Isolation, cDNA Sequences, and Biochemical Characterization of the Major Cyclosporin-binding Proteins of Toxoplasma gondii

Kevin P. High†‡§, Keith A. Joiner†¶ and Robert E. Handschumacher

From the Infectious Diseases Section, the Department of Medicine and the Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510

The activities of the immunosuppressive, antifungal compounds cyclosporin A (CsA), FK-506, and rapamycin are dependent upon high affinity binding proteins collectively termed immunophilins. We report the isolation, biochemical characterization, and amino acid sequences of two major CsA-binding proteins, cyclophilins, from the pathogenic protozoan, Toxoplasma gondii. The 18.5- and 20-kDa molecular mass proteins exhibit peptidylproline cis-trans-isomerase activity, which is inhabitable by 10 μM CsA. The amino acid sequences of these two proteins, deduced from cDNA clones, reveal up to 70% amino acid identity to previously isolated cyclophilins. The 18.5-kDa protein appears to be synthesized as a precursor with a 15 amino acid signal peptide. The amino-terminal region of the mature 20-kDa protein has significant homology to the B subunit of the calmodulin-dependent phosphatase, calcineurin. The two T. gondii cyclophilins are products of different genes and appear to have different subcellular distributions.

The T-cell immunosuppressive agents cyclosporin A (CsA), FK-506, and rapamycin have revolutionized solid organ and bone marrow transplantation (1–5). All three compounds, however, were initially described as antifungal agents (6–8). The immunosuppressive and antimicrobial effects of these compounds involve receptor proteins collectively termed immunophilins (9–12). Immunophilins catalyze the peptidylproline cis-trans-isomerase (PPIase) activity, which is inhabitable by CsA. Both the 18.5- and 20-kDa molecular mass proteins exhibit PPIase activity, which is inhabitable by CsA. The amino acid sequences of these two proteins, deduced from cDNA clones, reveal up to 70% amino acid identity to previously isolated cyclophilins. The 18.5-kDa protein appears to be synthesized as a precursor with a 15 amino acid signal peptide. The amino-terminal region of the mature 20-kDa protein has significant homology to the B subunit of the calmodulin-dependent phosphatase, calcineurin. The two T. gondii cyclophilins are products of different genes and appear to have different subcellular distributions.
For subcellular localization experiments, T. gondii prepared as above were resuspended in phosphate-buffered saline (with phenylmethylsulfonyl fluoride, leupeptin, and aprotinin as above) rather than CHAPS buffer. Organisms were frozen at 20 °C, thawed twice, and then lysed by vortexing for 20 s with one-half volume of glass beads (0.5 mm). The resulting lysates were spun at 10,000 × g. The pellet was extracted with 1.5% CHAPS for 45 min on ice (the supernatant fraction is referred to as the 10,000 × g pellet extract). The supernatant (hereafter, this fraction is referred to as the 10,000 × g supernatant extract) and the 10,000 × g pellet extract were both spun at 100,000 × g to remove particulates and processed further as described below.

Protein Purification/Preparation of CsA-Binding Proteins—T. gondii lysates were screened for CsA binding activity and the concentration of protein with high affinity for CsA determined using the LH-20 assay previously described (41, 42). The percentage of CsA-binding protein was then calculated by determining the total protein concentration by using an Applied Biosystems model 470A gas phase Sequenator with an on-line model 120A phenylthiohydantoin derivative analyzer. Additional sequences were obtained by cyanogen bromide elution of the 18.5-kDa protein bands from the phenylthiohydantoin fragment trypsin digestion. Peptides were separated on a 2.1 × 250-mm Vydac C-18 reverse phase column in a Hewlett Packard model 1090 high pressure liquid chromatography system as described previously (44). Peptide fragments were sequenced as above and matched against those recorded in the National Center for Biotechnological Information database (45, 46).

PPase Assay—PPase activity of T. gondii proteins eluted from the 8-ornithino-CsA column was assessed using fractions eluted with low pH buffer to avoid CsA inhibition. Isomerization of N-succinyl-Ala-Ala-Pro-Phel-p-nitroanilide) was measured in the chymotrypsin cleavage assay described by Kofron et al. (47) at 10 °C using 30 μM substrate and 60 μM chymotrypsin in 35 mM Hepes pH 8. T. gondii protein concentration was determined by amino acid analysis or Bio-Rad (Hercules, CA) protein assay standardized for these particular proteins based on amino acid analysis. CsA-mediated inhibition of PPase activity was determined by adding increasing amounts of CsA to 40% ethanol (not to exceed a final concentration of 0.4% ethanol) to the PPase assay. The mixture was then incubated at 10 °C for 20 min, followed by the addition of chymotrypsin and then substrate.

Production of DNA Probes by the Polymerase Chain Reaction (PCR) to Generate Primers Corresponding to Two Peptide Sequences in Each of the Major T. gondii Proteins were synthesized in the William Keck Foundation, Yale School of Medicine, Oligonucleotide Synthesis Laboratory. The sequences used for the 18.5-kDa protein were: 5'-TA(CT)- \[\text{ATGGAG(CTTA)ACAT(CT)GAG}3' \text{ (sense sequence)} \text{and 5'}-\text{GA(TGAT)CTTGCA(GA)}(GC)3' \text{ (antisense sequence)} \text{ corresponding to the derived peptide sequence YMDIDIDG} \text{VMDIDIDG} \text{ and GDFENH, respectively. The primer sequences used for the 20-kDa protein were: 5'-AT(AGG)AT(CTTA)AA(GA)CA(GC)3' \text{ (sense sequence) and 5'}-\text{(AAG)AAAGT(TCTT)TCGAC(CTGCG)CGC(AA)GT(TTCA)TTY(TC)TT}3' \text{ (antisense sequence). For DNA sequencing, the peptides were digested using PKK22 and treated using an Applied Biosystems model 470A gas phase Sequenator with an on-line model 120A phenylthiohydantoin derivative analyzer. DNA sequencing was performed in the William Keck Foundation, Yale School of Medicine, Biotechnology Resource Laboratory.}

Production of Anti-T. gondii Cyclophilin Antisera—Approximately 2 μg of each of T. gondii cyclophilin isolated as above were used as the partial source for the CHAPS-PAGE gels, and the gel piece containing both protein bands was sliced out and sequenced using the Sequenase kit (United States Biochemical, Cleveland, OH). Protein bands were cut out, and sequencing was performed in the William Keck Foundation/Yale School of Medicine, Protein and Nucleic Acid Chemistry Facility. NH2-terminal sequencing was accomplished using an Applied Biosystems model 470A gas phase Sequenator with an on-line model 120A phenylthiohydantoin derivative analyzer. Additional sequences were obtained by cyanogen bromide elution of the 18.5-kDa protein bands from the phenylthiohydantoin fragment trypsin digestion. Peptides were separated on a 2.1 × 250-mm Vydac C-18 reverse phase column in a Hewlett Packard model 1090 high pressure liquid chromatography system as described previously (44). Peptide fragments were sequenced as above and matched against those recorded in the National Center for Biotechnological Information database (45, 46).
FIG. 1. Purification of T. gondii cyclophilins. 10^9 parasites were lysed in CHAPS as described under “Experimental Procedures.” Whole T. gondii lysates (lane A) were passed through a 0.3-mI 8-ornithino-CsA column and the column washed with >20-column volumes of wash buffer. Fractions were eluted with 10 μg/ml CsA in wash buffer (lane B) or low pH buffer (lanes C, pH 3.5, and D, pH 3.5), and the eluates were concentrated as described under “Experimental Procedures” before SDS-PAGE. Fifty micrograms of T. gondii CHAPS lysate protein were loaded in lane A. Fifty percent of the total yield from 10^9 parasites was loaded in lanes C and D.

chemical Corp.) and [35S]-dATP (DuPont NEN) using T3 and T7 primers corresponding to plasmid DNA regions. Sequenced DNA was separated on 6% polyacrylamide gels and the gels exposed to Kodak-XAR film (Rochester, NY) after drying.

[35P]-labeled DNA probes were prepared by labeling of the specific PCR products above using the Random primer kit (Boehringer Mannheim) and [32P]dCTP (DuPont NEN) (specific activity, 107-10^8 dpm/μg DNA). Approximately 50,000 λ phage were plated and plaque lifts performed using nitricellulose membranes (Schleicher and Schuell). Filters were hybridized overnight with labeled probe at 42 °C in 50% formamide, 5 x Denhardt’s, 0.1% SDS, 6 x TEN (10 mM Tris, pH 8.0, 100 mM NaCl, 1 mM EDTA) with 50 ng/ml RNA. Filters were washed at 62 °C in progressively lower concentrations of SSC buffer with 0.1% SDS until background radioactive counts were minimized. The filters were then exposed to Kodak-XAR film at ~80 °C for 24–36 h. Positive plaques were identified, isolated, and serially screened two more times until all plaques were positive.

cDNA from positive clones was prepared as described above, the inserts cut out with EcoRI restriction endonuclease (New England Biolabs, Beverly, MA), and cloned into Bluescript KS- plasmid. The sequences of the inserts were determined using the Sequenase kit (U. S. Biochemical Corp.) as described above. Some DNA sequencing was performed in the William Keck Foundation DNA sequencing facility using an Applied Biosystems model 373A Sequencer. New primers for sequencing were synthesized as cDNA data was obtained (see Fig. 6 for base sequences used). Because the cDNA encoding the 20-kDa T. gondii CsA-binding protein contained an internal EcoRI site, two plasmids containing inserts were required to completely determine the cDNA sequence. Final sequencing of the cDNA encoding both major T. gondii CsA-binding proteins was accomplished from both stands.

RESULTS

T. gondii Has Two Major CsA-Binding Proteins—We first documented CsA binding activity in T. gondii lysates. As determined with the LH20 binding assay, CsA-binding proteins comprised 0.1–0.2% of all CHAPS-extractable proteins in whole T. gondii lysates. A one-step affinity purification on 8-ornithino-CsA, followed by SDS-PAGE analysis demonstrated two major protein bands at approximately 18.5 and 20 kDa molecular mass (Fig. 1, lane B). Three much lighter staining bands, faintly visible in Fig. 1 and perhaps representing proteins with lower CsA affinity, were consistently noted at approximately 60-, 40-, and 26-kDa molecular mass when 8-ornithino-CsA columns were not washed with at least 20-column volumes of buffer before elution. Elution of the 8-ornithino-CsA affinity column by progressively lowering the pH of the washing buffer from 3.5 to 3.3 successfully eluted the column without the need to add CsA and resolved the two major CsA-binding proteins (Fig. 1, lanes C and D). Further characterization of these two protein bands demonstrated that neither was glycosylated (data not shown). The isoelectric point of the 18.5-kDa protein was 5.7. Two isoforms were noted in the 20-kDa protein fraction eluted at pH 3.3, one with a pI of 7.05, the other at 7.80 (Fig. 2).

T. gondii CsA-Binding Proteins Are Not Due to Host Cell Protein Contamination—Two experiments were performed to demonstrate that these proteins were derived from T. gondii and not host cells. First, CsA-binding proteins were also isolated by 8-ornithino-CsA affinity chromatography from uninfected human foreskin fibroblast cells. The major CsA-elutable protein from human foreskin fibroblast cells migrated with a lower apparent molecular mass on SDS-PAGE (18 kDa) than the two T. gondii proteins (Fig. 3A). Second, immunoblot analysis with a 1:1000 dilution of affinity-purified anti-serum prepared against bovine thymus CyP-18 detected only recombinant human CyP-18 (HCyP-18) (Fig. 3B).

T. gondii CsA-Binding Proteins Partition into Different Cell Fractions—The distribution of the T. gondii CyPs was assessed in crude cell fractionation experiments. Parasites were lysed by two freeze/thaw cycles followed by vortexing with glass beads, then fractionated as described under “Experimental Procedures.” CyPs of both 18.5 and 20 kDa were present in the 10,000 × g supernatant extract. However, a 20-kDa protein that eluted at pH 3.3 predominated in the 10,000 × g pellet extract (Fig. 4).

T. gondii CsA-Binding Proteins are PPIases—Both T. gondii CsA-binding protein fractions demonstrated PPIase activity at concentrations comparable with mammalian CyPs (5–10 nm). The activity of both fractions was suppressed by CsA, with an IC50 for inhibiting solutions containing 10 nM T. gondii CyP of approximately 32 nm for the T. gondii CyP-18.5 fraction and 5 nm for the T. gondii CyP-20 fraction (Fig. 5).

Peptide Sequence Analysis of T. gondii CsA-Binding Proteins—The two T. gondii CsA-binding protein fractions were subjected to amino acid sequence analysis. NH2-terminal sequencing of the 18.5 kDa/pH 3.5 eluted protein yielded a 16-
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Fig. 3. Comparison of T. gondii and human CsA-binding proteins. Part A, Coomassie Blue-stained SDS-PAGE gel of CsA eluate from uninfected human foreskin fibroblast cell CHAPS lysates (lane A) or whole T. gondii CHAPS lysates (lane B) after purification on an 8-ornithino-CsA column. Part B, in panel I, recombinant human cyclophilin-18 (lane A) and T. gondii cyclophilins (lane B), isolated as described in the legend of Fig. 1, were probed using a 1:1000 dilution of polyclonal rat anti-??.

Fig. 4. Partitioning of T. gondii cyclophilins in disrupted parasites. 10⁶ organisms were disrupted by freezing and thawing twice, followed by vortexing with glass beads. The 10,000 × g supernatant and 10,000 × g pellet extracts were prepared from the resulting lysate as described under "Experimental Procedures" and CsA-binding proteins of each fraction purified on an 8-ornithino-CsA affinity column. Purified proteins were isolated by SDS-PAGE and transferred to nitrocellulose, and the filters were probed with polyclonal rat anti-T. gondii CyP antiserum. Panel I: lane A, 10,000 × g pellet extract eluted at pH 3.7; lane B, 10,000 × g pellet extract eluted at pH 3.5; lane C, 10,000 × g supernatant extract eluted at pH 3.3. Panel II: lane A, 10,000 × g supernatant extract eluted at pH 3.7; lane B, 10,000 × g supernatant extract eluted at pH 3.5; lane C, 10,000 × g supernatant extract eluted at pH 3.3.

Fig. 5. Inhibition of the PPIase activity of T. gondii CyPs by CsA. PPIase assays were performed using 75 μM substrate and 60 mg/ml chymotrypsin. The concentration of T. gondii CyP-18.5 or -20 used in each assay was 10 nm as determined by amino acid analysis or Bio-Rad protein assay standardized for these CyPs. However, the yield of active enzyme after low pH elution and refolding at neutral pH could not be determined due to limited amounts of total protein. Values shown are mean ± S.E. for three experiments at each concentration. Closed circles, T. gondii CyP-18.5; open circles, T. gondii CyP-20. The 50% inhibitory concentration (IC50) is 32 nm CsA for the 18.5-kDa T. gondii CyP fraction eluted at pH 3.5 and 5 nm CsA for the 20-kDa T. gondii CyP fraction eluted at pH 3.3.
FIG. 6. Complete cDNA and translated protein sequences of *T. gondii* CyP-18.6 (part A) and *T. gondii* CyP-20 (part B). Amino acid sequences of *T. gondii* CyPs were derived from cDNA sequencing as described under "Experimental Procedures" and peptide sequencing from isolated proteins (underlined amino acids). The internal EcoRI site in the *T. gondii* Cyp-20 cDNA sequence is also underlined. Outlined base sequences indicate DNA sense or antisense regions synthesized as primers for complete sequencing of both strands.

A

The *T. gondii* CyP-20 sequence was isolated as part of a large open reading frame with cDNA encoding 174 amino acids before the identified NH₂-terminal amino acid. There are 2 methionines encoded within this NH₂-terminal sequence, one immediately proximal to the NH₂-terminal residue determined by amino-terminal sequencing. The long open reading frame preceding the *T. gondii* CyP-20 sequence does not match any known protein or DNA sequence in the NCBI data base when searched using only the non-CyP coding region as the query sequence. Translation of the two alternate frames of this cDNA yields several stop codons in the second frame but only a single stop codon in the third frame. None of the peptide sequences derived from these translated cDNA frames (or the negative strand) matches any identified protein.

The most closely matched sequences for *T. gondii* CyP-20 are CyPs isolated from plants, primarily due to a 7-amino acid region commonly present in plant CyPs and mammalian CyP-40 (49,50) but absent in most other eukaryotic CyPs (Fig. 7). Close homology of the *T. gondii* CyP-20 sequence is also noted to other parasite sequences.

DISCUSSION

*Cyclophilins of T. gondii*—We have identified two major cyclosporin-binding proteins in the CsA-sensitive protozoan *T. gondii*. Biochemical characteristics and sequence homology clearly identify both of these proteins as CyPs. However, dif-
The differential association of the *T. gondii* CyPs with crude cell fraction extracts, homology of the NH$_2$-terminal region of the 20-kDa *T. gondii* CyP with mammalian calcineurin B, and the presence of an apparent signal sequence encoded in the CDNA of the 18.5-kDa CyP indicates that they may have unique and different functions.

Both *T. gondii* CyPs isolated in these experiments are abundant proteins and exhibit PPIase activity, which is inhibited by nanomolar concentrations of CsA. Based on molecular weight differences and distinct elution characteristics, there initially appeared to be only two proteins. The similar molecular weight of the two major bands identified by SDS-PAGE analysis first led us to speculate that one of the two proteins might represent a post-translationally modified version of the other. However, we detected no glycosylation of either protein, despite the later identification of several N-glycosylation sites in the amino acid sequence of both proteins. Isoelectric focusing clearly indicated that there were at least variant isoforms within the 20-kDa band and perhaps different proteins. Subsequent sequence analysis firmly established that at least 2 different genes encode *T. gondii* CyPs. The deduced amino acid sequences of the two *T. gondii* CyPs isolated indicate that they may have unique and different functions.

Based on the cDNA sequence, *T. gondii* CyP-18.5 may be initially synthesized with an amino-terminal signal sequence, which is subsequently cleaved. The presence of a single gene encoding two CyPs, one with a signal sequence, has been previously noted in *Neurospora crassa*, in which one gene encodes both the cytosolic and mitochondrial CyPs (16). The initial 15 amino acids identified in the amino-terminal signal sequence indicates the presence of a signal peptide encoded in the cDNA of the 18.5-kDa CyP, indicating identity to human calcineurin B subunit.

In isolectric focusing of the 20-kDa protein fraction eluted from the 8-ornithino-CsA column by pH 3.3 buffer (Fig. 7A), a tryptic fragment known to be essential for CsA binding (51) at position 129 of *T. gondii* Cyp-20 and position 123 of the mature *T. gondii* Cyp-18.5 (position 138 of the precursor protein). The other two parasitic CyPs previously isolated (19, 20) are homologous to both *T. gondii* CyPs, but the overall identity is greatest with *T. gondii* Cyp-20.

Based on the cDNA sequence, *T. gondii* Cyp-18.5 may be initially synthesized with an amino-terminal signal sequence, which is subsequently cleaved. The presence of a single gene encoding two CyPs, one with a signal sequence, has been previously noted in *Neurospora crassa*, in which one gene encodes both the cytosolic and mitochondrial CyPs (16). The initial 15 amino acids identified in the amino-terminal signal sequence indicates the presence of a signal peptide encoded in the cDNA of the 18.5-kDa CyP, indicating identity to human calcineurin B subunit.

The cDNA encoding *T. gondii* Cyp-20 is part of a much larger open reading frame with 520 bases 5' to the presumed initiation methionine that immediately precedes a proline identified as the NH$_2$-terminal residue by peptide sequencing. This extensive 5' region has no identity with previously reported CDNA or protein sequences. While this region could represent a cloning artifact, it might well encode a precursor protein or functionally related protein. Efforts to determine the identity and relationship of this sequence to the *T. gondii* Cyp-20 are ongoing.

The homology of the amino-terminal portion of the *T. gondii* Cyp-20 protein to the carboxyl-terminal region of the B subunit of human calcineurin is significant.
of the mammalian Ca\(^{2+}\)-calmodulin-dependent phosphatase calcineurin is remarkable, because the human CyP-CsA complex can inhibit the phosphatase activity of calcineurin (52, 53). This mechanism of action has been proposed by several authors to be critical to the CsA-mediated events that interrupt T-cell signaling (11, 53–56). T. gondii CyP-20 is identical to calcineurin in 11/20 amino acids of this region; eight of these 11 amino acids are also conserved in HCyP-18 (Fig. 7B). Studies of the three-dimensional interaction of the CsA-CyP complex with the A and B subunits of calcineurin, calmodulin, and Ca\(^{2+}\) are ongoing (57, 58). While there are no currently available data indicating that the region of homology between CyP and calcineurin B contributes to this association, further studies focused on this sequence are warranted.

**Subcellular Location and Function of the T. gondii CyPs—**An essential clue to the function of these proteins may lie in their subcellular location. The 20-kDa T. gondii CyP forms predominately in the 10,000 x g pellet extract, which would be expected to contain the pellicle and subcellular organelles, especially the rhoptries, dense granules, mitochondria, and nuclei (59). This suggests an organellar association of the cloned T. gondii CyP-20 species, perhaps in a modified form, or possibly the T. gondii CyP-18.5 precursor with the signal sequence intact. However, all disruption schemes of coccidian parasites (French press, glass beads, N\(_2\) cavitation bomb) have been shown to cause some organellar disruption (59), and definitive statements regarding the purity of these cell extracts are difficult. We have been unsuccessful in identifying the distribution of these proteins within the parasite and/or host cell using immunofluorescence microscopy and the polyclonal antisera prepared against both T. gondii CyPs. Precise definition of the subcellular location of the T. gondii CyPs will require extensive analysis using reagents or approaches that are beyond the scope of the current study.

**PPIase Participation in Microbial Pathogenesis—**The most well-defined microbial PPIase is the legionella MIP (macrophage infectivity potentiator) protein, an outer membrane virulence protein with high affinity for FK-506 that assists intracellular survival of the organism (31, 32). Experimental evidence suggests that MIP is important in an early event after legionella entry into the host cell (32). Another intracellular bacterium with a MIP-like protein, chlamydia, has recently demonstrated behavior similar to MIP(-) legionella in cell culture and the polyclonal antisum prepared against both T. gondii CyPs. Precise definition of the subcellular location of the T. gondii CyPs will require extensive analysis using reagents or approaches that are beyond the scope of the current study.

**References**

1. The Canadian Multicentre Transplant Study Group (1983) N. Engl. J. Med. 309, 809–815
2. The Canadian Multicentre Transplant Study Group (1986) N. Engl. J. Med. 314, 1219–1225
3. Stollar, D. B., Toto, S., Fung, J., Demetris, A., Venkataramanan, R., and Jain, A. (1989) Lancet 2, 1000–1004
4. Dreyfuss, M., Harri, E., Hofmann, M., Kohel, H., Pache, W., and Tscheter, H. (1976) Eur. J. Appl. Microbiol. 3, 125–133
5. Kino, T., Tantamaka, H., Hashimoto, M., Nishiyama, M., Goto, T., Okuhara, M., Koshaka, M., Aoki, H., and Imanaka, H. (1987) J. Antibiot. (Tokyo) 40, 1254–1259
6. Vezina, C., Kudelski, A., and Sehgal, S. N. (1975) J. Antibiot. (Tokyo) 28, 721–726
7. Hig, K. P., and Handschumacher, B. R. (1992) Infect. Agents Dis. 1, 121–135
8. Kim, J. H., and Perfect, J. R. (1989) Rev. Infect. Dis. 2, 677–690
9. Kellin, Y., Faouette, L., Bergmann, D. J., Levy, M. A., Cafferkey, R., Kosar, P. I., Johnson, R. R., and Livi, G. P. (1991) Mol. Cell. Biol. 11, 1718–1723
10. Schreiber, S. L. (1991) Science 251, 263–267
11. Schreiber, S. L., and Crabtree, G. R. (1992) Immunol. Today 13, 136–141
12. Tropschug, M., Barthelmess, I., and Neupert, W. (1999) Nature 342, 953–955
13. Fischer, G., Wittmann-Liebold, B., Lang, K., Kieferber, T., and Schmid, F. X. (1989) Nature 337, 476–478
14. Harding, M. W., Galat, A., Uhling, D. E., and Schreiber, S. L. (1989) Nature 341, 756–760
15. Takahashi, N., Hayano, T., and Suzuki, M. (1989) Nature 337, 473–475
16. Tropschug, M., Nixonson, F. W., Hartl, F. U., Kohler, H., Pflanzer, W., Wachter, E., and Neupert, W. (1988) J. Biol. Chem. 263, 14333–14440
17. Haendler, B., Keller, R., Hiestand, P. C., Kocher, H. P., Wegmann, G., and Mason, N. R. (1989) Gene (Amst.) 83, 39–46
18. Koser, P. I., Livi, G. P., Levy, M. A., Rosenberg, M., and Bergsma, D. (1990) Gene (Amst.) 96, 189–195
19. Lightowlers, M. W., Haralamabous, A., and Richard, M. D. (1989) Mol. Biochem. Parasitol. 36, 297–290
20. Argast, V. P., and Mitchell, G. F. (1991) J. Parasitol. 77, 660–664
21. Liu, J., and Walsh, C. T. (1996) Proc. Natl. Acad. Sci. U. S. A. 97, 4028–4032
22. Hayano, T., Takahashi, N., Kato, S., Makii, N., and Suzuki, M. (1991) Biochemistry 30, 3041–3048
23. Tran, P. V., Bamber, T. A., Kofskis, S. Z., and Nichols, B. P. (1990) J. Bacteriol. 164, 407–410
24. Herrler, M., Bang, H., Brune, K., Fischer, G., and Marahiel, M. A. (1992) FEBS Lett. 299, 231–234
25. Britzma, L., Chroboc, G., Boskian, K. A., and Parent, S. A. (1991) Mol. Cell. Biol. 11, 4166–4162
26. Tropschug, M., Wachter, E., Mayer, S., Schonbrunner, E. R., and Schmid, F. X. (1990) Nature 346, 674–677
27. Wiederecht, B., Britzma, L., Elliston, K., Sigal, N. H., and Siekierski, J. J. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 1029–1033
28. Samuels, R. A., and Gotschlich, E. C. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 1164–1168
29. Lundemose, A. G., Virkelsund, S., Fey, S. J., Larsen, P. M., and Christiansen, G. (1991) Mol. Microbiol. 5, 109–115
30. Lundemose, A. G., Kay, J. E., and Pearse, J. H. (1993) Mol. Microbiol. 7, 777–783
31. Cianciotto, N. P., Eisenstein, B. I., Mody, C. H., Thews, G. B., and Engleberg, N. C. (1990) Infect. Immun. 57, 1255–1262
32. Cianciotto, N. P., Eisenstein, B. I., Mody, C. H., and Engleberg, N. C. (1990) J. Infect. Dis. 162, 121–129
33. Fischer, G., Bang, H., Ludwig, B., Mann, K., and Hacker, J. (1992) Mol. Microbiol. 6, 1375–1383

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34. Mack, D. G., and McLeod, B. (1984) *Antimicrob. Agents Chemother.* **28**, 26–30
35. McCabe, R. B., Luft, B. J., and Remington, J. S. (1986) *Transplantation* **41**, 611–615
36. Porter, S., and Sande, M. (1992) *N. Engl. J. Med.* **327**, 1643–1648
37. Joiner, K. A., Fuhrman, S. A., Miettinen, H. M., Kasper, L. H., and Mellman, J. (1990) *Science* **249**, 641–646
38. Marks, W. H., Harding, M. W., Handschumacher, R., Marks, C., and Lorber, M. I. (1991) *Transplantation* **52**, 340–345
39. Sarrin, A. H., Harding, M. W., Jiang, F. R., Aftab, D., Handschumacher, R. E. (1992) *Transplantation* **54**, 904–910
40. Liu, J., and Walsh, C. T. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 4038–4043
41. Koletsy, A. J., Harding, M. W., and Handschumacher, R. E. (1986) *J. Immunol.* **137**, 1054–1059
42. Handschumacher, R. E., Harding, M. W., Rice, J., and Druge, R. J. (1984) *Science* **226**, 544–547
43. Kieffer, L. J., Thalhammer, T., and Handschumacher, R. E. (1992) *J. Biol. Chem.* **267**, 5503–5507
44. Stone, K. L., LoPresti, M. B., Williams, N. D., Crawford, J. M., Deangelis, R., and Williams, K. R. (1989) in *Techniques in Protein Chemistry* (Hugh, T. E., ed.) pp. 377–391, Academic Press, San Diego
45. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Liparian, D. J. (1989) *J. Mol. Biol.* **215**, 403–410
46. Gish, W., and Staler, D. (1993) *Nature Genetics* **3**, 266–272
47. Kofron, J. L., Kuzmic, P., Kissore, V., Colon-Bonilla, E., and Rich, D. H. (1991) *Biochemistry* **30**, 6127–6134
48. Sambrook, J., Fritsch, E. G., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
49. Kieffer, L. J., Song, T. W., Li, W., Osterman, B. G., Handschumacher, R. E., and Bayney, R. M. (1993) *J. Biol. Chem.* **268**, 12303–12310
50. Ratyczak, T., Carrello, A., Mark, P. J., Warner, B. J., Simpson, R. J., Moritz, R. L., and House, A. K. (1990) *J. Biol. Chem.* **266**, 13187–13192
51. Liu, J., Chen, C. M., and Walsh, C. T. (1991) *Biochemistry* **30**, 2306–2310
52. Liu, J., Farmer, J. D., Lane, W. S., Friedman, J., Weissman, I., and Schreiber, S. I. (1991) *Cell* **66**, 897–815
53. Liu, J., Albers, M. W., Wandless, T. J., Luan, S., Altberg, D., Belshaw, P., Cohen, P., MacKintosh, C., Rhee, C., and Schreiber, S. (1992) *Biochemistry* **31**, 3896–3901
54. Schreiber, S. L. (1992) *Cell* **70**, 365–368
55. O'Keefe, S. J., Tamura, J., Kincaid, R. L., Toep, M. J., and O'Neill, E. A. (1992) *Nature* **357**, 692–694
56. Clipstone, N. A., and Crabtree, G. R. (1992) *Nature* **357**, 695–697
57. Li, W., and Handschumacher, R. E. (1993) *J. Biol. Chem.* **268**, 14040–14044
58. Ryffel, B., Weitly, G., Murray, M., Eupster, H. P., and Car, B. (1993) *Biochem. Biophys. Res. Commun.* **194**, 1074–1083
59. Lerner, M. A., and Dubremetz, J. F. (1991) *Mol. Biochem. Parasitol.* **45**, 249–269
60. Foor, P., Parent, S. A., Morin, N., Dahl, A., Ramadani, N., Chrebet, G., Bastian, K., and Nielson, J. (1992) *Nature* **360**, 682–684
61. Collier, N. J., Baker, E. K., Stenzel, M. A., and Zaker, C. S. (1991) *Cell* **67**, 255–263
62. Schenerly, S., Shortridge, R. D., Larrivee, C. D., Ong, T., Otsuki, M., and Pak, W. L. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **86**, 5390–5394
63. Shieh, B.-H., Stenzel, M. A., Seavello, S., Harris, G. L., and Zaker, C. S. (1989) *Nature* **338**, 67–70
64. Lebeau, M., Masson, N., Herrick, J., Faber, I., Renoir, J., Radangi, C., and Baubier, E. (1992) *J. Biol. Chem.* **267**, 4281–4284
65. Pratt, W. B. (1990) *J. Biol. Chem.* **265**, 21455–21458
66. Pratt, W. B., Czar, M. J., Stancato, L. F., and Owens, J. K. (1993) *J. Steroid Biochem. Mol. Biol.* **46**, 269–279
67. Sanchez, E. R. (1990) *J. Biol. Chem.* **265**, 22077–22079
68. Sanchez, E. R., Faber, L. E., Henzel, W. J., and Pratt, W. B. (1990) *Biochemistry* **29**, 5145–5152
69. Tai, P. K., McArd, Y., Nakas, K., Wukin, N. G., Dubring, J. L., and Faber, L. E. (1988) *Biochemistry* **27**, 5266–5275
70. Bliska, J. B., Galan, J. E., and Falkow, S. (1993) *Cell* **73**, 903–920