The Mammal Family Tree

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EVOLUTION

The Mammal Family Tree

A combination of molecular and fossil data yields a well-resolved and well-dated phylogenetic tree for mammalian families.

Kristofer M. Helgen

Evolutionary biologists have long aimed to understand the relationships between the major groups of mammals and the pattern and timing of the evolution of these groups and their characteristics. On page 521 of this issue, Meredith *et al*. (*1*) report an important step toward this end by providing the first matrix-based molecular phylogeny that incorporates essentially all modern mammal families.

Because convergent evolution is widespread across the mammal tree of life, comparative morphology—the traditional workhorse of building classifications—often proved misleading in determinations of mammal relationships, leading to arguments about relationships between major groups of mammals (*2*). Some morphological classifications were long uncontroversial; all could agree that the primates, bats, or whales were natural groups. But what group of mammals was the closest relative of primates, or bats, or whales?

Already in 1945, George Gaylord Simpson recognized the value of genetic data in this field (*3*); 50 years later, analyses of large DNA sequence data sets led to well-resolved phylogenies involving all modern mammal orders (*4*, *5*). But no molecular phylogenetic study has examined the mammal tree of life comprehensively at the next level of taxonomic resolution, the family. This is what Meredith *et al*. have now achieved.

The family is the unit in the taxonomic hierarchy between order and genus. Family boundaries are largely uncontroversial among mammal taxonomists—a good indication that the family is a useful standard of classification. The bears, cats, and dogs each constitute a single mammalian family, as do the kangaroos, armadillos, elephants, shrews, deer, and beaked whales. Roughly 150 families of living and very recently extinct mammals are currently recognized (see the figure).

All in the family. The silky anteater of the American tropics (Cyclopedidae, **top left**), Kitti's hog-nosed bat from Southeast Asia (Craseonycteridae, **top right**), and the aye-aye of Madagascar (Daubentoniidae, **bottom**) are each classified in its own mammalian family. Meredith *et al*. have produced the first molecular phylogeny to include all living mammalian families.

Meredith *et al*. now include representatives encompassing this diversity in a phylogeny and generate enough molecular data for each family to produce a well-resolved tree. They incorporate a wealth of information from the mammalian fossil record to evaluate the timing and rate of evolutionary events, and make use of a "relaxed clock" approach that allows the tremendous variability in rates of evolution across the mammal tree of life to be taken into account. The study is a welcome advance on previous phylogenies, which are less complete or based on disparate data sources (*6*).

A next step will be to extend these comparisons beyond living mammal families. Most modern mammal families missing from the analyses of Meredith *et al*. are those that have become extinct in recent centuries, such as Thylacinidae (the thylacine) or Nesophontidae (Caribbean insectivores), for which no frozen tissue samples are available. Now that it is technologically feasible to generate genome-scale data sets from ancient DNA preserved in degraded sources such as museum skins, bones, and recent fossils (*7*, *8*), it should be possible to incorporate these families into this framework.

Another ambitious challenge will be to expand comprehensive comparisons beyond the level of families, to that of genera and species, bringing mammalian biodiversity into true phylogenetic focus. This is a daunting task, because no single research team will ever amass frozen tissue samples of the 1300 genera and nearly 6000 species of mammals currently recognized, many known from few specimens collected long ago. Here too, phylogeneticists will need to rely on next-generation approaches to generate data from molecules preserved in historical museum specimens (*9*). Specimens squirreled away in museum cabinets have never been more valuable to biology at large.

Another important goal will be to integrate the phylogeny of Meredith *et al*. with morphological and paleontological data for a wide array of living and fossil mammals such that long-extinct mammal lineages can be placed on the tree, and dating can be further refined. Most mammals that have ever lived are long extinct, their remains too old to yield DNA, and comparative morphology will be needed to place them on a backbone molec-

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ular phylogeny. The true shape of the mammal tree of life cannot be appreciated until many fossil lineages are placed on the tree. And molecular phylogenies cannot be dated effectively without well-selected calibrations drawn from the fossil record. Meredith *et al*. suggest that major Late Cretaceous events may have driven the diversification of modern mammal groups. These conclusions are exciting, but this will not be the final word on this subject. New fossil discoveries and interpretations can always clarify hypotheses regarding the timing of evolutionary events; for example, a recently discovered eutherian mammal fossil shows that marsupial and placental mammals diverged far earlier than previously established (*10*).

Less than a decade ago, paleontologists and molecular biologists waged fierce battles over preferred phylogenetic hypotheses. Today the two disciplines depend closely on one another to understand their data, with well-resolved phylogenies flowing from DNA labs, and clues that establish the antiquity and tempo of evolutionary events emerging from fossil studies. Meredith *et al*. illustrate beautifully the value of this collaboration. In the rising generation, more evolutionary biologists will have to be fluent in both disciplines.

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CANCER

BRCA1, Everything But the RING?

Genetically engineered mouse models reveal essential functions of the BRCA1 protein in tumor suppression.

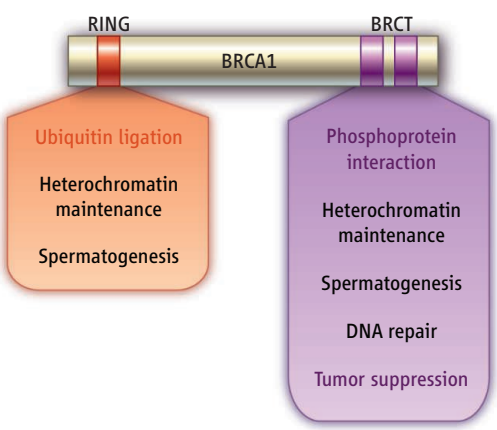
Roger A. Greenberg

Since its discovery in 1994, familial breast and ovarian cancer susceptibility gene *BRCA1* (breast cancer early onset gene 1) has been routinely sequenced in women with family histories for either malignancy (*1*). Genetic alterations are reported in a public database (http://research.nhgri.nih.gov/bic/), providing a wealth of information on pathogenic mutations in the *BRCA1* gene. Mutations found in either the BRCA1 amino or carboxyl terminus confer highly penetrant breast and ovarian cancer risk, suggesting that each domain within the BRCA1 protein plays an essential role in BRCA1-dependent DNA repair (*2*, *3*), thereby limiting cancer susceptibility. On page 525 of this issue, Shakya *et al*. (*4*) put this assumption to the test, using elegant in vivo models to show that phosphoprotein binding by the BRCA1 carboxyl-terminal domain is critical for DNA repair and tumor suppression, whereas E3 ligase activity at the amino terminus is not.

The BRCA1 protein is composed of several interaction surfaces, each represented by a specific domain. The first 110 amino acids of BRCA1 comprise the RING domain, the most common structural motif implicated in E3 ubiquitin ligase activity (*5*) (see the figure). This domain interacts with at least eight different E2 ubiquitin-conjugating enzymes in vitro to affect either mono- or polyubiquitylation of substrate proteins (*6*). At the opposite end of the protein lie the BRCA1 carboxyl-terminal (BRCT) tandem repeats, a motif that recognizes a phosphorylated serine consensus sequence in at least three different DNA repair protein complexes (*7*, *8*). Pathogenic mutations occur in either domain, suggesting that E3 ligase activity within the RING domain and phosphoprotein interactions at the BRCT repeats both contribute to tumor suppression.

However, many RING domain mutations result in considerable structural perturbation (*9*), confounding interpretation of whether E3 ubiquitin ligase activity plays an essential role in tumor suppression. To circumvent these concerns, Shakya *et al*. used genetically engineered mice in which isoleucine-26 in the BRCA1 RING domain was replaced with alanine (I26A). This mutation abrogates interaction with E2 enzymes as well as BRCA1 E3 ligase activity, while maintaining overall RING domain architecture (*10*). The authors also created a BRCT mutation (S1598F, which replaces serine-1598 with phenylalanine) that corresponds to a known cancer-causing allele in humans (S1655F) lacking phosphopeptide recognition. The BRCT mutation accelerated mammary or pancreatic carcinoma formation in three different mouse models. Surprisingly, the RING mutation did not result in any appreciable difference in tumor suppression compared to wild-type BRCA1. The BRCT amino acid change produced genomic instability in cells and tumors, whereas the RING mutation did not in either scenario, highlighting the intimate association of BRCA1 DNA repair and tumor suppression functions. Moreover, BRCA1 I26A cells did not display any diminution in either homology-directed DNA repair or ubiquitin foci (in response to ionizing radiation) (*4*, *11*), further implying that BRCA1 E3 ligase activity does not play a prominent role in response to DNA damage.

So what purpose does the evolutionarily conserved BRCA1 E3



Functional ends. Amino-terminal BRCA1 E3 ubiquitin ligase activity is not required for tumor suppression or DNA repair, in contrast to the BRCA1 carboxyl-terminal phosphoprotein binding domain.

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