

Genomics and Its Impact on Parasitology and the Potential for Development of New Parasite Control Methods

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ABSTRACT

Parasitic organisms remain the scourge of the developed and underdeveloped worlds. Malaria, schistosomiasis, leishmaniasis, and trypanosomiasis, for example, still result in a large number of human deaths each year worldwide, while drug resistance among nematodes still poses a major problem to the livestock industries. Genome projects involving parasitic organisms are now abundant, and technologies for the investigations of the parasite transcriptome and proteome are well established. There is no doubt the era of the “omics” is with parasitology, and current trends in the discipline are addressing fundamental biological questions that can make best use of the new technologies, as well as the vast amount of new data being generated. Will this become the “golden age of molecular parasitology,” leading to the control of parasitic diseases that have plagued mankind for hundreds of years? The primary aim of this paper is to review advances in the general area of parasite genomics, and to outline where the application of “omics” technologies can and have impacted on the development of new control methods for parasitic organisms.

INTRODUCTION

PARASITIC ORGANISMS remain the scourge of the developed and underdeveloped worlds. Malaria, Schistosomiasis, Leishmaniasis and Trypanosomiasis still result in a large number of deaths each year (Anonymous, 2002), while drug resistance in malaria is increasing as a public health problem (Nosten and Brasseur, 2002). Similarly, resistance in parasitic worms is increasing at such an alarming rate that many drugs will soon be rendered useless to the livestock industry (Sangster, 2001). It is therefore not surprising that genome projects on parasitic organisms are now abundant in the hope that new methods for the control of parasites will be forthcoming.

The purpose of this article is to review advances in the general area of parasite genomics, and to outline where the application of genomics and proteomics studies can impact on the development of new control methods for parasitic organisms. This review concentrates primarily on parasites of the phylum Apicomplexa, which includes malaria, *Toxoplasma*, *Neospora*, and *Eimeria*. All these are important either medically or to the livestock industry.

VACCINE DEVELOPMENT BEFORE THE GENOMICS ERA

Commercially, vaccines based on live parasites (some naturally attenuated) are available and used successfully and extensively around the world for a limited number of parasite species. For example, live vaccines are available against besnoitiosis in cattle (Bigalke *et al.*, 1974), *Toxoplasma*-induced abortion in sheep (Buxton and Innes, 1995), and coccidiosis in poultry (Chapman *et al.*, 2002).

In contrast, few inactivated or subunit vaccines based on recombinant proteins have been developed against parasites (Lightowlers *et al.*, 2000), but none for commercially important human or animal pathogens. There is great optimism, however, that the application of the “omics” technologies will deliver new candidates for development into vaccines, and so more attention has been placed on gene discovery processes and the role of their products in parasite biology.

Identifying vaccine candidates

In the past, prior knowledge of induction of protective immunity in the host has been an essential prerequisite of any par-

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asite vaccine program. For example, the malaria vaccine programs are founded primarily on two examples of immunity to malaria in humans: (1) naturally acquired immunity develops in those residing in malaria endemic areas (Carucci, 2001), and (2) irradiated sporozoites can protect humans against challenge. Of relevance to the animal health industry, it has long been recognised that *Eimeria* species induce a long-lasting, species specific immunity in chicken (Chapman *et al.*, 2002).

Previous approaches for identifying potential vaccine candidates have focussed on antigens which stimulate or are the target of host immunity (Jenkins, 2001) or molecules, which are vital to infection (Lustigman *et al.*, 2002). Identifying such molecules is not an easy task, given the complexity found in the diverse life cycle stages known to exist among parasites. Indeed, these life cycle stages of parasites typically present a plethora of antigens to the host immune system.

In some circumstances, the life cycle stages involved in the presentation of protective antigens to the host can be determined. For example, studies on precocious lines of *Eimeria* (Shirley and Bednik, 1997) demonstrated that lines with abbreviated life cycles could still induce protection (McDonald *et al.*, 1986, 1988). An inactivated vaccine (NeoGuard) based on tachyzoites of *Neospora caninum* was developed to prevent *Neospora*-associated abortion in cattle, and this was recently released in the United States and New Zealand (Reichel and Ellis, 2002).

Screening of expression libraries with immune sera (or monoclonal antibodies) has become probably the most widespread approach for identifying antigens recognized by the host antibody. This approach is limited by the fact that frequently humoral immunity is relatively unimportant in conferring protective immunity against a parasite. In addition, the approach has led to the characterisation of molecules in the past, which are typically abundant and highly expressed within the cell (Maizels *et al.*, 2001).

One of the best examples utilising this approach successfully, however, is the correlation of maternal antibody-based immunity in the chicken with protection from infection by *Eimeria maxima* (Wallach *et al.*, 1995). This led to the development of a vaccine using gametocyte antigens of this species, which are now the basis of a commercial vaccine (CoxAbic™).

Hidden antigens have shown great promise for some years now as vaccine candidates for parasitic nematodes. These are molecules that are normally hidden from the host immune system, and do not illicit an immune response during the course of a natural infection. Immunization with native proteins of this type can induce a strong protective immune response (Knox and Smith, 2001); however, commercial development of a subunit form of such a vaccine has not yet materialized.

Cell-mediated responses

For many parasitic diseases, the induction of cell-mediated immunity is paramount for successful vaccine production, and much has been learnt in recent years on the mechanisms and molecules which participate in these processes (Brake, 2002; Quinn *et al.*, 2002). A Th1 response is seen as vital for induction of protective immunity against the Apicomplexa, and therefore, selection of vaccine candidates which stimulate this type of immunity must warrant further consideration. One of the best

examples from the protozoa relating to identification of vaccine candidates through their interaction with the cell-mediated immune response was the identification of the LACK antigen (*Leishmania* homolog of receptors for activated C kinase) from *Leishmania major* (phylum Sarcomastigophora) as the main target of the Th2 response in mice which conferred susceptibility to leishmaniasis (Mougneau *et al.*, 1995; Schilling and Glaichenhaus, 2001). Vaccination of mice using protein or DNA of LACK generated substantial levels of species-specific protective immunity to *L. major* (Gurunathan *et al.*, 1997; Melby *et al.*, 2001).

In summary, attempts to develop vaccines against parasitic organisms have thus far concentrated on relatively few molecules, and more often or not, these targets were isolated by techniques which selected for immunogens which are reasonably abundant in the cell. Studies on the genomes of parasites have expanded the population of genes and proteins available for study, and the issue at hand now is how to make best use of the information available.

Laboratory models and screening

The development of a laboratory model of infection, incorporating the features of immunization schedules and challenge, greatly facilitates the screening of potential vaccine formulations. Animal models have now been developed for many pathogenic parasites of humans and animals. For example, cell free lysates of *Eimeria tenella* and *N. caninum* were shown to protect against coccidiosis in chickens and transplacental transmission of *N. caninum* in a mouse model, respectively (Karkhanis *et al.*, 1991; Liddell *et al.*, 1999).

ANALYSES OF THE PARASITE TRANSCRIPTOME

Initially, several approaches were used to define mRNA populations of parasitic organisms. Techniques such as differential display (Heard *et al.*, 1996; Schroeder *et al.*, 1998; Lau *et al.*, 2000; Cui *et al.*, 2001) and serial analysis of gene expression (Patankar *et al.*, 2001) have played a role in gene discovery, yet 5' sequencing of random cDNA clones to generate expressed sequence tags has proven to be the most important approach, limited only by the quality of the cDNA libraries generated (Blackwell, 1997; Ajioka, 1998; Abrahamsen, 1999; Blaxter *et al.*, 2002).

Clustering of expressed sequence tags (ESTs) and the generation of a consensus sequence for each cluster, greatly facilitates the ease by which data can be rapidly assembled to generate a complete gene sequence (Lawson, 1999). It is interesting to note that many of the abundant ESTs of Apicomplexa encode antigens that have been well characterized in the past (Table 1) such as those found in the micronemes, dense granules, and rhoptries.

Of importance is the accessibility of information and data in the public domain through a variety of internet-based resources, such as the Parasite Genome Internet Resource List (www.ebi.ac.uk/parasites/paratable.html), Parasite Genome Blast Server (www.ebi.ac.uk/blast2/parasites.html); Parasite Databases of Clustered Sequences (<http://ParaDB.cis.upenn.edu/>); Nembase

TABLE 1. SUMMARY OF THE 20 MOST ABUNDANT ESTs IN THREE COCCIDIAN SPECIES (<http://ParaDB.cis.upenn.edu>)^a

<i>Toxoplasma gondii</i>	<i>Neospora caninum</i>	<i>Eimeria tenella</i>
GRA7	GRA2	Antigen
GRA1	GRA7	Actophorin
GRA2	Uncharacterized cluster (neo_566)	Serine protease inhibitor
GRA6	MIC1	Uncharacterized cluster (Ceimqual_264)
P22	Uncharacterized cluster (neo_617)	MIC1
P30	GRA1	Uncharacterized cluster (Ceimqual_287)
HSP30	MIC10	Uncharacterized cluster (Ceimqual_521)
NTPase	P38	Uncharacterized cluster (Ceimqual_1487)
Uncharacterized cluster (Ctoxoqual4_276)	SUL1	Uncharacterized cluster (Ceimqual-953)
GRA5	Uncharacterized cluster (neo_824)	Uncharacterized cluster (Ceimqual-926)
Uncharacterized cluster (Ctoxoqual4_5857)	Serine protease inhibitor	MIC2
GRA3	NTPase	Calmodulin
SAG1	Serine protease	Uncharacterized cluster (Ceimqual_566)
Uncharacterized cluster (Ctoxoqual4_64)	Uncharacterized cluster (neo_378)	Uncharacterized cluster (Ceimqual_714)
H4 antigen	MIC4	Uncharacterized cluster (Ceimqual_775)
GRA5	Uncharacterized cluster (neo_150)	Uncharacterized cluster (Ceimqual_218)
ROP2	Uncharacterized cluster (neo_549)	Cytochrome <i>c</i> oxidase
Uncharacterized cluster (Ctoxoqual4_3035)	Uncharacterized cluster (neo_759)	TA4 antigen
GRA8	MIC6	Uncharacterized cluster (Ceimqual_758)
Uncharacterized cluster (toxoqual4_4452)	Uncharacterized cluster neo_287)	Uncharacterized cluster (Ceimqual_161)

^aGRA = dense granule protein; MIC = microneme protein; ROP = rhoptry protein.

(<http://nema.cap.ed.ac.uk/hematodeESTs/nembase.html>); and the *Plasmodium* and *Toxoplasma* genome resources (<http://plasmodb.org/PlasmoDB.shtml>); <http://ToxoDB.org/ToxoDB.shtml>).

Methods for searching sequence databases with new sequence data has proven to be fundamentally vital to parasite gene discovery programs. Algorithms like BLAST, when used in conjunction with large sequence datasets present in Genbank or other similar databases, readily assign a putative function through detecting sequence similarity. Of interest to vaccine development, however, is the very large number of parasite genes which exist for which no provisional identification can be made and about which nothing is as yet known (Table 1). Potentially, this also proves to be a difficulty, because of the high throughput approaches that need to be developed to assess such a large number of vaccine candidates.

Microarrays

Recently expression profiling by microarray analyses (Carucci, 2000; Cummings and Relman, 2000; Rathod *et al.*, 2002) has been reported from a small number of taxa (Hayward *et al.*, 2000; Mamoun *et al.*, 2001; Cleary *et al.*, 2002; Matrajt *et al.*, 2002; Singh *et al.*, 2002), and no doubt the popularity of this technique will increase in parasitology as resources (such as clones and arrays) become more widely available. Such studies are proving important in raising and testing hypotheses on the developmental biology of parasites, and the signalling pathways that control them.

There is nothing inherently unique about microarray data or its analysis. In a microarray experiment one is simply trying to identify which genes are the most “interesting” in terms of the experimental question, and these will usually be those that are either overexpressed or underexpressed (or transiently expressed) under the experimental conditions being

studied. However, the type of data analysis to be used will depend on the objective of the experiment. If the experimental question predicts an explicit pattern (e.g., differences in expression between developmental stages), then traditional statistical hypothesis testing is the best method for assessing whether the data support or refute that pattern. Alternatively, if the experimental question is about searching for unknown patterns among the samples (e.g., groups of genes that show coexpression) then multivariate pattern analysis is the best method for exploring the patterns that might exist in the data. A detailed discussion can be found in the introductory book by Knudsen (2002).

Statistical hypothesis testing is no different for microarray data than for any other type of data. However, there are issues that are particularly important for such data, and some of them have been discussed previously (Nadon and Shoemaker, 2002; Yang and Speed, 2002). Specialist computer programs have also been developed (Didier, 2002). Similarly, pattern analysis is a general mathematical technique in science, although there are many alternative methods. Some of these methods are briefly described in the context of microarray data analysis by others, so will not be discussed further (Hess *et al.*, 2001; Quackenbush, 2001; Raychaudhuri *et al.*, 2001; Sherlock, 2001). Eisen *et al.* (1998) describe a commonly used computer program for carrying out the analyses.

Microarrays are also being used to investigate changes in gene expression of host cells during parasite infection to investigate host response mechanisms (Blader *et al.*, 2001; Gail *et al.*, 2001). Real-time quantitative RT-PCR analyses, using fluorogenic 5' nuclease assays, or Taqman, is typically used to confirm and quantify gene expression levels (Blair *et al.*, 2002).

As yet, no reports have described simultaneous analyses of gene expression in the host as well as in the parasite as they interact with each other. This clearly would be a fascinating approach to adopt.

SEQUENCING OF PARASITE GENOMES

Table 2 lists all genome projects for parasites, being conducted through either the Sanger Centre (U.K.) (www.sanger.ac.uk/Projects/) or The Institute for Genome Research (USA) (www.tigr.org/tdb/parasites/). The approaches being adopted are similar in structure for each, concentrating on sequence assembly from whole genome shotgun sequencing approaches (Gardner, 2001). Initially, some of them began on a chromosome by chromosome basis, with different groups taking responsibility for individual chromosomes (Gardner *et al.*, 1998; Bowman *et al.*, 1999). Strategically, this approach was important because it facilitated the leverage of funds from a number of public and private bodies, and demonstrated proof of concept that parasite genomes could be sequenced. However, the power of shotgun sequencing realistically has made approaches based on individual chromosomes redundant for protozoa, and more dependent on the tools of bioinformatics for compiling and annotating the genome sequences generated. For example, the high AT content of the *Plasmodium falciparum* genome coupled with the recognized gene density of 20%, required the development and training of new gene finder algorithms such as GlimmerM (Salzberg *et al.*, 1999) and Phat (Cawley *et al.*, 2001). Even then, manual searches are proving just as effective in making robust gene predictions (Huestis and Fischer, 2001).

The genomes of parasitic worms, being much larger than those of protozoa, will take some considerable time to investigate, although EST programs are prevalent and rapidly defining the genetic component of these organisms. Projects to sur-

vey the genomes of *Brugia malayi* and *Schistosoma mansoni* are underway. The generation and analyses of such large volumes of data is daunting, yet it potentially provides many new exciting opportunities for gene discovery which may result in the development of new parasite control methods.

LINKAGE ANALYSES

The ability to perform classical genetic crosses using cloned lines of parasites of defined phenotype represents an outstanding advantage for investigating unique traits in some parasitic organisms. For example, acute virulence in *Toxoplasma gondii* is recognized as an inherited, multigenic trait (Howe *et al.*, 1996; Sibley *et al.*, 2002), precocious development in *Eimeria* was assigned to chromosome 2 (Shirley and Harvey, 2000), and a gene with a functional role in malarial gametocytogenesis was assigned to chromosome 12 (Guinet and Wellems, 1997). Such studies, in conjunction with pulsed-field gel electrophoresis and DNA markers not only confirm the number of linkage groups (and hence the number of chromosomes) present (Shirley *et al.*, 1990; Sibley and Boothroyd, 1992; Hays *et al.*, 1995), but also provides a framework on which genome sequences can be overlaid and correlated. Hence, positional cloning with the help of physical genetic maps and genome sequences, may materialize gene loci central to parasite development and pathogenesis.

HAPPY mapping is a relatively simple method for assigning and ordering DNA markers to chromosomes (Dear and Cook, 1993), and was first used to produce a linkage map of the genome of *Cryptosporidium parvum* (Piper *et al.*, 1998).

TABLE 2. SUMMARY OF PARASITE GENOME PROJECTS BEING CONDUCTED THROUGH THE SANGER INSTITUTE OR TIGR

Species	Number of chromosomes, genome size (Mb)	Comments ^a	Useful Web-sites ^b
<i>Plasmodium falciparum</i> ^f	14, 30	WG	www.plasmodb.org ; SI; TIGR
<i>Plasmodium yoelli</i> ^f		WG	TIGR
<i>Theileria annulata</i>	4, 10	WG	SI, TIGR
<i>Toxoplasma gondii</i>	11, 80	WG	http://ToxoDB.org/ToxoDB.shtml http://ParaDB.cis.upenn.edu/ SI, TIGR
<i>Eimeria tenella</i>	14, 60	WG	SI
<i>Trypanosoma brucei</i>	11+, 35	WG	http://parsun1.path.cam.ac.uk/ www.genedb.org/ SI, TIGR
<i>Trypanosoma cruzi</i>	35, 40	PG	www.dbbm.fiocruz.br/genome/tcruzi/tcruzi.html
<i>Leishmania major</i>	36, 33.6	WG	www.genedb.org ; SI, TIGR
<i>Entamoeba histolytica</i>	18, 20	WG	SI, TIGR
<i>Brugia malaya</i>	6,110	WG	www.nematode.net/ www.nematodes.org/html http://circuit.neb.com/fgn/filgen1.html www.wormbase.org/ SI, TIGR
<i>Schistosoma mansoni</i>		PG	www.nhm.ac.uk/hosted_sites/schisto/ TIGR

^aWG = whole genome; PS = partial genome; ^bSI = Sanger Institute. (www.sanger.ac.uk/Projects/); TIGR = The Institute for Genome Research (www.tigr.org/tdb/parasites/); ^cpartial shortguns are also being generated for *P. berghei*, *P. chabaudi*, and *P. knowlesi*.

More recently, it has found use in GAP closure in the *P. falciparum* genome where there was a paucity of markers for ordering of the contigs (Gardner, 2001). The main advantage of this approach for mapping genomes of lower eukaryotes is its relative simplicity, and so will find further widespread use in the study of protozoa.

COMPARATIVE GENOMICS

Comparisons of parasite genomes from closely related species is now providing valuable information not only on genome organization but also on gene function (Thompson *et al.*, 2001; Waters, 2002). In malaria research, karyotype analyses demonstrated conservation of chromosome structure amongst both rodent and human malarias (Janse *et al.*, 1994; Carlton *et al.*, 1999), and this work has aided the identification of homologous genes amongst species (Thompson *et al.*, 2001). In addition, genetic manipulation of *P. berghei* is somewhat more amenable than for *P. falciparum*, thereby facilitating functional studies through targeted disruption (Waters *et al.*, 1997).

Functional genomics may also prove valuable in the study of other protozoan parasites. For example, *in vitro* culture and transfection of *Eimeria* species of poultry is not yet possible, so genetic manipulation of its genome is difficult. Comparisons of genome sequences from different *Eimeria* species may assist in studies on gene identification and function. Similarly, *T. gondii* and *N. caninum* are closely related species, but relatively little is known about the genome of the latter. Sequencing and mapping of the *N. caninum* genome, and its comparison to that of *T. gondii*, may ultimately shed light on the unique biological features of this organism.

ANALYSES OF THE PARASITE PROTEOME

The investigation of protein–protein interactions is fundamentally central to the investigation of parasite–host interactions. The processes of host cell recognition, attachment, and invasion require the interaction of a large number of parasite surface and organelle specific molecules with those present on host cells (Hemphill, 1999). Determination of their identity and function can provide potential vaccine targets.

The key technologies behind the core of proteome analyses, namely two-dimensional gel electrophoresis coupled to mass spectroscopy and data base searching, have been described in detail elsewhere (Barrett *et al.*, 2000; Ashton *et al.*, 2001). Recently, large-scale analyses on parasite proteomes have been reported (Jefferies *et al.*, 2001; Cohen *et al.*, 2002), and the available data shows that it is unnecessary to possess whole genome sequence data to make proteomic analyses viable. Large numbers of proteins are being identified merely from database searches of peptide fragmentation data.

Post-translational modifications of proteins clearly exist, but this whole area is vastly underexplored from a vaccine viewpoint, and so presents many potential opportunities. Glycosylphosphatidylinositols have been extensively studied in protozoa for their role as membrane anchors and in cell signalling (Schofield and Tachado, 1996), and more recently their role in

activating a Toll-like receptor recognition system may have important implications for vaccine design (Campos *et al.*, 2001). Mice immunised with a synthetic GPI designed from malaria were substantially protected against malarial acidosis, pulmonary oedema, cerebral syndrome, and fatality (Schofield *et al.*, 2002).

Currently, there are few attempts to group genes and proteins into functional categories. The Parasite Proteome Server (www.ebi.ac.uk/parasites/proteomes.html) was the first attempt to do this, but other information sources are now being developed that may facilitate the incorporation and integration of additional biological information (Coppel, 2001). The InterPro database (www.ebi.ac.uk/interpro/), which is an assemblage of others for defining gene families, signatures, and domains, is proving useful in this respect along with Gene Ontology assessments (www.geneontology.org/). The elucidation of metabolic pathways will prove vital to the development of new control methods based on drugs.

GENETIC MANIPULATION OF PARASITES

The development of a wide range of molecular genetics tools for *T. gondii*, such as transformation and a variety of gene disruption technologies, has propelled this organism into the forefront of parasite genetics (Donald and Roos, 1993, 1995, 1998; Sibley *et al.*, 1993; Soldati, 1996; Boothroyd *et al.*, 1997; Roos *et al.*, 1997; Black and Boothroyd, 1998; Brecht *et al.*, 1999; Soete *et al.*, 1999; Knoll *et al.*, 2001; Sibley *et al.*, 2002). Although some of these techniques have also been developed for parasites such as *Plasmodium* species (de Koning-Ward *et al.*, 2000; Cowman *et al.*, 2002), and are probably easily transferable to others such as *N. caninum* (Howe and Sibley, 1997), advanced genetic manipulations are currently impossible for many others such as *Eimeria* or *Sarcocystis*. RNA interference methodologies, although available for *Trypanosoma brucei* (Ullu *et al.*, 2002), are not yet commonplace for the Apicomplexa (McRobert and McConkey, 2002).

Probably the main value to be gained from applying these techniques to the study of parasites is an advanced knowledge on the distribution, localization, and function of individual molecules. This can facilitate an assessment of their role in parasite virulence and pathogenesis. Many of the knock-outs generated typically show no discernable change in cell phenotype, and so many of these lines may not be rigorously pursued for further investigation. However, the creation of knock-out mutants, and the restoration of the gene and wild-type function back to these mutants, is required to fulfil a molecular version of Koch's postulates (Falkow, 1988).

VACCINES AND IMMUNOTHERAPIES

The generation of genome sequences facilitates access to all potential targets of immunity, so they can be used in a number of novel ways. The use of reverse vaccinology is growing, being increasingly dependent on the tools of bioinformatics (Rappuoli, 2000, 2001). Sequence databases are searched (mined) for molecules that are predicted to possess some novel trait, such as being secreted or membrane bound. Genes are identi-

fied coding for molecules which may play an important role in the parasite life cycle, pathogenesis, or immune evasion. Subsequently, these molecules are tested in animal models for their ability to induce protective immunity.

The development of high-throughput approaches has been needed to screen large numbers of vaccine candidates in a rational, unbiased fashion. The majority of these have used the DNA vaccination approach, with candidates being assessed individually or in pools (Almeida *et al.*, 2002). Expression library immunization, has also been used directly as a screening method in conjunction with animal models, for the identification of vaccine candidates (Melby *et al.*, 2000; Smooker *et al.*, 2000). This later approach is recognized to be limited in its ability to identify vaccine candidates because of the phenomenon of masking of protection, which can occur by individual members of the group.

The development of novel-live vaccines through the use of genetic manipulation technologies has exciting potential. To date, auxotrophic mutants of *Leishmania* and *T. gondii* created by knock-out strategies, have been shown to be attenuated and to be able to induce strong protective immunity (Titus *et al.*, 1995; Fox and Bzik, 2002). Issues such as persistence of the live vaccine, or reversion to the virulent phenotype within the vaccinated population, remain a concern, but nevertheless, these issues are potentially addressable.

IDENTIFICATION OF NEW DRUG TARGETS

Investigations into the genomes of parasitic protozoa have also identified many new, potentially exciting targets for chemotherapeutic treatment, such as enzymes of folate metabolism, the mannitol cycle, and polyamine biosynthesis, for example (Coombs and Muller, 2002). Some of the most exciting advances come from the characterization of the apicoplast, a novel remnant of a chloroplast which is essential for parasite survival (McFadden and Roos, 1999; Ralph *et al.*, 2001). Plastid genomes in Apicomplexa are similar to those found in bacteria, and some known drugs like Ciprofloxacin, selectively target the apicoplast (Fichera and Roos, 1997).

Another good example of drug development is the discovery of fosmidomycin as an antimalarial compound, and this has been described in some detail elsewhere (Anonymous, 2002). Briefly, mining of the malaria genome database demonstrated the parasite used the nonmevalonate pathway for isoprenoid synthesis, which is absent in humans. Inhibitors of this pathway had been available for some time, and through the use of mouse models were found to be effective antimalarial compounds (Jomaa *et al.*, 1999).

The identification of the shikimate pathway in Apicomplexa is also worth noting, since this pathway is missing in mammals and is a target for herbicides in plants (Roberts *et al.*, 2002). Indeed, glyphosphate, which targets 5-enolpyruvylshikimate 3-phosphate synthase, shows antiparasitic activity (Roberts *et al.*, 1998).

The large number of parasite genes and proteins which remain to be characterized imply that we know relatively little about the metabolic pathways of parasites and that many more remain to be discovered. Hence, it is highly likely that new drug targets will be identified in the future (Fairlamb, 2002).

SUMMARY

There is no doubt the era of the “omics” is with parasitology, and current trends in the discipline are addressing fundamental biological questions that can make best use of the new technologies, as well as the vast amount of new data being generated. Genes, currently uncharacterized, will be identified that are involved in the host–parasite interaction, and we will learn more about parasite biology and pathogenesis. Attempts to explain the diversity of phenotypic traits shown by parasites will occur using genome sequence data, and new parasite-specific biochemical pathways and mechanisms of drug resistance will be identified. In addition, the host response to parasites will be investigated, and the mechanisms used by parasites to evade immunity will be explained. This surely will become the “golden age of molecular parasitology,” leading to the control of parasitic diseases which have plagued mankind for hundreds of years.

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