A Type I Peritrophic Matrix Protein from the Malaria Vector
Anopheles gambiae Binds to Chitin

CLONING, EXPRESSION, AND CHARACTERIZATION

Zhicong Shen and Marcelo Jacobs-Lorena‡
From the Case Western Reserve University, School of Medicine, Department of Genetics, Cleveland, Ohio 44106-4955

(Received for publication, March 9, 1998, and in revised form, April 16, 1998)

Upon feeding, mosquito midguts secrete the peritrophic matrix (PM), an extracellular chitin-containing envelope that completely surrounds the blood meal. Because the malaria parasite must cross the PM to complete its life cycle in the mosquito, the PM is a potential barrier for malaria transmission. By antibody screening of an expression library we have identified and partially characterized a cDNA encoding a putative PM protein, termed Anopheles gambiae adult peritrophin 1 (Ag-Aper1). Ag-Aper1 is the first cloned PM gene from a disease vector. Northern analysis detected an abundant Ag-Aper1 transcript only in the adult gut, and not in any other tissues or at any other stages of development. The predicted amino acid sequence indicates that it has two tandem chitin-binding domains that share high sequence similarity with each other and also with the chitin-binding domain of an adult gut-specific chitinase from the same organism. The presumed ability of Ag-Aper1 to bind chitin was verified by a functional assay with the baculovirus-expressed recombinant protein. Ag-Aper1 did bind to chitin but not to cellulose, indicating that Ag-Aper1 binds chitin specifically. The double chitin-binding domain organization of Ag-Aper1 suggests that each protein molecule is able to link two chitin polymer chains. Hence, this protein is likely to act as a molecular linker that connects PM chitin fibrils into a three-dimensional network.

The peritrophic matrix (PM) is an extracellular layer that surrounds the food bolus in the guts of most arthropods (1–4). All PMs are composed of chitin, proteins, and proteoglycans (1–4). There are two types of PMs in insects. The type 1 PM is thick (usually between 2 and 20 μm) and is synthesized continuously from a specialized organ, the cardia (or proventriculus), which is located at the junction of the foregut and midgut. Mosquitoes and many other hematophagous insects produce type 2 PM during larval life and type 1 PM during adult life. Several type 2 larval PM proteins have been cloned (8–10). Little is known about the structural and biochemical differences between these two very different types of PM.

The function of the PM has not been clearly established. It is likely that the PM provides protection for midgut epithelial cells from damage from food particles, prevents infection from viruses, bacteria, and other pathogens, and also facilitates digestion by partitioning digestive enzymes and the ingested food between the endo- and the ecto-peritrophic compartments (1–4). The PM may also function as a partial barrier to toxins and other macromolecules. For example, exogenous chitinase significantly increases the toxicity of Bacillus thuringiensis’ delta toxin in Spodoptera littoralis, presumably by increasing PM permeability (11).

Human malaria is caused by protozoan parasites, Plasmodium sp. Malaria is one of the most serious global health problems, causing about 2 million deaths annually. Anopheline mosquitoes are the sole vectors for human malaria. Upon ingestion of an infected blood meal, Plasmodium gametes mate, develop into ookinetes, and about one day later, the ookinetes penetrate the PM and the gut epithelial cells. After traversing the gut epithelium and reaching the gut basal lamina, the ookinetes develop into oocysts. Each oocyst eventually releases thousands of sporozoites that invade the salivary glands and infect the next person when the mosquito bites again. The PM is a thick and dense layer that the Plasmodium parasite must traverse. Although it is known that the parasite secretes a chitinase to facilitate the penetration of the PM (12), it is unclear what role, if any, the PM plays in triggering chitinase secretion or whether secretion of other hydrolytic enzymes by the parasite is also required. The resolution of these issues will require the elucidation of the molecular composition and structure of the Anopheles PM. Here, we report for the first time, the cloning of a PM protein from a disease vector and propose that this PM protein plays a major role in the spatial organization of the PM.

MATERIALS AND METHODS

Preparation of the PM—An A. gambiae (G3 strain) colony was maintained as described previously (13). Adult mosquitoes (about 5 days old) were fed with a protein-free latex meal as described previously (7) to induce PM formation. PMs were dissected in PBS buffer 30 min after mosquitoes had a latex meal. Dissected PMs were transferred to a 1.5-ml centrifuge tube (kept on ice) with a fine glass pipette. After collecting PMs for about 30 min, the tube was centrifuged for 1 min at

* This work was supported by grants from the John D. and Catherine T. MacArthur Foundation and from the NIAID, National Institutes of Health. 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: Case Western Reserve University, School of Medicine, Dept. of Genetics, 10900 Euclid Ave., Cleveland, OH 44106-4955. Tel.: 216-368-2791; Fax: 216-368-3432; E-mail: mxj3@po.cwru.edu.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EBI Data Bank with accession number(s) AF030431.

The Journal of Biological Chemistry Vol. 273, No. 28, Issue of July 10, pp. 17665–17670, 1998
Printed in U.S.A.

This paper is available online at http://www.jbc.org
Antisera were collected after 6 injections at 2-week intervals. Complete Freund's adjuvant was used for the initial immunization and incomplete Freund's adjuvant for all boosts. For each immunization, about 100 μl of the antigen/adjuvant mixture (containing 40 PMs) per mouse were injected intraperitoneally. The antisera were collected after 6 injections at 2-week intervals.

SDS-PAGE Analysis—Dissected PMs were rinsed briefly with 50 mM Tris-HCl (pH 7.5) and centrifuged at 12,000 × g for 10 min. The pellet was then homogenized in H2O containing 1 mM phenylmethylsulfonyl fluoride using a disposable plastic pestle. After vortexing for 2 min, the sample was centrifuged at 12,000 × g for 10 min. The supernatant was the water-soluble fraction, and the pellet was the water-insoluble fraction. Both fractions were boiled for 10 min with SDS sample buffer containing mercaptoethanol. The proteins in both fractions were analyzed by SDS-PAGE (12% acrylamide) as described by Laemmli (14).

Western Blot Analysis of PM Proteins—Five guts from 4th-instar larvae or from adult mosquitoes 1, 3, 5, 7, and 9 d after eclosion, were dissected and kept frozen at −80 °C. After adding 50 μl of 2× SDS sample buffer (14), each sample was boiled for 5 min. An aliquot from each sample (10 μl) was analyzed by SDS-PAGE. After electrophoresis, the proteins were blotted onto a nitrocellulose membrane (Micron Separation, Inc., MA). PM proteins were detected with the anti-PM antisera. An alkaline phosphatase-conjugated goat anti-mouse IgG was used as the secondary antibody, and a chromogenic reaction was used to detect the bound antibodies according to the manufacturer's recommendations (Schleicher & Schuell).

Preparation of PM Antibodies—Five BALB/cJ mice were used to prepare polyclonal antisera. About 200 PMs dissected from latex-fed mosquitoes were homogenized in 250 μl of PBS and mixed with an equal volume of adjuvant. Complete Freund's adjuvant was used for the initial immunization and incomplete Freund's adjuvant for all boosts. For each immunization, about 100 μl of the antigen/adjuvant mixture (containing 40 PMs) per mouse were injected intraperitoneally. The antisera were collected after 6 injections at 2-week intervals.

RESULTS

Western Analysis of Anopheles PM Proteins—Mice were immunized with total adult PM proteins, and the antiserum was used to detect PM proteins in gut extracts. Three major bands were detected (Fig. 2). One is approximately 180 kDa, which may correspond to the 180-kDa water-extractable protein (Fig. 1, lane 1). The two other major bands of around 60 kDa may correspond to the major insoluble proteins shown in lane 1 of Fig. 1. The antiserum cross-reacts with some proteins from the larval gut (Fig. 2, lane 1). Although one of the larval proteins co-migrates with the ~180 kDa adult PM protein, another major cross-reacting protein migrates with an apparent molecular mass of over 220 kDa. There were no cross-reacting larval PM proteins in the range of 60 kDa. The results of the experiment in Fig. 2 also indicate that PM proteins are synthesized within the first day of adult life. Although the abundance of the 180-kDa protein does not appear to increase with age, the group of ~60-kDa proteins appears to increase in amount until day 4 after eclosion, when mosquitoes reach maturity for blood feeding. The finding that PM proteins are stored in the gut epithelium before blood feeding is consistent with the observation that the PM forms within minutes of feeding.

Cloning of a cDNA That Encodes a PM Protein—A gut cDNA
expression library was screened with the anti-PM serum. A cDNA clone, named Ag-Aper1 for Anopheles gambiae adult peritrophin 1, was obtained. It has an insert of 0.77 kilobase pairs and an open reading frame encoding 153 amino acids (Fig. 3). The cDNA has 27 base pairs of 5'-untranslated sequence and 254 base pairs of 3'-untranslated sequence. The polyadenylation signal (AATAAAA) is found 30 base pairs upstream of the poly(A) end. The deduced amino acid sequence has a highly hydrophobic N terminus, as expected for a secretory protein. According to von Heijne’s rules (19), the signal peptide cleavage site is predicted to be after Ala-17. The conceptual translation of the Ag-Aper1 cDNA predicts a protein of 15 kDa, pl of 4.0 with a high content of proline (17.5%), cysteine (8.8%), and aspartic acid (9.5%). The high proline content suggests that Ag-Aper1 has little secondary structure.

Western analysis with the same antiserum used to clone Ag-Aper1 did not detect a major protein at ~15 kDa (Fig. 1). This could be because of protein glycosylation.

**Domain Structure of Ag-Aper1**—The Ag-Aper1 amino acid sequence is internally repeated. The amino acid residues of the first and second halves of the protein are 41% identical and 60% conserved (Fig. 4), indicating that the protein is composed of two similar domains. Interestingly, the two domains also share high sequence similarity with the chitin-binding domain of AgChi-1, the gut-specific chitinase from A. gambiae (20) (Fig. 4). Sequence comparisons with different data bases by BLAST searching (21) revealed that Ag-Aper1 also shares low but significant similarity with several groups of proteins that interact (or are presumed to interact) with chitin. These include chitinases from nematodes (Caenorhabditis elegans (SwissProt accession no. Q11174, Nhan, N; Brugia malayi (22)), arthropods (Penaeus japonicus (23), Manduca sexta (24)), and insect larval type 2 PM proteins (Lucilia cuprina peritrophins 44 and 95 (8–9), Trichoplusia ni mucin (10)). The larval type 2 PM proteins have 5 putative chitin-binding domains, whereas Ag-Aper1 has only two.

**FIG. 2.** Western analysis of PM proteins from mosquito midguts. Midgut proteins were fractionated by SDS-PAGE, blotted onto a nitrocellulose membrane, and detected with a mouse polyclonal antiserum prepared against total PM proteins. Lane 1, guts from 4th-instar larvae; lanes 2–6, midguts from sugar-fed females aged for 1, 3, 5, 7, and 9 days, respectively. Migration of protein size markers (in kDa) is indicated on the left.

**FIG. 3.** Nucleic acid and deduced amino acid sequences of a cDNA clone encoding the Anopheles gambiae adult peritrophin 1 (Ag-Aper1). The arrowhead after alanine-17 indicates the putative signal peptide cleavage site. The putative polyadenylation signal (AATAAAA) is underlined.
Expression Pattern of Ag-Aper1 mRNA—Northern analysis was used to measure Ag-Aper1 mRNA expression at different developmental stages and in different tissues. The Ag-Aper1 mRNA was only detected in adult guts. RNA from adult carcass (whole body minus gut), pupae, or larvae yielded no detectable signal (Fig. 5). Therefore, Ag-Aper1 is an adult gut-specific gene. In the adult midgut, a strong signal was detected 1 day after eclosion (Fig. 6) even though the mRNA appears to be absent from pupae (Fig. 5). Thus, it is likely that Ag-Aper1 transcription is turned on soon after eclosion. Ag-Aper1 mRNA abundance does not change appreciably with age of the mosquito or as a function of time after feeding (Fig. 6). This expression pattern is in agreement with the profile of PM proteins detected by Western analysis (Fig. 2) but different from that of many digestive enzymes that are induced after blood feeding (25–28).

Genomic Organization of Ag-Aper1—Ag-Aper1 gene copy number was estimated by Southern blotting analysis of genomic DNA digested with either EcoRI or HindIII (Fig. 7). Two bands in the 6-kilobase pair region were detected in the EcoRI digest, whereas only one band was detected with the HindIII digest. Although the Ag-Aper1 cDNA does not have an EcoRI site, it is possible that the Ag-Aper1 gene has an intron with an EcoRI site. To investigate this possibility, we performed PCR with primers located at the 3´- and 5´-ends of the cDNA sequence. The size of the PCR product was the same using either the genomic DNA or the cDNA clone as the template (result not shown), indicating that no introns are present within this interval. When the Southern blot of Fig. 7 was rehybridized with the PCR product of genomic DNA it gave the identical pattern as with the cDNA probe (result not shown). Therefore, the Southern blot analysis suggests that they may be two genes (similar or identical) located within 7 kilobase pairs. However, we cannot rule out the possibility that there is only one Ag-Aper1 gene per haploid genome and that because of sequence polymorphism, one allele has an internal EcoRI site although the other does not.

The Recombinant Ag-Aper1 Protein Specifically Binds to Chitin—The structure of Ag-Aper1 (Fig. 4) suggested that this is a chitin-binding protein. This premise was tested with a functional assay. Ag-Aper1 was expressed in an insect cell line from a recombinant baculovirus and detected with the anti-peritrophic matrix serum as a protein of ~30 kDa (Fig. 8, lanes 2 and 9 in the lower panels). An extract from cells infected with a recombinant baculovirus expressing the green fluorescent protein was analyzed as a control (Fig. 8, lanes 1 and 8). The antiserum did not detect any proteins, even though more extract was loaded on the gels. This indicates that the antiserum detected specifically the recombinant Ag-Aper1 protein. The apparent molecular weight of the recombinant protein is higher than that calculated (15 kDa). This may be because of protein glycosylation.

The following experiments were to investigate whether Ag-Aper1 binds to chitin, and if so, whether binding is specific for this polymer. Total soluble proteins from cells expressing Ag-Aper1 were mixed with an insoluble matrix made of chitin (a polymer of N-acetylglucosamine subunits) or cellulose (a polymer of glucose subunits). The recombinant Ag-Aper1 protein appeared to bind to chitin efficiently, because no Ag-Aper1 was detected in the flow-through (Fig. 8, lane 3). The chitin matrix did not absorb proteins nonspecifically since most proteins were present in the flow-through (Fig. 8, upper panel, lanes 2 and 3). The bound proteins were eluted with a pH 2 buffer (Fig. 8, lane 6), but not with a pH 5 buffer (Fig. 8, lane 4). The interaction of Ag-Aper1 with chitin appears to be specific, because no Ag-Aper1 binding was detected to a cellulose matrix (Fig. 8, right panel). Virtually all of the Ag-Aper1 was detected in the flow-through from the cellulose matrix (Fig. 8, lower panel, lane 10). Two bands were detected in the pH 2 eluate by Coomassie Blue staining (Fig. 8, upper panel, lane 6). One migrates at about 30 kDa, which coincides with the mobility of the band detected by Western analysis (Fig. 8, lower panels). Therefore, this band is likely Ag-Aper1. The upper band also
cellulose matrix and rocked for 30 min. The matrices were then packed with chitin. The Sf21 insect cell line was infected with a recombinant baculovirus encoding the Ag-Aper1 protein. Total soluble proteins prepared from cells 60 h after infection were mixed with a chitin or a cellulose matrix and rocked for 30 min. The matrices were then packed into a column, and the proteins were sequentially eluted with 50 mM NaAc (pH 5.0) and with 50 mM glycine-HCl (pH 2.0). The two top panels show gels stained with Coomassie Blue; the two bottom panels are Western blots probed with an anti-peritrophic matrix serum. The two left panels illustrate the assay for chitin-binding; the two right panels illustrate the assay for cellulose binding. The double arrows indicate the position of migration of the Ag-Aper1 recombinant protein. Lanes 1 and 8, control experiment showing total soluble proteins from Sf21 cells infected with a recombinant baculovirus expressing the green fluorescent protein; lanes 2 and 9, total soluble proteins from Sf21 cells infected with a recombinant baculovirus encoding the Ag-Aper1 protein. This was the starting material for the binding experiments illustrated in lanes 3-7 and 10–13; lanes 3 and 10, flow-through (nonbinding proteins); lanes 4 and 11, proteins eluted with NaAc (pH 5.0); lanes 5 and 6, two fractions of proteins collected in the elution with glycine-HCl (pH 2.0) from chitin; lanes 12, combined fractions of elution with glycine-HCl (pH 2.0) from cellulose; lanes 7 and 13, proteins recovered from the matrices by boiling with 0.5% SDS. The migration of protein size markers (given in kDa) is shown on the right.

appears to be a chitin-binding protein, and originates either from the Sf21 cells or from the baculovirus itself. Boiling with SDS did not release a significant amount of protein from either matrix (Fig. 8, lanes 7 and 13). We conclude that Ag-Aper1 binds to chitin but not to cellulose.

**DISCUSSION**

**Significance of the Ag-Aper1 Two-domain Structure—**Chitin is a linear polymer of N-acetylglucosamine and a key structural component of the PM. In the presence of inhibitors of chitin synthesis, such as Dimilin or Polyoxin D, PM formation is repressed or completely blocked (1). Moreover, feeding mosquitoes with a meal containing chitinase completely blocks PM formation (29). Thus, chitin is an essential component of the PM. However, it is unknown how chitin fibers interact with proteins to form the PM. The characterization of the Ag-Aper1 gene has shed some light on this issue. Ag-Aper1 has two chitin-binding domains connected by a short linker sequence. This double-domain structure suggests that each Ag-Aper1 molecule can bind two chitin fibers and thus form a three-dimensional chitin-Ag-Aper1 network (Fig. 9). Growth of the PM would occur by addition of new chitin fibers and Ag-Aper1 to the existing network, as illustrated in Fig. 9.

The two chitin-binding domains of Ag-Aper1 are highly homologous to the chitin-binding domain of the Anopheles gut-specific chitinase (Fig. 4). In the case of chitinase, the chitin-binding domain may enhance enzyme function by facilitating the binding of the enzyme to the chitin fibers (Fig. 9). Although the Ag-Aper1 is presumed to be an essential component of PM synthesis and structure, the gut chitinase would play an inverse but equally important role of controlling PM turnover and porosity. Chitin hydrolysis is likely to serve two physiologically important roles: 1) control of PM thickness by balancing PM growth with degradation; and 2) increase PM porosity to support the heavy “molecular traffic” that occurs during digestion, digestive enzymes crossing the PM from the epithelium toward the food bolus and hydrolytic products traveling in the opposite direction.

**The Chitin-binding Domain As a Basic Module in Evolution—**The Ag-Aper1 protein has two chitin-binding domains that share significant amino acid sequence similarity. It is unlikely that these two domains have arisen independently by convergent evolution. Rather, they probably resulted from gene duplication. Furthermore, the high similarity among the chitin-binding domains of Ag-Aper1 and AgChi-1 suggests that they may be derived from a common ancestor. We propose that the chitin-binding domain is a basic module that combined with other protein sequences to generate new function or modify existing function.

**Ag-Aper1 As a Candidate Antigen for a Transmission-blocking Vaccine—**Elucidation of PM structure may help clarify how Plasmodium manages to pass through the PM. This knowledge could be used to develop novel strategies to control malaria transmission. For instance, the blood of sheep vaccinated with the L. cuprina larval PM (type 2) hinders larval development, probably because of the blockage of PM pores (32, 33). Although the adult mosquito forms a different type of PM (type 1), it is conceivable that antibodies to adult *Anopheles* PM proteins will have a similar effect on the PM. This effect could impede Plasmodium to cross the PM, prolong digestion time, and perhaps shorten the life span of the mosquito. The combination of antibodies against PM proteins and gut chitinase may have even more pronounced effects. Inhibition of chitinase may result in a thicker PM, which in turn inhibits *Plasmodium* development (20, 34). The cloning of genes encoding PM proteins may provide the tools to test these ideas.

**Use of the Ag-Aper1 Promoter to Drive the Expression of Foreign Anti-malaria Genes in Transgenic Mosquitoes—**With the advance of germ line transformation of mosquitoes (35), new approaches became possible for the control of malaria transmission. For instance, it may be possible to genetically engineer mosquitoes that express foreign genes that are toxic to *Plasmodium* (36). This approach requires the identification of a promoter capable of driving the expression of a foreign gene in the right tissue and at the right time. The gut is an excellent target, because *Plasmodium* must complete a complex developmental program while exposed to the relatively confined environment of the gut lumen. Most gut-specific genes that

---

**FIG. 8.** Recombinant Ag-Aper1 protein specifically binds to chitin. The Sf21 insect cell line was infected with a recombinant baculovirus encoding the Ag-Aper1 protein. Total soluble proteins prepared from cells 60 h after infection were mixed with a chitin or a cellulose matrix and rocked for 30 min. The matrices were then packed into a column, and the proteins were sequentially eluted with 50 mM NaAc (pH 5.0) and with 50 mM glycine-HCl (pH 2.0). The two top panels show gels stained with Coomassie Blue; the two bottom panels are Western blots probed with an anti-peritrophic matrix serum. The two left panels illustrate the assay for chitin-binding; the two right panels illustrate the assay for cellulose binding. The double arrows indicate the position of migration of the Ag-Aper1 recombinant protein. Lanes 1 and 8, control experiment showing total soluble proteins from Sf21 cells infected with a recombinant baculovirus expressing the green fluorescent protein; lanes 2 and 9, total soluble proteins from Sf21 cells infected with a recombinant baculovirus encoding the Ag-Aper1 protein. This was the starting material for the binding experiments illustrated in lanes 3-7 and 10–13; lanes 3 and 10, flow-through (nonbinding proteins); lanes 4 and 11, proteins eluted with NaAc (pH 5.0); lanes 5 and 6, two fractions of proteins collected in the elution with glycine-HCl (pH 2.0) from chitin; lanes 12, combined fractions of elution with glycine-HCl (pH 2.0) from cellulose; lanes 7 and 13, proteins recovered from the matrices by boiling with 0.5% SDS. The migration of protein size markers (given in kDa) is shown on the right.

**FIG. 9.** Proposed model for the participation of Ag-Aper1 and AgChi-1 (a gut-specific chitinase) in the formation and maintenance of PM structure. The two chitin-binding domains of the PM protein Ag-Aper1 cross-link chitin polymer chains to form a three-dimensional network, whereas the chitinase AgChi-1 binds to chitin and hydrolyzes it, resulting in local disruption and introduction of discontinuities in the network.
have been characterized to date reach peak expression and secrete their protein products into gut lumen relatively late after a blood meal, at a time when the lumen (and any parasites therein) is shielded from the secretory epithelium by a thick PM. Ag-Aper1 is different. Large amounts of Ag-Aper1 mRNA accumulate prior to the blood meal (Fig. 6), and its protein is presumed to be stored and released immediately after feeding. Thus, the Ag-Aper1 promoter (and its signal peptide) should be ideal for driving the expression of anti-malaria proteins in transgenic *Anopheles*.

REFERENCES

1. Peters, W. (1992) in Zoophysiology: Peritrophic Membranes (Bradshaw, S. D., Burggren, W., Heller, H. C., Ishii, S., Langer, H., Neuwiler, G., and Randall, D. J., eds) Vol. 130, Springer-Verlag, Berlin
2. Jacobs-Lorena, M., and Oo, M. M. (1996) in The Biology of Disease Vectors (Beatty, B. J., and Marquardt, W. C., eds) pp. 318–332, University Press of Colorado, Colorado
3. Tellam, R. (1996) in Biology of the Insect Midgut (Lehane, M. J., and Billingsley, P. F., eds) pp. 86–108, Chapman & Hall, London
4. Lehane, M. J. (1997) *Annu. Rev. Entomol.* 42, 148–154
5. Freyvogel, T. A., and Jaquet, C. (1965) *Acta Trop.* 18, 269–281
6. Ramos, A., Mahowald, A., and Jacobs-Lorena, M. (1994) *J. Exp. Zool.* 269, 269–283
7. Moskalyk, L. A., Oo, M. M., Jacobs-Lorena, M. (1996) *Insect Mol. Biol.* 5, 261–268
8. Elvin, C. M., Vuocolo, T., Pearson, R. D., East, I. J., Riding, G. A., Eisemann, C. H., and Tellam, R. L. (1996) *J. Biol. Chem.* 271, 8925–8935
9. Casu, R., Eisemann, C., Pearson, R., Riding, J. East, I., Donaldson, A., Cadogan, L., and Tellam, R. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 8939–8944
10. Wang, P., and Granados, R. R. (1997) *J. Biol. Chem.* 272, 16663–16669
11. Regev, A., Keller, M., Strizhov, N., Sneh, B., Prudovsky, I., Chet, I., Ginzberg, I., Konz-Kalman, Z., Konz, C., Schell, J., and Zilberstein, A. (1996) *Appl. Environ. Microbiol.* 62, 3581–3586
12. Huber, M., Cabib, E., and Miller, L. H. (1991) *Proc. Natl. Acad. Sci. U. S. A.* 88, 2807–2810
13. Lemos, F. J., Cornel, A. J., and Jacobs-Lorena, M. (1996) *Insect Biochem. Mol. Biol.* 26, 651–658
14. Laemmli, U. K. (1970) *Nature* 227, 680–685
15. Chomczynski, N., and Sacchi, N. (1987) *Anal. Biochem.* 162, 156–159
16. Feinberg, A. P., and Vogelstein, B. (1983) *Anal. Biochem.* 132, 6–13
17. Campbell, B. C., Bragg, T. S., and Turner, C. E. (1992) *Insect Biochem. Mol. Biol.* 22, 415–421
18. Freyvogel, T., and Staubli, W. (1985) *Acta Trop.* 22, 118–147
19. von Heijne, G. (1986) *Nucleic Acid Res.* 14, 4683–4690
20. Shen, Z., and Jacobs-Lorena, M. (1997) *J. Biol. Chem.* 272, 28895–28900
21. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) *J. Mol. Biol.* 215, 401–410
22. Fuhrman, J. A., Lane, W. S., Smith, R. F., Pessens, W. F., and Perler, F. B. (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 1548–1552
23. Watanae, T., Kono, M., Aida, K., and Nagasawa, H. (1996) *Mem. Mar. Biol. Biotechnol.* 5, 299–303
24. Kramer, K. J., Corpuz, I., Choi, H. K., and Muthukrishnan, S. (1993) *Insect Biochem. Mol. Biol.* 23, 691–701
25. Muller, H. M., Catteruccia, F., Vizioli, J., della Torre, A., and Crisanti, A. (1995) *Exp. Parasitol.* 81, 371–385
26. Muller, H. M., Crampton, J. M., della Torre, A., Sinden, R., and Crisanti, A. (1993) *EMBO J.* 12, 2891–2900
27. Muller, H. M., Vizioli, I., della Torre, A., and Crisanti, A. (1993) *Parasitologia (Rome)* 35 (suppl.) 73–76
28. Edwards, M. J., Lemos, F. J. A., Donnelly-Doman, M., and Jacobs-Lorena, M. (1997) *Insect Biochem. Mol. Biol.* 27, 1063–1072
29. Shahabuddin, M., Toyoshima, T., Aikawa, M., and Kaslow, D. C. (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 4266–4270
30. Deleted in proof
31. Deleted in proof
32. Willadsen, P., Eisemann, C. H., and Tellam, R. L. (1993) *Parasitol. Today* 9, 132–135
33. East, I. J., Fitzgerald, C. J., Pearson, R. D., Donaldson, R. A., Vuocolo, T., Cadogan, L. C., Eisemann, C. H., and Tellam, R. L. (1993) *Int. J. Parasitol.* 23, 221–229
34. Billingsley, P. P., and Rudin, W. (1992) *J. Parasitol.* 78, 430–440
35. Jasinskiene, N., Coates, C. J., Benedict, M. Q., Cornel, A. J., Rafferty, C. S., James, A. A., and Collins, F. H. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 3743–3747
36. Crampton, J. M., Warren, A., Lyckett, G. J., Hughes, M. A., Conley, I. P., and Eggleston, P. (1994) *Ann. Trop. Med. Parasitol.* 88, 3–12