Plasmodium, the causative agent of malaria, has to undergo sexual differentiation and development in anopheline mosquitoes for transmission to occur. To isolate genes specifically induced in both organisms during the early stages of Plasmodium differentiation in the mosquito, two cDNA libraries were constructed, one enriched for sequences expressed in differentiating Plasmodium berghei ookinetes and another enriched for sequences expressed in Anopheles stephensi guts containing invading ookinetes and early oocysts. Sequencing of 457 ookinete library clones and 652 early oocyst clones represented 175 and 346 unique expressed sequence tags, respectively. Nine of 13 clones represented 175 and 346 unique expressed sequences expressed in ookinetes and another enriched for late genes specifically induced in both organisms during ookinete differentiation and mosquito gut invasion. An-caspase-7, an Anopheles effector caspase, is proteolytically activated during Plasmodium invasion of the midgut. WARP, a gene encoding a Plasmodium surface protein with a von Willebrand factor A-like adhesive domain, is expressed only in ookinetes and early oocysts. An anti-WARP polyclonal antibody strongly inhibits (70–92%) Plasmodium development in the mosquito, making it a candidate antigen for transmission blocking vaccines. The present results and those of an accompanying report (Srinivasan, P., Abraham, E. G., Ghosh, A. K., Valenzuela, J., Ribeiro, J. M. C., Dimopoulos G., Kafatos F. C., Adams, J. H., and Jacobs-Lorena, M. (2004) J. Biol. Chem. 279, 5581–5587) provide the foundation for further analysis of Plasmodium differentiation in the mosquito and of mosquito responses to the parasite.

Malaria, the deadliest of the human parasitic diseases, is transmitted exclusively by Anopheles mosquito vectors. Plasmodium, the causative agent of malaria, has to complete a complex developmental program in the mosquito for transmission to occur (2, 3). Within minutes after a mosquito ingests an infected blood meal, gametocytes emerge from red blood cells and differentiate into male and female gametocytes. After fertilization, zygotes differentiate into ookinete that move within the blood bolus. After crossing the peritrophic matrix and the midgut epithelium, the ookinete lodge beneath the basal lamina, facing the hemocoel, and differentiate into oocysts. Each oocyst undergoes about 12 rounds of nuclear divisions to produce thousands of sporozoites that, upon oocyst maturation, are released into the hemocoel.

Difficulties with the development of an effective malaria vaccine and the emergence of drug-resistant parasites make the search for alternative weapons to fight the disease a critical priority. Little is known about the molecular mechanisms that direct parasite development in its mosquito host. The molecular dissection of Plasmodium development in the mosquito may lead to new targets for malaria control. Of the ~5000 predicted Plasmodium genes (4), only a few are known to be expressed specifically in ookinetes and oocysts (5). Proteome analysis of Plasmodium falciparum has identified a large number of proteins expressed in gametocytes and gametes (5, 6). Recent studies have begun to characterize the activation of mosquito innate immune responses during the course of Plasmodium development in the midgut (7–11). With the long term goal of understanding the molecular mechanisms that drive Plasmodium differentiation, we constructed cDNA libraries enriched for Plasmodium and Anopheles transcripts during early parasite development in the mosquito. Numerous novel Plasmodium and mosquito transcripts were isolated from these libraries. This work led to initial insights as to how the mosquito uses induction of cell death genes as a possible defense mechanism and to the identification of a candidate parasite antigen for transmission blocking vaccine.

**Experimental Procedures**

**Parasites and Mosquito Infection—**See the accompanying report (1). Ookinete Culture and Enrichment—Plasmodium berghei ookinetes were cultured in vitro and enriched as described (12). A typical enrichment yielded 70–80% ookinetes containing 15–20% blood-stage parasites.

**Subtraction Library Construction—**Total RNA was extracted using TRI reagent (Molecular Research Center, Inc.). Polyadenylated RNA was isolated using oligo(dT) (Stratagene) cellulose column chromatography. For enriched cDNA library construction, cDNA subtraction was carried out using the Clontech PCR select cDNA subtraction kit (catalog number K1804-1). Secondary PCR products were cloned into pGEMT-easy (Promega) and transformed into high efficiency DH5α Escherichia coli.
coli to yield the subtracted libraries. Two subtraction libraries, enriched ookinete and enriched early oocyst, were constructed (see “Results” for details).

cDNA Cloning and Sequencing—Individual clone inserts from the subtracted libraries were amplified using T7 and SP6 primers and sequenced on a CEQ 2000 DNA sequencing instrument (Beckman Coulter, Inc.) (1). The full-length cDNA for WARP and ancaspase-7 were isolated by screening a separate cDNA library made with the αTriplEx™ vector (Clontech) using RNAs extracted from pooled midguts dissected 24, 36, and 52 h after an infectious blood meal.

Clustering and Data Base Analysis—See the accompanying report (1). Sequences with significant similarity on BlastX (BlastX, e ≤ 10⁻⁴) were grouped based on the function of the homologous protein. Sequences with no significant BlastX similarity were grouped based on their BlastN (BlastN, e ≤ 10⁻¹⁰) similarity.

Northern Analysis and Hybridization—Northern analysis of total RNA was done as previously reported (13). A mosquito mitochondrial rRNA gene was used as a loading control (13).

Antibody Production, Immunoblotting, and Immunofluorescence—WARP was expressed as a glutathione S-transferase fusion protein (glutathione S-transferase fused to amino acids 45–303 of WARP) using the pGEX-4T1 expression vector (Amersham Biosciences) in E. coli strain BL21. The purified protein was used to raise anti-WARP antibody in a rabbit. Inhibition of oocyst formation and immunofluorescence assays were performed using anti-WARP IgG purified with the Immunopure A IgG purification kit (Pierce). Mosquito gut sheets were prepared by dissecting mosquito guts fed on infected or non-infected mice. Guts were opened longitudinally, and the resulting sheets were washed in PBS to remove blood. The sheets were fixed overnight in 4% paraformaldehyde at 4 °C, blocked for 2 h in PBS containing 4% bovine serum albumin, and incubated with WARP antibody (×1000 dilution). Antibody binding was detected with a fluorescein isothiocyanate-labeled goat anti-rabbit secondary antibody (Sigma).

Full-length ancaspase-7-His6 was expressed using the pET-15b expression vector in E. coli (BL21). The purified protein was used to raise anti-ancaspase-7 antibody in a rabbit. Antibody affinity-purified with immobilized ancaspase-7 protein using the AminoLink plus immobilization kit (Pierce, product #44884). For immunoblotting, 20 gut sheets (see above) from mosquitoes fed on infected or non-infected mice were suspended in 120 μl of 1× Laemmli buffer containing 4 μM urea and boiled for 10 min, and 12 μl were separated on 15% SDS-PAGE followed by a electrotransfer to a polyvinylidene fluoride membrane. A pre-stained protein ladder (Benchmark, Invitrogen) was used as molecular weight marker. The membrane was incubated with ancaspase-7 antibody (1:20,000), and the bound antibody was detected with a horseradish peroxidase-linked anti-rabbit immunoglobulin (Pierce, 1:30,000 dilution) by exposing the blots to x-ray films.

Passive Immunization—To measure inhibition of oocyst formation by passive immunization, a batch of 30–40 female mosquitoes (control) were fed a P. berghei-infected mouse for 20 min. Next, 1 mg of anti-WARP IgG (or pre-immune IgG as a control) was injected into the tail vein of the same mouse followed 20 min later by feeding of another batch of 30–40 mosquitoes (experimental). Fully engorged mosquitoes were selected, and the number of oocysts that formed in the gut of the surviving mosquitoes was counted 15 days later (14).

RESULTS

Subtraction Libraries—To construct an enriched ookinete library, blood-stage parasite cDNAs were subtracted from a pool of ookinetes cDNAs from four different developmental stages (6, 12, 18, and 24 h). The subtraction was expected to significantly reduce common cDNAs (e.g. housekeeping genes) and enrich for ookinete stage-specific cDNAs. Similarly, an enriched early oocyst library was constructed by subtracting midgut cDNAs from mosquitoes fed a non-infected blood meal plus blood-stage parasite cDNAs, from midgut cDNAs from mosquitoes fed a P. berghei-infected blood meal. This library was expected to be enriched for two classes of ESTs, ookinetes and early oocyst-specific ESTs and mosquito ESTs corresponding to genes induced by the parasite. To construct the enriched early oocyst library, guts were dissected at 24, 36, and 52 h after a non-infected or infected blood meal. Ookinete invasion of the midgut and the initial stages of ookinete differentiation into oocysts occurred during this period.

To assess efficiency of the enrichment procedure, Southern blots of cDNAs before and after enrichment were hybridized with a mosquito actin probe. A 7-fold weaker signal was obtained with the subtracted cDNA compared with non-subtracted cDNA, indicating that the enrichment procedure was effective (see Supplemental Fig. 1). Enrichment was further confirmed by PCR analysis of the enriched cDNAs using circumsporozoite- and thrombospondin-related adhesive protein (TRAP)-related protein (CTR), an ookinete-specific gene (15). A 4-fold stronger signal was observed with the enriched cDNA template than with the non-enriched template (see Supplemental Fig. 1).

Putative Ookinete ESTs—The 457 sequence reads from the enriched ookinete library could be grouped into 175 unique ESTs (Table I). Most of these (169 or 97%) had an (A+T) content higher than 55% (average 65%) and had homology with Plasmodium sequences in databases. This indicates that sequences with >55% (A+T) content have a high likelihood of being of Plasmodium origin (the library was constructed from in vitro transformed ookinetes, free of any mosquito tissues). Of the six (3%) ESTs with <55% (A+T), two were of mouse and four were of Plasmodium origin. The ESTs were further grouped based on their similarity with sequences in the NR and PlasmoDB databases (Fig. 1A). A high proportion (29%) of the ESTs had no similarity to proteins in the NR data base but had significant similarity to sequences in the Plasmodium genomic databases, indicating that these are novel Plasmodium genes expressed during ookinete development. Moreover, 18% of the ESTs had similarity only to the Plasmodium EST data base, not to any known protein sequences. These are presumed to represent genes expressed in blood stages or mature sporozoites, since all ESTs deposited to date in databases are from blood-stage parasites or from sporozoites. The remaining ESTs belonged to various functional classes, as illustrated in Fig. 1A. A complete tabulation of all genes is provided in Supplemental Table 1A.

Putative Early Oocyst ESTs—The 652 sequence reads from the enriched early oocyst library could be grouped into 346 unique ESTs (Table I). Of the 120 presumed Plasmodium ESTs (>55% A+T), 43 correspond to putative Plasmodium proteins with similarity to adhesive proteins, proteases and proteasomes, stress-related proteins, structural proteins, and pro-

### Table I

Characteristics of the enriched libraries

<table>
<thead>
<tr>
<th></th>
<th>Ookinete</th>
<th>Early oocyst</th>
</tr>
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<tbody>
<tr>
<td>Average size of inserts</td>
<td>380 bp</td>
<td>221 bp</td>
</tr>
<tr>
<td>Total number of sequences</td>
<td>457</td>
<td>652</td>
</tr>
<tr>
<td>Number of unique sequences</td>
<td>175 (38%)</td>
<td>346 (53%)</td>
</tr>
<tr>
<td>Unique sequences with &gt;55% (A+T)</td>
<td>169 (97%)</td>
<td>120 (35%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Mosquito</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>Unique sequences with &lt;55% (A+T)</td>
<td>6 (3%)</td>
<td>226 (65%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>Plasmodium</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Mosquito</td>
<td>0</td>
<td>194</td>
</tr>
<tr>
<td>Mouse</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>
teins involved in transcription and translation (Fig. 1B; Supplemen-
tal Table 1B). ESTs with similarity to known proteins on BlastX search were
grouped according to function of their homologues. The remaining ESTs were
searched (BlastN) for similarity to the PlasmoDB EST data base (Plasmodium
EST). All remaining ESTs had similarity to Plasmodium genomic sequences (GSS).
B, classification of 346 unique sequences from the early oocyst library (see also Ta-
ble I and Supplemental Table 1B). Sequences with (A+T) content higher or lower
than 55% are charted separately, with pie slices corresponding to presumed
Plasmodium sequences (>55% A+T) pulled out. Searches for similarity to known
proteins and to the PlasmoDB EST data base were done as in A. The remaining sequences either had similarity
to Plasmodium or Anopheles genomic sequences (GSS) or were classified as un-
knowns. Predicted protein refers to a se-
quence with similarity to genomic DNA
region predicted to code for a hypothet-
cal protein.

Putative Mosquito ESTs—Of the 226 presumed mosquito
unique ESTs (<55% A+T), 45 encode putative mosquito pro-
teins, apoptosis-related proteins, detoxification and antioxi-
dants, stress-related proteins, serpins, transmembrane proteins, proteins involved in carbohydrate and ornithine me-
tabolism, proteases and proteasomes, and proteins involved in transcription and translation (Fig. 1B, Supplemental Table
1B). An additional 64 had homology to Anopheles gambiae
genomic sequences encoding predicted proteins. Moreover, 71
had homology only to A. gambiae genomic sequences for which no open reading frame is predicted, suggesting that these are
novel mosquito genes. These results should facilitate the ongo-
ing annotation efforts. Although a number of the parasite ESTs
had matches to Plasmodium rRNA genes (Fig. 1, A and B), no
EST was identified with homology to mosquito rRNA. Possibly,
Plasmodium rRNAs were primed by oligo(dT) relatively effi-
ciently during reverse transcription because of its high (A+T)
content.

Expression Profiles—Seven randomly selected transcripts
from the ookinete library having no significant similarity to the
NR or Plasmodium EST databases were selected for expression analysis. As discussed below the presumed function of some of the gene products became known after the experiments were initiated. Fig. 2A shows the results obtained with five of the seven ESTs. The mRNA for Pbs21, an abundant ookinete specific protein, served as a control. O1228 showed similarity to an uncharacterized Plasmodium yoelii EST (H11001). O1198 shows similarity to an uncharacterized protein centrin 1 (17), was similar to O934. Expression of O2189 and O1198 also increased at the 2-h time point, whereas O1228 expression paralleled that of P. berghei ribosomal protein gene (Fig. 2, A and B). The expression profiles of O2091 and O1944 were similar to that of the ribosomal protein gene (data not shown), indicating that these may not have a specific function during ookinete differentiation. Thus, four of the seven ESTs from the ookinete library are up-regulated during ookinete differentiation.

The analysis of five putative Plasmodium genes (>55% A+T) from the early oocyst library is shown in Fig. 2B. These ESTs were selected based on their similarity to Plasmodium genomic sequences except for E3108, which had no significant similarity with sequences of any data base. Each of the five randomly selected genes has a unique expression pattern. E125 may be expressed only in blood stage parasites. E351 appears to be induced early (2 h), whereas E182, E294, and E3108 are induced at progressively later times after infection. The absence of hybridization to RNA from non-infected guts is in agreement with the initial assignment of these clones as Plasmodium ESTs.

Expression profiles of four putative mosquito genes (<55% A+T) from the early oocyst library are shown in Fig. 2C. Consistent with the tentative mosquito assignment, no signal is detected for any of the four sequences with blood stage parasite RNA and all were hybridized with RNA from mosquito guts that had ingested a non-infected blood meal. Interestingly, expression of E151, E2864, and E2998 is induced, whereas E2954 is repressed by the parasite (compare infected with non-infected). E2954 is similar to A. gambiae G12 (accession number Z22925). E2864, which has similarity to 1-Cys peroxiredoxin-like sequences, may function as an antioxidant enzyme to protect cells against oxidant-induced membrane damage (18).

An Anopheles Effector Caspase Gene Is Induced during Ookinete Invasion—Recent work suggested that apoptosis is triggered in midgut cells that are invaded by ookinetes (19, 20). In agreement with these observations, a group of genes showing similarity to apoptosis-related proteins was identified among early oocyst library sequences (Fig. 1B and Supplemental Table 1B). These included three caspases and one gene containing oxiredoxin-like sequences, which may function as an antioxidant enzyme to protect cells against oxidant-induced membrane damage (18).

An Anopheles Effector Caspase Gene Is Induced during Ookinete Invasion—Recent work suggested that apoptosis is triggered in midgut cells that are invaded by ookinetes (19, 20). In agreement with these observations, a group of genes showing similarity to apoptosis-related proteins was identified among early oocyst library sequences (Fig. 1B and Supplemental Table 1B). These included three caspases and one gene containing oxiredoxin-like sequences, which may function as an antioxidant enzyme to protect cells against oxidant-induced membrane damage (18).
mosquitoes fed a non-infected or a *P. berghei*-infected blood meal (Fig. 3C). In addition, a 19-kDa band was detected only in gut sheets from *P. berghei*-infected mosquitoes (Fig. 3C). This apparent proteolytic cleavage product appears to be due to parasite invasion in that the 19-kDa band was not detected either in guts from mosquitoes fed a non-infected blood meal (Fig. 3C) or in guts from mosquitoes fed a *P. berghei*-infected meal, dissected before the start of parasite invasion (15 h) or after invasion was completed (2 days) (data not shown). Moreover, detection of the 19-kDa band required high infection rates (mean oocyst intensity >380 oocysts/gut). These results suggest that Ancaspase-7 activation in the mosquito gut is linked to ookinete invasion of the midgut and occurs at both transcriptional and post-transcriptional levels.

The WARP Gene Plays an Essential Role in Early Parasite Development in the Mosquito—Subtraction library clones were analyzed with microarray experiments for *P. berghei* genes specific for mosquito stages of parasite development. One clone was further characterized. A full-length cDNA was isolated from a non-subtracted cDNA library prepared from mosquito guts dissected 24, 48, and 52 h after feeding with a *P. berghei*-infected blood meal. The cDNA was 1679 bp long and had a 303-amino acid-long open reading frame. While this work was in progress, the same gene was independently identified and named WARP (21). The putative protein has an amino-terminal signal sequence and a vWA-like domain (Fig. 4A). A BlastX search identified CTRP and TRAP as the closest related proteins. However, WARP contains only one vWA do-

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had a mean of 45 oocysts/gut. The probe was 32P-labeled WARP cDNA. CTRP, and TRAP genes.

B-infected mouse. /H11011] extracted at the indicated times after feeding on a

Fig. 4. Structure and expression of the WARP gene in ookinetes and in early oocysts. A, structure of the P. berghei (Pb) WARP, CTRP, and TRAP genes. B, Northern blot of A. stephensi gut RNA (3 μg of total RNA/line) extracted at the indicated times after feeding on a P. berghei-infected mouse. ~80% of the mosquitoes were infected and had a mean of 45 oocysts/gut. The probe was 32P-labeled WARP cDNA. An A. gambiae mitochondrial rRNA probe was used for the loading control. C, A. stephensi gut sheets were prepared 36 and 72 h after a non-infected or infected blood meal, as indicated. Sheets were fixed and incubated with WARP antibody, washed, and incubated with a FITC-labeled goat anti-rabbit antibody. Blue, DAPI-stained mosquito epithelial cell nuclei. Green, ookinetes (ok) or early oocysts (oc) stained by the anti-WARP antibody.

main (as opposed to six in CTRP) and does not have a predicted transmembrane domain (Fig. 4A).

Northern analysis of mosquito gut RNA at different times after ingestion of an infected blood meal revealed strong up-regulation of WARP mRNA abundance between the 12-h and 2-day time points (Fig. 4B). By 2 days the majority of the ookinetes have completed invasion of the midgut epithelium and have transformed into oocysts. A small, but reproducible decrease of WARP mRNA abundance was detected at about 1 day after blood ingestion (Fig. 4B). Moreover, WARP mRNA was not detectable at later stages of oocyst development (Fig. 4B) nor in blood-stage parasites (data not shown), indicating that WARP expression is restricted to ookinetes and early oocysts. An antibody raised against recombinant WARP detects ookinetes and early oocysts in midgut sheets prepared from mosquitoes fed an infected blood meal, confirming that the gene is expressed at both developmental stages (Fig. 4C). The WARP antibody did not recognize late oocysts (10 days) nor sporozoites isolated from mature midgut oocysts (results not shown).

We used passive immunization with anti-WARP antibodies to investigate possible roles that WARP may play in ookinete invasion and differentiation into oocysts. In four independent experiments, WARP antibody markedly reduced (69.5–92%) oocyst formation, whereas pre-immune sera had little or no effect (0–29.6%; Table II). These results indicate that the WARP protein is located on the surface of the parasite (accessible to antibodies) and that it plays an important role in the differentiation of ookinetes into oocysts, possibly mediating midgut invasion.

**DISCUSSION**

A major limitation for the study of *Plasmodium* development in the mosquito has been the difficulty to obtain and purify enough parasites at the appropriate stages of development. Another problem has been the difficulty of separating *Plasmodium* oocysts from mosquito midgut tissues, to which they are firmly attached. Until recently, in vitro differentiation of *P. berghei* was limited to the ookinete stages (12). Recently, progress has been made toward developing procedures for in vitro differentiation to later stages (22, 23), but these are not readily amenable for large-scale cultures. To circumvent some of these limitations we used subtractive hybridization, by which cDNAs from non-infected midguts were subtracted from cDNAs from infected midguts. The PCR-based enrichment protocol was successful, as shown by the efficient recovery of *Plasmodium* clones from an RNA population in which *Plasmodium* sequences constituted only a small proportion of the total. Moreover, the approach used in this study has the important advantage that it enriches not only for parasite cDNAs but also for mosquito cDNAs that are up-regulated in the presence of the parasite. One caveat is that the approach is not appropriate for the identification of genes, such as Pbs21 and Pbs25, which are under translational control (16). The enriched subtraction library clones are now being used for a microarray-based expression analysis of both mosquito and *Plasmodium* genes during the course of parasite development in the mosquito. These studies are expected to define more completely the transcriptional patterns of both novel genes and genes with a predicted function.

As presented here, several new *Plasmodium* genes that are expressed during ookinete and early oocyst stages of parasite development and mosquito genes that are up-regulated by the parasites were isolated. One group of sequences identified from the ookinete library has similarity to non-clathrin coat proteins, clathrin coat assembly proteins, and protein transport protein SEC24D (Supplemental Table 1A). In other organisms, these proteins have been implicated in vesicle formation and protein transport (24, 25), raising the possibility that these novel *Plasmodium* proteins are involved in formation of rhoptry and micronemes (organelles that store parasite secretory proteins) (3). Other genes identified in this study belong to the class of putative signaling proteins such as calcium-dependent protein kinase-3 (26), GTPase-activating protein (27), and protein-tyrosine phosphatase (28), suggesting a role in the transformation of zygote to ookinetes (Supplemental Table 1). Cysteine proteases, such as subtilisin-2 and bergapain (*P. berghei* homologue of falcipain), may participate in the process of ookinete invasion of the peritrophic matrix and the midgut epithelium (19).

Among the putative mosquito genes identified in this study is a group of genes involved in detoxification and antioxidant functions. These include the mosquito homologue of 1-cys peroxiredoxin, which has been implicated in protecting cells from oxygen radicals formed as a product of aerobic metabolism (29), and glutathione S-transferase (30).
apoptosis (19, 20). In agreement with this hypothesis, we found that Ancaspsae-7, an Anopheles effector caspase, is transcriptionally induced and proteolytically activated during parasite invasion of the mosquito gut. Caspases are made as zymogens and are present in most cells. In response to injury or developmental signals, these genes are transcriptionally (31) and/or translationally (32) activated. Proteolytic processing by upstream caspases activates zymogens that act on structural proteins in a process that eventually leads to cell death. An- 

defense against the invading parasite. In Drosophila, the caspase Dredd has been implicated in the defense against Gram-negative bacteria (33). Thus, caspase activation may be part of the mosquito defense against the invading parasite.

vWA domains are found in mammalian adhesion proteins such as integrin chains, numerous collagen types, and matrixins (34). CTRP, a vWA domain-containing ookinete protein, has been connected with ookinete gliding motility and mosquito gut infectivity (15) and shown to interact with laminin and collagen IV (35). Furthermore, TRAP, a vWA domain-containing protein, has been implicated in salivary gland invasion by sporozoites (36). It seems that the vWA domain acts as a cell-anchoring system for salivary gland and mammalian cell invasion by sporozoites (34). It is possible that during midgut invasion, WARP mediates ookinete attachment to the mosquito epithelium and during ookinete-to-oocyst differentiation, WARP mediates interactions with the mosquito basal lamina. Although WARP has a signal peptide, it does not have an obvious transmembrane or glycosylphosphatidylinositol anchor domain. The significant inhibition of oocyst formation when mosquitoes are fed on an infected mouse passively immunized with the anti-WARP antibody indicates that the antibody interferes with WARP function by recognizing the protein on the surface of the parasite and makes this a candidate antigen for a transmission-blocking vaccine. However, because the antigen used for immunization contained the WARP vWA domain, we cannot rule out that the observed effects are due to antibody interaction with other vWA domain-containing proteins.

In summary, we have used subtraction hybridization and large scale cDNA sequencing to identify parasite and mosquito genes expressed during P. berghei development in Anopheles stephensi. Northern blot analysis verified that 13 of the 18 sequences tested conformed to the expected specificity and temporal expression profile and confirmed the gene discovery value of the subtractive hybridization approach. A large proportion of the genes are novel and of unknown function. Initial experiments began to outline the role played by a mosquito caspase and a parasite WARP gene. Further functional characterization of genes from the libraries is an important challenge that may yield valuable weapons for the fight against malaria.

Acknowledgment—We are indebted to Marilyn Donnelly-Doman for expert technical assistance.

REFERENCES
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Ookinete and Mosquito Midgut Transcriptional Repertoire
