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Variability and Exclusion in Host and Parasite: Epigenetic Regulation of Ig and var Expression

Shira Fraenkel and Yehudit Bergman

The immune system generates highly diverse AgRs of different specificities from a pool of designated genomic loci, each containing large arrays of genes. Ultimately, each B or T cell expresses a receptor of a single type on its surface. Immune evasion by the malaria parasite Plasmodium falciparum is mediated by the mutually exclusive expression of a single member of the var family of genes, which encodes variant surface Ags. In this review, we discuss the similarities as well as the unique characteristics of the epigenetic mechanisms involved in the establishment of mutually exclusive expression in the immune and parasite systems. The Journal of Immunology, 2006, 177: 5767–5774.

The immune system is made up of a large spectrum of individual B and T cells, each of which express one specific AgR molecule. The DNA sequences that confer Ag specificity to the BCR and the TCR are assembled at seven different loci/clusters (three for the BCR: Ig H chain, κ, or λ L chains; four for the TCR: α, β, γ, and δ). Thus, each B or T cell has initially to choose one locus/cluster for recombination. In each cluster, recombination occurs between V (variable), J (joining), and, in some cases, D (diversity) region gene segments. Thus, once a cluster is chosen, the cell must select one of the V, J, and D gene segments for rearrangement. Rearrangement at each AgR locus is strictly regulated with respect to developmental timing (e.g., IgH before IgL) and lineage specificity (e.g., V_{H1} to DJ_{H1} rearrangement in B but not in T cells). Since every cell has two alleles for each of the seven receptor loci, the ultimate choice of one receptor type (Ig or TCR) per cell involves an extra process of selection of one allele, termed allelic exclusion. Allelic exclusion is controlled at the V(D)J rearrangement level by initially restricting recombination to only one allele in each cell. The above-described choices seem to be achieved through monoallelic epigenetic changes (1–4).

Epigenetics is also involved in selecting single members of gene families for expression in several other biological systems.

In this review, we will analyze the role of epigenetics in choosing one member of the var family of genes that encodes the malaria parasite Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) (5). During the proliferation phase of the parasite infection, expression of PfEMP1 switches from one var gene to another, giving rise to parasite antigenic variation and thus facilitating host immune evasion (6). The var switching process is regulated at the level of transcription and follows the rule that only one gene is expressed at a time in a single parasite (6). Similar to the Ig and TCR gene clusters, the var genes in P. falciparum genome also appear in several clusters. In parallel to the series of molecular decisions in developing B and T cells, a parasite cell would initially select a certain gene cluster, after which selection of a single var gene would have to take place. Unlike B and T lineage cells, allelic selection is irrelevant to the process since P. falciparum carries only a haploid genome in its human host.

The molecular mechanisms that control mutually exclusive expression are not completely understood in any eukaryotic system. In this review, we will discuss the transcriptional and epigenetic mechanisms used by the immune system to generate a repertoire of Abs, as well as those used by P. falciparum to evade attacks by host Abs. Expectantly, insight could be gained by comparing the unifying features of these single-gene expressing systems and by identifying the unique characteristics displayed by each.

The Ig system

Ordered Ig rearrangements during B cell differentiation. Both the unrearranged H and L chain loci (Fig. 1) are silenced in non-B lymphoid cells, as well as in early B cell precursors. In these cell types, the IgH and IgL chain loci are maintained in an extended conformation at the nuclear periphery, which is regarded as a repressive environment (7).

All genes are packaged in a basic nucleosome-repeat structure, but alterations in histone-tail modifications can greatly influence both local and higher-order chromatin confirmation

References:
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3. Abbreviations used in this paper: PfEMP1, Plasmodium falciparum erythrocyte membrane protein 1; TARE, telomere-associated repeat element.

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Repressive modifications such as histone H3 lysine 9 methylation (H3K9) can prevent histone acetylation (9) and can recruit chromodomain-containing factors such as HP1 that are probably involved in stabilizing a silenced state (9). The Ig loci are hypoacetylated and H3K9 methylated in non-B lymphoid progenitor cells and in hemopoietic cells outside the B lineage (reviewed in Ref. 3). Indeed, a recent study suggests that H3K9 methylation inhibits V(D)J recombination when directed to the promoter of an artificial, stably integrated recombination substrate (10).

One of the main mechanisms for mediating gene repression is DNA methylation (11), which is established at the time of embryo implantation by de novo methylation of all DNA except for CpG islands, and this state is preserved through all cell divisions. Consequently, immune receptor loci are initially configured in a repressed methylated state, and they remain this way until they are about to rearrange during B cell development.

**Ig rearrangement in pro-B cells.** During pro-B cell development, only the H chain genes undergo rearrangement (Fig. 2). These cells are characterized by relocation of the IgH alleles to central nuclear positions (7), changes in histone modification (12–16), antisense transcription along the entire VH gene cluster (17), and long-range contraction of the IgH locus (18, 19), which ultimately results in VH-DJH recombination. Two different silencing processes are involved in directing an ordered monoallelic IgH rearrangement. One regulates the sequence of the rearrangement in the IgH locus, i.e., DJH rearrangement.

![FIGURE 1.](http://www.jimmunol.org/) Genomic organization of the mouse IgH and Igκ chains and the *P. falciparum* var family of genes. Distances are not drawn to scale. A, IgH and Igκ loci. The IgH locus consists of ~150 functional VH gene segments, 12–13 DH gene segments, 4 JH gene segments, and a few constant region genes starting with the 5′ Cα exon. The entire locus spans ~3 Mb and resides near the mouse chromosome 12 telomere. The Igκ locus consists of ~100 functional Vκ gene segments, 5 Jκ gene segments, of which 4 are functional, and a single constant region gene. The entire locus spans ~3 Mb and resides in the mouse chromosome 6. B, The var gene family. The *P. falciparum* genome contains ~60 var genes found in clusters at either subtelomeric or chromosome central regions. The chromosome end structure is conserved, comprising TAREs 1–6. The subtelomeric var genes are the most telomere–proximal genes located 3′ to TARE6 (Rep20). Also shown is the conserved structure of the var genes, with a long 5′ exon followed by a short 3′ exon.

![FIGURE 2.](http://www.jimmunol.org/) IgH and Igκ configurations in B cell development. At the pro-B cell stage, both H chain alleles undergo D-to-J gene recombination, whereas the Igκ alleles are in their germline configuration. Following transition to the pre-B cell stage, one of the IgH alleles has recombined, and the productive rearrangement results in the IgH-protein pairing with the surrogate light chains λ5 and VpreB, which, together with other subunits, form the pre-BCR complex. The cell surface expression of the pre-BCR leads to epigenetic changes occurring at the Igκ locus that culminate in Igκ rearrangement on one of the two κ alleles, after which, transition to the immature B cell stage takes place. A productive κ rearrangement enables surface expression of the BCR. See text for discussion.
before VH-to-DJH, and the other ensures that the second rearrangement step occurs on one allele only.

Initially, the D_{1H}-C_{µ} region becomes hyperacetylated while the V_{1H} gene segments remain hypoacetylated (20). This apparently accounts for D_{1H} to J_{1H} recombination occurring first during B cell development. To activate V_{1H} genes for rearrangement, the region requires the removal of repressive methylating marks such as methylation of histone H3 on lysine 9. Evidently, Pax5, a transcription factor required for B cell commitment, is necessary and sufficient for the removal of H3K9 methylation in the V_{1H} locus (21). Moreover, V_{1H} genes have to become packaged with nucleosomes enriched with histone H3 methylated on lysine 4 and lysine 27 (for a subset of V_{1H}), as well as enriched with acetylated histones. Both methylation of H3K27 and acetylation of H3 and H4 are dependent on IL-7R signaling (reviewed in Ref. 22). Furthermore, histone acetylation was specifically attributed to the IL-7 responsive transcription factor STAT5 functioning as a signal-dependent coactivator (23).

Regulation of VH-to-DJH rearrangement varies between D_{1H}-proximal and D_{1H}-distal region, with the proximal V_{1H} being a preferable target for rearrangement and a more promiscuous one (12, 13, 15, 19, 20, 24, 25). Following DJ rearrangement, genic and intergenic antisense transcripts are generated throughout the IgH V region on both alleles (17). These transcripts appear to be long, extending across several genes, and are rapidly lost from both alleles following VDJ recombination. It still has not been determined whether these antisense transcripts participate in opening up a large chromatin domain for possible rearrangement, or vice versa, they participate in RNA-dependent silencing.

There is little mechanistic data for allelic epigenetic differences that will help in discriminating between the two IgH alleles. One very interesting process that occurs in pro-B cells is the long-range contraction or compaction of the IgH locus, which draws the VH region near the IgH DJ cluster and thus facilitates the V-to-DJ recombination (18, 19). Interestingly, one of the studies reported that these loops occur monoallelically and thus could account for choosing one allele to undergo V-to-DJ recombination (26).

As for the κ chain gene, both alleles become centrally located and disassociated from heterochromatic regions (7), produce germline transcripts (27), and are packaged with acetylated histones (14), even though the DNA is still methylated (1), and thus refractory to the recombination machinery that targets the H chain locus.

**Ig rearrangement in pre-B cells.** Successful rearrangement of the IgH locus leads to cell surface expression of the µ protein as part of the pre-BCR, which functions as an important checkpoint to signal proliferative expansion of populations of large pre-B cells to induce subsequent differentiation to small-pre-B cells, in which the κ chain gene rearranges (Fig. 2). In small pre-B cells, RAG proteins are re-expressed and thus silencing mechanisms must be involved in restricting IgH rearrangement. Inhibition of VH-to-DJH recombination involves several mechanisms, including the decontraction of both IgH loci, reduced accessibility and lower levels of histone acetylation at V_{1H} segments (mostly of distal V_{1H}), and allelic recruitment to repressive centromeric domains (reviewed in Ref. 4). Based on the observation that germline V_{1H}558 gene segments can revert to a hypoacetylated state in the absence of replication, active histone deacetylation probably occurs. Reduction in IL-7-R signaling at the pre-B stage most likely underlies histone deacetylation and centromeric recruitment as treatment of B cells with IL-7 interferes with centromeric recruitment of the IgH allele, while simultaneously inducing histone acetylation of the distal V_{1H} genes (12). Thus, several mechanisms may preclude the DJ-rearanged allele from further rearranging following the re-expression of the RAG proteins in small pre-B cells.

Igκ recombination that specifically initiates in pre-B cells is regulated through targeted changes in chromatin accessibility (28, 29). For example, recombination signal sequences in the Jκ region are only subject to cleavage in the nuclei of pre-B cells (30). To achieve this level of accessibility, the κ locus seems to undergo a programmed process that releases multiple layers of repression in a stepwise manner. The Igκ gene becomes preferentially packaged into an active chromatin structure characterized by histone acetylation and methylation of histone H3K4 (13, 14, 31). Furthermore, the locus undergoes DNA looping and locus contraction, which most probably facilitates VJ recombination as well (18).

It is pertinent to ask how the Igκ allele choice is dictated in pre-B cells. There are several epigenetic molecular mechanisms that enforce this phenomenon, resulting in allelic exclusion (Fig. 3). It seems that allelic exclusion in pre-B cells is mediated by a predetermined “instructive” mechanism that begins early in development when the Igκ alleles first become asynchronously replicated (32). Once established, this differential state is then maintained in a clonal manner in somatic cells, but only during pre-B cell development are the consequences of this marking established. It seems that the asynchronous replication directs one of the κ alleles to heterochromatin, while causing the other to become packaged with highly acetylated histones. Experimental data indicate that the late replicating allele is almost always (80%) repositioned to heterochromatin, whereas the early replicating allele remains detached from heterochromatin and is preferentially DNA-demethylated. Relocalization to heterochromatin involves association with the heterochromatin protein HP1 (14) and Ikaros, which forms multimers that colocalize with foci of pericentromeric heterochromatin in pre-B cells (33, 34). It is thought that in this way it may be involved in the transcriptional inactivation of specific genes in the B lymphoid lineage (35).

Interestingly, the aforementioned changes in histone modifications occur preferentially on the nonheterochromatic allele, but it is still not clear whether heterochromatinization occurs before or as a consequence of these changes. It seems that the Igκ gene remains fully methylated almost throughout pre-B cell development (14). However, the active chromatin structure and most probably Igκ nuclear repositioning are involved in directing the demethylation machinery to one allele in each cell, shortly after the proper demethylation trans-acting factors become available during the transition to B cells (36). Thus, by late stages of pre-B cell development, the two κ alleles adopt completely different chromatin structures, and in keeping with these data, germline Igκ expression, which is biallelic in pre-B cells (27), actually becomes monoallelic at about this stage of differentiation (37). This monoallelic germline transcription could be due to a limiting concentration of a transcription factor that binds preferentially to the nonheterochromatinized allele, thus reinforcing the previously instructed epigenetic marks.
Several trans-acting factors can influence the epigenetic status of the locus, as well as actively participate in facilitating the recombination process itself. Pax5 is known to perform such dual functions. It was recently shown that in addition to its ability to induce proper chromosomal configuration and chromatin structure, it is able to facilitate RAG-mediated recombination through direct binding to individual VH coding regions and to the RAG1-RAG2 complex (38). Specific cis-regulatory elements involved in demethylation or histone acetylation have been identified (reviewed in Refs. 39 and 40). Recently, a novel cis-acting element that negatively regulates rearrangement in the IgH/H9260 locus has been identified (41). The element, termed Sis, specifies the targeting of IgH/H9260 in pre-B and B cells to centromeric heterochromatin, associates with Ikaros, and thus probably participates in IgH allelic exclusion.

Editing in B cells. Immature B cells are screened for autoreactivity in the bone marrow environment so that the mature B cell population is generally devoid of cells reactive with self-Ags. These cells can undergo receptor editing, which involves secondary Ig rearrangement(s) that generate a new AgR with an innocuous specificity. This process uses the same recombination machinery that mediates primary V(D)J joining and occurs mainly at the Igκ locus through Vκ-to-Jκ secondary rearrangement. In addition, receptor editing also occurs, at a low frequency, at the IgH locus through Vκ-to-Jκ rearrangement. Thus, epigenetic silencing of the Ig loci must be reversed to allow for RAG-mediated recombination. Indeed, when B cells are treated with IL-7, centromeric recruitment is prevented and histone hyperacetylation of VH genes is induced (12, 18). This mechanism enables reversal of the initial choice and exemplifies the built-in plasticity of this system.

The var system
As stated above, the variant Ags in *P. falciparum* are the PfEMP1 family, which is encoded by the var family of genes. Each genome contains ~60 members of this family, interspersed in groups at subtelomeric regions and in several groups at chromosome central loci (42) (Fig. 1). The overall structure of *P. falciparum* chromosome ends is conserved, with blocks of repeated DNA defined as telomere-associated repeat elements (TAREs/Rep20) found internal to the telomere. Subtelomeric var genes are the most telomere-proximal genes and are located downstream of TARE6/Rep20 (5). The repetitive element
Reprom20 appears to be associated with var gene control since truncated chromosomes, in which the entire subtelomeric region including Rep20 is deleted, do not silence adjacent genes (43). Also of interest for the analysis is the typical var gene organization. All var genes share a two-exon structure, with the 5’ exon encoding the variable extracellular segment of the PfEMP1 protein and the 3’ exon encoding the cytoplasmic tail. The var intron present between the two exons has a highly conserved structure and is implicated in var gene regulation (see below).

Cis-regulatory elements that control var expression

Var expression has been shown to be controlled at the level of transcriptional initiation, and DNA rearrangement, which is crucial for Ig expression, is not involved (5, 6). As for cis-regulatory elements, var promoters were shown to play a cardinal role in regulating mutually exclusive gene expression. Var genes are flanked by one of several upstream sequences, upsA, upsB, upsC, and upsE, where upsA- and upsB-type promoters are used by the subtelomeric var genes, the upsC-type sequences are used by the chromosome-central var genes, and the upsE uniquely controls the var2CSA gene (5). Different mechanisms responsible for regulating var promoters in internal as opposed to subtelomeric regions were found (44, 45).

It is not clear whether the var intron participates in regulating var mutually exclusive expression (46, 47). Yet, the intron sequences apparently do harbor an insulator activity that antagonizes spreading of chromatin activation into neighboring loci (48). This agrees with previous results, suggesting that the upsC-promoter-intron interaction has a boundary function to protect var genes in chromosome-central clusters from activation through nearby euchromatic regions and neighboring var genes (49).

Promoters from active or silenced var genes are capable of driving expression of episome-based reporter genes, suggesting that all var promoters are competent for transcription at one time or another and that the majority of them are silenced by epigenetic factors. Importantly, it was shown that a single active var promoter is sufficient to silence endogenous var genes and to ensure mutually exclusive expression of a single var gene (48, 50). Together, it is reasonable to suggest that interplay between the promoter and intronic cis-regulatory elements generates a stably inherited silenced chromatin environment.

Epigenetic regulation of var expression

How is a single var gene elected and kept active while the rest are maintained in a silenced state? It has been demonstrated that epigenetic regulation plays an important role in setting up this process. In the reported experimental system, a transgene inserted in the subtelomeric region of a P. falciparum chromosome can be reversibly silenced independent of the promoter sequences driving its expression. Sites in the promoter that are sensitive to micrococcal nuclease digestion in the active state are protected against digestion when the promoter is silenced, indicating that chromatin packaging at the repressed transgene locus is more compact than at the actively transcribing promoter (51).

Differential subnuclear localization is probably critical to var gene regulation. Both subtelomeric and internal var genes were found to be localized to the nuclear periphery (52). Fluorescence in situ hybridization analysis demonstrated that a reporter gene occupied different positions in the perinucleus, depending on its state of transcriptional activation, and that two different selectable markers had significantly higher chances of colocalization when both were actively transcribed (51). Thus, the data indicate that transcription at the periphery of the nucleus may be confined to privileged zones and movements of var loci within the periphery could either enable or disable their access to the spatially restricted transcriptional machinery. One possible model to explain var mutually exclusive expression is the existence of a unique perinuclear compartment that is responsible for transcription of a single var locus. It is tempting to suggest that this compartment contains a cis-regulatory element such as an enhancer localized to the privileged zone, which is able to trans enhance transcription from a single promoter. Switching in var transcription would then occur by competition of silenced var promoters for occupancy of this compartment.

Several chromatin modifications which lead to the silencing of the var genes have been discovered (45). One of these is heterochromatinization involving the P. falciparum homologue of the yeast Silent Information Regulator 2 protein (PfSir2). PfSir2 colocalizes with telomeric clusters, whereas it is absent from the electron-sparse nuclear interior, and from central var clusters. Moreover, it was shown that PfSir2 propagates from telomers > 50 kb into the chromosome coding region. Rep20 sequences and subtelomeric var promoters are bound by PfSir2. In contrast, no PfSir2 was associated with two expressed blood-stage genes, which are located ~100 kb from the end of the telomer (51).

Reversible covalent modifications of histones were shown to play a role in var gene transcriptional regulation as well. It was demonstrated that the promoter of an active var gene was packaged with hyperacetylated histones H3 and H4, while the histones associated with the silenced var genes were hypoacetylated (45). Furthermore, an inverse correlation between the presence of PfSir and enrichment of nucleosomes with acetylated histones was observed. Since the yeast Sir2 is known to be a histone deacetylase, it was reasonably suggested that PfSir2 deacetylates histones packaging the nontranscribed var genes. This is probably followed by recruitment of additional silencing factors and the establishment of a heterochromatin-like environment preventing transcriptional activation. Indeed, disruption of PfSir2 leads to increased transcription of members of the var gene family located in the subtelomeric regions, a result that is consistent with the association of PfSir2 to the telomeric regions of P. falciparum chromosomes (45).

Previous studies described an AT-rich region inside the intron of var genes which harbors a promoter activity (49, 53). These sterile promoters apparently give rise to noncoding RNA that may take part in repressing var expression, in a manner similar to Xist RNA-dependent X chromosome inactivation. Yet, a recent study did not find a role for antisense RNA in the transcriptional regulation of var genes (54). In addition, it is possible that competition between the 5’ and the intronic var promoters for either limiting amounts of transcription factors, or entrance into a few privileged perinuclear location(s), could serve as an auxiliary epigenetic repressing mechanism, contributing to the expression of one var gene of 60 possible candidates.

The Ig loci and the var family

This review describes two essentially different systems that share a common mode of gene regulation in which mutually exclusive
expression takes place (Fig. 3 and Table I). In both systems, the cell has to choose one cluster of genes for either recombination (Ig) or transcription (var), and from each cluster one gene segment has to be further selected. Choosing one var gene of 60 for expression is reminiscent of choosing 1 of the 150 or 100 V_h or V_l segments, respectively, for rearrangement. A related common feature is that gene usage is not completely random. For example, certain families of V_h were found to be preferentially expressed in neonatal B cells (55) and, accordingly, switching to some var genes occurs more frequently than to others (56).

Notably, both the Ig and the var systems are quite plastic in nature. The parasite cells of P. falciparum keep changing their choice of which PfEMP1 to express, making their decision rather temporary. Yet, editing at the immature B cell stage (57), as well as back-differentiation of mature B cells accompanied by novel rearrangements (58), suggests a high plasticity of the immune system as well.

Both Ig allelic exclusion and var mutual exclusion can be explained by a stochastic mechanism. At the Ig loci, several lines of evidence suggest that the process is likely to comply with a stochastic decision that instructs later choices that take place in committed B lineage cells (14, 31). According to this model, one of the two alleles is selected for recombination at an early developmental stage, a choice that is manifested through asynchronous DNA replication (31). Subsequently, numerous known (described above) and possibly unknown epigenetic mechanisms are involved in maintaining the allelic asymmetry before rearrangement in B cells. The ever-changing var gene choice is likely to follow the stochastic model, which assumes that most genes are equal candidates for each choice.

Both processes involve activation of the otherwise silenced target gene, which becomes distinguished from the silenced counter allele or member genes. The Ig and the var genes differentially associate with euchromatin and with heterochromatin according to their state of activation. For both systems, studies have shown that silenced genes are present in a more compact packaging than their activated counterparts, and in the two systems, histone H3 and H4 hyperacetylation characterizes activity, whereas hypoacetylation is linked with a state of inactivity (13, 20, 45). One note of caution for both systems should be considered. It is not clear whether hyperacetylation of one var or one Ig in a cell is the cause or the result of choosing them. It could be the case that histone acetylases are recruited to a specific target either randomly or by a previous instructive mark. However, it is equally probable that histone acetylation occurs as a consequence of repositioning to a privileged active nuclear zone.

Furthermore, once a selection is made, each system uses distinct mechanisms to disable subsequent selections from being made. In large pre-B cells, for instance, RAG1/2 expression is down-regulated, the V_H region becomes hypoacetylated, and the locus decontracts (4, 18). In parallel, at subtelomeric locations, a var gene that is no longer expressed becomes occupied by PfSir2, which participates in heterochromatin assembly and probably harbors a histone-deacetylase activity of its own, ensuring that a stably silenced state is maintained (45). However, while termination of the rearrangement process depends on negative feedback generated by signaling of pre- and B cell membrane receptors, the mutually exclusive manner of expression at the var gene family relies entirely on noncoding elements of the P. falciparum genome. Moreover, while DNA-demethylation precedes Igk rearrangement and is essential for the rearrangement to take place (1, 2, 14), methylation patterns are largely indistinguishable between active and inactive var genes, although subtle differences may still exist (6). This is in agreement with the highly reversible nature of the var decision, which precludes long-term modifications such as DNA demethylation.

Numerous studies indicate that expression in both systems is directed by cis-acting sequences that play both positive and negative roles. Another common feature of the mutually exclusive expression involves timely transcription of sterile transcripts. At the Ig loci, noncoding RNA is transcribed from both DNA strands, giving rise to germline transcripts and to antisense RNA (17, 27). This sterile transcription is restricted to a time window just before rearrangement takes place (17, 28). In the var system, the assumed noncoding transcripts are generated from the so-called intronic promoters. In both systems, the physiological role of these transcripts is not known. They could simply be byproducts of a permissive chromatin configuration or could play a more active role in directing mutually exclusive expression (52).

Finally, and most intriguingly, gene choice in both systems operates through regulated movements of the target genes within the nucleus. Change in the position of the Ig loci is known to precede rearrangement events. At the pro-B cell stage the H and κ chain loci dissociate from the nuclear periphery, which is regarded as a repressive environment, to become centrally located (7). Furthermore, during pre-B cell differentiation, one Igκ allele is recruited to heterochromatin while the other remains centrally located and away from heterochromatin, apparently making it a better target for the recombination machinery (14). Activation of a single var gene also involves its movement in the nucleus, and more specifically, the activated gene occupies privileged zones at the nuclear periphery, which are transcription permissive. Colocalization of active markers in the perinucleus is in agreement with the limiting transcription zone hypothesis, which states that competition over these specialized zones governs P. falciparum mutual exclusive expression.

### Conclusions

We have examined, in some detail, the mechanisms used in two biologically remote systems to achieve a similar goal, namely the expression of one single copy of a gene in any given cell. In the vertebrate immune system, Ig expression from one single allele is used to allow for the generation of an infinitely diverse yet
highly specific array of active Ig genes. In the cells of P. falciparum, only one cell surface var gene is expressed at a time to avoid detection and targeting by the immune system’s ever evolving Ag specificity. In both systems, mutually exclusive expression is apparently achieved by differential chromatin modifications and unequal nuclear localization. In the future, privileged nuclear zones should be studied more at the molecular level to determine whether they harbor a cis-regulatory master element that enables transcription and rearrangement. In addition, a new understanding of interchromosomal pairing, as well as involvement of noncoding RNA, has added a new dimension to the understanding of other mutually exclusive choices (59, 60). It will be of great interest to discover whether these themes also apply to Ig and var allelic exclusion.

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5774  BRIEF REVIEWS: EPIGENETIC REGULATION OF Ig AND var EXPRESSION

Plasmodium falciparum var genes are regulated by two regions with separate promoters, one upstream of the coding region and a second within the intron. *J. Biol. Chem.* 278: 34125–34132.


