estimated rates of change were 10^{-6} and 10^{-10} per cell per generation. The mutation rate for mammalian somatic cells is in the range 10^{-6} to 10^{-8} per cell per generation and for bacterial cells 10^{-6} to 10^{-7} per locus per replication cycle (*14*). The calculated rates of change from K^+ to K^- and from K^- to K^+ may reflect mutational events. However, because of the difficulty in calculating the rates of change for *P. falciparum* grown in vitro and the paucity of genetic information on this organism (*5*), these numbers should be interpreted with reservation.

The mechanism by which *P. falciparum* changes its K^+ phenotype is speculative. However, because the K^+ strain derived from the K^- clone produced a protein of different molecular weight than that produced by the original K^+ clone, a genetic rearrangement may have occurred in the K^+ phenotypic revertant. Indeed, genetic rearrangement has already been implicated in controlling the expression of S-antigens in *P. falciparum* (*15*) and surface proteins of *Trypanosoma brucei* (*16*). However, the observed change in molecular weight of the knob-associated protein could be explained by other genetic mechanisms, such as suppression or phenotypic reversion of a point mutation or changes in protein expression or stability.

Other investigators have suggested that the surfaces of *P. falciparum*–infected cells can change antigenically and functionally and that such changes are mediated by the spleen in infected monkeys (*17, 18*). Our results show that the K^+ phenotype can change in vitro independent of immunological selection and that this phenotypic change is reversible. These results suggest that *P. falciparum* has the potential to change considerably both in vitro and under immunological stress in vivo. By mediating surface changes in the cell that it infects, the parasite may elude the immunological response of an individual vaccinated against malaria. This potential for change should be seriously considered in developing a synthetic vaccine for malaria.

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A Gradient of Sequence Divergence in the Human Adult α-Globin Duplication Units

Abstract. *The nucleotide sequences of the two 5′-homology blocks of human α-globin gene duplication units were determined. The sequence difference between the two blocks is essentially zero in the 5′ portions, and increases gradually toward the 3′ ends until it reaches a value of 18 percent. This gradient of sequence divergence is similar to the distribution of the frequencies of gene conversion along several loci in* Ascobolus *and yeast. Hot spots for initiation of gene correction processes appear to exist near the 5′ ends of the human α-globin duplication units. The data provide the physical evidence for polar gene correction process in a mammalian genome.*

Sequence homology among members of a eukaryotic multigene family may be maintained by two genetic recombination processes: gene conversion and homologous but unequal crossing-over (*1*). The duplicated human adult α-globin genes, α2 and α1, provide an excellent system for exploring the molecular mechanism of these processes in mammalian cells. These loci are contained within tandemly arranged, highly homologous duplication units (*2, 3*). Each unit spans approximately 4 kilobases (kb) of DNA. Despite the age of the duplication event [before the time of primate divergence (*3*)], three long blocks of sequence homology (X, Y, and Z in Fig. 1), separated by three nonhomologous sequences (I, II, and III in Fig. 1), have been preserved (*2, 4, 5*). As discussed earlier (*4, 5*), this mosaic structure suggests that gene correction between the two duplication units, by gene conversion or unequal crossing-over, or both, acts on segments of DNA. To gain further insight into this segmental correction process, we have completely sequenced DNA from the two X homology blocks which are located in the 5′ regions of the duplication units.

The map in Fig. 1A shows the general organization of the tandem duplication containing the adult α2 and α1 globin genes. The nucleotide sequences of all the α-like globin genes and their immediate flanking regions have been studied (*5–7*). Much of the intergenic DNA sequence of this gene cluster has also been determined recently (*4, 5, 8*). In the regions of the duplication units, the nucleotide sequences of the homology blocks Y and Z, the nonhomology blocks I, II, and III, and a small portion of the X blocks have been determined. From these data, Hess *et al.* (*4*) and Michelson and Orkin (*5*) have proposed the following evolutionary scenario for the generation of the present-day human α-globin gene duplication units: (i) insertion of an ancestral α-globin gene; (ii) insertion of an Alu family repeat in the 5′-flanking region of the ancestral α-globin gene; (iii) tandem duplication of a 4-kb DNA fragment containing the ancestral α-globin gene and the Alu family repeat; (iv) concerted evolution of the duplicated DNA segments by gene conversion or unequal crossing-over (or both); (v) interruption of gene correction by nonhomologous DNA insertion/deletions; and (vi) segmental sequence correction.

The major portions of the X block sequences were not determined in the above-mentioned studies. These data are of both evolutionary and clinical importance, because a complete picture of the sequence organization would provide essential information about the DNA recombination processes occurring between the two duplication units. These processes are responsible for both gene correction and the α-globin gene deletion

in individuals with α-thalassemia 2 (*9*).

The strategy of nucleotide sequencing of the X blocks is described in Fig. 1B. The two sequences are aligned in Fig. 2A for comparison. There are 54 base differences over the entire lengths of the X blocks, which are 1440 and 1338 base pairs (bp), respectively. These differences include 36 transitions, 16 transversions, and two deletion/insertions. The guanine (G) and cytosine (C) contents of different regions within the X blocks vary between 32 and 66 percent. There is no obvious relationship between the GC contents and the sequence divergence.

Although the overall sequence divergence between the two blocks is only 4 percent, the distribution of these differences is not random. As shown in the nucleotide sequences of Fig. 2A and in the histogram of Fig. 2B, there are only three base differences in the first 800 bp of DNA. The divergence then increases gradually toward the 3′ ends of the two X blocks until it reaches a value of approximately 18 percent. This gradient of sequence divergence of the X blocks is not found in the homology blocks Y and Z. In the two Y blocks (165 bp and 191 bp, respectively), where the total divergence is 14 percent, there is no obvious gradient of divergence (*4*). In the Z blocks, there are 26 base differences (1.5 percent divergence) in addition to the 7-bp insertion/deletions within the second intron (*5*). Nineteen of these differences are located in the 3′ untranslated regions. Two are in the second introns. The clustering of base differences in the 3′ untranslated regions was attributed to the inhibition of branch migration by the 7-bp insertion in the second intron of α1 gene. Gene correction can be affected by nonhomologous sequences to different extents for different genes in different species (*4, 5*).

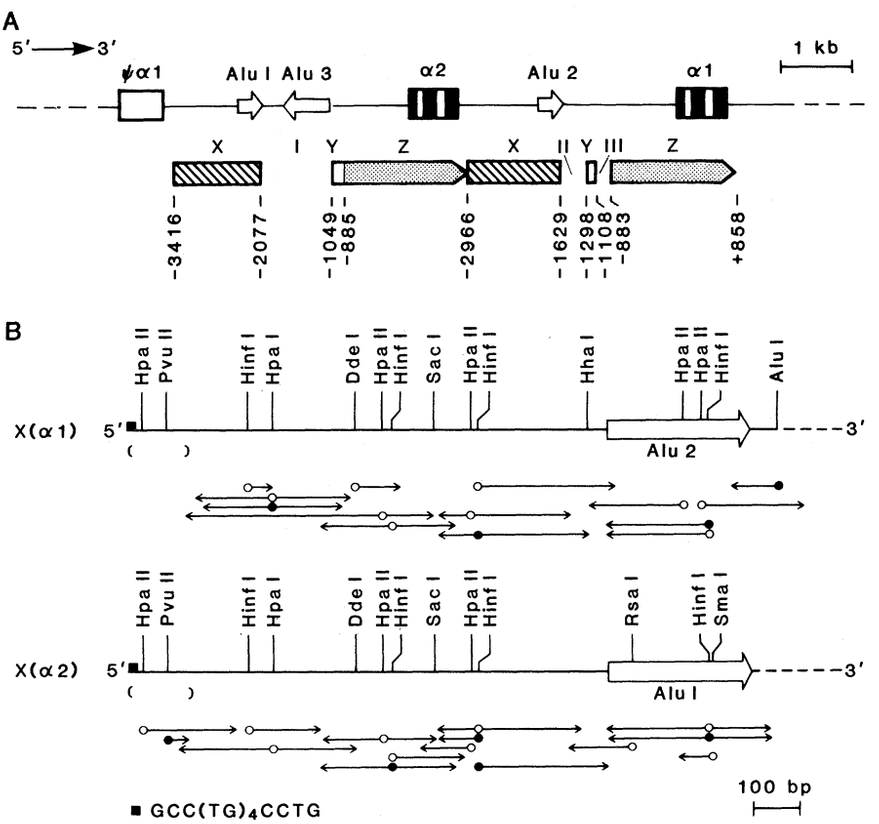


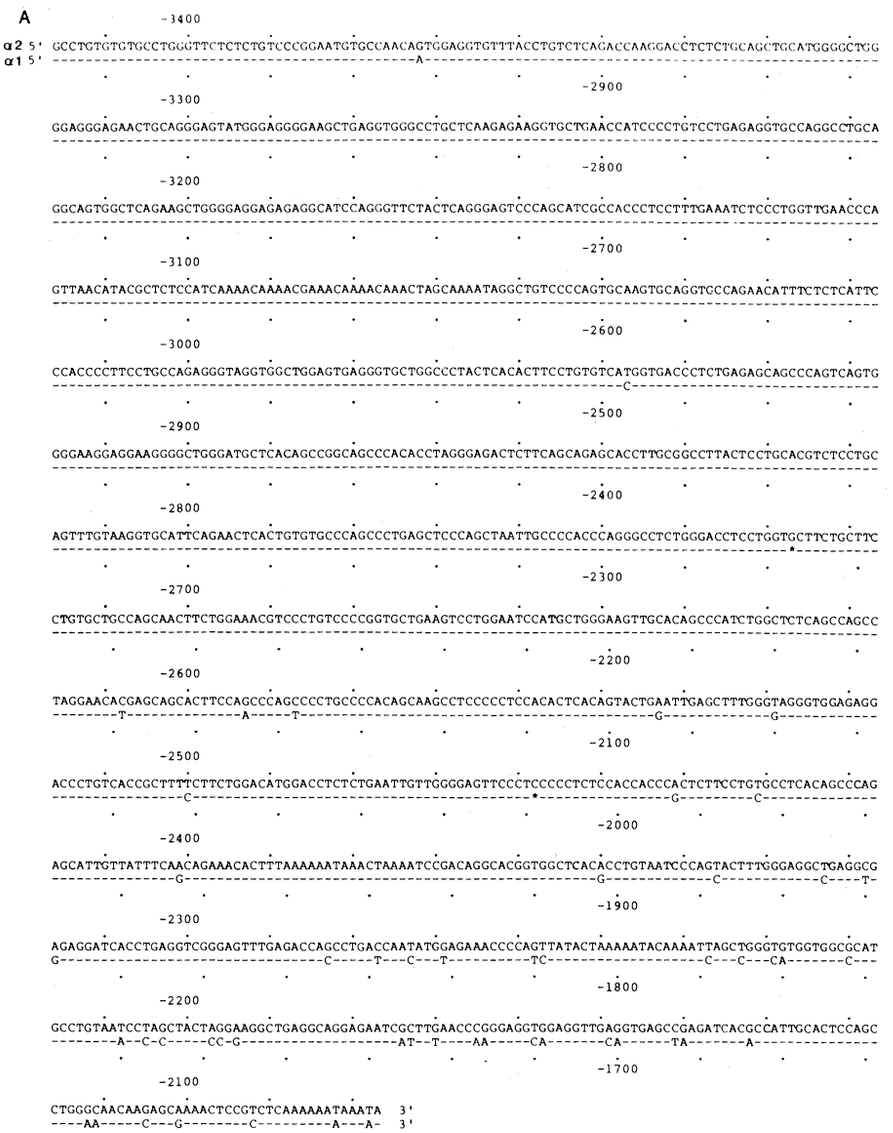
Fig. 1. (A) Sequence organization of the human adult α-globin gene locus. The linkage map of ψα1-α2-α1 is derived from (*2*). The black and blank boxes show the exons and introns, respectively, of the two adult α-globin genes. The three homology blocks X, Y, and Z, and the nonhomology blocks I, II, and III are shown below the linkage map. The numbers on the boundaries of each block show their positions relative to the 5′ end of α2 or α1 messenger RNA. They are derived from earlier sequence data (*4, 5*) and from the data of this study (see text and Fig. 2). A minus sign indicates that the position is upstream, and a plus sign indicates that it is downstream from the capping site of messenger RNA. The 5′ ends of the X blocks and the 3′ ends of the Z blocks contain the sequence 5′-GCC(TG)$_4$CCTG and define the boundaries of the duplication units (*6*). The two monomeric Alu family repeats (Alu 1 and Alu 2) located in the 3′ portions of the X blocks, and the dimeric Alu repeat (Alu 3) inserted in the nonhomology block I (*4*) are also shown in the figure. (B) Sequencing strategy of the two X blocks. The appropriate restriction fragments were isolated from plasmid subclones pRHα2 or pHBψα1, ^{32}P-labeled at one of the 5′ (●) or 3′ (○) ends and sequenced by the chemical cleavage method of Maxam and Gilbert (*22*). The nucleotide sequences contained within the parentheses on the 5′ ends of the X blocks have been determined previously by Proudfoot and Maniatis (*6*). The nucleotide sequences of the entire X blocks are shown in Fig. 2A.

The pattern of sequence divergence in the X blocks (Fig. 2) fits with the predictions of the "polaron hypothesis" for eukaryotic gene conversion (*10–13*). According to this hypothesis, events leading to conversion begin at a preferred site and proceed in one direction until they terminate randomly. The three characteristics of the model (specific initiation, polarity, and random termination) were inferred from the extensive genetic studies of gene conversion in lower eukaryotes such as *Ascobolus* and yeast (*10–12*). For example, Rossignol *et al.* (*11*) and Fogel *et al.* (*12*) have shown that certain regions within a gene are more likely to undergo gene conversion than others. Furthermore, gene conversion frequencies between different alleles gradually decrease from one end of the locus to the other. They also concluded that increased frequencies of gene conversion are due to increased heteroduplex DNA formation. Thus, hot spots for the initiation of heteroduplex formation and a directional or polar recombination process must exist in these species. Our sequence data of the human α-globin gene region suggest that a polar gene correction process also exists in the mammalian species.

Gene correction of repeated genetic elements can be accomplished by gene conversion, unequal crossing-over, or both. Both of these processes require prior heteroduplex formation by either symmetric (*14*) or asymmetric strand exchange (*15*). One notion is that the strand exchange is accomplished by the introduction of single-stranded DNA nicks and subsequent invasion of one duplex molecule by the single-strand DNA from the other duplex. The data in Fig. 2B suggest a specific initiation site of gene correction near the 5′ ends of the X blocks. This sequence may be recognized and nicked by an enzyme complex with endonuclease activity. Because the gene correction proceeds only in the 3′ direction, the Meselson-Radding model (*15*) predicts that the nick leading to the recombination would be located on the strand coding for α-globin. In the Holliday model (*14*), the nick could occur on either strand. Of course, a fixed site of initiation of gene correction need not be a hot spot for nicking (*16*). Instead, it could be a binding site for the enzyme complex with endonuclease activity. The

enzyme complex may recognize the initiation site and then migrate along the DNA duplex before making nicks at random positions removed from the initiation site. Heteroduplex formation and gene correction can then start in either direction from those nicks. Although there is still no biochemical evidence to differentiate between the two possibilities, we favor the first scheme of specific initiation of gene correction of the X blocks. That is, a specific nick is generated near the 5′ end of the X block (or blocks), in particular, within or very close to the sequence GCC(TG)$_4$CCTG (T, thymine). This is supported by the following hypothetical evolutionary history. Since this sequence defines the boundaries of both duplication units (*6*), it is most likely that in ancient time, an unequal crossing-over between the GCC(TG)$_4$CCTG sequences located on the ends of two 4-kb elements on sister chromatids or homologs created the tandemly arranged, α-globin gene duplication units (*17*). Homology between the two units was maintained during evolution by a correction process until long nonhomologies were created by insertion/deletion. The correction process then proceeded segmentally. These evolutionary considerations together with our sequence analysis of the X blocks strongly suggest that the sequence GCC(TG)$_4$CCTG may have been a hot spot for nicking by specific endonucleases throughout the evolution of the adult α-globin gene region. It is interesting that (TG)$_n$-containing sequences can adopt Z DNA conformation under supercoil strain and have been suggested to be hot spots for DNA recombination (*18*). Our data could also be interpreted in terms of other models of DNA recombination such as the double-strand-break repair mechanism (*19*).

The low frequency of sequence divergence in the 5′ portions of the two X blocks (three differences in approximately 800 bp) implies that the last sequence correction event of the X blocks could have occurred as recently as 0.8 million years ago, assuming that (i) noncoding DNA diverges at a rate of 1 percent per 2.2 million years (*20*), and (ii) the sequence organization of the particular adult α-globin gene locus we have studied is representative of the human population. Since the formation of the heteroduplex presumably begins at the 5′ ends, as discussed above, and progresses toward the 3′ ends, one possible reason for the higher divergence toward the 3′ ends is the instability of heteroduplex formation in those regions (*21*). This could result because nonhomology blocks I and II at the 3′ sides of the X blocks inhibit the homologous pairing of chromosomes during meiosis or meitosis. If the heteroduplex formation does initiate from the 5′ end of the X blocks, the histogram in Fig. 2B suggests that the



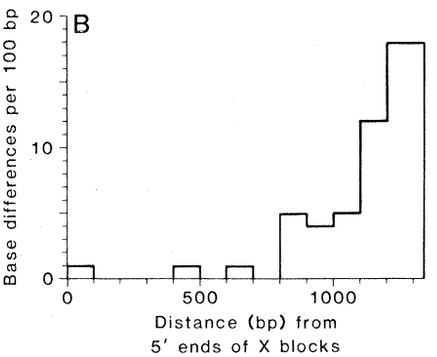


Fig. 2. (A) Comparison of nucleotide sequences of the two X blocks as determined in Fig. 1B. The complete nucleotide sequence of the X block upstream from the α2-globin gene (−2077 to −3416) is shown in the upper line. The lower line shows the sequence of the X block upstream from the α1-globin gene (−1629 to −2966). A dash indicates that the nucleotide is identical to the corresponding one in the upper line. Positions with base deletions are indicated by an asterisk. The last 350 bp DNA of each X block overlaps the sequences shown in figure 2 of (*4*). Some differences can be found between these overlapped regions. However, we consider the sequences reported here are accurate ones since they have been determined by chemically sequencing both strands of the DNA duplex. (B) Histogram showing the distribution of base differences (including transitions, transversions, and deletions/insertions) between the two X blocks. The sequenced regions are divided into 100-bp intervals and compared. The 5′ boundaries of the X blocks are positioned on the left end of the histogram. The sequence divergence increases gradually from the 5′ ends of the X blocks to the 3′ ends (see text for details).

heteroduplex may be stable for at least 800 bp. In any case, a calculation similar to the one described above indicates that the 3′ portions of X blocks have diverged for as long as 40 million years since the last gene correction event. This value is close to that of the Y blocks. We proposed earlier that a large DNA insertion/deletion (200 to 1000 bp) may inhibit gene correction (*4*). Hence, the genetic rearrangement events that generated the nonhomology blocks I and II in the human adult α-globin gene region may have occurred 40 million years ago.

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Rapid Expulsion of *Trichinella spiralis* in Suckling Rats

Abstract. *Orally administered* Trichinella spiralis *muscle larvae were rapidly expelled by rat pups suckling an immune dam. The immunity was delivered in the milk; substantial resistance was conferred on normal rat pups suckled for only 24 hours by a* Trichinella*-immune foster mother. The pups were protected by oral or systemic administration of specific serum antibodies. When infused into a normal lactating dam, these antibodies accumulated in the serum of her suckling pups.*

The immune response to the enteric helminth *Trichinella spiralis* involves several distinct but synergistic responses to different phases of the parasite's life cycle (*1*). A dramatic response in appropriately immunized adult rats is manifested in the prompt rejection of up to 99 percent of orally administered *T. spiralis* muscle-stage larvae (*1*). This rapid expulsion is a major defensive response against reinfection; yet the mediator of the expulsion process has not been identified, nor is it known how infectious larvae are displaced from their intestinal niche. To express rapid expulsion, adult rats must be exposed to the enteric phase

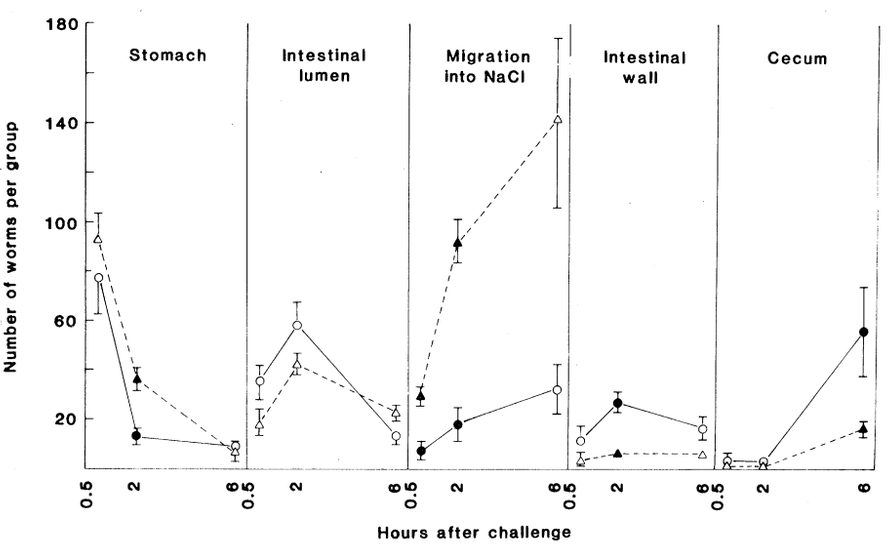

Fig. 1. Kinetics of rapid expulsion in infant rats. Five compartments of the gastrointestinal tract of 14- to 16-day-old AO rats were examined for *T. spiralis* 30 minutes, 2 hours, or 6 hours after oral challenge with 200 *T. spiralis* larvae. Dams were infected with 1000 larvae 28 days before being bred. Pups were separated from the dams 2 to 4 hours before being challenged. They were killed by cervical dislocation and the stomach, intestine, and cecum were removed. The intestines were rinsed with 0.85 percent NaCl and the wash was saved for enumeration of luminal worms. They were then slit open and incubated in 0.85 percent NaCl for 5 hours at 37°C. Worms that migrated into the medium were collected and counted. The intestinal tissue was digested overnight at 37°C with 1 percent pepsin HCl; worms freed by this procedure were also counted. The stomach and cecum were rinsed with 0.85 percent NaCl and the wash was examined for worms. Worms appearing in the cecum had been expelled, while worms appearing in the migratory compartment were considered to have established themselves in the intestine. The mean number of worms per compartment for two or three pups from each of three to six litters was calculated for each time point. Significant differences between treatment groups were determined with the Wilcoxon rank-sum test. For simplicity, pooled means and standard errors are shown for each point. Symbols: (△) nonimmune pups and (○) immune pups; shading indicates significant differences ($P < 0.05$) between groups.