

There is also the question of why the absence of IRC is enough to cause mortality. ROS have been widely studied for their role in signaling, tissue injury, regulation of vascular tone, and control of inflammation. High levels of ROS have been implicated in long-term effects like aging and DNA damage (reviewed in Fang, 2004). But what makes the ROS so toxic to flies? Is the oxidative stress enough to induce tissue damage and death? Or are the ROS involved in signaling to specific mechanisms that initiate programmed cell death? Recent literature shows other evidence of immune-induced signaling molecules having a harmful effect on fly survival (Brandt et al., 2004). In vertebrates, there is a widely held belief that while a controlled immune response is a good thing, too much of an immune response is not. This may be a telling demonstration in the fly of a similar paradigm.

**Subhamoy Pal and Louisa Wu**  
Center for Biosystems Research  
University of Maryland Biotechnology Institute  
5115 Plant Sciences Building  
College Park, Maryland 20742

#### Selected Reading

- Basset, A., Khush, R.S., Braun, A., Gardan, L., Boccard, F., Hoffmann, J.A., and Lemaitre, B. (2000). *Proc. Natl. Acad. Sci. USA* 97, 3376–3381.
- Basset, A., Tzou, P., Lemaitre, B., and Boccard, F. (2003). *EMBO Rep.* 4, 205–210.
- Brandt, S.M., Dionne, M.S., Khush, R.S., Pham, L.N., Vigdal, T.J., and Schneider, D.S. (2004). *PLoS Biol.* 2(12), e418 10.1371/journal.pbio.0020418.
- De Gregorio, E., Spellman, P.T., Rubin, G.M., and Lemaitre, B. (2001). *Proc. Natl. Acad. Sci. USA* 98, 12590–12595.
- Fang, F.C. (2004). *Nat. Rev. Microbiol.* 2, 820–832.
- Ha, E.-M., Oh, C.-T., Ryu, J.-H., Bae, Y.-S., Kang, S.-W., Jang, I.-h., Brey, P.T., and Lee, W.-J. (2005). *Dev. Cell* 8, this issue, 125–132.
- Macpherson, A.J., and Harris, N.L. (2004). *Nat. Rev. Immunol.* 4, 478–485.
- Silverman, N., Zhou, R., Erlich, R.L., Hunter, M., Bernstein, E., Schneider, D., and Maniatis, T. (2003). *J. Biol. Chem.* 278, 48928–48934.

Developmental Cell, Vol. 8, January, 2005, ©2005 Elsevier Inc. All rights reserved. DOI 10.1016/j.devcel.2004.12.011

## Genetic Basis of Human Brain Evolution: Accelerating along the Primate Speedway

Using novel variations of traditional methods, Dorus et al. report in the December 29<sup>th</sup> issue of *Cell* that diverse genes involved in neural biology (particularly those critical in development) show higher rates of protein evolution in primates than in rodents—particularly in the lineage leading to humans.

A larger and “more complex” brain is regarded as the cardinal feature that distinguishes us from other primates. Indeed, the biological basis for this difference is among the most interesting questions in human evolution. It is reasonable to think that human-specific changes of genes expressed in the nervous system are a major factor, and it has now become feasible to approach this matter from a genetic perspective. The recently popular approach has been to make comparisons between humans and our closest evolutionary relative, the chimpanzee, with whom we share near-identity of most protein sequences (Olson and Varki, 2003; Varki, 2004). In fact, there is already evidence for human-specific changes in expression of multiple genes in the brain (reviewed in Preuss et al., 2004) and for increased frequency of human-specific amino acid changes in genes that are determinants of brain size (reviewed in Varki, 2004) as well as in others that appear important to nervous system function (Enard et al., 2002; Burki and Kaessmann, 2004; Grossman et al., 2004).

In looking for accelerated evolution of specific genes, most groups have used a well-known parameter, the Ka/Ks ratio (Li, 1997), which compares rates of nonsynonymous (amino acid changing) and synonymous (non-amino acid changing) substitutions in the coding regions of genes. However, writing in the December 29<sup>th</sup> issue of *Cell*, Dorus et al. point out that human-chimpanzee comparisons of Ka/Ks ratios suffer from high stochastic uncertainty and reduced statistical power resulting from studying the very small differences between such closely related sister species. To avoid this problem, they instead use more distantly related Old World monkey (macaque) sequences to assess acceleration in the primate lineage and a rodent sequence pair (rat and mouse) as an outgroup—only then narrowing down their attention to ape- and human-specific changes. Also, rather than studying all possible genes, they followed “Sutton’s Law,” focusing specifically on genes that are exclusively or predominantly expressed in the nervous system, and/or known to mediate nervous system-specific functions, and/or associated with developmental disorders of the nervous system (Sutton’s Law is so named because when asked why he robbed banks, the bank robber Willie Sutton supposedly responded, “Because that’s where the money is!”).

A problem the researchers faced is that genes important in a highly complex organ like the brain tend to be under strong functional constraints, such that amino acid changes are not easily tolerated. Thus, essentially all of the genes they studied had Ka/Ks ratios of <1. Since Ka/Ks >1, =1, and <1 are traditionally assumed to support positive selection, neutral evolution, and purifying selection, respectively, their result did not appear

significant at first glance. However, they noted that the Ka/Ks ratios were much higher in the primate pair than in the rodent pair, with the differences clearly being statistically significant. Furthermore, the trend was strongest in nervous system development-related genes rather than other genes involved in routine physiological operation and maintenance. Moreover, upon then adding in chimpanzee sequences, they showed that the primate lineage leading to humans showed the highest degree of acceleration of protein evolution. Taken together, these data suggest that accelerated evolution of nervous system development-related genes very likely contributed to brain enlargement and “complexity” in primates in general and in humans in particular. In addition to showing this overall evolutionary trend, the work also points to specific candidate genes for further examination in exploring human brain evolution and function. The overall conclusion is that human-specific brain features were established by changes not only in regulatory regions (causing changes in gene expression) (Preuss et al., 2004) but also in the amino acid coding regions of many genes (presumably causing functional changes).

A few caveats are in order. The approach taken excluded primate-specific genes such as *GLUD2* (Burki and Kaessmann, 2004), genes that underwent significant human-specific changes like *FOXP2* (Enard et al., 2002) and *AH11* (Ferland et al., 2004), and those that were specifically inactivated in humans (see listing in Varki, 2004). Needless to say, such genes could also be very important in explaining unusual features of the human brain. Also, using the entire coding region to generate Ka/Ks values may miss the identification of functional domain-specific accelerated evolution (Ferland et al., 2004; Sonnenburg et al., 2004).

Dorus et al. take great pains to rule out “relaxation of constraint” as an explanation for the higher primate Ka/Ks ratios, rather than “positive” or “adaptive” evolution. While their analyses and logic appear convincing, one should consider the possibility that *both* phenomena might be relevant to human brain evolution and are not necessarily mutually exclusive. A relaxation of constraint typically assumes release from strong functional constraints against amino acid changes and is considered a signal of neutral or nearly neutral evolution, i.e., changes occur because they don’t matter one way or the other. In contrast, an adaptive change of preexisting function is the classic view of positive selection, indicating importance for evolution. However, in the case of the primate brain, it is possible that relaxation of constraint is actually one mechanism to provide *opportunity* for adaptive evolution, i.e., the positive elimination or release of stringency of preexisting functions might actually allow one to gain new functions. Indeed, unlike rodents, primates in general and humans in particular are characterized by a prolonged period of postnatal brain development, during which the development of functions (and presumably of neural connections) can be influenced by environmental and cultural factors. Thus, positive adaptive outcomes of brain evolution might actually be facilitated by relaxation of constraint during postnatal brain architectural changes. Such a scenario could also help explain the much greater variation in primate encephalization and brain anatomy discussed by the authors.

No one doubts the importance of brain enlargement in

human evolution, and the data of Dorus et al. contribute toward understanding it at the molecular level. However, brain size increases during human evolution stopped >200,000 years ago, long before the documented appearance of modern human behaviors such as formed art and complex tools, which apparently emerged only ~70,000 years ago and then spread across the world (Mellars, 2004). Also, there is an unclear relationship between modern brain size and cognitive function. Furthermore, children who have undergone hemispherectomy (removal of half of the brain) can later have normal or nearly normal cognitive function (Pulsifer et al., 2004). Thus, it seems very likely that additional important genetic changes have occurred in human evolution that were not related to brain size, e.g., the human-specific mutation creating a potential phosphorylation site in *FOXP2* gene that was fixed in the human population ~200,000 years ago (Enard et al., 2002). Of course, in examining evolutionary events that occurred during this very recent period, we must distinguish biological evolution from human cultural history. Some apparently universal human behaviors could actually be conferred by postnatal cultural factors that now happen to be universal to all living human populations. Thus, assuming 100% human literacy in the year 2100, an alien anthropologist studying humans might conclude that reading and writing are genetically hard-wired in the human brain. Of course, this begs the question posed long ago by Alfred Russel Wallace: how is a brain that was evolved tens of thousands of years ago capable of taking on complex new functions such as reading and writing that are recently derived from human cultural practices?

Finally, we address the term “complexity.” Primates do appear to have a larger number of cortical subdivisions than most other mammals examined, and these areas are accompanied by highly elaborate system of interconnections. However, primates are complex along some dimensions, but not others (Preuss, 2005). For example, the olfactory system seems to be stripped down in anthropoid primates, compared to other mammals. Furthermore, we know remarkably little about primate nervous systems relative to those of rodents, and even much less about humans (Preuss, 2005). Does the human nervous system actually have a higher density of neurons? Does it actually have more connections per neuron? Are there specific neuronal cell types completely unique to humans? Even such fundamental questions remain unanswered today. Thus, the genetic approach must be complemented by the classical approach toward human and primate neuroanatomy and neurobiology, even while taking into account the effects of environment and culture on brain development.

**Toshiyuki Hayakawa, Tasha K. Altheide,  
and Ajit Varki**  
Glycobiology Research and Training Center  
Department of Medicine  
Department of Cellular and Molecular Medicine  
University of California, San Diego  
La Jolla, California 92093

## Selected Reading

- Burki, F., and Kaessmann, H. (2004). *Nat. Genet.* 36, 1061–1063.
- Dorus, S., Vallender, E.J., Evans, P.D., Anderson, J.R., Gilbert, S.L., Mahowald, M., Wyckoff, G.J., Malcom, C.M., and Lahn, B.T. (2004). *Cell* 119, 1027–1040.
- Enard, W., Przeworski, M., Fisher, S.E., Lai, C.S., Wiebe, V., Kitano, T., Monaco, A.P., and Paabo, S. (2002). *Nature* 418, 869–872.
- Ferland, R.J., Eyaid, W., Collura, R.V., Tully, L.D., Hill, R.S., Al-Nouri, D., Al-Rumayyan, A., Topcu, M., Gascon, G., Bodell, A., et al. (2004). *Nat. Genet.* 36, 1008–1013.
- Grossman, L.I., Wildman, D.E., Schmidt, T.R., and Goodman, M. (2004). *Trends Genet.* 20, 578–585.
- Li, W.H. (1997). *Molecular Evolution* (Sunderland, MA: Sinauer Associates).
- Mellars, P. (2004). *Nature* 432, 461–465.
- Olson, M.V., and Varki, A. (2003). *Nat. Rev. Genet.* 4, 20–28.
- Preuss, T.M. (2005). What is it like to be a human? In *The Cognitive Neurosciences*, Third Edition, M.S. Gazzaniga, ed. (Cambridge, MA: MIT Press), pp. 5–22.
- Preuss, T.M., Caceres, M., Oldham, M.C., and Geschwind, D.H. (2004). *Nat. Rev. Genet.* 5, 850–860.
- Pulsifer, M.B., Brandt, J., Salorio, C.F., Vining, E.P., Carson, B.S., and Freeman, J.M. (2004). *Epilepsia* 45, 243–254.
- Sonnenburg, J.L., Altheide, T.K., and Varki, A. (2004). *Glycobiology* 14, 339–346.
- Varki, A. (2004). *Nat. Genet.* 36, 1034–1036.

Developmental Cell, Vol. 8, January, 2005, ©2005 Elsevier Inc. All rights reserved. DOI 10.1016/j.devcel.2004.04.009

## Pardon Me—No Access without Ubiquitin

**Spatial separation of ubiquitin conjugation pathways contributes to target-specific ubiquitination. Recently, Plafker et al. reported that importin 11-dependent nuclear import of the ubiquitin-conjugating enzyme UbcM2 occurs only if the latter is charged with ubiquitin. This interesting finding describes a link between nuclear transport pathways and ubiquitin and reveals a novel mechanism for localizing components of the ubiquitin system within the cell.**

The small polypeptide ubiquitin serves as a protein modifier, which is covalently attached to the  $\epsilon$ -amino groups of internal lysine residues of substrates. Ubiquitin conjugation usually requires the successive action of three enzyme classes: ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3). The E1 and all members of the class of E2 enzymes are “charged” when they form a thioester bond between an active site cysteine residue within the enzyme and the C-terminal glycine of ubiquitin. Most ubiquitin ligases function as substrate recognition factors that mediate interaction between the E2 and the substrate. In these cases, distinct E2/E3 combinations define substrate specificity. E2 and E3 enzymes may also be part of larger protein complexes. One example for such a multisubunit E3 ligase is the SCF (Skp1-Cullin-F-box protein) complex (Cardozo and Pagano, 2004). At least one class of E3s (HECT-domain ligases) also forms a thioester reaction intermediate and directly ubiquitinates substrates.

At least two different principles allow regulation of specific ubiquitination: first, modification of the substrate (e.g., by phosphorylation), which in turn uncovers signals for ubiquitination by the E3 ligase. Second, restriction of ubiquitination pathways to certain compartments can alter the half-life of a protein dependent on

its subcellular location. Examples are the ubiquitin-dependent degradation of proteins from the endoplasmic reticulum, where the involved E2 and E3 enzymes are largely located at the cytosolic surface of the membrane (Jarosch et al., 2002), and the nucleus-specific degradation pathways of Far1p and Mat $\alpha$ 2p in yeast (Blondel et al., 2000; Lenk and Sommer, 2000). Here, Far1p ubiquitination is limited to the nucleus since the F-box protein Cdc4p, which is part of the Far1p-recognizing SCF complex, is localized to this compartment. Other components of this complex, Skp1p, the Cullin Cdc53p, but also the involved E2 enzyme Cdc34p, appear to be evenly distributed throughout the cytoplasm and the nucleus. Furthermore, the turnover of the transcription factor MyoD is restricted to the nucleus, as it has been shown in higher eukaryotes (Lingbeck et al., 2003). Evidence for compartment-specific localization also comes from the analysis of the E3 ligase Nedd4. It contains nuclear export signals that prevent accumulation of this protein in the nucleus (Hamilton et al., 2001). These results show that proper sorting of components of the conjugation machinery is crucial for the function of specific ubiquitination pathways.

In their work, Plafker and coworkers investigate the nuclear import of UbcM2 via the transport receptor importin 11 (Plafker et al., 2004). Previous studies revealed that UbcM2 binds only weakly to importin 11 in vivo. They now demonstrate that only the enzymatically active form of UbcM2, which forms a thioester bond with ubiquitin, is allowed to enter the nucleus. In microinjection experiments, mutant forms of UbcM2, which lack the active site cysteine and are thus unable to be charged with ubiquitin, fail to reach the nucleus. Importantly, Plafker et al. also demonstrate that UbcM2 binds importin 11 in vitro when incubated with cellular extracts. This association requires ATP and depends on the activity of the E1 enzyme. The exchange of the active site cysteine of UbcM2 with arginine abolishes this interaction. Finally, coimmunoprecipitation experiments demonstrate that importin 11 specifically interacts with ubiquitin-charged forms of UbcM2 and two other ubiquitin-conjugating enzymes of the same class of E2 enzymes.

These exciting results suggest that the linkage of ubiquitin via a thioester plays an important role in directing