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Of apes and men

How do our brains differ from those of our closest living relatives, the great apes (bonobos, chimpanzees, orangutans and gorillas)? Despite our persistant curiosity about the issue, even very basic questions remain unresolved, such as whether differences in cognitive abilities emerged from the addition of new types of neurons and cortical areas or enlargement of otherwise similar components.

In a new study, Svante Pääbo and colleagues[1] offer a fresh approach to the problem by comparing gene and protein expression on a large scale between ape and human brains. The study is provocative and raises many new questions. Nevertheless, given the sheer complexity of neural structures, linking these molecules to changes in circuits and behavior may prove very difficult. More modest goals, however, seem achievable.

The authors removed gray matter from the left prefrontal lobe of adult humans, chimpanzees, orangutans and macaques (which had died of natural causes), and then used oligonucleotide and cDNA arrays to compare gene expression in the brain, liver and blood. Although non-human primate gene chips are not available, the authors were able to use probes based on human sequences, because primate genomic DNA sequences are highly conserved (over 98% identity for coding regions).

By summing differences in mRNA levels over all genes for each tissue, the authors report that for blood leukocytes and liver, the human expression pattern was more similar to that of chimpanzees than to that of macaques. This result was expected—humans are more closely related to chimpanzees than chimpanzees are to macaques. However, in brain tissue, the chimpanzee and macaque expression patterns were more similar to each other than to the human pattern. The authors conclude that human evolution included large and rapid changes in gene expression levels in the brain compared with other organs.

Human and chimpanzee brains differ substantially, yet their DNA sequences do not. Thus the general conclusion that large changes in gene expression might account for brain differences is not necessarily surprising. The goal now is to use this information about expression differences to understand what changes at the level of DNA led to a brain with different functional properties.

First, it will be necessary to verify that the reported gene expression patterns are stable and reproducible. This is important because microarray technology is still rapidly evolving—as are the bioinformatics tools that are necessary to interpret the results. For example, the oligonucleotide chip technique indicated considerable within-species variation: one human brain sample differed as much from the other human samples as it did from chimpanzee brain. Furthermore, it will be critical to determine whether the observed changes reflect 'recent' (and less informative) transcription events associated with learning and memory, for example, or more profound interspecies differences.

Given the serious ethical issues involved in experimenting on great apes, similar comparative experiments are unlikely to be done during development. This limitation is unfortunate because early development is when gene activity can profoundly influence overall brain structure, and differences in early gene activity probably drive evolutionary changes. However, such experiments are possible in monkeys, and they would help to focus more traditional comparative studies. For example, using slice cultures of embryonic human, macaque and mouse brains, Letinic and Rakic[2] recently reported a migratory pathway from the telencephalon to the diencephalon that exists in humans but is not apparent in macaque or rodent brain; this pathway seems to contribute to the expansion of particular brain regions in humans. (Because great apes were not examined, it is not known whether this pathway is unique to humans.) The authors conclude that small changes in migratory guidance cues during evolution could lead to the expansion of human-specific brain structures. Identifying the molecular basis of such differences would be an important advance.

A major challenge will be to understand what underlies the differences in gene expression profiles. Differences in promotor regions of genes and other cis-acting regulatory regions will surely contribute, and it may be possible to identify these. However, changes in RNA levels may result from other factors, such as transactivating proteins. A detailed comparison of the human and chimpanzee genome sequences will be of great use here—and although progress on chimpanzee sequencing has so far been limited to chromosome 21, it now seems likely that substantial resources will be committed to this project.

Most importantly, conclusions based on informatics approaches will need to be placed into a broader biological context. Even within a small area of the brain, the interpretation of averaged mRNA abundance levels can be complicated by large differences in the expression of given transcripts between different cell types. Therefore, several groups are using array technologies together with techniques that offer high spatial resolution. For example, David Anderson and colleagues[3] combined microarray experiments with *in situ* hybridization to identify genes that respected anatomically defined subdivisions of the amygdala. Such gene expression domains also defined subdivisions that were not readily apparent with anatomical staining techniques. Other groups are using microarrays and *in situ* hybridization with similar goals of identifying transcripts expressed preferentially in various neocortical areas. This 'molecular anatomical' information could be used in comparative studies to understand the relationship between human and great ape cortical areas, and perhaps to identify regions that have evolved very recently in humans.

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2. Letinic, K. & Rakic, P. *Nat. Neurosci.* **4**, 931–936 (2001).
3. Zirlinger, M., Kreiman, G. & Anderson, D. J. *Proc. Natl. Acad. Sci. USA* **98**, 5270–5275 (2001).