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Abstract

A combination of sequence analysis and functional assays has revealed steps in functional innovation through the divergence of paralogs

Significance and context

Colobine monkeys are unusual among primates in that they eat leaves rather than fruits and animal flesh; the leaves are digested by fermentation by symbiotic foregut bacteria. Fossil evidence suggests that this primate subfamily acquired its leaf-eating habit approximately 10 million years ago. Leaf-eating colobine monkeys, like ruminants, are believed to acquire nitrogen through RNase-mediated digestion of RNA from rapidly growing bacterial symbionts. The colobines have a large amount of foregut RNase. Using sequence analysis, Zhang *et al*. have documented steps in the origin and directional evolution of the ribonuclease gene *RNASE1B*, a paralog (gene duplicate) of the *RNASE1* found in colobines but not in the fruit- and insect-eating hominoids, prosimians, New World monkeys, or any of the other Old World monkeys. Enzyme functional assays revealed that RNASE1B, unlike the ancestral RNASE1, achieves maximum efficiency in the acidic colobine foregut. This study provides key insights into how gene duplication and divergence can facilitate evolutionary innovation and adaptation to new ecological niches.

Key results

Amplification and sequencing of the ribonuclease *RNASE1* gene from various primates revealed one copy in all of the primates studied except the douc langur (*Pygathrix nemaeus*), a leaf-eating colobine, which appeared to have two copies. A phylogenetic tree constructed from all the *RNASE* sequences found in 16 primates confirmed a gene duplication in the colobines, producing a paralog *RNASE1B* from the ancestral *RNASE1* sequence. The douc langur has retained both sequences. The topology of the tree was robust and consistent with known primate phylogeny. Sequence analysis revealed that the colobine-specific paralog has an intron that is shared with *RNASE1*, suggesting that the new gene was produced by DNA duplication, rather than by retrotransposition of a processed RNA transcript. The authors considered the alternative hypothesis, that the two colobine sequences represent two alleles at a polymorphic locus rather than a duplication event, but deemed it implausible given the large (7.8%) divergence at the level of protein sequence.

Comparisons of colobine *RNASE1* and *RNASE1B* with the *RNASE1* of appropriate other primates as outgroups did not lead to rejection of the null hypothesis that these regions are evolving in accordance with a molecular clock, and suggested that the gene duplication occurred approximately 4.2 million years ago, about 10.8 million years after the divergence of the colobines from their closest relatives, the cercopithecine monkeys. In contrast to the non-coding regions, coding regions of *RNASE1B* revealed a high ratio of amino-acid replacement to silent nucleotide changes (K_a/K_s), suggesting positive selection. The rate of radical changes (amino-acid substitutions resulting in a change of the charge of the protein) was in vast excess of the rate of conservative changes. This pattern is inconsistent with the typically conservative mode of substitution in mammalian proteins. Moreover, all seven of these substitutions contributed to an increase in the negative charge of the protein, resulting in a net change in charge from +8.8 to +0.8 at neutral pH. This provides strong evidence of directional selection favoring negative charge.

Functional assays revealed that the colobine-specific RNASE1B achieves maximal efficiency at pH 6, which is within the pH range of the colobine foregut. This is in contrast to RNASE1, which functions optimally at the pH 7.4 characteristic of the human small intestine. Thus, the preponderance of negative substitutions in the evolution of RNASE1B seems directly related to the enzyme's role in the low-pH colobine foregut. By contrast, site-directed mutagenesis revealed that seven of the nine substitutions that distinguish the sequence of *RNASE1B* from that of *RNASE1* reduce its efficiency in degrading double-stranded RNA, a task of the ancestral enzyme. It appears that the evolutionarily innovative features of RNASE1B arose at the direct expense of efficiency in its ancestral function.

Reporter's comments

A plausible story for the evolution of *RNASE1B* emerges from the authors' data. Colobine monkeys diverged from the cercopithecines around 15 million years ago, and leaf-eating emerged among the colobines roughly 5 million years later. *RNASE1B* was produced via gene duplication, and was apparently maintained from the start by purifying selection, as it increased ribonuclease concentration, a fitness-conferring trait for gut fermenters. Mutations that diminished the enzyme's ability to degrade double-stranded RNA but did not increase negative charge were presumably either neutral or selected against; in turn, mutations that diminished RNA-degrading activity but increased the protein's negative charge and thus its efficacy at lower pH were selected for, as RNASE1 still retained the capacity for the ancestral function of degrading double-stranded RNA. This resulted in accumulation of a set of radical substitutions in RNASE1B.

As well as revealing a fascinating story about the molecular processes underlying the evolution of the colobine monkey subfamily, Zhang *et al*. have used sequence analysis and functional assays to reveal details of the natural history of a species. Such studies stand to contribute to our understanding of the mechanisms responsible for the preservation of gene duplicates, and other major processes in genome evolution.

Table of links

References

1. Zhang J, Zhang Y, Rosenberg HF: Adaptive evolution of a duplicated pancreatic ribonuclease gene in a leaf-eating monkey. Nat Genet. 2002, 30: 411-415. 1061-4036