eral, it seems reasonable to expect that the members of such large families of DNA-binding proteins may have several conserved contacts with the DNA backbone, and that closely related members may also have conserved contacts with one or more bases.

REFERENCES AND NOTES

1. M. Ptashne, *A Genetic Switch* (Cell Press, Cambridge, MA, 1986).
2. S. R. Jordan and C. O. Pabo, *Science* **242**, 893 (1988).
3. J. E. Anderson, M. Ptashne, S. C. Harrison, *Nature* **326**, 846 (1987).
4. A. K. Aggarwal, D. W. Rodgers, M. Drottar, M. Ptashne, S. C. Harrison, *Science* **242**, 899 (1988).
5. R. T. Sauer, R. R. Yocum, R. F. Doolittle, M. Lewis, C. O. Pabo, *Nature* **298**, 447 (1982).
6. For the data from 8.0 to 2.5 Å, the *R* factor is 0.204 for the λ complex and 0.179 for the 434 complex.
7. T. A. Steitz, D. H. Ohlendorf, D. B. McKay, W. F. Anderson, B. W. Matthews, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3097 (1982).
8. D. H. Ohlendorf, W. F. Anderson, M. Lewis, C. O. Pabo, B. W. Matthews, *J. Mol. Biol.* **169**, 757 (1983).
9. A. Mondragon, S. Subbiah, S. C. Almo, M. Drottar, S. C. Harrison, *ibid.* **205**, 189 (1989).
10. C. O. Pabo and R. T. Sauer, *Annu. Rev. Biochem.* **53**, 293 (1984).
11. J. S. Richardson and D. C. Richardson, *Science* **240**, 1648 (1988).
12. W. G. J. Hol, P. T. van Duijnen, H. J. C. Berendsen, *Nature* **273**, 443 (1978).
13. N. C. Seeman, J. M. Rosenberg, A. Rich, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 804 (1976).
14. A. Mondragon and S. C. Harrison, unpublished results.
15. D. H. Ohlendorf, W. F. Anderson, R. G. Fisher, Y. Takeda, B. W. Matthews, *Nature* **298**, 718 (1982).
16. H. C. M. Nelson and R. T. Sauer, *J. Mol. Biol.* **192**, 27 (1986).
17. A. Johnson, thesis, Harvard University, Cambridge, MA (1980).
18. R. Bushman, J. E. Anderson, S. C. Harrison, M. Ptashne, *Nature* **316**, 651 (1985).
19. Z. Otwinowski *et al.*, *ibid.* **335**, 321 (1988).
20. C. Wolberger, Y. Dong, M. Ptashne, S. C. Harrison, *ibid.*, p. 789.
21. Y. Q. Qian *et al.*, *Cell* **59**, 573 (1989).
22. A. Laughon and M. P. Scott, *Nature* **310**, 25 (1984).
23. J. C. W. Shepherd, W. McGinnis, A. E. Carrasco, E. M. DeRobertis, W. J. Gehring, *ibid.*, p. 70.
24. M. P. Scott, J. W. Tamkun, G. W. Hartzell, *BBA Rev. Cancer* **989**, 25 (1989).

25. Supported by the Howard Hughes Medical Institute and by grants from the NIH (GM 31471 to C.O.P.; GM 29109 to S.C.H. and M. Ptashne). We thank R. T. Sauer for helpful discussions and thank K. Kolish for help preparing this manuscript.

22 September 1989; accepted 24 January 1990

An Intron in the Genes for U3 Small Nucleolar RNAs of the Yeast *Saccharomyces cerevisiae*

EVELYNE MYSLINSKI, VÉRONIQUE SÉGAULT, CHRISTIANE BRANLANT*

The origin of the intervening sequences (introns), which are removed during RNA maturation, is currently unknown. They are found in most genes encoding messenger RNAs, but are lacking in almost all small nuclear (sn)RNAs. One exceptional snRNA (U6) is part of the spliceosomal machinery that is involved in messenger RNA maturation. It has been suggested that its intron arose as a result of incorrect splicing of a messenger RNA precursor. This study revealed the presence of an intron, with the characteristic features of nuclear introns from precursors to messenger RNA, in the two genes coding for *Saccharomyces cerevisiae* U3 snRNA. The branch point was GACTAAC instead of the TACTAAC sequence found in all yeast introns examined so far. As U3 is a nucleolar snRNA required for maturation of ribosomal RNA, its intron could not have been acquired from aberrant messenger RNA processing in a spliceosome.

THE NUCLEI OF VERTEBRATE CELLS contain metabolically stable small nuclear RNAs (snRNAs). Five of the major ones, denoted U1, U2, U4, U5, and U6 snRNAs, are involved in the splicing of pre-mRNAs (*1*). The small nuclear ribonucleoproteins (snRNPs) corresponding to these five RNAs react with antisera of the Sm serotype from patients with the autoimmune rheumatic disease lupus erythematosus (*2*). Another major snRNA species, denoted U3, is located in the nucleolus (*3*). Domain A (*4*) containing the binding site for Sm-antigen proteins is absent in U3 snRNA, and so U3 snRNP does not react with Sm antibodies. Observation of hydrogen bonding between U3 snRNA and nucleolar 28*S* pre-ribosomal RNA (pre-rRNA) suggested an involvement of U3 snRNA in pre-rRNA maturation (*3, 5*), and several models of base-pairing have been proposed (*1*).

Yeast nuclear genes with intervening sequences (introns) interrupting the coding regions are rare and most of them encode ribosomal proteins. Although the pre-mRNA splicing pathway in yeast is similar to that in higher eukaryotes, some features of introns are significantly different in yeast. First, all known yeast pre-mRNA introns contain the sequence TACTAAC in which lariat formation occurs. Second, the sequence at the 5′ end of introns is significantly more variable in mammalian than in yeast mRNA, the 5′ sequence GTATGT being highly conserved in yeast introns.

The yeast *Saccharomyces cerevisiae* contains a large variety of snRNAs (*6*). Two of them, snR17A and snR17B, correspond to vertebrate U3 snRNA (*7*). Unlike other yeast snRNAs, which are encoded by single-copy genes, snR17 is encoded by two genes having similar nucleotide sequences (*7*). Before starting a study of snR17 function by site-directed mutagenesis, we sequenced *Saccharomyces cerevisiae* snR17 at the RNA level and discovered that both snR17A and snR17B genes contain an intron.

The nucleotide sequence of *S. cerevisiae* snR17 from the strain named by the American Type Culture Collection ATCC 28383 (*8*) was determined by both the chemical method for RNA sequencing (*9*) and the enzymatic method based on primer extension with reverse transcriptase (*10*). The RNA extracted from mechanically disrupted cells was separated by ultracentrifugation on a sucrose gradient. The reverse transcriptase sequencing was performed with the 4*S*–8*S* RNA mixture recovered from the gradient, by means of synthetic deoxyoligonucleotides (Fig. 1a). The chemical method was used on 3′ end-labeled, purified snR17 RNA (*11*). SnR17B RNA was not detected by either method. Nevertheless, the presence of two distinct snR17 genes in our strain was confirmed by Southern blot analysis of total DNA (Fig. 2). The size of the two hybridizing bands observed with Eco RI restriction endonuclease digestion fits with the physical map established for snR17A and snR17B genes (*7*). Therefore, as previously observed (*7*), snR17B gene is only poorly expressed in this strain.

There was good correlation between our RNA sequence and that for the snR17A gene, except for the 5′ end of the molecule, where the sequence obtained by reverse transcriptase was completely different from that deduced for the gene (Fig. 1). Thus, either snR17A had a different sequence in our strain or snR17A gene had an intron. According to this second hypothesis, the 5′ terminal sequence CGACGUACUUCA found by reverse transcriptase analysis (Fig. 1b) should belong to a first exon.

We isolated an snR17 gene from an *S. cerevisiae* ATCC 28383 genomic library, using 3′ end-labeled snR17 as a probe, and determined the nucleotide sequence of the

Laboratoire d'Enzymologie et de Génie Génétique, Université de Nancy I, URA CNRS 457, BP 239,54506 Vandoeuvre Les Nancy, Cedex, France.

*To whom correspondence should be sent.

coding region and a long 5′ flanking region (*12*). Except for one additional G residue in the RNA and three mutations upstream of the coding region (Fig. 1a), the sequence of the isolated gene was identical to that previously determined for snR17A gene (*7*). In the additional upstream DNA sequence, we observed the sequence CGACGTACTTCA corresponding to the 5′ end of the RNA. This coding segment is separated from the rest of the coding region by a 157-nucleotide intervening sequence. The sequences at the 5′ and 3′ extremities of this intervening sequence are identical to the consensus sequence found at the extremities of introns from *S. cerevisiae* pre-messengers. A nucleotide sequence very similar to the branch site of *S. cerevisiae* nuclear introns (*13*) is present close to the 3′ extremity (Fig. 1a). Taken together, these observations show that the snR17A gene has an intron located 14 nucleotides from the initiation site of transcription.

Reverse transcriptase does not identify the two nucleotides after the cap structure as they should be 2′-O-methylated. The 3′ end of the coding region of snR17A (determined by the chemical method for RNA sequencing) is located four nucleotides downstream of the position proposed by Hughes *et al.* (*7*).

The intron of the snR17A gene is located between two segments that are highly conserved throughout U3 snRNA evolution. One segment has previously been described as box A (*7, 14*), whereas the second one, deduced from this study, is denoted box A′ (Fig. 3). The tetranucleotide sequence where the intron is inserted is the same in *Schizosaccharomyces pombe* and *S. cerevisiae* but differs from those used by the other eukaryotes shown.

Porter *et al.* (*14*) proposed that the secondary structure of the 5′ region of U3 snRNA is different in higher and lower eukaryotes. The sequence of mature snR17 RNA is compatible with the model proposed for *S. pombe* but the corresponding structure is not very stable. The sequence previously deduced from the snR17 genes, which corresponds to unspliced RNA, was also found to be compatible with the structure proposed for *S. pombe* U3 snRNA (*14*). It will be necessary to determine the secondary structure from mature and unspliced snR17 RNA in order to understand snR17 RNA maturation.

Although snR17B had not been sequenced at the RNA level, it was interesting to look for a putative intron in the snR17B gene. Hughes *et al.* (*7*) sequenced a long upstream DNA region for snR17B. Comparison of snR17A and snR17B DNA sequences (Fig. 1a) reveals that the two genes have a similar architecture. In the snR17B gene, the sequence GTCGACGTACTTCA coding for the 5′ end of the RNA is also separated from the rest of the coding region by an intervening sequence. This intervening sequence is shorter than that of the snR17A gene: 130 nucleotides instead of 157. Its 5′ and 3′ end sequences fit the consensus sequences for 5′ and 3′ ends of *S. cerevisiae* nuclear introns, and a branch site is also present. In addition to the classical consensus sequences, the two introns have additional common sequences: (i) a hexanucleotide sequence close to the 5′ recognition site, (ii) the sequence in between the branch site and the 3′ recognition site, and (iii) an octanucleotide sequence preceding the branch point (Fig. 1a). Inspection of a large number of *S. cerevisiae* introns, including introns of ribosomal protein genes (*13*), revealed that these three sequences are not found in other introns and may have a specific role in U3 pre-snRNA maturation (*15*).

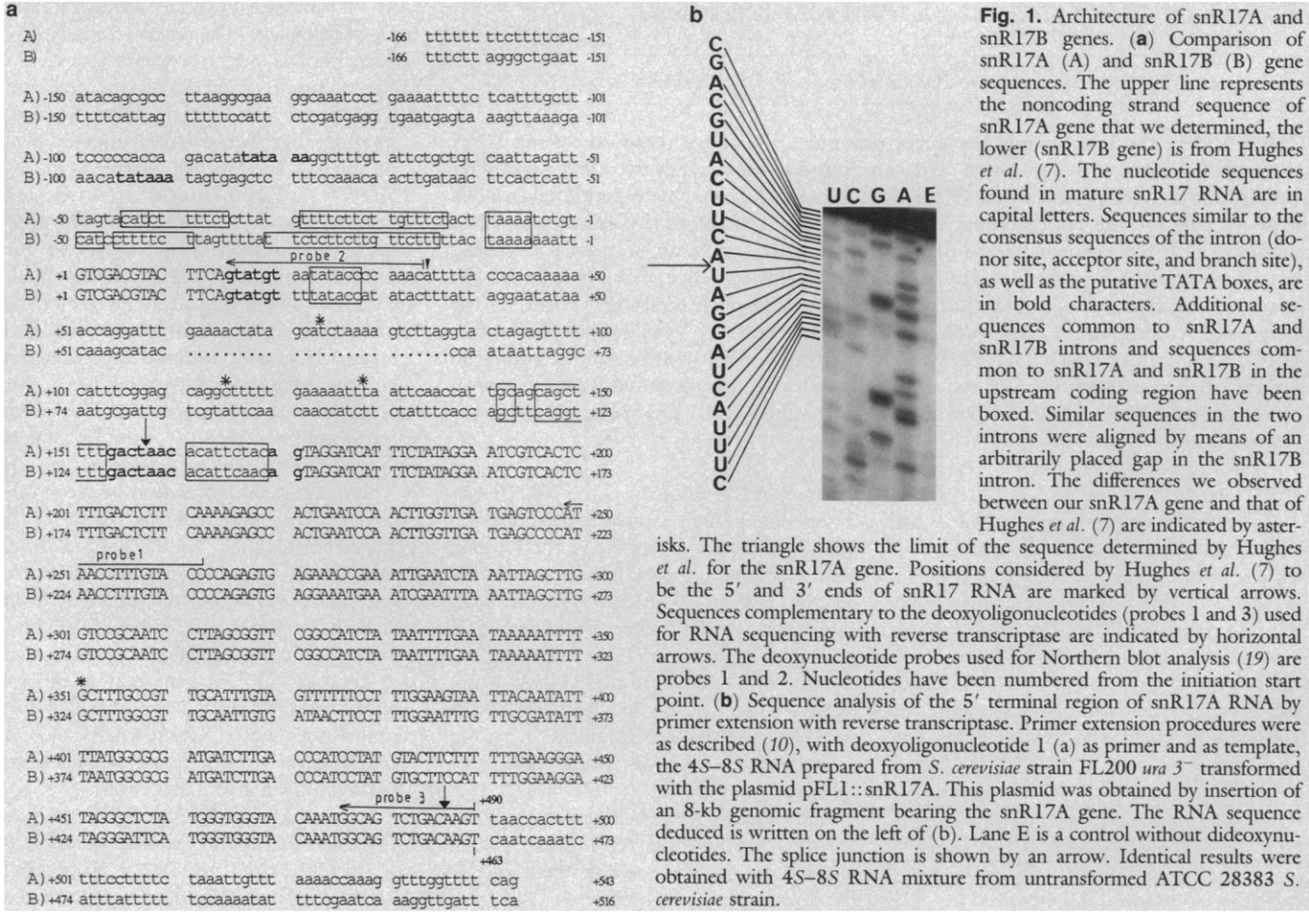

Fig. 1. Architecture of snR17A and snR17B genes. (**a**) Comparison of snR17A (A) and snR17B (B) gene sequences. The upper line represents the noncoding strand sequence of snR17A gene that we determined, the lower (snR17B gene) is from Hughes *et al.* (*7*). The nucleotide sequences found in mature snR17 RNA are in capital letters. Sequences similar to the consensus sequences of the intron (donor site, acceptor site, and branch site), as well as the putative TATA boxes, are in bold characters. Additional sequences common to snR17A and snR17B introns and sequences common to snR17A and snR17B in the upstream coding region have been boxed. Similar sequences in the two introns were aligned by means of an arbitrarily placed gap in the snR17B intron. The differences we observed between our snR17A gene and that of Hughes *et al.* (*7*) are indicated by asterisks. The triangle shows the limit of the sequence determined by Hughes *et al.* for the snR17A gene. Positions considered by Hughes *et al.* (*7*) to be the 5′ and 3′ ends of snR17 RNA are marked by vertical arrows. Sequences complementary to the deoxyoligonucleotides (probes 1 and 3) used for RNA sequencing with reverse transcriptase are indicated by horizontal arrows. The deoxynucleotide probes used for Northern blot analysis (*19*) are probes 1 and 2. Nucleotides have been numbered from the initiation start point. (**b**) Sequence analysis of the 5′ terminal region of snR17A RNA by primer extension with reverse transcriptase. Primer extension procedures were as described (*10*), with deoxyoligonucleotide 1 (a) as primer and as template, the 4*S*–8*S* RNA prepared from *S. cerevisiae* strain FL200 *ura 3*⁻ transformed with the plasmid pFL1::snR17A. This plasmid was obtained by insertion of an 8-kb genomic fragment bearing the snR17A gene. The RNA sequence deduced is written on the left of (b). Lane E is a control without dideoxynucleotides. The splice junction is shown by an arrow. Identical results were obtained with 4*S*–8*S* RNA mixture from untransformed ATCC 28383 *S. cerevisiae* strain.

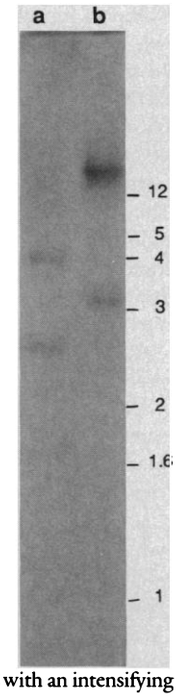

Fig. 2. Southern blot analysis of total DNA. Total *S. cerevisiae* DNA was digested with Eco RI (lane a) or Pst I (lane b), separated by electrophoresis on an 0.8% agarose gel, and transferred on a nitrocellulose filter. Positions of size markers (1-kb ladder, Bethesda Research Laboratories) are on the right in kilobases. Hybridization was performed at 37°C in a buffer containing 50% formamide, 5× saline sodium citrate (SSC), 20 m*M* sodium phosphate (*p*H 6.5), 5× Denhardt's solution, and DNA probe (a coding region fragment, +204 +412 positions, inserted in M13mp9 DNA) labeled to high specific activity by the random priming method (*25*). After hybridization for 18 hours, the filters were washed two times for 30 min each in 2× SSC at 37°C. Autoradiography was carried out at −80°C with an intensifying screen.

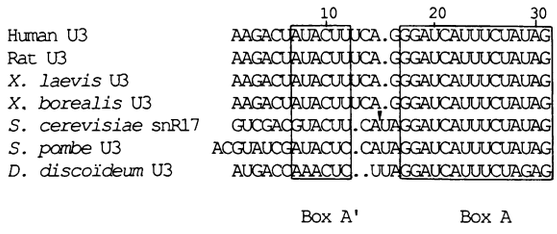

Fig. 3. Comparison of the 5′ terminal region of U3 snRNAs from various organisms. The nucleotide sequence of the 5′ region of *S. cerevisiae* snR17 RNA is aligned with those of human (*26*), rat (*27, 28*), *Xenopus laevis* (*29*), *X. borealis* (*29*), *S. cerevisiae* (*7*), *S. pombe* (*14*), and *Dictyostelium discoideum* (*30*). The triangle indicates the junction point of exons in snR17 RNA. The evolutionarily conserved sequences are boxed: box A was previously described (*7, 14*) and box A′ is deduced from this alignment.

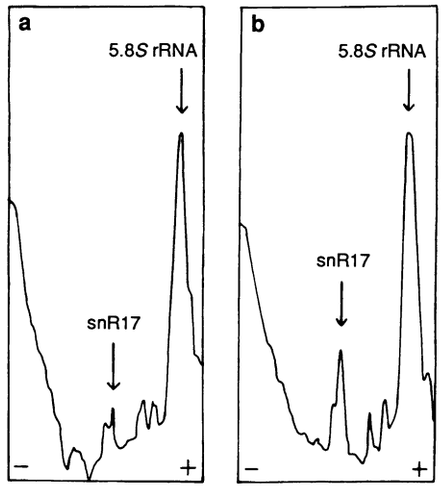

Fig. 4. Comparison of snRNA composition of untransformed (**a**) and transformed (**b**) *S. cerevisiae* cells. The *S. cerevisiae* FL200 strain *ura 3*$^-$ was transformed with the pFL1::snR17 plasmid. RNAs from transformed and untransformed *S. cerevisiae* strain FL200 *ura 3*$^-$ cells were phenol-extracted and fractionated on 10 to 30% sucrose gradients, and 500 μg of each 4*S*–8*S* RNA mixture recovered from the gradient was fractionated in parallel by electrophoresis on a 15% polyacrylamide gel in the presence of 8*M* urea. The gel was stained with methylene blue and scanned with an ultroscan densitometer (LKB). The tracings obtained with untransformed and transformed cells are represented in (a) and (b), respectively; 5.8*S* RNA was used as an internal reference. The positions of snR17 and 5.8*S* RNAs are indicated by an arrow. Transformed cells contained about 2.5 times as much snR17 as untransformed cells, as determined from the difference of peak areas in transformed and untransformed cells relative to 5.8*S* RNA. The relative amounts of the two RNAs with mobilities intermediary between those of snR17 RNA and 5.8*S* RNA remained constant in transformed and untransformed cells.

The branch site has an unusual structure: GACTAAC, instead of the TACTAAC, that until now, was found in all *S. cerevisiae* nuclear introns (*13*). The fact that the sequence GACTAAC can act as a branch site in *S. cerevisiae* introns is not surprising, since Watts *et al.* (*16*) showed that a *Drosophila* alcohol dehydrogenase intron with a branch-point sequence AACTAAC can be correctly spliced in *S. cerevisiae*. SnR17 introns may constitute a special class of *S. cerevisiae* nuclear introns. Parker and Patterson (*17*) have already distinguished two classes of fungal nuclear introns, denoted 3′S and 3′L. Most *S. cerevisiae* introns belong to the 3′L class. SnR17 introns have features common to both classes: the short distance between the branch point and the AG at the 3′ junction is characteristic of 3′S introns, whereas their 3′ end sequence fits perfectly the consensus sequence [(U or A) APyAG] for 3′L introns.

To demonstrate that an snR17 gene containing an intron is correctly expressed and spliced in vivo, *S. cerevisiae* strain FL200 *ura 3*$^-$ was transformed with a pFL1 plasmid (*18*) in which the snR17A gene was inserted (pFL1::snR17A). Transformation with this plasmid resulted in an increase in snR17 RNA in cells (transformed cells contained about 2.5-fold as much snR17 RNA as control cells) (Fig. 4). Northern blot analysis (*19*) of the RNA isolated from transformed cells was performed with deoxynucleotide probes for the coding region (probe 1) and the intron sequence (probe 2). (Probes 1 and 2 are represented in Fig. 1a). Only probe 1 hybridized with mature snR17 RNA, indicating that there was rapid intron excision and degradation. This was confirmed by the result of the reverse transcriptase sequence analysis with probe 1 as a primer and the 4*S*–8*S* RNA mixture from transformed cells as a template. The sequence obtained corresponded to that of mature snR17 RNA; no intermediary product lacking the 5′ terminal sequence and no pre-snR17 RNA were detected (Fig. 1b).

A putative TATA box is located 84 nucleotides from the site of transcription initiation in the snR17A gene and 96 nucleotides from the snR17B initiation site (Fig. 1a). These distances are not unusual for *S. cerevisiae* genes (*20*). In addition to the TATA box, the upstream regions of the two snR17 genes display sequence homology between positions −45 and −6 from the transcription initiation start point. These two sequences may represent binding sites for transcription factors required for snR17 gene expression.

An intron was recently observed in the gene for the nucleoplasmic U6 snRNA from *S. pombe* (*21*). This intron is very short compared to those of snR17 genes and shares no homology with them except the consensus sequences. Brow and Guthrie (*22*) proposed that the *S. pombe* U6 intron was not present in the ancestral gene but, as a result of splicing, had been acquired more recently in evolution by insertion of a pre-mRNA intron in U6 snRNA. This kind of hypothesis cannot be postulated for snR17 snRNA since it is not a spliceosomal RNA. Therefore, if the intron in snR17 RNA is not an ancestral intron, how was it acquired?

U3 snRNA genes are transcribed by RNA polymerase II in vertebrates (*23*), as is true for mRNAs. The exceptional presence of an intron similar to pre-mRNA introns in an snRNA that is also transcribed by RNA polymerase II suggests that a link may exist between transcription by RNA polymerase II and maturation of introns in a spliceosomal structure. At first sight, the observation of an intron in the *S. pombe* U6 snRNA gene would seem to contradict this statement. Nevertheless, it should be pointed out that U6 RNA transcription is complex: although performed by RNA polymerase III, it uses some RNA polymerase II promoter elements (*24*). Further investigations will be needed to determine whether the presence of such elements in the gene may play a role in allowing intron excision in the transcript.

REFERENCES AND NOTES

1. J. A. Steitz *et al.*, in *Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles*, M. L. Birnstiel, Ed. (Springer-Verlag, Berlin, 1988), pp. 115–154.
2. M. R. Lerner and J. A. Steitz, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5495 (1979).
3. H. Busch, R. Reddy, L. Rothblum, Y. C. Choi, *Annu. Rev. Biochem.* **51**, 617 (1982).
4. C. Branlant *et al.*, *EMBO J.* **1**, 1259 (1982).
5. J. P. Calvet and T. Pederson, *Cell* **26**, 363 (1981).
6. N. Riedel *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8097 (1986).
7. J. M. Y. Hughes, D. A. M. Konings, G. Cesareni, *EMBO J.* **6**, 2145 (1987).
8. F. Lacroute, *J. Bacteriol.* **95**, 824 (1979).
9. D. A. Peattie, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1760 (1979).
10. L. Hu Qu, B. Michot, J. P. Bachellerie, *Nucleic Acids Res.* **11**, 5903 (1983).
11. SnRNAs from the 4S–8S RNA mixture recovered from a sucrose gradient centrifugation of total yeast RNA extract were selected by means of their special cap structure with a 2,2,7-trimethylguanosine residue [P. Bringmann, J. Rinke, B. Appel, R. Reuter, R. Lührmann, *EMBO J.* **2**, 1129 (1983)]. They were immunoprecipitated with antibodies to m3G, which were bound to protein A–Sepharose [A. Krol, J. P. Ebel, J. Rinke, R. Lührmann, *Nucleic Acids Res.* **11**, 8583 (1983)]. The snRNA mixture was then 3′ end-labeled, and snR17 RNA was purified by electrophoresis on a 15% polyacrylamide gel.
12. The nucleotide sequence of the snR17 gene from the strain ATCC 28383 was determined by the Sanger method [F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977)] with deoxyoligonucleotides as primers, and as template, subfragments inserted in phage M13mp9.
13. J. L. Teem *et al.*, *Nucleic Acids Res.* **12**, 8295 (1984).
14. G. L. Porter, P. J. Brennwald, K. A. Holm, J. A. Wise, *ibid.* **16**, 10131 (1988).
15. V. Ségault, E. Myslinski, C. Branlant, unpublished results.
16. F. Watts, C. Castle, J. Beggs, *EMBO J.* **2**, 2085 (1983).
17. R. Parker and B. Patterson, in *Molecular Biology of RNA: New Perspectives*, M. Inouye and B. S. Dudock, Eds. (Academic Press, New York, 1987), pp. 133–149.
18. M. R. Chevallier *et al.*, *Gene* **11**, 11 (1980).
19. E. Myslinski, V. Ségault, C. Branlant, unpublished observations.
20. K. Struhl, *Cell* **49**, 295 (1987).
21. T. Tani and Y. Ohshima, *Nature* **337**, 87 (1989).
22. D. A. Brow and C. Guthrie, *ibid.*, p. 14.
23. T. S. Ro Choi, B. K. Raj, L. M. Pike, H. Busch, *Biochemistry* **15**, 3823 (1976).
24. P. Carbon *et al.*, *Cell* **51**, 71 (1987).
25. A. P. Feinberg and B. Vogelstein, *Anal. Biochem.* **132**, 6 (1983).
26. K. A. Parker and J. A. Steitz, *Mol. Cell. Biol.* **7**, 2899 (1987).
27. D. Suh, H. Busch, R. Reddy, *Biochem. Biophys. Res. Commun.* **137**, 1133 (1986).
28. J. L. Stroke and A. M. Weiner, *J. Mol. Biol.* **184**, 183 (1985).
29. S. A. Gerbi, C. Jeppesen, B. Stebbins-Boaz, M. Ares, Jr., *Cold Spring Harbor Symp. Quant. Biol.* **52**, 709 (1987).
30. J. A. Wise and A. Weiner, *Cell* **22**, 109 (1980).
31. We thank R. Lührmann for his gift of antibodies to m3G, F. Lacroute for providing us with the *S. cerevisiae* ATCC 28383 genomic bank, and strain FL200 *ura* 3^- and with pFL1 plasmid and for helpful discussion, J. Bayeul and E. Habermacher for technical assistance. V.S. was supported by the Ministère de la Recherche et de l'Enseignement supérieur.

21 August 1989; accepted 26 December 1989

Control of the Interferon-Induced 68-Kilodalton Protein Kinase by the HIV-1 *tat* Gene Product

SOPHIE ROY, MICHAEL G. KATZE, NEIL T. PARKIN, ISAAC EDERY,* ARA G. HOVANESSIAN, NAHUM SONENBERG

The *tat*-responsive region (TAR) of the human immunodeficiency virus–1 (HIV-1) exhibits a trans-inhibitory effect on translation in vitro by activating the interferon-induced 68-kilodalton protein kinase (p68 kinase). Productive infection by HIV-1 was shown to result in a significant decrease in the amount of cellular p68 kinase. The steady-state amount of p68 kinase was also reduced in interferon-treated HeLa cell lines stably expressing *tat*, as compared to the amount of the kinase in interferon-treated control HeLa cells. Thus, the potential translational inhibitory effects of the TAR RNA region mediated by activation of p68 kinase may be downregulated by *tat* during productive HIV-1 infection.

THE HIV-1 *tat* GENE PRODUCT trans-activates viral gene expression (*1*) and is essential for HIV-1 replication (*2*). Its mode of action has been proposed to be either transcriptional, post-transcriptional, or a combination of both (*3*). TAR is present at the 5′ end of all HIV-1 mRNAs (*4*) and assumes a stable stem and loop structure in vitro, as determined by RNA nuclease mapping (*5*). When fused to a heterologous mRNA, TAR exhibits a strong inhibitory effect on translation in cell-free extracts and *Xenopus* oocytes (*6*). Translational inhibition is partly due to a trans-inhibitory effect mediated by the activation of the interferon-induced, double-stranded (ds) RNA-dependent protein kinase (*7*), also termed dsI, DAI, or p68 kinase. This results in autophosphorylation of the kinase (*8*), which then catalyzes the phosphorylation of the α subunit of eukaryotic initiation factor–2 (eIF-2), with subsequent inhibition of protein synthesis (*9*). The p68 kinase is one of many proteins induced by interferon; its activation has been suggested to be important in the establishment of the antiviral state mediated by interferon (*10*).

Activation of p68 kinase and subsequent inhibition of protein synthesis can be outcomes of viral infection (*11, 12*). However, a number of eukaryotic viruses have developed strategies to escape the inhibitory effects caused by activation of p68 kinase (*12, 13*). Consequently, we examined the possibility that HIV-1 regulates the expression of p68 kinase. The human T-lymphoid cell line CEM was infected with the LAV isolate (*14*) of HIV-1 under conditions in which >90% of cells express viral protein 3 days after infection, as determined by indirect immunofluorescence. One to 5 days after infection, cell extracts were prepared and analyzed by protein immunoblotting for viral proteins, p68 kinase, and actin (Fig. 1). Viral proteins p24, p55, p66, gp120, and gp41 were prominent as early as 2 days after infection, as determined with the use of serum from an individual with acquired immunodeficiency syndrome (AIDS) (Fig. 1A). The amount of p68 kinase in infected cells, measured with a monoclonal antibody to the kinase (*15*), did not significantly differ from that in mock-infected cells during the first 2 days after infection (an increase in the amounts of p68 kinase was observed on day 2 in both mock- and HIV-1–infected cells, possibly because the medium was changed) but was reduced by day 3 and virtually undetectable at 4 and 5 days after infection (Fig. 1B). The decline in the amount of p68 kinase occurred before the appearance of virus-induced cytopathic effects, which were generally apparent 7 days after infection. The decrease in p68 kinase is unlikely to reflect a general proteolysis, because the amount of actin remained relatively stable over the time course of infection, with only a slight decrease detected after 3 days (Fig. 1B). This difference cannot be explained by different half-lives of the proteins, as the half-lives of p68 kinase and actin are 6 to 7 hours and 12 to 16 hours, respectively, as determined by immunoblotting of CEM cells treated with the protein synthesis inhibitor anisomycin (*16*). Thus, productive HIV-1 replication is associated with a decrease in the amount of cellular p68 kinase.

It is conceivable that one of the viral trans-acting proteins is responsible for the

S. Roy, N. T. Parkin, I. Edery, Department of Biochemistry, McGill University, Montreal, Canada, H3G 1Y6.
M. G. Katze, Department of Microbiology, School of Medicine, Regional Primate Research Center, University of Washington, Seattle, WA 98195.
A. G. Hovanessian, Unité D'Oncologie Virale, Institut Pasteur, Paris 75724, France.
N. Sonenberg, Department of Biochemistry and McGill Cancer Center, McGill University, Montreal, Canada, H3G 1Y6.

*Present address: Department of Biology, Brandeis University, Waltham, MA 02554.