**METHODS**

**Parasites and cell extracts**

The pleiomorphic *T. brucei* strain TREU927, which has the most complete genome sequence at present [54], was employed for production of slBF and PCF. Three biological replicates of each stage were used (see **Additional File 1, Table S1**). slBF were grown in irradiated Wistar rats following injection of 108 parasites derived from stabilates following IACUC approved protocols. The parasites were harvested on day 3 at a parasitemia of 5×107-1×108 cells per ml. Only parasite populations with greater than 99% slender cells were used. After harvest, the blood was centrifuged and the buffy coat extracted and placed into 20 ml HMI-9 medium (without serum) pre-warmed to 37°C. To arrest translation, cycloheximide was added to 100 g/ml and incubated for 2 minutes at 37°. To rapidly chill the cells, 300 ml of ice-cold phosphate buffered saline with glucose (PSG) was added and the cells were pelleted at 4°. Parasites from 2-3 animals infected from the same culture of *in vitro* grown parasites were pooled and lysates prepared as described below. Microscopic analysis showed that rat white blood cells represented less than 1% of the population. For cBF, a derivative of *T. brucei* monomorphic strain Lister 427 was grown *in vitro* in HMI-9 medium [63] and harvested when the cultures were between 8×105 and 1.6×106 parasites/ml. The cultures were centrifuged for 10 minutes at 900×g, resuspended in 25 ml of pre-warmed serum-free medium, treated with cycloheximide and rapidly chilled as above. Three biological replicates of these *in vitro* derived cBF were used for comparative purposes. We grew strain 927 PCF in SDM79 medium containing glucose [64], with 2-4×109 parasites being harvested in mid-log phase (density of 5×106-1.2×107 cells/ml). The initial large volume of culture was centrifuged at 5000×g for 5 minutes at room temperature and the pellet resuspended in 50 ml of medium lacking serum. The parasites were incubated for 2 min in cycloheximide as above, rapidly chilled by the addition of 250 ml PSG, and collected by centrifugation.

Cell pellets were resuspended in Buffer A (10 mM Tris pH 7.4, 300 mM KCl, 10 mM MgCl2, plus protease inhibitors [62]) to approximately 1.3×109 cells/ml. Approximately one-third of the sample was placed into TRIzol (Life Technologies) for RNA extraction following the manufacturer’s suggested protocol. To the remainder, one-sixth volume of buffer A containing 0.2M sucrose and 1.2% Triton N-101 was added and the samples were homogenized (30 strokes using a chilled dounce with a 0.004-0.006 inch clearance pestle). After transfer to a pre-chilled microfuge tube, the samples were clarified by centrifugation in a microfuge at 15,000 rpm for one minute. The supernatant was withdrawn, pooled if needed, and then aliquots flash frozen in liquid nitrogen for storage at -70°C. These extracts were then used for ribosome footprinting or polysome gradients*.* Polysome analysis was performed as previously described [62].

**Library preparation and sequencing**

*Ribosome footprinting*. Preliminary experiments established the appropriate conditions for RNAse I treatment of lysates (**Additional File 2,** **Figure S1A**). After thawing on ice, RNase I (Ambion) was added at 30 units/OD260 of lysate. Samples were then incubated for 1 hour at room temperature. RNase digestion was stopped by adding 400 units RNasin (Promega). Samples were them layered over a 1 ml 1M sucrose cushion prepared in buffer A and ribosomes were pelleted by centrifugation for 4 hours at 70,000×g in an SW55 rotor. After removing the supernatant, the ribosomal pellet was resuspended in 500 l buffer A with 10 mM EDTA replacing the MgCl2 to dissociate the ribosomes (**Additional File 2,** **Figure S1B**). The protected fragments were then separated from contaminating larger ribosomal RNA fragments by passage through an Amicon Ultra-4 or YM-100 column with 100,000 MW cut-off. The RNA in the flow-through (400 μl) was extracted with phenol:CHCl3:isoamyl alcohol and the RNA precipitated.

*mRNA libraries.* Poly(A)+ RNA was isolated using Dynabeads mRNA Direct (Life Technologies). RNA was fragmented as described [36] and fragments between 30 and 70 nucleotides isolated. For a detailed protocol on generating sequencing libraries for both the ribosome protected and fragmented mRNA library see Ingolia *et al*. [65]. Briefly, following dephosphorylation the adapter Linker-1 (IDT) was ligated to the 3’ end of the fragment and the ligated product gel purified. The adapter was used for priming reverse transcription with the primer RP\_index\_RT (all primers are provided in **Additional File 1, Table S5**). Following gel purification the cDNA was circularized with Circ Ligase (Epicenter Biotechnologies). Circles containing ribosomal RNA were subtracted using biotinylated primers at 10 M. The final library was generated by PCR using RP\_index\_PCR\_forward and one of the RP\_index reverse primers.

*SL RNA-seq libraries*. Libraries enriched for the 5’ ends of mRNAs were constructed from three biological samples of strain 927 (two PCF and one slBF of the biological samples above), as described previously [66]. In brief, RNA was prepared and cDNA synthesized using primer Random5. Second strand synthesis was primed using SL\_2nd primer3, which matches the 3’ *T. brucei* SL sequence. The sequencing library was generated by PCR using the primer Multi-PCR P2 and one of the RP\_index\_PCR\_reverse primers.

All libraries were sequenced using Illumina GA II machines at the High Throughput Genomics Unit at the University of Washington to generate ~36 nt reads using the proprietary Illumina read 1 sequencing primer (Rd1 SP) for fragmented mRNA and ribosome profiling libraries, or a custom sequencing primer (SL\_SEQ\_Primer2) for the SL RNA-seq libraries, as well as the Illumina indexing sequencing primer (Index SP).