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A *Pan*-oramic view: insights into hominoid evolution through the chimpanzee genome

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The draft sequence of the second primate genome, of the common chimpanzee *Pan troglodytes*, is now complete. Together with its sibling species the bonobo *Pan paniscus*, chimpanzees are our closest living relatives and the availability of their genome sequence provides countless opportunities for evolutionary studies. Determining the mechanisms and identifying the evolutionary forces that have shaped the genomes of *Homo* and *Pan* will be challenging, because they will depend on the identification of relevant genetic changes against a vast background of neutral differences. Linking differences between the genomes to phenotypic consequences in both species and their respective evolutionary trajectories will require the collaboration of researchers from diverse fields, ranging from genomics to behavioral sciences. Interpreting these genomic comparisons will depend crucially on expanding the chimpanzee phenotype data set and on the availability of high-quality tissue samples.

Since the celebrations of the completion of the human genome project in 2001 [1], research teams have focused their efforts on sequencing the genomes of other species, among them, that of our closest living relative, the common chimpanzee *Pan troglodytes*. Here, I discuss the promises and limitations of the availability of this latest genome sequence, and argue for the urgent need for new phenotypic data on chimpanzees and other great apes.

The draft chimpanzee sequence has been publicly available since November 2003 from GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html) the Nucleotide Sequence Database of the European Molecular Biology Laboratory (EMBL-Bank; http://www.ebi.ac.uk/genomes/eukaryota.html) and the DNA Data Bank of Japan (DDBJ; http://www.ddbj.nig.ac.jp/). The alignment of the sequence with the human genome can be viewed at the server of the University of California Santa Cruz (http://genome.cse.ucsc.edu/cgi-bin/hgGateway?org=Chimp). This second hominoid genome is the first complete non-human primate genome against which human genetic features can be compared (Box 1).

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The suggestion by Darwin and Huxley that African great apes are the closest living relatives of humans [2,3] has been confirmed by comparative molecular studies. The recently completed draft sequence of the chimpanzee genome adds to a series of studies, including immunological studies of blood proteins [4,5], amino acid sequences [6], cytogenetics [7], genomic DNA comparisons [8,9], mitochondrial X-linked autosomal gene sequencing [10–13] and gene expression patterns [14]. These and additional studies have shown that humans and chimpanzees are sibling species with a divergence time between hominid and chimpanzee–bonobo (panid) lineages of 5–6 million years [15,16]. The question first posed by King and Wilson [8] remains: how did humans and chimpanzees, two species with differences at the molecular level that one would expect to find between mammalian sibling species, evolve such dramatically different phenotypes that one would expect to find between mammalian families? King and Wilson suggested that regulatory changes explain this apparent paradox, although this is probably only part of the answer.

Long-term field studies of chimpanzee behavior have documented behavioral traits, such as tool use, culture and warfare, which were all formerly considered to be hallmarks of humanity [17,18]. Behavioral comparisons between humans and chimpanzees led many researchers to highlight the close similarity of the species, whereas others have focused on the profound differences between the cognitive capacities of the two species [19,20].

More-detailed recent molecular and genetic data are also beginning to uncover differences between the genomes of these two species [21–23]. Identifying relevant differences (i.e. non-neutral changes), and discovering the biological or phenotypic consequences of them, are important research endeavors [24–26].

Although the initial report from the chimpanzee genome-sequencing consortium has yet to be published, there are several publications that already shed light on interesting differences between the human and chimpanzee genomes. A recent analysis of sequences of 20 000 human–chimpanzee gene alignments identified several candidate genes that experienced human or chimpanzee-specific selection [27]. The potential benefits of the chimpanzee genome project for biomedical research and

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Box 1. Summary of current state of chimpanzee genome data

By November 2003, 95% of the chimpanzee genome had 4X sequence coverage [i.e. each nucleotide position is covered by at least four different reads from randomly amplified (shotgun) sequencing fragments]. The data comprise ~300 000 contigs (defined as a set of sequence reads that are related by overlap of their sequences; Figure I). The total contig length is 2.73 Gb, spanning 3.02 Gb, which can be assembled into 37 849 supercontigs. Supercontigs are scaffolds that are an ordered and oriented list of sequence islands that ideally approximate a chromosome but which are usually much shorter and contain gaps as well as less reliable sequences based only on single strand reads (Figure I). As of April 2004, there were 235 296 bacterial artificial chromosome (BAC) Ends (chimpanzee genome fragments inserted into bacterial artificial chromosomes) on the GenBank database.

The genome has been assembled from shotgun sequencing using the ARACHNE algorithm [59]. Whereas most of the assembled segments can be aligned with the human genome without much difficulty, tiling the draft sequence of the chimpanzee genome against the human genome might be introducing a bias because chimpanzee sequences are forced against the scaffold of the human sequence. This is of special concern owing to regions of hominoid genomes that are enriched for duplicated and palindromic (inverted duplications) sequences [60].

Most large-scale comparisons of chimpanzee and human genome sequences reveal that, in those areas of the genome that can be aligned, sequences of both genomes differ by 1.2% [12,13,27], when insertions and deletions (indels) are not considered. There is evidence that, in terms of genome evolution, time has not stood still for chimpanzees, the genome of which appears to have undergone a level of change that is comparable with that in humans since their common ancestor 5–6 million years ago [27]. Thus, the phenotypic conservativeness observed among the great apes not including humans is not due to genomic stasis. It has recently been pointed out that the human and chimpanzee genomes differ by as much as 5% if one includes insertion deletion differences in the analysis, for which there are no good models of mutation rate and mechanism [22,61,62]. Furthermore, there are large fragments of chimpanzee genomic DNA that seem to lack counterparts in the human genome [13]. Segments of the human genome lacking a counterpart in chimpanzees also exist [33,63–65] but it is still unclear how many contain functional genes (i.e. those that are expressed and translated into functional proteins). How to interpret such 'missing chunks' in terms of genetic distance poses an interesting new problem. A consortium of scientists headed by Yoshiyuki Sakaki has produced a very high fidelity BAC clone based sequence for the smallest chimpanzee chromosome 22, the counterpart of human chromosome 21. Comparisons of this high-quality sequence with that from the shotgun assembly will enable one to estimate the degree of (im)precision associated with the much more efficient shotgun assembly. The unpublished sequence is viewable at https://chimp22-pub.gsc.riken.go.jp/ [13]. A comparison of large palindromic segments on the Y chromosome was published in 2003 [66].

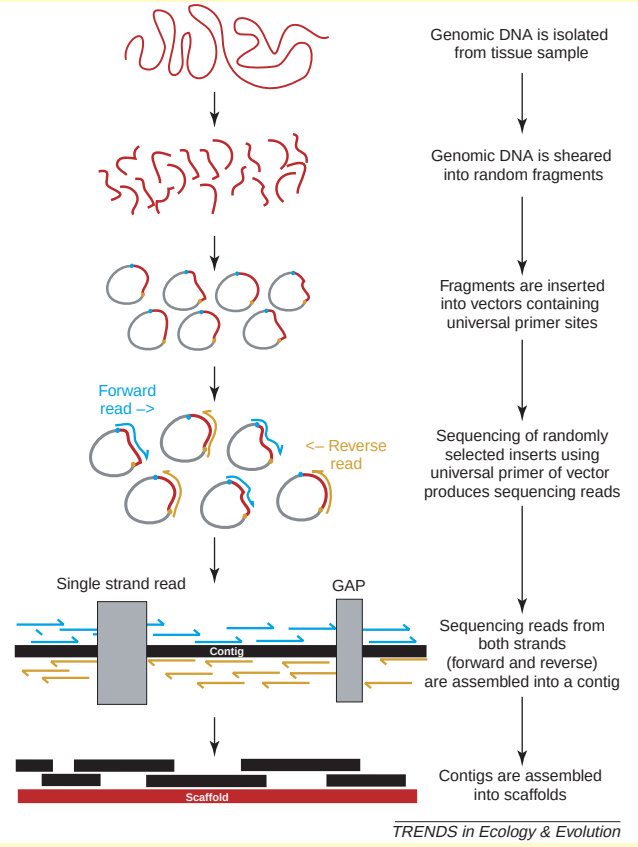

Figure I.

for evolutionary insights have been highlighted [25,26], and two recent reviews addressed the state of comparative human–chimpanzee genomics just before the exponential increase in numbers of comparative genetic and genomic studies [28,29].

Dynamic changes mediated by genomic elements

The gap between classic cytogenetics and molecular genetics is rapidly being bridged. With the use of genomic probes and fluorescent *in situ* hybridization, the detailed organization of chromosomes is being revealed [30]. Major cytogenetic changes between humans and chimpanzees include three in the hominid lineage and seven in the panid lineage [7]. In addition, there are large numbers of minor changes that appear to be mediated by mobile elements and segmental duplications. Recently, it has been suggested that chromosomal rearrangements have a potential role to play in driving sequence divergence and speciation, based on the fact that chromosomal regions with rearrangements appear to show higher divergences [31]. Other researchers have questioned such a role as a result of the comparison of cDNA sequence and expression divergence patterns of genes on rearranged and collinear chromosomes [32]. Detailed genomic comparisons that take into account intraspecific variation will be needed to address these conflicting arguments. The degree to which segmental duplications and mobile elements (i.e. retroviruses and transposons) contribute to changes in genome architecture and associated changes in gene evolution and expression is one of the exciting areas of genomics. The first study of genome-wide gene duplications in hominoids based on cDNA samples has just been published [33], which reports a bias for increase in copy number among genes with a lineage-specific difference in copy number in the human lineage (143 out of 140 differences). It remains to be seen how many of these differences involve expressed pseudogenes rather than functional genes. Nevertheless, even expressed pseudogenes, by affecting translational efficiency, might have effects on the final expression levels of the functional copies.

Hot spots, indels and suspicious (interesting) changes

Frequent mutations are caused by the strand slippage-mediated expansion and contraction of variable number tandem repeats (e.g. microsatellite loci) and it has been suggested that such mechanisms affect human and chimpanzee genomes differentially [34]. It appears that triplet repeat expansion (e.g. polyglutamine tracts) in genes that are associated with several human diseases, such as spinocerebral ataxia, show variation in repeat length in chimpanzees versus humans, predisposing humans to the pathogenic effects of extreme expansion [35]. Only a small fraction of the single nucleotide substitutions, and insertions and deletions (indels), which range in size from a single to thousands of nucleotides, is likely to affect gene function. Mobile elements change genome sequences through insertions of copies, and their impact on gene function and regulation ranges from no effect to sudden loss of function, change in expression or even compromised chromosomal stability.

Olson and Varki [26] have proposed a less-is-more hypothesis, whereby the unique human phenotype is derived from a hominoid ancestor by the differential loss of gene activity (i.e. humans represent a degenerate ape). Phenotypically, humans appear as 'degenerate apes' with respect to the loss of most of their body hair, and increased muscle strength, skeletal robustness, copulatory plugs and the penis bone (baculum). Contrary to the less is more hypothesis, humans also seem to have gained more of a uniquely hominoid brain cell type (spindle neurons) in the anterior cingulate cortex, a brain region that is associated with cognitive function, especially that relating to the perception of others and their feelings [36]. The genetic basis for the difference in spindle number remains unknown and, as with any phenotypic trait, could involve novel genes, novel expression patterns of existing genes, or a combination thereof.

At the genetic level, an increasing number of genes that are inactivated in humans but still intact in the great apes are being identified. These include the genes encoding the T-cell receptor (*TCRGV10*; GenBank Accession no. NG_001336 [37]), the sialic acid-modifying enzyme cytidine monophospho-*N*-acetyl-neuramic acid hydroxylase (*CMAH* GenBank Accession no. BC022302 [38]), the endogenous sialic acid-binding immunoglobulin-like lectin 1 (*SIGLECL1*; GenBank Accession no. NM_033329 [57]), keratin hair acidic pseudogene 1 (*KRTHAP1*; GenBank Accession no. Y16795 [39]), myosin heavy polypeptide 16 (*MYH16*; GenBank Accession no. NR_002147 [40]), as well as numerous olfactory genes [41]. The sample size is still small and the analysis of the chimpanzee genome will shed light on whether increased gene loss is characteristic of the hominid lineage. By contrast, there is only one reported gain of a functional gene in humans, the Y-linked protocadherin 11 gene (*PCDH11Y*; GenBank Accession no. NM_032973 [42]). Recent loss of gene function in humans might be due to mutations, such as Alu element-mediated deletions (e.g. in *CMAH* [43]). Such mutations have only small effects on genomic sequence divergence and might have occurred too recently to have led to detectable changes in non-synonymous:synonymous substitution ratios (Ka:Ks), a common measure of selection. It is likely that several mechanisms account simultaneously for the differing evolution of human and chimpanzee traits involving not only regulatory changes of transcription factors (e.g. *FOXP2*; GenBank Accession no. NC_000007) or their target sequences, but also loss or gain of gene function through duplication and/or translocation.

The effects of nucleotide sequence differences are nested in a series of contextual levels, including: (i) the immediate genomic neighborhood; (ii) the other allele of the same locus (for autosomal genes); (iii) other genes with which the sequence or its transcripts interacts either as a result of gene regulation or transcript function; (iv) maternal effects, through the presence of mRNA in egg cytoplasm and in sperm; (v) maternal effects during gestation; (vi) social environment during development; and (vii) epigenetic effects (i.e. imprinting or parental-origin effects [44]. In spite of the limited number of imprinted genes, some of these are functionally important for development, making them good candidates for highlighting human–chimpanzee differences.

Where to expect changes

A recent study by Kitano *et al.* [45] has estimated the average number of amino acid substitutions between humans and chimpanzees to be 0.6 amino acid changes per gene. If this is representative of most genes, then every second gene will have experienced an amino acid substitution. Given that a large fraction of genes are transcription factors, even small (single amino acid) mutations could have effects on the binding of regulatory regions or other members of a regulatory complex. Evolution might affect genes very locally, such as the binding pockets of specific proteins, causing such effects to be easily missed when comparing whole-gene sequences. An example is the domain-specific functional adaptation of a sialic acid-binding endogenous lectin of the human innate immune system (SIGLEC) [46]. The number of reported instances of adaptive evolution based on Ka:Ks ratio calculation for whole genes is rapidly increasing. Although providing interesting candidates for genes involved in lineage-specific adaptations, these cases will have to be studied for their effects on function (by the expression in cell culture and functional assays, such as those performed on SIGLEC molecules [46,47]. Unfortunately, functional studies are done more easily with proteins that can be assayed for function *in vitro* than with genes for transcription factors. Speculations about the biological systems that are likely to show important genetic differences between humans and chimpanzees are discussed in Box 2.

From genomic architecture to gene expression

A more difficult level of investigation is the level of gene expression, where shades of gray (subtle changes in expression) rather than black-white (function or dysfunction of gene) might be determining species-specific traits. We are witnessing the beginning of comparative gene expression and proteomic studies in tissues of humans and great apes [14,48–51]. They have provided evidence in humans for higher rates of change in expression, and for

Box 2. Speculating about the nature of important differences between humans and chimpanzees

Lineage-specific evolution might explain several differences between humans and chimpanzees.

Genes associated with timing and rate of growth and development

Given the differences in developmental schedule between humans and chimpanzees, most notably the delayed maturation of young humans, it is safe to expect that development is an area in which many genetic differences might be identified (e.g. difference in thyroid hormone metabolisms described for adults [48] might be even greater during early development).

Genes associated with the reproductive system and sexual selection

Among the most rapidly evolving proteins are many reproductive proteins [67]. Chimpanzees, unlike humans, have a mating system where females mate with multiple males during each periovulatory period. Comparisons between human and chimpanzees reveal that related evolutionary selection pressures affect investment in testicular tissue and sperm morphology [68]. It has been argued that many of the allegedly uniquely human characteristics, such as language, cognitive capacity and symbolic representation, have been influenced by sexual selection [42]. Although the X chromosome carries only 6% of all genes, these are more likely to be involved in speciation events than are autosomal genes [69]. One of the hypotheses for the existence of imprinting involves conflict between male and female interests [70] and, given the stark differences between human and chimpanzee mating systems, it is possible that the imprinting process also differs between them.

Recognition systems

There are at least three other areas of rapid evolution that each involve recognition systems:

(i) Mother–offspring recognition during pregnancy involving placentation, and the immunological detente that this entails;

(ii) Host–pathogen and host–symbiont coevolution, both of which are likely to have left different footprints in the genomes of humans and chimpanzees (these footprints would be expected in genes that affect both the innate and the adaptive immune systems as well as the major interfaces with microbes, such as the major epithelia that all have highly glycosylated ciliated cells and secrete glycoconjugates-rich mucins. Comparisons of genes involved in assembling such glycoconjugates of epithelia are likely to reveal many differences (e.g. [71]); and

(iii) The cognitive system involving the many traits that are typical of humans and their societies. Cognitive functions are affected by many genes that are expressed in tissues other than the brain, particularly during development (e.g. gene for masticatory muscle fibres [41]). The cognitive system includes human language, which depends on cognitive capacity, vocal production and the auditory perception of language.

the general up regulation of numerous genes in brain tissue, when compared with non-central nervous system tissues. These expression studies have helped identifying genes that are linked to functional or metabolic categories, and will trigger studies of the candidate genes and the metabolic pathways that they affect. These studies have relied on access to rare tissue samples, which explains the small sample sizes used for great ape tissues. It also illustrates the need for high-quality great ape tissue samples and for cDNA resources derived from those. A cDNA resource will also be invaluable for predicting amino acid sequences of proteins when conducting proteomic studies and for *in vitro* expression of chimpanzee proteins for structural studies.

A complicating factor for comparative gene expression studies is that relatively modest changes of expression are not easily detected. Furthermore, differences in levels of expression in cell types with low numbers in a tissue sample (e.g. the columnar epithelium of a mucosa, or a particular cell type in the brain) could easily be drowned by similar mRNA levels in more abundant cell types. Finally, comparisons of the degree to which the same genes are subject to alternative splicing in humans and chimpanzees might help identify differences in function. However, as with mutational differences, it is likely that much of the variability in alternative splicing products is effectively neutral [52].

Challenges and limitations

Many of the differences between humans and chimpanzees are linked to different developmental schedules, including the comparatively delayed developmental schedule of humans [53]. A promising strategy could be to look for genes that are expressed in juvenile chimpanzees only, but show continued expression in adult humans. Obtaining samples enabling the study of gene expression during chimpanzee development will be difficult, given the widespread stricture on captive breeding and the ethical issues involved.

It will also be important to consider intraspecific variation. With the exception of microsatellite loci and some major histocompatibility complex (MHC) class 1 genes [54,55], most comparative studies of human and chimpanzee genetic variation have reported levels of diversity that are up to four times greater in chimpanzees. By contrast, a recent study of 50 nuclear non-coding non-repetitive loci has contradicted these observations by documenting an average of only twice the amount of variation in chimpanzees compared with humans [56]. Data on more loci in a larger sample of chimpanzees of known subspecies are needed to resolve this apparent paradox. Accounting for the existing intraspecific variation in chimpanzees is essential to avoid false leads when detecting differences in the genomes of only a few individuals. Unlike the situation in humans, the existence of at least three different subspecies in chimpanzees, and a bonobo sibling species, provides a natural experiment in hominoid genome divergence over relatively short evolutionary time (i.e. 2 million to a few hundred thousand years).

The need for a 'phenome' project for chimpanzees and other apes

The study of hominoid evolution and the basis for human chimpanzee differences will generate an acute demand for data on chimpanzee and other great ape biology. Compared with abundance of human biomedical data, our knowledge about chimpanzees remains limited. There is an overwhelming consensus that invasive experiments on great apes are no longer ethical, given that they are self-aware beings with complex emotional lives [57]. There are thousands of chimpanzees and other great apes in captivity and an urgent need for a coordinated effort to provide the best veterinary care for these animals,

including full necropsies upon the death of an animal. Such an effort has been initiated in the USA under the Great Ape Phenome Project (GAPP) of the UC San Diego Project for Explaining the Origin of Humans (http://origins.ucsd.edu/). These efforts aim to establish a network of facilities caring for chimpanzees and other apes and to provide support for the collection of post-mortem samples for histopathology, biochemical studies and nucleic acid preparation. Any collection of samples from live apes will follow protocols as used with human volunteers, with the notable exception of prior informed consent. Such an ethical stance is logical, and indeed essential, given that most captive chimpanzees and other great apes will be cared for by people who have strong convictions about the status of our close relatives. The GAPP has also initiated the first online Museum of Comparative Anthropogeny (MOCA), a database of all known and/or alleged human–great ape differences [58].

Last, but not least, my colleagues and I frequently discuss how to translate the upsurge of interest in comparative biology of humans, chimpanzees and other great apes into enhanced support for apes in captivity and in the wild. There might be ways in which those of us benefiting from precious ape samples could divert part of our own support to benefit the living animals, by supporting chimpanzee sanctuaries (http://www.panafricanprimates.org) and wild chimpanzee conservation (http://www.wildchimps.org/). This will for the most part, depend on individual acts by members of the scientific community and the public alike.

Conclusions

With the availability of the chimpanzee genome, we might now be able to compare the genetic underpinnings of humans and our closest living relative. Because data on chimpanzee biology are so limited compared with our knowledge of human biomedicine, the correct interpretation of genomic comparisons will come to rely heavily on expanding the biological data sets about chimpanzees and other great apes. It will be crucial to consider genomic differences in light of well documented organismal differences to pinpoint the meaningful (non-neutral) genetic changes that have come to define the evolution of the human lineage. The need for more information about living great apes illustrates the utilitarian facet of why great apes are considered world heritage species (http://www.4greatapes.com/).

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