A Trypanosome Mitochondrial RNA Polymerase Is Required for Transcription and Replication*

Received for publication, January 22, 2002, and in revised form, February 14, 2002
Published, JBC Papers in Press, February 21, 2002, DOI 10.1074/jbc.M100662200

Jayleen Grams‡, James C. Morris§, Mark E. Drew§, Zefeng Wang§, Paul T. Englund§, and Stephen L. Hajduk‡¶

From the ‡Department of Biochemistry and Molecular Genetics, Schools of Medicine and Dentistry, University of Alabama, Birmingham, Alabama 35294 and the §Department of Biological Chemistry, Johns Hopkins School of Medicine, Baltimore, Maryland 21205

Understanding mitochondrial transcription is a requisite first step toward understanding the regulation of mitochondrial gene expression in kinetoplastids. Here we report the identification and functional characterization of a mitochondrial RNA polymerase (mtRNAP) from Trypanosoma brucei, the first trans-acting factor involved in kinetoplast mitochondrial transcription to be identified. Using sequences conserved among the catalytic domains of the single-subunit mtRNAPs, we were able to obtain a full-length sequence for a candidate mtRNAP from T. brucei. Sequence comparison indicates that it shares homology in its catalytic domain with other single-subunit mtRNAPs, including functionally conserved residues that are identical in all single-subunit RNAPs. We used RNA interference to functionally knock out the gene product to determine whether the candidate gene represents an mtRNAP. As predicted for a mitochondrial specific RNA polymerase, reduction of the gene product resulted in a specific decrease of mitochondrial versus nuclear transcripts. Additionally, similar to the mtRNAP of other organisms, the mtRNAP characterized here is involved in replication of the mitochondrial genome. Thus, based on sequence comparison and functional studies, we have cloned an mtRNAP from trypanosomes.

The mitochondrial genome of kinetoplastids is unusual in that it is comprised of large and small circular DNAs, maxicircles and minicircles, respectively, catenated into a giant network called kinetoplast DNA (1–3). Little is known of the mechanisms regulating gene expression of either maxicircles or minicircles. In Trypanosoma brucei, the 23-kb maxicircles represent the typical mitochondrial DNA, coding for rRNAs and several proteins involved in mitochondrial respiration. Similar to other organisms, numerous polycistronic transcripts have been detected, indicating that maxicircles are transcribed polycistronically (4–6). No maxicircle promoter has yet been identified, although a precursor extending at least 1200 nucleotides upstream of the 12 S rRNA has been detected and may represent an initiation site for transcription (7).

Many maxicircle genes encode cryptic transcripts that require the post-transcriptional insertion or deletion of uridines to produce a functional mRNA, which is a process known as RNA editing (Ref. 8; for reviews see Ref. 9–11). The information for the editing of maxicircle transcripts is provided by small RNAs termed guide RNAs (gRNAs). With the exception of gMurfII-1 and gMurfII-2 (guide RNAs 1 and 2, respectively, for maxicircle unidentified reading frame II) found on the maxicircle, gRNAs are encoded by the second component of the kinetoplast, the minicircles (12–14). In T. brucei, minicircles are ~1-kb circular DNA molecules, and there are several thousand copies/kinetoplast network. Despite some conserved features, they are heterogeneous in sequence and provide the 250–300 sequence classes necessary to edit maxicircle transcripts (15, 16).

Each minicircle of T. brucei contains three or four potential gRNA transcription units, and minicircles also appear to be transcribed polycistronically (17). Like maxicircles, no promoter has been identified for minicircles. However, each gRNA transcription unit is flanked by 18-bp imperfect inverted repeats that have been proposed to function in gRNA expression, because transcription initiates 31–32 bp downstream of the 5’ inverted repeat at the conserved sequence 5’-RYAYA-3’ in the gRNA gene (12). The function of the 18-bp inverted repeats in transcriptional initiation, termination, or processing remains to be determined.

In addition to a lack of knowledge concerning cis-acting sequences within the mitochondrial genome, neither the mitochondrial RNA polymerase (mtRNAP) nor any transcription factor has been identified in trypanosomes. The search for cis-elements or trans-acting factors involved in kinetoplastid mitochondrial transcription has been hindered by the lack of a functional assay to either test putative promoter elements or purify the transcriptional complex. In contrast to the multisubunit RNAPs of the nucleus, the catalytic domains of mtRNAPs in all organisms identified to date are related to the single-subunit RNA polymerases, similar to the T7 bacteriophage polymerase (18–20). Using conserved sequences in the catalytic domains of single-subunit RNA polymerases, a sequence was identified in the trypanosome genome data base as a candidate fragment of mtRNAP (21). As expected because mtRNAP would be required in active mitochondria, functional knockout of the candidate gene was lethal in procyclic form trypanosomes. Recently, a full-length sequence has been re-

* This work was supported by National Institutes of Health Grant AI21401 (to S. L. H.), Medical Scientist Training Program Grant 5T32GM08361 (to J. G.), and National Institutes of Health Grant GM27608 (to P. T. E.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed. Tel.: 205-934-6033; Fax: 205-934-0758; E-mail: shajduk@uab.edu.

1 The abbreviations used are: gRNA, guide RNA; mtRNAP, mitochondrial RNA polymerase; RNA-pol, RNA polymerase; RNAi, RNA interference; dsRNA, double-stranded RNA; ISP, Rieske iron sulfur protein; COI and COII, cytochrome oxidase subunits I and II, respectively; Cyb, apocytochrome b; ND1 and ND4, NADH dehydrogenase subunits 1 and 4, respectively; RT, reverse transcriptase.

© 2002 by The American Society for Biochemistry and Molecular Biology, Inc.
reported that represents the same candidate mtRNA (22). However, there remains no functional demonstration that it is a mtRNA.

In this paper, we report the sequence of the full-length gene for a candidate mtRNA in *T. brucei*. Comparison of the predicted amino acid sequence of the putative *T. brucei* mtRNA with those of other organisms provides evidence that we have identified an mtRNA and that it is the same candidate mtRNA reported earlier (21, 22). We demonstrate that the gene is in fact a mitochondrial specific RNA polymerase through functional analyses using RNA interference (RNAi). RNAi is a phenomenon by which the introduction of double-stranded RNA results in the specific degradation of a transcript sharing the same sequence. Although the mechanism of RNAi is a subject of intense study, RNAi is also rapidly becoming a convenient method of genetic manipulation (for reviews see Refs. 23–27). As predicted for an mtRNA, functional knockout resulted in a specific decrease of mitochondrial transcripts versus nuclear RNAs. Additionally, we find a corresponding decrease in maxicircle abundance, suggesting that as in other organisms, the *T. brucei* mtRNA also functions in replication of the mitochondrial genome. With the identification of a mtRNA, functional assays can now be developed to determine promoter sequences and other trans-acting factors involved in mitochondrial transcription of the kinetoplast.

**EXPERIMENTAL PROCEDURES**

**Growth of Cells and Preparation of RNA**—Procyclic *T. brucei* TREU 667 cells were grown at 26°C in Cunningham medium supplemented with 10% fetal bovine serum (28). RNA was isolated using TriPure isolation reagent (Roche Molecular Biochemicals), and poly(A) RNA was selected using a Dynabeads mRNA purification kit (Dynal A. S.) according to manufacturer's instructions. The RNAi vector and cell line has been described previously (21). Briefly, procyclic *T. brucei* strain 29-13 (a gift from Drs. Elizabeth Wirtz and George Cross), which harbors integrated genes for T7 RNA polymerase and the tetracycline repressor, were transfected with the stem-loop vector into which a 470-bp fragment of the mtRNAP was cloned in opposite orientation. RT-PCR was performed as previously described to GeneScreen Plus nylon membranes using a dot blot apparatus. 32P-Radiolabeled probes were made by random priming PCR products with 2 ng of template DNA and 0.1 mg/ml phleomycin. The cells were induced with 100 μg/ml hygromycin C; and 0.1 mM NaCl, 100 mM EDTA) and resuspended in NET-100 at a density of 2 × 107 cells/ml. The cells lysate was extracted with phenol/chloroform, DNA was precipitated with 2-propanol, and the pellet was washed with 70% ethanol.

DNA from 1 × 106 cell equivalents was treated with 0.15 μl HCl for 10 min, denatured in 0.4 M NaOH, 25 mM EDTA, and then transferred to GeneScreen Plus nylon membranes using a dot blot apparatus.

**RESULTS**

**Cloning a Candidate Mitochondrial RNA Polymerase**—Mitochondrial RNA polymerases share significant sequence homology in their catalytic domains with the single-subunit RNA polymerases such as that of T7 bacteriophage (18). Using conserved sequences in the catalytic domain, we identified a 470-bp sequence in the trypansomome genome data base (www. tigr.org/tdb/mdb/tdb) as a candidate fragment of the *T. brucei* mtRNA. Using the combined methods of RT-PCR and genomic PCR, we were then able to identify an open reading frame of 3,428 nucleotides, preceded by a stop codon −200 nucleotides upstream and terminating with a pair of stop codons (Fig. 1). There are a number of potential candidates for the initiating methionine downstream of the 5′ end stop codon. Because a 39-nucleotide spliced leader RNA is trans-spliced onto the 5′ end of all trypansomome nuclear encoded mRNAs, we used RT-PCR with spliced leader RNA and gene-internal primers to identify the initiating methionine. The splice site mapped to 29 nucleotides upstream of a methionine, resulting in a relatively short 5′-untranslated region. Only one site was obtained in three clones, and this site agrees with the consensus splice acceptor site, containing an upstream polypyrimidine-rich tract and being preceded immediately by an AG dinucleotide (Fig. 1, underlining and dots beneath sequence, respectively). Two sites of polyadenylation at 9 and 50 nucleotides after the 3′ end stop codon were mapped using RT-PCR with poly(T) and gene internal oligonucleotides (Fig. 1, arrows). Using the original 470-bp fragment in Southern mapping suggest the gene is single-copy, and an ~4 kb transcript detected by Northern blot is in congruence with the predicted open reading frame (data not shown).

**The Predicted Protein Shares Homology with Other mtRNAPs**—The full-length protein has a predicted molecular mass of 144 kDa. Comparison of the predicted amino acid sequence of the putative *T. brucei* mtRNA with those of other organisms supports that we have identified a mitochondrial specific RNA polymerase (Fig. 2A). First, because the gene is
encoded by the nucleus, we would expect the protein to contain a mitochondrial localization signal to be imported into the mitochondrion. Indeed, the amino acid sequence of the candidate mtRNAP contains a predicted mitochondrial localization signal of 30 amino acids at the N terminus. Other than containing a mitochondrial localization signal, mtRNAPs do not share homology in their N termini, and this region accounts for most of the size variability among them. Similarly, the N terminus of the T. brucei protein accounts for its size variability when compared with other mtRNAPs.

Second, the mtRNAPs of all organisms identified to date are conserved in their catalytic domains, delineated as subdomains III–X (18, 32). The putative T. brucei mtRNAP shares overall amino acid sequence homology (≈50% identity and ≈70% similarity) with other mtRNAPs in these subdomains, including many residues that are identical (Fig. 2B). In addition to overall sequence homology, amino acids that are present in the active site and are thought to be involved in catalytic function of the single-subunit RNAPs have been mapped for T7 RNAP (33). These are identical in all mtRNAPs and are present in the T. brucei sequence (Fig. 2B). For example, Asp537 and Asp812 of T7 RNAP are involved in binding metal ions at the active site (34, 35); Lys631 and His811 may facilitate phosphodiester bond formation (34). Residue His 811 along with Phe 882 appear to function in ribonucleotide binding (33–36), and residue Tyr 639 is involved in discrimination of the enzyme for rNTPs versus dNTPs (37, 38). Thus, sequence comparison with other mtRNAPs supports that the gene is a T. brucei mtRNAP. It is

FIG. 1. Nucleotide and predicted amino acid sequences of the candidate mtRNAP in T. brucei. Arrowhead, mapped splice site immediately preceding the 3′ acceptor site; underlining, poly pyrimidine-rich tract; dots under sequence, AG dinucleotide; asterisks, in-frame stop codons; arrows, polyadenylation sites.
Mitochondrial RNA polymerases share homology in their catalytic domains with single-subunit RNA polymerases. A, diagram comparing the *Triticum aestivum* (wheat), *Saccharomyces cerevisiae* (yeast), and *Homo sapiens* (human) mtRNAPs and T7 bacteriophage RNA polymerase with the candidate mtRNAP in *T. brucei*. MLS, mitochondrial localization signals as predicted by MitoProt II 1.0d4 (www.mips.biochem.mpg.de/cgi-bin/proj/medgen/mitofilter). Open boxes, nonconserved N termini; hatched boxes, conserved catalytic domains among single-subunit RNA polymerases. B, amino acid sequence comparison of the catalytic domains of indicated organisms. *T. brucei*; *T. aestivum*; *S. cerevisiae*; *H. sapiens*; T7, T7 bacteriophage; green, similarity in at least 60% of sequences; yellow, identity in at least 60% of sequences; blue, identity in all; 1, residues involved in coordination of divalent metal ions in the active site; 2, residues involved in phosphodiester bond formation; 3, residues involved in binding rNTPs or in rNTP discrimination.

**Fig. 2.** Mitochondrial RNA polymerases share homology in their catalytic domains with single-subunit RNA polymerases. A, diagram comparing the *Triticum aestivum* (wheat), *Saccharomyces cerevisiae* (yeast), and *Homo sapiens* (human) mtRNAPs and T7 bacteriophage RNA polymerase with the candidate mtRNAP in *T. brucei*. MLS, mitochondrial localization signals as predicted by MitoProt II 1.0d4 (www.mips.biochem.mpg.de/cgi-bin/proj/medgen/mitofilter). Open boxes, nonconserved N termini; hatched boxes, conserved catalytic domains among single-subunit RNA polymerases. B, amino acid sequence comparison of the catalytic domains of indicated organisms. *T. brucei*; *T. aestivum*; *S. cerevisiae*; *H. sapiens*; T7, T7 bacteriophage; green, similarity in at least 60% of sequences; yellow, identity in at least 60% of sequences; blue, identity in all; 1, residues involved in coordination of divalent metal ions in the active site; 2, residues involved in phosphodiester bond formation; 3, residues involved in binding rNTPs or in rNTP discrimination.
also apparent from sequence analysis that we have, using an independent method, identified an identical candidate mtRNAP recently reported by another group (22).

RNA Interference Analysis of the Putative mtRNAP—RNAi is a recently discovered phenomenon in which introduction of a specific dsRNA into cells results in the degradation of the specific cellular mRNA (for reviews see Refs. 23–27). As such, it is rapidly becoming a powerful tool for the genetic manipulation of trypanosomes (21, 39, 40). A major advantage of using RNAi to produce functional knockouts is that only partial sequence is necessary to induce the specific degradation of any given target mRNA. Hence, Wang et al. (21) cloned the 470-bp sequence obtained from the data base into an RNAi vector and subsequently integrated it into a nontranscribed region of the genome to produce a stable cell line. Expression of the dsRNA is regulated by a tetracycline-inducible promoter. After induction of dsRNA with tetracycline, there is a ~95% reduction in transcript abundance of an ~4-kb mRNA after 24 h, and there is growth inhibition 6 days after induction followed by cell death (21).

To determine whether our candidate gene was in fact the mtRNAP, we wanted to further analyze the phenotype of these cells. Similar to what has been reported, we find the cells stop dividing 3–4 days after tetracycline induction, eventually succumbing to cell death ~6 days after induction (Fig. 3A). A lethal phenotype was expected because mitochondrial function is required in procyclic cells. Likewise by Northern blot analysis, the ~4-kb transcript of the candidate mtRNAP had decreased by ~80% after 24 h of tetracycline induction, whereas dsRNA production was already near its maximal levels (Fig. 3B). Reduction of the ~4-kb transcript occurs rapidly after tetracycline induction as seen in a time course, with 50% reduction in transcript abundance as early as 7 h post-induction (data not shown). After 3 days of tetracycline induction, there is a 95% reduction in transcript abundance. On days 1–3 after RNAi induction we observed faint bands migrating slightly faster than the full-length mtRNAP transcript that may represent partially degraded mtRNAP mRNA (Fig. 3B).

If the candidate gene is a mtRNAP, we would predict that a functional knockout of the mtRNAP would result in a specific decrease in mitochondrial transcripts versus those of the nucleus. To test this, RNA was isolated from cells after 0–3 days of tetracycline induction and analyzed by Northern blot. For nuclear transcripts, the mRNA levels of α-tubulin, the nuclear RNA polymerase II (RNAP II), and a protein that is imported into the mitochondrion, the Rieske ISP, were assayed (Fig. 4A). The steady-state abundance of all three nuclear transcripts remained comparable with preinduction with tetracycline (0 days of tetracycline induction).

In contrast to the nuclear transcripts, the abundance of all mitochondrial transcripts, cytochrome oxidase subunits I and II (COI and COII, respectively), Cytb, NADH dehydrogenase subunits 1 and 4 (ND1 and ND4, respectively) decreased over 3 days after tetracycline induction (Fig. 4B). The kinetics of mRNA decrease were variable for the different maxicircle genes. The simplest pattern occurred for COI and ND1, with mRNA steadily decreasing over the course of 3 days. Transcript levels of Cytb and ND4 are not as straightforward, as they actually increase by 36 and 85%, respectively, after 1 day of tetracycline induction. The cause for this increase is unknown. The steady-state abundance of COII only modestly increases by 6%, so that the profile of COII may result from a slower turnover rate of the COII mRNA versus that of COI and ND1 mRNAs. A larger COII transcript also increases after tetracycline induction. This larger transcript has previously been described as either a polycistronic precursor or the result of the addition of a long poly(A) tail (41, 42). That this transcript increases with overall declining mRNA levels may indicate a coordination of transcription and RNA processing. Despite the differences in the rate of decline, the steady-state abundance of all mitochondrial mRNAs examined decreased considerably with functional knockout of the candidate mtRNAP. The difference in the reduction rates of the candidate mtRNAP transcript (Fig. 3B) versus that of the mitochondrial transcripts (Fig. 4B) probably reflects the active degradation of the ~4-kb transcript by RNAi versus the variation in mRNA turnover rates for the other transcripts following loss of mtRNAP function. The finding that functional knockout of the ~4-kb transcript results in the specific decrease in mitochondrial versus nuclear transcripts is consistent with the gene representing an mtRNAP of T. brucei.

Analysis of Maxicircle and Minicircle Abundance—The mtRNAP of other organisms functions in the transcription of the mitochondrial genome as well as in initiation of DNA replication. In both humans and yeast, replication of one mitochondrial DNA strand utilizes an RNA primer made by the mtRNAP, whereas replication of the other strand uses primers generated by a mitochondrial primase (for review see Ref. 43).
To determine whether the *T. brucei* mtRNAP is also involved in replication of the kDNA, the abundance of minicircles and maxicircles was determined using dot blot analysis with specific probes. After 3 days of tetracycline induction, the abundance of minicircles remains relatively unchanged at 97.9% of preinduction levels, whereas maxicircle abundance decreases to 57% of preinduction levels (Fig. 5). After 4 days of tetracycline induction, both minicircle and maxicircle abundance continues to decrease reaching 85.6 and 30.7% of preinduction levels, respectively. However, after 4 days of tetracycline induction, the level of nuclear transcripts also decreases (data not shown), suggesting that the cells have become compromised so that the decrease in minicircle abundance may not be directly attributable to loss of the mtRNAP. Taken together, these results suggests that the mtRNAP is involved in maxicircle replication but not minicircle replication.

**DISCUSSION**

To understand mitochondrial gene regulation in trypanosomes, we must understand transcription of the unusual kinetoplast DNA. However, no *cis*-elements or *trans*-acting factors had been characterized for the transcription of either component of kinetoplast DNA. Using conserved sequences among mtRNAPs, we identified a 470-bp fragment in the *T. brucei* genome data base representing a portion of a candidate mtRNAP. RT-PCR and genomic PCR then enabled us to obtain full-length sequence of the gene. Sequence analysis indicates that it shares overall homology in its catalytic domain, including functionally conserved residues, with other single-subunit RNA polymerases. As expected for an mtRNAP, functional knockout of the gene product using RNAi resulted in the specific decrease of mitochondrial versus nuclear transcripts. Additionally, functional knockout of the mtRNAP resulted in a decrease in maxicircle abundance. This suggests that as in other organisms, the *T. brucei* mtRNAP also functions in replication of the mitochondrial genome, specifically that of maxicircles. Thus, sequence comparison and functional studies indicate that we have cloned an mtRNAP in trypanosomes.

In the simplest model of kinetoplastid mitochondrial transcription, maxicircles and minicircles would share promoter...
elements and transcriptional complexes. Alternatively in a more complicated system, different promoter elements could vary in their ability to recruit a transcriptional complex or could recruit different trans-acting factors, utilizing distinct mtRNAPs or transcription factors. We show that the mtRNAP described here transcribes numerous genes on the maxicircle, but we were unable to analyze minicircle transcription, probably because of the relatively low abundance of gRNAs. Thus, we were unable to determine whether the mtRNAP described here functions in minicircle transcription. However, we were able to demonstrate that this mtRNAP is involved in replication of the maxicircles but not of the minicircles. This suggests that replication of minicircles may involve a different mtRNAP or perhaps the mtDNA primase previously reported (48).

Although it is possible that two mtRNAPs transcribe the two distinct components of the kinetoplast, this would be unprecedented in mitochondria. However, it has been shown that chloroplasts contain both an eubacteria-like RNAP, coded by the chloroplast genome (PEP for plastid-encoded polymerase) and a single-subunit RNAP, coded by the nucleus (NEP for nuclear-encoded polymerase) and imported into the chloroplast (for reviews see Refs. 44 and 45). Reduc-tion of the maxicircle transcripts results in cell death after 6 days of tetracycline induction. This suggests that, if there is another RNAP, the two are not functionally redundant, and any minicircle-specific RNA polymerase cannot compensate in transcription of the maxicircle. Furthermore, data base searches using the conserved catalytic domain failed to identify another RNAP, the mtRNAP has been found in the mitochondrial genome of jacobid flagellates (46, 47). However, this is based solely on genetic data, and it is unknown whether these produce a functional mitochondrial polymerase and/or whether these organisms also contain a single-subunit mtRNAP.

Regardless of whether there is more than one RNAP in the trypanosome mitochondrion, we have demonstrated the specific reduction of several maxicircle transcripts and in maxicircle abundance following functional knockout of the putative mtRNAP. Kinetic analysis suggests that the reduction of mitochondrial transcripts is not simply a result of a decrease in the maxicircle abundance. With a decrease in maxicircle abundance to 87% after 1 day of tetracycline induction, there is a decrease of COI and ND1 transcripts to 65.4 and 53.6%, respectively. After 2 days of tetracycline induction, maxicircle abundance has decreased to 77.5%, whereas COI and ND1 transcripts have decreased to 17.4 and 29%, respectively. Furthermore, the transcript levels of Cyb and ND4 surprisingly increase by 36 and 85%, respectively, after 1 day of tetracycline induction. We cannot explain the initial increase in the abundance of these transcripts. However, during the first 24 h post-induction of RNAs, the transcript abundance of mtRNAP is reduced ~80% (Fig. 4B), suggesting that there remains a potentially active pool of mtRNAP. The initial increase in these transcripts may then reflect a preferential recruitment of residual transcriptional complexes to the Cyb or ND4 promoters over the promoters utilized in the transcription of COI, COII, and ND1.

We do not know how the mtRNAP is involved in maxicircle replication. One possibility is that the mtRNAP synthesizes nascent transcripts that serve as leading strand primers at the origin of maxicircle replication. Alternatively, the mtRNAP may synthesize the primers for Okazaki fragments for lagging strand replication. Another mitochondrial protein was isolated, from a related kinetoplastid, with primase activity in vitro (48). The biological function of this protein and its possible relationship to the mtRNAP has not been established. Finally, it is possible that the mtRNAP is not a primase but that maxicircle transcription may stimulate DNA replication as reported for bacterial lambda (49).

Identification of cis- and trans-acting factors in the transcription of kinetoplast DNA is the requisite first step toward understanding kinetoplast gene expression. With identification of the first factor in mitochondrial transcription, functional assays may now be developed to aid in the search for promoter elements and other trans-acting factors and to begin to dissect how transcription and RNA processing are coordinated in the regulation of gene expression.

Acknowledgments—We thank members of the Hajduk laboratory and Robert Sabatini for useful discussion and criticism.

REFERENCES

1. Klingbeil, M. M., Drew, M. E., Liu, Y., Morris, J. C., Motyka, S. A., Saxowsky, T. T., Wang, Z., and Englund, P. T. (2001) *Protozool.* **132,** 255–262
2. Shapira, T. A., and Englund, P. T. (1995) *Annu. Rev. Microbiol.* **49,** 117–143
3. Simpson, L. (1986) *Int. Rev. Cytol.* **99,** 119–179
4. Feagin, J. E., Jasmer, D. P., and Stuart, K. (1985) *Nucleic Acids Res.* **13,** 4577–4596
5. Read, L. K., Myler, P. J., and Stuart, K. (1992) *J. Biol. Chem.* **267,** 1123–1128
6. Koslowsky, D. J., and Yahampath, G. (1997) *Mol. Biochem. Parasitol.* **90,** 89–94
7. Micheleotti, E. F., Harris, M. E., Adler, B., Torri, A., and Hajduk, S. L. (1992) *Mol. Biochem. Parasitol.* **53,** 41–42
8. Benne, R., van den Burge, J., Brakenhoff, J. P., Sloof, P., van Boom, J. H., and Tromp, M. C. (1986) *Cell* **46,** 819–826
9. Alfonzo, J. D., Thiemann, O., and Simpson, L. (1997) *Nucleic Acids Res.* **25,** 3751–3759
10. Stuart, K., Allen, T. E., Heidmann, S., and Seiwert, S. D. (1997) *Microbiol. Mol. Biol. Rev.* **61,** 105–120
11. Hajduk, S. L., and Sabatini, R. S. (1998) in *Modification and Editing of RNA* (Groujean, H., and Benne, R., eds) pp. 377–393, ASM Press, Washington, DC
12. Pollard, V. W., Rohrer, S. P., Micheleotti, E. F., Hancock, K., and Hajduk, S. L. (1990) *Cell* **63,** 783–790
13. Sturm, N. R., and Simpson, L. (1990) *Cell* **61,** 879–884
14. van der Spek, H., Arts, G. J., Zwaal, R. R., van den Burge, J., Sloof, P., and Benne, R. (1991) *EMBO J.* **10,** 1217–1224
15. Steinert, M., and van Assel, S. (1988) *FEMS Microbiol. Lett.* **53,** 3–7
16. Grams, J., McManus, M. T., and Hajduk, S. L. (2000) *EMBO J.* **19,** 5525–5532
17. Masters, B. S., Stohl, L. L., and Clayton, D. A. (1987) *Cell* **51,** 89–99
18. Cermakian, N., Ikeda, T. M., Cedergren, R., and Gray, M. W. (1996) *Nucleic Acids Res.* **24,** 648–652
19. Cermakian, N., Ikeda, T. M., Miramontes, P., Lang, B. F., Gray, M. W., and Cedergren, R. (1997) *J. Mol. Biol.* **265,** 671–681
20. Wang, Z., Morris, J. C., Drew, M. E., and Englund, P. T. (2000) *J. Biol. Chem.* **275,** 40174–40179
21. Clement, S. L., and Koslowsky, D. J. (2001) *Gene (Amst.)* **272,** 209–218
22. Fire, A. (1999) *Trends Genet.* **15,** 358–363
23. Bass, B. (2001) *Nature* **411,** 235–238
24. Hammond, S. M., Caudy, A. A., and Hannon, G. J. (2001) *Nat. Rev. Genet.* **2,** 110–119
25. Sharp, P. A. (2001) *Genes Dev.* **15,** 485–490
26. Tuschl, T. (2001) *Chem. Biochem.* **2,** 239–245
27. Cunningham, I. (1977) *J. Protozool.* **24,** 325–329
28. Zwiebel, J. C. B. M., Ouellette, M., ten Asbroek, A. L. M. A., Kieft, R., Bommer, A. M. M., Clayton, C. E., and Borst, P. (1990) *EMBO J.* **9,** 2791–2801
29. Ntombo, J. M., and Englund, P. T. (1985) *J. Biol. Chem.* **260,** 5574–5579
30. Wang, Z., and Englund, P. T. (2001) *EMBO J.* **20,** 4674–4683
31. Li, J., Maga, J. A., Cermakian, N., Cedergren, R., and Feagin, J. E. (2001) *Mol. Biochem. Parasitol.* **113,** 261–267
32. Sousa, R., Chung, Y. J., Rose, J. P., and Wang, B.-C. (1993) *Nature* **364,** 593–599
33. Osuni-Davis, P. A., de Aguilera, M. C., Woody, W. R., and Woody, A.-Y.-M. (1992) *J. Mol. Biol.* **226,** 679–686
34. Woody, A.-Y.-M., Eaton, S. S., Osuni-Davis, P. A., and Woody, W. R. (1996) *Biochemistry* **35,** 144–152
35. Cermakian, N., Ikeda, T. M., Miramontes, P., Lang, B. F., Gray, M. W., and Cedergren, R. (1997) *J. Mol. Biol.* **265,** 671–681
36. Wang, Z., Morris, J. C., Drew, M. E., and Englund, P. T. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 14687–14692
37. Shi, H., Djikeng, A., Mark, T., Wirtz, E., Tschudi, C., and Ullu, E. (2000) *RNA* **6,** 1069–1076
41. Michelotti, E. F., and Hajduk, S. L. (1987) *J. Biol. Chem.* 262, 927–932
42. Feagin, J. E., and Stuart, K. (1988) *Mol. Cell. Biol.* 8, 1259–1265
43. Lecrenier, N., and Foury, F. (2000) *Gene* (Amst.) 246, 37–48
44. Iglot, G. L., and Kossel, H. (1992) *Crit. Rev. Plant Sci.* 10, 525–558
45. Gray, M. W., and Lang, B. F. (1998) *Trends Microbiol.* 6, 1–3
46. Lang, B. F., Burger, G., O’Kelly, C. J., Cedergren, R., Golding, G. B., Lemieux, C., Sankoff, D., Turmel, M., and Gray, M. W. (1997) *Nature* 387, 493–497
47. Lang, B. F., Seif, E., Gray, M., O’Kelly, C. J., and Burger, G. (1999) *J. Eukaryot. Microbiol.* 46, 320–326
48. Li, C., and Englund, P. T. (1997) *J. Biol. Chem.* 272, 20787–20792
49. Learn, B., Karzai, A. W., and McMacken, R. (1993) *Cold Spring Harb. Symp. Quant. Biol.* 58, 389–402