Ancestral capture of syncytin-Car1, a fusogenic endogenous retroviral envelope gene involved in placentation and conserved in Carnivora

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AUTHOR SUMMARY

In most mammals, an essential component of the placenta is a continuous layer of cells fused together into a structure known as the syncytiotrophoblast (1). Mechanisms underlying its formation remained mysterious until the discovery of genes of retroviral origin, named syncytins (2). Syncytins are envelope (env) genes captured from ancestral retroviruses that integrated into the chromosomes of the infected host and have been transmitted in a Mendelian manner since that time (3) (Fig. P1). These genes have retained the capacity to trigger cell membrane fusion. Only five such genes have so far been characterized in higher primates, muroids (the mouse), and the rabbit. We previously showed through genetically modified animals that the mouse syncytin-A and syncytin-B genes drive the formation of the two mouse syncytiotrophoblast layers and that they are essential for embryonic development (4, 5).

Therefore, these genes raise a paradox. They were acquired by chance, and yet, they are necessary for a fundamental function in some mammals—the development of the placenta. We also propose that these genes were pivotal in the emergence of placental animals, which implies that syncytins should be found in all such mammals. We offer support for this hypothesis by identifying syncytin genes in animals not previously shown to possess them and belonging to the Laurasiatheria superorder, namely cats and dogs.

In the present study, we have searched for syncytin genes within a major clade of the Laurasiatheria superorder, namely the Carnivora. Evolutionarily, the Laurasiatheria diverged from the Euarchoontoglires, mammals in which syncytins have previously been found, about 100 Mya in the Cretaceous Era. The genomes of two Carnivora representative species—the dog and the cat—have been entirely sequenced, aiding these investigations. Moreover, placentation can be extensively studied in these two species thanks to the availability of appropriate biological tissues. We searched for candidate syncytin genes in their genomes using in silico computer analyses based on some specific signatures contained in all env genes. In this analysis, we identified 11 putative retroviral env genes within the dog and cat genomes. One of these genes, syncytin-Car1, in each species was shown to operate specifically in the placenta, which was assayed using quantitative RT-PCR, a method for amplifying the levels of genetic material so that they may be measured.

The identified env gene proved to be integrated at precisely the same—orthologous or corresponding—position within both...
species’ genomes. It was also found to be conserved at the same position in all of the 26 Carnivora species that we further investigated for this gene, indicating that it was captured ~80 Mya (Fig. P1). A refined analysis of the ratio of nonsynonymous to synonymous mutations in these 26 orthologous genes proved that they have been subject to purifying selection (i.e., the removal, over the course of evolution, of gene variants that harm fitness). This finding is expected for a bona fide gene with a physiological role. We next showed that they still behave as fusogenic (i.e., eliciting membrane fusion) retroviral env genes, because they could substitute for the env gene of present day retroviruses in pseudotype infection assays. Finally, in situ hybridization experiments carried out on dog and cat placenta sections showed specific expression of the gene in the fused syncytiotrophoblast layer within the maternofetal interface (Fig. P1). This finding is consistent with a role for this gene in the formation of the Carnivora placenta.

We, therefore, identified a gene in Carnivora that possesses all of the characteristic features of a bona fide syncytin: it encodes a fusogenic protein of retroviral origin, it is specifically expressed in the placenta, and it has been conserved over 80 My of evolution. The syncytin-Car1 gene is the oldest syncytin gene identified to date.

Altogether, our results strongly support the notion that retroviral infections have resulted in several independent captures of genes that were then positively selected for during convergent evolution. It also shows that genomic and genetic analyses cannot assume that all mammalian genes evolved from primitive genes common to most living species. Analysis must also take into account the stochastic de novo acquisition of exogenous genes from parasites, which are possibly the source of dramatic evolutionary changes. The transition from egg-laying animals to placental mammals might be one illustrative example of such a genomic “coup de force.”