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Sex-specific and blood meal-induced proteins of *Anopheles gambiae* midguts: analysis by two-dimensional gel electrophoresis

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Published: 11 February 2003

Received: 15 November 2002

Malaria Journal 2003, 2:1

Accepted: 11 February 2003

This article is available from: <http://www.malariajournal.com/content/2/1/1>

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Abstract

Background: *Anopheles gambiae* is the main vector of *Plasmodium falciparum* in Africa. The mosquito midgut constitutes a barrier that the parasite must cross if it is to develop and be transmitted. Despite the central role of the mosquito midgut in the host/parasite interaction, little is known about its protein composition. Characterisation of *An. gambiae* midgut proteins may identify the proteins that render *An. gambiae* receptive to the malaria parasite.

Methods: We carried out two-dimensional gel electrophoresis of *An. gambiae* midgut proteins and compared protein profiles for midguts from males, sugar-fed females and females fed on human blood.

Results: Very few differences were detected between male and female mosquitoes for the approximately 375 silver-stained proteins. Male midguts contained ten proteins not detected in sugar-fed or blood-fed females, which are therefore probably involved in male-specific functions; conversely, female midguts contained twenty-three proteins absent from male midguts. Eight of these proteins were specific to sugar-fed females, and another ten, to blood-fed females.

Conclusion: Mass spectrometry analysis of the proteins found only in blood-fed female midguts, together with data from the recent sequencing of the *An. gambiae* genome, should make it possible to determine the role of these proteins in blood digestion or parasite receptivity.

Background

Anopheles gambiae is the main vector of the human malaria parasite, *Plasmodium falciparum*, in Africa. The number of cases of malaria, and their severity, have increased, leading to an increase in the social and economic burden of this disease [1,2]. The development of drug resistance in parasites and insecticide resistance in mosquitoes has contributed to this situation. Malaria incidence could be reduced by controlling parasite transmission by the

mosquito. The sporogonic development of *Plasmodium*, from gamete to oocyst formation, takes place in the lumen and epithelium of the mosquito midgut. Mosquito-specific factors probably determine the outcome of this sporogonic development. Indeed, xanthurenic acid, produced by the mosquito, is important for the exflagellation of parasite microgametes [3,4]. Trypsin, produced in the mosquito's digestive tract, probably activates parasite chitinase(s), facilitating the passage of the parasite

through the peritrophic matrix surrounding the parasite-containing blood meal in the mosquito [5,6]. Recent studies have shown that early sporogonic stages of *Plasmodium* parasite modulate the mosquito midgut immune response [7–9]. This suggests that certain immune molecules could be used to inhibit the development of *Plasmodium* in transgenic mosquitoes [10,11]. These studies, and others, were based on analyses of mRNA production; very little has been published concerning proteome analysis for mosquito midguts [7–9,12,13].

As the early phase of *Plasmodium* sporogonic development occurs at the same time as blood-meal digestion, the physiology and biochemistry of this process have been extensively studied [14–18]. Indeed, several digestive enzymes secreted within the midgut lumen have been characterised [17,19–21]. However, very few studies have focused on characterisation of the proteins of the mosquito midgut epithelium. Using monoclonal antibodies, Lal *et al.* [22] recently identified a set of midgut proteins that may be involved in *Plasmodium* development. Ghosh *et al.* [23] screened a phage display library and selected a peptide that recognised midgut protein(s), as yet unidentified. The production of this peptide in transgenic *Anopheles stephensi* mosquitoes reduced the development of *Plasmodium berghei* oocysts on the mosquito midgut wall [24].

We analysed the midgut protein profile of female *An. gambiae* by two-dimensional (2-D) gel electrophoresis. Midguts were isolated 19 h after feeding on uninfected human blood. This time course corresponds to the early phase of ookinete interaction with midgut cells in mosquitoes fed on the blood of gametocyte carriers. We compared the profile obtained with those from the midguts of males and females not fed on blood. We identified a set of proteins that were specifically produced and regulated in females following blood ingestion. The recently determined sequence of the *An. gambiae* genome could be used in the analysis of these proteins and their potential functions, and transgenesis may provide a new tool for studying the involvement of mosquito proteins in *Plasmodium* sporogonic development.

Methods

Mosquito rearing and blood-feeding

An. gambiae strain G3 was reared at 26°C, in conditions of 80% relative humidity and a 12/12 h light/dark cycle. For mass rearing, female mosquitoes were allowed to feed on the blood of an anaesthetised rabbit. The blood-fed females used in this analysis were first starved for 12 h and then fed on uninfected human blood, using a membrane-feeding device [25]. Unfed or partially fed females were discarded.

Midgut preparation

All dissections were performed on ice, in PBSI (phosphate-buffered saline containing 1 mM EDTA and 1 mg/ml Pefabloc®). Midguts were dissected from 4-day-old sugar-fed male and female mosquitoes. Midguts were isolated from blood-fed mosquitoes 19 hours after blood-feeding. Midguts from blood-fed mosquitoes were opened by a longitudinal incision and thoroughly rinsed in ice-cold PBSI to remove all traces of peritrophic matrix and gut contents. Dissected midguts were stored at -80°C until processing.

Protein extraction

Proteins were extracted as previously described [26]. Briefly, sixty midguts were placed in 150 µl of extraction buffer (0.6% sodium-dodecyl sulphate (SDS), 0.2% β-mercaptoethanol, 10 mM Tris-HCl pH 8.0) supplemented with a cocktail of protease inhibitors (1 µg/ml antipain; 1 µg/ml aprotinin; 1 mM EDTA; 100 µM TPCK; 1 µg/ml leupeptin; 1 mg/ml Pefabloc®; 1 µg/ml pepstatin (Boehringer Mannheim). Samples were homogenized with a Wheaton-33® Potter-Elvehjem tissue grinder, transferred to an Eppendorf tube and boiled for 4 minutes. The samples were centrifuged for 10 minutes at 9,000 g, 4°C, and the supernatant was transferred to a fresh tube. Proteins were concentrated and SDS and β-mercaptoethanol eliminated by precipitation with acetone as follows: 1.5% 1 N NaOH and 9 volumes of ice-cold acetone were added to each protein sample. Samples were incubated for 3 hours or overnight at -20°C and centrifuged for 10 minutes at 9,000 g, 4°C. Pellets were dried for 1 hour under vacuum, using a "speed vac" (Savant) and resuspended in either distilled water, for protein determination, or sample buffer (9.95 M urea; 4% NP-40; 2% ampholines; 100 mM dithiothreitol), for 2-D electrophoresis. For each extract, proteins were precipitated from two separate aliquots: a 30 µl aliquot for protein determination, and a 120 µl aliquot for 2-D electrophoresis.

Protein determination

Protein concentrations were determined in duplicate, using the Lowry-SDS method and microtitre plates [27,28]. Bovine serum albumin was used as a standard (1 to 20 µg). Absorbance at 630 nm was measured with a Biotek EL 311 spectrophotometer.

Electrophoresis

Two-dimensional gel electrophoresis was performed essentially as described by Garrels [29], with the modifications of Laurent-Winter *et al.* [30]. For the first dimension (isoelectric focusing, IEF), we used gradient gels covering the range pH4 to pH8 (ampholines, Millipore Inc.), which were run for 20,000 Vh. We used 10% acrylamide gels for the second dimension. Gels were silver stained as described by Morrissey [31]. For each sample, 30 µg of

protein was run on the gel and molecular weight markers were applied in a separate slot. For each type of midgut (male, sugar-fed female and blood-fed female), at least three independent sample preparations were used and at least three independent gel analyses performed.

Data analysis

Silver-stained gels were examined by eye, using a light box. Protein extracts were compared in pairs by superimposing the corresponding 2-D gels. Differences in the pattern of spots between two extracts were scored if reproduced in at least three independent experiments. We considered only differences involving the presence or absence of a particular spot but differences in the intensities of spots for proteins with identical migration patterns were also observed.

Spots and their corresponding proteins were classified into three categories: a) constitutive – detected in midgut extracts from both sugar-fed and blood-fed females; b) BDP – detected only in blood-fed mosquitoes; c) SDP – detected only in sugar-fed mosquitoes.

Results and Discussion

Midgut proteins extracted from male and female mosquitoes were analysed by 2-D gel electrophoresis. Gels were silver stained and protein profiles compared in pairs. At least three independent samples were analysed for each physiological condition. A representative example of the 2-D gel patterns observed is presented in Figure 1, with panels A and B corresponding to sugar-fed and blood-fed female mosquitoes, respectively, and panel C, to sugar-fed male mosquitoes. All extracts from male and female mosquitoes gave approximately 375 well-resolved spots. We identified a number of protein spots displaying a specific pattern of expression, with differences either between males and females or between sugar-fed and blood-fed females mosquitoes.

Sex-specific proteins from *An. gambiae* midguts

Comparison of 2-D electrophoresis profiles for male and female mosquitoes (Figure 1) revealed that the midguts of males and females differ by only a few proteins (Figure 2). Ten proteins were identified as specifically produced in male midguts and not in female midguts, whereas 23 proteins were identified as female-specific proteins. Some of these female-specific proteins were produced only after a sugar meal (SDP, for sugar meal-dependent proteins), some were produced only after a blood meal (BDP, for blood-meal-dependent proteins), and some were produced after both types of meal (constitutively expressed). Eight other proteins were common to the midguts of sugar-fed males and females and were absent from the midguts of blood-fed females. We took into account only differences in the presence/absence of protein spots, al-

though some spots, such as spots A and B, also reproducibly displayed differences in intensity (Figure 1, panel A).

Although midgut morphology differs between male and female mosquitoes, the cellular structure of the midgut is almost identical in males and females, consisting of columnar and regenerative cells [32–34]. The morphological differences may reflect specific functions, and may imply differences at the molecular level.

Both male and female mosquitoes feed on nectar. The ten male-specific proteins are therefore more likely to be involved in male-specific functions or structures than in nectar processing, whereas the eight proteins shared by sugar-fed males and females and absent from blood-fed females are probably involved in sugar processing. In contrast, as male mosquitoes never ingest blood, the BDP detected in the midguts of blood-fed females but not in males are presumably involved in blood digestion, a female-specific function.

Cazares-Raga *et al.* [35] analysed the sex-specific proteins and proteases present in the midguts of *Anopheles albimanus* mosquitoes. They detected approximately 150 well-resolved spots and found that the protein profiles of midguts from males and females differed considerably. The overall profile obtained with midguts from *An. albimanus* males was similar to that obtained in this study with midguts from *An. gambiae* males. However, the pattern for *An. albimanus* females mostly included low-molecular weight proteins. In our experimental conditions, profiles of this type were invariably associated with proteolysis of the sample, resulting in the accumulation of proteins in the lower part of the gel.

Identification of midgut proteins regulated upon blood ingestion

Comparison of protein patterns from sugar-fed and blood-fed female *An. gambiae* revealed a limited number of differences. Five of the 23 female-specific proteins (Figure 2) were produced in the midguts of both sugar-fed and blood-fed females; these proteins were classified as constitutive proteins. Eight proteins were present only in sugar-fed females (SDP). The ten remaining proteins were present in the midguts of females only after a blood meal (BDP). In addition to differences in the presence/absence of protein spots, we also observed reproducible differences in spot intensity for another small set of proteins. The amount of these proteins either decreased (spots A & B panel A) or increased (spots C-F, panel B) upon blood-feeding.

Proteins specifically produced in blood-fed mosquitoes and proteins overproduced upon blood-feeding are likely to be involved in blood digestion or in synthesis of the

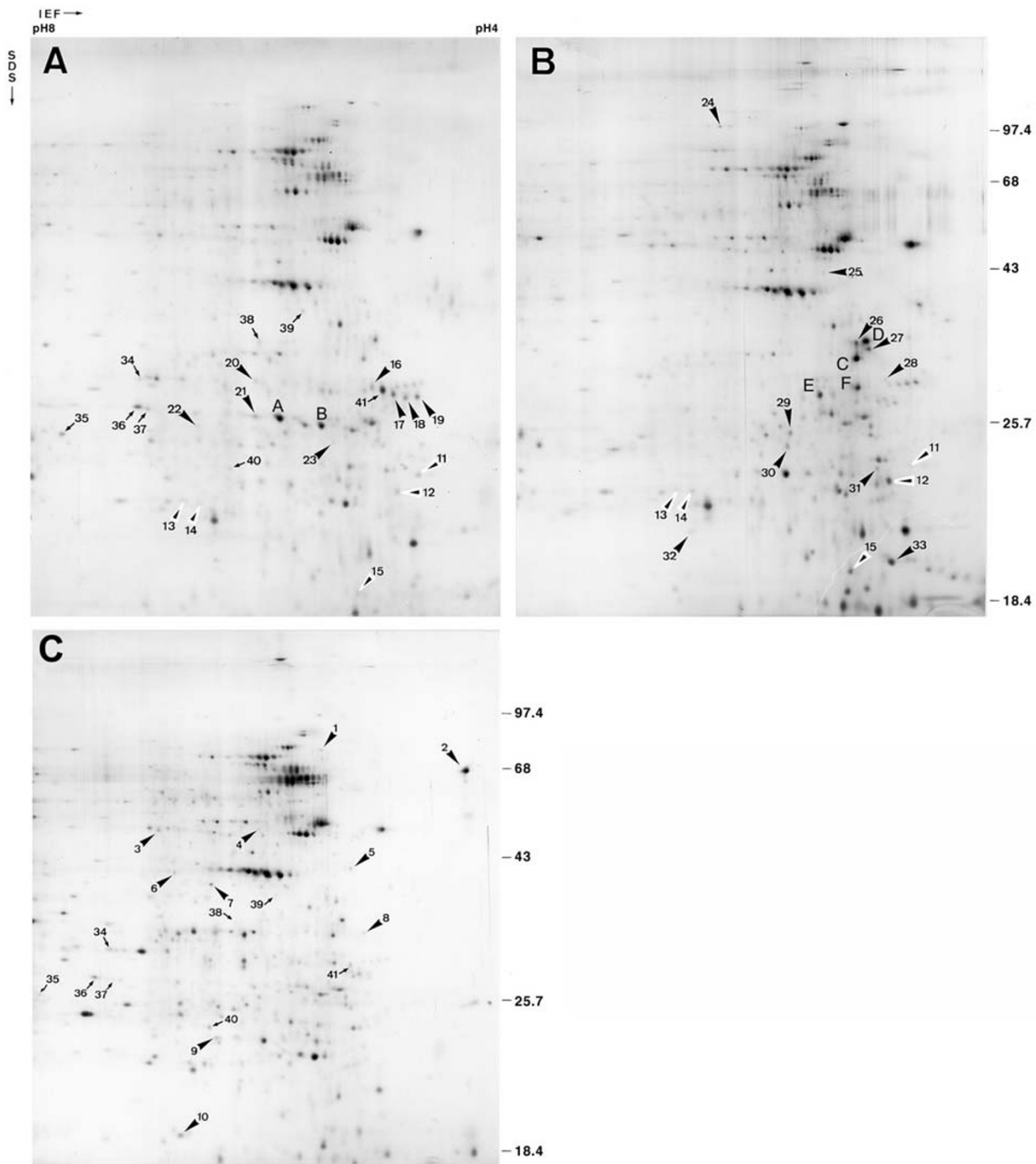


Figure 1

Silver-stained 2-D gels of *Anopheles gambiae* midgut proteins: **(A)** Sugar-fed females. **(B)** Blood-fed females. **(C)** Males. Black arrowheads: sugar-meal-dependent proteins (panel A), blood-meal-dependent proteins (panel B), male-specific proteins (panel C); white and black arrowheads: constitutive female-specific proteins (panels A and B); thin arrows: proteins detected in both sugar-fed females and males (panels A and C). Numbers refer to proteins described in Figure 2; lettered spots are discussed in the main text. The molecular weights of standards (kDa) are indicated on the right-hand side of the gels.

Male	Sugar-fed Female	Blood-fed Female	Number of spots	Effect of the meal	Spot Number
-	+	+	5	Constitutive	11 - 15
-	+	-	8	SDP	16-23
-	-	+	10	BDP	24-33
+	-	-	10	-	1-10
+	+	-	8	SDP	34-41
+	+	+	358	None	not numbered

Figure 2

Midgut protein differences between male and female *Anopheles gambiae* mosquitoes. Spot number refers to the labelling used in Figure 1. SDP denotes a protein detected in sugar-fed mosquitoes that was absent from blood-fed mosquitoes; BDP: absent from sugar-fed mosquitoes and present in blood-fed mosquitoes; constitutive: present in both blood-fed and sugar-fed mosquitoes [Female-specific proteins: dark shaded fields; Male-specific proteins: light shaded fields].

peritrophic matrix that surrounds the blood meal. Alternatively, some of these proteins may be involved in defence reactions directed against micro-organisms ingested along with the blood meal. We conducted our analysis 19 h after feeding. At this time, *Plasmodium* ookinetes interact with midgut cells if the mosquito fed on *Plasmodium* gametocyte-containing blood; some of the BDP may therefore be involved in host-parasite interactions as well. Indeed, in a well-adapted host-parasite system, such as that constituted by *P. falciparum* and *An. gambiae*, the parasite probably makes use of pre-existing host molecules to ensure its development at a lower cost to the host. If this is indeed the case, then some of the BDP identified here may be of relevance to *P. falciparum/An. gambiae* midgut interactions.

In previous studies of differences between *An. stephensi* strains refractory and susceptible to infection by *P. falciparum*, we also detected sugar-meal and blood-meal-de-

pendent proteins in female mosquitoes [26]. The main difference between the refractory and susceptible *An. stephensi* lines was the larger number of BDP in the susceptible line. This observation is consistent with the possible involvement of some midgut BDP in *Plasmodium/Anopheles* midgut interactions.

Conclusions

Overall, in found 16 SDP in the midguts of *An. gambiae* females: eight were female-specific and the other eight were present in both males and females. We also found ten BDP. All the BDP were female-specific, as males do not feed on blood. The characterisation of BDP by mass spectrometry of protein spots isolated from gels will be greatly facilitated by the recent completion of the whole genome sequence of *An. gambiae* [36]. This will undoubtedly provide additional information as to whether these proteins are involved in blood digestion or in the development of the malaria parasite. The characterisation of female-specif-

ic proteins may also be useful for the identification of blood meal-inducible, female-specific promoters, which could be used to drive the expression of genes encoding proteins impairing the development of *Plasmodium* parasites in transgenic mosquitoes [37].

Authors' contributions

GP carried out mosquito manipulation and dissection, protein preparation and gel analyses. C.L.-W. carried out 2-D gel electrophoresis. FR provided the scientific environment and CB conceived and supervised the experimental work and the writing of the manuscript.

Acknowledgements

We would like to thank Jean-Claude Jacques for technical assistance and Nadia Monnier for rearing mosquitoes. This work was supported in part by fellowships awarded to G.P. (MRE, FRM and CRG) and research funds from the Pasteur Institute and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR).

References

- Greenwood B and Mutabingwa T **Malaria in 2002**. *Nature* 2002, **415**:670-2
- Sachs J and Malaney P **The economic and social burden of malaria**. *Nature* 2002, **415**:680-5
- Billker O, Lindo V, Panico M, Etienne AE, Paxton T, Dell A, Rogers M, Sinden RE and Morris HR **Identification of xanthurenic acid as the putative inducer of malaria development in the mosquito**. *Nature* 1998, **392**:289-292
- Garcia GE, Wirtz RA and Rosenberg R **Isolation of a substance from the mosquito that activates *Plasmodium* fertilization**. *Mol Biochem Parasitol* 1997, **88**:127-135
- Shahabuddin M and Kaslow DC ***Plasmodium*: Parasite chitinase and its role in malaria transmission**. *Exp Parasitol* 1994, **79**:85-88
- Shahabuddin M, Lemos FJA, Kaslow DC and Jacobs-Lorena M **Antibody-mediated inhibition of *Aedes aegypti* midgut trypsin blocks sporogonic development of *Plasmodium gallinaceum***. *Infect Immun* 1996, **64**:739-743
- Dimopoulos G, Seeley D, Wolf A and Kafatos FC **Malaria infection of the mosquito *Anopheles gambiae* activates immune-responsive genes during critical transition stages of the parasite life cycle**. *EMBO J* 1998, **17**:6115-6123
- Dimopoulos G, Muller H, Levashina EA and Kafatos FC **Innate immune defense against malaria infection in the mosquito**. *Curr Opin Immunol* 2001, **13**:79-88
- Tahar R, Boudin C, Thiery I and Bourgoon C **Immune response of *Anopheles gambiae* to the early sporogonic stages of the human malaria parasite, *Plasmodium falciparum***. *EMBO J* 2002, **21**:6673-6680
- Kokoza V, Ahmed A, Cho WL, Jasinskiene N, James AA and Raikhel A **Engineering bloodmeal-activated systemic immunity in the yellow fever mosquito, *Aedes aegypti***. *Proc Natl Acad Sci U S A* 2000, **97**:9144-9
- Raikhel AS, Kokoza VA, Zhu J, Martin D, Wang S-F, Li C, Sun G, Ahmed A, Dittmer N and Attardo G **Molecular biology of mosquito vitellogenesis: from basic studies to genetic engineering of antipathogen immunity**. *Insect Biochem Mol Biol* 2002, **32**:1275-1286
- Shen Z, Dimopoulos G, Kafatos FC and Jacobs-Lorena M **A cell surface mucin specifically expressed in the midgut of the malaria mosquito *Anopheles gambiae***. *Proc Natl Acad Sci U S A* 1999, **96**:5610-5
- Oduol F, Xu JN, Niare O, Natarajan R and Vernick KD **Genes identified by an expression screen of the vector mosquito *Anopheles gambiae* display differential molecular immune response to malaria parasites and bacteria**. *Proc Natl Acad Sci U S A* 2000, **97**:11397-11402
- Billingsley PF and Hecker H **Blood digestion in the mosquito *Anopheles stephensi* Liston (Diptera: Culicidae): activity and distribution of trypsin, aminopeptidase and α -glucosidase in the midgut**. *J Med Entomol* 1991, **28**:865-871
- Chadee DD and Beier JC **Blood-digestion kinetics of four *Anopheles* species from Trinidad, West Indies**. *Ann Trop Med Parasitol* 1995, **89**:531-540
- Chege GM and Beier JC **Blood acquisition and processing by three *Anopheles* (Diptera: Culicidae) species with different innate susceptibilities to *Plasmodium falciparum***. *J Med Entomol* 1998, **35**:319-23
- Clements AN **The biology of mosquitoes**. London: Chapman and Hall 1992,
- Lehane MJ and Billingsley PF **The Insect Midgut**. Chapman and Hall 1996,
- Graf R, Raikhel AS, Brown MR, Lea AO and Briegel H **Mosquito trypsin: immunocytochemical localisation in the midgut of blood-fed *Aedes aegypti* (L.)**. *Cell Tissue Res* 1986, **245**:19-27
- Muller HM, Vizioli I, della Torre A and Crisanti A **Temporal and spatial expression of serine protease genes in *Anopheles gambiae***. *Parassitologia* 1993, **35**:73-6
- Müller HM, Crampton JM, Dellatorre A, Sinden R and Crisanti A **Members of a trypsin gene family in *Anopheles gambiae* are induced in the gut by bloodmeal**. *EMBO J* 1993, **12**:2891-2900
- Lal AA, Patterson PS, Sacci JB, Vaughan JA, Paul C, Collins WE, Wirtz RA and Azad AF **Anti-mosquito midgut antibodies block development of *Plasmodium falciparum* and *Plasmodium vivax* in multiple species of *Anopheles* mosquitoes and reduce vector fecundity and survivorship**. *Proc Natl Acad Sci U S A* 2001, **98**:5228-33
- Ghosh AK, Ribolla PE and Jacobs-Lorena M **Targeting *Plasmodium* ligands on mosquito salivary glands and midgut with a phage display peptide library**. *Proc Natl Acad Sci U S A* 2001, **98**:13278-81
- Ito J, Ghosh A, Moreira LA, Wimmer EA and Jacobs-Lorena M **Transgenic anopheline mosquitoes impaired in transmission of a malaria parasite**. *Nature* 2002, **417**:452-5
- Tchuinkam T, Mulder B, Decherer K, Stoffels H, Verhave JP, Cot M, Carnevale P, Meuwissen JHET and Robert V **Experimental infections of *Anopheles gambiae* with *Plasmodium falciparum* of naturally infected gametocyte carriers in Cameroon - factors influencing the infectivity to mosquitoes**. *Trop Med Parasitol* 1993, **44**:271-276
- Prévot GI, Laurent-Winter C, Feldmann AM, Rodhain F and Bourgoon C **Two-dimensional gel analysis of midgut proteins of *Anopheles stephensi* lines with different susceptibility to *Plasmodium falciparum* infection**. *Insect Mol Biol* 1998, **7**:375-383
- Harrington CR **Lowry protein assay containing sodium dodecyl sulfate in microtiter plates for protein determinations on fractions from brain tissue**. *Anal Biochem* 1990, **186**:285-287
- Markwell MAK, Haas SM, Bieber LL and Tolbert NE **A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples**. *Anal Biochem* 1978, **87**:206-210
- Garrels JI **Quantitative two-dimensional gel electrophoresis of proteins**. *Meth Enzymol* 1983, **100**:411-423
- Laurent-Winter C, Fougère-Deschattre C and Weiss MC **Identification of polypeptides whose presence correlates with retention or loss of an albumin extinguisher chromosome in rat hepatoma-mouse L cell fibroblast microcell hybrids**. *Differentiation* 1994, **55**:225-232
- Morrissey JH **Silver stain for proteins in polyacrylamide gels: a modified procedure with enhanced uniform sensitivity**. *Anal Biochem* 1981, **117**:307-310
- Hecker H, Freyvogel TA, Briegel H and Steiger R **The ultrastructure of midgut epithelium in *Aedes aegypti* L. (Insecta, Diptera) males**. *Acta Trop* 1971, **28**:275-290
- Hecker H, Freyvogel TA, Briegel H and Steiger R **Ultrastructural differentiation of the midgut epithelium in female *Aedes aegypti* L. (Insecta, Diptera) imagines**. *Acta Trop* 1971, **28**:80-104
- Hecker H **Structure and function of midgut epithelial cells in Culicidae mosquitoes (Insecta, Diptera)**. *Cell Tissue Res* 1977, **184**:321-341
- Cazares-Raga FE, Sanchez-Contreras ME, Rodriguez MH and Hernandez-Hernandez FC **Sex-specific proteins and proteases present in the midguts of *Anopheles albimanus* (Diptera: Culicidae)**. *J Med Entomol* 1998, **35**:184-6
- Holt RA, Subramanian GM, Halpern A, Sutton GG, Charlab R, Nusser DR, Wincker P, Clark AG, Ribeiro JM and Wides R **The ge-**

nome sequence of the malaria mosquito *Anopheles gambiae*.
Science 2002, **298**:129-49

37. Grossman GL, Rafferty CS, Clayton JR, Stevens TK, Mukabayire O and Benedict MQ **Germline transformation of the malaria vector, *Anopheles gambiae*, with the piggyBac transposable element.** *Insect Mol Biol* 2001, **10**:597-604

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