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Pernicious plans revealed: *Plasmodium falciparum* genome wide expression analysis

Manuel Llinás* and Joseph L DeRisi

The asexual intraerythrocytic developmental cycle (IDC) of *Plasmodium falciparum* is responsible for the majority of the clinical manifestations of malaria in humans. Although malaria has been studied for over a century, the elucidation of the full genome sequence of *P. falciparum* has now allowed for in-depth studies of gene expression throughout the entire intraerythrocytic stage. As the mainstays of anti-malarial chemotherapy become increasingly ineffective, we need a deeper understanding of fundamental plasmodial bioregulatory mechanisms to successfully subvert them. Recent gene expression studies have begun to examine different aspects of the IDC and are providing key insights into the basic mechanisms of *Plasmodium* gene regulation and are helping to define gene functions. However, to date, no transcription factor has been fully characterized from *Plasmodium* and the definitive identification of *cis*-acting regulatory elements along with their corresponding *trans*-acting partners is still lacking. The characterization of the transcriptome of *P. falciparum* is the first major step towards the understanding of the genome wide regulation of gene expression in this parasite. IDC expression data for almost every gene in the *P. falciparum* genome can now be publicly queried at http://plasmodb.org and http://malaria.ucsf.edu. The results of these studies suggest promising leads for identifying novel targets for anti-malarial therapeutics and vaccines in addition to providing a solid foundation for the ongoing elucidation of plasmodial gene expression.

Addresses
Department of Biochemistry and Biophysics, UC San Francisco, 600-16th Street, BOX 2240, San Francisco, California 94143-2240, USA
*e-mail: manuel@derisilab.ucsf.edu

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Abbreviations
EST expressed sequence tag
IDC intraerythrocytic developmental cycle
ORF open reading frame

Introduction

Malaria is one of the most prevalent diseases worldwide affecting upwards of 400 million persons, and causing over 2.5 million deaths annually [1]. Of the four known human *Plasmodium* species, *P. falciparum* is the most lethal. Despite numerous large-scale attempts at malaria control, both at the parasite and vector level, *P. falciparum* continues to present a major health burden worldwide mainly owing to widespread drug resistance. This fact has renewed the urgency for the identification of new drug targets for chemotherapy and/or vaccine development.

The lifecycle of *Plasmodium* consists of three major stages, the mosquito stage, the liver stage, and the intraerythrocytic stage. The intraerythrocytic developmental cycle (IDC) or red blood cell stage is remarkably short, varying from 48 h (*P. ovale*, *P. vivax*, and *P. falciparum*) to 72 h (*P. malariae*) in length between the four human species. During this cycle, *Plasmodia* undergo an identical series of morphological changes [2] to complete iterative cycles of parasite replication, escape from red blood cells, and re-infection within the host (Figure 1).

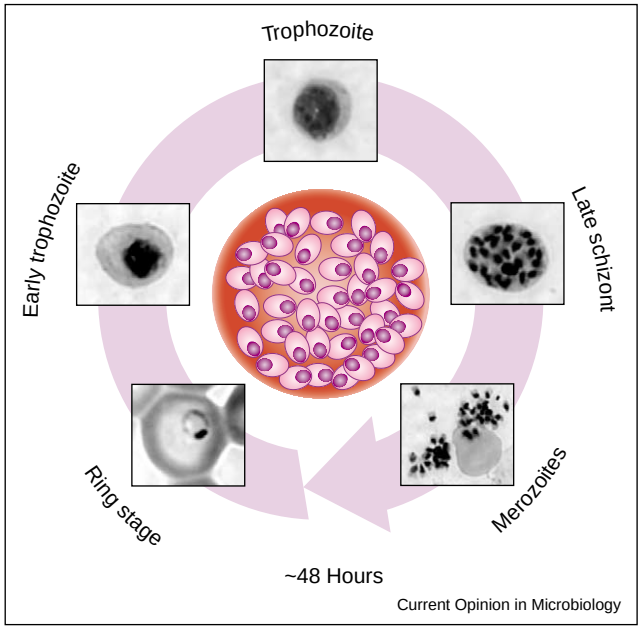
These morphological transformations imply that a high degree of regulation must exist to check and ensure that proteins necessary throughout the IDC are present at the appropriate times for precise developmental progression. However, little is known about the mechanisms for gene regulation in *Plasmodia*. The most comprehensive data regarding gene regulation in *P. falciparum* is for the *var* genes, which are located mostly in the sub-telomeric regions of chromosomes and has led to models of gene silencing with regard to antigenicity and immune evasion [3]. While there are several reports of *cis* acting regulatory elements [4–7], the proteins that interact with these regions remain unidentified. Moreover, *cis*-acting element specificity, genomic distribution, and contribution to the IDC remains unclear. Furthermore, while stage-specific transcripts have been widely identified, an inducible promoter has yet to be described.

As the sequences of new parasite genomes become available, there is an increasing demand for whole genome approaches to better characterize these organisms. In this review, we focus on the various methodologies that have been applied toward characterizing gene regulation in the developmental lifecycle of *Plasmodium falciparum* and how these approaches have evolved with the availability of the completed genome.

Pre-genome transcriptome analysis

In 1996, the *P. falciparum* genome sequencing project was launched [8] between three institutions: The Institute for Genomic Research (TIGR), Stanford University, and the

Figure 1



The *Plasmodium* intraerythrocytic developmental cycle (IDC).

Sanger Center. As sequence information was gathering, several genomic efforts were pursued to investigate transcriptional differences between the various stages of development. The first large scale *P. falciparum* study was accomplished using a DNA microarray constructed using a mung bean nuclease-generated genomic library containing 3648 random sequences [9]. By this method, stage-specific transcripts were identified by analyzing mRNA expression differences between the asexual trophozoite stage and the sexual gametocyte stage. This study was quickly followed by the application of serial analysis of gene expression (SAGE) measuring 4866 genes in an asynchronous population of parasites during the intraerythrocytic stage of development [10,11] and demonstrated that many of the genes in *P. falciparum* were likely to be expressed during this stage. Using a 944 element expressed sequence tag (EST)-based DNA microarray, Ben Mamoun *et al.* [12] captured the first glimpse of the temporal changes in gene expression throughout the asexual developmental process. While these studies provided the first hints of transcriptional differences between the major stages of *P. falciparum* development, they were limited in scope as they used relatively small cDNA libraries, subsets of ESTs, or asynchronous parasite populations.

As individual chromosome sequences became available [13,14], new approaches were undertaken to provide more complete analyses of gene expression. One such study used a short oligonucleotide Affymetrix array based only on the full coding sequence of Chromosome 2 [15]. In this case, three IDC stages were compared: two days post-synchronization (ring), 20–24 h thereafter (trophozoite), and again 16 h later (schizont). The results demonstrated that one-third of the expressed transcripts detected from Chromosome 2 were differentially transcribed across the three time points measured. In another study, Bozdech *et al.* [16•] assembled all of the available incomplete, un-annotated sequence, carried out gene predictions using TIGR's GlimmerM tool [17] and verified that 70-mer long oligonucleotide based DNA microarrays could accurately determine transcriptional differences throughout the genome between the trophozoite and schizont stages. This study identified over 800 genes that were differentially transcribed between these two morphological IDC stages. The detected changes in gene expression identified in these recent studies highlight the importance of synchronized parasites to the study of gene expression in *P. falciparum*.

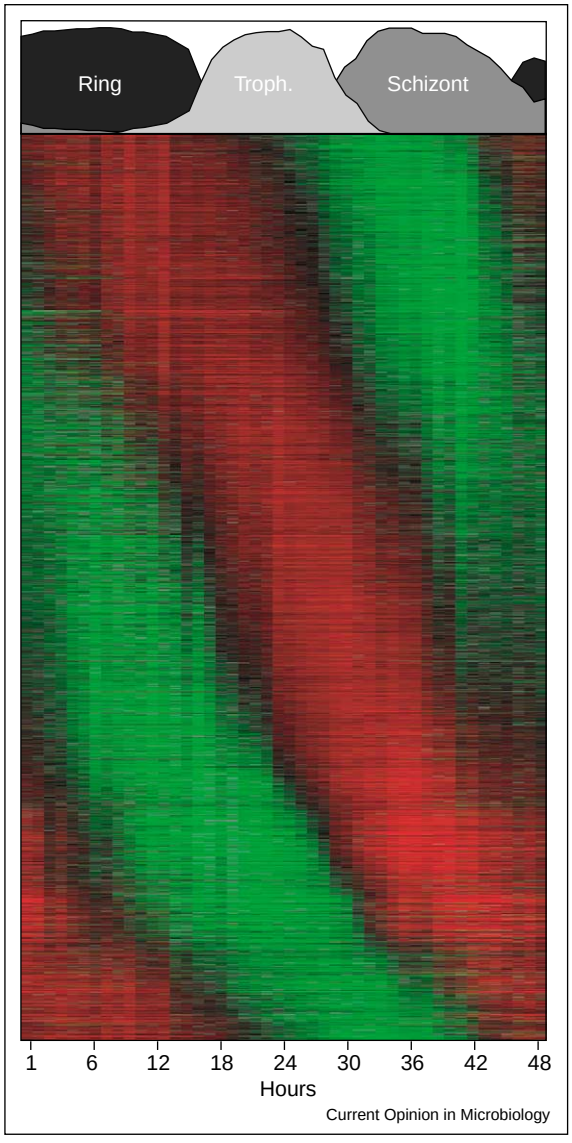
Whole genome approaches

In 2002, the complete sequence for the 3D7 strain of *P. falciparum* was published [18••]. The genome is 22.8 Mb in size and contains approximately 5400 open reading frames (ORFs) distributed across 14 chromosomes. In addition, the genome contains a 6 kb linear mitochondrial genome encoding three proteins plus rRNAs as well as a 35 kb circular plastid genome with over 60 ORFs [19]. Some remarkable features of the genome are the high A/T content, approximately 80% in coding and 90% in non-coding regions, and the high proportion (greater than 60%) of predicted ORFs with no homology in other organisms. With the completion of the genome, genome-wide approaches could now be applied to the study of this parasite.

Two whole-genome microarray platforms have emerged with full-genome coverage, providing the first glimpse of the full transcriptome of *P. falciparum* [20••,21••]. These studies both demonstrated that most of the 5400 predicted ORFs are utilized during the IDC implying a high degree of overlap in the metabolic requirements of the intraerythrocytic, mosquito, and liver stages of development. The work by Le Roch *et al.* [21••] includes a broad analysis of the *P. falciparum* transcriptome, examining six representative timepoints from the 48 h IDC, one sample each from the red blood cell invading merozoite stage, stage IV and V gametocytes, and sporozoites from mosquito salivary glands. The data samples from stages outside the IDC suggest that approximately 200 genes may be specifically transcribed in gametocytes, while only 41 transcripts are likely to be sporozoite-specific. Twenty percent of all predicted transcripts were characterized as IDC-specific. Overall, less than half of the genes were classified as cell-cycle regulated, and approximately half of the data was further grouped into 15 general functional clusters.

The study by Bozdech and Llinás *et al.* [20••] specifically details the IDC at one-hour time intervals and provides a level of resolution that highlights the periodic nature of most *P. falciparum* transcripts (Figure 2). The observed cascade of gene expression during the 48 hour IDC is suggestive of a 'just in time' mode of control whereby genes are only activated as their biological function becomes necessary to the parasite with respect to a fixed order. The data also demonstrate that most genes are induced maximally at a time when they are essential to the parasite, after which the genes are downregulated. Over 80% of the genes are regulated in this manner throughout the IDC, suggesting that *P. falciparum* contains very few housekeeping genes that are constitutively expressed throughout the IDC.

Figure 2



The transcriptome of *P. falciparum* demonstrates a cascade of gene expression throughout the IDC. Each row represents the expression profile for an individual gene [20••].

A major outcome of the *P. falciparum* transcriptome is the potential for further categorization of the 60% of the genome encoding genes of unknown functionality. This will undoubtedly aid in the future identification of novel targets for anti-malarial therapies and vaccine strategies. The strong predictive power of the data could provide an initial categorization of genes throughout the IDC as early, mid or late stages. The assignment of genes to stages based on their phase (timing) of expression as well as pattern of gene expression will begin to suggest the processes that these genes are involved in. Recently, the subcellular localization for various proteins involved in heme biosynthesis has been identified and, unlike in any other organism, this process appears to occur both in the plastid and the mitochondrion [22]. Coincidentally, the gene expression patterns of genes specific to a given organelle also display similar expression patterns. For example, δ-aminolevulinic acid dehydratase (PF14_0381) and uroporphyrinogen decarboxylase (MAL6P1.76), which are targeted to the plastid, are maximally expressed at 23 hours post invasion, whereas δ-aminolevulinic acid synthetase (PFL2210w), coproporphyrinogen oxidase (PF11_0436), and protoporphyrinogen oxidase (PF10_0275), all mitochondrial enzymes, are expressed much earlier with peak expression at 14 hours [20••]. This observation supports previous data suggesting that proper timing of gene expression is essential for accurate subcellular localization of proteins in *P. falciparum* [23].

A preliminary analysis of major metabolic functions including transcription, translation, glycolysis, ribonucleotide synthesis, deoxyribonucleotide synthesis and the TCA cycle, identified a striking correlation in gene expression for genes of similar functions [20••]. Furthermore, the induction of gene expression for these different functions is highly correlated with specific windows of time within the IDC. For example, upon invasion of the red blood cell, among the first genes to be induced are those that comprise the transcriptional machinery itself, including the recognizable subunits of RNAP II, TFIIB, TFIID, and TFIIH. This wave of induction is closely followed by genes involved in cytoplasmic translation. An interesting observation is that the induction of transcripts for genes involved in ribonucleotide synthesis and deoxyribonucleotide synthesis is offset by approximately 10 hours. In this manner, perhaps the parasite more efficiently utilizes the limited pools of nucleotide precursors only as absolutely required by focusing initially on mRNA production prior to DNA replication. Likewise, at the later stages of the IDC, during the mid to late schizont stage, a similar pattern of gene induction is measured for gene products known to be necessary to merozoite invasion. These merozoite-specific genes

are comprised of approximately 40% surface antigens, 30% kinases and phosphatases, and 10% proteases plus others. It is remarkable that all of the genes previously known to have an assigned role in merozoite invasion are also co-regulated at the transcriptional level, typified by the well-characterized merozoite surface protein (MSP), rhoptry, and erythrocyte binding antigen family of genes (for complete list see [20••]). Of course, there are numerous 'hypothetical proteins' of unknown function that follow similar patterns of induction and which deserve further exploration as candidates involved in merozoite invasion.

An interesting implication of the transcriptional cascade is that, in reality, distinct developmental stages, thought to be marked by transcriptional bursts throughout the IDC may not really exist. Rather, this transcriptional cascade likely reflects a continuum of development. Thus, the morphological ring, trophozoite, and schizont stages, while useful for microscopy, may not have a real transcriptional basis as distinct developmental states.

All analysis of gene regulation in *Plasmodium* is subject to a background of ongoing change as the natural course of the IDC unfolds. The data from the one-hour time resolution of the IDC transcriptome emphasizes that results from studies examining transcriptional changes using asynchronous cultures may be difficult to interpret. One must always ask the critical question concerning differential regulation in *Plasmodium*: is this a natural consequence of the IDC or is this actually altered transcription? This is particularly important given the large magnitude of transcriptional changes that may occur over a few-hour interval as part of the ongoing IDC. Laboratories interested in determining expression changes in mutant backgrounds, different strains, and environmental response studies should be urged to conduct timecourse experiments with synchronized parasites for such analyses. When addressing issues of transcript expression changes, it is not enough to examine stages roughly, based solely on the various morphological changes observed by staining cells, but rather, it is critical to examine the on-going developmental cycle at the highest possible resolution available. Using the high-resolution 48 h timecourse, one can anchor new data within the IDC to determine a relative timepoint equivalent and infer placement of a given experiment within the global IDC.

The *Plasmodium* community is fortunate to have excellent resources available for the annotation and distribution of all sequence, genomic and transcriptome data available at http://plasmodb.org [24,25•]. This site provides detailed information on all *Plasmodium* genomes as they are being sequenced and provides the necessary tools to query datasets in multiple ways. In addition, a linked site, http://malaria.ucsf.edu allows users to specifically query the Bozdech and Llinás *et al.* [20••] transcriptome dataset.

Transcriptional regulation in *P. falciparum*

The main questions that arise from these transcriptome studies are related to how *P. falciparum* manages to control gene expression. How is the temporal transcriptional cascade of the IDC driven? What mechanism imposes the strict 48 h timing and how does this organism function with apparently very few transcription factors?

There are many scenarios for how transcriptional regulation may be occurring in *Plasmodium*, and it is likely to be a multi-faceted process. One possibility, which has been seen in other parasites such as *Leishmania* spp. [26], could be that the parasite uses poly-cistronic messages to time the expression of relevant genes. However, there is scant evidence for this as only 14 groups with three or more co-expressed genes could be found anywhere along the *P. falciparum* linear chromosomes [20••]. In contrast to nuclear encoded genes, it seems that transcription from the 35 kb *P. falciparum* plastid genome occurs via separate transcriptional machinery that does produce poly-cistronic messages [19,20••]. An alternative possibility is that anti-sense messages might contribute significantly to the control of gene expression. While evidence for anti-sense has been reported, it has not been analyzed at the whole-genome level [11,27,28]. Lastly, it may very well be that there is no activated or repressed transcription or that transcriptional regulation may occur via an as of yet unidentified set of factors. A bioinformatic analysis of the putative *P. falciparum* orthologs to the yeast transcriptional complex and associated factors reveals a marked absence of RNA polymerase II mediator components (N Wilson and J DeRisi, personal communication).

It has been demonstrated [29–31] that environmental perturbation studies which elicit transcriptional responses can be used to identify not only functional relationships for sets of genes with similar transcription profiles, but also detailed information about regulation *per se*, including the identification of promoter elements. Recent studies have been undertaken to investigate environmental perturbations and their effect on transcription in *P. falciparum*. Temperature shift studies, which have great significance owing to febrile episodes experienced by affected individuals, suggest that the rate of transcription of A- and S-type rRNAs varies as a function of temperature [32]. Likewise, experiments addressing transcriptional changes caused by alterations in glucose levels have identified stage-specific changes in rRNA levels and over 550 other genes [33]. Using asynchronous cultures, changes in gene expression caused by chloroquine exposure have also recently been reported using serial analysis of gene expression (SAGE) [34]. Such experiments have great potential, as these approaches are likely to identify transcriptionally related groups of genes and

aid in promoter identification. To begin characterizing inter-strain transcriptional differences, we are currently studying the full IDC transcriptomes of the three strains 3D7, HB3, and Dd2 to identify potential differences in gene expression between strains of *P. falciparum* that are of different geographical origin and drug-resistance backgrounds. Overall, the transcriptional cascade is extremely well preserved and differences may lead to the identification of inter-strain variability (M Llinás, unpublished).

Conclusions

As more *Plasmodium* genomes are sequenced, it will become increasingly important to identify differences between species that may aid in elucidating essential genes by comparative genomics. Just as in yeast, with the 5+ divergent fungal species that have been sequenced, hopefully comparisons can be made to elucidate regulatory sequences [35,36]. At present *P. yoelii* has been sequenced to five-fold coverage [37] and other genomes are on the way including *P. berghei*, *P. chabaudi*, *P. knowlesi*, *P. reichenowi* and *P. vivax*. In addition, with the IDC transcriptome now available, further annotation of the genome can be accomplished by incorporating transcriptional data to confirm and/or correct predicted ORFs and intergenic regions. For example, there are various 'missing genes' such as the well-characterized histidine-rich protein 2 (HRP2) [38] that do not appear in the current release of the *P. falciparum* genome. Also, expression profiles for 'unannotated' features of the genome will assist in identifying correct gene models. Interestingly, for some ORFs with multiple exons, not all exons are detected in the microarray analyses. Of course, this could be due to sequence differences (deletions or insertions) between strains, but may also warrant further inspection of the current genome.

It is now time to begin testing the transcriptional control mechanisms in *P. falciparum*. First, using a combination of bioinformatic approaches and genome sequence analysis for putative transcription factors, it will be necessary to better define the promoters. Second, with the information from the IDC studies, it should now be possible to design stage-specific promoters for any timepoint during the IDC. With knock-in and knockout approaches becoming more feasible, candidate promoter constructs can be generated and tested *in vivo* [39,40]. Finally, applying biochemical approaches to purify factors associated with the genome by chromatin immunoprecipitation should identify key binding sites throughout the genome. As the understanding of transcriptional regulation improves, we should begin to address the related and important issues of post-transcriptional regulation, mRNA decay rates, splicing, and translation rates. These have all been successfully executed in model organisms. We need to take cues from the work done on model organisms and extend these tools to the study of *P. falciparum* biology.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Sachs J, Malaney P: **The economic and social burden of malaria**. *Nature* 2002, **415**:680-685.
2. Bannister LH, Hopkins JM, Fowler RE, Krishna S, Mitchell GH: **A brief illustrated guide to the ultrastructure of *Plasmodium falciparum* asexual blood stages**. *Parasitol Today* 2000, **16**:427-433.
3. Flick K, Chen Q: **var genes, PfEMP1 and the human host**. *Mol Biochem Parasitol* 2004, **134**:3-9.
4. Militello KT, Dodge M, Bethke L, Wirth DF: **Identification of regulatory elements in the *Plasmodium falciparum* genome**. *Mol Biochem Parasitol* 2004, **134**:75-88.
5. Dechering KJ, Kaan AM, Mbacham W, Wirth DF, Eling W, Konings RN, Stunnenberg HG: **Isolation and functional characterization of two distinct sexual-stage-specific promoters of the human malaria parasite *Plasmodium falciparum***. *Mol Cell Biol* 1999, **19**:967-978.
6. Horrocks P, Lanzer M: **Mutational analysis identifies a five base pair cis-acting sequence essential for GBP130 promoter activity in *Plasmodium falciparum***. *Mol Biochem Parasitol* 1999, **99**:77-87.
7. Voss TS, Kaestli M, Vogel D, Bopp S, Beck HP: **Identification of nuclear proteins that interact differentially with *Plasmodium falciparum* var gene promoters**. *Mol Microbiol* 2003, **48**:1593-1607.
8. Hoffman SL, Bancroft WH, Gottlieb M, James SL, Burroughs EC, Stephenson JR, Morgan MJ: **Funding for malaria genome sequencing**. *Nature* 1997, **387**:647.
9. Hayward RE, Derisi JL, Alfadhli S, Kaslow DC, Brown PO, Rathod PK: **Shotgun DNA microarrays and stage-specific gene expression in *Plasmodium falciparum* malaria**. *Mol Microbiol* 2000, **35**:6-14.
10. Munasinghe A, Patankar S, Cook BP, Madden SL, Martin RK, Kyle DE, Shoaibi A, Cummings LM, Wirth DF: **Serial analysis of gene expression (SAGE) in *Plasmodium falciparum*: application of the technique to A-T rich genomes**. *Mol Biochem Parasitol* 2001, **113**:23-34.
11. Patankar S, Munasinghe A, Shoaibi A, Cummings LM, Wirth DF: **Serial analysis of gene expression in *Plasmodium falciparum* reveals the global expression profile of erythrocytic stages and the presence of anti-sense transcripts in the malarial parasite**. *Mol Biol Cell* 2001, **12**:3114-3125.
12. Ben Mamoun C, Gluzman IY, Hott C, MacMillan SK, Amarakone AS, Anderson DL, Carlton JM, Dame JB, Chakrabarti D, Martin RK *et al.*: **Co-ordinated programme of gene expression during asexual intraerythrocytic development of the human malaria parasite *Plasmodium falciparum* revealed by microarray analysis**. *Mol Microbiol* 2001, **39**:26-36.
13. Gardner MJ, Tettelin H, Carucci DJ, Cummings LM, Aravind L, Koonin EV, Shallom S, Mason T, Yu K, Fujii C *et al.*: **Chromosome 2 sequence of the human malaria parasite *Plasmodium falciparum***. *Science* 1998, **282**:1126-1132.
14. Bowman S, Lawson D, Basham D, Brown D, Chillingworth T, Churcher CM, Craig A, Davies RM, Devlin K, Feltwell T *et al.*: **The complete nucleotide sequence of chromosome 3 of *Plasmodium falciparum***. *Nature* 1999, **400**:532-538.
15. Le Roch KG, Zhou Y, Batalov S, Winzeler EA: **Monitoring the chromosome 2 intraerythrocytic transcriptome of *Plasmodium falciparum* using oligonucleotide arrays**. *Am J Trop Med Hyg* 2002, **67**:233-243.
16. • Bozdech Z, Zhu J, Joachimiak MP, Cohen FE, Pulliam B, DeRisi JL: **Expression profiling of the schizont and trophozoite stages of *Plasmodium falciparum* with a long-oligonucleotide microarray**. *Genome Biol* 2003, **4**:R9.

This paper describes the 70mer oligo array methodology as applied to expression differences between trophozoites and schizonts.

17. Salzberg SL, Pertea M, Delcher AL, Gardner MJ, Tettelin H: **Interpolated Markov models for eukaryotic gene finding**. *Genomics* 1999, **59**:24-31.

18. •• Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW, Carlton JM, Pain A, Nelson KE, Bowman S *et al.*: **Genome sequence of the human malaria parasite *Plasmodium falciparum***. *Nature* 2002, **419**:498-511.
The authors provide a comprehensive overview of the genome sequencing effort.

19. Wilson RJ, Denny PW, Preiser PR, Rangachari K, Roberts K, Roy A, Whyte A, Strath M, Moore DJ, Moore PW *et al.*: **Complete gene map of the plastid-like DNA of the malaria parasite *Plasmodium falciparum***. *J Mol Biol* 1996, **261**:155-172.

20. •• Bozdech Z, Llinas M, Pulliam BL, Wong ED, Zhu J, DeRisi JL: **The Transcriptome of the Intraerythrocytic Developmental Cycle of *Plasmodium falciparum***. *PLoS Biol* 2003, **1**:E5.
This paper describes the genome wide transcriptional profile of the intraerythrocytic lifecycle of *Plasmodium falciparum* at one-hour time intervals, in addition to comparative genomic hybridization analysis and plastid expression.

21. •• Le Roch KG, Zhou Y, Blair PL, Grainger M, Moch JK, Haynes JD, De La Vega P, Holder AA, Batalov S, Carucci DJ *et al.*: **Discovery of gene function by expression profiling of the malaria parasite life cycle**. *Science* 2003, **301**:1503-1508.
The authors analyzed expression for seven timepoints during the intraerythrocytic lifecycle in addition to gametocyte and sporozoite stage samples.

22. Sato S, Clough B, Coates L, Wilson RJ: **Enzymes for heme biosynthesis are found in both the mitochondrion and plastid of the malaria parasite *Plasmodium falciparum***. *Protist* 2004, **155**:117-125.

23. Triglia T, Healer J, Caruana SR, Hodder AN, Anders RF, Crabb BS, Cowman AF: **Apical membrane antigen 1 plays a central role in erythrocyte invasion by *Plasmodium* species**. *Mol Microbiol* 2000, **38**:706-718.

24. Kissinger JC, Brunk BP, Crabtree J, Fraunholz MJ, Gajria B, Milgram AJ, Pearson DS, Schug J, Bahl A, Diskin SJ *et al.*: **The *Plasmodium* genome database**. *Nature* 2002, **419**:490-492.

25. Bahl A, Brunk B, Crabtree J, Fraunholz MJ, Gajria B, Grant GR, Ginsburg H, Gupta D, Kissinger JC, Labo P *et al.*: **PlasmoDB: the *Plasmodium* genome resource. A database integrating experimental and computational data**. *Nucleic Acids Res* 2003, **31**:212-215.
This article represents the definitive description of the malaria genome resource database.

26. Myler PJ, Beverley SM, Cruz AK, Dobson DE, Ivens AC, McDonagh PD, Madhubala R, Martinez-Calvillo S, Ruiz JC, Saxena A *et al.*: **The Leishmania genome project: new insights into gene organization and function**. *Med Microbiol Immunol (Berl)* 2001, **190**:9-12.

27. Kyes S, Christodoulou Z, Pinches R, Newbold C: **Stage-specific merozoite surface protein 2 antisense transcripts in *Plasmodium falciparum***. *Mol Biochem Parasitol* 2002, **123**:79-83.

28. Noonpakdee W, Pothikasikorn J, Nimitsantiwong W, Wilairat P: **Inhibition of *Plasmodium falciparum* proliferation *in vitro* by antisense oligodeoxynucleotides against malarial topoisomerase II**. *Biochem Biophys Res Commun* 2003, **302**:659-664.

29. Gasch AP, Huang M, Metzner S, Botstein D, Elledge SJ, Brown PO: **Genomic expression responses to DNA-damaging agents and the regulatory role of the yeast ATR homolog Mec1P**. *Mol Biol Cell* 2001, **12**:2987-3003.

30. Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, Storz G, Botstein D, Brown PO: **Genomic expression programs in the response of yeast cells to environmental changes**. *Mol Biol Cell* 2000, **11**:4241-4257.

31. Causton HC, Ren B, Koh SS, Harbison CT, Kanin E, Jennings EG, Lee TI, True HL, Lander ES, Young RA: **Remodeling of yeast genome expression in response to environmental changes**. *Mol Biol Cell* 2001, **12**:323-337.

32. Fang J, McCutchan TF: **Thermoregulation in a parasite's life cycle**. *Nature* 2002, **418**:742.

33. Fang J, Zhou H, Rathore D, Sullivan M, Su XZ, McCutchan TF: **Ambient glucose concentration and gene expression in *Plasmodium falciparum***. *Mol Biochem Parasitol* 2004, **133**:125-129.

34. Gunasekera AM, Patankar S, Schug J, Eisen G, Wirth DF: **Drug-induced alterations in gene expression of the asexual blood forms of *Plasmodium falciparum***. *Mol Microbiol* 2003, **50**:1229-1239.

35. Kellis M, Patterson N, Endrizzi M, Birren B, Lander ES: **Sequencing and comparison of yeast species to identify genes and regulatory elements**. *Nature* 2003, **423**:241-254.

36. Cliften P, Sudarsanam P, Desikan A, Fulton L, Fulton B, Majors J, Waterston R, Cohen BA, Johnston M: **Finding functional features in *Saccharomyces* genomes by phylogenetic footprinting**. *Science* 2003, **301**:71-76.

37. Carlton JM, Angiuoli SV, Suh BB, Kooij TW, Pertea M, Silva JC, Ermolaeva MD, Allen JE, Selengut JD, Koo HL *et al.*: **Genome sequence and comparative analysis of the model rodent malaria parasite *Plasmodium yoelii yoelii***. *Nature* 2002, **419**:512-519.

38. Noedl H, Wongsrichanalai C, Miller RS, Myint KS, Looareesuwan S, Sukthana Y, Wongchotigul V, Kollaritsch H, Wiedermann G, Wernsdorfer WH: ***Plasmodium falciparum*: effect of antimalarial drugs on the production and secretion characteristics of histidine-rich protein II**. *Exp Parasitol* 2002, **102**:157-163.

39. Crabb BS: **Transfection technology and the study of drug resistance in the malaria parasite *Plasmodium falciparum***. *Drug Resist Updat* 2002, **5**:126-130.

40. Gardiner DL, Skinner-Adams TS, Spielmann T, Trenholme KR: **Malaria transfection and transfection vectors**. *Trends Parasitol* 2003, **19**:381-383.