Comparative sequencing of human and chimpanzee MHC class I regions unveils insertions/deletions as the major path to genomic divergence

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Despite their high degree of genomic similarity, reminiscent of their relatively recent separation from each other (∼6 million years ago), the molecular basis of traits unique to humans vs. their closest relative, the chimpanzee, is largely unknown. This report describes a large-scale single-contig comparison between human and chimpanzee genomes via the sequence analysis of almost one-half of the immunologically critical MHC. This 1,750,601-bp stretch of DNA, which encompasses the entire class I along with the telomeric part of the MHC class III regions, corresponds to an orthologous 1,870,955 bp of the human HLA region. Sequence analysis confirms the existence of a high degree of sequence similarity between the two species. However, and importantly, this 98.6% sequence identity drops to only 86.7% taking into account the multiple insertions/deletions (indels) dispersed throughout the region. This is functionally exemplified by a large deletion of 95 kb between the virtual locations of human MICA and MICB genes, which results in a single hybrid chimpanzee MIC gene, in a segment of the MHC genetically linked to species-specific handling of several viral infections (HIV/SIV, hepatitis B and C) as well as susceptibility to various autoimmune diseases. Finally, if generalized, these data suggest that evolution may have used the mechanistically more drastic indels instead of the more subtle single-nucleotide substitutions for shaping the recently emerged primate species.

The draft sequence of the human genome is now at the final stages of being transformed into a definitive blueprint available for high-resolution comparative analysis (1). Perhaps the most biologically enticing comparative genomics experiment would be the one between our genome and that of our closest evolutionary relative, the chimpanzee (Pan troglodytes). The chimpanzee is believed to share ∼98.77% nucleotide and >99% amino acid identity with us (2, 3). However, there are important biomedical (as well as obvious morphological and cognitive) differences between the two species, which thus far have eluded any molecular explanation within this supposedly 1% diversity segment. Among these are our differential handling of a number of infectious agents, e.g., HIV (progression to AIDS) (4), late complications of hepatitis B and C (5, 6), as well as susceptibility to Plasmodium falciparum (7), which are of utmost public health importance. The molecular basis of these distinctive traits is thought to be in large part encoded within the MHC, where MHC class I molecules sample pathogen-derived antigenic peptides for recognition by the CD8+ T cell receptor expressing cytotoxic T cells (8).

We have already reported the complete sequence and gene map of the 3.7-Mb human chromosome 6p21.3-located MHC (alternatively called the human leukocyte antigen or HLA) gene complex (9, 10). This is a gene-rich (224 identified loci) highly polymorphic (with some MHC genes having >400 alleles) genomic segment that is associated with a myriad (>100) of mostly autoimmune but also infectious disorders for which our molecular knowledge, for the most part, remains rudimentary. It is precisely this extremely high level of MHC polymorphism and heterozygosity that is believed to confer a selective advantage to the host in encountering the extraordinarily diverse pathogen-derived antigenic repertoire (8). The human MHC is composed of three distinct regions, designated from the centromere to the telomere as the class II, III, and I regions. The telomeric 1.8-Mb class I region harbors two notable (but not only) multicopy gene families, HLA and MIC (10, 11), which are thought to have arisen from repeated gene duplications (10, 12) and which engage a host of critical immune receptors: the T cell receptor as well as Ig and lectin-like inhibitory and activatory receptors (13, 14).

Despite the facts that structural and/or functional orthologues for all human HLA genes have been found in chimpanzee (15–19) (HLA-A/B/C/E/F/G vs. Patr-A/B/C/E/F/G) and that there is no doubt that the MHC biology between these two close species is nearly interchangeable, the genomic architecture of our closest MHC relative is unknown, although it is assumed to be closely linear to that of human. Our aim was to capitalize on our detailed knowledge of the human HLA region to jump-start a large-scale comparative genomic analysis with regard to that of the chimpanzee (P. troglodytes) MHC (called Patr). Not only will the chimpanzee MHC sequence provide an in-depth analysis of this important genomic region between two such closely related species, but it also has the intrinsic power to unravel the molecular basis for some important biological differences between us and the chimpanzee. In this regard, we present 1.75 Mb of continuous genomic sequence linking the Lymphotatin B (LTB) gene in the telomeric area of the class III region to Pair-F locus (chimpanzee HLA-F orthologue) at the telomeric end of the MHC class I region.

Materials and Methods

Bacterial Artificial Chromosome (BAC) Clones and Construction of a Contig Map. Two BAC libraries, RPCI-43 and CHORI-251, constructed from white blood cells of the same male chimpanzee, were used for the present study. The sequences reported in this paper have been deposited in the GenBank database (accession nos. AB100082–100087 and BA000041).

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Abbreviation: BAC, bacterial artificial chromosome.
zee, were obtained from the BACPAC Resource Center at the Children’s Hospital Oakland Research Institute (Oakland, CA). Hybridization screenings were performed following the recommended protocols. Hybridization probes, $4.110^2$ kb in length, were PCR-generated from the human HLA and MIC genes (exons 2–4) as well as several MHC-based sequence tagged sites by using cloned human genomic DNA as a template. The final contig map was constructed by comparison with the complete sequence of the human MHC (9, 10).

DNA Sequencing and Analysis. Fourteen chimpanzee BAC clones that covered $\approx 1.75$ Mb from the LTB to Patr-F genes were completely and bidirectionally shotgun sequenced with an average redundancy of $7.0$ $\times$, which was sufficient for assembly and analysis of the entire sequence using previously established procedures (10, 20). The chimpanzee sequence was compared with our previously published human sequence (GenBank accession nos. AP000502–000521) (10, 20). Sequence alignments were performed and homologies determined by using the programs contained within the GENETYX Ver. 11 (www.sdc.co.jp/genetyx) and DNASIS (Hitachi, Tokyo) software packages. Dot matrix analysis was performed by using HARRPLOT Ver. 2 as part of the GENETYX package. The nucleotide diversity (21) profile was constructed after determining the percent nucleotide difference between the human and chimpanzee sequences for a sliding window of 1 kb. The diversity profile was then drawn by using the graphics output of Microsoft EXCEL. All indels were removed from the alignments to standardize the number of nucleotides examined within each window. Finally, repetitive elements were identified within the contiguous sequences by using the REPEATMASKER webserver (A. F. A. Smit and P. Green, http://ftp.genome.washington.edu/cgi-bin/RepeatMasker).

Results and Discussion

Comparative MHC Genomics Reveals Indels as the Major Evolutionary Driving Force Between Human and Chimpanzee. The 1,750,601-bp MHC sequence was obtained from 14 overlapping chimpanzee BAC clones and encompasses the telomeric part of the class III as well as the entire Patr class I regions linking LTB, 70-kb centromeric to the class III/class I boundary, to Patr-F at the telomeric end of the class I region. The length of the entire Patr class I region proper, from Patr-MIC to Patr-F, is 1,671,882 bp, $\approx 95$ kb shorter than the corresponding 1,796,912-bp HLA class I region. Fig. 1 depicts a detailed comparative genomic map between these two MHC regions. Analysis of the repeat content reveals an occupancy rate of 52.03% of the region by such sequences as compared with 51.11% for the human counterpart. These were respectively composed of 17.66% (chimpanzee) and 16.79% (human) short interspersed elements (SINEs), 17.87% (chimpanzee) and 18.10% long interspersed elements (LINEs), and 12.98% (chimpanzee) and 12.88% LTR elements. A detailed breakdown of the repeat content of the entire region is provided in Table 1, which is published as supporting information on the PNAS web site, www.pnas.org). As expected, there is considerable similarity between the genomic organization of human and chimpanzee MHCs. The chimpanzee sequence contains 41 putative coding genes and 59 noncoding or pseudogenes, which are matched with orthologous loci in identical orientations within the human MHC (9, 10). Interestingly, a detailed sequence analysis revealed the existence of 64 indels, each $>100$ bp in length (Fig. 1).
of these indels include repetitive elements, such as Alu, LINE, LTR, etc., as well as the frequent insertion of the repeated sequence, SVA, within the chimpanzee sequence. Importantly, the indels were directly responsible for the major differences observed between the two species. These include the loss of three human pseudogenes, DHRFP, HCGII-4, and MICF, and the presence of only a single chimpanzee MHC gene in the region corresponding to the two human functional MICA and MICB genes, at the centromeric end of the class I region. This single chimpanzee MHC gene, Patr-MIC, was therefore produced as result of a large 95-kb deletion between the corresponding human MICA and MICB genes following a scenario that we reconstitute below.

A 95-kb Deletion Between the Human MICA and MICB Genes Leads to the Generation of a Single Chimeric Patr-MIC Gene. The human MICA and MICB genes are believed to result from a genomic duplication that occurred ~33–44 million years ago (Mya) (22, 23), hence well before the separation of the chimpanzee from the human lineage ~6 Mya (24) (Fig. 2A). Therefore, we asked from which ancestral MIC gene (A or B) this single Patr-MIC gene was originated, i.e., which MIC gene was deleted from the chimpanzee genome? Because the human MICA and MICB genes have a relatively high sequence similarity, it was not possible to settle the issue by dot plot analysis (data not shown). Consequently, and to thoroughly address this question, we performed both structural as well as similarity analyses of the human and chimpanzee MIC genes. Structural analysis showed that the 5’ flanking sequences and the first intron of the Patr-MIC gene have retained all of the signature retroelements characteristic of the orthologous region of human MICA, whereas the 3’ flanking sequences of the Patr-MIC display all of the characteristic retroelements of the human MICB (Fig. 2B). In accordance with this retroelement profiling, similarity investigations unveiled that exon 1 to intron 2 of Patr-MIC show greater sequence similarity with corresponding regions of MICA rather than with MICB, whereas the opposite is seen for Patr-MIC exons 5 and 6 as well as the 3’ noncoding region (Table 2, which is published as supporting information on the PNAS web site). Finally, the polymorphic (GCT)n (n = 4, 5, 6, 9, 10) short-tandem repeat, which exists only within the fifth exon (transmembrane domain) of MICA but not MICB (19), is also absent from the same exon in Patr-MIC. However, because the sequences between exon 3 and intron 4 of the Patr-MIC are equally homologous to orthologous regions within MICA and MICB, one could not establish the exact position of the recombinational event. Nevertheless, on the basis of sequence differences, we have narrowed the recombination breakpoint down to a segment located between the ends of MICA’s second and MICB’s fourth introns.

The existence of a single functional MIC gene centromeric of the major classical class I locus, Patr-B here, is not exclusive to chimpanzee, because it has been recognized in other primates, including humans. The gorilla, indeed, appears to have only one MHC gene with a strong sequence similarity to the human MICA (25, 26). In humans, individuals carrying the HLA-B*4801 allele (rare in Caucasians but more common in Northeast Asians as well as Native Americans) have lost the MICA locus also due to a large 100-kb genomic deletion surrounding and including this locus (albeit the genomic breakpoints are distinct from those observed in chimpanzee) (27, 28). All in all, it is quite intriguing that an equal-sized deletion involving this very same region and genes (MICA/B) has happened at distinct points in time in several different primate species. This very phenomenon might also be the reason why rodents are devoid of MIC genes, because the putative location of functional mouse MICA and MICB genes, the segment linking H2-D (equivalent to HLA-B or Patr-B) and BAT1, is substantially shortened compared with the human MHC:40 instead of 173 kb (11, 29, 30). The molecular basis of the existence of such an apparently “deletion-prone” segment between MICA and MICB remains to be established, but this could be due to the existence of a HERV-L sequence, which contains a 2.5-kb AT-rich insertion in its 5’ LTR, which might therefore serve as a recombination hot spot (23, 31).

Nucleotide, Amino Acid, and Structural Similarities Between Human and Chimpanzee Orthologous Sequences. Fig. 3 compiles our similarity analysis with respect to nucleotide and amino acid diversity among 35 orthologous human/chimpanzee genes identified here, of a total of 41 putative coding sequences. The average nucleotide and amino acid identities were 98.9% and 98.3%, respectively (Table 3, which is published as supporting information on the PNAS web site). This relatively lower amino acid identity might be the result of positive selection aimed to maintain genetic polymorphism in the MHC (that is MHC class I) genes (32). Indeed, once genes were divided into MHC (hereafter designating MHC class I or MHC-I) and non-MHC loci, it was found that sequence identities were 99.3%/99.1% (nucleotide/amino acid) for the 28 non-MHC genes and “only” 97.1%/95.0% for the seven MHC-I genes, including the solo...
As expected, the genomic segments surrounding the MHC genes, except for the nonclassical HLA/Par-G loci, reveal continuous high diversity profile, especially around the classical class I loci. This high degree of nucleotide variation may be the result of positive selection, the existence of multicopied sequences as well as hitchhiking effect due to the accumulative effect of balancing selection acting on the MHC loci in linkage disequilibrium (33). In contrast, the 35 kb surrounding HLA/Par-G genes is highly conserved, displaying only a 0.9% nucleotide difference in contrast to the situation next to other MHC genes. This low level of nucleotide variation between HLA/Par-G genes might be in connection with the biology of the HLA/Par-G molecule implicated at maternofetal immunity. Finally and interestingly, the diversity profile between the chimpanzee and human sequences closely resembles that previously obtained between different human MHC haplotypes (33–37).

Interestingly, once the indels are taken into account, the above-observed 98.6% sequence identity drops to only 86.7% (substitution, 1.4%; indels, 11.9%). This indel-included 86.7% identity may be a better representation of whole-genome sequence similarity between the human and the chimpanzee, as confirmed by a recently published study comparing a number of fragmented chimpanzee sequences with their human counterparts (38).

**Fig. 4.** Diversity profile between human and chimpanzee MHC. The aligned sequence (excluding indels) is shown along the horizontal axis and the percent nucleotide differences calculated per kb of nonoverlapping windows are shown along the vertical axis. The relative positions of the coding (red box), noncoding (gray box), and MHC (blue box) sequences are shown along the horizontal axis.

**Comparative Nucleotide Diversity Profiling.** A “nucleotide diversity (substitution: single-nucleotide polymorphism) profile” was generated across the entire 1.68-Mb gap-free (indels excluded) aligned genomic sequence by using a sliding window of 1,000 bp (Fig. 4). The average degree of nucleotide identity between the chimpanzee and the human for this region (again excluding indels) is 98.6%, which is similar to the earlier estimation of 98.77% (1.23% nucleotide difference) (7). However, this nucleotide difference is not constant across the entire MHC. For instance, within the two non-MHC gene-rich clusters (Fig. 4, left, LTB to BAT1 gene; center, IEX-1 to HSR1 gene), it is of ~0.7%, which is five to nine times less than the average nucleotide difference of 6.7–3.5% around the classical MHC genes. This variation in nucleotide difference implies again that purifying selection is acting to maintain conservation much more strongly throughout these non-MHC gene-rich clusters (including their intergenic regions), whereas in contrast, the classical class I gene regions (including their intergenic sequences) show a lower degree of similarity, probably as a result of overdominant selection necessary to maintain polymorphism (33).

As expected, the genomic segments surrounding the MHC genes, except for the nonclassical HLA/Par-G loci, reveal
percentages of the total substitutions between them, but G to N (9.1%) and A to T (6.1%) gave higher and lower percentages as compared with G to T and A to C as well as those obtained in the previous studies, respectively (38, 39). Further, although the MHC gene regions tend to maintain a high degree of genetic polymorphism, the ratios within nucleotide substitutions from and to each base were almost the same between the MHC class I (multicopy) gene and non-MHC (single-copy) gene regions (Fig. 5, which is published as supporting information on the PNAS web site).

In summary, this work reports the sequence of one-half of the chimpanzee MHC, which to date represents the longest continuous sequence within this species, our closest evolutionary relative. Comparative genomics with the orthologous human MHC class I region unveiled a wealth of information, the most salient being the existence of a large number of indels that appear to be the main driving force behind the observed differences between the two species. Hence our perceived sequence divergence of only 1% between these two species appears to be erroneous, because this work, along with another recently published analysis, puts both species much further apart, >10% here and ~5% in another recently published study (40), albeit the latter study compared shorter segments of both genomes. This relatively high and previously unexplained degree of sequence divergence might have functional implications not only within the coding sequences itself but also within regulatory elements (41, 42). Within the MHC per se, the most notable effect of indels appears to be the generation of a single chimeric Patr-MHC by fusion of MICA and MICB. This, along with other indels as well as nucleotide substitutions [which could be dubbed “transspecies single-nucleotide polymorphisms (SNPs)”], might therefore directly contribute to the genetic difference between these two closely linked species with regard to susceptibility to a number of infectious as well as autoimmune disorders, most of which are primarily linked to the MHC. The study of these transspecies SNPs might further help to pinpoint the most ancient and perhaps functionally relevant human SNPs among the increasing numbers that are being continuously identified.

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![Fig. 6. Grouping of nucleotide substitutions.](image)

**Fig. 5.** Structural differences between human and chimpanzee sequences. (A) Diversity profile of nucleotide differences between the human and chimpanzee genomic sequences using a sliding window of 1,000 bp across the entire 1.68-Mb aligned genomic sequence, including single-nucleotide indels. The aligned sequence is shown along the horizontal axis, and the percent nucleotide differences calculated per 1 kb of nonoverlapping windows are shown along the vertical axis. Diversity profiles of a single-nucleotide indel and substitutions by transition and transversion are indicated by blue, pink, and yellow lines, respectively. The relative positions of the MHC class I (multicopy) gene (brown) and non-MHC (single-copy) gene (light blue) regions are shown along the horizontal axis. (B) Alteration of percentages of continuous indels that override 2-bp length.


Table 1. Analysis of repeat content

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