Differential Expression of B1-containing Transcripts in Leishmania-exposed Macrophages

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Abstract

When the parasitic protozoan Leishmania infect host macrophage cells, establishment of the infection requires alteration in the expression of genes in both the parasite and the host cells. In the early phase of infection of macrophages in vitro, Leishmania exposure affects the expression of a group of mouse macrophage genes containing the repetitive transposable element designated B1 sequence. In Leishmania-exposed macrophages compared with unexposed macrophages, small (~0.5 kilobase) B1-containing RNAs (small B1-RNAs) are down-regulated, and large (1–4 kilobases) B1-containing RNAs (large B1-RNA) are up-regulated. The down-regulation of small B1-RNAs precedes the up-regulation of large B1-RNAs in Leishmania-exposed macrophages. These differential B1-containing gene expressions in Leishmania-exposed macrophages were verified using individual small-B1-RNA and large B1-RNA. The differential expressions of the B1-containing RNAs at the early phase of Leishmania-macrophage interaction may associate the establishment of the leishmanial infection.

Protozoan parasites of the genus Leishmania cause a diverse group of human diseases designated leishmaniasis, which affects over 12 million individuals annually in over 80 countries (1). Leishmania are transmitted from infected sandfly vectors to the mammalian hosts when the fly takes a blood meal (1). Leishmania exist as extracellular flagellated promastigotes in the fly, and as intracellular non-motile amastigotes in host macrophages (1). Amastigotes multiply and kill macrophages when the infection is established.

There seem to be two key steps in establishing the leishmanial infection: the expression of the virulent factors on the Leishmania (1–8), and interference with the immune response of the macrophages (9–13). Leishmania promastigotes express surface molecules such as gp63 and lipophosphoglycan (1–8), which may act as the virulent factors. Lipophosphoglycan and gp63, for example, participate in (i) binding the macrophages, (ii) phagocytosis by macrophages, and (iii) protecting Leishmania from lysis by complement factors, from damages caused by reactive oxygen species, and from proteolysis by hydrolytic enzymes in macrophage phagolysosomes (1–8). Leishmania also affect the expression of cytokines and the activation of the helper T lymphocytes in macrophages in order to suppress the macrophage inflammatory responses (9–13). When leishmanial infection is established, the expression of pro-inflammatory cytokines such as interleukin (IL)1 and IL-12 are
decreased, and anti-inflammatory cytokines such as IL-10 and transforming growth factor β are increased (9–13). These altered cytokine expressions involve the response of T helper lymphocyte 2 (Th2) and inactivate the Th1 response, leading to the suppression of the inflammatory response of macrophages during the leishmanial infection (9–13). It is, however, not known what gene expressions initiate to trigger these immune responses in the establishment of leishmanial infection.

This study shows that B1-containing RNAs in mouse macrophages are regulated in the early phase of *Leishmania*-macrophage interaction. The mammalian genome commonly contains small interspersed repetitive elements (SINEs) such as the B1 sequence in rodents and the Alu sequence in primates (14–17). In rodent chromosomes, B1 sequences are about 150 base pairs (bp), consist of around 80,000 repetitive transposable elements (14), and are highly homologous with the Alu sequence in primates (15). SINEs emerged about 60 million years ago, which is also considered the time when mammals emerged, and have rapidly diverged in each mammalian species (18). The ancestor sequence of SINEs is known as the 7SL-RNA, which is the RNA component in the signal recognition particle participating in the transport of newly synthesized proteins from the cytoplasm to the endoplasmic reticulum (19–21).

SINEs are known to be transcribed into two types of SINE-containing RNAs: small SINE-containing RNAs such as small B1-RNAs, and large SINE-containing RNAs such as large B1-RNAs (22–36). The small SINE-RNAs are known to be synthesized by polymerase III (pol III), which recognizes the internal promoter elements inside the SINE sequence. The small SINE-RNAs usually contain many sizes of 5′-flanking regions (22), so that the sizes of pol III-directed small B1-RNAs, for example, range from 0.2 to 0.4 kilobases (23). Since these SINE-RNAs are associated with polyadenylated nuclear RNAs and heterogeneous nuclear RNAs, their role is thought to be related to the processing of other RNAs (24, 25). Small SINE-RNAs are not only transcribed, but are also processed as small cytoplasmic poly(A)⁺ SINE-RNAs (23, 26). The expression of the poly(A)⁺ small Alu-RNAs are known to increase in cells after such stresses as heat shock (27), inhibition of protein synthesis (27), viral infection (28, 29), and UV irradiation (30). These poly(A)⁺ small Alu-RNAs are also shown as inhibitors of RNA-dependent protein kinase (PKR) (31). The activated PKR phosphorylates eukaryotic translation initiation factor 2, leading to the inhibition of translation; it also phosphorylates inhibitor of nuclear factor-κB, leading to activation of the transcription of many genes (32). Despite these studies, the role of small B1-RNAs is not clearly defined. SINEs also exist at the 3′-UTR of some pol II transcripts such as large B1-RNAs and large Alu-RNAs (33–36). The role of the SINEs in the 3′-UTR is also not clear, although some evidence suggests that B1 sequences enhance the stability of pol II transcripts (36).

The extent to which early gene expression leads to the establishment of leishmanial infection is poorly understood. In the present study, the expression of B1-containing RNAs was studied in *Leishmania*-exposed macrophages in an attempt to understand the role of differential macrophage gene expression in the establishment of leishmanial infection.

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1The abbreviations used are: IL, interleukin; SINE, short interspersed element; bp, base pair(s); PCR, polymerase chain reaction; DD-RT-PCR, differential display reverse transcriptase-PCR; RT-PCR, reverse transcriptase-PCR; EST, expressed sequence tag; TFIIIS, transcription factor IIS; 5′-RACE, 5′-rapid amplification of cDNA end; pol II, RNA polymerase II; pol III, RNA polymerase III; PKR, RNA-dependent protein kinase; UTR, untranslated region.

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EXPERIMENTAL PROCEDURES

Cell Cultures

Leishmania amazonensis (LV78) and mouse macrophage cells (J774G8) were obtained from Dr. K.-P. Chang (Chicago Medical School, Chicago, IL). Leishmania promastigotes were maintained at 25 °C in Medium 199 (Life Technologies, Inc.) containing 10% heat-inactivated fetal bovine serum (37, 38). Monolayers of cultured macrophage cells were incubated at 37 °C with Leishmania promastigotes (ratio of macrophage:Leishmania = 1:10) for 1–6 h in RPMI 1640 medium (Life Technologies, Inc.) containing 20% heat-inactivated fetal bovine serum (37, 38).

Isolation of RNA

Total macrophage RNA was isolated using the Promega RNAgents total RNA isolation system. The isolated RNA (1–1.5 mg) was treated with 0.1 unit/ml RNase-free DNase I (Life Technologies, Inc.) for 30 min at 37 °C in a mixture of 10 units of human placental RNase inhibitor, 10 μM Tris-HCl, pH 8.3, 50 μM KCl, and 2.5 μM MgCl₂. The RNAs were then extracted with phenol/chloroform (3:1), precipitated with ethanol, and eluted with H₂O.

DD-RT-PCR Analysis

An aliquot (0.3 μg) of DNase-treated RNA was denatured at 65 °C for 5 min in a 30-μl mixture of 50 μM Tris-HCl, pH 8.3, 75 μM KCl, 3 μM MgCl₂, 10 μM DTT, 51.3 μM each dNTP, 3 μM dinucleotide-anchored oligo(dT) primers. This mixture was incubated at 37 °C for 10 min to allow the primer to anneal, and then incubated with Superscript II reverse transcriptase (Life Technologies, Inc.) for 1 h at 37 °C. This mixture was then incubated at 95 °C for 5 min to inactivate the reverse transcriptase. An aliquot of the synthesized cDNA (1 μl) was used in 20 μl of the cDNA amplification in a mixture of 0.5 μM arbitrary primers (10-mers), 2.5 μM dinucleotide-anchored oligo(dT) primers, 2 μM each of dGTP, dTTP, and dCTP, 10 μCi of [α-33P]dATP, 0.5 unit of AmpliTaq DNA polymerase (Perkin-Elmer) with the following PCR conditions: 5 cycles (30 s (94 °C), 1 min (40 °C), and 1 min (72 °C)), 35 cycles (30 s (94 °C), 1 min (42 °C), and 1 min (72 °C)), and 1 cycle (10 min (72 °C)) (39–41). The PCR products (4 μl) were denatured for 2 min at 80 °C and loaded onto 6% denaturing polyacrylamide gels. The gels were run at 75 watts for 2 h, dried onto Whatman 3MM paper, and exposed to x-ray film for 48 h at −70 °C. The amplified 3′-region of cDNAs designated 3′-expressed sequence tags (ESTs) were identified on the autoradiogram, excised from dried gel, and rehydrated in 100 μl of water at 100 °C for 15 min. The dissolved 3′-ESTs were amplified by two rounds of PCR in the same condition as described above. The amplified products were electrophoresed in 2% low melting agarose gel, purified, subcloned into the pCR-II plasmid vector (Invitrogen), and sequenced.

Northern Blot Analysis

The isolated total RNAs (5 μg) from Leishmania-exposed and -unexposed macrophages were electrophoresed on a 1.2% formaldehyde agarose gel (42), transferred onto a nylon membrane (Schleicher & Schuell), and hybridized with the following probes using Rapid-hyb Buffer (Amersham Pharmacia Biotech). To make a probe from a differentially expressed 3′-EST named YU7-EST in DD-RT-PCR, 25 ng of YU7-EST was excised from the pCR-II plasmid vector, purified, and labeled with [α-32P]dCTP (NEN Life Science Products) using the Random Primer labeling system (Life Technologies, Inc.). Unincorporated dNTPs were removed by centrifugation through Sephadex G-50 spin column (Amersham Pharmacia Biotech). To make the transcription factor IIS (TFIIS) probe, the coding region (720 bp) of TFIIS was amplified by PCR using a primer set (5′- CCTCGGGCACCAAGCACTTC-3′ and 5′-GCTGATCTGAGACCAGAAGGC-3′) and

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the templates, reverse transcribed macrophage cDNAs. These primers were designed from the TFIIS sequence in Ehrlich ascites tumor cells described by Hirashima et al. (43). The labeling method was the same as that for YU7-EST. To make the B1 probe, B1 sequence of YU7-EST was amplified by PCR using a primer set (5′-TGGTGGGACACGCCTTTAATC-3′ and 5′-TTTTTGGGGATGGGAGGGTTC-3′) and the template, YU7-EST recombinant pCR-II. The amplified B1 sequence (150 bp) was cloned into pCR-II vectors, and labeled using the PCR labeling system (Life Technologies, Inc.). To make gene-specific probe, gene-specific region of YU7-EST was amplified using a primer set (5′-GGTCATAGCAGTATGAGATGGAAACG-3′ and 5′-TTAAAGGCGTGTCCCAACACAC-3′), cloned into pCR-II vectors, and labeled with the same method as in the labeling of the B1 probe.

Macrophage Library Screening

mRNAs were isolated from J774G8 macrophage cells using QuickPrep micro mRNA purification kit (Amersham Pharmacia Biotech); cDNAs were then synthesized using ZAP Express cDNA synthesis kit (Stratagene). The cDNAs were cloned into a phage vector, ZAP Express (Stratagene). The constructed library was screened by using the TFIIS probe.

5′-Rapid Amplification of cDNA End (RACE)

The 5′-RACE was performed by using the protocol provided by Life Technologies, Inc., with the following modifications. The total RNA (1 μg) was reverse transcribed using two primers synthesized from YU7-EST: (i) 5′-TTTTTGGGGATGGGAGGGTTC-3′ designed from the region between the B1 sequence and the poly(A)+ region, and (ii) 5′-GTGATTTCTTGGACACTGAGAACGCTTG-3′ designed from the gene-specific region. These two primers generate two kinds of cDNA pools in the reverse transcriptase reaction. These cDNA pools were purified with the Sephadex G-50 column (Amersham Pharmacia Biotech) to eliminate these primers and to recover small (under 500 base pairs) cDNAs. These two kinds of cDNA pools were tailed by adding cytosine residues at the 5′ ends by using terminal transferase (Life Technologies, Inc.), and amplified by PCR using a primer set (5′-CACTGAGAACAGCTTGAGAAAG-3′ designed from YU7-EST and oligo(G)-linked amplification primer provided by Life Technologies, Inc.). PCR was performed in a reaction mixture containing 0.5 μM of each primer, 200 μM each dGTP, dTTP, dATP, and dCTP, 0.5 unit of AmpliTaq DNA polymerase (Perkin-Elmer) with the following conditions: 1 cycle (2 min (94 °C)), 35 cycles (1 min (94 °C), 1 min (55 °C), 2 min (72 °C)), and 1 cycle (7 min (72 °C)). The primary PCR products were further amplified by a nested gene-specific primer (5′-GCTTCCGAATCTAGTGCTATGACCC-3′) designed from YU7-EST and the amplification primer without the oligo(G) anchor (Life Technologies, Inc.). The secondary PCR products were purified, cloned into pCR-II (Invitrogen), and sequenced.

RT-PCR Analysis

DNase-treated total RNA (5 μg) from Leishmania-exposed and -unexposed macrophages were reverse transcribed using oligo(dT) primers as described above for the DD-RT-PCR analysis. The synthesized cDNAs (1 μl) were amplified by PCR using the following primer set: FP (5′-CCTTCCACATGGTGCCCAAG-3′), forward primer from 5′-RACE product and CP (5′-CTTCCGAATCTAGTGCTATGACCC-3′), reverse primer from the connecting region between YU7-EST and the 5′-RACE product. The other reverse primer, BP (5′-TCGAACGCAAAATCTGGCGCCGG-3′) from the B1 region of YU7-EST, was also used with the forward primer to amplify the cDNAs. The amplification condition was the same as that of the PCR amplification used in the 5′-RACE described above.
RESULTS

Leishmania-exposed Macrophages Contain Less YU7-EST than Control Macrophages

To analyze differential gene expressions in the early phase of leishmanial infection, poly(A)+ RNAs in Leishmania-exposed and -unexposed mouse macrophages were compared by DD-RT-PCR analysis. The autoradiogram of DD-RT-PCR showed that a 3'-EST designated YU7-EST was amplified in control macrophages, but not in Leishmania-exposed macrophages (Fig. 1A). The YU7-EST was subcloned and sequenced (Fig. 1B). The underlined nucleotides in Fig. 1B are the arbitrary primer sequence (5′-TCGGTCATAG-3′) and the complementary sequence of the dinucleotide-anchored oligo(dT) primer (5′-TTTTTTTTTTTGG-3′), which were used for cDNA amplification in the DD-RT-PCR analysis.

YU7-EST Contains B1 Sequence at the 3′ End

YU7-EST has a homologous region with more than 7,000 mouse gene sequences detected in GenBank™; this homologous region was identified as B1 sequences. The components of YU7-EST are 105 bp of gene-specific region, 151 bp of B1 sequence, and 11 bp of part of poly(A)+ region (Fig. 1, B and C). The gene-specific region did not have any significant homology with sequences in GenBank™.

Small B1-RNAs Are Down-regulated, and Large B1-RNAs Are Up-regulated in Leishmania-exposed Macrophages

YU7-EST was used as a probe in Northern analysis to compare the hybridization pattern with total RNAs from Leishmania-exposed and -unexposed macrophages. As shown in Fig. 2A, the YU7-EST probe hybridized with many RNA species, but hybridizing RNAs were somewhat different in size in the Leishmania-exposed versus -unexposed macrophage RNAs: small (~0.5 kilobase) RNAs (small B1-RNAs) were down-regulated and large (1–4 kilobases) RNAs (large B1-RNAs) were up-regulated, when macrophages were exposed to Leishmania for 2 h (Fig. 2A).

To analyze the hybridization pattern further, the gene-specific sequence and the B1 sequence of YU7-EST were separately hybridized with RNAs isolated from macrophages which were exposed to Leishmania for up to 6 h. Northern analysis with the B1 probe (Fig. 2B) showed a hybridization pattern similar to that in Fig. 2A; small B1-RNAs were down-regulated, and large B1-RNAs were up-regulated in Leishmania-exposed macrophages. The up-regulation of large B1-RNAs in Northern analysis with the YU7-EST probe (Fig. 2A) is more striking than that seen in Northern analysis with the B1 probe (Fig. 2B). This time-course experiment showed that the down-regulation of small B1-RNAs occurred prior to the up-regulation of large B1-RNAs in Leishmania-exposed macrophages (Fig. 2B). Northern analysis with the gene-specific probe did not show any significant hybridization on the Northern autoradiogram.

The Full Length of the YU7-RNA

By using 5′-RACE analysis, the extended 5′-region of YU7-EST was identified to be 147 bp (Fig. 3A); the full length of the YU7-RNA transcript was estimated to be 398 bases by connecting the nucleotide sequences of 5′-RACE product and YU7-EST (Fig. 3B). RT-PCR was performed to confirm whether this expected YU7-RNA exists in macrophages. The primer sets FP/BP and FP/CP generated the expected sizes of PCR products (Fig. 3C). These products were sequenced and confirmed to be part of YU7-cDNAs. As shown in Fig. 3C, these RT-PCR products were down-regulated in Leishmania-exposed macrophages. This result confirmed that YU7-RNA is one of the small B1-RNAs, and supported the data in Fig. 2. In the B1 sequence of the YU7-RNA, the putative A-box (5′-GGTGTGGT-3′) and B-box

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(5′-GTTCGAGGC-3′) for the pol III promoter region were identified by alignment with 10 other known cytoplasmic polyadenylated small B1-RNAs described by Maraia et al. (44).

**A Large B1-RNA, TFIIS mRNA, Is Overexpressed in Leishmania-exposed Macrophages**

To verify the overexpression of the large B1-containing RNAs in the Leishmania-exposed macrophages, TFIIS, a general transcription elongation factor, was selected as a representative of pol II-directed large B1-RNA from the BLAST result list aligned with YU7-EST, because some TFIIS isoforms contain the B1 sequence at the 3′-UTR (43, 45–48). To analyze the expression of the TFIIS in the macrophages of our experimental system, macrophage cDNA library was constructed using J774G8 mRNAs, and then screened with the TFIIS probe. The library screening confirmed that the majority of the TFIIS mRNAs in J774G8 cells contained B1 sequence at the 3′-UTR. Northern analysis with TFIIS probe showed that the TFIIS mRNA was up-regulated in the Leishmania-exposed macrophages (Fig. 4). These data are consistent with the idea that small B1-RNAs are decreased while large B1-RNAs are increased in macrophages during the early phase of leishmanial interaction.

**DISCUSSION**

It has been thought that the many repeats of SINEs such as B1 and Alu sequences serve some physiological roles, but this has not been proven (49). Two important findings were established by this study relating to the SINE-RNAs. First, the pool size of small B1-RNAs is down-regulated in Leishmania-exposed mouse macrophages. Second, the pool size of the pol II-directed large B1-RNAs is up-regulated in Leishmania-exposed macrophages. The down-regulation of small B1-RNAs precedes the up-regulation of large B1-RNAs, and this alteration occurs within a short period (1–6 h) of Leishmania-macrophage co-incubation.

The mechanisms underlying the regulation of small and large B1-RNAs were not explored in this study. However, the stabilization of SINE-RNAs has been reported to result from the functional alteration of chromatin structure (50), strength of the internal promoter (26), DNA methylation (51), and posttranscriptional stabilization (52). The rapid and transient macrophage gene expressions during the early phase of leishmanial infection have not been extensively reported.

There is evidence that the pool size of small SINE-RNAs is altered when cells are treated with specific conditions (28, 29, 52). In virus-infected cells, for example, small Alu-RNAs are up-regulated in human cells (28, 29). When cells are incubated with wild type p53 (a tumor suppresser protein), the small Alu-RNAs are down-regulated (52). Chu et al. (31) also showed evidence indicating that small Alu-RNAs have a potential to be inhibitors for PKR. PKR is known to be induced in stressed cells by such factors as UV irradiation and viral infection (32, 53). The activated PKR phosphorylates eukaryotic translation initiation factor and inhibitor of nuclear factor-κB (32). In this manner, the activated PKR inhibits translation and stimulates the transcription in host cells. The activation of PKR seems to be needed for the inhibition of the viral protein translation for host survival, and viruses indeed possess the mechanism to inhibit the activation of PKR for their survival (54). The transient increase of the small Alu-RNAs after viral infection seems to inhibit the PKR, which may bring the cells back to their normal state. It is intriguing to speculate that the increase in small SINE-RNAs may be related to the recovery of “damaged cells” to “normal cells.” For example, the decrease in small B1-RNAs during leishmanial interaction may be a signal for damaged cells, such that the decrease of the small B1-RNAs may inhibit the recovery from damaged cells to normal cells. Leishmanial interaction may inhibit the induction of the small B1-RNAs in macrophages; this would keep macrophages in a non-viable state while leishmanial infection is established. However, not much detail is known about how leishmanial interaction affects the down-regulation of these pol III-directed synthesis of small B1-RNAs.
in macrophages. The knowledge of this mechanism will be helpful in our understanding of how *Leishmania* sends signals to macrophages at the initial phase of infection.

Concerning pol II-directed transcripts, many have the B1 sequence mainly at the 3′-UTR (33–36). The role of the B1 sequence at the 3′-UTR is not clear, although some evidence suggests that it enhances the stability of pol II transcripts (36). We selected TFIIS as a representative of the large B1-RNAs because TFIIS is general transcription factor (43, 45–48) and TFIIS mRNA is expected to be commonly expressed in many cell types.

Do the down-regulation of small B1-RNAs and the up-regulation of large B1-RNAs involve B1-binding proteins in *Leishmania*-exposed mouse macrophages? It has been reported that the families of SINE-RNAs have conserved secondary structures (18, 19, 44), which serve as binding sites for SINE-binding proteins (55–59). In our experimental system, the expression of the small B1-RNAs was abundant in the control macrophages compared with that in *Leishmania*-exposed macrophages. This observation may indicate that most of B1-binding proteins are occupied on the small B1-RNAs in control macrophages. Hypothetically, a decrease of small B1-RNAs in *Leishmania*-exposed macrophages may make more B1-binding proteins available in the macrophage cytoplasm; these could then bind to the B1 sequence at the 3′-UTR of pol II transcripts and stabilize them in *Leishmania*-exposed macrophages.

Acknowledgments

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Fig. 1. DD-RT-PCR analysis showing B1-containing 3′-EST, YU7-EST, in the control macrophages but not in Leishmania-exposed macrophages

A, autoradiogram of DD-RT-PCR. Total RNAs (0.3 μg) from Leishmania-exposed and -unexposed mouse macrophages were compared using DD-RT-PCR analysis. One of the 3′-EST, designated YU7-EST, was amplified by DD-RT-PCR in the control macrophages (lane M), not in Leishmania-exposed macrophages (lane L+M). B, the nucleotide sequence of YU7-EST. YU-7-EST was recovered from polyacrylamide gel in DD-RT-PCR analysis, and the nucleotides were sequenced. Underlined nucleotides are 10-mer of the arbitrary primer and the complementary sequence of dinucleotide-anchored oligo(dT)primer used in DD-RT-PCR analysis. C, the schematic representation of YU7-EST. YU7-EST contains gene-specific sequence at 5′ end and B1 sequence at the 3′ end just before poly(A)^+ tail.
Fig. 2. Small B1-RNAs are down-regulated, and large B1-RNAs are up-regulated in *Leishmania*-exposed macrophages

A, Northern blot analysis with YU7-EST as a probe. *Upper panel*, ethidium bromide-stained RNAs in formaldehyde agarose gel. *M*, control macrophages; *L*, *Leishmania*-exposed macrophages (2-h exposure). *Lower panel*, Northern autoradiogram. The transferred RNAs from agarose gel onto the nylon membrane were hybridized with YU7-EST probe. B, Northern blot analysis with B1 probe at different times after *Leishmania* exposure. *Upper panel*, ethidium bromide-stained RNAs in formaldehyde agarose gel. Macrophages (10^6 cells) were exposed to *Leishmania* (10^7 cells) for 1, 2, 4, and 6 h. *Lower panel*, Northern autoradiogram. The transferred RNAs on the nylon membrane were hybridized with the B1 probe.
Fig. 3. Characterization of YU7-RNA

A, nucleotide sequences of 5′-RACE product (147 bp) and the primer from YU7-EST used in the 5′-RACE. The reverse primer from YU7-EST was extended on the cDNA templates that were reverse transcribed from DNase-treated total macrophage RNAs. The extended fragment was amplified, cloned into a cloning vector, and sequenced. B, full length of the transcript, YU7-RNA, was calculated to be 398 bases by connecting the 5′-RACE product and YU7-EST. On this expected YU7-RNA, four primers were designed for RT-PCR: FP, forward primer from the 5′-RACE product; CP, reverse primer designed from the connection between the 5′-RACE product and YU7-EST; BP, reverse primer designed from B1 sequence; RP, oligo(dT) primer. C, ethidium bromide-stained RT-PCR product in agarose gel. Total RNAs were reverse transcribed with the oligo(dT) primer, RP. The synthesized cDNAs were amplified with two primer sets, FP/BP and FP/CP. Both RT-PCR amplifications generated 314- and 164-bp bands, as expected. S, 100-bp ladder DNA size standard.
Fig. 4. Up-regulation of TFIIS in *Leishmania*-exposed macrophages

A, ethidium bromide-stained macrophage RNAs in formaldehyde agarose gel. B, Northern autoradiogram with TFIIS probe. The transferred RNAs from agarose gel onto the nylon membrane were originally hybridized with YU7-EST probe (Fig. 2A). The YU7-EST probe was stripped from the RNA blot and re-hybridized with the TFIIS probe.