CHAPTER II

REVIEW OF LITERATURE

1.Life cycle

Malaria parasite alternates between two hosts during its complex life cycle. It is transmitted by a large number of female anopheline species, in which the sexual cycle takes place. Development of the parasite also takes place in the vertebrate host, including man, thereby starting the asexual cycle (Figure 1).

1.1 Stages in the human host

During its asexual stage the parasites injected to the host by a mosquito bite is a haploid organism (Walliker, 1982; Walliker, 1983; Janse et al., 1986). Within a short time the sporozoites enter the bloodstream and invade the hepatocytes where they undergo exoerythrocytic schizogony, resulting in asexual proliferation of tissue-stage merozoites. When the process of merogony is complete, the parenchyma cells burst and the merozoites are released into the bloodstream. Different species have different pathways eg. P. vivax ; some of them remain in the hepatic cells. By rapidly invading the

erythrocytes, these merozoites mature through rings, trophozoites and schizonts which produce many new merozoites. The red blood cells erupt, liberating the merozoites which immediately invade other red blood cells, thereby reinitiating further erythrocytic cycles in the recipient.

1.2 Stages in the mosquitos

Some asexual parasites in human erythrocytes can, occasionally, undergo "sexual" differentiation into immature microgametocytes (male) and macrogametocytes (female) which become mature within 7-8 days. These gametocytes are ingested by the mosquito vector,and the micro- and macrogamonts are released from the red blood cells. During this process, the macrogamonts mature into macrogametes and are then ready for fertilization. The microgamonts undergo a further process of maturation called "exflagellation" during which rapid DNA replication and cell division occurs to form a microgametes. The flagellated cells swim through the midgut of the vector in quest of the macrogametes. After fertilization, the gamete nuclei fuse, producing the diploid zygotes. These undergo meiosis to develop into motile ookinetes, which penetrates the midgut wall to form oocysts within the outer wall. The oocysts mature and finally forms a legion of haploid sporozoites. These elongated, fusiform-shaped sporozoites emerge from the oocyst and migrate throughout the body of the mosquito and eventually enter the lumen of the

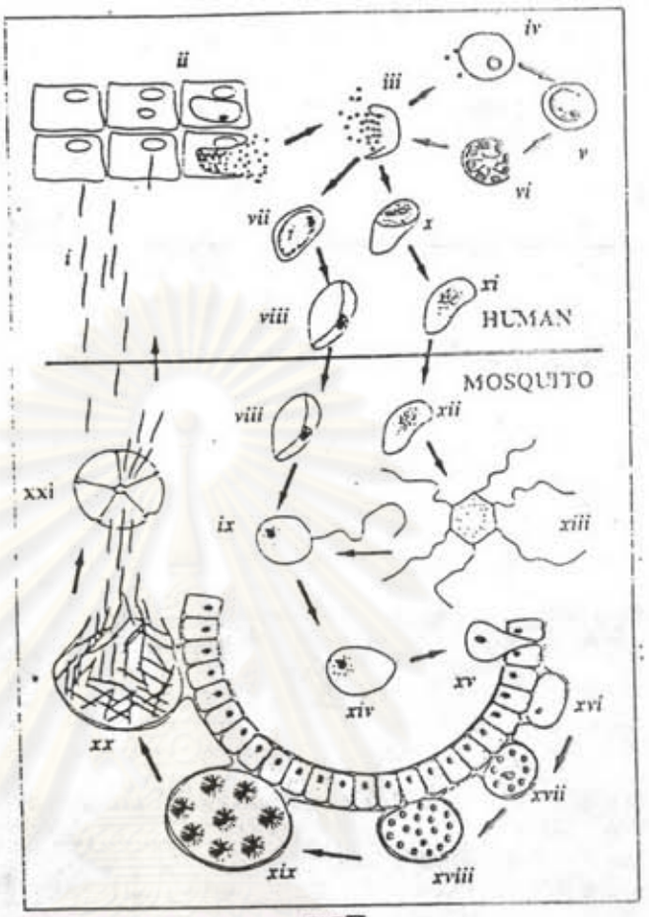


FIG 1

The life cycle of *P. falciparum*. Sporozoites (i) enter the blood stream of the host, invade hepatocytes (ii) where they undergo schizogony; merozoites are released which invade red blood cells and mature through the ring (iv), trophozoite (v), and schizont stages (vi). Merozoites are released (iii), which reinvade red cells (iv); the invading merozoites can then develop as before or can undergo 'sexual' differentiation into immature macro- (vii) or microgametocytes (x). These mature (viii, xi) and are taken up by the feeding mosquito. The microgametocyte undergoes rapid DNA replication and cell division to form flagellated cells (xiii); these are released and one invades the macrogametocyte to form the diploid zygote (ix), which undergoes meiosis to develop into the ookinete (xv). This stage invades the wall of the mosquito gut to develop into the oocyst (xvi) which undergoes sporogony (xvii–xix) to produce large numbers of haploid sporozoites (xx) which invade the salivary glands of the mosquito (xxi) from where they are injected into the human host and the cycle recommences.

Figure 1 The life cycle of P. falciparum (Foote and Kemp, 1989)

salivary glands. Here they may remain until they are inoculated into the human host via the next bloodmeal of the Anopheles vector (Foote and Kemp, 1989; Cornelissen, 1988).

In the past decade few studies have been made on the basic genetics of malaria parasites. This is mainly because the organisms undergo a complex life cycle. With the development of methods to maintain erythrocytic stages of P. falciparum in continuous culture (Trager and Jensen, 1976), parasites then become available to be investigated.

Before studies at the DNA sequence level were done characterization studies using a wide variety of approaches had clearly identified types of genetic diversity in Plasmodium as described below.

2. Characterization of malaria parasites

Identification of malaria parasites has been based on morphological, biological, and biochemical characters. Using biochemical methods, the following characters have been investigated :-

(1) Enzyme typing

Starch gel and cellulose acetate gel electrophoresis of enzymes have been used for characterizing malaria parasites to establish the extent of genetic variation among isolates

collected from different countries (Carter and McGregor, 1973; Thaithong et al., 1981).

Six enzymes have been principally studied:-

Glucose-phosphate isomerase (GPI - EC 5.3.1.9.)

6-phosphogluconate dehydrogenase (PGD - EC 1.1.1.44)

Lactate dehydrogenase (LDH - EC 1.1.1.27)

NADH-dependent glutamate dehydrogenase (GDH - EC 1.4.1.4)

Adenosine deaminase (ADA - EC 3.5.4.4)

Peptidase E (PEP - EC 3.4.11)

Enzyme typing has been helpful in identifying natural mixtures of parasites. An example was isolate T9, which contained parasites characterized by both types of GPI-1 and GPI-2 (Rosario, 1981). After cloning, two types of parasite were obtained. Moreover, they were good markers as they are stable over many generations, and because of the haploidy of the blood parasites, interpretation of their variation was straightforward.

(2) Protein variation

Tait (1981) used the two-dimentionalgel electrophoresis system of O' Farrell to examine protein variation in P. falciparum parasites labelled with ^{35}S-methionine. Proteins were separated according to their molecular weights and isoelectric points. The gel was then examined by autoradiography and many distinct spots appeared on the gel . These proteins have been designated 1,2,3,... according to their positions and

the variant forms of each protein have been given as letters of the alphabet A, B, C,...(e.g. 1A, 1B, 2A, 2B, etc.). Two-dimensional protein screening had an advantage over enzyme typing because more than 100 parasite proteins have been detected at any one time; this was of obvious value when only minor differences existed between organisms. Seven proteins were found polymorphic exhibiting variant forms in different isolates examined. Furthermore, it did not exhibit host protein markers , as mature redcells did not synthesize proteins (Fenton et al., 1985). This approach was valuable in differentiating isolates or clones of the same isolate (Pinsawasdi et al.,1987), however, great skill and long time was required to obtain the results by this method.

(3) Antigens

Studies have been made on soluble S-antigens as well as antigens detected by monoclonal antibodies and immunofluorescent techniques. Considerable diversity occurred among parasite populations (Wilson, 1980). In P. falciparum such diversity appeared not to have any regional basis, similar forms of antigen being found in different countries. The development of procedures for cloning and sequencing many antigens had expedited these studies (Ardeshir et al., 1985)

(4) Molecular genetics

The application of recombinant DNA technology to study of P. falciparum and, to a lesser extent , other species of Plasmodium has resulted in rapid advances in understanding of genetic diversity in malaria at the molecular level. Characteristics of DNA have been studied in several species of Plasmodium by several authors as described below.

Analysis of composition of plasmodial DNA by measurements of buoyant density and melting temperatures indicates a low G+C content (Gutteridge et al., 1971; Pollack etal., 1982; McCutchan et al., 1984). Both G+C content and gene hybridization studies indicate that the parasites can be arranged into 3 distinct groups : those with a single component DNA containing 18% G+C (P. falciparum, P. berghei, P. lophurae), those with asingle component DNA containing 30% G+C (P. knowlesi, P. fragile), and those with both DNA components (P. cynomolgi, P. vivax).

Goman et al. (1982) have prepared clones of repetitive DNA of P. falciparum and studied the hybridization patterns of one clone with nuclear DNA of 2 isolates. Different hybridization patterns were found in each isolate, showing that there were different DNA arrangements in their genomes. Estimates of its genome size have ranged from 10^7 to ~3.8×10^8 base pairs (Hough-Evans and Howard, 1982; Pollack et al., 1982).

The introduction of pulsed field gradient gel electrophoresis has allowed us to study Plasmodium chromosomal

DNA, and it has become clear that there is considerable variation in chromosome size from one parasite clone to another.

3. Pulsed field gradient gel electrophoresis (PFGE)

Gel electrophoresisis is widely used in DNA analysis on the basis of size, charge, or conformation. However, the conventional gel electrophoretic techniques are limited to molecules less than 20 kilobases (kb) in size due to the sieving properties of the gel matrix. Previous attempts to extend the range of gel electrophoresis relied on simply reducing the concentration of agarose gel (Fangman,1978). Unfortunately, this has unwanted band-spreading effects, and low-percentage agarose gels are difficult to handle. In any event, such ordinary techniques were unable to deal with intact large and complex DNA.

A novel approach to karyotypic analysis has been provided recently by the technique of pulsed field gradient gel electrophoresis (PFGE), which allows the fractionation of DNA molecules ranging in size from 10 to more than 2500 kb (Schwartz et al., 1983; Schwartz and Cantor, 1984). It is a new way of using a gel matrix and an electric field to separate large molecules.

In PFGE, DNA molecules are forced to continually change the direction in which they are moving. The rate at which a

distorted DNA coil can change its configuration is known to be extremely dependent on the molecular weight. In free solution this forms the basis of the viscoelastic relaxation technique. In a gel matrix no detailed physical modeling of the reorientation of DNA coils has yet been reported. Nevertheless, it seems clear that the shorter the DNA coil (which occupies only a short path through the gel pores), the faster the ability to alter the direction. Thus, an introduction of new electrode configurations that generate electric fields in alternating orientations has allowed the separation of large molecules. In a typical PFGE, molecules are subjected to alternating pulses of current in 2 directions perpendicular to each other (Schwartz and Cantor, 1984 ; Carle and Olson,1984). The net motion will be along the line bisecting the two field directions. Usually the two alternate fields are applied for equal time intervals, called the pulse time (figure 2). The intact molecules are then size-fractionated by PFG electrophoresis using pulses of appropiate duration (figure 3). Not only does this allow for the determination of the sizes and number of chromosomes, but also cloned DNA molecules can be hybridized to blots of the chromosomes, thereby mapping the corresponding genes to the chromosomes. Furthermore, the chromosomes embedded in agarose can be digested with restriction enzymes before and/or after electrophoresis, thereby permitting the construction of genetic maps (Kemp et al., 1990).

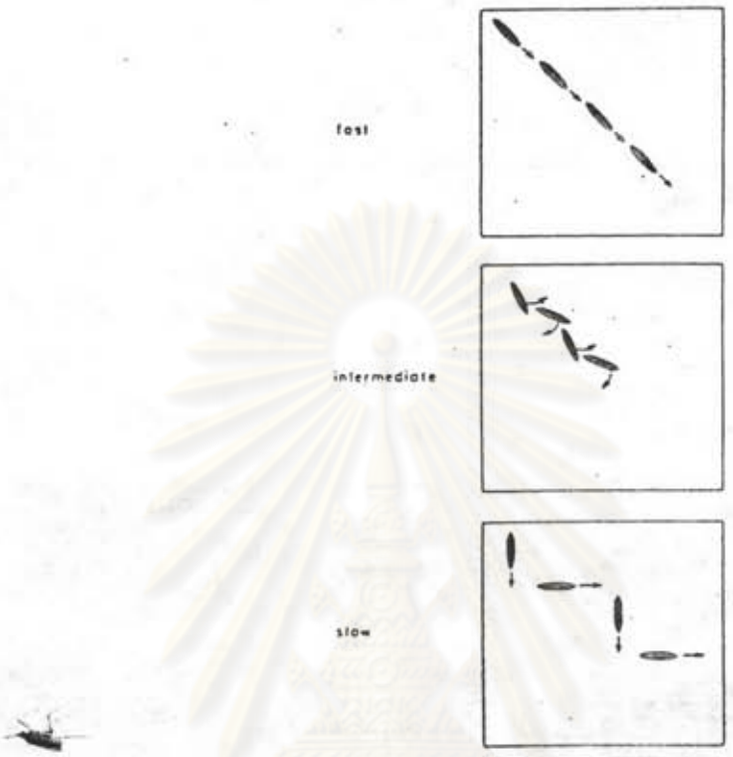


Figure 2 Schematic illustration of DNA migration in response to alternate pulsed perpendicular electric fields. In the top panel of pulse time is much slower than the DNA reorientation time; in the center panel the two times are comparable; in the bottom panel the pulse time is much faster than the reorientation time (Smith et al., 1987).

DNA fragments prior to initial pulsation.

Positions of fragments after start of initial pulse.

Positions of fragments at end of first pulse.

Positions of fragments after start of second pulse.

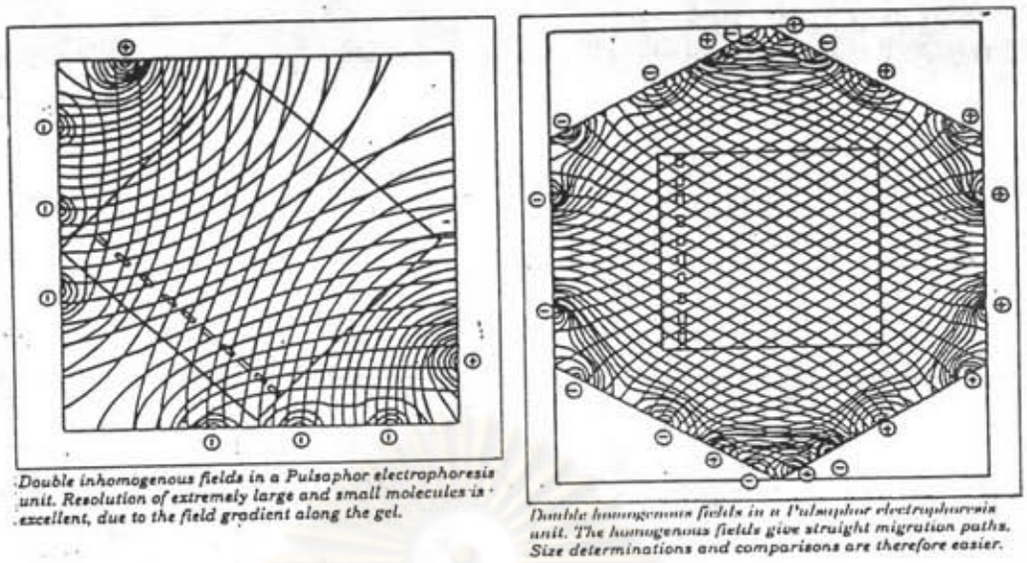
Positions of fragments at end of second pulse. Note that the separation time is achieved along a bisecting line between the angles generated by the two pulsed electric fields.

Figure 3 Proposed separation mechanism for PFGE.

The introduction of the contour-clamped homogeneous electric field (CHEF) apparatus of Chu et al.,(1986) allowed fractionation of the chromosomes in straight tracks (Peterson et al., 1988;Cowman and Lew, 1989; Sheppard et al.,1989; Tanaka et al., 1990), a very useful technical improvement of reorientation angle of the hexagonal electrode (figure 4 and 5).

4. Genetics by PFGE

Analysis of the chromosomes of P. falciparum and other species of Plasmodium has been hindered by the failure of chromosomes to condense into discrete entities during metaphase and the complexities of the life cycle. To circumvent these problems, a novel approach has been provided recently by the technique of pulsed field gradient gel electrophoresis (PFGE), allowing the fractionation of DNA molecules of 30 - 3,000 kilobases (kb) (Schwartz et al., 1983). This range encompassed the sizes of intact chromosomal DNA molecules from eukaryotes such as yeast (Schwartz and Cantor, 1984; Snell and Wilkin, 1986), trypanosomatids (Van der Ploeg, 1984a,b), Leishmania spp.(Spithill and Samaras, 1985),Giardia lambia (Adam et al., 1988) and Plasmodium spp., e.g. P. falciparum (Kemp et al., 1985; Van der Ploeg et al., 1985) P. vivax (Langsley et al.,1988), P. chabaudi (Sharkey et al., 1988; Cowman and Lew, 1989) , P. berghei (Ponzi et al., 1990), P.vinckei (Sheppard et al.,1989).



Double inhomogenous fields in a Pulsaphor electrophoresis unit. Resolution of extremely large and small molecules is excellent, due to the field gradient along the gel.

Double homogeneous fields in a Pulsaphor electrophoresis unit. The homogeneous fields give straight migration paths. Size determinations and comparisons are therefore easier.

Figure 4 Illustration of the electrode configurations used in the double inhomogeneous and double homogeneous model of PFGE.

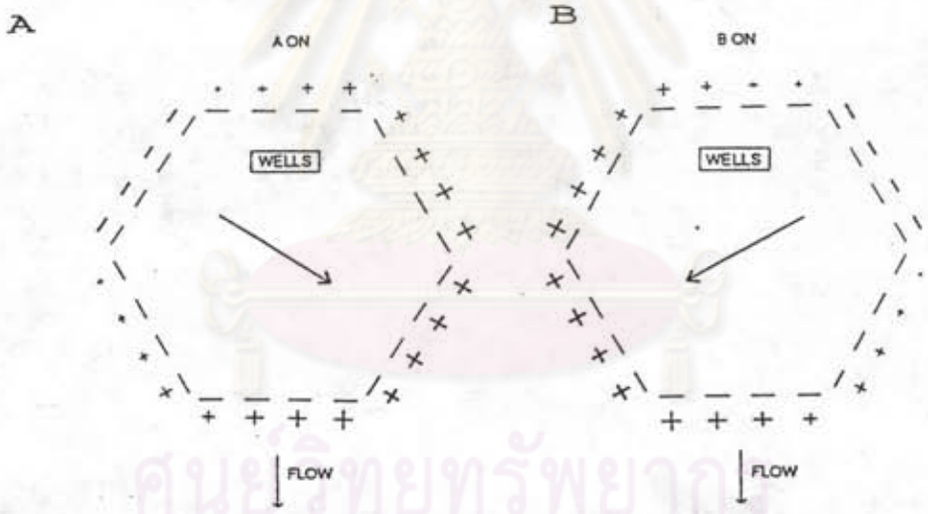


Figure 5 Voltage clamping by the CHEF-DR II system. (a)Relative electrode potentials and field vector when A channel of the electric field switcher is on. (b)Relative potentials and field vector when B channel is on. The average vector during the run is in the direction of the arrow marked flow.

By using PFGE, the earliest studies from cultures of independent isolates of P. falciparum separated chromosomes into at least 7 discrete size groups (Kemp et al., 1985; Van der Ploeg et al., 1985). This analysis also revealed surprising size polymorphisms among equivalent chromosomes of different isolates; in some cases, a difference amounting to ~20% of the length of chromosome. Subsequently , karyotype profile of P. chabaudi were demonstrated by Langsley et al. (1987). From this study, 11 chromosomes were revealed. Finally , the separation conditions of the chromosomal DNA were improved and the resolved number of chromosomes probably rose up to 14 (Kemp et al., 1987; Wellems et al., 1987) ranging in size from 800 to 3,500 kb.

This number of chromosomes was consistent with that of kinetochores in the mitotic spindles of a blood stage schizont visualized by electron microscopy (Prensier and Slomianny,1986). Thus it may now be assumed that both methods faithfully reflect the probable chromosome number. There was a clear evidence from genetic crosses of P.falciparum that the bloodstage parasite was haploid (Walliker et al.,1987). Studies on DNA synthesis in P. berghei using microfluorometry have provided additional evidence that sporozoites, ring forms, young trophozoites and mature microgametes possessed a similar quantity of DNA, assumed to be the haploid amount (Janse et al., 1986).

Fourteen chromosomes ranged from 600 to 3000 kilobases in size and were polymorphic: homologous chromosomes in independent isolates varied frequently and dramatically in size (Kemp et al., 1985; Van der Ploeg et al., 1985). These polymorphisms were observed in cloned lines of P. falciparum in culture and in isolates taken directly from infected individuals under conditions that precluded selection in vitro (Corcoran et al., 1986). Moreover, in a cloned line, the chromosomes were of identical size in rings, trophozoites and schizonts (Corcoran et al., 1986). Although the karyotype of a clone was relatively stable during culture and so provided a useful marker for different clones, some size variation occurred in vitro, most commonly from intrachromosomal events rather than interchromosomal translocations events (Kemp et al., 1990).

From the studies cited above it is clear that chromosomal polymorphisms can occur during mitotic growth of the parasite and that stability of a particular karyotype is both strain-dependent and subjected to environmental growth conditions. A prior study (Walliker et al., 1987) demonstrated that chromosomal polymorphisms could also arise at meiosis. In that study, the cross-fertilization in mosquito of 2 distinct clones differing in numerous characteristics of P. falcipaum resulted in the appearance of nonparental karyotypes when analysed after cloning of the progeny blood stage parasites.

The molecular karyotypes of three rodent malaria species,

P. chabaudi (Langsley et al.,1987; Sharkey et al., 1988) and P.berghei (Ponzi et al, 1990) as well as P. vinckei (Sheppard et al., 1989), have also been studied by means of PFGE. Each species appeared to have 14 chromosomes (Sheppard et al., 1989), ranging in size from approximately 730 to >2,000 kb. Many probes representing cloned P. falciparum antigens failed to hybridize to P. chabaudi DNA (Sheppard et al., 1989).

Chromosome size variation has been found in P. vivax (Langsley et al.,1988). Each of the 15 different isolates which were obtained directly from infected patients exhibited a different chromosome migration pattern. This hinted that a high level of polymorphism prevailed in wild populations of P. vivax and that PFGE can be used on a large scale for the epidemiological analysis of wild parasite populations.

Further mapping of the chromosomes was performed by digesting the chromosomes with restriction enzymes that cut the parasite DNA infrequently. These macrorestriction fragments could then be separated by PFGE, transferred to filters and hybridized with various probes. To clarify the mechanisms that underlied chromosome size polymorphisms, many chromosomes from several different P. falciparum isolates have been extensively mapped (Corcoran et al., 1988; Sinnis and Wellems, 1988; Foote et al., 1989; Wellems et al., 1987). The available evidence suggests that the major polymorphic regions in P. falciparum chromosomes were the subtelomeric regions (Corcoran

et al., 1988; Patarapotikul and Langsley, 1988). From observation of the restriction maps forchromosome1 and 2 of 6 cloned lines of P. falciparum , the subtelomeric zones were found to be polymorphic and to coincide with the location of a repetitive element (rep20). Additionally, it was shown that deletions of rep20 generated clones of the parasites that were bereft of rep20 on one or both ends of chromosomes 1 or 2 and larger deletions removed telomere-proximal genes as well (Corcoran et al., 1988). In several cases, size differences between homologous chromosomes of the order of 100 kilobases or more were shown to result in a concomitant loss of genes encoding antigens. P. falciparum cultured in vitro often lost the ability to produce knobs on the infected red cells. It is now clear that in many knoblessisolates the gene encoding knob-associated histidine-rich protein (KAHRP) , normally located on chromosome 2 , is completely (Corcoran et al., 1986) or partially (Pologe and Ravetch, 1986) deleted from the genome. The histidine-rich protein genes were also subjected to deletions, for instance, HRP-III or designated SHARP (Stahl et al., 1985) gene was present on the chromosome of one isolate but absent from that of the other because the gene was deleted from the terminus ofchromosome 13 (Wellems et al., 1987). Thus, size polymorphisms could be generated by deletions of repetitive DNA or of coding sequences.

Long-range restriction maps of chromosome 4 from 2

parent clones and 1 progeny clone have shown that, while the overall structure and organization of chromosome 4 from each clone were similar, large-scale variation occurred within a few hundred kilobase pairs of the chromosome ends ,and that crossover between 2 parent chromosomes explicated for an intermediate-size chromosome of the progeny which contained restriction sites characteristic of the left end of one parent and of the right end of the other (Sinnis and Wellems,1988). It was also evident that blocks of rep20 could vary in copy number; for example, two fragments bearing rep20 sequences on chromosome 2 of one parent were considerably shorter and hybridize to a much lesser extent than their counterparts in the progeny and the other parent. Hence, it was proposed that chromosome length polymorphisms were the consequence of homologous recombination between expandable subtelomeric repeat units (Corcoran et al., 1988).

Thereupon PFGE, which has proven to be a powerful method of karyotype analysis, has also been used to examine the relationship between Plasmodium chromosomes in different isolates. Drug resistance, an important genetic characteristic of some isolates of P. falciparum, may also be connected to some types of chromosomal variation, and this is the main theme of this study.

5. DHFR and pyrimethamine

Several drugs are available for the treatment of malaria. These can be classified into 4 groups :-

1) Antifolates (e.g. pyrimethamine, proguanil)

2) Sulphonamides (e.g. sulphadiazine, sulphadoxine)

3) Quinine, 4-amino-quinolines (e.g. chloroquine) and 4-quinoline methanols (e.g. mefloquine)

4) 8-amino-quinolines (e.g. primaquine)

All these drugs were primarily active against the blood forms of the parasite, 8-amino-quinolines being the only drugs fully effective against exo-erythrocytic stages, and normally used only for treatment of those forms.

Extensive use of the drugs has resulted in a selection of resistant parasites. The main problems of drug-resistance in P.falciparum concern resistance to pyrimethamine and chloroquine because these two drugs are the most widely used. The present interest focuses on pyrimethamine anti-malarial drugs.

Pyrimethamine (figure 6) has been freely available since 1952 as an effective antimalarial agent (Cook, 1986).It is a 4-amino analog of dihydrofolate and acts as a specific inhibitor of dihydrofolate reductase (DHFR- 5, 6, 7, 8- tetrahydrofolate : $NADP^+$ oxidoreductase [E.C. 1.5.1.3]) of malarial parasites. Plasmodium depends upon DHFR activity for dTMP production, and

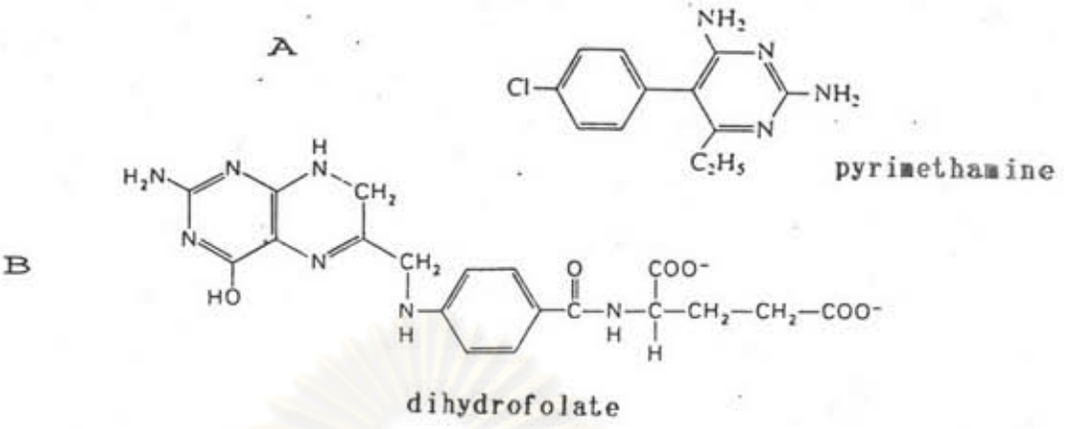


Figure 6 Structure of pyrimethamine compared with its natural substrate, dihydrofolate. (Cook, 1986 and Stryer, 1988.)

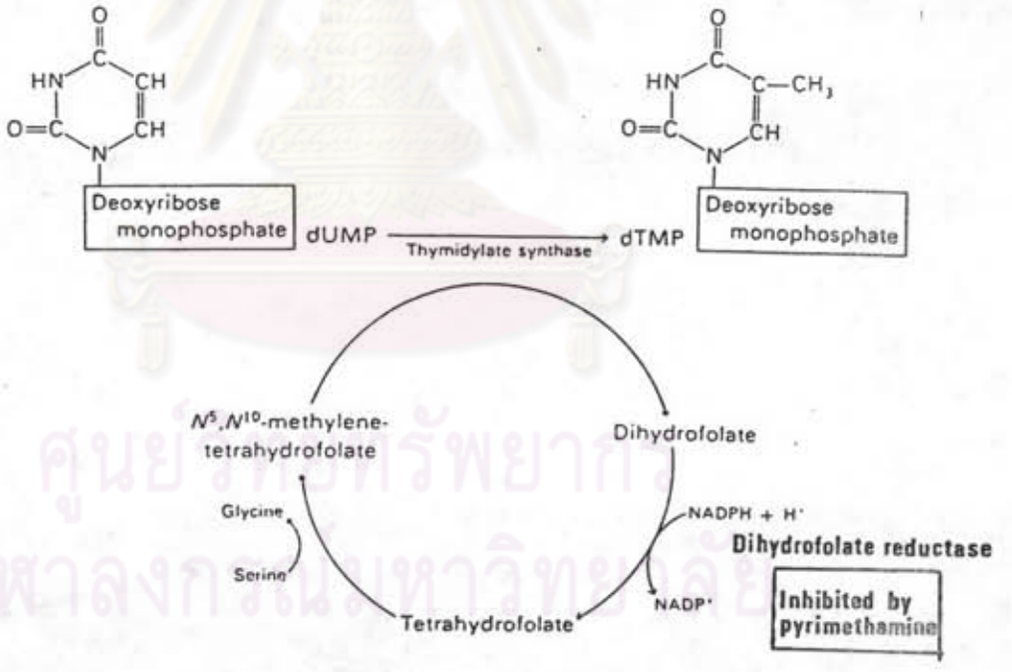


Figure 7 Site of action of pyrimethamine drug in folate metabolic pathway. (This illustration is modified from Stryer, 1988.)

hence DNA synthesis, and also transfer of 1-carbon units in other metabolic pathways (figure 7). The affinity of pyrimethamine for the parasite enzyme has been found to be 2,000-fold greater than for the host DHFR (Ferone et al., 1969; Ferone, 1970). Accordingly, this difference in sensitivity is the basis of the chemotherapeutic effectiveness of the drug in treatment of malaria infection (Sirawaraporn and Yuthavong, 1984).

An important feature of the DHFR activity in many protozoa, including P. falciparum, resided on a bifunctional peptide, associated with thymidylate synthetase (TS-5, 10-methylenetetrahydrofolate : dUMP C- methyltransferase [E.C. 2.1.1.45]) activity (Garrett et al., 1984).

Analysis of the DHFR-TS gene of P. falciparum indicated that the deduced bifunctional enzyme contained 608 amino acids (71,682 daltons). The coding region for DHFR-TS contains no intervening sequences and has a high A+T content (75%). The DHFR domain, in the amino-terminal portion of the protein, is joined by a 94-amino acid junction sequence to the TS domain in the carboxyl-terminal portion of the protein (figure 8). Both domains of P. falciparum are more homologous to eukaryotic than to prokaryotic forms of the enzymes (Bzik et al., 1987)

Numerous pyrimethamine-resistant (pyr^r) parasite mutants have now been isolated and studied. Investigations in P. berghei revealed an enhancement of DHFR activity and the

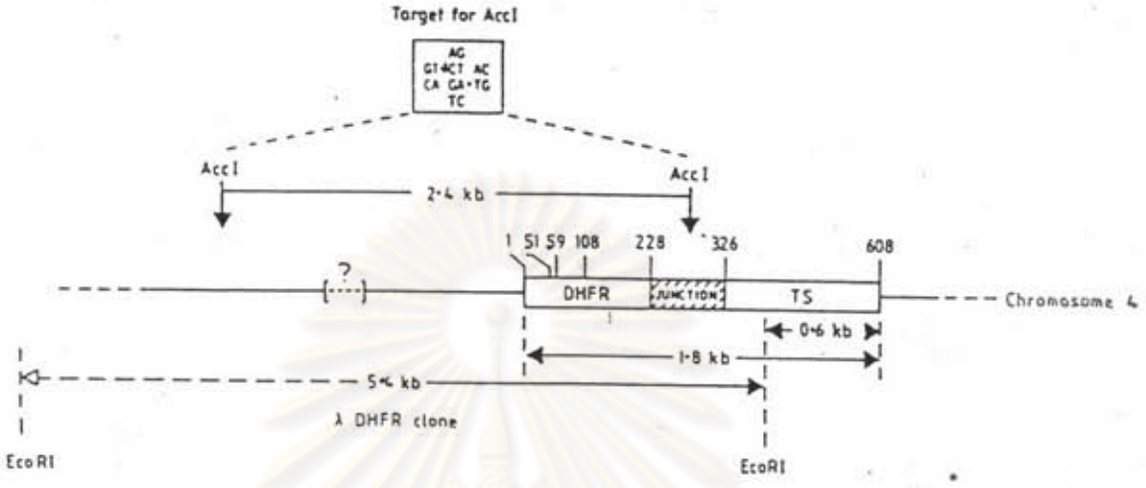


Figure 8 Organisation of the gene for DHFR-TS. Targets and sequence recognised by the restriction enzyme AccI in DNA are shown. Important amino acid positions are numbered on the gene. Positions 51, 59 and 108 are candidate sites for amino acid changes affecting pyrimethamine sensitivity (Peterson et al., 1989, Snewin et al., 1988). The origin of the EcoRI DHFR clone is described by Snewin et al. (1988). The position of a putative deletion responsible for AccI RFLP is indicated:[].(Chan et al., 1989).

mutations within the gene could account for the pyrimethamine resistance (Ferone, 1970; Diggens et al.,1970). In P. chabaudi, the mechanism of pyrimethamine resistance did not involve an increase in enzyme content, but was attributable to the changes in the properties of the DHFR enzyme which resulted in a large decrease in drug binding in pyr^r clones (Sirawaraporn and Yuthavong,1984).

Prior to the advent of PFGE , resistance to pyrimethamine in P. falciparum was reported to be ascribed to 2 mechanisms. First was an amplification of the DHFR-TS gene and second was the structural changes in the DHFR-TS enzyme which affecting the uptake of the drug.

Inselburg et al.(1987) have isolated a pyr^r strain of P. falciparum that demonstrated accumulation of the drug as efficiently as its pyr^s parent clone. Nonetheless, one pyr^r mutant clone exhibited a 5-10 fold higher uptake of pyrimethamine and overproduced the DHFR-TS protein than pyr^s clone, but the level of DHFR enzyme activity was equal. The genomic DNA of this pyr^r clone was shown to contain at least twice much DHFR-TS specific DNA than either another pyr^r or pyr^s clone. These results hinted that some of the DHFR-TS genetic material was associated with a gene duplication. However, in parasite lines derived from parasites which have acquired pyrimethamine resistance in the field, kinetics studies have yielded no evidence for DHFR overproduction in any of the pyr^r

strains (Walter, 1986; Chen et al, 1987). Instead, these studies in which an affinity to the drug was decreased suggested that a mechanism involved a structural alteration of DHFR. This was congruent with the results of Dieckmann and Jung (1986), showing that uptake of the drug was identical in both pyr^s and pyr^r strains of P. falciparum and no metabolite of pyrimethamine was found in either strain. DHFR from the pyr^r strain was 300 times less susceptible to inhibition by pyrimethamine than the enzyme from the pyr^s strain. They concluded that altered properties of plasmodial DHFR were the only ostensible mechanism responsible for pyrimethamine resistance in their study.

The arrival of PFGE with other recombinant DNA technology, for instance, DNA sequencing and polymerase chain reactions method, has facilitated an identification of those amino acids involved in pyrimethamine resistance.

Sequencing of the DHFR-TS gene from the pyr^s clone FCR3 (Bzik et al., 1987) and from a number of pyr^r clones have implicated the mechanism of pyrimethamine resistance associated with an accumulation of point mutations (Cowman et al.,1988; Peterson et al., 1988; Snewin et al., 1989; Tanaka et al., 1990).

Cowman et al. (1988) reported that the DHFR-TS gene was present as a single copy on chromosome 4 in all pyr^s and pyr^r isolates tested. Copy number analysis has confirmed the presence of a single DHFR-TS gene in K1(Snewin et al., 1989). Therefore, the molecular basis of pyrimethamine resistance in the parasites

tested was not amplification of the DHFR-TS gene. It was shown that the degree of resistance to pyrimethamine correlated with point mutations from Thr-108 / Ser-108 to Asn-108 in the gene encoding DHFR-TS of virtually all resistant isolates analysed so far (Peterson et al., 1988; Cowman et al, 1988; Snewin et al, 1989). Recently, DHFR sequences of 4 pyr^r mutants were demonstrated the presence of the point mutation at amino acid 223 from Phe to Ser (Tanaka et al., 1990).

In chloroquine resistance of P. falciparum studied by using PFGE (Foote and Kemp,1989),the multidrug resistance gene of P. falciparum (pfmdr) showed to be amplified in several resistant lines and pfmdr transcription levels were increased (Foote et al., 1989). While under the conditions of mefloquine pressure, it was found that generalized modifications of genomic structure in response to the growth environment might explain for chromosome size variations of mefloquine-resistant P. falciparum (Wellems et al., 1988).

This was not necessarily the case for other malaria parasites studied by PFGE. The DHFR gene in P. chabaudi was normally found on chromosome 7 (Sheppard et al., 1989). Nevertheless, the pyr^r parasites no longer contained the original chromosome 7,instead they had 2 smaller derivatives of chromosome 7, both carrying a copy of DHFR-TS gene. This chromosomal rearrangement and duplication of a portion of chromosome 7 provided the first example of a parasite increasing

its karyotype from 14 to 15 chromosomes (Cowman and Lew, 1989).

In PFGE study here, thus, chromosome 4 of the resistant parasites are anticipated to be different from those of the sensitive ones if the mechanism of pyrimethamine resistance involves DHFR gene amplification. Alternatively, if the size of chromosome 4 of both sensitive and resistant clones shows no variation, the mechanism responsible for such resistance might be due to point mutation or another causes.To confirm that the mechanism of pyrimethamine resistance is correlated to DHFR gene amplification, the copy number of DHFR gene in resistant clones should be more than the sensitive ones when studied by dot blot analysis.