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MINIREVIEWS

Antigenic Variation in *Plasmodium falciparum*: Gene Organization and Regulation of the *var* Multigene Family▽

Sue A. Kyes,[1] Susan M. Kraemer,[2]† and Joseph D. Smith[2,3]*

Molecular Parasitology Group, Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DS, United Kingdom[1]; Seattle Biomedical Research Institute, 307 Westlake Avenue North, Suite 500, Seattle, Washington 98109-5219[2]; and Department of Pathobiology, University of Washington, Seattle, Washington 98195[3]

Plasmodium falciparum imposes an enormous burden upon the developing world, with 300 to 500 million cases and 1 to 2 million deaths per year (94). Despite extensive research efforts, development of parasite drug resistance is a growing problem, and an effective vaccine is still lacking. Individuals living in areas of high *P. falciparum* transmission acquire protective immunity to severe malaria during early childhood after only a few symptomatic infections yet remain susceptible to uncomplicated disease and asymptomatic infection into adulthood (65). Thus, sterile immunity that prevents infection may never develop, but significant antidisease immunity is acquired relatively rapidly. While the protective targets of antidisease immunity are largely unknown, the parasite variant antigens exposed at the erythrocyte surface are considered strong candidates.

Key virulence factors and prime candidates for antidisease vaccines have been identified in a family of clonally variant surface antigens collectively termed *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), encoded by about 60 *var* genes per haploid genome (9, 40, 92, 97). *P. falciparum*-infected erythrocytes (IEs) bind host endothelium and other host cells, in turn sequestering infected cells away from the spleen, which would otherwise destroy them. Switching of *var* gene expression allows the parasite to modify the antigenic and functional properties of IEs, thereby evading immunity and affecting infection outcome.

How antidisease immunity could be achieved rapidly against variant surface antigens is a deep mystery. Unraveling the basis for this protection represents a promising direction for antidisease malaria vaccines. This review considers how *var* gene organization may shape the functional and antigenic properties of PfEMP1 variants and regulate their expression during infection.

* Corresponding author. Mailing address: Seattle Biomedical Research Institute, 307 Westlake Avenue North, Suite 500, Seattle, WA 98109-5219. Phone: (206) 256-7384. Fax: (206) 256-7229. E-mail: joe.smith@sbri.org.

† Present address: Malaria Research Institute, Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, Room E5638, 615 N. Wolfe St., Baltimore, MD 21205.

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CHROMOSOMAL ORGANIZATION OF *var GENES*

The *var* gene repertoires have been compared in three geographically diverse *P. falciparum* clones: 3D7, HB3, and IT4/25/5 (IT4), representing Africa, Central America, and Southeast Asia, respectively (52). Of the three, *var* repertoire coverage is most complete for the genome reference isolate, 3D7 (40). HB3 has been sequenced to 8× genome coverage (105), and IT4 *var* genes were identified by targeted gene cloning. Overall, 3D7 has 61 *var* genes, HB3 has 54 full and partially sequenced *var* genes (including six pseudogenes), and 48 full and partially sequenced *var* genes have been cloned from IT4.

Despite having distinct geographic origins, the general organization of *var* genes appears to be similar between isolates. In 3D7, approximately two-thirds of the 61 *var* genes are located at subtelomeric regions of the 14 chromosomes, with the remainder in chromosome central regions (40). Each chromosome end typically contains one, two, or three *var* genes, followed by a group of *rif*, *stevor*, and other multigene families. Many subtelomeric regions have two *var* genes arranged in tail-to-tail orientation relative to each other with one or more *rif* genes in between (Fig. 1A). Chromosome central *var* genes can appear singly or in groups that are nearly always tandem arrays (head to tail), containing from three to seven *var* genes in genomes sequenced to date. Superimposed on this general organization, the chromosomal location and transcription orientation of a *var* gene can be predicted from its 5′ noncoding region sequence (106). Based upon sequence similarity, the 5′ promoter regions can be defined into four major upstream (Ups) sequence groups, UpsA, UpsB, UpsC, and UpsE. The former UpsD is now grouped with UpsA (52). The functional significance of these different promoter types is unclear. However, in all three parasite genomes where the gene location has been mapped, UpsC *var* genes are chromosome central, UpsB *var* genes are either subtelomeric and transcribed away from the telomere or chromosome central in tandem arrays with other UpsB or UpsC *var* genes, and UpsA and UpsE-type *var* genes are subtelomeric but transcribed towards the telomere in the opposite direction to UpsB *var* genes (Fig. 1A). There is only one known exception, a UpsA *var* gene that is predicted to be in a central chromosome cluster in HB3 (52), but this may represent a sequence assembly artifact. This overall conservation of *var* gene organization based on 5′ noncoding sequences, in an organism where one-to-one pairings between true alleles might only occur in meiotic selfing, may reflect either recombinational

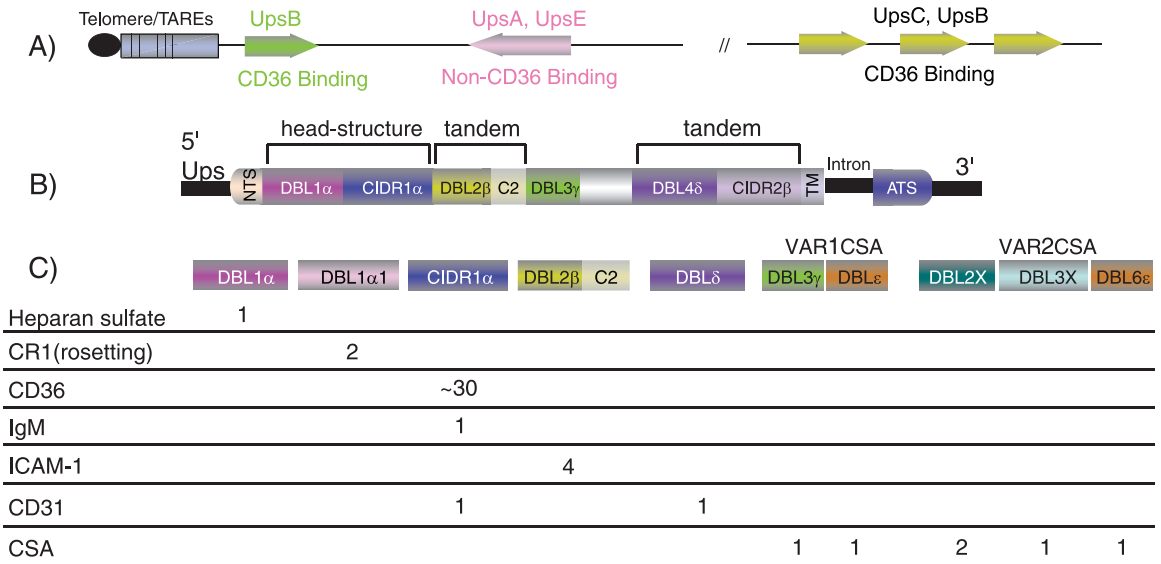

FIG. 1. Chromosomal organization of *var* genes. (A) *var* genes are classified according to upstream promoter type and are found at the ends of most of the 14 *P. falciparum* chromosomes and clustered in internal regions on chromosomes 4, 7, 8, and 12. Arrows indicate the direction of transcription. TAREs, telomere associated repeat elements. (B) The extracellular binding region of the PfEMP1 proteins consists of four domain types: NTS, DBL, CIDR, and C2. DBL and CIDR domains are classified according to sequence similarity. (C) Relatively few PfEMP1 adhesion traits have been mapped to specific domains. The numbers of sequences with mapped adhesion domains are indicated for each host receptor-domain interaction (16, 17, 39, 80, 84, 85, 95).

constraints and/or host selection pressures. Functional requirements for controlled *var* gene expression in each chromosomal location might also select for the conservation and particular arrangement of *var* promoter types.

var GENE DIVERSITY, RECOMBINATION HIERARCHIES, AND EVOLUTION

While recent studies have made great progress in investigating genetic diversity across the *P. falciparum* genome (45, 48, 71, 105), these studies have not been able to address the highly diverse and largely nonallelic *var* gene family. Thus, the mechanisms driving *var* gene diversity remain only partially understood. *Plasmodium* parasites are haploid during the vertebrate stage of infection and replicate asexually by mitotic division. Sexual (meiotic) recombination only occurs during the mosquito stage of the infection. Ectopic gene recombination among *var* genes occurs during meiosis (34, 98) and potentially during mitotic division, although the latter has not been definitively shown. Sequence comparisons and restriction fragment length polymorphism analysis of parasite crosses and population studies suggest that both small (~100 to 200 nucleotides) and larger recombination events contribute to *var* gene evolution (7, 13, 34, 52, 98, 100, 108). Also, recent studies have shown that higher rates of recombination events occur in the chromosomal regions near or surrounding *var* genes (70, 71), further confirming the role of recombination in generating *var* gene diversity.

P. falciparum telomeres form four to seven clusters of chromosome ends, called "bouquets" (34). It has been speculated that telomere end clustering may allow *var* genes with similar 5′-flanking sequence and gene orientation to preferentially line up and facilitate recombination within the different *var* groups (53, 59, 88). This may involve some members of the *rif* multigene family, because many *rif* genes are linked head to head with UpsA *var* genes, and the entire intergenic region containing both genes' promoters is highly conserved (59). Repertoire-wide comparisons of 3D7, HB3, and IT4 show that coding sequences of UpsA *var* genes have diverged from UpsB and UpsC *var* genes (52). While the extent to which subtelomeric group B and central group C *var* genes might be recombining is unknown, central *var* genes have been shown to be located within the nuclear periphery (78) and may be colocalizing with telomere bouquets (107). However, it is uncommon to find larger segments of gene similarities, greater than 500 bp, shared between subtelomeric and central *var* genes (52), suggesting that exchange between these groups may be infrequent. These sequence relationships support the hypothesis that recombination preferentially occurs within genes that have a common genome location and gene orientation and is also likely influenced by 5′-flanking region and gene coding similarity (Fig. 2). This recombination hierarchy may be shaping the *var* gene repertoire and influencing the evolution of the family.

The *var* gene repertoire also includes three unusual semi-conserved *var* genes (*var1CSA*, *var2CSA*, and type 3 *var*) that are found in most parasite isolates. Interestingly, these distinctive genes share little sequence identity with other members of the *var* gene family and may therefore primarily undergo self-self recombination (52). Remarkably, homologs of *var1CSA* and *var2CSA* are present in the chimpanzee malaria parasite *Plasmodium reichenowi* (100), although it is believed to have diverged from *P. falciparum* ~5 to 7 million years ago (29). Therefore, these isolate-transcendent genes have ancient origins and may be under special selection to be maintained in the parasite population.

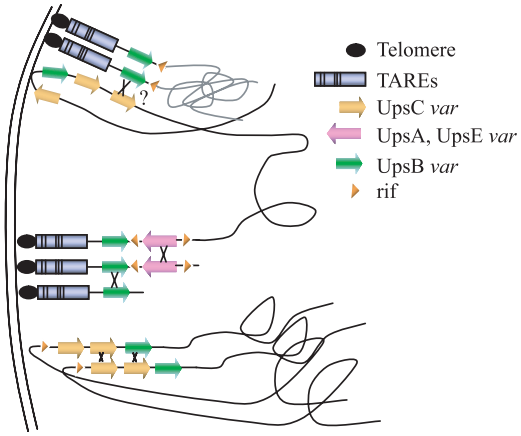

FIG. 2. Model of recombination among *var* genes. Clustering of telomeres and central *var* groups may facilitate ectopic recombination events between *var* genes with a similar Ups sequence, gene orientation, and chromosomal location. These events may occur during meiosis and possibly mitosis. The prevalence of recombination between central and subtelomeric *var* genes remains to be established (indicated by a question mark). UpsA *var* genes are highly distinct from other *var* groups, suggesting they mainly recombine among themselves. Only those *rif* genes immediately adjacent to *var* genes are indicated; *stevors* are generally not found directly adjacent to *var* genes. (Modified from reference 53 with permission of the publisher.)

It has been hypothesized that an original ancestral *var* gene was duplicated and diverged into three main *var* groups (A, B, and C) and subsequently into transitional groups (59). This interpretation is supported by analyses showing similar categories of *var* genes in all three parasite isolates (Fig. 3A). Interestingly, sequence comparisons of *var2CSA* alleles from a global collection of parasite isolates including *P. reichenowi* revealed high levels of gene mosaicism and extensive overlap of polymorphic segments (100). Thus, ancient polymorphic segments have been carried forward into existing parasite populations, possibly from a burst of polymorphism that occurred in the common progenitor to these isolates. Small stretches of shared polymorphism have also been observed between less-conserved *var* genes and potentially contribute to cross-reactive antibody responses (15, 108). These findings suggest that recombination of slowly mutating polymorphic segments has an important role in *var* gene diversification and that recombining genes may be more likely to share antigenic relationship. Furthermore, ancestral polymorphism may maintain antigenic similarities between otherwise diverse PfEMP1 variants even across global isolates.

Figure 4 shows a possible scenario of *var* gene evolution in which a gene duplication event occurred very early in the evolution of the gene family, followed by gene diversification and inversion into conserved orthologs, such as *var1CSA* and *var2CSA* and the *var* groups, before *P. falciparum* and *P. reichenowi* split. Future studies testing if similar patterns of *var* groupings exist in *P. reichenowi* as in *P. falciparum* will be useful in shedding light on the origins and evolution of the *var* gene family.

PfEMP1 PROTEIN STRUCTURE AND FUNCTION

PfEMP1 proteins are encoded in two exons, with exon 1 encoding the exposed extracellular portion (Fig. 1B) (97). This region is extremely variable in both sequence and length, although it consists of a few fundamental building blocks put together with some minimal rules (93). A short region of the N terminus (NTS) contains sequence features sufficient for transport beyond the parasitophorous vacuole that surrounds the intraerythrocytic parasite (42, 66). Other regions of the PfEMP1 protein may assist in final transport to the erythrocyte membrane (50). The remainder of the extracellular region consists of two main adhesion domains: Duffy binding like (DBL) and cysteine-rich interdomain regions (CIDR), which are classified according to sequence similarity.

The diverse exon 1 structures of PfEMP1 variants can be categorized by their domain combinations, typically ranging from two to seven DBL domains and one or two CIDR domains. Thirty-one different architectural types were described in the three sequenced parasite *var* repertoires (52), with certain tandem domain combinations consistently preserved (DBLα-CIDR1, DBLβ-c2, and DBLδ-CIDR) (Fig. 1). Most PfEMP1 variants have a semiconserved protein head structure consisting of NTS-DBLα-CIDR1 domains (40).

While seven protein architectural types are shared among the three isolates, most PfEMP1 proteins have overall amino acid identities of less than 50% in individual domains, even among proteins of the same architectural type (52). In addition, there is minimal overlap of DBLα tags in population surveys of parasite isolates (7, 13, 31, 100). The only exceptions are the three isolate-transcendent *vars*, which have identities greater than 75% over multiple domains (53). The vast antigenic diversity of PfEMP1 proteins in the parasite population may help explain why individuals are repeatedly susceptible to *P. falciparum* infections and never develop sterilizing immunity. Nevertheless, although the diversity of variant antigens is indeed large, hyperimmune human sera from distinct geographic regions are able to recognize IEs from East or West Africa (2), suggesting that some epitopes are globally related, possibly due to ancestral polymorphism and gene recombination.

Despite having distinct *var* repertoires, the three parasite genomes have approximately the same number of genes in each *var* group (Fig. 3A). However, the ratio of small to large PfEMP1 proteins differs between isolates, and there is an overrepresentation of distinct architectural types in the different genotypes (Fig. 3B). Consistent with the idea that *var* genes may be functionally and structurally diversifying under a gene recombination hierarchy, UpsA PfEMP1 variants tend to have larger, more complex domain architectures and encode a distinct protein head structure. In addition, the UpsA group lacks the type 1 PfEMP1 protein architecture, which is otherwise the most common PfEMP1 type in all three parasite genomes (Fig. 3B) (52).

Relatively few PfEMP1 adhesion traits have been mapped to specific domains or proteins (summarized in Fig. 1B). The best-characterized binding interaction is between the CIDR1 domain and the host receptor CD36 (8). Considered the primary receptor for IE binding to blood microvessels, the CIDR1 domains from UpsB and UpsC PfEMP1 proteins tend to bind CD36 (80). In contrast, the UpsA PfEMP1 proteins tend not to bind CD36, due either to primary sequence differences in CIDR1 in the UpsA-type-associated PfEMP1 (80) or complete lack of CIDR domains in type 3 *var* and *var2CSA* (40). Significantly, *var2CSA* is specifically upregulated in infected erythrocytes that have switched away

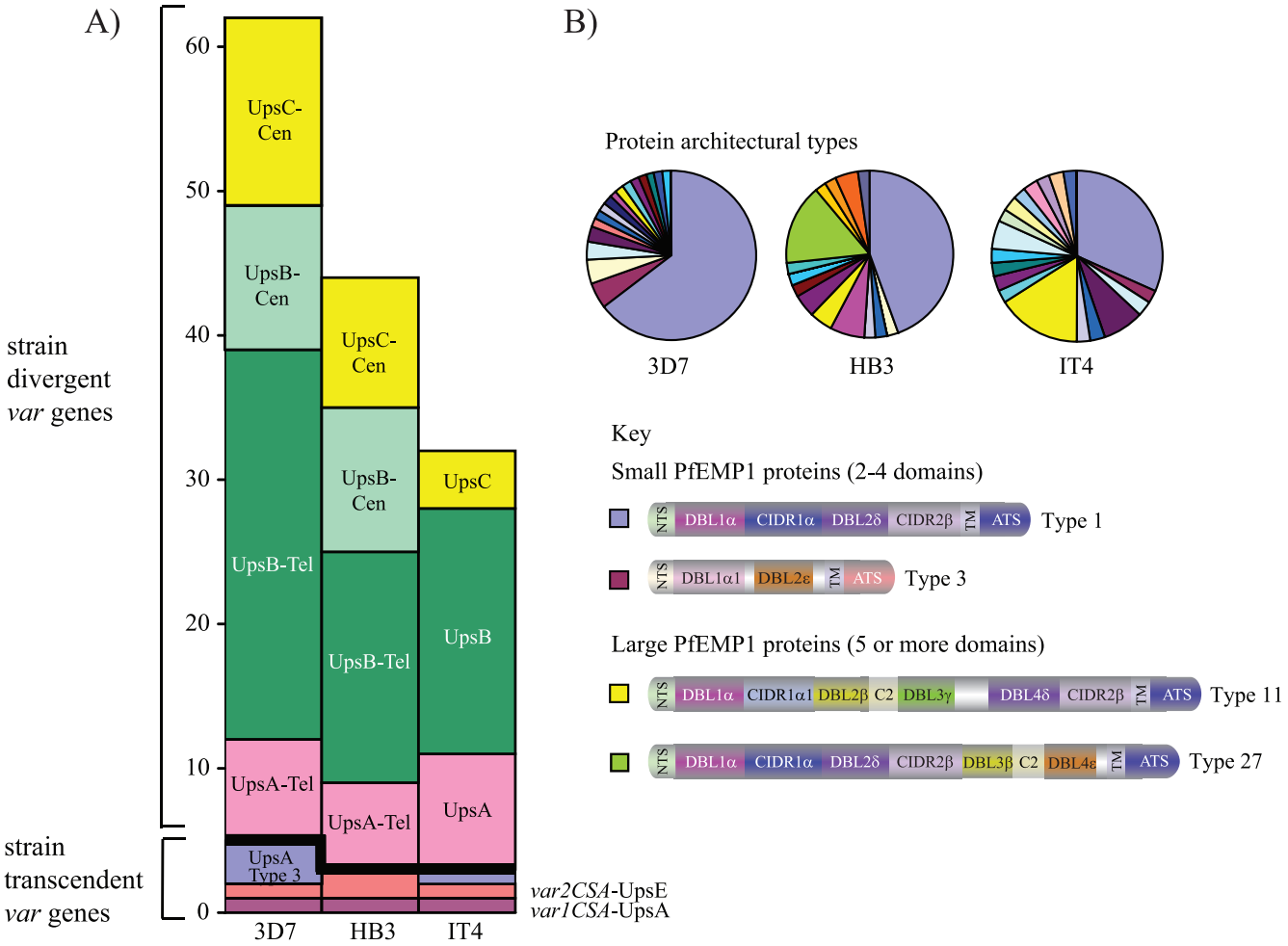

FIG. 3. The *var* gene repertoire in three geographically diverse parasite isolates. (A) Based upon the current *var* gene sets, there are approximately the same proportions of UpsA, UpsB, and UpsC *var* genes in the three parasite isolates. Central (Cen) and telomeric (Tel) chromosome locations are known for all 3D7 and most HB3 *var* genes, but few IT4 *var* genes have been mapped to chromosomal regions. While most *var* genes have a nonallelic relationship, the *var* repertoire contains three isolate-transcendent genes (*var1CSA*, *var2CSA*, and type 3 *var*). The type 3 *var* gene is lacking in HB3 but is present in all other parasite isolates that have been examined (100). (B) PfEMP1 proteins have been characterized into different protein architectural types on the basis of domain composition (40). The pie chart shows the distribution of different types in the three parasite genomes. At the bottom, type 1 and type 3 are small PfEMP1 architectural types with four or fewer extracellular domains. Interisolate comparisons show that type 1 is the most common PfEMP1 architectural type and that the ratio of small to large PfEMP1 proteins differs between isolates. Type 11 and type 27, denoted by yellow and green boxes, are examples of larger PfEMP1 proteins which are overrepresented in IT4 and HB3 isolates, respectively (see pie chart above).

from CD36 binding and sequester in the placenta (36, 86). There is considerable interest in understanding whether specific groups of PfEMP1 proteins may have different roles in binding and disease and how this may relate to *var* groups and recombination potential.

var GENE EXPRESSION DURING INFECTION AND DISEASE

Of the different malaria disease syndromes, the role of PfEMP1 proteins is best understood for pregnancy-associated malaria (PAM) (reviewed in references 38 and 81). During pregnancy, women who have previously developed malaria immunity become susceptible to IEs that bind low-sulfated chondroitin sulfate A (CSA) in the placenta (3, 36). After one or two pregnancies, women develop protection to the placental form of the disease. This protection is correlated with the development of antibodies that recognize placental parasites from different geographical regions (26, 37, 96), suggesting that the surface molecules expressed by placental infected erythrocytes may have unique and conserved features that may be utilized in the development of a pregnancy malaria vaccine.

One such "conserved" antigen and possible vaccine candidate has been identified, UpsE-associated *var2CSA*. VAR2CSA is conserved at approximately 70 to 80% amino acid identity across global isolates (53, 87, 100) and is transcriptionally upregulated in placental isolates and parasites selected to bind CSA (23, 24, 39, 87, 101). Disruption of *var2CSA* causes infected erythrocytes to lose their ability to bind CSA (25, 104). Furthermore, Salanti et al. showed that high levels of anti-VAR2CSA antibodies correlated with a lower risk of delivering low birth weight children (86).

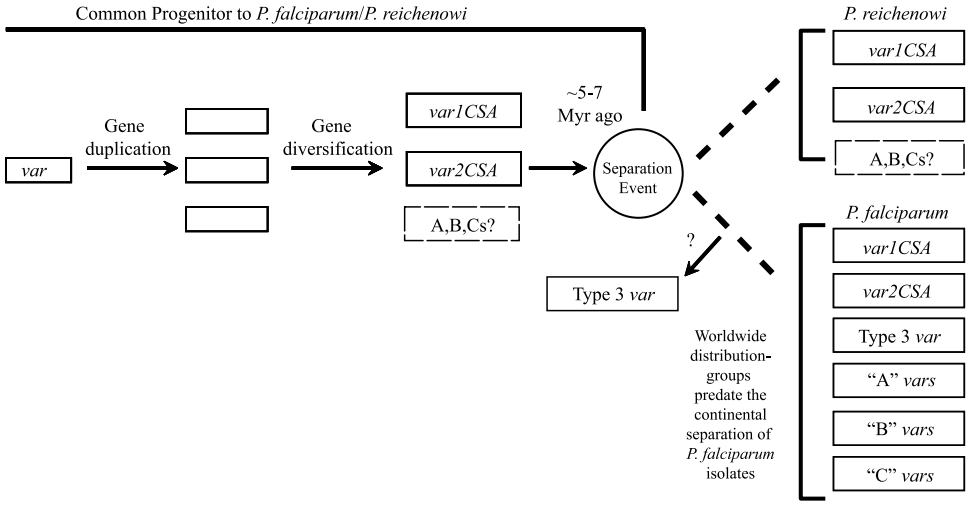

FIG. 4. Hypothetical model of *var* gene evolution. *var* genes are confined to human malaria (*P. falciparum*) and chimpanzee malaria (*P. reichenowi*) and appear to have evolved in the common progenitor to these organisms, presumably after acquiring the DBL adhesion domain from erythrocyte invasion ligands found in diverse *Plasmodium* species (1). An original ancestral *var* gene may have evolved by gene duplication, gene inversion (A and B *var* groups), and gene diversification into the present *var* groupings. The three main *var* groups (A, B, and C) are found in parasite isolates from Africa, Southeast Asia, and Central America, indicating this division predates the continental separation of *P. falciparum* isolates. The presence of A, B, and C *var* groups in *P. reichenowi* has not yet been established. The variant antigen repertoire also contains three unusual strain-transcendent *var* genes (*var1CSA*, *var2CSA*, and type 3 *var* genes). While *var1CSA* and *var2CSA* have ancient origins, a type 3 *var* gene has not been identified in *P. reichenowi*. However, there is only one partially sequenced *P. reichenowi* isolate, and it is possible the gene may have been deleted from the reference isolate.

Detailed serological comparisons have shown that the human immunoglobulin G response against PAM appears to be highly focused on polymorphic regions in VAR2CSA (6, 10, 20). Significantly, sequence analysis indicates there is extensive overlap of VAR2CSA polymorphism between globally diverse parasite isolates and that polymorphic loops assume a relatively limited diversity of basic types (11, 100). Whether PAM immunity is conferred by a repertoire of antibodies that collectively recognize polymorphic epitopes shared between different VAR2CSA alleles, or if low level antibody responses develop to highly conserved but "cryptic" epitopes, is the subject of investigation.

Similar to PAM disease, it is being investigated whether variant surface antigen types associated with other malaria disease syndromes may be antigenically restricted. Severe childhood malaria encompasses several disease syndromes (severe anemia, cerebral malaria, respiratory distress, and hypoglycemia) and has been linked to sequestration of infected erythrocytes to many tissues (63). Also, severe malaria has been associated with parasite phenotypes such as rosetting (binding of infected erythrocytes to uninfected erythrocytes), adhesion to brain microvasculature, and autoagglutination (clumping of infected erythrocytes bridged by platelets) (14, 73, 76, 79, 83, 102). To determine if specific PfEMP1 proteins are important to one or more of the severe malaria syndromes, researchers are analyzing *var* gene expression during infections, characterizing the antibody response to the infected erythrocyte surface, and investigating the binding properties of PfEMP1 proteins.

To date, only five studies representing a total of 88 patients with differing forms of severe malaria have been done to investigate the types of *var* gene(s) that are expressed during disease (13, 47, 49, 58, 82). Differences in epidemiology, severe disease characterization, and *var* classification make comparisons across studies difficult. In three studies, expressed DBLα sequence tags were classified by the number of cysteines encoded (49) and other features (13, 58). Genes with two cysteines in this region (2cys/DBLα1 type) are likely to represent UpsA *var* genes, or a small subgroup of UpsB *var* genes (58), whereas those with four cysteines are either UpsB or UpsC. So far, expression of 2cys/DBLα1/UpsA sequence variants correlates with rosetting phenotype (13, 58), with severe cerebral malaria in children (58) and noncerebral severe malaria in adults (49). Real-time PCR analysis determining the expression of *var* genes with the different promoter types showed a correlation between expression of both UpsA and UpsB *var* and severe malaria cases in Tanzanian children (82), but in Papua New Guinea only UpsB *var* expression correlated with severe disease (47). However, the latter study was in an area where ~79% of the population is deficient in complement receptor 1 (19), a major receptor for infected erythrocyte rosetting, and the rosetting phenotype does not associate with severe disease (5). Therefore, it is possible that human genetic polymorphisms in cytoadhesion receptors may influence PfEMP1 disease associations. Although strict correlations between any group of *var* genes and disease status have not been found, expression of both UpsA *var* and UpsB *var* has been associated with severe disease at different geographic sites.

Although many different parasite genotypes are potentially virulent (22, 65, 69), severe malaria syndromes are a relatively infrequent complication of malaria infections (estimated to be about 1% of infections) (65), suggesting that isolate-transcendent disease immunity can develop rapidly. Given the limited overlap of variant antigen repertoires, with only three known isolate-transcendent variants, it can be questioned whether PfEMP1 immunity is an important factor in the rapid development of disease immunity. Although the variant antigen

repertoire is vast, serological evidence suggests that the variant surface antigens associated with disease may be antigenically restricted (12, 13, 46, 74). By analogy to PAM immunity, we hypothesize that a "patchwork" epitope relationship between disease-promoting PfEMP1 variants could contribute to antigenic cross-reactivity, especially *var* genes in the same gene recombination group, because these are more likely to share regions of overlapping polymorphism. Therefore the concept of a *var* gene recombination hierarchy has implications for investigating antidisease immunity and vaccine development. To test this hypothesis it will be necessary to develop a better understanding of the sequence and antigenic relationship of PfEMP1 disease variants (13, 46, 62, 64).

CONTROL OF *var* GENE TRANSCRIPTION AND SWITCHING

Information about *var* gene regulation may provide further insight into the factors influencing gene expression during infection or lead to drug interventions that could prevent PfEMP1 expression. *var* genes are expressed in a mutually exclusive manner, with only one PfEMP1 protein expressed by any individual parasite (60, 89). The relatively limited *var* repertoire in any haploid, clonal parasite population requires that antigenic variation take place at a rate sufficient to maintain infection in face of the acquired host immune response, but not so rapidly that the repertoire is exhausted before the parasite can be transmitted via mosquito bite to another host. Whether the parasite is able to prolong blood-stage infections by *var* mutation/recombination is unknown, as is the relative contribution of mitotic (vertebrate host) versus meiotic (mosquito host) processes to *var* diversification. Antigenic variation for PfEMP1 comprises both memory for expression of the same variant in most progeny parasites and switching to expression of new types at variable rates (43). The paradigm for study of antigenic variation in protozoan parasites is the variant surface glycoprotein (*vsg*) of *Trypanosoma brucei* (99). With few parallels between the two parasites' antigenic variation systems, and the big picture describing *var* gene regulation still uncertain, at least a few details are now clear.

***var* gene expression is stage specific and regulated by in situ epigenetic mechanisms.** While *P. falciparum* parasites have a 48-h blood-stage asexual life cycle, full-length *var* transcripts are only detected up to about 20 h postinvasion (56). The *var* protein, PfEMP1, is synthesized early and relies on a slow trafficking pathway to arrive at the IE surface (54). One variant, *var1CSA*, is unusual in that it is transcribed constitutively in all parasites, even as a truncated pseudogene (PFE1640w in 3D7), and thus falls outside the controls of mutually exclusive gene expression (57). Whether the protein encoded by *var1CSA* is exposed at the red cell surface is unknown, and its role in antigenic variation is currently unclear.

Unlike *T. brucei vsg*, *P. falciparum var* genes do not undergo rearrangement or gene conversion into an active expression site in order to be switched on, although there are examples of *var* gene deletion accompanied by adjacent *var* gene activation (21, 44). Specific *var* gene expression is activated in situ (89, 92) and is controlled at the level of transcription initiation soon after the parasite invades a red blood cell (55, 90).

Critical features of *var* gene exclusive expression have been identified through transfection of parasites with plasmids containing both (i) a *var* 5′ promoter driving a drug resistance marker, and (ii) a second promoter in the form of either the *var* intron or a heterologous promoter driving a second drug resistance marker (28, 107). By selecting for expression from the *var* 5′ promoter/drug resistance marker, all endogenous *var* gene expression is shut down. The *var* 5′ promoter-driven drug resistance marker appears to "occupy" the single *var* expression site, filling the place normally occupied by an endogenous 5′ *var* promoter. Depending on the interpretation, the presence of the *var* 5′ promoter is either sufficient for the drug resistance marker to be counted as a *var* gene by the exclusive expression mechanism (107) or the interaction between the 5′ *var* promoter and a second promoter (*var* intron or heterologous promoter) is required (28; reviewed in reference 32). Either way, the strict counting mechanism relies not on negative feedback from the *var* protein product itself but on noncoding information. Although the stage-specific expression of *var* intron transcripts suggested that they may have a potential role in *var* silencing, it has not been possible to establish a relationship between these transcripts and the active or silent state of a *var* locus (55, 56, 77, 97).

Location within the nucleus: role in control of expression. All *var* genes, regardless of chromosomal location or transcription status, appear to physically reside at the nuclear periphery (27, 67, 78, 107), usually in telomeric clusters (34). By using parasite populations known to be highly homogeneous for expression of a single *var* gene, it has been shown that the active *var* gene moves away from the "silent" cluster to another position at the nuclear periphery (78). However, from studies of parasites transfected with various episomally maintained telomere-homing constructs or drug resistance genes under control of a *var*-specific promoter (27, 67, 107), it appears that active *var* promoters can associate in the bouquet structures.

Whatever the explanation for these differences, the idea that an actively transcribed *var* locus undergoes nuclear repositioning to an active transcription location is reminiscent of the *T. brucei* expression site body (72), the extranucleolar site of *vsg* transcription. Although it is well-established that the polycistronic *vsg*-containing mRNA is transcribed by RNA polymerase I, it has recently been shown that *var* genes are transcribed by RNA polymerase II (55, 90), like all other protein-coding genes that have been investigated in *P. falciparum* (68). Moreover, any active *var* gene would usually be linked to at least one silent *var* gene within several kilobase pairs (15 single *var* genes versus 46 *var* genes in groups in the 3D7 genome). Therefore, mechanisms must exist to prevent transcriptional upregulation of nearby *var* loci following nuclear repositioning. The physical movement of a *var* gene to a transcription-permissive region of the nuclear periphery cannot universally explain mutually exclusive regulation: further layers of control must operate.

Chromatin structure. Histone modifications can influence gene expression by altering DNA accessibility or recruiting other nonhistone proteins (51). Chromatin structure may hold some clues to *var* gene regulation, as histone hypoacetylation correlates with *var* gene silencing (35). The *P. falciparum* homologue of the histone deacetylase SIR2 (PfSIR2) protein associates with silent *var* 5′ promoter types UpsE and UpsB, but not UpsC (35), consistent with a telomere-silencing association for this protein. Knock-out of the Pf*Sir2* gene results in

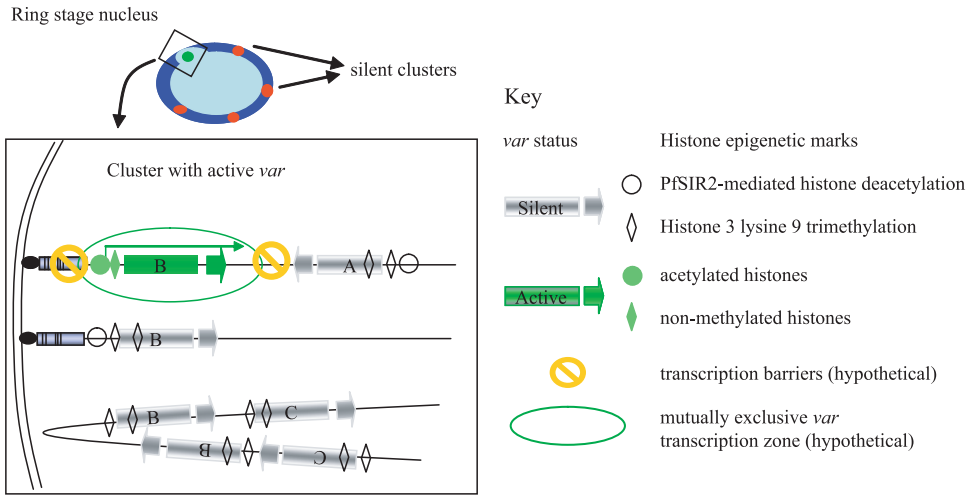

FIG. 5. Location and transcription of *var* genes. Transcription of a single *var* gene promoter begins almost immediately after parasite invasion of erythrocytes (ring stage) and continues until the parasite matures to late ring/early trophozoite stage (~16 to 20 h postinvasion). The top diagram shows a hypothetical ring-stage parasite nucleus. Most subtelomeric/central *var* clusters are silent (red dots) and are predicted to be in regions of electron-dense heterochromatin in the nuclear periphery. The active *var* locus (green dot) repositions to a region of less electron density. PfSIR2, a histone deacetylase, has been found associated with silent UpsB and UpsA promoters and may affect the packing of the chromatin, as in the yeast model organism. Memory of the *var* gene transcribed in ring parasite stages must be transferred to most or all of the progeny, with only a small rate of switching at the next cycle of intraerythrocytic development. It has been hypothesized that histone 3 lysine 9 trimethylation, which is increased at silent *var* loci, may be a memory mark.

activation of only certain subtelomeric *var* genes, some UpsA and the UpsE-type *var* genes (27), which suggests that the UpsB-type *var* genes are subject to a further layer of silencing. Recent studies in yeast and *T. brucei* have shown links between the factors involved in telomere gene silencing (including SIR2) and DNA repair (4, 61, 75, 103). These connections raise the possibility that factors involved in regulating the transcription of *var* genes may also facilitate recombination between family members.

The modified histone H3K9me3 (H3 trimethylated at lysine 9) appears to be an epigenetic marker for both subtelomeric and central *var* gene silencing (18). The inheritance of this unmethylated pattern in progeny parasites could provide a marker to trace switching events and provides yet another clue leading to the gene-specific control mechanisms.

Rate of gene switching: "slow" or "fast." Observation of parasite *var* gene switching in vitro has shown that different *var* genes switch on and off at different rates, resulting in an apparent overall hierarchy that remains to be defined. Switch rates are intrinsic to each *var* gene sequence and presumably to its associated noncoding sequence. Although Horrocks and colleagues found no correlation between switch rates and 5′ *var* promoter type (43), the study was done in the IT4 isolate, for which there was limited sequence information at that time. Transgenic parasites that have switched all *var* genes off due to occupation of the single *var* expression site by a drug resistance marker are an exciting new tool for studying switch rate phenomena (28, 107). With this approach it has been shown that *var* genes have different intrinsic switch rates that correlate with chromosomal location and that *var* genes located in central chromosomal regions had extremely stable expression patterns but that subtelomerically located *var* genes readily switched to alternative *var* loci (33).

Expression of PfEMP1 proteins at other developmental stages of the parasite life cycle. While the function of PfEMP1 proteins is best understood during the asexual cycle in erythrocytes, *var* gene expression is not limited to this stage of parasite development. PfEMP1 proteins are also expressed by early stage gametocyte-infected erythrocytes (41, 91), the transmissible form of the parasite that is infective to mosquitoes. PfEMP1 tryptic fragments were also identified in mosquito salivary gland sporozoites (30). Therefore, PfEMP1 proteins may have multistage functions, including a role in cytoadhesion and transmission of gametocyte-infected erythrocytes and potentially other stages.

In summary, mutually exclusive expression of *var* genes appears to involve multiple layers of control, potentially including nuclear repositioning, histone modifications, and interaction between the *var* 5′ promoter and *var* intron promoter (Fig. 5). These and other as-yet-unidentified processes may be involved in packaging of chromatin into functional domains with defined boundaries, the regulation of which may be the key to control of *var* gene expression. Additional mechanisms are required to explain switching of *var* gene expression in progeny cells and stage specificity. The relative influence of various control mechanisms may be different for specific *var* groups. Thus, the conserved organization of *var* genes within the parasite genome may be, to some extent, maintained by requirements for tightly regulated *var* gene expression.

CONCLUSIONS

PfEMP1 proteins have a central role in *P. falciparum* immune evasion and pathogenesis. While considered strong candidates for disease intervention, we are only beginning to glimpse the complex relationship between PfEMP1 expression,

disease, and immune acquisition. The best-understood disease syndrome is pregnancy malaria, which has been associated with a uniquely conserved PfEMP1 variant, termed VAR2CSA (81, 86). Whether other severe malaria syndromes are similarly restricted in PfEMP1 variants or antigenic diversity of protective epitopes is the subject of intense investigation. Recent progress in understanding *var* gene organization suggests that gene recombination hierarchies may have an important role in shaping the antigenic and functional properties of PfEMP1 variants. Further information on both the acquisition of immunity to PfEMP1 variants and PfEMP1 virulence phenotypes may lead to potential disease interventions.

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