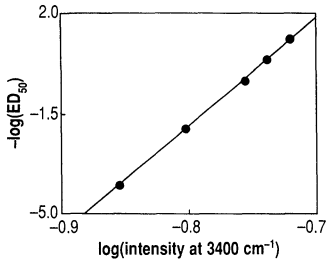

Fig. 4. Linear relation between the logarithm of the O–H stretching intensity in the alkanol-DPPC complex at 3400 cm^{-1} and the negative logarithm of the brine shrimp ED_{50}. Brine shrimps, *Artemia salina*, were captured at the Great Salt Lake in Utah. The brine shrimps' motions were monitored in a 5 by 4 inch shallow glass container by a video camera, interfaced with a digital video adapter XV-D300 (Sony) and an A-D converter, and recorded on a floppy disk of an NEC PC-9801 computer. Their position in the *x-y* plane was digitized every 0.5 s, and 50 data points were analyzed for each scan. The alkanol concentration that reduced shrimp movement to 50% of the control without alkanols was interpolated and designated as ED_{50}. Long-chain alkanols were dissolved in a small amount of ethanol and added to the aqueous phase. The solvent ethanol concentration was well below the anesthetizing concentration.

thetic actions to the conformational change of the lipid tails, hence, the membrane core property. Nevertheless, interfacial hydrogen bond–breaking action of anesthetics is shown in various systems. Release of surface-bound water by anesthetics has been shown in proteins, nonionic and ionic surfactant micelles, phospholipid vesicles, and multilamellar phospholipid suspensions (*8*). Because of the high polarizability, apolar anesthetics, such as cyclopropane, are also shown to break hydrogen bonds (*7*). The increased hydrophobicity of the interface caused by dehydration, together with the conformational relaxation of membranes and proteins, appears to be the cause of anesthesia. The precise mechanism of how these changes induce the state of unconsciousness remains to be elucidated.

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A Mn^{2+}-Dependent Ribozyme

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An RNA hairpin identical in sequence with the one formed during autocyclization of the 414-nucleotide *Tetrahymena* intervening sequence undergoes strand scission at a specific site in the presence of Mn^{2+}. In addition to representing one of the smallest and simplest ribozymes possible, strand scission occurs readily under physiological conditions, is unaffected by the presence of Mg^{2+}, and displays salt, *p*H, and temperature optima of potential use in exploiting Mn^{2+} as a regulatory switch in intact cells. The chemistry of strand scission of the RNA hairpin is described, as is the Mn^{2+}-dependent solvolysis of a 231-nucleotide RNA transcript containing this structural motif.

A NUMBER OF RNA CATALYSTS HAVE been discovered in recent years; perhaps the best known is the group I ribosomal RNA intron from *Tetrahymena*, which mediates the formation of mature RNA by self-catalyzed processing of the initial RNA transcript (*1*). Cleavage and ligation involve Mg^{2+}-dependent transesterification with nucleophilic attack at the phosphodiester bond by an external (*2*) or internal (*3*) guanine nucleotide 3′-OH group, with concomitant release of an RNA strand possessing a terminal 3′-OH group. Viroid, virusoid, and satellite RNAs from a number of sources (*4, 5*) and human hepatitis delta virus (*6*) also undergo self-catalyzed cleavage in site-specific, Mg^{2+}-dependent processes. Cleavage of the phosphodiester bond occurs by nucleophilic attack of the adjacent 2′-OH group. The products, which include an oligonucleotide 2′,3′-cyclic phosphate and a 3′-fragment having a 5′-OH terminus, are identical with those formed when RNA strand scission is mediated by alkaline hydrolysis, aqueous Pb^{2+} or Zn^{2+}, or some ribonucleases (*7, 8*). Relatively small oligonucleotides have been made that mimic the catalytic behavior of the plant RNAs (*9*); a few of these have been designed to effect sequence-specific cleavage of target RNAs (*9, 10*).

Our interest in defining novel chemical strategies for polynucleotide strand scission prompted us to focus on the role of Mg^{2+} in RNA processing and on the possible involvement of other metals. The foregoing processes all require Mg^{2+}, and proposed reaction intermediates (*11–13*) suggest its direct participation in processes such as (i) activation of phosphorus toward nucleophilic attack, (ii) correct spatial ordering of reaction components, and (iii) general acid-base catalysis. Polynucleotides can bind Mn^{2+} (*14*), and Mn^{2+} may sometimes substitute for Mg^{2+} in RNA processing systems (*8, 9, 13*), consistent with its similar ionic radius and divalent charge. However, unlike Mg^{2+}, which binds to RNA through phosphate oxygens, Mn^{2+} can form complexes involving both the phosphate oxygens and RNA bases (*15*).

Because RNA structure per se can contribute to the feasibility of processing, we sought to identify a structural element that might be stabilized by Mn^{2+} binding or hydrolysis. In this context, the 15-nucleotide (nt) hairpin excised from the 5′ end of the 414-nt *Tetrahymena* intron during autocyclization (*1*) was of special interest. In addition to its formation by Mg^{2+}-catalyzed phosphoryl transfer at the 3′ end (*1*), it was anticipated (*16*) that the hairpin would have

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rather modest stability, suggesting the possibility of additional stabilization through appropriate metal ion binding. As noted, Mn^{2+} might impact stabilization by binding an RNA base in addition to a phosphate ester (*15*). We report that this RNA hairpin undergoes facile cleavage in the presence of Mn^{2+}, and that strand scission is Mn^{2+}-dependent.

Two RNA transcripts were studied initially. One of these, 31 nucleotides in length, included a sequence (nucleotides 13 to 28) identical with the first 16 nucleotides of the 414-nt *Tetrahymena* intervening sequence (*1*). The other substrate, transcribed from the same expression plasmid after linearization with a different restriction endonuclease, included an additional 16 nucleotides capable of forming a second hairpin of identical size but greater predicted (*16*) stability. The generally labeled 47- and 31-nt RNA transcripts (*17*) (Fig. 1) were incubated in 10 m*M* $MnCl_2$ (tris-acetate buffer, *p*H 7.5). After 2 hours in the presence of Mn^{2+}, the 47-nt RNA substrate underwent strand scission to give two new bands corresponding to 34- and 13-nt products. Analogous incubation of the shorter RNA substrate (lanes 1 and 2) provided 18- and 13-nt products when Mn^{2+} was present (*18, 19*). Since the first 31 nucleotides in the two RNA substrates were identical, strand scission of each had probably occurred at a common site 13 nucleotides from the 5′ end, as was established by sequence analysis of the position of cleavage (see below) and by demonstrating that when the same RNA transcripts were 5′ end–labeled, the sole product observable by autoradiography was 13 nucleotides in length.

The remarkable selectivity of cleavage and the location of the cleavage site at the base of a hairpin suggest that the key recognition element is the hairpin structure. The lack of cleavage of the second hairpin in the 47-nt transcript demonstrates that not all hairpins of this size are cleaved by Mn^{2+}. Further, the lack of effect on cleavage of the additional nucleotides in the 47-nt substrate suggests that secondary structure distant from the actual site of cleavage does not participate in the strand scission process. Additionally, a DNA oligonucleotide containing the same sequence as the substrate RNAs was not hydrolyzed by Mn^{2+} (*20*), and the addition of 5*M* urea to the RNA strand scission reaction (to denature the putative hairpin) largely suppressed RNA strand scission by Mn^{2+}.

The sizes of the RNA cleavage products were estimated initially by simple comparison with comigrating polynucleotides of known lengths. To establish definitively the position of cleavage, we treated 5′-^{32}P end-labeled 31-nt substrate with $MnCl_2$ and analyzed the migration of the radiolabeled product in comparison with other samples of the same substrate treated with alkali or subjected to partial digestion with a series of base-specific ribonucleases (*21*). The 5′-^{32}P end-labeled fragment comigrated with a band producted by ribonuclease T_1 (RNase T_1, G-specific) cleavage of the same substrate (Fig. 2), implying that G was the 3′-terminal nucleotide. Comparison with the primary sequence of the substrate RNA implies that the product (lane 2) is GAAUACAAGCUUG>p. The presence of a 2′3′-cyclic phosphate at the 3′-terminus of the product was inferred from its comigration with the bands resulting from digestion of the same substrate with alkali and RNase T_1 (*22*). Thus Mn^{2+}-promoted strand scission of the RNA hairpin seems to involve nucleophilic attack of the 2′-OH group of guanosine$_{13}$ on the phosphodiester bond connecting G_{13} and A_{14}, affording a 2′,3′-cyclic phosphate at the 3′-terminus of the 13-nt product and a free 5′-OH at the 5′-terminus of the 18- or 34-nt product (compare Figs. 1 and 2).

A plausible mechanism for the strand scission reaction is shown in Fig. 3 (*23*); this posits the formation of a Mn^{2+} complex with N-7 of A_{28} (*24*). As shown, strand scission results from Mn^{2+}-hydroxide promoted deprotonation of the 2′-OH group on G_{13}. In support of the role of nucleoside 28 in RNA cleavage, it was found in preliminary experiments that a 31-nt RNA sub-

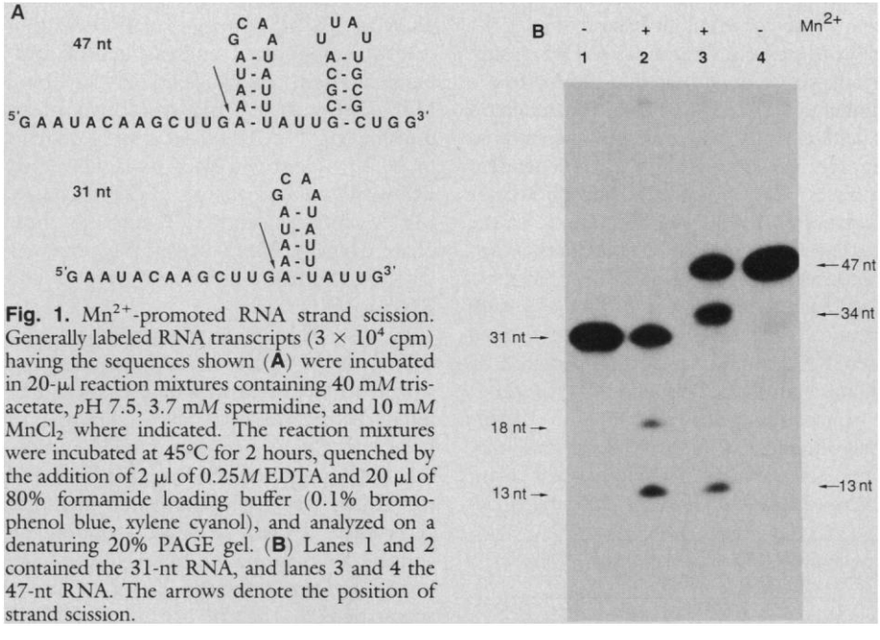

Fig. 1. Mn^{2+}-promoted RNA strand scission. Generally labeled RNA transcripts (3×10^4 cpm) having the sequences shown (**A**) were incubated in 20-μl reaction mixtures containing 40 m*M* tris-acetate, *p*H 7.5, 3.7 m*M* spermidine, and 10 m*M* $MnCl_2$ where indicated. The reaction mixtures were incubated at 45°C for 2 hours, quenched by the addition of 2 μl of 0.25*M* EDTA and 20 μl of 80% formamide loading buffer (0.1% bromophenol blue, xylene cyanol), and analyzed on a denaturing 20% PAGE gel. (**B**) Lanes 1 and 2 contained the 31-nt RNA, and lanes 3 and 4 the 47-nt RNA. The arrows denote the position of strand scission.

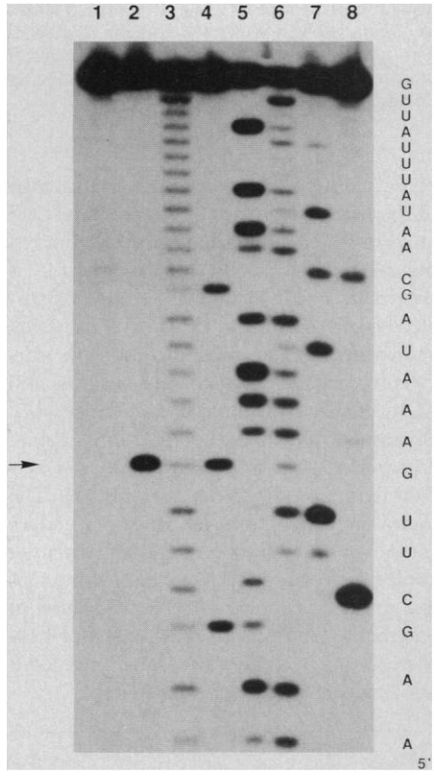

Fig. 2. Analysis of the position of RNA strand scission. The 31-nt RNA transcript (5.5×10^5 cpm) was incubated in 10 μl of 40 m*M* tris-acetate, *p*H 7.5, containing 100 m*M* NaCl and 3.7 m*M* spermidine, with (lane 2) or without (lane 1) 10 m*M* $MnCl_2$. The reactions were incubated at 45°C for 30 min, quenched by the addition of loading buffer (10*M* urea, 1.5 m*M* EDTA, 0.05% bromophenol blue, xylene cyanol), heated to 90°C for 1 min, and quick-chilled before application to a 20% PAGE sequencing gel. Nucleotide sequence analysis was determined by partial digestion with base-specific ribonucleases (*21*): lane 3, alkaline hydrolysis; lane 4, RNase T_1 (G); lane 5, RNase U_2 (A > G); lane 6, RNase Phy M (U + A); lane 7, RNase Bc (U + C); and lane 8, RNase CL3 (C).

strate differing only in that it contained uridine in position 28 failed to undergo detectable Mn^{2+}-catalyzed strand scission. It may be noted that the orientations of A_{28} and the reactive OH group attached to Mn^{2+} in Fig 3 are arbitrary; space-filling models indicate that catalysis could also occur with adenosine$_{28}$ in the *anti*-configuration coordinated to Mn^{2+} having the reactive OH as an axial ligand. The possible participation of exocyclic amino groups of the RNA bases in Mn^{2+} coordination at position 28 was underscored by the finding that 31-nt transcripts containing guanosine and cytidine at position 28 also underwent Mn^{2+}-promoted cleavage at G_{13}, albeit not as efficiently as the substrate containing A_{28}.

RNA strand scission was detectable at Mn^{2+} concentrations as low as 0.25 m*M*; cleavage was optimal at 5 to 10 m*M* Mn^{2+}. Strand scission was measured over the *p*H range 6.6 to 8.4. No hydrolysis could be detected at *p*H 6.6 within 2 hours, but the extent of strand scission increased steadily with increasing *p*H and became maximal near physiological *p*H. The formation of Mn^{2+}-dependent cleavage products was complete within 2 hours at *p*H 7.5. At *p*H 8.1, additional bands characteristic of nonspecific cleavage were apparent, and at *p*H 8.4 RNA degradation was completely nonspecific, even at shorter (10-min) incubation times (*25*). When RNA strand scission was carried out at *p*H 7.8 at each of several temperatures, the extent of cleavage increased with increasing temperature, but the positional specificity of strand scission was optimal only up to 45°C and diminished dramatically above that temperature. Even at 59°C, much shorter (10-min) incubations yielded the initial formation of two products 18 and 13 nucleotides in length. Strand scission diminished steadily with decreasing temperature and was not detectable below 23°C.

The facility of strand scission actually increased with increasing NaCl concentration, as might be expected from the lessening of charge repulsion associated with the phosphodiester moieties, and was unaffected by added Mg^{2+} (up to 25 m*M*), indicating that Mg^{2+} will not compete with Mn^{2+} for the essential Mn^{2+}-RNA binding sites. When strand scission was carried out at *p*H 7.5 and 45°C with the 31-nt RNA as a substrate, the expected 13- and 18-nt products appeared to an increasing extent as a function of time (half-time, $t_{1/2} \sim 25$ min) and constituted the only significant products formed within 2 hours. Thus the rate of strand scission was well within the range noted for other catalytic RNAs (*1–11*).

Many features of the Mn^{2+}-catalyzed strand scission reaction described above suggest its potential use in experimental systems that model regulation of gene expression at the level of transcriptional control, such as by controlling the rate of destruction of specific (messenger) RNAs as they are transcribed. Although detailed studies of the strand scission of larger RNA transcripts have yet to be carried out, we have investigated the Mn^{2+}-promoted strand scission of a 5′-^{32}P end-labeled 231-nt RNA transcript containing the same sequence at the 5′ end as the two transcripts described above. Treatment of this transcript produced a band that comigrated with the 13-nt fragment (Fig. 4).

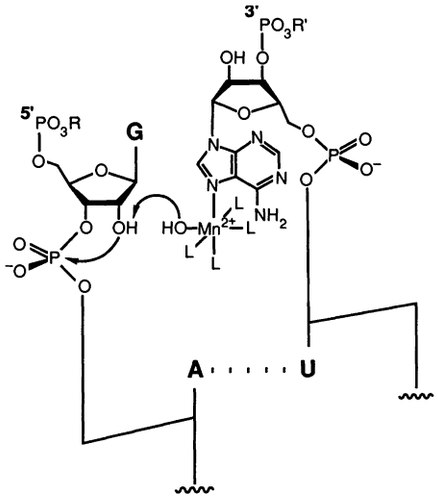

Fig. 3. A possible molecular mechanism for Mn^{2+}-mediated scission of the RNA hairpins.

We note that Mn^{2+} is concentrated from culture media by many growing cells (*26*) and can be well tolerated by intact cells when Mg^{2+} is also present (*26–28*). Although mutagenic at high concentrations (*28*), Mn^{2+} is actually required for cell growth and performs a number of essential biochemical functions (*29*). Thus the Mn^{2+}-specific transformation described here could in principle be used in intact cells to control the concentrations of specific RNA substrates.

Of the RNA strand scission reactions characterized to date, the present system most nearly resembles that of the viroid and satellite RNAs from the perspectives of reaction conditions required (temperature, *p*H, and metal ion requirement) and chemical products formed. However, unlike those plant RNAs, which require relatively complex secondary structures to achieve hydrolytic cleavage, the present system requires only a single RNA hairpin and uses a metal ion whose presently known physiological functions (*29*) are more limited than those of Mg^{2+}. The simplicity of the structural motif described here and the promotion of strand scission by a metal ion compatible with function in intact biological systems provide the motivation for more precise definition of the structural requirements that promote strand scission and the extent to which this transformation can obtain when the same motif is present within larger (messenger) RNA transcripts. The system described here potentially provides a simple chemical switch that may be used as an exogenous agent to model regulation of gene expression at the level of transcriptional control.

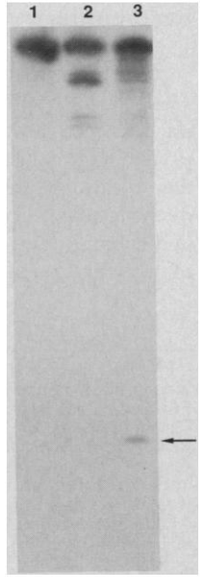

Fig. 4. Sequence-selective cleavage of a 231-nt RNA transcript containing the same sequence at the 5′-end as the 31- and 47-nt RNAs. Reactions (lanes 2 and 3) were carried out in 40 m*M* tris-acetate, *p*H 7.5, containing 100 m*M* NaCl, 3.7 m*M* spermidine, 5′-^{32}P end-labeled RNA (3×10^5 cpm) and 10 m*M* $MnCl_2$ (lane 3 only). The reactions were incubated at 45°C for 1 hour and then analyzed on a polyacrylamide gel as indicated in the legend to Fig. 1. Lane 1 contained an untreated 231-nt RNA transcript that was analyzed by PAGE without incubation. The arrow indicates the position of migration of the 13-nt fragment obtained by Mn^{2+}-promoted cleavage of the 31-nt RNA substrate.

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17. Generally labeled RNA transcripts 47 and 31 nucleotides in length were transcribed from a pSP64 plasmid containing the required sequence inserted between the Hind III and Eco RI sites; transcription was effected with SP6 RNA polymerase + [α-^{32}P]CTP (cytidine 5′-triphosphate) following plasmid linearization with Hha I or Bal I, respectively. The RNAs were purified by 10% polyacrylamide gel electrophoresis (PAGE; 7*M* urea, 90 m*M* tris-borate, *p*H 8.0, with 2 m*M* EDTA). The 5′ end–labeled transcripts were prepared by successive treatments of the unlabeled transcripts with calf intestinal phosphatase, and then T_4 polynucleotide kinase + [γ-^{32}P]ATP (adenosine triphosphate). Purification was effected by 20% PAGE.
18. The RNA transcripts were not processed by Mg^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Ba^{2+}, or Ca^{2+}. Incubation in the presence of Fe^{2+}, Zn^{2+}, or Sm^{2+} resulted in nonspecific degradation. Although initial experiments suggested that Mn^{2+}-dependent RNA strand scission was facilitated by spermidine, more recent experiments have indicated that no spermidine is required. As anticipated, 25 m*M* EDTA completely inhibited RNA processing.
19. That RNA strand scission was not the result of some protein contaminant having ribonuclease activity was shown by (i) demonstration that strand scission of the hairpin was insensitive to proteinase K and (ii) the finding that a chemically synthesized 31-nt RNA substrate identical in sequence with the RNA derived by transcription also underwent Mn^{2+}-dependent strand scission.
20. See also J. J. Butzow and G. Eichhorn, *Nature* **254**, 358 (1975).
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22. The chemical nature of Mn^{2+}-promoted strand scission was also studied by degradation of the derived products. The 31-nt RNA substrate prepared by transcription in the presence of [α-^{32}P]ATP was treated with Mn^{2+}, and the 13-nt product was isolated following PAGE separation. Digestion of the product with nuclease P_1 afforded a mixture of mononucleotides, which was analyzed by polyethyleneimine chromatography in comparison with authentic standards and shown to contain a strong band for [^{32}P]5′-AMP (adenosine 5′-monophosphate) and a weaker band corresponding to [^{32}P]pG>p, as predicted. The formation of radiolabeled pG>p reinforced the inference (Fig. 2) that the 3′-terminus of the 13-nt product contained a 2′,3′-cyclic phosphate. Furthermore, since the source of radiolabel in the 31-nt substrate was [α-^{32}P]ATP, the labeled phosphate in pG>p must have derived from an adjacent adenine nucleotide which, after hydrolytic cleavage from the labeled phosphate, formed the 5′ end of the 18-nt product (compare with Fig. 1). In a parallel experiment the 18-nt product, derived from a substrate that had not been radiolabeled, was treated successively with T_4 polynucleotide kinase + [γ-^{32}P]ATP and then with nuclease P_1. As anticipated, the sole radiolabeled product detectable by thin-layer chromatographic analysis was [^{32}P]5′-AMP, verifying that adenosine (containing a free 5′-OH group) was the nucleotide at the 5′ end of the 18-nt product.
23. In the context of this proposed mechanism, we note that Mn^{2+}-supported proton dissociation from a β-hydroxyethyliminodiacetic acid chelate at a pK_a value 2.2 units lower than that of the respective Mg^{2+} chelate [S. Chaberek, Jr., R. C. Courtney, A. E. Martell, *J. Am. Chem. Soc.* **74**, 5057 (1952)], which provides a further basis for the observed metal specificity of RNA strand scission.
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25. Although the reason for nonspecific degradation was not investigated, on the basis of observations in other systems it could well reflect formation of a reactive Mn^{2+}-hydroxide complex at the higher *p*Hs [see, for example, M. I. Page, in *The Chemistry of Enzyme Action*, M. I. Page, Ed. (Elsevier, Amsterdam, 1984), pp. 243–246, and references therein] or the oxidation of Mn(II) in the presence of tris-buffer with concomitant reduction of dioxygen [W. D. Hobey and J. A. Prybyla, *Clin. Chem.* **24**, 2206 (1978)]. Site-specific cleavage occurred at *p*H 7.5 even when the reaction was run under argon in the absence of O_2.
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Expression of a Zinc Finger Gene in HTLV-I– and HTLV-II–Transformed Cells

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Gene products encoded by the human T cell leukemia virus (HTLV) types I and II mediate transformation by the transactivation of cellular genes necessary for proliferation, probably including transcriptional regulatory factors. By searching for factors that may control proliferation, a zinc finger gene (225) was identified that was constitutively expressed in all HTLV-I– or HTLV-II–transformed cell lines examined, whereas in normal T cells it was only transiently expressed after mitogenic stimulation. The 225 gene was also constitutively expressed in two HTLV-I–transformed helper T cell clones, but not in the parental cell lines. Thus this putative cellular transcriptional factor, which was abnormally expressed in retrovirus-infected cells, may have a role in transformation.

ACTIVATION OF RESTING PERIPHERAL T lymphocytes by antigen or mitogen initiates a cascade of sequential and interregulated transcriptional events that ultimately result in DNA synthesis, lymphocyte proliferation, and differentiated immunologic function (*1*). The regulation of this activation program and the identification of genetically responsive genes that are essential to the proliferative response has been approached in our laboratory by cloning inducible genes from a cDNA library generated during the early stages of T lymphocyte activation. More than 60 distinct gene transcripts representing genes activated during the transition of quiescent (G_0) cells through the early prereplicative (G_1) stage of the cell cycle were identified. These transcripts presumably include transcriptional regulatory molecules that control the initial stages of the response to cellular activating agents (*2*). Some of the early regulatory genes involved in mitogen or antigen-induced T cell activation and division may also participate in the initiation and maintenance of cell proliferation after transformation of T lymphocytes by HTLV-I and HTLV-II. These lymphotropic retroviruses are associated with specific T cell malignancy;

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