

might provide more than just markers for tumor subsets. Perhaps studying colon cancers with CIMP versus those clustered for other hypermethylated genes, within the context of cell renewal events, might help define the cellular origins of subsets of colorectal (Fig. 1) and other cancers. As we argue the merits of CIMP, we may emerge wiser about the origins of cancer, and this is yet more good news for basic and translational epigenetic research.

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## Monkey see, monkey do

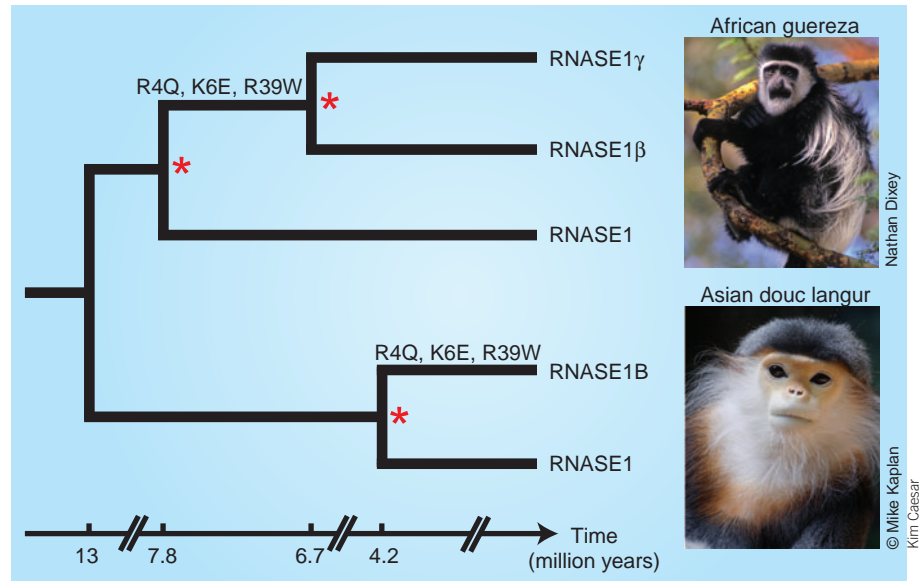
Benjamin Prud'homme & Sean B Carroll

**A new study shows that the independent adaptation to a ruminant lifestyle in two leaf-eating monkeys relied on parallel amino acid substitutions in ribonuclease gene duplicates. This discovery suggests that, given similar initial conditions, proteins may repeatedly follow similar adaptive evolutionary paths.**

The precise course of evolution is certainly unpredictable. Nevertheless, repeated patterns and trends are observed in the living world<sup>1</sup>. For instance, all kinds of cave-dwelling animals are commonly blind and unpigmented, and island species are generally reduced in size compared to their continental close relatives. Other famous examples of repeated evolution include the origin of flippers in penguins and dolphins; of wings in birds, bats and pterosaurs; and of similar pigmentation patterns among insects and vertebrates. While evolution is immensely creative, the pervasiveness of repeated evolution suggests that some particular phenotypic 'solutions' may be easier (i.e., more likely) to evolve than others in response to similar selective conditions<sup>2</sup>. How, then, does this translate at the genetic and molecular level? To what extent is the course of adaptive evolution constrained or channeled to preferentially follow certain mutational paths? On page 819 of this issue, Jianzhi Zhang sheds light on these questions and reports on the independent adaptive evolution of duplicate pancreatic ribonucleases that followed parallel evolutionary paths in leaf-eating monkeys<sup>3</sup>.

### Independent digestive adaptations

Asian and African colobine monkeys have a specialized diet of leaves. Similar to other ruminants, they use symbiotic bacteria in their intestine to



**Figure 1** Parallel adaptive amino acid substitutions in ribonuclease duplicates in two leaf-eating monkeys. Duplications of a ribonuclease gene occurred independently in the Asian douc langur and the African guereza lineages (red stars). The fine-tuning of the duplicates' activity evolved through parallel amino acid substitutions (indicated on branches). Images courtesy of Nathan Dixey and Mike G. Kaplan (<http://www.mikekaplan.com>).

ferment the leaves and then further process the metabolic compounds recovered from the bacteria with various enzymatic activities. One key enzyme in this process is a pancreatic ribonuclease (RNase) that is transported to the acidic environment of the small intestine where it degrades the large amount of RNA released by bacteria, allowing the recycling of nitrogen.

Zhang discovered that, while non-colobine primates have a single copy of the RNase gene, the Asian colobine, the douc langur, has two copies<sup>4</sup> and the African colobine guereza has

three copies of the gene. Phylogenetic and statistical analyses clearly indicate that independent duplications of an ancestral RNase gene occurred in each colobine group after the African and Asian lineages diverged 13 million years ago (Fig. 1). In each colobine lineage, one of the duplicates, RNASE1, remained almost or completely unchanged and thus retained a nondigestive function of degrading double-stranded RNA (dsRNA), presumably an antiviral mechanism acting in many tissues.

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In contrast, the other duplicates, RNASE1B in douc langur and RNASE1 $\beta$  and RNASE1 $\gamma$  in guereza, became highly expressed in the pancreas and experienced more amino acid substitutions than expected under neutral evolution, a strong signature of positive darwinian selection. Strikingly, three parallel amino acid substitutions occurred in the two lineages (Fig. 1), a pattern that chance alone cannot explain. *In vitro* functional assays showed that most of the amino acid replacements, and in particular the three parallel substitutions, dramatically affected the catalytic activity of the enzyme on dsRNA but also shifted the pH for the maximal ribonucleolytic activity from 7.4 to 6.3–6.7. Hence, the parallel amino acid substitutions fine-tuned the activity of the enzyme to the relatively low pH of the colobine's small intestine.

Altogether, these results strongly suggest that a suite of similar changes evolved independently in African and Asian colobines (gene duplications, preservation of the essential nondigestive function, regulatory changes driving strong pancreatic expression and amino acid replacements including three parallel substitutions) as an adaptive response to a common selective pressure for a high ribonucleolytic activity in an acidic environment resulting from a ruminant lifestyle.

### Molecular evolution repeated

Evolution results from the interplay between chance (random mutations) and necessity (directional selection). At the molecular level, the probability of evolution repeating itself as

a response to a common selective condition depends on the mutational target size and the functional consequences of the mutations. Obviously, many different mutational events can disrupt the function of a particular coding sequence or *cis*-regulatory element, and thus, it is not unexpected to find instances where the independent inactivation of gene function has occurred by different mutational paths<sup>5–7</sup>. However, when it comes to the fine-tuning of existing function or creating a new one, a growing body of work suggests that the range of enabling mutations might be more limited and depend on the structure and the history of the molecule<sup>8–10</sup>.

Many molecules have strong structure–function requirements. For instance, the photosensitive opsin molecules are tuned to particular wavelengths by specific key residues. As a result, the independent evolution of color vision in primates<sup>11</sup> or ultraviolet vision in distinct bird lineages<sup>12</sup> occurred through repeated changes of precisely the same amino acids. In contrast, for other molecules, different amino acid changes can have similar functional consequences. As in colobine monkeys, the RNase gene of barnyard ruminants, such as cows, is duplicated, and one copy is fine-tuned to the acidic environment of the intestine. However, independent adaptations in monkeys and cows resulted from different amino acid substitutions, and functional studies have shown that parallel changes would not necessarily have had the same effect in both lineages<sup>13</sup>.

All proteins in every lineage accumulate different sets of amino acid substitutions that do

not alter the function of the molecule. However, these neutral changes can affect the repertoire of mutations that may enable the evolution of new functions, thus constraining the mutational trajectories of adaptive evolution<sup>10,14</sup>. Therefore, it is expected that the more divergent the sequences of two proteins are, the more likely it is that they will independently acquire a similar function through different amino acid substitutions. Conversely, when different protein sequences are very similar and independently face a strong pressure for a specific requirement, the chances are greater that they will repeatedly follow the same adaptive evolutionary path. While replaying the tape of phenotypic life has been proposed to produce a different story<sup>15</sup>, the tape of protein evolution might be more reproducible and predictable than previously appreciated<sup>10</sup>.

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## GATA1s goes germline

Gina Mundschau & John Crispino

**Acquired somatic mutations in the transcription factor *GATA1* are a defining feature of acute megakaryocytic leukemia in children with Down syndrome. A new study shows that similar inherited *GATA1* mutations do not promote leukemia in the absence of trisomy 21 but lead to defects in multiple hematopoietic lineages.**

The discovery that *GATA1*, a gene on the X chromosome encoding a blood-specific transcription factor, is mutated in virtually every case of acute megakaryocytic leukemia (AMKL) in children with Down syndrome (DS) provided the first insight into the molecular basis of this com-

plex disease<sup>1</sup>. Characteristic mutations abrogate expression of full-length *GATA1* protein and lead to the production of a shorter isoform, *GATA1s*<sup>2–5</sup>. What has remained unclear is the functional interplay between *GATA1* mutations and trisomy 21 in normal and malignant hematopoiesis. On page 807 of this issue, Luciana Hollanda and colleagues<sup>6</sup> report the remarkable discovery of a family with a germline *GATA1* mutation similar to those seen in DS-AMKL. Affected males generate only the *GATA1s* isoform and exhibit anemia and trilineage dysplasia, but they do not

develop leukemia. This valuable finding demonstrates that *GATA1* mutations of this type are insufficient to initiate leukemogenesis in the absence of trisomy 21, and it provides insight into the function of *GATA1s* in hematopoiesis and leukemogenesis (Fig. 1).

### Coming up short

Normal human hematopoietic cells express both full-length *GATA1* and *GATA1s*<sup>7</sup>. The full-length protein contains an N-terminal transcription activation domain and two zinc

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