4, pre-dephosphorylated tRNA in 15-min and 30-min incubations, respectively. Lanes 5 through 8, phosphatase treatment of material in lanes 1 through 4, respectively. Lane 9, phosphotransfer product produced in vitro. Lane 10, phosphatase treatment of material in lane 9. Dephos. tRNA, dephosphorylated tRNA.

Because Appr > p is an unexpected product of dephosphorylation of ligated tRNA, *Xenopus* oocytes were used to determine if this same molecule is produced in vivo. Zillmann and co-workers (*6*, *17*) observed that all of the yeastlike tRNA splicing enzymes are conserved in HeLa cell extracts and that *Xenopus* extracts have an activity that dephosphorylates tRNA in an NAD-dependent manner. We therefore microinjected ligated tRNA with a labeled 2′-phosphate into *Xenopus* oocytes and monitored the fate of the splice junction 2′ phosphate. Concomitant with 2′ phosphate removal, material that was phosphatase resistant and that comigrated with the phosphotransfer product was produced from ligated tRNA and not from dephosphorylated tRNA (Fig. 4). The in vivo product comigrated in three chromatographic systems with phosphotransfer product produced in vitro (*10*). As expected, treatment of the phosphotransfer product generated in oocytes with pyrophosphatase and phosphatase produced a molecule that comigrated with r > p generated from the in vitro phosphotransfer product. These results imply that the phosphotransferase studied in vitro is the cellular enzyme responsible for the final step of tRNA splicing.

On the basis of the known chemistry of NAD, a mechanism for production of Appr > p can be envisioned. Transfer of the phosphate from the 2′ position of tRNA to the 2″ position on the ribose of the NMN moiety could be the first step. This would be followed by cyclization at the 1″ position with concomitant release of nicotinamide. The first step is consistent with the specificity of the phosphotransferase for 2′ phosphates. The second step of this proposed mechanism requires a phosphate oxygen to act as a nucleophile, similar to the way in which the phosphate oxygen of P_i acts in the nucleoside phosphorylase reaction to attack the 1′ position of inosine and release hypoxanthine (*12*). The proposed displacement of nicotinamide from NAD is similar to that observed in ADP-ribosylation (*18*), where a nucleophilic residue of a protein attacks this bond, and in cyclic ADP-ribose formation (*19*), where the N-6 amino group of adenine attacks this bond.

The splicing of tRNA in yeast is complex, involving a minimum of seven steps and requiring at least three nucleotide cofactors—guanosine triphosphate, ATP, and NAD (*3*, *5*). The result of splicing is a mature-length tRNA and the cellular molecule ADP-ribose 1″-2″ cyclic phosphate, described here. On the basis of the number of intron-containing tRNA molecules in yeast, the steady-state concentration of the phosphotransfer product would be of the order of 10 μM in the absence of turnover (*1*, *20*). A minimum of two steps are required to return Appr > p to known metabolic pathways, one involving opening of the cyclic phosphate. There is evidence for a specific cyclic phosphodiesterase responsible for opening the phosphotransfer product to its 1″ phosphate derivative in yeast extracts (*14*). This complexity of tRNA splicing in yeast is striking compared to the much simpler splicing mechanism that coexists with this pathway in some higher eukaryotes (*6*, *17*, *21*). HeLa cells contain both a yeastlike ligation-dephosphorylation system and a simple ligation pathway that bypasses the need for 2′ phosphate removal because the ligase acts with different chemistry. Presumably, both ligase pathways have a cellular function. This redundancy has fueled speculation that the yeastlike pathway is required to produce Appr > p so that it can function in some other cellular capacity.

REFERENCES AND NOTES

1. M. R. Culbertson and M. Winey, *Yeast* **5**, 405 (1989); H. van Tol and H. Beier, *Nucleic Acids Res.* **16**, 1951 (1988); A. K. Hopper and N. C. Martin, in *Molecular and Cellular Biology of the Yeast Saccharomyces: Gene Expression*, E. W. Jones, J. R. Pringle, J. R. Broach, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1992), chap. 3, pp. 99–141; E. M. Phizicky and C. L. Greer, *Trends Biochem. Sci.* **18**, 31 (1993).
2. C. L. Peebles, R. Gegenheimer, J. Abelson, *Cell* **32**, 525 (1983); V. M. Reyes and J. Abelson, *ibid.* **55**, 719 (1988); E. Szekely, H. G. Belford, C. L. Greer, *J. Biol. Chem.* **263**, 13839 (1988); M. I. Baldi, E. Mattoccia, E. Burfardeci, S. Fabbri, G. P. Tocchini-Valentini, *Science* **255**, 1404 (1992).
3. G. Knapp, R. C. Ogden, C. L. Peebles, J. Abelson, *Cell* **18**, 37 (1979); C. L. Greer C. L. Peebles, R. Gegenheimer, J. Abelson, *ibid.* **32**, 537 (1983); E. M. Phizicky, R. C. Schwartz, J. Abelson, *J. Biol. Chem.* **261**, 2978 (1986); E. M. Phizicky, S. A. Consaul, K. W. Nehrke, J. Abelson, *ibid.* **267**, 4597 (1992); S. K. Westaway, H. G. Belford, B. L. Apostol, J. Abelson, C. L. Greer, *ibid.* **268**, 2435 (1993).
4. S. M. McCraith and E. M. Phizicky, *Mol. Cell. Biol.* **10**, 1049 (1990).
5. ———, *J. Biol. Chem.* **266**, 11986 (1991).
6. M. Zillmann, M. A. Gorovsky, E. M. Phizicky, *ibid.* **267**, 10289 (1992).
7. T. W. Reed and I. B. Wilson, in *The Enzymes*, B. D. Boyer, Ed. (Academic Press, New York, 1971), vol. 4, chap. 17; H. N. Fernley, *ibid.*, chap. 18.
8. G. W. Rushiky and H. A. Sober, *J. Biol. Chem.* **238**, 371 (1963); T. Uchida and F. Egami, *J. Biochem.* **61**, 44 (1967).
9. Purified RNase T2 product (Fig. 3) was treated with 30 mM carbodiimide [1 = ethyl-3(3-dimethyl-aminopropyl)-carbodiimide-HCl] in 10 μl of reaction buffer with 100 mM MES [2-(*N*-morpholino)-ethane sulfonic acid] (pH 5.5) at 37°C for 60 min (*13*).
10. The comigration of small molecules is based on the behavior in some combination of the following systems: (i) one of four concentrations of LiCl (0.2, 0.5, 0.75, and 1.0 M) (*22*); (ii) 1.2 M $NaHCO_2$ (pH 3.5) (*23*); and (iii) 0.75 M Tris and 0.4 M HCl (*24*).
11. J. W. Little, *Anal. Biochem.* **48**, 217 (1972).
12. O. H. Lowry and J. A. Lopez, *J. Biol. Chem.* **162**, 421 (1946); H. M. Kalckar, *ibid.* **167**, 477 (1947).
13. D. C. Sogin, *J. Neurochem.* **27**, 1333 (1976).
14. G. M. Culver, S. A. Consaul, W. Filipowicz, E. M. Phizicky, unpublished results.
15. K. Tyc, C. Kellenberger, W. Filipowicz, *J. Biol. Chem.* **262**, 12994 (1987).
16. We treated r > p with 160 mM HCl in 10 μl of reaction buffer at 45°C for 30 min and neutralized it before analysis. Periodate treatment was done as described (*25*).
17. M. Zillmann, M. A. Gorovsky, E. M. Phizicky, *Mol. Cell. Biol.* **11**, 5410 (1991).
18. R. J. Collier, in *ADP-Ribosylating Toxins and G Proteins: Insights into Signal Transduction*, J. Moss and M. Vaughan, Eds. (American Society for Microbiology, Washington, DC, 1990), chap. 1.
19. N. Rusinko and H. C. Lee, *J. Biol. Chem.* **264**, 11725 (1989); H. C. Lee, T. F. Walseth, G. T. Bratt, R. N. Hayes, D. L. Clapper, *ibid.*, p. 1608.
20. C. Waldron and F. Lacroute, *J. Bacteriol.* **122**, 855 (1975).
21. W. Filipowicz and A. J. Shatkin, *Cell* **32**, 547 (1983); F. A. Laski, A. Z. Fire, U. L. RajBhandary, P. A. Sharp, *J. Biol. Chem.* **258**, 11974 (1983).
22. K. Randerath and E. Randerath, *Methods Enzymol.* **12A**, 323 (1967).
23. R. A. Barton, A. Schulman, E. L. Jacobson, M. K. Jacobson, *J. Chromatogr.* **130**, 145 (1977).
24. B. R. Bochner and B. N. Ames, *J. Biol. Chem.* **257**, 9759 (1982).
25. H. C. Neu and L. A. Heppell, *ibid.* **239**, 2927 (1964).
26. R. Kierzek, G. M. Culver, E. M. Phizicky, D. H. Turner, unpublished results.
27. N. Michaud and D. S. Goldfarb, *J. Cell Biol.* **112**, 215 (1991).

28. We thank M. Bruist, W. Filipowicz, L. Tabak, and O. Uhlenbeck for advice; B. Bambara, S. Butler, B. Grayhack, T. Platt, and L. Tabak for comments on the manuscript; and D. Goldfarb for microinjection facilities. Supported by NSF grant MCB-9118571 (E.M.P.), Oral Cellular and Molecular Biology Training grant DE07202-02 (G.M.C.), Public Health Service grants GM 22939 (to D.H.T.) and GM 25349 (to A. Senior for support of R.D.L.).

16 February 1993; accepted 17 May 1993