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Reviews

Progress in molecular parasitology

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Summary. Substantial progress has been made in the last ten years in understanding the structural and functional organization of parasitic protozoa and helminths and the complex physiological relationships that exist between these organisms and their hosts. By employing the new powerful techniques of biochemistry, molecular biology and immunology the genomic organization in parasites, the molecular basis of parasite's variation in surface antigens and the biosynthesis, processing, transport and membrane anchoring of these and other surface proteins were extensively investigated. Significant advances have also been made in our knowledge of the specific and often peculiar strategies of intermediary metabolism, cell compartmentation, the role of oxygen for parasites and the mechanisms of antiparasitic drug action. Further major fields of interest are currently the complex processes which enables parasites to evade the host's immune defense system and other mechanisms which have resulted in the specific adaptations which enabled parasites to survive within their host environments. Various approaches in molecular and biochemical parasitology and in immunoparasitology have been proven to be of high potential for serodiagnosis, immunoprophylaxis and drug design.
Key words. Molecular parasitology; *Trypanosoma; Leishmania; Plasmodium;* helminths; antigenic variation; membrane proteins; kinetoplast DNA; metabolism; bioenergetics; tubulin; hemoglobin; anthelmintics.

Introduction

During the first half of this century the study of molecular aspects of parasitic organisms was a largely neglected research area which lagged far behind the biochemical knowledge about bacteria and vertebrates. During World War II the increasing public recognition of diseases caused by both protozoan parasites and helminths initiated a turning point in biochemical parasitology. A major goal of the increased and improved parasitological research was that a detailed understanding of the molecular differences between parasites and their hosts, and a comprehensive knowledge of the crucial molecular properties of parasites, would offer interesting opportunities for rational approaches to antiparasitic chemotherapy. As a result of these activities, significant advances were achieved over the next 25 years, in particular in the areas of intermediary metabolism, including the identification and characterization of enzymes and metabolic pathways, and of metabolic regulation, membrane transport, the role of oxygen for parasites and the mechanisms of antiparasitic drug action (see von Brand[113]). Although the potential chemotherapeutic significance of these studies remained to be demonstrated the associated increase in knowledge helped molecular parasitology to emerge from its infancy.

A further change in parasitology has occurred in the last 10–15 years. On the one side, application of powerful and precise new techniques in biochemistry and biophysics has made possible more detailed studies in the established fields of parasite biochemistry, including work on intermediary metabolism, enzymology, bioenergetics and the structural and functional organization of membranes[5,44]. Simultaneously, recent developments in molecular biology and immunology, such as the cloning of genes, their expression in bacteria and hybridoma techniques, have opened up a completely new molecular area in parasitology[2,115]. By employing these technologies, and the ability to sequence both proteins and nucleic acids, we are now in a position to study the antigenicity of parasites in more detail, to isolate and characterize parasite genes and analyze their organization and expression, and thus increase the knowledge of molecular genetic aspects of parasitology. Monoclonal antibodies and other immunological techniques, because of their exquisite specificity, have proved to be invaluable tools for purifying and characterizing parasite proteins. It is now possible to localize parasite antigens precisely within cells, and antigen isola-

tion and purification on both analytical and preparative scales has become feasible. These new approaches seem to be worth pursuing, not only because of their potential for serodiagnosis and immunoprophylaxis but also for a better understanding of the intimate molecular interplay that exists between parasites and their hosts.

With the new sophisticated and powerful techniques of biochemistry and molecular biology many problems of parasitology have become more approachable and this, in turn, has attracted scientists from other research disciplines. The result of these considerable efforts was a number of real advances which ultimately set the stage for our present knowledge in molecular parasitology, and I am convinced that this field will now grow rapidly along the lines of modern biology. The evidence for this change is clear. New journals devoted to parasite biochemistry, molecular biology and immunology have appeared, and more papers on molecular aspects of parasitology are published in established journals of biology. Also greater availability of funds for research in this area can be recognized.

Undoubtedly, this rapidly increasing progress has relied heavily on the development and dissemination of in vitro culture methods, in particular for protozoan parasites. The continuous culture of *Plasmodium falciparum* in red blood cells, as established by Trager and Jensen in 1976[106], is one of the most important. Another is the development of an in vitro culture system for the bloodstream form trypanosomes of the *brucei* complex by Hirumi[50] and by Hill[49] and their colleagues in the late 1970s. This technique ultimately led to the reproduction of the entire life cycle of African trypanosomes in vitro. Very recently an in vitro model for the cultivation of the hepatic phase of *P. falciparum* was developed by Mazier et al.[75]. Although this culture system does require further refinement, it documents that the complete liver schizogony of the malaria parasite can be obtained outside its host.

Primarily because of the advances made in in vitro techniques, research on molecular aspects of *Trypanosoma* and *Plasmodium* species has tremendously increased, and in particular, because of its unique metabolic and structural organization, *Trypanosoma brucei* has interested scientists remote from medical and field problems and who have used this protozoan as biological model for biochemical as well as molecular genetical studies. Evidence for the significance of molecular studies on trypanosomes and malarial parasites is the fact that in recent years papers on these species with emphasis on molecular aspects constitute almost half of the total number of articles published in the area of molecular parasitology. In view of the wealth and diversity of material which has accumulated in that field it is impossible to summarize all the major recent advances within the available space. Instead, I have selected examples which, I feel, constitute most important approaches and which illustrate the present status of the work.

Molecular and membrane biology of parasites

1. Antigenic variation and chromosomal organization in trypanosomes

A revolutionary discovery in molecular parasitology was undoubtedly the elucidation of the biochemical and, at least to some extent, the genetic basis of antigenic variation of African trypanosomes. In the mammalian host, this unique process enables the protozoan population to evade elimination by the immune system. The capacity to undergo antigenic variation is associated with the presence of a surface coat covering the parasite's cell membrane, which was visualized first by Vickerman[112] in 1969 in transmission electron microscopy as an electron-dense layer some 12–15 nm thick. Further elegant and outstanding work by Cross[20] in the mid-1970s has shown that this surface coat is made up of a matrix of about ten million molecules of a single species of glycoprotein, known as the variant surface glycoprotein (VSG). Subsequently a large number of other scientists entered the area of VSG research, which has led to a considerable growth of literature on this subject. Major advances were achieved in our understanding of the biochemical, physicochemical and immunological properties of VSGs and their biosynthesis and processing[6,8,13,27] (fig. 1). New data derived from different investigators[11,36] suggest that anchoring of the VSG in the cytoplasmic membrane is mediated by a phospholipid, identified as *sn*-1,2-dimyristoyl-3-phosphoglycerol. The release of the surface coat from bloodstream trypanosomes during transformation to procyclic forms is obviously catalyzed by a membrane-bound phospholipase C-like hydrolase[11]. This enzyme is able to convert membrane-bound VSGs to soluble VSGs and 1,2-dimyristin.

Comparison of cDNA sequences has revealed extensive differences among VSGs but the major antigenic sites within the 360-amino acid variable region (fig. 1) have not been determined. Some progress toward this goal has come recently from X-ray-crystallographic studies done by Freymann et al.[38]. These authors have obtained crystals from the soluble form of VSGs exhibiting an approximate molecular mass of 120–130 kD. The crystal structure of the amino-terminal domain of one of the dimers has been resolved to 6 Å and the structure of the entire crystallized protein has been characterized[38].

The switching of the antigenic types of trypanosomes seem to reflect the successive transcriptional activation of single VSG genes from a large repertoire containing possibly more than 500 genes[27]. As a result of extensive studies in a number of laboratories, activation of some

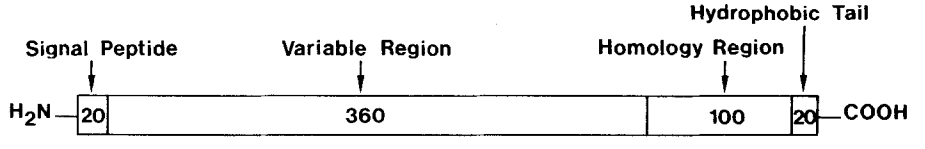

Figure 1. Structure of the nascent protein sequence of a representative VSG (after Donelson and Turner[27]). These proteins are composed of about 500 amino acids.

genes was found to be accomplished by duplication of a previously silent basic copy gene and insertion of this expression-linked copy near a chromosomal telomere where it is expressed (duplicative transposition or gene conversion mechanism); while other genes, always located near a telomeric site, are expressed without being duplicated and translocated[67] (reciprocal gene recombination). Recently Myler and his colleagues[80] have reported on a VSG gene which can be activated with and without duplication. The authors suggested that utilization of both mechanisms for activation of a VSG gene may allow its more frequent expression. From another recent finding on the simultaneous activation of two VSG gene transcription units in an exceptional trypanosome, Cornelissen et al.[16] have postulated a model for the control of VSG gene transcription. A comparably interesting observation was obtained from analyses of trypanosomes during antigenic switching[32]. It was found that the organisms simultaneously expressed both pre- and post-switch VSGs uniformly on their surface membrane.

Although antigenic variation in trypanosomes has been extensively studied in the last ten years, the precise mechanisms triggering the switch from one VSG gene to another and the control mechanisms of the multiple VSG gene expression sites remain to be elucidated. Furthermore, our understanding still does not provide an explanation for the restricted size of the metacyclic antigen pool and for the phenomenon of predominance. All these mechanisms appear to be so complex and flexible that attempts to develop a vaccine against trypanosomiasis may remain unsuccessful.

In conjunction with the work on antigenic variation other outstanding discoveries were made on the molecular and cell biology of kinetoplastid flagellates. A new approach to the elucidation of the chromosomal organization of these organisms was recently provided by a novel technique, termed pulsed field gradient gel electrophoresis[39,93], whose extraordinary resolving power allows the fractionation and analysis of chromosome-sized DNA molecules of up to at least 2000 kilo-base pairs (kb) in size. Recent application of this method to parasitology research has resulted in interesting new insights into the ploidy, size and number of chromosomes within the genome of trypanosome and *Leishmania* species[108,110]. Most of the species investigated were found to contain at least 20 chromosomes with an average size of 2000 kb, but large differences were observed between these species in chromosome-sized DNA molecules and in the distribution of particular genes (tubulin genes, rRNA genes, mini-exon sequences)[39,108]. As an example, *T. vivax* was found to contain no DNA smaller than 2000 kb, while *T. brucei* and *T. congolense* showed an abundance ($\sim$ 100) of mini-chromosomes (50–150 kb) and several small chromosomes (200–2000 kb). As most of the mini-chromosomes contain telomeric VSG genes and are possibly subject to frequent DNA rearrangements, it has been suggested that these genomic elements play an important role in the regulation of VSG gene expression[109]. However, in view of the comparative analysis of the chromosomal organization of trypanosomes, as obtained with the new electrophoretic technique, the presence of telomeric VSG genes in mini-chromosomes does not appear to be vital for the mechanism of antigenic variation. In addition to the above findings, pulsed field electrophoretic analysis has shown remarkable variations in the size-distribution of small chromosomes in different isolates of *T.brucei*[108]. It is thus likely that the novel electrophoretic method has opened up new possibilities for the identification of subspecies and strains indistinguishable by other techniques. Very recently this method was also extended to malaria parasites[52].

2. Surface enzymes and survival mechanisms of leishmanial parasites

Another example demonstrating the superior role of the parasite surface structure is *Leishmania donovani,* a kinetoplastid flagellate residing, in the mammalian host, in the phagolysosomal system of macrophages. The mechanisms whereby these protozoans survive and multiply in the metabolically hostile environment of phagocyte cells is still not completely understood. It has been proposed that the non-susceptibility of the organism to oxygen metabolites, such as superoxide anions or hydrogen peroxide, is due to the fact that the parasite may suppress the production of the toxic compounds generated in its natural environment[74]. A very exciting observation, which may explain the evasion abilities of leishmanial parasites, is related to the presence of acid phosphatases, which are found to be distributed over the entire surface of *L. donovani* promastigotes[41]. Recently, the purification and characterization of three acid phosphatases derived from the external membrane of this parasite were reported by Remaley and his associates[88]. The predominant species of these enzymes is a 128 kD protein which catalyzes the dephosphorylation of a variety of sugar phosphomonoesters as well as phosphorylated liver pyruvate kinase. Although the physiological role of this membrane-bound phosphatase has yet to be established, several interesting possibilities have been hypothesized. Since there is evidence that phagocytic cell activation is controlled by the phosphorylation of membrane proteins, the ability of the enzyme(s) to dephosphorylate phosphoproteins may provide an explanation of how the leishmanial parasite escapes inactivation by the host's metabolic defense mechanisms. A most important observation which would support this idea was the recent finding that the leishmanial acid phosphatase inhibited superoxide anion production by human neutrophils and macrophages[89]. Another suggestion of how the leishmanial membrane-bound phosphatases might facilitate the establishment of the parasitic infection may be related to the possible ability of the enzymes to inactivate lysosomal hydrolases[88], a further class of potential tools for intracellular parasite killing.

3. Mitochondrial DNA of kinetoplastid flagellates

The kinetoplast DNA (kDNA) found within the single mitochondrion of *Trypanosoma* and *Leishmania* is an area in parasitology which has also been extensively studied with the methods developed in molecular biology. As discussed in a number of excellent review articles[9,99], this DNA was shown to distinguish the members of the order Kinetoplastida from other flagellated protozoa and was the first extranuclear DNA to be discovered

in eukaryotes. kDNA represents 5–30% of the total cell DNA mass and is composed of several 20–40 kb circular DNA molecules termed maxicircles and thousands of 1–3 kb circular DNA molecules termed minicircles.

Maxicircle DNA was shown to be homogeneous in sequence and actively transcribed. Sequences of the 9S and 12S mitochondrial RNA genes from *T. brucei* have been published[31] and De la Cruz et al.[23] have reported on the sequence of the 9S rRNA gene from *L. tarentolae,* and a secondary structure model for the kinetoplast small rRNA was proposed. In the maxicircle, several genes have been tentatively identified by hybridization with mitochondrial DNA sequences from other organisms[99]. The cytochrome oxidase subunits I and II, ATPase and apocytochrome *b* coding sequences have now been identified in maxicircles of *L. tarentolae* und *T. brucei,* primarily by Hill and Stuart and their colleagues[51,84]. Although to date attempts to isolate kinetoplast ribosomes and to demonstrate mitochondrial protein synthesis have generally been unsuccessful, the results illustrate the functional homology between maxicircles and mitochondrial DNA of other organisms. Recently the mechanisms which regulate the differential expression of respiratory-chain enzymes during the life cycle of *T. brucei* have been investigated by Feagin et al.[34,35]. These authors found that the control of enzyme production involves differential expression of mitochondrial genes between bloodstream and procyclic stages of the parasite.

A fundamental mystery of kDNA is still the function of the minicircles, which represent a unique form of mitochondrial DNA not only because of their small size but also because they are responsible for the network structure of kDNA. Minicircle DNA has been suggested to play a role in the segregation of the maxicircles or in the changes occurring during the life cycle of the parasite[99]. A possible significance of this DNA in antigenic variation is not yet excluded, and the theory that minicircles have no function but consist of 'selfish' DNA remains to be ruled out. In view of the rapid sequence evolution of minicircle DNA, restriction digests of this DNA can be useful for stock and strain identification of kinetoplastid flagellates and may thus become a helpful tool in epidemiological studies.

4. Surface antigens and histidine-rich proteins of malaria parasites

Numerous other examples where parasite surface proteins with antigenic properties have been identified and successfully isolated and characterized are found in malaria research. A number of groups[21,28–30,40,45] have recently achieved expression of cloned genes encoding sporozoite and bloodstage proteins of various *Plasmodium* species. One type of these antigens is the membrane-associated protein, which uniformly and densely covers the surface of the plasmodial sporozoite and which was termed circumsporozoite (CS) protein (fig. 2). Antigenically, these proteins, like the protective response they elicit, have been found to be stage- and species-specific in all studies to date. All of these and other malarial surface proteins investigated so far contain short repetitive sequences of amino acids, and a high percentage of the molecule consists of these repeats. The presence of these repeated epitopes suggests that more than one antibody molecule might be necessary to inactivate the protein which, in turn, would increase the chance of survival for the parasite. Such a possible function of the CS protein as an immune decoy protein has been discussed by Godson et al.[40].

In 1983 Ellis and his associates[28] cloned the gene for the CS antigen from *P. knowlesi* sporozoites and in 1984 a number of other groups[21,30] reported on the cloning of the gene for the CS antigen from the human malaria parasite *P. falciparum*. According to the results obtained by Dame et al.[21], a major portion of the *P. falciparum* protein consists of 41 tandem repeats of tetrapeptides, 37 of which are identical (fig. 2). Interestingly, the method used by Dame et al.[21] to clone the CS antigen gene depended on a new technique developed by McCutchan et al.[76]. This method is based on the finding that mung bean nuclease is able to cut the genomic DNA of malaria parasites at positions before and after genes but not within gene-coding regions. This enabled the investigators to clone the intact gene for the CS, and also other plasmodial proteins, from genomic rather than complementary DNA, which has the impressive advantage that no mRNAs of the sporozoite are required. The gene can be directly obtained from the DNA of a erythrocytic form that can be grown in culture.

In the meantime, a high degree of conservation of the CS protein gene among different strains of *P. falciparum*[116] and a striking homology of certain regions of this antigen between different *Plasmodium* species have been recognized[21], which suggests conservation for a sporozoite function such as reception for liver invasion. If this region is also conserved in other human malaria parasites and is exposed to the immune system, immunization with this region from *P. falciparum* may give protection against other species of human malaria. Meanwhile, proteins composed of 16, 32 and 48 tandem copies of a tetrapeptide repeating sequence found in the CS protein of *P. falciparum* were expressed at high levels in *Escherichia coli*[118]. These recombinant proteins have proven immunogenicity and were found to block sporozoite invasion of human hepatoma cells in vitro[3]. Thus, specific sequences of the CS protein as synthesized by recombi-

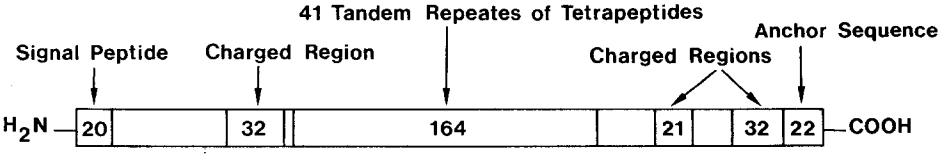

Figure 2. Structure of the CS protein from *P. falciparum* as deduced from the nucleotide sequence data of Dame et al.[21]. The symbolic drawing shows the various regions of the 412 amino acid-containing protein sequence.

nant DNA techniques appear currently to be the most appropriate candidates for the development of a malaria vaccine.

Of the more than 200 different surface proteins which have been investigated from the intraerythrocytic stages of malaria parasites as well as from the infected red blood cell, many are being evaluated to see whether any is suitable for use in developing a merozoite vaccine. In recent work by Hall et al.[46] a 190 kD major surface antigen was purified from cultured *P. falciparum* and its synthesis and processing were investigated. It has been suggested that this protein may play a role in merozoite invasion and that its proteolytic cleavage may be part of the mechanism which drives merozoite invasion[46]. Interestingly, Hall and his colleagues[45] have succeeded in cloning part of the gene for the 190 kD *P. falciparum* antigen and expressing it in *E. coli*. Recently another blood-stage malaria antigen has been cloned and expressed in *E. coli* by Cheung et al.[14] and the complete nucleotide sequence encoding a dominant immunogen of the external surface of *P. falciparum* schizont and ring-stage trophozoites has been identified by Stahl et al.[97]. As deduced from the nucleotide structure the latter protein contains, like the CS antigens, multiple tandem repeats of oligopeptides flanked by regions rich in charged amino acids.

Another protozoan protein extensively studied with the techniques of molecular biology and immunology is the histidine-rich protein (HRP) from *P. lophurae*. This unique protein was first purified from cytoplasmic granules of trophozoites of the avian parasite by Kilejian in 1974[53]. In this stage the protein accumulates until it comprises at least 50% of the total cellular protein mass. Its molecular mass is 45 kD and it was found to exhibit the unique feature of containing approximately 70% histidine[53,56]. The biological function of this protein is not yet understood but it has been hypothesized that it may have a function in merozoite penetration[54]. It has also been suggested that the related HRP from the human malaria parasite *P. falciparum* plays a role in the formation of the knob-like protrusions which develop in the surface membranes of infected erythrocytes[69]. Electron microscopic studies have demonstrated that these protrusions serve as points of attachment between infected blood cells and endothelial cells. An understanding of the composition of these knobs may therefore be essential to the understanding of the molecular basis of sequestration of the malaria parasite. To date only the HRP from the avian malaria parasite has been purified and the discovery of its primary structure and genomic organization has been reported by Ravetch and his colleagues[86]. The structure of this protein is of particular interest since it displays immunochemical cross-reactivity to the *P. falciparum* HRP, and may thus have relevance for protective immunity[55].

5. Molecular biology of helminths

Recombinant DNA and hybridoma technologies have, in the meantime, also been applied to studying surface antigens and to cloning antigen genes from worm parasites. As for the protozoans, this may open up a powerful approach to the development of defined antigen vaccines, and will aid in the investigation of the strategies helminths have evolved for evading the vertebrate host's immune response. Cell-free synthesis of surface antigens and their bacterial expression have been achieved for a number of helminths, including *Schistosoma mansoni*[4,42,57,65,103], *Taenia taeniaeformis*[10], *Onchocerca volvulus*[104] and most recently *Toxocara canis*[100]. cDNA clones encoding helminth antigens were isolated from a library constructed from adult *S. mansoni* mRNA[15]. These may eventually be used to express these proteins in sufficient quantities for their potential usefulness for vaccination[18] to be evaluated.

Studies on parasite membranes are, of course, not only important because of their antigenic properties. As the parasite surface represents the intimate interface between the host and the invader, this structure can be considered as a major site responsible for the maintenance of the complex host-parasite relationship. Investigations into the dynamic aspects of protozoan and helminth surface structures are therefore very valuable for our understanding of this interrelation, and studies in this direction include the processes involved in membrane synthesis and turnover, the recycling and shedding of surface layers and the transport of water, ions and nonelectrolytes. Since membrane turnover and modulation may be critical in protecting the parasite from damage caused by immune components or toxic metabolites synthesized by the host, membrane studies also provide exciting approaches to the problems associated with the mechanisms promoting parasite survival and evasion.

In the last two decades extensive studies on many aspects of parasite membrane biology have been conducted in a number of laboratories, which has resulted in an enormous accumulation of knowledge. The dynamic nature of the helminth surface has now been documented at the molecular level[85]. Nematodes have been shown to shed surface antigens in vitro and the complex tegumental structure of schistosomes, which mediates important physiological and immunological interactions with the host, has been extensively studied in several research groups by electron microscopy, enzymological and radiolabeling techniques[77,85,95]. One of the most puzzling aspects in the study of host-parasite relationships in schistosomiasis is the ability of the parasite to overcome the host's defence mechanisms. Intrinsic changes occur in the surface structure during schistosome development which render them refractory to immune killing mechanisms[94,95]. These developmental changes in the surface structure have not been detected in polypeptides but in lipids and carbohydrates, and they correlated with the protection of the parasite against the immune effector mechanisms[91,94,111]. In addition, other escape strategies seem to be operative at the level of the schistosomulum's outer surface. The complex network of these possibilities and the host effector mechanisms have been studied in great detail primarily by Capron and his colleagues[12,43]. Further analyses of the larval tegument, whose separation was very recently achieved for the first time by Levi-Schaffer and her colleagues[70], may shed further light on the invasive capacity and non-susceptibility of this parasite.

Biochemistry of parasites

Another large area of molecular parasitology in which our knowledge has expanded remarkably is intermediary

metabolism, including bioenergetics and studies on enzymes and other proteins. From the numerous organisms investigated a few examples will be considered to illustrate recent work in this field.

1. Aerotolerant anaerobic protozoans

One group of parasites which has been quite extensively investigated are the trichomonad flagellates which, together with the parasitic *Amoeba* and *Giardia* species, have been classified as aerotolerant anaerobes[72,112,117]. A peculiarity among eukaryotic organisms is that these protozoans lack morphologically recognizable mitochondria and such molecular and metabolic attributes as the Krebs cycle, cytochromes and oxidative phosphorylation[79,117]. Most unusual is also the fact that trichomonad flagellates are able to eliminate reducing equivalents in the form of molecular hydrogen[79]. The nature of the pathway of hydrogen formation was of considerable interest to molecular parasitologists who found that it is similar to that observed in strict anaerobes[79]. As in some anaerobic prokaryotes, pyruvate, the product of glycolysis, is oxidatively decarboxylated to acetate by a ferredoxin-linked enzymatic reaction[73] and the substrate-derived reducing equivalents are subsequently donated to protons to form hydrogen. As illustrated in figure 3, the consecutive reaction steps involved in pyruvate oxidation are catalyzed by a pyruvate:ferredoxin oxidoreductase and a hydrogenase, respectively. Most importantly, hydrolysis of acetyl CoA, the primary product of pyruvate oxidation, was found to be coupled to energy conservation via substrate level phosphorylation[79]. Another feature unique to trichomonad flagellates is the fact that all the steps involved in anaerobic pyruvate oxidation are localized in large cytoplasmic granules. Although the presence of these organelles in the cytoplasm of trichomonads has been well known for decades their functional significance was discovered only in the 1970s by Lindmark and Müller[71] who named these structures hydrogenosomes. Hydrogenosomes resemble in their morphology microbodies of aerobic cells; in their biochemistry, however, they differ from peroxisomes and also from mitochondria to such an extent that they can be regarded as organelles sui generis.

2. African trypanosomes

Similar outstanding discoveries on metabolic aspects were made with the trypanosomes. In 1977 Opperdoes and Borst[82] found that in the bloodstream form of *T. brucei* a number of glycolytic enzymes are localized in special membrane-bounded microbody-like organelles which they called 'glycosomes'. These organelles are homogeneous in size and measure about 0.3 µm in diameter. It has been estimated that a *T. brucei* cell contains between 200 and 300 glycosomes[81]. Since their discovery these organelles have been found in all major representatives of trypanosomatid flagellates[48].

More recently, the metabolic significance of the glycosomes was largely elucidated, in particular by the work done by Opperdoes and his colleagues[83]. Unlike in the cells of other organisms, in trypanosomatid flagellates the first seven enzymes of the glycolytic pathway, catalyzing the degradation of glucose to 3-phosphoglycerate, are harbored in the glycosome. The presence of the major portion of the glycolytic pathway inside an organelle is a property unique to these organisms. Although there appears to be a close physical association among the glycolytic enzymes within the glycosome, there is as yet no firm conclusion about whether or not these enzymes are aggregated in a specific way with specific substrate channellings[1]. In the meantime, the simultaneous purification of four of these enzymes has been reported by Misset and Opperdoes[78]. Of these enzymes, hexokinase showed properties significantly different from those of its mammalian counterpart. In contrast to the results of the latter authors other investigators have also found significant differences in regulatory properties with trypanosome phosphofructokinase[19]. In the laboratory of Opperdoes protein crystals of several of the purified glycosomal enzymes are currently being grown, and their three-dimensional structure will be elucidated by X-ray diffraction. Also, genomic libraries have been constructed and several parallel routes are being followed to identify DNA sequences coding for the glycolytic enzymes (Opperdoes, pers.commun.). It is to be hoped that the metabolic and enzymatic peculiarities of the trypanosome may offer opportunities for the design of safer and more effective agents for controlling trypanosomiasis.

Progress has also been made in our knowledge of the metabolic alterations that occur during the life cycle of African trypanosomes[7]. It now seems clear that as bloodstream trypomastigotes of *T. brucei* differentiate into procyclic vector forms, they switch from a predominantly glycosomal to a more mitochondrial type of energy metabolism[83]. The stimuli eliciting the dramatic morphological and metabolic changes and the precise sequence of events occurring during trypanosome transformation and the control mechanisms and genetic basis underlying this unique biological process are currently being investigated in a number of laboratories.

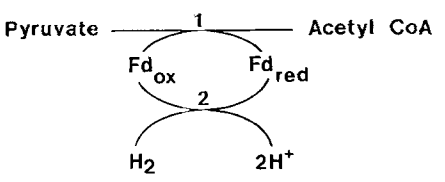

Figure 3. Oxidative decarboxylation of pyruvate in hydrogenosomes of trichomonad flagellates[79]. 1=Pyruvate:ferredoxin oxidoreductase; 2=hydrogenase; Fd=ferredoxin.

3. Helminths

Several major advances in the biochemistry of parasites have also been made with the helminths. Various enzymes from these metazoan parasites have been purified to homogeneity and their kinetic and regulatory properties have been investigated[17,37,63,64,98,101,102]. These analyses, in conjunction with other biochemical studies, have led to the elucidation of the predominant energy conserving routes present in worm parasites (fig. 4). Examples are the pathways for the formation of propionate and branched-chain volatile fatty acids. As a result of our own studies[61], and work by other investigators[92], propio-

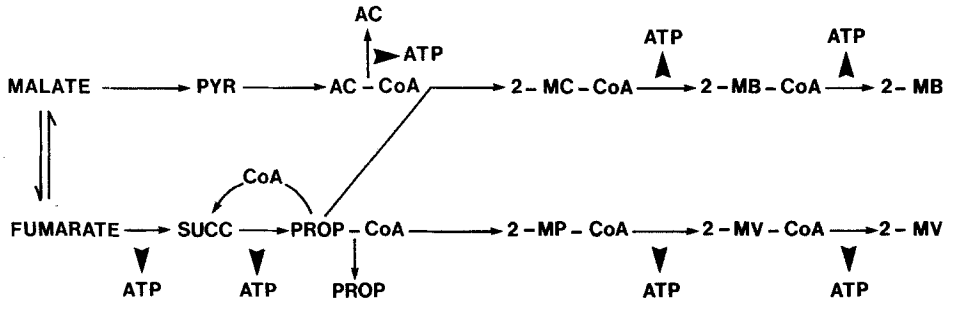

Figure 4. Sites of energy generation in muscle mitochondria of *Ascaris suum*[59]. Malate is the major mitochondrial substrate and is derived from glucose or glycogen, respectively. ATP synthesis was clearly demonstrated for the pathways of fumarate reduction and succinate decarboxylation. All other sites of energy generation indicated in the scheme are still hypothetical. Abbreviations are explained in the above reference.

nate formation from succinate, a major step in carbohydrate catabolism in helminths, was shown to be associated with energy conservation. Recently, Komuniecki and his colleagues[62,63] have purified two enzymes from *Ascaris* muscle, an electron transferring protein and an enoyl CoA reductase, both of which are involved in the synthesis of the nematode's methyl-branched volatile acids. Most interestingly, evidence was obtained that this pathway can be coupled to electron transport phosphorylation in a way similar to the pathway of fumarate reduction[90].

In the last two decades the major sites of energy generation in helminths have been identified[58,59,92]. The adult stages of these parasites seem to place greater emphasis on multiple fermentations and anaerobic electron transport processes to meet their energy requirements[58,92]. As outlined in figure 4, energy conservation mediated by these strategies would display a clear advantage over the simple pathway of lactate fermentation, thus rendering the parasite more versatile and flexible in responding to its often peculiar environmental conditions. The significance of oxygen for the bioenergetics of adult helminths is still not completely understood, but recent studies suggest that oxygen may play a greater role in this respect than was originally thought[58].

Another interesting area which has recently attracted molecular parasitologists concerns the alterations in metabolism occurring during the development of a helminth and the mechanisms initiating the switches from one pathway to another. An example is the liver fluke, *Fasciola hepatica* which, in its early liver-parenchymal stage, was found to have a predominantly aerobic metabolism capable of complete substrate degradation to CO_2 and water. Recent in vitro experiments by Tielens et al.[105] have demonstrated that in 3-week-old flukes the Krebs cycle is largely suppressed, but concomitantly a pathway resulting in the formation of acetate becomes the predominant oxidative pathway. However, after arrival of the flukes in their definitive environment, the bile ducts, the organism has to rely almost exclusively on anaerobic redox processes, similar to those shown in figure 4, to meet its energy demands. Unfortunately, there is little evidence so far about the ways in which these metabolic switches are controlled. As a consequence of the progress of in vitro culturing techniques, this central question is currently being investigated not only for helminths but also for protozoans, like *T. brucei*.

A last area of molecular parasitology which I would like to touch is that of non-enzymatic proteins. These include the microtubule proteins which, together with their genetic basis, have been investigated to some extent in parasitic organisms[22,66]. In our laboratory, microtubule protein was purified from the intestine of *A. suum*[60]. As revealed by gel electrophoresis, the molecular mass of this protein was similar to that reported for tubulin from other sources but it was found to react more sensitively towards benzimidazole carbamate anthelmintics than does mammalian brain tubulin. This finding may suggest that the selective toxicity of these important drugs is primarily based on differential binding affinities between parasite and host microtubule protein.

Other examples are the hemoglobins of helminths. Although a number of these heme proteins have been characterized in the past, by far the most extensive studies have been carried out on the monomeric hemoglobin from *Dicrocoelium dendriticum*, a trematode residing in the bile ducts of sheep and cattle[107]. This hemoglobin proved to be the most primitive animal hemoglobin characterized to date. Its nearly completed sequence shows various unusual features and its very low sequence homology to other hemoglobins suggests that it represents a very old hemoglobin folding in the evolutionary scale. Recently, crystals of this protein were obtained by Smit et al.[68,96] and its atomic organization was studied by nuclear magnetic resonance spectroscopy and other techniques. In the near future an electron density map of the trematode hemoglobin will be calculated to study the globin folding evolution. Differences between the structures of the oxygenated and other forms of the protein should reveal the structural features responsible for its extremely high oxygen affinity[26].

Summary

Substantial progress has been made in the last years in understanding the structural and functional organization of parasites and the molecular mechanisms underlying the intimate, complex and delicately balanced host-parasite interactions. Undoubtedly, membrane-bound parasite antigens, parasite surface coats and antigenic variation of parasites are major areas in current parasitological research. These and other important advances have been, at least to some extent, discussed in the present review but countless others of equivalent significance and quality could only be touched within the page limits of this report or have not been mentioned at all.

Examples of interesting subjects not considered in this review are the mini-exon sequences found in trypanosomatid flagellates[24,25] and the recently-discovered novel cofactor required by glutathione reductase, termed trypanothione[33], found in the same group of organisms. Other molecular peculiarities of parasites are the pyrophosphate-dependent enzymes of *Entamoeba histolytica*[87] and the multiplicity of routes for salvage in pyrimidine and purine metabolism in parasites[47,114]. Of high value are also studies done in the areas of neuro-biochemistry of helminths, the transport mechanisms in plasma and tegumental membranes of parasites and the molecular modes of drug action.

Of course, in all fields of molecular parasitology a lot of details still remain to be filled in. Particularly important in this respect appear to be investigations on the factors which control the differentiation and development of parasitic organisms, analyses of the genomic organization in parasites, the genetics of the variation in surface antigens of parasites, and the biosynthesis, processing and transport of these proteins through the cell membrane. Of high priority are also studies on the molecular mode of action of antiparasitic drugs, on the complex processes which enable parasites to evade the host's immune defense system, and on other adaptive mechanisms which have made parasites so successful in their hosts.

Clearly, the primary aim of our studies on molecular aspects of parasites should be to identify ways in which new, powerful methods for the control of parasitic diseases, such as drugs and vaccines, can be developed. On the other hand, the variety of unique metabolic systems and biochemical and genetic mechanisms evolved by parasites to survive in their immunologically and metabolically hostile environments have also opened up a fascinating research field of general biological interest. Studies on the molecular organization in parasites are likely to be of great value for studies on the elucidation of complex life processes observed in other biological systems.

*This paper is based on a review presented at a workshop on Molecular Parasitology, organized by the Swiss Society of Tropical Medecine and Parasitology at the University of Neuchâtel, March 1985.

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