43-Million-Year-Old Gene Fusion Likely Protected Primates from Viruses

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Paleovirology is a relatively new branch of Evolutionary Biology dedicated to the study of ancient viruses and their impact on host biology. Host genomes are amazing adept at challenging viral infection both short-term (immediate immune responses) and long-term (co-evolution with viral proteins). The existence of ancient genomic changes in host anti-viral factor encoding genes can have strong implications about the viruses that infected the primate lineage. These genomic changes, usually advantageous at the time of their birth, helped to shape the modern primate immune system.

Of the plethora of genes that contribute to a host's innate immune response to viral infection, the protein encoded by the *TRIM5*gene is of particular importance. *TRIM5* is a retroviral restriction factor that limits the life cycle of several retroviruses by binding the viral capsid protein. Modifications in the*TRIM5* locus in a range of primate and non-primate species throughout evolution has made *TRIM5* better able to inhibit viral infection. These modifications include point mutations in the capsid-binding coiled-coil domain of *TRIM5*, as well as novel *TRIM5*gene fusions, that allowed TRIM5 to retain robust capsid binding. *TRIMCypA1*, a recently discovered *TRIM5* gene fusion event with retrotransposed *Cyclophilin A (CypA)* that was identified in owl monkeys, occurred 4.5-6 million years ago (Mya). This *TRIM5*chimeric protein has potent activity against several retroviruses including HIV-1. Another *TRIMCyp* gene fusion, *TRIMCypA2*, occurred in macaque species around the same time period to generate a second independent *TRIM5* chimera. These fusions of TRIM5 with CypA enhanced the capsid binding activity of TRIM5, giving rise to a novel antiviral defense activity. A recent paper published by the Malik and Emerman Labs (Basic Sciences Division) reveals two additional *TRIMCyp* fusions that predate*TRIMCypA1* and *TRIMCypA2* by nearly 37 million years.

To determine if other *TRIMCyp* gene fusions existed in primate genomes, Malfavon-Borja *et. al.* sequenced the *TRIM5* locus from primate fibroblast cells for the presence of *CypA* retrogenes. Along with *CypA2* (present exclusively in macaques), the authors were surprised to discover two additional *TRIM* gene fusions they named *CypA3* and *CypA4*. Similar to *CypA2*, *CypA3* was present in the macaque species examined but was also found in the more ancestral gibbons lineage. Since both gibbons and Old World monkeys have *CypA3* retrogenes, this suggested that the

ancestral *CypA3* retrogene was acquired in primates at least 32 Mya based on phylogeny. However, further phylogenetic analysis revealed two additional features of this novel gene fusion: (1) *CypA3*was at least 43 million years old and (2) likely functioned for 10 million years before it was pseudogenized.

Given the genomic presence of *TRIMCypA3*, it was likely that this *TRIMCyp* gene fusion was transcribed to produce a protein with novel anti-viral activity. The researchers probed total mRNA isolated from primate fibroblasts by RT-PCR and found *TRIMCypA3*transcripts from a variety of species. Although present, the *TRIMCypA3* sequencing data revealed deleterious stop codons in*TRIMCypA3* from present-day macaques. The presence of pseudogenizing mutations in *TRIMCypA3* acquired since its birth indicated that its function as a viral restricting factor had ceased to be necessary in primates as time advanced. However, the expression of a nearly full-length *TRIMCypA3* transcript in gibbons indicated that the chimeric protein might once have had a viral restricting function.

In a final line of investigation, the researchers expressed the *TRIMCyp* chimeras in tissue culture cells and tested their ability to inhibit viral infection by ancient paleolentiviruses and modern lentiviruses (e.g. HIV-1 and HIV-2). For these experiments, owl monkey *TRIM5* was fused to a *CypA3* sequence deemed to be present based on phylogenetic analyses 32 Mya (TRIM5-32myoCypA3) or to the parental *CypA* sequence from which *CypA3* was derived (TRIM5-parental CypA; see figure). Astonishingly, TRIM5-parental CypA displayed a broad and potent antiviral activity, but TRIM5-32myoCypA3 did not show antiviral activity against any of the lentiviruses tested. These results indicate that the most ancient TRIM5-CypA fusion protein could restrict a broad range of retroviruses, but that subsequent evolution refined its activity to a limited number of ancient retroviral capsids. Thus, *TRIMCyp3* likely arose to combat a viral pathogen encountered by primate ancestors 43 Mya.

Altogether, the research by Bordia *et. al.* confirm that studying the genetic changes that occurred in host retroviral restriction factors throughout evolution can shed light on viral encounters that challenged primate health millions of years ago. The techniques and ideas generated by the researchers will fuel paleovirology, a burgeoning field of science less than a decade old. When asked about future directions of research, Dr. Malik states “We are pursuing other retrogene insertions in the human and related genomes. While these have been previously ignored/dismissed as pseudogenes, it is clear that among them might reside powerful new antiviral functions."

Malfavon-Borja R, Wu LI, Emerman M, Malik HS. 2013. Birth, decay, and reconstruction of an ancient *TRIMCyp* gene fusion in primate genomes. *Proc Natl Acad Sci*. 110: E583–E592.

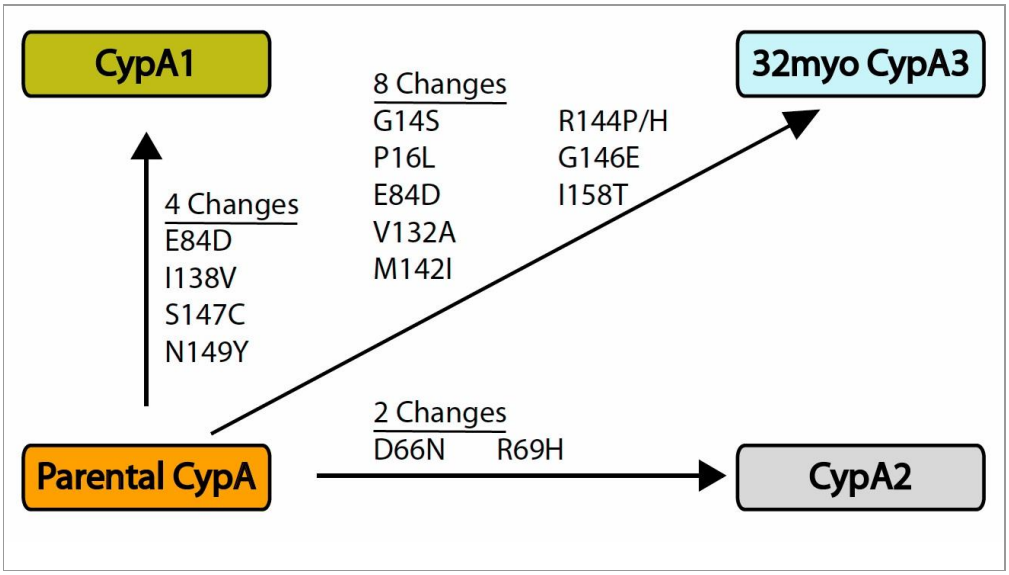

Image obtained from the manuscript

Genotyping and phylogenetic analysis revealed amino acid changes in parental CypA that occurred throughout evolution. Four point mutations in CypA1 narrowed the broad antiviral activity of parental CypA. With similar logic, Malfavon-Borja et. al. argue that the 8 amino acid changes in the novel CypA3 gene fusion they have identified generated a viral restrictive factor with specificity for a limited number of (ancient) viruses.