Supplementary Material

Construction of TAP Plasmids

For construction of the LtREL1-TAP vector, restriction sites for BamHI and XbaI were introduced during PCR of the LtREL1 to the 5' and 3' ends, respectively using the following oligos:

5' primer with BamHI site added, 5'- AAGGATCCATGCGTCGACTGGCACTGCGTTG-3'

3' primer with XbaI site added, 5'-TTTCTAGACTGCGCCTCTGCCTTCTCCTGC-3'

The TAP cassette was amplified from pBS1479 (Puig et al., 2001) with the introduction of XbaI and MunI sites at the 5' and 3' ends, respectively using the following oligos:

5' primer with XbaI site added, 5'-TCTAGAGGATCCATGGAAAAGAGAAGATGG-3'

3' primer with MunI site added, 5'-CAATTGGGTTGACTTCCCCGCGGAATTCGC-3'

These two fragments were co-ligated into the BamHI/MunI-digested pCATSUP1 vector (Alfonzo et al., 1999), a derivative of the pX expression vector (LeBowitz et al., 1990).

To construct the LtREL2-TAP vector, the full length LtREL2 gene was PCR amplified from the genomic insert and XmaI and XbaI sites introduced at the 5' and 3' ends using the following primers:

5' primer with XmaI site added, 5'-CCCGGGATGCTTCGGCGATG-3'

3' primer with XbaI site added, 5'- TCTAGAATAGCACGATTTTCTGTCCGCCTCC-3'

This PCR fragment was then ligated into the p26-TAP vector (Aphasizhev et al., 2002a) digested with XmaI and XbaI.

To construct the LmLC-4-TAP vector, restriction sites for BamHI were introduced to the 5' and 3' ends during PCR of the LmLC-4 using the following oligos:

5’ primer, 5’-CGGGATCCATGACGATGAAGAGGCGGATCGG-3’

3’ primer, 5’-CGGGATCCTTTATCCGTAAATGGCAAAACG-3’

This PCR fragment was then ligated into the BamHI site of the p26-TAP vector (Aphasizhev et al., 2002).

The constructs were verified by sequencing and transfected into *L. tarentolae* (UC strain) by electroporation. Selection was performed with 200 μg/ml of Geneticin (Gibco) on agar plates containing BHI medium (Difco) supplemented 10% fetal calf serum, 10 μg/ml hemin and 0.8% folic acid (S. Sbicego and L. Simpson, unpublished).

The sequences of all constructs in Genbank format can be obtained at the following web site: http://www.hhmi.ucla.edu/simpson/vectors/new/newvectors.htm

TAP Purification

Mitochondria (400 mg protein) were lysed on ice for 15 min in 12 ml of TMK buffer (20 mM Tris-HCl, pH 7.6, 60 mM KCl, 10 mM $MgCl_2$) plus 0.5% NP40. One tablet of Complete proteinase inhibitors (Roche) was added directly to the extract. After centrifugation at 200,000 g for 10 min, the pellet was sonicated three times for 10 sec in 12 ml of the same buffer without detergent and the centrifugation repeated. Supernatants from both extraction were pooled and incubated with 1 ml of IgG Sepharose FF (AP Biotech) for 2 hr in batch, transferred to three disposable 1 ml columns (Pierce) and each column was washed sequentially with 25 ml of TMK buffer plus 0.1% of NP40 and 10 ml of TEV proteinase cleavage buffer (20 mM Tris-HCl, pH 7.6, 60 mM KCl, 10 mM $MgCl_2$, 0.1% NP40, 1 mM DTT). One ml of the same buffer with 100 U of TEV proteinase was added to each column and columns were incubated overnight with

constant mixing. The IgG resin was drained and washed with 2 ml of CB buffer (20 mM Tris-HCl, pH 7.6, 60 mM KCl, 10 mM $MgCl_2$, 1 mM imidazsole, 10 mM β-mercaptoethanol, 2 mM $CaCl_2$, 0.1% NP40). Eluted material was incubated with 1 ml of calmodulin agarose (Stratagene) for 1-2 hr, transferred to two disposable 1 ml columns (Pierce), washed with 20 ml of CB buffer and eluted with 4 ml of 20 mM Tris-HCl, pH 7.6, 60 mM KCl, 10 mM $MgCl_2$, 2 mM EGTA, 2 mM CHAPS. Eluted proteins were concentrated with Slide-A-Lyzer solution (Pierce) to 300 µl and loaded on 10-30% glycerol gradients with 20 mM Tris-HCl, pH 7.6, 60 mM KCl, 10 mM $MgCl_2$, 2 mM EGTA, 2 mM CHAPS. Sedimentation was performed in the SW41 rotor (Beckmann) for 20 hr at 30,000 RPM. Fractions of 750 µl were collected from the top of the gradient using an Isco UA-6 fractionator.

Mass Spectrometric Analysis

The recovered tryptic peptides were adsorbed onto µC18 ZipTips (Millipore, Bedford, MA), washed with 0.1% TFA, then eluted with 3-4 µL of 50% ACN/0.1% TFA. One µl of the tryptic peptide mixture from each gel band was combined with an equal volume of matrix solution and allowed to dry on the MALDI target. The matrix solution used was a 10 g/L solution of alpha-cyano-4-hydroxycinnamic acid in 50% acetonitrile/50% 0.1% aqueous TFA. All mass spectrometric measurements were performed on an Applied Biosystems (Foster City, CA) 4700 Proteomics Analyzer, which is a tandem time-of-flight instrument (TOF/TOF) with a MALDI ion source (Bienvenut et al., 2002). Normal reflector spectra were acquired first to determine the masses of the peptides of interest. Trypsin autolysis peaks were used to calibrate the mass scale, typically giving masses to better than 10 ppm accuracy. MS/MS CID spectra were acquired

manually on selected peptides, using air as the collision gas. Default calibration of the mass scale was used for all MS/MS spectra, which typically provided fragment masses accurate to <0.1 Da.

Peptide sequences were determined by manual interpretation of the MS/MS spectra. Calculated masses of the sequences determined were checked against the experimental accurate masses to verify that they were consistent. In most instances (about 60-70%), I and L could be distinguished by the presence of *w* ions. Q and K could usually be determined from the accurate mass of the whole peptide. The sequences determined were searched using Protein Prospector (UCSF) against the nr NCBI database (7/30/02) as well as the parasite genome databases at:

http://www.genedb.org

Western Blotting

Electrophoretic transfer on nitrocellulose membrane from SDS gels was performed for 1.5 hr and for 10 hr from native gels in Mini Trans-Blot cells (Biorad) in 25 mM Tris, 190 mM glycine, 10% methanol at 80 V. Gels and membrane were pre-soaked in transfer buffer for 10 min prior to blotting. Immunodetection was performed with affinity purified rabbit antibody and SuperSignal West Pico chemiluminescent substrate (Pierce) by standard techniques. Membrane blocking and antibody incubations were done in 5% milk in PBS buffer, pH 7.5, with 0.05% Tween 20 for 1 hr, and all washes were in 1xPBS/0.05% Tween 20.

References

Alfonzo,J.D., Blanc,V., Estevez,A.M., Rubio,M.A., and Simpson,L. (1999). C to U editing of the anticodon of imported mitochondrial $tRNA^{Trp}$ allows decoding of the UGA stop codon in Leishmania tarentolae. *EMBO J.,* **18**.

Aphasizhev,R., Aphasizheva,I., Nelson R.E. and Simpson,L. (2002). A 100 kDa complex of two RNA-binding proteins from mitochondria of Leishmania tarentolae catalyzes RNA annealing and interacts with several editing components. *RNA,* **In press**.

Bienvenut,W.V., Deon,C., Pasquarello,C., Campbell,J.M., Sanchez,J.C., Vestal,M.L., and Hochstrasser,D.F. (2002). Matrix-assisted laser desorption/ionization-tandem mass spectrometry with high resolution and sensitivity for identification and characterization of proteins. *Proteomics,* **2**, 868-876.

LeBowitz,J.H., Coburn,C.M., McMahon-Pratt,D., and Beverley,S.M. (1990). Development of a stable Leishmania expression vector and application to the study of parasite surface antigen genes. *Proc. Natl. Acad. Sci. U. S. A.,* **87**, 9736-9740.

Puig,O., Caspary,F., Rigaut,G., Rutz,B., Bouveret,E., Bragado-Nilsson,E., Wilm,M., and Seraphin,B. (2001). The tandem affinity purification (TAP) method: a general procedure of protein complex purification. *Methods,* **24**, 218-229.